silenciamiento tanto simple shFOXO, como doble shRNA/shFOXO produce igualmente un aumento en la expresión de FUS. Sin embargo, no se ven diferencias de expresión de FUS entre el silenciamiento doble shFUS/shFOXO con respecto al silenciamiento simple shFUS.

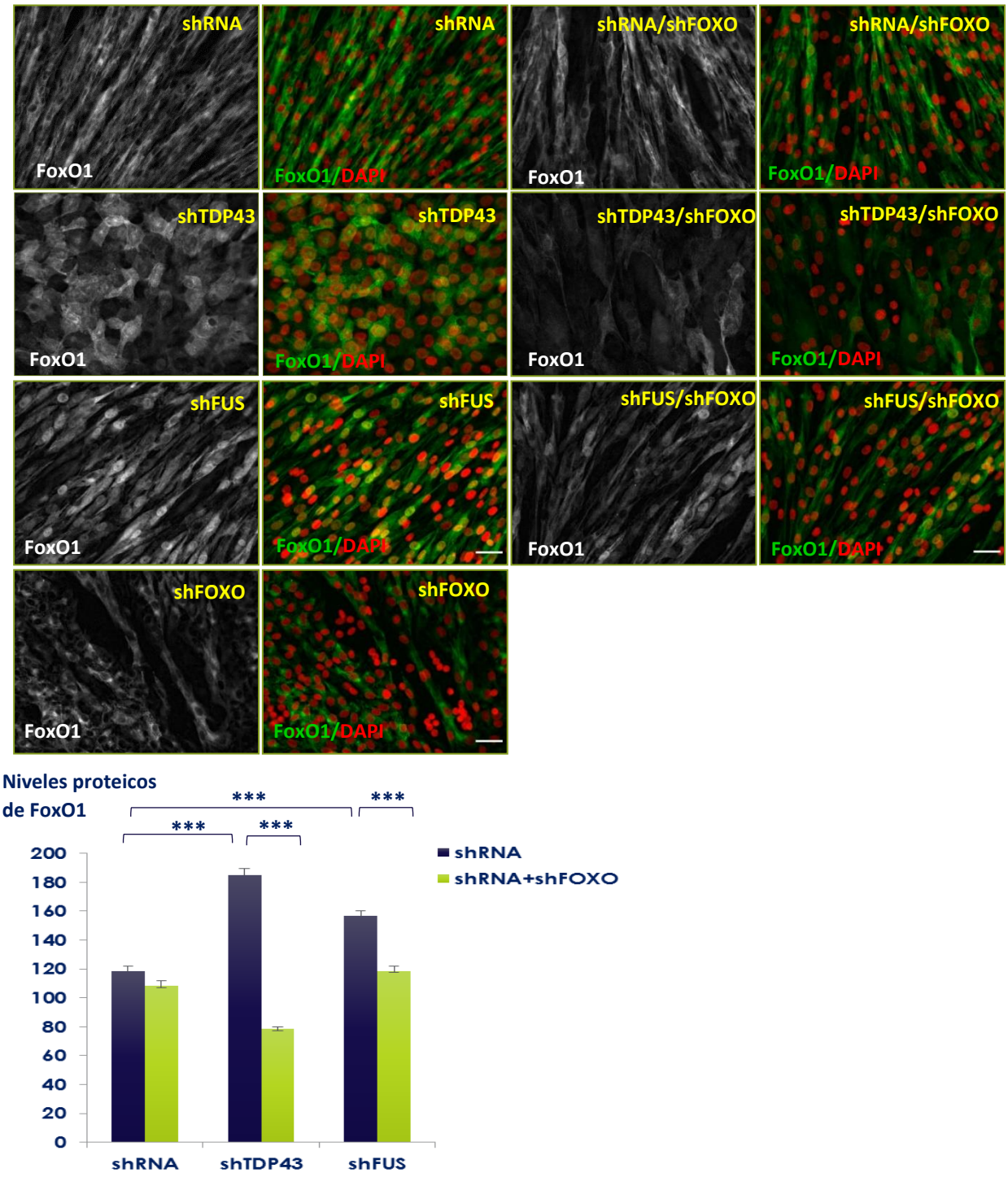

Figura 5.15. Análisis de la expresión de FoxO 1 en las líneas con silenciamiento tanto simple como doble shRNA, shRNA/shFOXO, shTDP43, shTDP43/shFOXO, shFUS, shFUS/shFOXO mediante Image J. Análisis de 40 célulass por grupo (n=3). FoxO1 (blanco/verde), DAPI (rojo). Prueba t-Student, (n=3) *p<0,05. **p<0,01. ***p<0,001. Escala 38 µm.

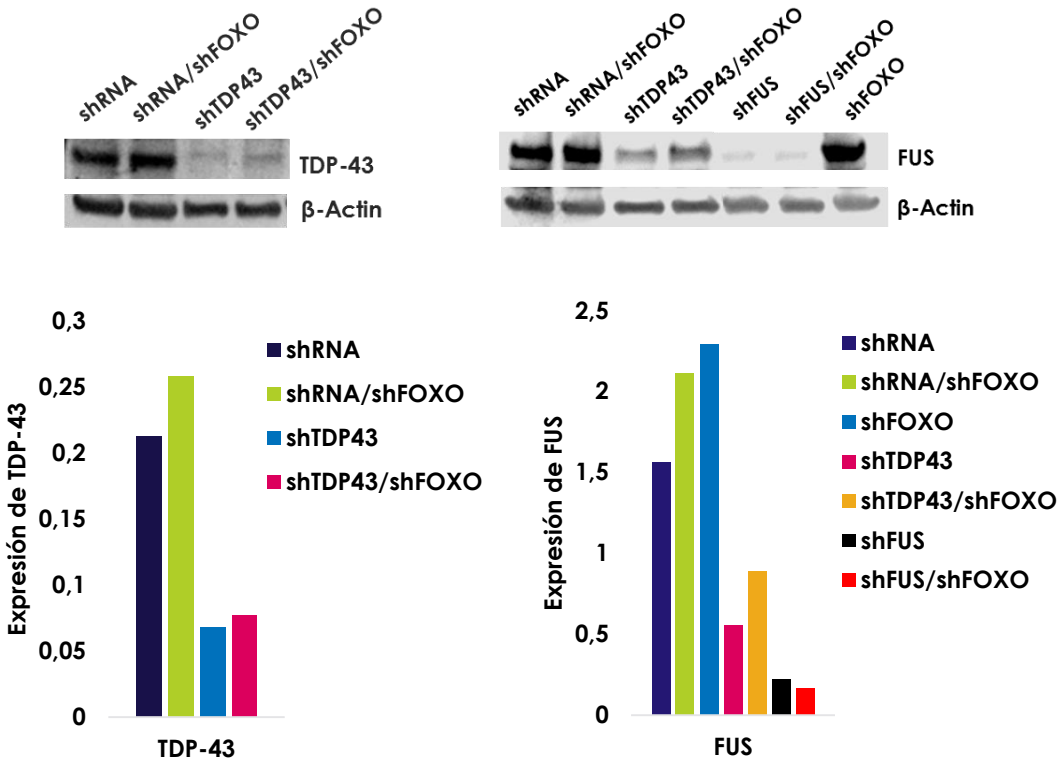

Figura 5.16. Análisis mediante Western blot de las proteínas silenciadas y la relación existente entre TDP-43, FUS y FoxO, (n=2).

Con respecto a la capacidad de diferenciación, el tratamiento con shFOXO, que es específico para los genes *FOXO* 1, 3 y 4, aumenta el índice de fusión en las líneas shTDP43 y shFUS de forma significativa. En la línea shTDP43 el cambio es el más dramático: de un índice de fusión nulo, se pasa a una fusión del 21% gracias a la inhibición génica de FoxO. Este hecho indica que el silenciamiento de los genes *FOXO* restaura la capacidad de diferenciación que se pierde al silenciar *TARDBP* y, por tanto, sugiere que ambos genes, *TARDBP* y *FOXO,* en la regulación de la diferenciación muscular, están mecanísticamente relacionados. Por otra parte, teniendo en cuenta que el silenciamiento de FUS produce un defecto en diferenciación, que el silenciamiento de *TARDBP* disminuye la expresión de la proteína FUS, y que el silenciamiento de *FOXO* en shTDP43 aumenta la expresión de FUS, se puede hipotetizar que existe una relación directa entre TDP-43 y FUS a través de FoxO. Asimismo, el silenciamiento de *FOXO* en shFUS aumenta

significativamente el índice de fusión de mioblastos desde un 18% hasta un 28%, con lo que, de forma independiente a TDP-43, la inactivación de *FOXO* en shFUS duplica la capacidad de diferenciación en dicha línea **(figura 5.17 y 5.18)**. El silenciamiento de *FOXO* en la línea control shRNA no aumenta significativamente el índice de fusión.

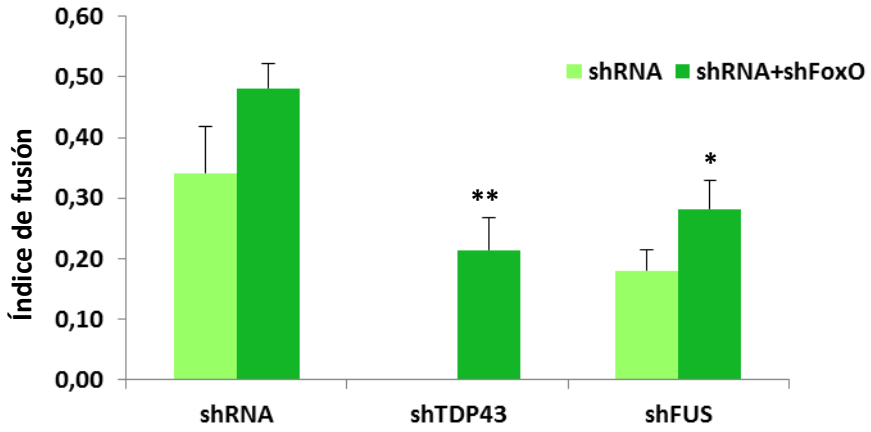

Figura 5.17. Aumento del índice de fusión con el silenciamiento de *FOXO* en mioblastos con silenciamiento de TDP43 y FUS. Prueba t-Student *p<0,05. **p<0,01. ***p<0,001, (n=4).

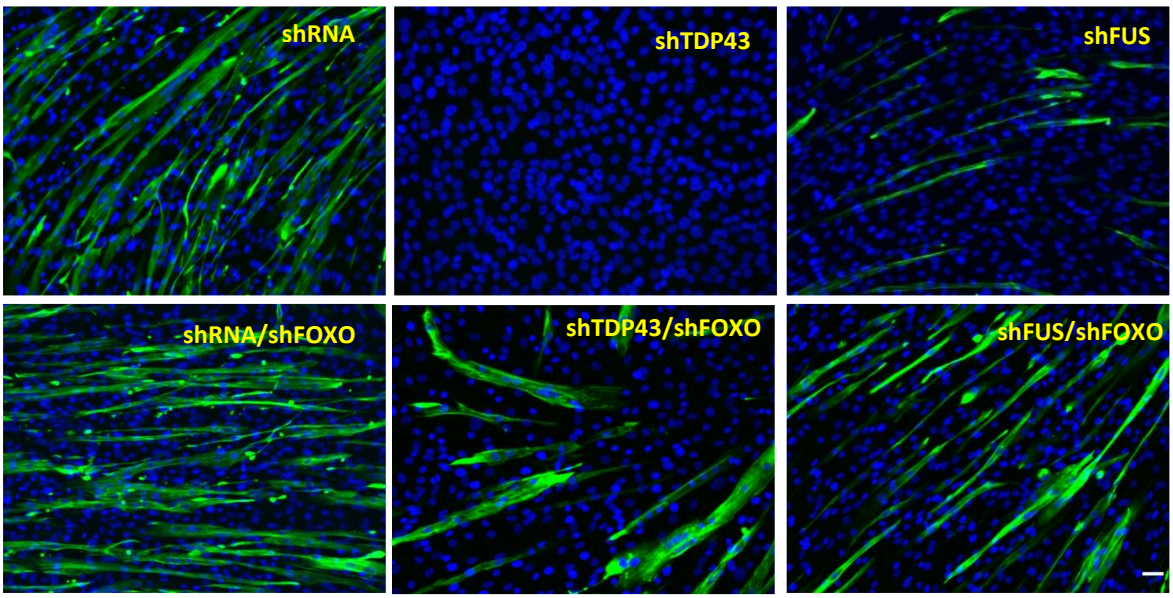

Figura 5.18. Inmunofluorescencia del marcador de miotubos MyHC (verde) y DAPI (azul) en las líneas silenciadas de TARDBP y FUS con respecto al control de silenciamiento. Escala 20 µm.

En las imagénes de microscopía óptica se observa la coexistencia de mioblastos, miocitos y miotubos de la línea silenciada doblemente para *TARDBP* y *FOXO* (**figura 5.19**; flechas rojas). Sin embargo, en el silenciamiento simple de *TARDBP*, únicamente se observan mioblastos en (flechas azules). Estas diferencias fenotípicas no se explican por los niveles de TDP-43, puesto que la expresión de TDP-43 es similar en las líneas shTDP43 y shTDP43/shFOXO (alrededor de un 75% de reducción de TDP-43; **figura 5.16**). Por otro lado, en el silenciamiento simple de *FOXO*, los miotubos son más gruesos en comparación con el control shRNA (flechas blancas) **(figura 5.19).**

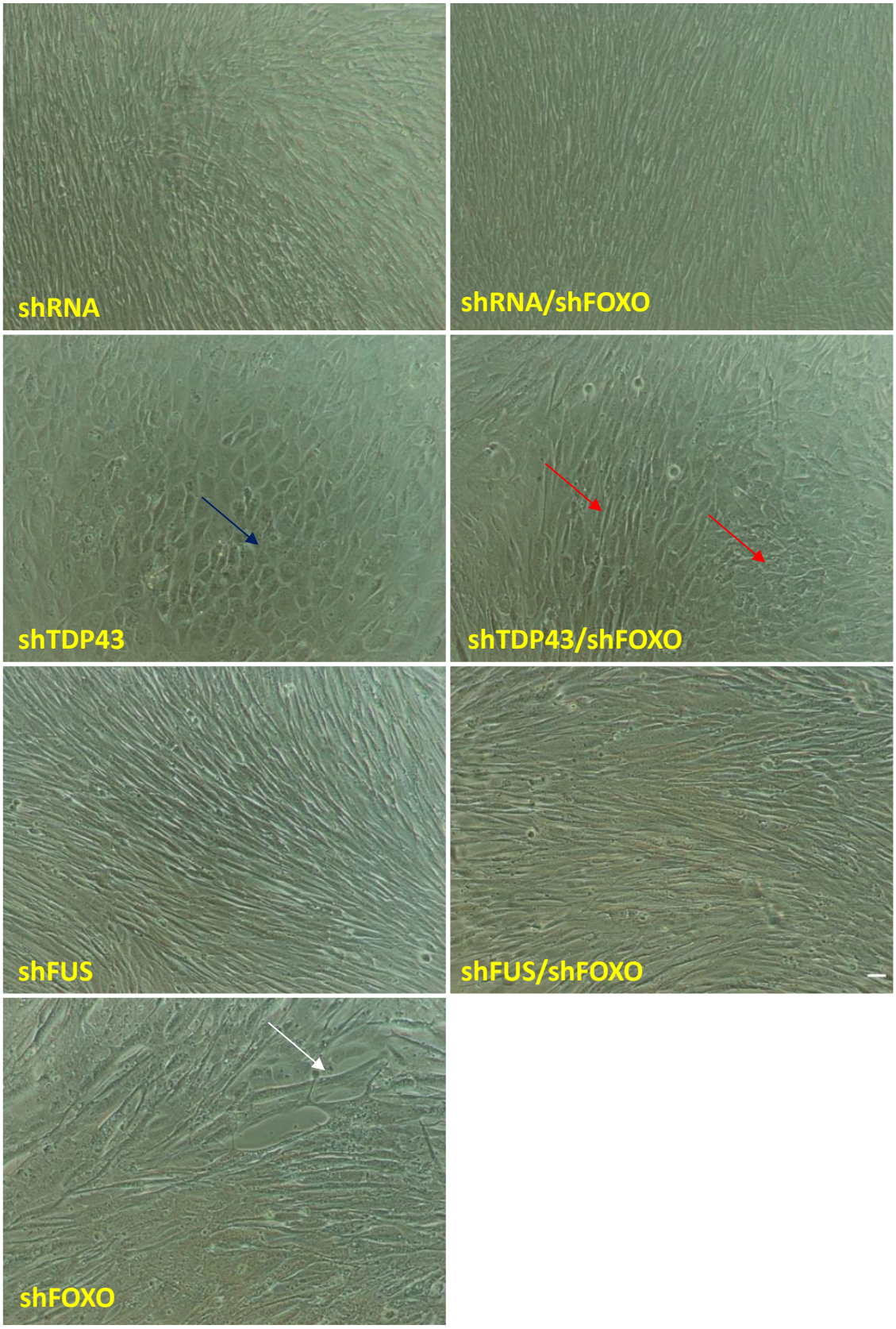

Figura 5.19. Diferenciación celular vista por microscopía óptica de simples y dobles silenciamientos. Las mioblastos defectivos en FoxO1 y FoxO3 son más gruesos (flechas blancas). Presencia de dos estadías de diferenciación simultáneamente en shTDP43 (flechas rojas). Mioblastos sin alineación (flechas azules). Escala 19 µm.

Discusión

1. El tejido muscular tiene como predominante el metabolismo glucolítico y puede ser un buen modelo para estudiar los cambios metabólicos en la ELA

2. El silenciamiento de los genes mendelianos de la ELA induce una profunda perturbación metabólica-energética muscular que afecta preferentemente a la glucolisis

3. La pérdida de función de TDP-43 en el músculo está relacionado con las vías de señalización pro-atrofia independiente de la denervación

4. Los genes mendelianos relacionados con la ELA están implicados en el proceso de diferenciación muscular

5. Los factores de transcripción FoxO están relacionados con el papel de TDP-43 en el músculo y pueden tener relación con la ELA

6. La inhibición de FoxO1 y FoxO3 puede ser una potencial diana terapéutica para el tratamiento de la ELA

7. Proyecciones de futuro

1. EL TEJIDO MUSCULAR TIENE COMO PREDOMINANTE EL METABOLISMO GLUCOLÍTICO Y PUEDE SER UN BUEN MODELO PARA ESTUDIAR LOS CAMBIOS METABÓLICOS EN LA ELA

En este trabajo hemos podido demostrar la importancia de la activación metabólica, tanto la vía glucolítica como la oxidativa, a lo largo del proceso de diferenciación muscular y, de manera más específica, el aumento del potencial metabólico glucolítico en el momento de fusión celular. En 2008, Fulco y colaboradores, ya demostraron que la correcta funcionalidad de la glucolisis era esencial para permitir la diferenciación celular siendo la vía precursora de intermediarios metabólicos para el proceso de síntesis proteica necesaria para dicha diferenciación (Fulco *et al.*, 2008).

La glucolisis es el proceso catabólico de la glucosa catalizado por múltiples enzimas que actúan secuencialmente y que está regulada por la disponibilidad de glucosa, por los niveles hormonales de insulina, adrenalina y glucagón (Boiteux and Hess, 1981; Melkonian and Schury, 2019), y por la actividad de sus enzimas mediante modificaciones covalentes y regulaciones alostéricas. En el alosterismo, una molécula se une de forma no covalente a la enzima e induce un cambio de conformación espacial de la misma, de tal manera que expone u oculta su sitio de unión al sustrato regulando así el proceso de catálisis (Monod, Wyman and Changeux, 1965).

En esta investigación, hemos confirmado la idea de que en la ELA podría haber un problema en el metabolismo de la glucosa ya que los pacientes estudiados presentan evidencias clínicas muy sugestivas de una alteración en la glucolisis. También, hemos utilizado como modelo de estudio los efectos del silenciamiento de una serie de genes (*TARDBP, FUS* y *SOD1*) implicados en formas mendelianas de la esclerosis lateral amiotrófica (ELA) en el tejido muscular tanto a nivel de los procesos metabólicos como en sus efectos sobre el proceso de diferenciación. Esto se ha hecho tanto en líneas de mioblastos humanos como en un modelo en *Drosophila* con un silenciamiento parcial de estos genes en tejido muscular *(datos del grupo, no publicados)*. Además, se han estudiado ambos fenómenos, metabolismo

energético y diferenciación miogénica, en dos muestras de tejido muscular de pacientes, uno con una forma familiar de ELA y otro con una forma esporádica. En todos los casos y, mediante diferentes técnicas, se han estudiado los mecanismos moleculares por los que la pérdida de función de los genes *TARDBP, FUS* y *SOD1* generan cambios en el tejido muscular, presumiendo que estos cambios podrían estar presentes en el músculo de pacientes con ELA y que podrían ser, en algunos casos, muy precoces y previos a la afectación de la motoneurona (MN).

Como limitación del modelo hay que recordar que a diferencia de lo que ocurre con las mutaciones en humanos (donde puede verse una gama diferente de efectos de las mutaciones con ganancia o pérdida de función) (Lee, Lee and Trojanowski, 2011b; Jucker and Walker, 2013, 2018; Koppers *et al.*, 2015b; Walker and Jucker, 2015; Cascella, Capitini, Fani, Christopher M Dobson, *et al.*, 2016), el proceso de silenciamiento analiza el impacto de la pérdida de función completa (o casi completa, asumiendo que el silenciamiento no es del todo completo). Esta pérdida de función de estos genes reproduce un evento patogénico temprano de la ELA (Mancuso and Navarro, 2015), correspondiente a un proceso de vaciado nuclear de estas proteínas que ocurre en concomitancia con la formación de gránulos de estrés y eventualmente los agregados tóxicos. Realmente, tanto la formación de agregados tóxicos como la pérdida de función de TDP-43, FUS y SOD1, constituyen dos elementos interconectados en un ciclo secuencial en el tiempo que se retroalimenta, pero en el que es difícil determinar cuál de ellos es el evento inicial en el tiempo y cuál la consecuencia patológica.

2. EL SILENCIAMIENTO DE LOS GENES MENDELIANOS DE LA ELA INDUCE UNA PROFUNDA PERTURBACIÓN METABÓLICA ENERGÉTICA MUSCULAR QUE AFECTA PREFERENTEMENTE A LA GLUCOLISIS

En el modelo de silenciamiento hemos podido demostrar que los efectos del mismo, cuando se silencian *TARDBP* o *FUS*, son similares a los de una

inhibición farmacológica con 2-desoxi-D-glucosa (2-dg), y que este efecto genera una disfunción de los sistemas aerobio y anaerobio que son los encargados de mantener los niveles de NAD^+ en el citoplasma de la célula. Hemos confirmado igualmente en los dos pacientes, el caso familiar y el esporádico, que los mioblastos obtenidos mediante biopsia de tibial anterior y deltoides, respectivamente, presentan defectos en la glucolisis así como en la capacidad mitocondrial de obtener energía. Esta afectación, que viene a confirmar los datos obtenidos en los experimentos de silenciamiento, podría explicar la pérdida de la capacidad de diferenciación (ver más adelante), y que ambas vías metabólicas podrían ser secundarias a la pérdida de función de TDP-43 o de FUS.

2.1. Alteraciones del metabolismo oxidativo

En el modelo celular de silenciamiento, los mioblastos defectivos en TDP-43, FUS o SOD1 presentan un metabolismo anaerobio y aerobio defectuoso, con niveles de ATP, tanto los obtenidos por la vía glucolítica como de la oxidativa, reducidos. El silenciamiento de *TARDBP* es el que produce la alteración metabólica más notoria entre los tres genes estudiados. Esta depleción genera mitocondrias menos eficaces, las cuales presentan una menor eficacia en el acoplamiento electroquímico de la mitocondria. El desorbitado consumo máximo de O_2 en estas mitocondrias junto con los niveles de ATP obtenidos por unidad de O_2, indican que estas mitocondrias no son eficaces y que el gran consumo de O_2 se debe a que el gradiente electroquímico se disipa por otra vía diferente de la ATPsintasa. Este acoplamiento ineficaz podría ser la causa del gran número de mitocondrias, aunque de pequeño tamaño, observado mediante microscopia electrónica en los mioblastos defectivos para TDP-43, cuyo silenciamiento genera un aumento en la expresión del PGC1-a (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha), regulador de la biosíntesis de mitocondrias (Puigserver, 2005). Este hecho podría sugerir la existencia de una desregulación en proteínas mitocondriales responsables de este

desacople como posteriormente demostramos ya que el silenciamiento de *TARDBP* induce un aumento en la expresión genética de una proteína desacoplante que impide la entrada de protones por la ATPsintasa, la UCP3 *(uncoupled protein 3)* (Erlanson-Albertsson, 2003). Por otro lado, en ratones *SOD1*$^{(G83R)}$ en músculo no denervado, se han encontrado niveles de UCP3 elevados, por lo que podemos pensar que esta sobreexpresión de UCP3 en musculo esquelético podría formar parte constitutiva de la fisiopatología de la ELA (Dupuis *et al.*, 2003).

2.2. Alteraciones en la glucolisis anaerobia

No obstante, a pesar de la notoriedad de los cambios mitocondriales, las consecuencias del silenciamiento génico, concordantes con los hallazgos en las biopsias musculares de los pacientes, señalan una afectación preferente de la vía glucolítica. Este defecto glucolítico se puede explicar con la reducción de la expresión de los enzimas integrantes de los sistemas de comunicación metabólica entre la mitocondria y el citosol, las lanzaderas, encargadas de regenerar el NAD^+ citosólico. En los mioblastos defectivos en TDP-43 hemos encontrado niveles muy disminuidos del enzima citosólica glicerol 3-fosfato deshidrogenasa 1 (GPD1), que transforma la DHAP en glicerol 3-fosfato regenerando rápidamente el NAD^+ citosólico y que es crucial para la glucolisis en la reacción catalizada por la gliceraldehído 3-fosfato deshidrogena (MacDonald and Marshall, 2000). Este hecho explicaría por sí mismo la deficiencia glucolítica de estos mioblastos. No obstante, otros cambios de expresión en enzimas implicados en la regeneración del coenzima van en la misma dirección; así, la disminución de UCP2, enzima que transporta malato y oxalacetato de la mitocondria al citosol, reduce el abastecimiento de esqueletos tetracarbonados en el citoplasma necesarios para la regeneración de NAD^+ que se produce a través de la reaccion catalizada por MDH1 (malato deshidrogenasa 1), que junto con la enzima GOT1 (glutamatooxalacetato transaminasa 1) constituyen los enzimas de la lanzadera de malato-aspartato que a su vez,

también se encuentran disminuidas en estos mioblastos. El perfil metabolómico de estos mioblastos medido por cromatografía líquida de alta resolución (HPLC) y resonancia magnética nuclear (RMN), con niveles reducidos de los subproductos de las reacciones que catabolizan dichos enzimas, apoya este hecho. De igual manera, el transportador responsable de suministrar el a-cetoglutarato necesario para la reacción catalizada por GOT1, esto es, el transportador OGC1, está también notablemente reducido en los mioblastos defectivos en TDP-43, lo cual genera una nueva dificultad añadida para la regeneración del NAD^+ citosólico.

Tomadas en conjunto todas estas evidencias experimentales apuntarían a que un descenso del NAD^+ citosólico podría ser el responsable principal de la deficiencia glucolítica observada en los mioblastos tras el silenciamiento de *TARDBP*.

Una manera de vincular el defecto de función de TDP-43 con la alteración de la glucolisis podría derivar de la conocida función de esta proteína en el procesamiento, estabilización, transporte y traducción de los mRNAs. De la misma manera que los miogránulos necesarios para la regeneración muscular (diferentes de los gránulos de estrés), observados por Vogler y colaboradores, contienen TDP-43 y mRNAs de proteínas sarcoméricas para su traducción (Vogler *et al.*, 2018), se podría especular que dichos miogránulos también podrían contener los mRNAs de enzimas metabólicas, que desaparecerían tras el silenciamiento de *TARDBP* comprometiendo, así, el metabolismo glucolítico.

En el caso de los mioblastos defectivos en FUS, la glucolisis también está afectada aunque la cantidad de enzimas alteradas que participan en la regeneración del NAD^+ es menor que tras el silenciamiento de *TARDBP*, pudiendo ser compensatorio a la falta de FUS. Al igual que TDP-43, según los resultados, FUS regula la expresión de los enzimas GOT1 y MDH1, con lo que su defecto comprometería igualmente la regeneración de NAD^+.

Por otra parte, a pesar de que los niveles de lactato deshidrogenasa no están afectados, y por tanto la fermentación láctica no se encuentra aparentemente alterada en el silenciamiento de *FUS*, según los datos de

nuestro estudio, la entrada masiva de piruvato a la mitocondria debida a la sobreexpresión del transportador MPC1 podría generar una pérdida de sustrato para dicha enzima y, por tanto, afectar el flujo basal de la fermentación láctica, la cual es otra fuente importante de regeneración de NAD^+ citosólico.

A diferencia del mecanismo propuesto para explicar las consecuencias del silenciamiento de *TARDBP* y *FUS*, la vinculación de la depleción de SOD1 con la alteración de la glucolisis es más complicada de explicar. El hallazgo del efecto metabólico que la deficiencia de SOD1 produce en músculo no parece casual, en el sentido de que ya ha sido reportado que la pérdida de función de esta proteína genera problemas en el metabolismo de la glucosa en otros tejidos como el hígado (Wang, Jiang and Lei, 2012a). Dada la función de la proteína SOD1 en el aclaramiento del estrés oxidativo, los efectos de su depleción en la regulación del metabolismo energético podrían vincularse con un incremento de la oxidación de las proteínas y los ARNm codificantes de enzimas metabólicas, lo que impediría su correcta traducción a proteínas. Es sabido que los modelos animales *knock out* para SOD1 no reproducen los fenotipos motores con la misma intensidad que los modelos por sobre-expresión de SOD1 mutante, y de ahí que la patología asociada a SOD1 es comúnmente atribuible más a la toxicidad de los agregados citosólicos de SOD1 que a la pérdida de función de la enzima (Reaume *et al.*, 1996). Sin embargo, el silenciamiento de SOD1 produce denervación de la unión neuromuscular y axonopatía distal en ratones adultos (Flood *et al.*, 1999; Shefner *et al.*, 1999), hecho que invita a postular que la pérdida de función de SOD1, además de su agregación, podrían participar de forma conjunta en la denervación de la neurona motora. A esta conjetura le apoyan una serie de trabajos que demuestran pérdida de actividad dismutasa en la mayoría de las mutaciones de SOD1 asociadas a ELA (Saccon *et al.*, 2013), así como las concentraciones elevadas de productos derivados del estrés oxidativo que se han encontrado tanto en el líquido cefalorraquídeo (LCR), como en el suero como en los tejidos en pacientes de las formas esporádica y familiar (Pedersen *et al.*, 1998;

Bogdanov *et al.*, 2000; Simpson *et al.*, 2004; Mitsumoto *et al.*, 2008). A pesar de ello, nuestro trabajo no aporta datos para sostener un mecanismo concreto, por lo que para comprender mejor el papel de SOD1 en el metabolismo energético, sería necesario profundizar en el análisis de los enzimas clave para este proceso y establecer si la regulación de su expresión, es atribuible, y en qué proporción en cada caso, a las proteínas TDP-43, FUS y SOD1.

Tomados los datos en conjunto, se podría hipotetizar que el aumento de estrés oxidativo, causante de la formación de gránulos de estrés (Riancho *et al.*, 2014), provocaría una detención reversible de la maquinaria de traducción de los mRNAs codificantes de las enzimas metabólicas. Dado que el silenciamiento de *TARDBP* provoca una disminución de la propia proteína y de FUS, cuya posición funcional se encontraría aguas abajo de una misma vía metabólica, ambas estarían relacionadas con el proceso.

Otro hallazgo fundamental de nuestro trabajo, que viene a corroborar nuestra hipótesis, es el papel central de UCP2 en este proceso ya que su silenciamiento reproduce los hallazgos del silenciamiento de *TARDBP* y *FUS*, tanto por los niveles de ROS aumentados (dato no mostrado), como por los defectos metabólicos, con una reducción significativa del factor modulador de la diferenciación muscular, MyoD, lo que convierte a UCP2 en otra potencial diana para el tratamiento de la ELA.

En efecto, la función de UCP2 puede modularse bien farmacológicamente (Zhong *et al.*, 2018) o bien mediante compuestos lipídicos de cadena media (Lou *et al.*, 2014; Jez, Garlid and Jabu, 2018), por lo que una acción dietética usando compuestos lipídicos ricos en ácidos grasos saturados de cadena media tendría todo el sentido.

2.3. El papel de NAD^+ y sus posibilidades como diana terapéutica

Como se viene especulando, la resultante de todos estos procesos metabólicos en mioblastos con silenciamiento de genes ELA podría ser la reducción de la disponibilidad del NAD^+ citosólico. De hecho, el NAD^+ es un

elemento fundamental para el metabolismo anaerobio, y en general para la homeostasis energética de la célula; y se ha relacionado su deficiencia con los procesos de envejecimiento y neurodegeneración a través del metabolismo de las sirtuínas (Imai and Yoshino, 2013; Gariani *et al.*, 2016; Goody and Henry, 2018). Aunque no están del todo claros los mecanismos por los que la disminución de NAD^+ actúa en este contexto, parece que en la ELA podría ser por la pérdida de su capacidad neuroprotectora (Belenky, Bogan and Brenner, 2007; Fang *et al.*, 2016; Chini, Tarragó and Chini, 2017; Katsyuba and Auwerx, 2017).

El NAD^+ es un activador alostérico del enzima gliceraldehído 3-fosfato deshidrogenasa (G3PDH). También es un co-sustrato de las reacciones de acetilación y ADP-ribosilación (Goody and Henry, 2018). NAD^+ tiene también un papel en la diferenciación muscular a través de las sirtuínas, que es controvertido (Goody and Henry, 2018). Según algunos autores, la sirtuína 1 (SIRT1) inhibe la diferenciación de mioblastos a miotubos en condiciones de deprivación de glucosa (Fulco *et al.*, 2008). Por otro lado, diferentes autores sostienen que es la sirtuína 3 (SIRT3) la que presentaba un punto de máxima expresión en el momento máximo de confluencia con el inicio de la fusión, lo que sugiere que sería SIRT3 quien regulara los pasos iniciales de la diferenciación celular, más concretamente en el momento de fusión. La implicación de la SIRT3 en este proceso se validó en ese mismo trabajo, al comprobar que el silenciamiento genético de SIRT3 en células murinas, provocaba la anulación de la diferenciación celular con una marcada reducción en el índice de fusión de mioblastos, así como de la expresión de MyoD y miogenina (Myog) entre otras (Khalek *et al.*, 2014).

Por otro lado, restaurar los niveles de NAD^+ es algo factible a través de precursores del mismo como la nicotinamida ribosido (NR) (Schöndorf *et al.*, 2018; Yoshino, Baur and Imai, 2018). Los estudios preliminares con NR muestran que la administración oral de este precursor es segura y que su absorción es completa y lineal (Airhart *et al.*, 2017; Martens *et al.*, 2018; Elhassan *et al.*, 2019), alcanzándose valores significativos del compuesto tanto en suero como en plasma así como del NAD^+ en el tejido muscular

(Elhassan *et al.*, 2019). En el caso de la ELA, se ha publicado recientemente un estudio piloto que recoge una mejoría clínica o estabilización en algunos pacientes con ELA tratados con una combinación de NR junto con un antioxidante como el pterostilbeno, y un suplemento dietético con aceite de coco rico en ácidos grasos saturados de cadena media (de la Rubia *et al.*, 2019). Curiosamente estos ácidos grasos tienen la capacidad de inducir una sobreexpresión de UCP2 (Lou *et al.*, 2014).

Desde un punto de vista clínico, la implicación magna de la vía glucolítica en la ELA, que por otro lado hemos confirmado en los enfermos, podría ser una de las razones de la resiliencia de la musculatura oculomotora (MOE) a verse afectada en la ELA. Estas fibras musculares tienen una preferencia por el metabolismo oxidativo, lo que explicaría su escasa afectación en la ELA comparativamente con las miopatías mitocondriales donde están preferentemente afectados (Porter *et al.*, 2001). De hecho, en los músculos oculomotores extrínsecos, se expresa predominantemente la isoforma de la lactato deshidrogenasa que transforma el lactato en piruvato, la LDH-B (Andrade and McMullen, 2006), que es la isoforma propia de las estirpes celulares oxidativas, como las neuronas y el corazón, que utilizan el lactato como fuente de energía y no tanto la glucosa a través de la glucolisis (Doherty and Cleveland, 2013; Urbańska and Orzechowski, 2019).

2.4. Limitaciones derivadas del modelo. Vías futuras de estudio

A pesar de las evidencias experimentales que soportan la hipótesis de que los genes mendelianos de la ELA tienen un papel determinante sobre la glucolisis muscular, ello no demuestra que estos cambios sean los eventos primarios en la génesis de la enfermedad en humanos o que de existir, estos cambios sean previos e inductores de la muerte neuronal. Para poder demostrar lo primero no es factible hacerlo directamente en los pacientes con ELA esporádica ya que cuando manifiestan los síntomas es cuando son diagnosticados, cuando la muerte de las motoneuronas es ya patente y avanzada, pero quizás podría demostrarse en biopsias seriadas en

pacientes portadores pre-sintomáticos de familias con mutaciones ELA identificadas en esos genes. Alternativamente, para demostrar lo segundo, sería necesario comprobar los efectos de la inhibición de estos genes en un modelo completo de músculo con inervación (organoide), y ver si los cambios en la glucolisis muscular inducen de manera retrógrada cambios en la neurona con aparición de depósitos de TDP-43 neuronales. Además, si tenemos en cuenta que esta explicación patogénica puede no ser aplicable para todos los casos de ELA, sería de interés averiguar si existen cambios similares en el metabolismo energético de la glía que pudiesen, mediante un mecanismo alternativo similar, explicar la muerte de las motoneuronas alimentadas por esa glía.

3. LA PÉRDIDA DE FUNCIÓN DE TDP-43 EN EL MÚSCULO ESTÁ RELACIONADO CON LAS VÍAS DE SEÑALIZACIÓN PRO-ATROFIA INDEPENDIENTE DE LA DENERVACIÓN

En el caso del paciente con una ELA familiar observamos una disminución de TDP-43 nuclear en los mioblastos, que se correspondería con la pérdida de función, mientras que, en el caso de la ELA esporádica, apreciamos una sobreexpresión de TDP-43, pero con una localización anormal perinuclear. En este caso, la presencia de agregados citoplasmáticos, positivos para TDP-43 y14-3-3, una chaperona presente en los agregados citoplasmáticos de las MN en la ELA, en células que presentan simultáneamente una localización perinuclear de TDP-43 (Umahara *et al.*, 2016), fortalece la idea de la existencia de una afectación muscular intrínseca, causada por la proteinopatía TDP-43 de forma independiente a la denervación de la MN. Otros estudios recientes han confirmado la presencia de agregados citoplasmáticos de TDP-43 en la musculatura axial e incluso en células miocárdicas de pacientes con ELA (Cykowski *et al.*, 2018; Mori *et al.*, 2019). En relación a la localización perinuclear de TDP-43, se ha observado la misma localización, en forma de un halo rodeando al núcleo, en la miositis

por cuerpos de inclusión previo a la acumulación de TDP-43 en vesículas sarcoméricas similares a las identificadas también en los mioblastos del paciente con ELA esporádica (Salajegheh *et al.*, 2009).

Así como la presencia de estos agregados refuerza la posibilidad de que el músculo juegue un papel importante en la enfermedad, la localización cercana de estos agregados en forma de vesículas de secreción cerca de la membrana plasmática genera expectativas sobre un presunto modelo de propagación de la proteinopatía de forma anterógrada desde el músculo a la MN, tal y como describen los posibles modelos priónicos de propagación en la ELA (McKinley *et al.*, 1991; Liu-Yesucevitz *et al.*, 2010; Münch, O'Brien and Bertolotti, 2011; Jucker and Walker, 2013; Kim *et al.*, 2013; Ayers *et al.*, 2016; McAlary *et al.*, 2019; Prasad *et al.*, 2019).

4. LOS GENES MENDELIANOS RELACIONADOS CON LA ELA ESTÁN IMPLICADOS EN EL PROCESO DE DIFERENCIACIÓN MUSCULAR

Otra conclusión de nuestro trabajo es que aportamos un mecanismo por el que se podrían los explicar los problemas en la diferenciación muscular que presentan los pacientes con ELA. Diversas investigaciones previas han afirmado la existencia de anormalidades en las células musculares en pacientes con ELA, con la subsiguiente afectación del proceso de diferenciación de mioblastos a miotubos (Pradat *et al.*, 2011; Scaramozza *et al.*, 2014), pero se desconocía que el mecanismo subyacente al proceso de diferenciación podía ser una alteración metabólica de la glucolisis.

4.1. Dinámica de TDP-43 y FUS en los procesos de diferenciación

En nuestro trabajo hemos detectado pequeños gránulos citoplasmáticos de TDP-43 por inmufluorescencia durante la fusión y la diferenciación de los mioblastos, pero al ser un modelo diferente, en el que se genera un daño muscular y se estudia la regeneración muscular, no ha sido posible

reproducir los resultados reportados por Vogler y colaboradores por fraccionamiento y Western blot. Esta discordancia también podría deberse a una menor sensibilidad del anticuerpo utilizado para el Western blot y/o a que la cantidad de TDP-43 que se transloca al citoplasma en nuestro modelo, es indetectable por esta técnica (Vogler *et al.*, 2018).

Por el contrario, se ha observado una salida masiva de FUS del núcleo durante la fusión y diferenciación de los mioblastos tanto por técnicas de inmunofluorescencia como por experimentos de fraccionamiento celular y Western blot. Con estos resultados se hipotetiza que FUS podría participar de igual forma en los procesos de diferenciación muscular, aspecto que debería estudiarse de manera específica en el futuro. Por ejemplo, sería interesante determinar si FUS y TDP-43 co-localizan en esas estructuras. En condiciones fisiológicas o tras daño muscular inducido, tal y como se ha señalado, el proceso de diferenciación celular conlleva una salida de TDP-43 y FUS del núcleo al citoplasma, donde podrían formar parte de los miogránulos, con FUS como un nuevo elemento relevante e integrante de éstos.

Aunque la participación de TDP-43, FUS y SOD1 en la regulación de la diferenciación muscular ha quedado reflejada en este trabajo, su impacto y el mecanismo por el cual influyen en este proceso, son diferentes. La diferenciación anormal de los mioblastos deficientes en SOD1 podría deberse al aumento del estrés oxidativo que presentan estos mioblastos. El cambio metabólico que se va produciendo a lo largo de la diferenciación, desde un predominio inicial de lo glucolítico hacia lo oxidativo en las etapas finales, conlleva a un aumento en la expresión de SOD1 como se ha demostrado en la línea celular *wild type*. SOD1 parece actuar por otra vía ya que su silenciamiento no genera variaciones de MyoD, aunque si presenta una alteración en la diferenciación produciendo miotubos aberrantes. Ello podría explicarse de manera alternativa por la pérdida de función de SOD1 con un aumento secundario de estrés oxidativo que podría generar defectos en la fusión (Sugihara *et al.*, 2018). De hecho, en el modelo de ratón *SOD1*$^{(G93A)}$ (en estadíos sintomáticos y denervación patente), se ha

visto, sin tener en cuenta el aumento de la proliferación de las células satélite con la denervación, que la proliferación de éstas era menor y que algunos factores miogénicos estaban reducidos (Manzano *et al.*, 2013). En cambio, la pérdida absoluta y relativa de la capacidad de diferenciación tanto de los mioblastos defectivos en TDP-43 como en FUS, además del cambio de localización celular de estas proteínas durante el proceso, sugiere una participación directa de ambas ribonucleoproteínas en la diferenciación muscular. El hecho de que MyoD, una de las proteínas reguladoras de la diferenciación muscular disminuya al silenciar TDP-43 y FUS, (y no tras silenciar SOD1) sugiere que el defecto de estas proteínas comprometería las fases iniciales del proceso. Existen varios trabajos que confirman que la depleción genética de TDP-43 podría conducir a una detención del crecimiento celular que podríamos extrapolar a una alteración en la la diferenciación a miotubos y formación de miofibras, *in vitro,* así como el fallo en la regeneración muscular tras daño muscular (Chiang *et al.*, 2010; Murphy *et al.*, 2011). Con todo, en el caso de FUS, las evidencias de su implicación en la diferenciación celular muscular son, a pesar de tener unas funciones similares a TDP-43, más escasas (Ansseau *et al.*, 2016; Homma *et al.*, 2016).

Además de las evidencias obtenidas en los experimentos de localización y expresión subcelular, la relación de TDP-43, FUS y SOD1 con la diferenciación muscular puede justificarse por el impacto de su deficiencia en el metabolismo energético celular. Las células musculares, tanto en proliferación como en diferenciación, necesitan tener energía para sintetizar rápidamente biomasa en forma de nucleótidos, proteínas y lípidos, y con ello sostener el crecimiento y división celular (Ryall, 2013). Para este cometido, la glucolisis, aunque es relativamente ineficiente, proporciona una serie de ventajas importantes para las células, incluidas la capacidad de generar rápidamente ATP en respuesta a cambios agudos de demanda energética (Pfeiffer, Schuster and Bonhoeffer, 2001), e intermediarios glucolíticos necesarios para la biosíntesis de nuevas biomoléculas: lípidos, aminoácidos y nucleótidos, siendo estos últimos a través de la vía de las pentosas fosfato (Li *et al.*, 2016). Es por esta función biosintética

precisamente por la cual la glucolisis es la vía metabólica preferente de las células tumorales para mantener su fenotipo proliferativo (Warburg, Wind and Negelein, 1927).

4.2. Relaciones entre metabolismo energético y miogénesis

En este trabajo se ha demostrado también la relación directa que existe entre el correcto funcionamiento de la vía glucolítica y el proceso de fusión y diferenciación de mioblastos. Utilizando la deprivación de glucosa con el tratamiento de 2-dg, se ha comprobado que la inhibición de la glucolisis bloquea la formación de miotubos a partir de mioblastos *wild type*. Este bloqueo puede estar producido por el descenso en la expresión de MyoD que, como se ha podido comprobar, la misma inhibición de la glucolisis produce.

Esta afirmación se ha corroborado en este estudio realizado en condiciones fisiológicas, el cual revela en el momento de la fusión celular la sobreexpresión del enzima regulador de la glucolisis por excelencia, PFKFB3 (PFK2), y la posterior reducción de éste una vez producida la fusión. Curiosamente, los silenciamientos de los 3 genes, *TARDBP, FUS y SOD1*, inducen un descenso en la expresión de dicho enzima, lo cual podría ser desde un punto de vista funcional uno de los principales motivos por los que estos mioblastos defectivos para TDP-43, SOD1 y FUS pierden la capacidad de diferenciación por un déficit energético. Otra asociación interesante a destacar es la regulación de dicha enzima por acción de UCP2, la cual según Sreedar y cols. activa a la PFK2 vía AKT (Sreedhar *et al.*, 2017). La pérdida de función de TDP-43 genera estos tres eventos que actúan sinérgicamente en el descenso del metabolismo anaerobio; disminución de la expresión de PFK2, por un lado, de UCP2 por otro, y en consecuencia, disminución en la activación de PFK2 vía AKT. Sin embargo, la depleción de TDP-43 o FUS genera un aumento en la expresión del otro enzima regulador de la glucolisis, la PFK1 (PFKM). Esta sobreexpresión que se ve tanto a nivel de mRNA como de proteína, podría ser un mecanismo de compensación

de la célula para intentan solventar el defecto glucolítico de algún modo. Avalando esta hipótesis, existen estudios en los que se demuestra la existencia de niveles elevados de *PFKM* tanto en tejido medular de pacientes con proteinopatía TDP-43 como en motoneuronas derivadas de IPSCs (*induced pluripotent stem cells*) creadas a partir de fibroblastos de estos pacientes (Manzo *et al.*, 2019). Asimismo, en el mismo trabajo y en el modelo de *Drosophila* de proteinopatía TDP-43 condicional para motoneurona, la sobreexpresión de PFKM rescataba el fenotipo motor (Manzo *et al.*, 2019). Otros efectos del silenciamiento de *TARDBP o FUS* en la expresión proteica en los mioblastos sería el aumento de expresión de las enzimas reguladoras de la vía glucolítica piruvato quinasa y hexoquinasa 2, respectivamente.

A pesar de que no existen demasiados argumentos para explicar la depleción de SOD1 como causa de esta alteración metabólica, no por ello deja de ser un efecto interesante o previsto. En los mioblastos defectivos en SOD1 hay una reducción tanto de transcritos de PFK1 como de su expresión proteica. Este dato podría explicar la deficiencia glucolítica observada en estos mioblastos por un mecanismo parcialmente diferente al de los otros dos genes. Para avalar este mecanismo diferencial de SOD1 hay evidencias experimentales que han demostrado un cambio de metabolismo de glucolitico a oxidativo en ratones mutantes para SOD1 (Browne *et al.*, 2006; Miyazaki *et al.*, 2012). Palamiuc y colaboradores ya advirtieron una disminución en la actividad enzimática y en la expresión del mRNA de *PFK1* en músculo de ratones SOD1 (G86R) en estadios presintomáticos y avanzados de la enfermedad (Palamiuc *et al.*, 2015). En contra de esta visión, en otro estudio reciente realizado con ratones SOD1 (G93A) se vio que la expresión de mRNA de las enzimas PFK1, PFK2 y PK aumentaba (Dobrowolny *et al.*, 2018). Por lo tanto, la controversia sobre el mecanismo por el que el defecto de SOD1 influye en la glucolisis permanece abierta.

5. LOS FACTORES DE TRANSCRIPCIÓN FOXO ESTAN RELACIONADOS CON EL PAPEL DE TDP-43 EN EL MÚSCULO Y PUEDEN TENER RELACIÓN CON LA ELA

5.1. Papel funcional de FoxO en el metabolismo

Algunos factores de transcripción que se translocan al núcleo en condiciones de estrés celular para regular la expresión de proteínas pro-atróficas, forman parte de uno de los mecanismos de reprogramación génica más importantes que dictan los procesos de diferenciación celular. Unos de los factores de transcripción estudiados en el presente trabajo como presuntos partícipes de los mecanismos reguladores de la diferenciación miogénica en respuesta al silenciamiento de TDP-43 y FUS son los miembros de la familia FoxO. En mamíferos, la familia de los los factores de transcripción FoxO (*forkhead box protein O*), está constituida por FoxO1 (FKHR), FoxO3 (FKHRL1), FoxO4 (AFX) y FoxO6, que, aunque se expresan ampliamente en el organismo, presentan expresión tejido dependiente. FoxO6 es el de menor homología con el resto y más específico de cerebro, mientras que FoxO1 está ampliamente expresado en músculo, hígado y páncreas (Hedrick *et al.*, 2012). Estos factores de transcripción están relacionados con la atrofia muscular y juegan un papel importante en diversas funciones fisiológicas celulares que incluyen la proliferación, la supervivencia, el ciclo celular y el metabolismo (Gross, van den Heuvel and Birnbaum, 2008; Milan *et al.*, 2015). Por otra parte, los factores FoxO también son necesarios en las diferentes etapas del desarrollo embrionario; ratones deficientes en FoxO1 mueren en su etapa embrionaria debido al desarrollo incompleto del sistema vascular (Hosaka *et al.*, 2004). Otra función atribuida a los factores FoxO, más concretamente a FoxO3, es el correcto desarrollo y mantenimiento del sistema reproductor (Castrillon *et al.*, 2003). Sin embargo, existen evidencias que atribuyen aspectos perjudiciales de la sobre-activación desregulada de estos factores. Por ejemplo, el aumento de expresión de FoxO1 causado por la reducción en la actividad de SIRT1

induce el desarrollo de disfunciones en las células del endotelio vascular en presencia de valores de glucosa altos en sangre (Arunachalam *et al.*, 2014). Según nuestras investigaciones, tanto en los mioblastos deficientes en TDP-43 como en los deficientes en FUS, FOXO1 se encuentra sobreexpresado en el núcleo, lo que cual invita a especular que la puesta en marcha de los fenómenos atróficos, entre ellos, la degradación de proteínas a través del sistema ubiquitin-proteasoma, podría deberse a la desregulación de estos factores, provocando eventualmente la anulación del proceso de diferenciación celular.

Los factores de transcripción FoxO son activados por los factores tipo TGF-β y son inhibidos por la señalización de la insulina, SGK (*serum and glucocorticoid-induced kinase*) y de factores de crecimiento, de ahí su implicación en dichas funciones celulares dependientes de éstos (McFarlane *et al.*, 2006). Cuando se activa la vía de PI3K-AKT, los factores FoxO se fosforilan en residuos conservados y se localizan fuera del núcleo evitando así su actividad transcripcional. En ausencia de insulina, en periodos de alta necesidad de energía o en presencia de estrés oxidativo, los factores FoxO se translocan al núcleo activando la expresión de genes metabólicos y relacionados con el mantenimiento de la proteostasis y disminución del estrés oxidativo. Además de las fosforilaciones por AKT y SGK, los factores FoxO pueden ser modificados postraduccionalmente por diversos estímulos externos, siendo fosforilados en otros residuos diferentes por las quinasas AMPK, JNK, MST,ERK, p38, o MAPK (Essers *et al.*, 2004; Lehtinen *et al.*, 2006; Greer *et al.*, 2007; Calnan and Brunet, 2008). Asimismo, también son regulados por metilación, acetilación, ubiquitinación y desacetilación a través de las sirtuínas (Yamagata *et al.*, 2008; Wang *et al.*, 2013).

En el hígado, FoxO1 media la expresión de genes involucrados en el metabolismo de la glucosa y de los lípidos. En condiciones de ayuno, donde FoxO1 está localizado en el núcleo, activa la expresión de enzimas gluconeogénicas (Altomonte *et al.*, 2003; Matsumoto *et al.*, 2007). Por otra parte, en estados de resistencia a insulina, se necesitan niveles más altos de insulina para compensar el defecto de la señalización (Asada *et al.*, 2007).

Esto se consigue promoviendo una hiperplasia de las células beta del páncreas que es inhibida por la actividad de FoxO1, con lo que se estaría evitando así la adaptación secretora de la insulina (Nakae *et al.*, 2002; Hribal *et al.*, 2003).

El músculo absorbe el 80-90% de la glucosa por acción de la insulina (de Lange *et al.*, 2007). El mantenimiento de esta vía es fundamental para el mantenimiento de la homeostasis de la glucosa en el organismo.

En el músculo esquelético, FoxO1 junto a FoxO3 son los que más se expresan y ambos activan la expresión de las proteínas pro-atróficas Atrogina-1 y Murf-1, que promueven la pérdida de masa muscular a través de la degradación de proteínas, entre ellas MyoD (con lo cual detienen también el proceso de diferenciación). Además, FoxO1 está considerado como un represor de la diferenciación de mioblastos a miotubos (McCarthy and Esser, 2010; Löw, 2011; Schakman *et al.*, 2013; Schiaffino, Kenneth A Dyar, *et al.*, 2013; Sanchez, Candau and Bernardi, 2014) Asimismo, la activación de FoxO1 aumenta tanto la expresión de miostatina, que a su vez inhibe la expresión de MyoD en el núcleo (Langley *et al.*, 2002). Por lo tanto, la activación de FoxO1 bloquea, a través de MyoD por diferentes vías sinérgicas, el programa miogénico y la diferenciación de mioblastos a miotubos (Accili and Arden, 2004; Xu *et al.*, 2017). Aunque el mecanismo molecular íntimo sigue siendo desconocido, FoxO1 podría jugar un papel activador en la fusión de los mioblastos (Xu *et al.*, 2017). Sin embargo, se ha visto que la ganancia de función de FoxO1 en mioblastos múridos inmortalizados C2C12, produce una pérdida de la capacidad de diferenciación a miotubos, mientras que la inhibición constitutiva de su actividad transcripcional, mediante la supresión del dominio de transactivación del gen, aumenta la expresión de marcadores de diferenciación, tales como la miogenina y la miosina (MyHC). En este estudio se demostró que la regulación de la diferenciación miogénica mediada por FoxO1 se da a través de la vía PI3K-AKT (Hribal *et al.*, 2003).

Los resultados del presente trabajo demuestran que existe una relación directa entre la activación de estos factores de transcripción y los

silenciamientos de *TARDBP* y *FUS* tanto en mioblastos humanos control inmortalizados como en los mioblastos primarios de los dos pacientes estudiados. En primer lugar, habiendo una translocación nuclear de FoxO1 en los mioblastos defectivos en TDP-43 y FUS, que está ausente en los mioblastos control, shRNA, y en los mioblastos shSOD1, la activación de FoxO1 en el núcleo conduce a la sobreexpresión de las proteínas ligasas fundamentales del sistema ubiquitin-proteasoma, Atrogina-1 y Murf-1, y conduce a presentar niveles bajos de MyoD.

En un estudio realizado en ratones mutantes SOD1 (G93A) se observó un aumento de la translocación de FoxO1 mediada por un incremento de ROS en células endoteliales de la barrera hemato-medular que producía una disminución en las proteínas de las uniones estrechas de dicha barrera (Meister *et al.*, 2015), lo que sugería una desorganización de la barrera promovida por FoxO1. Sin embargo, de acuerdo a nuestros resultados, en nuestro modelo de silenciamiento de SOD1, a pesar de que los niveles de estrés oxidativo están aumentados, pueden existir otro tipo de efectos desconocidos que pudieren estar contrarrestando la translocación de Foxo1 al núcleo. Sin duda, sería interesante estudiar, estos mecanismos compensatorios puesto que podrían dar explicación a la falta de alteraciones en la diferenciación en este modelo celular (Calnan and Brunet, 2008; Gross, van den Heuvel and Birnbaum, 2008; Meister *et al.*, 2015). De acuerdo a nuestros resultados, matizaríamos esa afirmación en el sentido de que para la activación de FoxO1 en el núcleo es posible que sea necesaria la presencia de varios otros tipos de estrés celular, además del oxidativo.

FoxO3, por otro lado, se localiza en el núcleo de los mioblastos control y su presencia va disminuyendo a medida que avanza la diferenciación muscular. Este hecho indicaría que la actividad de FoxO3 nuclear podría ser inhibitoria de este proceso. Esta idea se ve reforzada con los resultados obtenidos con el *microarray* de expresión donde FoxO1, y sobre todo FoxO3, son de los primeros en la lista de los factores de transcripción que predicen los cambios transcripcionales tras el silenciamiento de *TARDBP*. Esto se

refuerza por el hecho de que en dicho estudio transcripcional, dos de las vías más influyentes en la regulación de los factores FoxO, como son PI3K-AKT y TGF-β, presentan los cambios más consistentes tras el silenciamiento de *TARDBP*. La translocalización nuclear de FoxO1 contemplada tanto en los mioblastos con pérdida de función de TDP-43 y FUS como en los mioblastos de pacientes con ELA fortalecen la idea de la sobreactivación de FoxO1 como no solo un posible evento celular característico de la patología de la ELA sino también un factor condicionante de la vulnerabilidad selectiva de la neurona motora. En este sentido, se ha estudiado recientemente que una de las vías más activas en las unidades oculomotoras es la de PI3K-AKT (Allodi *et al.*, 2019), por lo que de estos hallazgos se intuye que los mecanismos que garanticen la regulación fina de la activación FoxO pueden jugar un papel en la resiliencia a la patología de la ELA.

Esta posible relación de los factores de transcripción de la familia FoxO con la ELA y especialmente con TDP-43 no es totalmente inédita. Así, en un estudio de expresión con *microarray*, se encontró un patrón de expresión genética diferente entre pacientes y controles. Estas diferencias se acentuaban en funciones celulares relacionadas con la integridad del sarcómero y el metabolismo oxidativo, siendo uno de los genes que más se sobreexpresaba en los pacientes era FoxO1 (Bernardini *et al.*, 2013). Además, algunos trabajos previos han demostrado que TDP-43 puede regular la actividad transcripcional de los factores FoxO. Cuando la célula se encuentra en un estado de reposo sin ningún tipo de estrés celular, TDP-43 se encuentra localizado predominantemente en el núcleo y ejerce un control negativo en los factores FoxO inhibiendo su actividad transcripcional (Zhang *et al.*, 2014) **(figura13)**.

Sin embargo, en situaciones de estrés celular TDP-43 se transloca al citoplasma para formar parte de los gránulos de estrés, compuestos por otras proteínas y transcritos de RNA (Taylor, Brown and Cleveland, 2016), de tal manera que se paraliza el proceso de síntesis de proteínas como se ha comentado anteriormente. En estos gránulos de estrés se encuentra una

proteína tipo *scaffold* que aparece también en los agregados citoplásmicos de TDP-43 en la ELA, la proteína 14-3-3 (Umahara *et al.*, 2016).

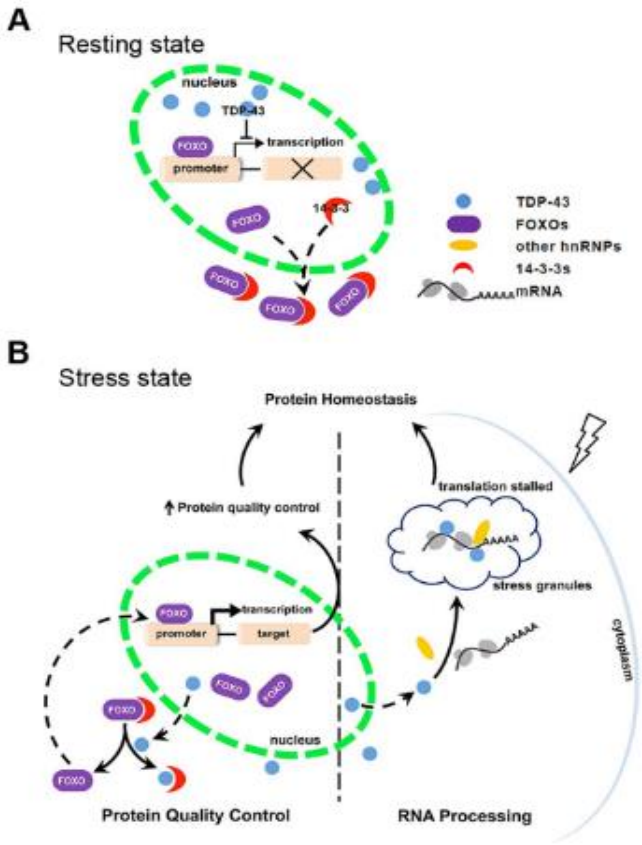

Figura 13. Diagrama esquemático de la regulación de la actividad de FoxO por TDP-43. Modificada de Zhang et al., 2014.

Akt fosforila a los factores FoxO e induce su unión con la proteína 14-3-3 para prevenir su translocación al núcleo e impedir la transcripción de genes que promueven la apoptosis (Arimoto-Ishida*et al.*, 2004; S. Wang *et al.*, 2013). Tanto TDP-43 como los factores FoxO compiten por esta proteína cuando TDP-43 migra al citosol en respuesta a un estrés celular, y es entonces cuando FoxO puede ser liberado de 14-3-3 y consecuentemente traslocar al núcleo para activar su actividad transcripcional. Esta regulación transcripcional promueve la actividad del sistema ubiquitin-proteasoma y la autofagia para resolver el estrés celular. Por esta razón, los factores FoxO son necesarios como una respuesta fisiológica celular para mantener la homeostasis celular. De hecho, se ha demostrado el papel de estos factores tanto en el

envejecimiento y el cáncer, promoviendo la longevidad y como gen supresor, respectivamente (Dansen and Burgering, 2008). En una situación aguda de pérdida de función nuclear de TDP-43 por su salida al citoplasma, disminuyen los niveles de proteínas mal plegadas debida a la activación de FoxO. En este sentido TDP-43 actúa como un interruptor de respuesta fisiológica al estrés que subyace a la etiología y la patología de las enfermedades degenerativas relacionadas con TDP-43.

Esta secuencia patogénica se podría hipotetizar diciendo que es probable que en las proteínopatías tipo TDP-43, como la ELA y la demencia frontotemporal (DFT), exista un tipo de estrés celular que inicialmente es agudo y que se intenta resolver mediante la translocación de TDP-43 al citoplasma, con el objeto de formar los gránulos de estrés y así paralizar de forma reversible la traducción de proteínas. Simultáneamente, la célula utilizaría la translocación de FoxO al núcleo para activar los sistemas de degradación de proteínas, UPS (*Ubiquitin proteosome system*) y la autofagia, para resolver dicho estrés. El mantenimiento crónico de este estrés sin resolverse en el tiempo, como puede ocurrir en situaciones en las que se pierde la capacidad funcional celular, por ejemplo, en el envejecimiento, la pérdida de función de TDP-43 nuclear debida a su salida de este compartimento, podría ser perjudicial a largo plazo (Zhang *et al.*, 2014). Además, la persistencia de los gránulos de estrés podría conducir a la formación de agregados insolubles similares a las fibras amiloides con propiedades de propagación de tipo prionoide (Pan *et al.*, 1993; Jucker and Walker, 2013; Walker and Jucker, 2015). Ello implicaría la sobreactivación de los factores FoxO, que promueven la senescencia e incluso la muerte celular. En esta situación, la célula activa una cascada de eventos moleculares que destinan a la célula a su final inevitable (Eijkelenboom and Burgering, 2013).

Como limitaciones de esta explicación fisiopatológica de la enfermedad, hay que recordar que el silenciamiento de *TARDBP* y *FUS* es un modelo experimental reduccionista que no tiene en cuenta los efectos mediados por la agregación citosólica de dichas proteínas, por lo que no se puede afirmar, por el momento, que estos mecanismos estén conservados de

forma total en pacientes con ELA. Este modelo más bien reflejaría lo acontecido en la célula durante un estrés celular mantenido en el tiempo. Sorprendentemente, como ha quedado demostrado en esta investigación, el hecho de silenciar *TARDBP* y *FUS* genera un estrés celular en sí mismo, probablemente debido a esa pérdida de función procesadora y estabilizadora de RNA, que conlleva precisamente a la movilización de los factores FoxO. Por lo tanto, los eventos patogénicos que se producen debido a una sobreactivación de los factores FoxO estarían reforzados por la propia pérdida de función de los genes *TARDBP* y *FUS* al salir persistentemente del núcleo y agregarse. Esta situación generaría unas condiciones muy desfavorables para la viabilidad celular y establecería un ciclo de eventos patogénicos que compondrían un círculo vicioso de degeneración. De hecho, esto podría explicar la rápida progresión de la enfermedad, como si al cruzar un hipotético umbral se desencadenase una cascada de eventos que, acumulados, acelerarían un proceso de degeneración celular.

Un hecho de trascendencia, aunque necesite de una confirmación posterior con nuevos casos, es que en el paciente con la forma familiar, aquellos mioblastos que expresan una mayor localización de FoxO1 en el núcleo son precisamente aquellos que localizan menos TDP-43 nuclear. Aunque no se ha podido observar la posible translocación de TDP-43 al citoplasma, la relación entre la disminución de TDP-43 nuclear con la concomitante pérdida de función y los factores FoxO queda manifestada y sería muy importante confirmar este hecho en pacientes adicionales.

5.2. FoxO y miogénesis

Con respecto a otros efectos de la activación de estos factores de transcripción, FoxO1 también participa en la especificación del tipo de fibras musculares. Kitamura y colaboradores demostraron que la ablación condicional de FoxO1 bajo el control del promotor de miogenina en ratones, cambia la distribución del tipo de fibra, aumentando las fibras que

contienen MyoD, predominante en fibras rápidas (Kitamura *et al.*, 2007), y disminuye la formación de fibras musculares que contienen miogenina, que es el factor miogénico predominante en fibras lentas (Hughes *et al.*, 1993). Este cambio de tipo de fibra, que es un cambio del tipo de metabolismo predominante, podría explicarse por el efecto inhibidor de FoxO1 en la expresión de MyoD. Este cambio de tipo de fibra se puede explicar también en base al factor de transcripción PGC1-a (*peroxisome proliferator-activated receptor-γ*), que es un co-activador directo de FoxO1 además de activar a PPAR-γ en el músculo, que es requerido para el metabolismo oxidativo, la biogénesis mitocondrial y la formación de fibras de contracción lenta (Puigserver *et al.*, 2003). De hecho, el silenciamiento de TDP-43 en mioblastos produce una sobreexpresión de este co-activador, que junto con el cambio metabólico de glucolítico a oxidativo que induce este silenciamiento, fortalece la relación conceptual entre FoxO1 y TDP-43. Igualmente, la insulina y Akt inactivan FoxO1 y con ello inhiben la actividad promotora de PGC1-a (Daitoku *et al.*, 2003). En los mioblastos deficientes en TDP-43, la vía PI3K-AKT es defectuosa, FoxO1 se encuentra en el núcleo y hay una sobreexpresión de PGC1-a, hechos que refuerzan la relación FoxO1-TDP43-PGC1-a. Por otro lado, en las fibras lentas oxidativas PGC1-a está altamente expresado, precisamente debido a los requerimientos energéticos oxidativos que caracterizan este tipo de fibra. En ratones transgénicos en los que se activó la expresión de PGC1-a bajo control del promotor de creatina quinasa, se observó un cambio de tipo de fibra, por el que los músculos normalmente ricos en fibras de tipo 2 se volvieron más rojos por el aumento de mioglobina y de las enzimas mitocondriales (Lin *et al.*, 2002). Esta misma situación podría estar ocurriendo en pacientes con ELA tras el aumento de PGC1-a observado en fibroblastos de pacientes con la expansión en C9orf72 (Onesto *et al.*, 2016).

5.3. FoxO y atrofia muscular en la ELA

La atrofia muscular en la ELA se debe fundamentelmente a la denervación de la neurona motora. Sin embargo, los resultados generados en este trabajo abren la puerta a conjeturar sobre la existencia de mecanismos pro-atrofia intrínsecos en el músculo de ELA originados por un desajuste de la homeostasis de la energía. Debido a que la atrofia muscular por la denervación esta mediada por los factores FoxO, la sobreactivación de éstos como signo patognomónico de la enfermedad y la posibilidad de que fuera únicamente causada por la denervación retrógrada en la ELA, podría quedar en entredicho. Hay un único estudio que reporta una sobreexpresión de FoxO1 en el musculo de pacientes con ELA (Bernardini *et al.*, 2013), pero no se reporta el grado de denervación de ese músculo impidiendo concluir si la sobreexpresión observada de FoxO1 se debía precisamente a la denervación o si se trataba de un evento primario. Por eso, siendo ésta una limitación en el estudio muscular, resultados positivos de FoxO1 nuclear en biopsias de músculo denervado podrían conllevar a una interpretación confusa. Cuando hay denervación, se activa la expresión de Atrogina-1 y Murf-1, activándose la degradación de proteínas. De hecho, se han encontrado niveles reducidos de la proteína quinasa B, PKB (regulador de la vía de señalización de la insulina), y elevados de Atrogina-1 en músculo de pacientes con ELA (Léger *et al.*, 2006). Finalmente, la sobreexpresión de FoxO1 en el músculo esquelético inhibe la síntesis de proteínas mediada por mTOR (Southgate *et al.*, 2007). De igual manera, se ha visto que ratones *knock-out* para estas ligasas (MurF-1 y Atrogina-1) protegen de la atrofia producida por la denervación (Bodine *et al.*, 2001). Todo ello sugiere que el músculo denervado podría mantener su estructura si se inhiben los factores que activan Atrogina-1 y Murf-1; ello proporcionaría múltiples posibilidades terapéuticas en diversas condiciones neurológicas con denervación central o periférica. Por lo tanto, el tratamiento de los procesos pro-atróficos, que en gran medida son mediados por la activación patológica de factores tales como Atrogina-1 y Murf-1, en el músculo de ELA podría aliviar el desgaste

muscular asociado a esta enfermedad neurodegenerativa, y en última instancia, podría incluso fortalecer la función neuromuscular ayudando a frenar el proceso degenerativo. De los hallazgos obtenidos en el presente trabajo y los datos bibliográficos, la inhibición de los factores FoxO es una posibilidad terapéutica razonable para frenar dichos eventos atróficos y mejorar la funcionaliad neuromuscular.

6. LA INHIBICIÓN DE FOXO1 Y FOXO3 PUEDE SER UNA POTENCIAL DIANA TERAPÉUTICA PARA EL TRATAMIENTO DE LA ELA

6.1. FoxO y su papel en la neurodegeneración

Muchas enfermedades neurodegenerativas tienen en común la presencia de agregados tóxicos, como la enfermedad de Alzheimer, la enfermedad de Huntington, el Parkinson y la ELA (Jucker and Walker, 2013). En este contexto, los sistemas de degradación tanto de proteínas mal plegadas como de orgánulos deteriorados, la autofagia y el sistema ubiquitin-proteasoma, juegan un papel fundamental en el reciclamiento fisiológico de esos componentes celulares (Wells *et al.*, 2019). Los factores FoxO ejercen un peso fundamental en la activación de estos sistemas de degradación (Salih and Brunet, 2008). Por eso, la respuesta fisiológica mediada por FoxO ante un estrés celular metabólico, proteostático y oxidativo es necesaria para el mantenimiento de la viabilidad celular. De hecho, en condiciones de estrés oxidativo, los factores FoxO inducen la autofagia y la degradación de acúmulos tóxicos promoviendo la supervivencia celular (Eijkelenboom and Burgering, 2013; Maiese, 2015; Brown and Webb, 2018).

6.2. FoxO y cambios metabólico-energéticos en el músculo de pacientes con ELA

En este trabajo hemos demostrado que los factores FoxO son dinámicos durante el proceso de diferenciación en condiciones fisiológicas y cómo

esta actividad está relacionada con los cambios metabólicos que suceden a lo largo del proceso de diferenciación. Precisamente en el momento en el que los mioblastos comienzan a alinearse para su fusión, la localización de FoxO1 es citoplásmica y los niveles de FoxO3 nucleares disminuyen. En ese mismo punto, el metabolismo energético se intensifica priorizando, en el momento de aparición de los primeras miotubos, el potencial metabólico anaeróbico sobre el oxidativo. Esto significa que la localización citoplasmática de FoxO1 y la disminución nuclear de FoxO3, podrían estar ligadas al aumento del metabolismo energético para que la fusión pueda llevarse a cabo y proceder con la consiguiente diferenciación celular. De igual forma, la localización citoplasmática de los factores FoxO proporcionaría a la célula las proteínas necesarias para la diferenciación que se encuentran reguladas por estos factores como MyoD (corroborado en los resultados con el aumento de MyoD durante la fusión celular).

El aumento de la capacidad de diferenciación celular con la inhibición de los factores FoxO ha quedado demostrado en este estudio tanto en los mioblastos primarios de pacientes con ELA como en los mioblastos defectivos de TDP-43 y FUS por separado, en los cuales los niveles de FoxO1 nucleares y la activación transcripcional de FoxO1 y FoxO3 están significativamente incrementados de manera basal. Teniendo en cuenta que el silenciamiento de *TARDBP* conlleva a una disminución de la expresión de la proteína FUS, al tratar estas células con el inhibidor de FoxO, parte de esa expresión defectiva se recupera. Es decir, la disminución en la expresión de FUS en situaciones de pérdida de función de TDP-43 podría estar mediada por la activación de FoxO. Por lo tanto, FoxO podría ser también un intermediario en la interacción TDP-43-FUS y su inhibición, por tanto, podría contribuir, de manera significativa, a reducir la toxicidad dependiente de TDP-43. Además, la inhibición de FoxO rescata en gran medida el fenotipo metabólico defectuoso en dichas estirpes celulares. Muchos de los enzimas que participan en la regeneración del NAD^+ y que estaban disminuidos en los mioblastos con silenciamiento de *TARDBP* y *FUS* aumentan su expresión con el tratamiento inhibitorio de FoxO. Por ejemplo,

en los mioblastos defectivos para TDP-43 con disminución de la enzima citosólica GPD1, propia del metabolismo de las fibras rápidas tipo 2b, aumenta considerablemente con el tratamiento inhibidor de FoxO. También ocurre con las enzimas de la lanzadera de malato-aspartato como la MDH1, GOT1 y los transportadores Aralar1/AGC1 y OGC1 que aumentan su expresión en diferentes grados en todos los silenciamientos. Asimismo, el perfil metabolómico medido por RMN corrobora la reducción de los niveles de Asp con el inhibidor de FoxO debido a su consumo activado por las enzimas citosólicas de la lanzadera de malato-aspartato. Con todo ello, se podría concluir que la inhibición de los factores FoxO estimula el metabolismo energético para salir del estado de senescencia que caracteriza a la activación de dichos factores. El efecto más notorio de este tratamiento es la recuperación del enzima UCP2 y la GPD1, de la lanzadera de glicerol, en los mioblastos silenciados para *TARDBP* y *FUS* que presentaban una reducción moderada de estas enzimas. Este aumento de expresión de UCP2, dada su acción como antioxidante y regulador metabólico (Jez, Garlid and Jabu, 2018), podría ir acompañado de una reducción de los radicales libres cuyos niveles en los mioblastos silenciados son elevados. De igual forma, el enzima regulador por excelencia de la vía glucolítica, PFKFB3 (PFK2), también aumenta, incluso en los mioblastos silenciados para SOD1 que también mejora su fenotipo metabólico aunque con limitaciones.

6.3. La inhibición de FoxO como estrategia terapéutica en la ELA

De manera más concreta, de acuerdo a los resultados de nuestro trabajo, la sobreactivación de FoxO en músculo no denervado y previo a la neurodegeneración podría establecer la base sobre la que se podría constituir una diana de tratamiento para la ELA. Los mioblastos son células que no se encuentran bajo la influencia directa de esta denervación y son, como revelan los datos, los que precisamente muestran la activación y translocación de los factores FoxO al núcleo. En este trabajo se han estudiado por un lado los mecanismos moleculares por los que, en

mioblastos inmortalizados bajo ninguna influencia neuronal, la pérdida de función de TDP-43, SOD1 y FUS conduce a una disfunción muscular. Además, se ha probado dicha disfuncionalidad en los mioblastos primarios de pacientes procedentes de músculo no denervado. Aun así, el cultivo de mioblastos por sí mismo no depende de la viabilidad de la motoneurona, pues se trata de una célula independiente a ésta y aunque el mioblasto reciba en condiciones fisiológicas influencia trófica neuronal, el cultivo *in vitro* tanto de los mioblastos control, de los pacientes con ELA esporádica y familiar, y de los mioblastos silenciados se ha realizado en las mismas condiciones sin dichos factores tróficos. Por ello podemos presumir que las diferencias observadas son por el efecto del propio silenciamiento en un caso y de la patología en sí misma en el caso de los mioblastos de los pacientes. Finalmente, podemos concluir que la activación observada de FoxO1 en las células procedentes de pacientes es presumiblemente independiente de la denervación o de cualquier proceso inflamatorio al que pudieran estar expuestas en los pacientes.

Diversos estudios han demostrado que la inhibición o el silenciamiento génico de FoxO1 o FoxO3 producen numerosos efectos beneficiosos para el sistema nervioso: 1) protegen de la muerte de la microglía por el estrés oxidativo (Shang *et al.*, 2009a) y por la exposición de β-amiloide (Shang *et al.*, 2009b); 2) su inhibición promueve los efectos protectores mediados por los receptores glutamatérgicos metabotrópicos (Chong, Li and Maiese, 2006); 3) disminuyen a través del estradiol el daño isquémico que produce la activación de FoxO (Won, Ji and Koh, 2006); 4) la acción trófica de neurotrofinas conlleva la inhibición de estos factores FoxO (Zheng, Kar and Quirion, 2002; Zhu *et al.*, 2004; Anitha *et al.*, 2006); y 5) el aumento de la supervivencia de neuronas producido por el precursor de NAD^+, se produce a través de la estabilización de la fosforilación inhibitoria de FoxO3 (Chong, Lin and Maiese, 2004). A todas estas evidencias beneficiosas de la inhibición de los factores FoxO se añaden, además, las evidencias de la inducción de la apoptosis en neuronas (Lehtinen *et al.*, 2006; Hou *et al.*, 2011; Zhong Chong *et al.*, 2011; Qi *et al.*, 2013; W. Wang *et al.*, 2013) y la atrofia muscular por

parte de estos factores (Salih and Brunet, 2008; Schiaffino, Kenneth A. Dyar, *et al.*, 2013; Milan *et al.*, 2015; Mcloughlin *et al.*, 2019), para constituir una razón importante por la que la inhibición de FoxO en un momento específico de la patología, cuando los efectos de la sobreactivación de FoxO desencadena muerte neuronal y atrofia muscular, podría funcionar como estrategia terapéutica para la ELA.

Para confirmar que los efectos son por la inactivación parcial de los factores FoxO y no por un efecto inespecífico, se han silenciado los factores FoxO simultáneamente, es decir, los factores FoxO1 y FoxO3 en los mioblastos que presentan valores elevados de actividad transcripcional y de expresión nuclear de dichos factores. Por lo tanto, son los mioblastos defectivos para TDP-43 y FUS los que han sido mayoritariamente silenciados para *FOXO*. El silenciamiento no ha sido completo con lo que se beneficia la respuesta fisiológica mínima que dichos factores proporcionan para el mantenimiento de la proteostasis celular, en términos de activación del UPS (sistema ubiquitin-proteasoma).

Tanto los efectos de la depleción de TDP-43 como la depleción de FUS en la diferenciación celular como el metabolismo energético se corrigen en gran medida con el silenciamiento de FoxO.

Este resultado ratifica a FoxO como mecanismo principal generador de la toxicidad derivada de la pérdida de función de estos genes asociados con la ELA.

Sobre esta base podemos deducir que la alteración metabólica de los mioblastos humanos secundaria al silenciamiento de *TARDBP* y *FUS*, y en mioblastos primarios de pacientes con ELA, podría tener su origen en la sobreactivación de los factores FoxO, y que esta sobreactivación de FoxO derivada del estrés celular que ocasiona la pérdida de función de TDP-43 y FUS, podría ser un hecho diferencial y central en los mioblastos de pacientes. Por lo tanto, la activación crónica perjudicial de los factores FoxO podría considerarse como uno de los mecanismos de patogenicidad en la ELA, que conlleva a la célula muscular a disminuir su capacidad para obtener energía en detrimento de su propia supervivencia. Existen evidencias previas que

avalan la participación de los factores FoxO en la regulación del metabolismo energético. Por ejemplo, la sobreexpresión de FoxO1 en el músculo esquelético en ratones, conduce a una pérdida de masa corporal, pérdida de masa muscular, disminución del tamaño de fibras de tipo I y II, y a un incorrecto control glucémico tras la administración de glucosa por vía oral e insulina por vía intraperitoneal (Kamei *et al.*, 2004). La sobreexpresión de FoxO1 en células múridas C2C12, incrementa la expresión de la lipoproteína lipasa (LPL) cuyo pape funcional es hidrolizar en el músculo los triglicéridos plasmáticos en ácidos grasos, destinados a la β-oxidación (Kamei *et al.*, 2003). Con ello se estimula el catabolismo lipídico propiciando el cambio de tipo de fibra muscular, de glucolítica a oxidativa, con una disminución de la musclina, un nuevo factor secretor descubierto propio del músculo esquelético y específico de fibras de tipo IIB (glucolíticas) (Banzet *et al.*, 2007; Yasui *et al.*, 2007). Asimismo, en condiciones de ayuno y ejercicio, la activación de FoxO1 en el músculo esquelético promueve la expresión de genes implicados en el metabolismo energético de los lípidos, como el transportador de ácidos grasos FAT/CD36, generando una transición metabólica desde la oxidación de carbohidratos a la oxidación de lípidos (Bastie *et al.*, 2005). En condiciones de baja disponibilidad de nutrientes, inanición o necesidad de energía, los bajos niveles de insulina en sangre propios de este tipo de estados metabólicos y con ello, la ausencia de señalización de insulina, producen en el hígado una activación de los factores FoxO que aumenta los niveles de glucosa en sangre a través de la activación de la expresión de las enzimas de la glucogenolisis y la gluconeogénesis: los enzimas glucosa 6-fosfatasa y fosfoenol-piruvatocarboxiquinasa, respectivamente.

Curiosamente, todos estos efectos fruto de la sobreactivación de FoxO que condiciona la regulación metabólica en las condiciones de necesidad de energía, coinciden con las evidencias experimentales de una adaptación hipercatabólica presente en los pacientes con ELA: una resistencia a la insulina e intolerancia a la glucosa, un aumento en el metabolismo lipídico

con la consecuente pérdida del tejido adiposo subcutáneo, la pérdida de masa muscular y atrofia, y el cambio de tipo de fibra muscular.

6.4. AS1842856: potencial fármaco anti-ELA

Por todo ello, en este trabajo se propone la inhibición de los factores FoxO como potencial diana terapéutica para resolver los efectos patogénicos metabólicos y miogénicos que se originan por su sobreactivación en situaciones de estrés crónico. Hasta que no se esclarezcan los eventos causativos iniciales que llevan a que la respuesta fisiológica no consiga resolver el estrés celular, y hasta que no se desentrañen los factores precipitantes de ese estrés celular, la inhibición de los factores FoxO podría ser una diana terapéutica a verificar.

Como prueba de concepto de esta propuesta, en esta investigación hemos utilizado para la inhibición de los factores FoxO la molécula AS1842856, una oxodihidroquinolina permeable a las células que inhibe preferentemente la actividad transcripcional de FoxO1 sobre la de FoxO3 y FoxO4, con un porcentaje de inhibición del 70%, 20% y 3%, respectivamente. Esta molécula se une a la forma no fosforilada activa e impide su unión al DNA y con ello la transcripción de sus genes diana. Aunque no impida la translocación nuclear de FoxO1 esta inhibición aumenta la fusión de mioblastos considerablemente. La dosis utilizada es la establecida que produce una inhibición del 50% de FoxO1. Con este grado de inactivación se evitan los posibles efectos perjudiciales que la pérdida de función de estos factores pudieran desencadenar como la pérdida de proteostasis celular por el bloqueo de la autofagia y del sistema ubiquitin-proteasoma.

A pesar de que hay estudios en los que consideran que los factores FoxO podrían activar una fase tardía de la diferenciación de los mioblastos (Bois and Grosveld, 2003; Hakuno *et al.*, 2011; Xu *et al.*, 2017), existen otras evidencias de que la activación constitutiva de FoxO1 en el núcleo impide la fusión y la diferenciación de los mioblastos, y que el silenciamiento de FoxO1 o de los factores FoxO1, FoxO3 y FoxO4 simultáneamente activa la

fusión y expresión de MyHC (Hribal *et al.*, 2003). Esto conlleva a pensar que en las diferentes etapas de la diferenciación muscular, la contribución de los factores FoxO es diferente. En las primeras fases en las que, como se ha demostrado en este trabajo, el metabolismo glucolítico es necesario, los factores foxO estarían inactivados y en el citoplasma. Sin embargo, en las fases finales de la diferenciación, cuando la preferencia metabólica es más oxidativa, los factores foxo podrían estar más activados, tal y como demuestran algunos estudios. Es por esta razón, por la que el inicio de la diferenciación celular mediada por la MyoD, se encontraría afectada tras el silenciamiento de *TARDBP, FUS* y *SOD1 y* en los mioblastos de pacientes.

6.5. Algunos riesgos teóricos inherentes a la terapia inhibidora de FoxO

A pesar de todo ello, la inhibición de FoxO como estrategia terapéutica podría tener como efecto secundario la inducción de una proliferación celular en otros sistemas, por lo que su utilidad como aproximación terapéutica debería intentar focalizar el efecto exclusivamente en el tejido muscular. De hecho, los factores FoxO actúan como represores de tumores por su función antiproliferativa y proapoptótica (Myatt and Lam, 2007; Katoh *et al.*, 2013; Farhan *et al.*, 2017) y tienen actividad antitumoral controlando la expresión de genes responsables de la interrupción del ciclo celular (Bouchard *et al.*, 2004, 2007). Este *rol* antitumoral de FoxO se podría ratificar con los efectos metabólicos que la sobreactivación de FoxO acarrea. En la investigación actual se ha demostrado que en un modelo de silenciamiento de *TARDBP* y *FUS* cuyo impacto en la célula no es otro que la translocación de FoxO1 al núcleo, la inhibición de los factores FoxO recupera la glucolisis de forma importante, siendo esta vía metabólica característica del metabolismo preferente en las células tumorales. También hay evidencias que relacionan la pérdida de función de TDP-43 con la disminución de la proliferación e invasividad de las células cancerígenas (Guo *et al.*, 2015). De todas formas, la relación epidemiológica de la ELA con el cáncer es

controvertida. Algunos estudios relacionan positivamente la ELA como factor de riesgo de padecer cáncer y viceversa, mientras que otros estudios indican un menor riesgo de padecer cáncer en pacientes diagnosticados con ELA (Fang *et al.*, 2013; Freedman *et al.*, 2013; Taguchi and Wang, 2017). Por todo ello, se debería evitar la inhibición de FoxO en células del organismo que no tuvieran una sobreactivación patológica de FoxO para evitar desarrollar tumores en otros lugares. La inhibición selectiva de FoxO en el músculo esquelético de pacientes con ELA tendría que ser una terapia dirigida que serviría tanto como tratamiento de los síntomas originados por la atrofia muscular como preventivo, antes de la aparición de la atrofia muscular en fibras glucolíticas.

7. PROYECCIONES DE FUTURO

El hecho de que los mioblastos de los pacientes y los genéticamente silenciados presenten alteraciones concordantes en la glucolisis, debería inducir la búsqueda de biomarcadores en suero u orina que reflejen esta situación. Si la pérdida de función de TDP-43 y FUS desregula la actividad de los sistemas reguladores de NAD^+, y sabiendo que más del 95% de los casos de ELA presentan agregados citoplasmáticos de TDP-43, un posible marcador en sangre u orina podría ser la determinación de este coenzima.
Otra conclusión de futuro de los resultados de este trabajo es que la activación de los factores FoxO en el núcleo podría considerarse como un biomarcador tisular de la enfermedad. Teniendo en cuenta el efecto activador de FoxO que produce la neurodegeneración en las fibras musculares maduras, las células musculares estudiadas deberían ser mioblastos independientes de la influencia neuronal y con ello, de la neurodegeneración. Asimismo, se tendría que incrementar el número de muestras de pacientes expuestas al tratamiento con el inhibidor de FoxO y también aplicarlo en diferentes modelos *in vivo* para demostrar la

implicación del músculo en la neurodegeneración con silenciamientos condicionales músculo-específico.

Con todo ello, este trabajo aporta evidencias suficientes para iniciar investigaciones más complejas en la línea de demostrar el posible origen muscular de la ELA, la toxicidad de los agregados de TDP-43 a través de la sobreactivación de los factores FoxO, y determinar el efecto terapéutico del tratamiento inhibitorio de FoxO dirigido al músculo esquelético en modelos animales así como el impacto de dicha terapia dirigida, sobre las neuronas.

Conclusiones

I. Los pacientes con ELA presentan un retraso en la producción de lactato en las prueba de ejercicio bajo isquemia que evidencia la existencia de un trastorno subclínico del metabolismo glucolítico.

II. La pérdida de función de TDP-43, FUS y SOD1 interfiere en el metabolismo energético muscular tanto aerobio como anaerobio, aunque de manera preferente en este último.

III. Los defectos glucolíticos causados por la pérdida de función de TDP-43 y FUS en el músculo están acompañados por una fuerte reducción de PFK2 que no es compensada ni por el incremento de PFK1, en ambos casos, ni por el incremento de la piruvato quinasa y hexoquinasa 2, en el silenciamiento de *TARDBP* y *FUS*, respectivamente.

IV. La pérdida de función de TDP-43 y FUS interfiere en la capacidad miogénica de los mioblastos humanos relacionada principalmente con la alteración glucolítica.

V. Los defectos glucolíticos que la deficiencia de TDP-43 y FUS causa en los mioblastos proliferativos, se explican al menos parcialmente por el deterioro de la maquinaria de reciclaje del NAD+ citosólico.

VI. El silenciamiento en células musculares de *TARDBP* provoca efectos similares al silenciamiento de *FUS*, pero no ocurre lo mismo con *SOD1*, lo que sugiere que las dos primeras proteínas tienen funciones estrechamente relacionadas.

VII. Los efectos deletéreos del silenciamiento de *SOD1* en células musculares, apuntan a un efecto relacionado con el incremento del estrés oxidativo y sus posibles efectos sobre el proceso de fusión muscular.

VIII. Los datos avalan que UCP2 puede tener un papel fundamental en regulación de la glucolisis, así como en los niveles de MyoD, lo que le convierte en otra potencial diana terapeútica en la ELA.

IX. La alteración metabólica de los mioblastos humanos secundaria a la pérdida de función de TDP-43 y FUS es concordante con la observada en los mioblastos primarios de pacientes con ELA, la cual podría tener su origen en la sobreactivación de los factores FoxO que podría ser un hecho diferencial y central en la patogenia de los disturbios metabólico-energéticos observados en los mioblastos de pacientes con ELA.

X. La traslocación de FoxO al núcleo, tras la pérdida de función de TDP-43, FUS y SOD1 en el músculo esquelético, pone en marcha un proceso de degradación muscular, bloqueando el programa de diferenciación a través de MyoD y activando la señal pro-atrofia muscular a través del incremento de Atrogina-1 y Murf-1.

XI. El silenciamiento de *FOXO* es capaz de corregir los defectos miogénicos y metabólicos en mioblastos humanos en los que se ha silenciado experimentalmente *TARDBP* y *FUS*.

XII. La inhibición de FoxO mediante el inhibidor específico AS1842856 es efectiva en la recuperación de los defectos de la glucolisis y del proceso de diferenciación observados en este modelo y no provoca ningún efecto tóxico celular a las concentraciones utilizadas. No obstante, dado que su uso sistémico podría tener, como efecto secundario, un incremento del riesgo oncogénico, su uso debería intentar testarse en una aproximación terapéutica focalizada en el tejido muscular.

XIII. Como conclusión general, el impacto de la pérdida de función de los genes *TARDBP, FUS* y *SOD1* en el metabolismo muscular, así como los hallazgos tanto clínicos como bioquímicos encontrados en el músculo de pacientes con ELA, sugieren una participación activa de este tejido en la patogenia de la enfermedad independiente del efecto pro-atrofia muscular secundario al proceso denervativo de las motoneuronas.

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Bibliografía

1. *A Biomarker Study to Evaluate MN-166 (Ibudilast) in Subjects With Amyotrophic Literal Sclerosis (ALS) ClinicalTrials.gov*. Available at: https://clinicaltrials.gov/ct2/show/NCT02714036?term=ibudilast&cond=ALS&draw=2&rank=1 (Accessed: 8 February 2020).
2. Abe, K., Aoki, M., Tsuji, S., Itoyama, Y., Sobue, G., Togo, M., Hamada, C., Tanaka, M., Akimoto, M., Nakamura, K., Takahashi, F., Kondo, K., Yoshino, H., Abe, K., Aoki, M., Tsuji, S., Itoyama, Y., Sobue, G., Togo, M., Hamada, C., Sasaki, H., Yabe, I., Doi, S., Warita, H., Imai, T., Ito, H., Fukuchi, M., Osumi, E., Wada, M., Nakano, I., Morita, M., Ogata, K., Maruki, Y., Ito, K., Kano, O., Yamazaki, M., Takahashi, Y., Ishiura, H., Ogino, M., Koike, R., Ishida, C., Uchiyama, T., Mizoguchi, K., Obi, T., Watanabe, H., Atsuta, N., Aiba, I., Taniguchi, A., Sawada, H., Hazama, T., Fujimura, H., Kusaka, H., Kunieda, T., Kikuchi, H., Matsuo, H., Ueyama, H., Uekawa, K., Tanaka, M., Akimoto, M., Ueda, M., Murakami, A., Sumii, R., Kudou, T., Nakamura, K., Morimoto, K., Yoneoka, T., Hirai, M., Sasaki, K., Terai, H., Natori, T., Matsui, H., Kotani, K., Yoshida, K., Iwasaki, T., Takahashi, F., Kondo, K. and Yoshino, H. (2017) 'Safety and efficacy of edaravone in well defined patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled trial', *The Lancet Neurology*. Lancet Publishing Group, 16(7), pp. 505–512. doi: 10.1016/S1474-4422(17)30115-1.
3. Abreu, P. (2018) 'Bioenergetics mechanisms regulating muscle stem cell self-renewal commitment and function', *Biomedicine and Pharmacotherapy*. Elsevier, 103(February), pp. 463–472. doi: 10.1016/j.biopha.2018.04.036.
4. Adams, C. R., Ziegler, D. K. and Lin, J. T. (1983) 'Mercury intoxication simulating amyotrophic lateral sclerosis.', *JAMA*, 250(5), pp. 642–3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6864963 (Accessed: 3 February 2020).
5. Aksoy, P., White, T. A., Thompson, M. and Chini, E. N. (2006) 'Regulation of intracellular levels of NAD: A novel role for CD38', *Biochemical and Biophysical Research Communications*, 345(4), pp. 1386–1392. doi: 10.1016/j.bbrc.2006.05.042.
6. Al-Chalabi, A. (2014) 'High-calorie diets in amyotrophic lateral sclerosis', *The Lancet*. Lancet Publishing Group, pp. 2028–2030. doi: 10.1016/S0140-6736(14)60270-1.
7. Al-Chalabi, A., Andersen, P. M., Nilsson, P., Chioza, B., Andersson, J. L., Russ, C., Shaw, C. E., Powell, J. F. and Leigh, P. N. (1999) 'Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis', *Human Molecular Genetics*. Oxford University Press, 8(2), pp. 157–164. doi: 10.1093/hmg/8.2.157.
8. Al-Chalabi, A., Calvo, A., Chio, A., Colville, S., Ellis, C. M., Hardiman, O., Heverin, M., Howard, R. S., Huisman, M. H. B., Keren, N., Leigh, P. N., Mazzini, L., Mora, G., Orrell, R. W., Rooney, J., Scott, K. M., Scotton, W. J., Seelen, M., Shaw, C. E., Sidle, K. S., Swingler, R., Tsuda, M., Veldink, J. H., Visser, A. E., van den Berg, L. H. and Pearce, N. (2014) 'Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study', *The Lancet Neurology*, 13(11), pp. 1108–1113. doi: 10.1016/S1474-4422(14)70219-4.
9. Al-Chalabi, A. and Hardiman, O. (2013) 'The epidemiology of ALS: a conspiracy of genes, environment and time', *Nature Reviews Neurology*,

9(11), pp. 617–628. doi: 10.1038/nrneurol.2013.203.
10. Al-Chalabi, A., Jones, A., Troakes, C., King, A., Al-Sarraj, S. and Van Den Berg, L. H. (2012) 'The genetics and neuropathology of amyotrophic lateral sclerosis', *Acta Neuropathologica*, pp. 339–352. doi: 10.1007/s00401-012-1022-4.
11. Al-Chalabi, A. and Lewis, C. M. (2011) 'Modelling the effects of penetrance and family size on rates of sporadic and familial disease.', *Human heredity*, 71(4), pp. 281–8. doi: 10.1159/000330167.
12. Al-Saif, A., Al-Mohanna, F. and Bohlega, S. (2011) 'A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis', *Annals of Neurology*, 70(6), pp. 913–919. doi: 10.1002/ana.22534.
13. Albery, W. J. and Knowles, J. R. (1977) 'Efficiency and evolution of enzyme catalysis.', *Angewandte Chemie (International ed. in English)*, 16(5), pp. 285–93. doi: 10.1002/anie.197702851.
14. Allodi, I., Nijssen, J., Benitez, J. A., Schweingruber, C., Fuchs, A., Bonvicini, G., Cao, M., Kiehn, O. and Hedlund, E. (2019) 'Modeling Motor Neuron Resilience in ALS Using Stem Cells.', *Stem cell reports*. Cell Press, 12(6), pp. 1329–1341. doi: 10.1016/j.stemcr.2019.04.009.
15. Alonso, R., Pisa, D., Fernández-Fernández, A. M., Rábano, A. and Carrasco, L. (2017) 'Fungal infection in neural tissue of patients with amyotrophic lateral sclerosis', *Neurobiology of Disease*. Academic Press Inc., 108, pp. 249–260. doi: 10.1016/j.nbd.2017.09.001.
16. Åman, P., Panagopoulos, I., Lassen, C., Fioretos, T., Mencinger, M., Toresson, H., Höglund, M., Forster, A., Rabbitts, T. H., Ron, D., Mandahl, N. and Mitelman, F. (1996) 'Expression Patterns of the Human Sarcoma-Associated GenesFUSandEWSand the Genomic Structure ofFUS', *Genomics*, 37(1), pp. 1–8. doi: 10.1006/geno.1996.0513.
17. Amthor, H., Otto, A., Vulin, A., Rochat, A., Dumonceaux, J., Garcia, L., Mouisel, E., Hourde, C., Macharia, R., Friedrichs, M., Relaix, F., Zammit, P. S., Matsakas, A., Patel, K. and Partridge, T. (2009) 'Muscle hypertrophy driven by myostatin blockade does not require stem/precursor-cell activity', *Proceedings of the National Academy of Sciences*, 106(18), pp. 7479–7484. doi: 10.1073/pnas.0811129106.
18. An, H., Skelt, L., Notaro, A., Highley, J. R., Fox, A. H., La Bella, V., Buchman, V. L. and Shelkovnikova, T. A. (2019) 'ALS-linked FUS mutations confer loss and gain of function in the nucleus by promoting excessive formation of dysfunctional paraspeckles.', *Acta neuropathologica communications*, 7(1), p. 7. doi: 10.1186/s40478-019-0658-x.
19. Andersen, P. M. and Al-Chalabi, A. (2011) 'Clinical genetics of amyotrophic lateral sclerosis: What do we really know?', *Nature Reviews Neurology*. Nature Publishing Group, pp. 603–615. doi: 10.1038/nrneurol.2011.150.
20. Arai, T., Hasegawa, M., Akiyama, H., Ikeda, K., Nonaka, T., Mori, H., Mann, D., Tsuchiya, K., Yoshida, M., Hashizume, Y. and Oda, T. (2006) 'TDP-43 is a component of ubiquitin-positive tau-negative inclusions in frontotemporal lobar degeneration and amyotrophic lateral sclerosis', *Biochemical and Biophysical Research Communications*, 351(3), pp. 602–611. doi: 10.1016/j.bbrc.2006.10.093.
21. Araki, K., Araki, A., Honda, D., Izumoto, T., Hashizume, A., Hijikata, Y., Yamada, S., Iguchi, Y., Hara, A., Ikumi, K., Kawai, K., Ishigaki, S., Nakamichi, Y., Tsunekawa, S., Seino, Y., Yamamoto, A., Takayama, Y., Hidaka, S.,

Tominaga, M., Ohara-Imaizumi, M., Suzuki, A., Ishiguro, H., Enomoto, A., Yoshida, M., Arima, H., Muramatsu, S.-I., Sobue, G. and Katsuno, M. (2019) 'TDP-43 regulates early-phase insulin secretion via CaV1.2-mediated exocytosis in islets.', *The Journal of clinical investigation*, 130, pp. 3578–3593. doi: 10.1172/JCI124481.

22. Armon, C. (2004) 'Excess incidence of ALS in young Gulf War veterans.', *Neurology*, 63(10), pp. 1986-7; author reply 1986-7. doi: 10.1212/wnl.63.10.1986.
23. Armstrong, G. A. B. and Drapeau, P. (2013) 'Loss and gain of FUS function impair neuromuscular synaptic transmission in a genetic model of ALS.', *Human molecular genetics*, 22(21), pp. 4282–92. doi: 10.1093/hmg/ddt278.
24. Arsenijevic, D., Onuma, H., Pecqueur, C., Raimbault, S., Manning, B. S., Miroux, B., Couplan, E., Alves-Guerra, M. C., Goubern, M., Surwit, R., Bouillaud, F., Richard, D., Collins, S. and Ricquier, D. (2000) 'Disruption of the uncoupling protein-2 gene in mice reveals a role in immunity and reactive oxygen species production.', *Nature genetics*, 26(4), pp. 435–9. doi: 10.1038/82565.
25. Arvidson, B. (1992) 'Inorganic mercury is transported from muscular nerve terminals to spinal and brainstem motoneurons', *Muscle & Nerve*, 15(10), pp. 1089–1094. doi: 10.1002/mus.880151006.
26. Asfour, H. A., Allouh, M. Z. and Said, R. S. (2018) 'Myogenic regulatory factors: The orchestrators of myogenesis after 30 years of discovery', *Experimental Biology and Medicine*. SAGE Publications Inc., 243(2), pp. 118–128. doi: 10.1177/1535370217749494.
27. Ayers, J. I., Fromholt, S. E., O'Neal, V. M., Diamond, J. H. and Borchelt, D. R. (2016) 'Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways', *Acta Neuropathologica*. Springer Verlag, 131(1), pp. 103–114. doi: 10.1007/s00401-015-1514-0.
28. Ayyasamy, V., Owens, K. M., Desouki, M. M., Liang, P., Bakin, A., Thangaraj, K., Buchsbaum, D. J., LoBuglio, A. F. and Singh, K. K. (2011) 'Cellular model of Warburg effect identifies tumor promoting function of UCP2 in breast cancer and its suppression by genipin.', *PloS one*. Edited by A. Lewin, 6(9), p. e24792. doi: 10.1371/journal.pone.0024792.
29. Bai, P., Cantó, C., Oudart, H., Brunyánszki, A., Cen, Y., Thomas, C., Yamamoto, H., Huber, A., Kiss, B., Houtkooper, R. H., Schoonjans, K., Schreiber, V., Sauve, A. A., Menissier-De Murcia, J. and Auwerx, J. (2011) 'PARP-1 inhibition increases mitochondrial metabolism through SIRT1 activation', *Cell Metabolism*, 13(4), pp. 461–468. doi: 10.1016/j.cmet.2011.03.004.
30. Baloh, R. H., Rakowicz, W., Gardner, R. and Pestronk, A. (2007) 'Frequent atrophic groups with mixed-type myofibers is distinctive to motor neuron syndromes.', *Muscle & nerve*, 36(1), pp. 107–10. doi: 10.1002/mus.20755.
31. Barbeito, A. G., Martinez-Palma, L., Vargas, M. R., Pehar, M., Mañay, N., Beckman, J. S., Barbeito, L. and Cassina, P. (2010) 'Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS', *Neurobiology of Disease*, 37(3), pp. 574–580. doi: 10.1016/j.nbd.2009.11.007.
32. BARRIS, R. W. (1953a) 'Hyperinsulinism and neuromuscular disorders; a consideration of the association of pancreatic adenoma with wasting

states.', *California medicine*, 78(3), pp. 224–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/13032800 (Accessed: 3 February 2020).

33. BARRIS, R. W. (1953b) 'Pancreatic adenoma (hyperinsulinism) associated with neuromuscular disorders.', *Annals of internal medicine*, 38(1), pp. 124–9. doi: 10.7326/0003-4819-38-1-124.
34. Baskoylu, S. N., Yersak, J., O'Hern, P., Grosser, S., Simon, J., Kim, S., Schuch, K., Dimitriadi, M., Yanagi, K. S., Lins, J. and Hart, A. C. (2018) 'Single copy/knock-in models of ALS SOD1 in C. elegans suggest loss and gain of function have different contributions to cholinergic and glutamatergic neurodegeneration.', *PLoS genetics*, 14(10), p. e1007682. doi: 10.1371/journal.pgen.1007682.
35. Bassett, T., Bach, P. and Chan, H. M. (2012) 'Effects of methylmercury on the secretion of pro-inflammatory cytokines from primary microglial cells and astrocytes', *NeuroToxicology*, 33(2), pp. 229–234. doi: 10.1016/j.neuro.2011.10.003.
36. Bé Langer, M., Allaman, I. and Magistretti, P. J. (2011) 'Cell Metabolism Review Brain Energy Metabolism: Focus on Astrocyte-Neuron Metabolic Cooperation'. doi: 10.1016/j.cmet.2011.08.016.
37. Beghi, E., Logroscino, G., Chiò, A., Hardiman, O., Millul, A., Mitchell, D., Swingler, R. and Traynor, B. J. (2010) 'Amyotrophic lateral sclerosis, physical exercise, trauma and sports: Results of a population-based pilot case-control study', *Amyotrophic Lateral Sclerosis*, 11(3), pp. 289–292. doi: 10.3109/17482960903384283.
38. Bendotti, C., Marino, M., Cheroni, C., Fontana, E., Crippa, V., Poletti, A. and De Biasi, S. (2012) 'Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response.', *Progress in neurobiology*, 97(2), pp. 101–26. doi: 10.1016/j.pneurobio.2011.10.001.
39. Van den Bergh, R., Swerts, L., Hendrikx, A., Boni, L. and Meulepas, E. (1977) 'Adipose tissue cellularity in patients with amyotrophic lateral sclerosis.', *Clinical neurology and neurosurgery*, 80(4), pp. 226–39. Available at: http://www.ncbi.nlm.nih.gov/pubmed/216513 (Accessed: 23 July 2019).
40. Bernardini, C., Censi, F., Lattanzi, W., Barba, M., Calcagnini, G., Giuliani, A., Tasca, G., Sabatelli, M., Ricci, E. and Michetti, F. (2013) 'Mitochondrial Network Genes in the Skeletal Muscle of Amyotrophic Lateral Sclerosis Patients', *PLoS ONE*, 8(2). doi: 10.1371/journal.pone.0057739.
41. Blasco, H., Mavel, S., Corcia, P. and Gordon, P. H. (2014) 'The Glutamate Hypothesis in ALS: Pathophysiology and Drug Development', *Current Medicinal Chemistry*. Bentham Science Publishers Ltd., 21(31), pp. 3551–3575. doi: 10.2174/0929867321666140916120118.
42. van Blitterswijk, M., DeJesus-Hernandez, M., Niemantsverdriet, E., Murray, M. E., Heckman, M. G., Diehl, N. N., Brown, P. H., Baker, M. C., Finch, N. A., Bauer, P. O., Serrano, G., Beach, T. G., Josephs, K. A., Knopman, D. S., Petersen, R. C., Boeve, B. F., Graff-Radford, N. R., Boylan, K. B., Petrucelli, L., Dickson, D. W. and Rademakers, R. (2013) 'Association between repeat sizes and clinical and pathological characteristics in carriers of C9ORF72 repeat expansions (Xpansize-72): a cross-sectional cohort study.', *The Lancet. Neurology*, 12(10), pp. 978–88. doi: 10.1016/S1474-4422(13)70210-2.
43. Blot, S., Poirier, C. and Dreyfus, P. A. (1995) 'The mouse mutation muscle

deficient (mdf) is characterized by a progressive motoneuron disease.', *Journal of neuropathology and experimental neurology*, 54(6), pp. 812–25. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7595654 (Accessed: 5 February 2020).

44. Bodine, S. C., Latres, E., Baumhueter, S., Lai, V. K., Nunez, L., Clarke, B. A., Poueymirou, W. T., Panaro, F. J., Na, E., Dharmarajan, K., Pan, Z. Q., Valenzuela, D. M., DeChiara, T. M., Stitt, T. N., Yancopoulos, G. D. and Glass, D. J. (2001) 'Identification of ubiquitin ligases required for skeletal muscle atrophy.', *Science (New York, N.Y.)*, 294(5547), pp. 1704–8. doi: 10.1126/science.1065874.
45. Boeynaems, S., Bogaert, E., Van Damme, P. and Van Den Bosch, L. (2016) 'Inside out: the role of nucleocytoplasmic transport in ALS and FTLD', *Acta Neuropathologica*. Springer Verlag, pp. 159–173. doi: 10.1007/s00401-016-1586-5.
46. Bogdanov, M., Brown, R. H., Matson, W., Smart, R., Hayden, D., O'Donnell, H., Flint Beal, M. and Cudkowicz, M. (2000) 'Increased oxidative damage to DNA in ALS patients', *Free Radical Biology and Medicine*, 29(7), pp. 652–658. doi: 10.1016/S0891-5849(00)00349-X.
47. Boillée, S., Vande Velde, C. and Cleveland, D. W. (2006) 'ALS: A Disease of Motor Neurons and Their Nonneuronal Neighbors', *Neuron*, 52(1), pp. 39–59. doi: 10.1016/j.neuron.2006.09.018.
48. Boillée, S., Yamanaka, K., Lobsiger, C. S., Copeland, N. G., Jenkins, N. A., Kassiotis, G., Kollias, G. and Cleveland, D. W. (2006) 'Onset and progression in inherited ALS determined by motor neurons and microglia.', *Science (New York, N.Y.)*, 312(5778), pp. 1389–92. doi: 10.1126/science.1123511.
49. Boiteux, A. and Hess, B. (1981) 'Design of glycolysis.', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 293(1063), pp. 5–22. doi: 10.1098/rstb.1981.0056.
50. Bonaldo, P. and Sandri, M. (2013) 'Cellular and molecular mechanisms of muscle atrophy', 39, pp. 25–39. doi: 10.1242/dmm.010389.
51. Bosco, D. A., Lemay, N., Ko, H. K., Zhou, H., Burke, C., Kwiatkowski, T. J., Sapp, P., McKenna-Yasek, D., Brown, R. H. and Hayward, L. J. (2010) 'Mutant FUS proteins that cause amyotrophic lateral sclerosis incorporate into stress granules', *Human Molecular Genetics*, 19(21), pp. 4160–4175. doi: 10.1093/hmg/ddq335.
52. Bosco, D. A., Morfini, G., Karabacak, N. M., Song, Y., Gros-Louis, F., Pasinelli, P., Goolsby, H., Fontaine, B. A., Lemay, N., McKenna-Yasek, D., Frosch, M. P., Agar, J. N., Julien, J.-P., Brady, S. T. and Brown, R. H. (2010) 'Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS', *Nature Neuroscience*, 13(11), pp. 1396–1403. doi: 10.1038/nn.2660.
53. Bouchard, C., Lee, S., Paulus-Hock, V., Loddenkemper, C., Eilers, M. and Schmitt, C. A. (2007) 'FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of Arf.', *Genes & development*, 21(21), pp. 2775–87. doi: 10.1101/gad.453107.
54. Bouchard, C., Marquardt, J., Brás, A., Medema, R. H. and Eilers, M. (2004) 'Myc-induced proliferation and transformation require Akt-mediated phosphorylation of FoxO proteins.', *The EMBO journal*, 23(14), pp. 2830–40. doi: 10.1038/sj.emboj.7600279.
55. Bouillaud, F. (2009) 'Biochimica et Biophysica Acta UCP2 , not a physiologically relevant uncoupler but a glucose sparing switch impacting

ROS production and glucose sensing', *BBA - Bioenergetics*. Elsevier B.V., 1787(5), pp. 377–383. doi: 10.1016/j.bbabio.2009.01.003.

56. Boylan, K., Levine, T., Lomen-Hoerth, C., Lyon, M., Maginnis, K., Callas, P., Gaspari, C. and Tandan, R. (2016) 'Prospective study of cost of care at multidisciplinary ALS centers adhering to American Academy of Neurology (AAN) ALS practice parameters', *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*. Taylor and Francis Ltd, 17(1–2), pp. 119–127. doi: 10.3109/21678421.2015.1091478.
57. Bradley, W. G. (2015) 'The John Walton Muscular Dystrophy Research Centre in the University of Newcastle and the BMAA Theory of Motor Neuron Disease.', *Journal of neuromuscular diseases*. Edited by H. Lochmüller, 2(s2), pp. S77–S81. doi: 10.3233/JND-150090.
58. Brodmann, K. (1909) *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues.* Available at: https://ia800403.us.archive.org/30/items/b28062449/b28062449.pdf (Accessed: 27 June 2019).
59. Brooks, B. R. (1994) 'El escorial World Federation of Neurology criteria for the diagnosis of amyotrophic lateral sclerosis', in *Journal of the Neurological Sciences*, pp. 96–107. doi: 10.1016/0022-510X(94)90191-0.
60. Brooks, B. R., Miller, R. G., Swash, M. and Munsat, T. L. (2000) 'El Escorial revisited: Revised criteria for the diagnosis of amyotrophic lateral sclerosis', *Amyotrophic Lateral Sclerosis*, 1(5), pp. 293–299. doi: 10.1080/146608200300079536.
61. BROWN, I. A. (1954) 'Chronic mercurialism; a cause of the clinical syndrome of amyotrophic lateral sclerosis.', *A.M.A. archives of neurology and psychiatry*, 72(6), pp. 674–81. Available at: http://www.ncbi.nlm.nih.gov/pubmed/13206485 (Accessed: 3 February 2020).
62. Brüel, A., Christensen, E., Qvortrup, K., Tranum-Jensen, J. and Geneser, F. (2014) *Geneser Histología*, *Editorial Médica Panamericana*.
63. BUNINA, T. L. (1962) '[On intracellular inclusions in familial amyotrophic lateral sclerosis].', *Zhurnal nevropatologii i psikhiatrii imeni S.S. Korsakova (Moscow, Russia : 1952)*, 62, pp. 1293–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14016841 (Accessed: 27 June 2019).
64. Byrne, S., Heverin, M., Elamin, M., Bede, P., Lynch, C., Kenna, K., MacLaughlin, R., Walsh, C., Al Chalabi, A. and Hardiman, O. (2013) 'Aggregation of neurologic and neuropsychiatric disease in amyotrophic lateral sclerosis kindreds: A population-based case-control cohort study of familial and sporadic amyotrophic lateral sclerosis', *Annals of Neurology*, 74(5), pp. 699–708. doi: 10.1002/ana.23969.
65. Cabanes, C., Bonilla, S., Tabares, L. and Martínez, S. (2007) 'Neuroprotective effect of adult hematopoietic stem cells in a mouse model of motoneuron degeneration', *Neurobiology of Disease*, 26(2), pp. 408–418. doi: 10.1016/j.nbd.2007.01.008.
66. Callaghan, B., Feldman, D., Gruis, K. and Feldman, E. (2011) 'The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications.', *Neuro-degenerative diseases*, 8(1–2), pp. 1–8. doi: 10.1159/000315405.
67. Calof, A. L. and Reichardt, L. F. (1984) 'Motoneurons purified by cell sorting

respond to two distinct activities in myotube-conditioned medium.', *Developmental biology*, 106(1), pp. 194–210. doi: 10.1016/0012-1606(84)90075-7.

68. Campion, D. R. (1984) 'The muscle satellite cell: A review', *International Review of Cytology*, VOL. 87, pp. 225–251. doi: 10.1016/s0074-7696(08)62444-4.
69. Cangiano, A., Buffelli, M., Busetto, G., Tognana, E. and Pasino, E. (1997) 'Studies on anterograde trophic interactions based on general muscle properties.', *Archives italiennes de biologie*, 135(4), pp. 331–41. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9270895 (Accessed: 5 August 2019).
70. Cao, Q., Boyer, D. R., Sawaya, M. R., Ge, P. and Eisenberg, D. S. (2019) 'Cryo-EM structures of four polymorphic TDP-43 amyloid cores.', *Nature structural & molecular biology*, 26(7), pp. 619–627. doi: 10.1038/s41594-019-0248-4.
71. Capitanio, D., Vasso, M., Ratti, A., Grignaschi, G., Volta, M., Moriggi, M., Daleno, C., Bendotti, C., Silani, V. and Gelfi, C. (2012) 'Molecular signatures of amyotrophic lateral sclerosis disease progression in hind and forelimb muscles of an SOD1G93A mouse model', *Antioxidants and Redox Signaling*, 17(10), pp. 1333–1350. doi: 10.1089/ars.2012.4524.
72. Cascella, R., Capitini, C., Fani, G., Dobson, C. M., Cecchi, C. and Chiti, F. (2016) 'Quantification of the Relative Contributions of Loss-of-function and Gain-of-function Mechanisms in TAR DNA-binding Protein 43 (TDP-43) Proteinopathies.', *The Journal of biological chemistry*. American Society for Biochemistry and Molecular Biology Inc., 291(37), pp. 19437–48. doi: 10.1074/jbc.M116.737726.
73. Cascella, R., Capitini, C., Fani, G., Dobson, C. M., Cecchi, C. and Chiti, F. (2016) 'Quantification of the Relative Contributions of Loss-offunction and Gain-of-function Mechanisms in TAR DNA-binding Protein 43 (TDP-43) Proteinopathies', *Journal of Biological Chemistry*. American Society for Biochemistry and Molecular Biology Inc., 291(37), pp. 19437–19448. doi: 10.1074/jbc.M116.737726.
74. Casteignau, A., Fontan, A., Morillo, A., Oliveros, J. A. and Segales, J. (2006) 'Clinical, Pathological and Toxicological Findings of a Iatrogenic Selenium Toxicosis Case in Feeder Pigs', *Journal of Veterinary Medicine Series A*, 53(6), pp. 323–326. doi: 10.1111/j.1439-0442.2006.00830.x.
75. Charcot, J. and Joffory, A. (1869) 'Deux cas d'atrophie musculaire progressive avec lesions de la substance grise et des faisceaux antero-lateraux de la moelle epiniere'. Available at: https://www.scienceopen.com/document?vid=090ec17c-1f69-4675-9617-a58ccc3477ca (Accessed: 27 June 2019).
76. Chattopadhyay, M., Durazo, A., Hui, S., Strong, C. D., Gralla, E. B., Whitelegge, J. P. and Selverstone, J. (2008) 'Chattopadhyay M, 2008.pdf', 105(48), pp. 2–7. doi: 10.1073/pnas.0807058105.
77. Cheah, B. C., Vucic, S., Krishnan, A. and Kiernan, M. C. (2010) 'Riluzole, Neuroprotection and Amyotrophic Lateral Sclerosis', *Current Medicinal Chemistry*. Bentham Science Publishers Ltd., 17(18), pp. 1942–1959. doi: 10.2174/092986710791163939.
78. Chemello, F., Bean, C., Cancellara, P., Laveder, P., Reggiani, C. and Lanfranchi, G. (2011) 'Microgenomic analysis in skeletal muscle: expression signatures of individual fast and slow myofibers.', *PloS one*.

Edited by S. Melov, 6(2), p. e16807. doi: 10.1371/journal.pone.0016807.

79. Chen, A. K.-H., Lin, R. Y.-Y., Hsieh, E. Z.-J., Tu, P.-H., Chen, R. P.-Y., Liao, T.-Y., Chen, W., Wang, C.-H. and Huang, J. J.-T. (2010) 'Induction of amyloid fibrils by the C-terminal fragments of TDP-43 in amyotrophic lateral sclerosis.', *Journal of the American Chemical Society*, 132(4), pp. 1186–7. doi: 10.1021/ja9066207.

80. Chew, J., Gendron, T. F., Prudencio, M., Sasaguri, H., Zhang, Y.-J., Castanedes-Casey, M., Lee, C. W., Jansen-West, K., Kurti, A., Murray, M. E., Bieniek, K. F., Bauer, P. O., Whitelaw, E. C., Rousseau, L., Stankowski, J. N., Stetler, C., Daughrity, L. M., Perkerson, E. A., Desaro, P., Johnston, A., Overstreet, K., Edbauer, D., Rademakers, R., Boylan, K. B., Dickson, D. W., Fryer, J. D. and Petrucelli, L. (2015) 'Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits.', *Science (New York, N.Y.)*, 348(6239), pp. 1151–4. doi: 10.1126/science.aaa9344.

81. Chiò, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E. and Traynor, B. G. (2009) 'Prognostic factors in ALS: A critical review', *Amyotrophic Lateral Sclerosis*, pp. 310–323. doi: 10.3109/17482960802566824.

82. Chiò, A., Mora, G., Moglia, C., Manera, U., Canosa, A., Cammarosano, S., Ilardi, A., Bertuzzo, D., Bersano, E., Cugnasco, P., Grassano, M., Pisano, F., Mazzini, L. and Calvo, A. (2017) 'Secular trends of amyotrophic lateral sclerosis: The Piemonte and Valle d'Aosta register', *JAMA Neurology*. American Medical Association, 74(9), pp. 1097–1104. doi: 10.1001/jamaneurol.2017.1387.

83. Cirulli, E. T., Lasseigne, B. N., Petrovski, S., Sapp, P. C., Dion, P. A., Leblond, C. S., Couthouis, J., Lu, Y. F., Wang, Q., Krueger, B. J., Ren, Z., Keebler, J., Han, Y., Levy, S. E., Boone, B. E., Wimbish, J. R., Waite, L. L., Jones, A. L., Carulli, J. P., Day-Williams, A. G., Staropoli, J. F., Xin, W. W., Chesi, A., Raphael, A. R., McKenna-Yasek, D., Cady, J., De Jong, J. M. B. V., Kenna, K. P., Smith, B. N., Topp, S., Miller, J., Gkazi, A., Al-Chalabi, A., van den Berg, L. H., Veldink, J., Silani, V., Ticozzi, N., Shaw, P. J., Baloh, R. H., Appel, S., Simpson, E., Lagier-Tourenne, C., Pulst, S. M., Gibson, S., Trojanowski, J. Q., Elman, L., McCluskey, L., Grossman, M., Shneider, N. A., Chung, W. K., Ravits, J. M., Glass, J. D., Sims, K. B., Van Deerlin, V. M., Maniatis, T., Hayes, S. D., Ordureau, A., Swarup, S., Landers, J., Baas, F., Allen, A. S., Bedlack, R. S., Harper, J. W., Gitler, A. D., Rouleau, G. A., Brown, R., Harms, M. B., Cooper, G. M., Harris, T., Myers, R. M., Goldstein, D. B., Hardiman, O., McLaughlin, R. L., Mazzini, L., Blair, I. P., Williams, K. L., Nicholson, G. A., Al-Sarraj, S., King, A., Scotter, E. L., Troakes, C., Vance, C., D'Alfonso, S., Duga, S., Corrado, L., ten Asbroek, A. L., Calini, D., Colombrita, C., Ratti, A., Tiloca, C., Wu, Z., Asress, S., Polak, M., Diekstra, F., van Rheenen, W., Danielson, E. W., Fallini, C., Keagle, P., Lewis, E. A., Kost, J., Sorarù, G., Bertolin, C., Querin, G., Castellotti, B., Gellera, C., Pensato, V., Taroni, F., Cereda, C., Gagliardi, S., Ceroni, M., Lauria, G., de Belleroche, J., Comi, G. P., Corti, S., Del Bo, R., Turner, M. R., Talbot, K., Pall, H., Morrison, K. E., Esteban-Pérez, J., García-Redondo, A. and Muñoz-Blanco, J. L. (2015) 'Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways', *Science*. American Association for the Advancement of Science, 347(6229), pp. 1436–1441. doi: 10.1126/science.aaa3650.

84. Cistaro, A., Pagani, M., Montuschi, A., Calvo, A., Moglia, C., Canosa, A.,

Restagno, G., Brunetti, M., Traynor, B. J., Nobili, F., Carrara, G., Fania, P., Lopiano, L., Valentini, M. C. and Chiò, A. (2014) 'The metabolic signature of C9ORF72-related ALS: FDG PET comparison with nonmutated patients', *European Journal of Nuclear Medicine and Molecular Imaging*, 41(5), pp. 844–852. doi: 10.1007/s00259-013-2667-5.

85. Clarke, B. A., Drujan, D., Willis, M. S., Murphy, L. O., Corpina, R. A., Burova, E., Rakhilin, S. V, Stitt, T. N., Patterson, C., Latres, E. and Glass, D. J. (2007) 'The E3 Ligase MuRF1 degrades myosin heavy chain protein in dexamethasone-treated skeletal muscle.', *Cell metabolism*, 6(5), pp. 376–85. doi: 10.1016/j.cmet.2007.09.009.
86. Cohen, B. and Caroscio, J. (1983) 'Eye movements in amyotrophic lateral sclerosis.', *Journal of neural transmission. Supplementum*, 19, pp. 305–15. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6583314 (Accessed: 21 August 2019).
87. Cohen, S., Lee, D., Zhai, B., Gygi, S. P. and Goldberg, A. L. (2014) 'Trim32 reduces PI3K-Akt-FoxO signaling in muscle atrophy by promoting plakoglobin-PI3K dissociation', *Journal of Cell Biology*. Rockefeller University Press, 204(5), pp. 747–758. doi: 10.1083/jcb.201304167.
88. Collis, W. J. and Engel, W. K. (1968) 'Glucose metabolism in five neuromuscular disorders.', *Neurology*, 18(9), pp. 915–25. doi: 10.1212/wnl.18.9.915.
89. Cooney, G. J. and Dawson, A. G. (1979) 'Effects of indomethacin on respiration and the alpha-glycerolphosphate shuttle in rat kidney mitochondria.', *Biochemical pharmacology*, 28(7), pp. 1067–70. doi: 10.1016/0006-2952(79)90305-8.
90. Court, F. A., Gillingwater, T. H., Melrose, S., Sherman, D. L., Greenshields, K. N., Morton, A. J., Harris, J. B., Willison, H. J. and Ribchester, R. R. (2008) 'Identity, developmental restriction and reactivity of extralaminar cells capping mammalian neuromuscular junctions', *Journal of Cell Science*, 121(23), pp. 3901–3911. doi: 10.1242/jcs.031047.
91. Csibi, A., Cornille, K., Leibovitch, M.-P., Poupon, A., Tintignac, L. A., Sanchez, A. M. J. and Leibovitch, S. A. (2010) 'The translation regulatory subunit eIF3f controls the kinase-dependent mTOR signaling required for muscle differentiation and hypertrophy in mouse.', *PloS one*. Edited by M. V. Blagosklonny, 5(2), p. e8994. doi: 10.1371/journal.pone.0008994.
92. Cykowski, M. D., Powell, S. Z., Appel, J. W., Arumanayagam, A. S., Rivera, A. L. and Appel, S. H. (2018) 'aggregates in the axial skeletal muscle of patients with sporadic and familial amyotrophic lateral sclerosis'. Acta Neuropathologica Communications, 43, pp. 1–15.
93. Dadon-Nachum, M., Melamed, E. and Offen, D. (2011) 'The "dying-back" phenomenon of motor neurons in ALS', *Journal of Molecular Neuroscience*, pp. 470–477. doi: 10.1007/s12031-010-9467-1.
94. DeBerardinis, R. J., Mancuso, A., Daikhin, E., Nissim, I., Yudkoff, M., Wehrli, S. and Thompson, C. B. (2007) 'Beyond aerobic glycolysis: transformed cells can engage in glutamine metabolism that exceeds the requirement for protein and nucleotide synthesis.', *Proceedings of the National Academy of Sciences of the United States of America*, 104(49), pp. 19345–50. doi: 10.1073/pnas.0709747104.
95. Deda, H., Inci, M. C., Kürekçi, A. E., Sav, A., Kayihan, K., Özgün, E., Üstünsoy, G. E. and Kocabay, S. (2009) 'Treatment of amyotrophic lateral sclerosis patients by autologous bone marrow-derived hematopoietic stem cell

transplantation: A 1-year follow-up', *Cytotherapy*. Elsevier Inc., 11(1), pp. 18–25. doi: 10.1080/14653240802549470.

96. Deng, H. X., Chen, W., Hong, S. T., Boycott, K. M., Gorrie, G. H., Siddique, N., Yang, Y., Fecto, F., Shi, Y., Zhai, H., Jiang, H., Hirano, M., Rampersaud, E., Jansen, G. H., Donkervoort, S., Bigio, E. H., Brooks, B. R., Ajroud, K., Sufit, R. L., Haines, J. L., Mugnaini, E., Pericak-Vance, M. A. and Siddique, T. (2011) 'Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia', *Nature*, 477(7363), pp. 211–215. doi: 10.1038/nature10353.
97. Desport, J. C., Preux, P. M., Magy, L., Boirie, Y., Vallat, J. M., Beaufrère, B. and Couratier, P. (2001) 'Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis.', *The American journal of clinical nutrition*, 74(3), pp. 328–34. doi: 10.1093/ajcn/74.3.328.
98. Diekstra, F. P., Saris, C. G. J., van Rheenen, W., Franke, L., Jansen, R. C., van Es, M. A., van Vught, P. W. J., Blauw, H. M., Groen, E. J. N., Horvath, S., Estrada, K., Rivadeneira, F., Hofman, A., Uitterlinden, A. G., Robberecht, W., Andersen, P. M., Melki, J., Meininger, V., Hardiman, O., Landers, J. E., Brown, R. H., Shatunov, A., Shaw, C. E., Leigh, P. N., Al-Chalabi, A., Ophoff, R. A., van den Berg, L. H. and Veldink, J. H. (2012) 'Mapping of Gene Expression Reveals CYP27A1 as a Susceptibility Gene for Sporadic ALS', *PLoS ONE*. Edited by M. P. van der Brug, 7(4), p. e35333. doi: 10.1371/journal.pone.0035333.
99. Dobrowolny, G., Lepore, E., Martini, M., Barberi, L., Nunn, A., Scicchitano, B. M. and Musarò, A. (2018) 'Metabolic changes associated with muscle expression of SOD1G93A', *Frontiers in Physiology*. Frontiers Media S.A., 9(JUL). doi: 10.3389/fphys.2018.00831.
100. Dodge, J. C., Treleaven, C. M., Fidler, J. A., Tamsett, T. J., Bao, C., Searles, M., Taksir, T. V, Misra, K., Sidman, R. L., Cheng, S. H. and Shihabuddin, L. S. (2013) 'Metabolic signatures of amyotrophic lateral sclerosis reveal insights into disease pathogenesis'. doi: 10.1073/pnas.1308421110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1308421110.
101. Dohrmann, U., Edgar, D., Sendtner, M. and Thoenen, H. (1986) 'Muscle-derived factors that support survival and promote fiber outgrowth from embryonic chick spinal motor neurons in culture', *Developmental Biology*, 118(1), pp. 209–221. doi: 10.1016/0012-1606(86)90089-8.
102. Dohrmann, U., Edgar, D. and Thoenen, H. (1987) 'Distinct neurotrophic factors from skeletal muscle and the central nervous system interact synergistically to support the survival of cultured embryonic spinal motor neurons', *Developmental Biology*, 124(1), pp. 145–152. doi: 10.1016/0012-1606(87)90467-2.
103. Dols-Icardo, O., García-Redondo, A., Rojas-García, R., Sánchez-Valle, R., Noguera, A., Gó mez-Tortosa, E., Pastor, P., Hernández, I., Esteban-Pérez, J., Suárez-Calvet, M., Antó n-Aguirre, S., Amer, G., Ortega-Cubero, S., Blesa, R., Fortea, J., Alcolea, D., Capdevila, A., Antonell, A., Lladó, A., Munoz-Blanco, J. L., Mora, J. S., LucíaGalán-Dá vila, De Rivera, F. J. R., Lleó, A. and Clarimón, J. (2014) 'Characterization of the repeat expansion size in C9orf72 in amyotrophic lateral sclerosis and frontotemporal dementia', *Human Molecular Genetics*, 23(3), pp. 749–754. doi: 10.1093/hmg/ddt460.
104. Dormann, D. and Haass, C. (2011) 'TDP-43 and FUS: A nuclear affair', *Trends in Neurosciences*, pp. 339–348. doi: 10.1016/j.tins.2011.05.002.
105. Drechsel, D. A., Estévez, A. G., Barbeito, L. and Beckman, J. S. (2012) 'Nitric

oxide-mediated oxidative damage and the progressive demise of motor neurons in ALS.', *Neurotoxicity research*, 22(4), pp. 251–64. doi: 10.1007/s12640-012-9322-y.

106. Dupuis, L., Aguilar, J. G. De, Echaniz-laguna, A. and Eschbach, J. (2009) 'Muscle Mitochondrial Uncoupling Dismantles Neuromuscular Junction and Triggers Distal Degeneration of Motor Neurons', 4(4). doi: 10.1371/journal.pone.0005390.
107. Dupuis, L., Pradat, P., Ludolph, A. C. and Loeffl, J. (2011a) 'Energy metabolism in amyotrophic lateral sclerosis', pp. 75–83. doi: 10.1016/S1474-4422(10)70224-6.
108. Dupuis, L., Pradat, P., Ludolph, A. C. and Loeffl, J. (2011b) 'Energy metabolism in amyotrophic lateral sclerosis', pp. 75–82. doi: 10.1016/S1474-4422(10)70224-6.
109. Eaglehouse, Y. L., Talbott, E. O., Chang, Y. and Kuller, L. H. (2016) 'Participation in Physical Activity and Risk for Amyotrophic Lateral Sclerosis Mortality Among Postmenopausal Women', *JAMA Neurology*, 73(3), p. 329. doi: 10.1001/jamaneurol.2015.4487.
110. Elden, A. C., Kim, H.-J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M. M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P. J., Rüb, U., Auburger, G., Trojanowski, J. Q., Lee, V. M.-Y., Van Deerlin, V. M., Bonini, N. M. and Gitler, A. D. (2010) 'Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS.', *Nature*, 466(7310), pp. 1069–75. doi: 10.1038/nature09320.
111. Elden, A. C., Kim, H. J., Hart, M. P., Chen-Plotkin, A. S., Johnson, B. S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M. M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P. J., Rüb, U., Auburger, G., Trojanowski, J. Q., Lee, V. M. Y., Van Deerlin, V. M., Bonini, N. M. and Gitler, A. D. (2010) 'Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS', *Nature*, 466(7310), pp. 1069–1075. doi: 10.1038/nature09320.
112. Ermilova, I. P., Ermilov, V. B., Levy, M., Ho, E., Pereira, C. and Beckman, J. S. (2005) 'Protection by dietary zinc in ALS mutant G93A SOD transgenic mice.', *Neuroscience letters*, 379(1), pp. 42–6. doi: 10.1016/j.neulet.2004.12.045.
113. van Es, M. A., Veldink, J. H., Saris, C. G. J., Blauw, H. M., van Vught, P. W. J., Birve, A., Lemmens, R., Schelhaas, H. J., Groen, E. J. N., Huisman, M. H. B., van der Kooi, A. J., de Visser, M., Dahlberg, C., Estrada, K., Rivadeneira, F., Hofman, A., Zwarts, M. J., van Doormaal, P. T. C., Rujescu, D., Strengman, E., Giegling, I., Muglia, P., Tomik, B., Slowik, A., Uitterlinden, A. G., Hendrich, C., Waibel, S., Meyer, T., Ludolph, A. C., Glass, J. D., Purcell, S., Cichon, S., Nöthen, M. M., Wichmann, H.-E., Schreiber, S., Vermeulen, S. H. H. M., Kiemeney, L. A., Wokke, J. H. J., Cronin, S., McLaughlin, R. L., Hardiman, O., Fumoto, K., Pasterkamp, R. J., Meininger, V., Melki, J., Leigh, P. N., Shaw, C. E., Landers, J. E., Al-Chalabi, A., Brown, R. H., Robberecht, W., Andersen, P. M., Ophoff, R. A. and van den Berg, L. H. (2009) 'Genome-wide association study identifies 19p13.3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis.', *Nature genetics*, 41(10), pp. 1083–7. doi: 10.1038/ng.442.
114. Estevez, A. O., Mueller, C. L., Morgan, K. L., Szewczyk, N. J., Teece, L., Miranda-Vizuete, A. and Estevez, M. (2012) 'Selenium induces cholinergic

motor neuron degeneration in Caenorhabditis elegans', *NeuroToxicology*, 33(5), pp. 1021–1032. doi: 10.1016/j.neuro.2012.04.019.

115. Ezzi, S. A., Urushitani, M. and Julien, J.-P. (2007) 'Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation', *Journal of Neurochemistry*, 102(1), pp. 170–178. doi: 10.1111/j.1471-4159.2007.04531.x.
116. Falconi, M., Iacovelli, F. and Desideri, A. (2013) 'A structural modeling approach for the understanding of initiation and elongation of ALS-linked superoxide dismutase fibrils.', *Journal of molecular modeling*, 19(9), pp. 3695–704. doi: 10.1007/s00894-013-1896-7.
117. Falkowska, A., Gutowska, I., Goschorska, M. and Nowacki, P. (2015) 'Energy Metabolism of the Brain , Including the Cooperation between Astrocytes and Neurons , Especially in the Context of Glycogen Metabolism', pp. 25959–25981. doi: 10.3390/ijms161125939.
118. Falone, S., Mirabilio, A., Carbone, M. C., Zimmitti, V., Di Loreto, S., Mariggiò, M. A., Mancinelli, R., Di Ilio, C. and Amicarelli, F. (2008) 'Chronic exposure to 50Hz magnetic fields causes a significant weakening of antioxidant defence systems in aged rat brain', *The International Journal of Biochemistry & Cell Biology*, 40(12), pp. 2762–2770. doi: 10.1016/j.biocel.2008.05.022.
119. Fang, F., Ingre, C., Roos, P., Kamel, F. and Piehl, F. (2015) 'Risk factors for amyotrophic lateral sclerosis', *Clinical Epidemiology*, 7, p. 181. doi: 10.2147/CLEP.S37505.
120. Fang, F., Kwee, L. C., Allen, K. D., Umbach, D. M., Ye, W., Watson, M., Keller, J., Oddone, E. Z., Sandler, D. P., Schmidt, S. and Kamel, F. (2010) 'Association Between Blood Lead and the Risk of Amyotrophic Lateral Sclerosis', *American Journal of Epidemiology*, 171(10), pp. 1126–1133. doi: 10.1093/aje/kwq063.
121. Farg, M. A., Sundaramoorthy, V., Sultana, J. M., Yang, S., Atkinson, R. A. K., Levina, V., Halloran, M. A., Gleeson, P. A., Blair, I. P., Soo, K. Y., King, A. E. and Atkin, J. D. (2014) 'C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia, regulates endosomal trafficking.', *Human molecular genetics*, 23(13), pp. 3579–95. doi: 10.1093/hmg/ddu068.
122. Farhan, M., Wang, H., Gaur, U., Little, P. J., Xu, J. and Zheng, W. (2017) 'FOXO signaling pathways as therapeutic targets in cancer', *International Journal of Biological Sciences*. Ivyspring International Publisher, pp. 815–827. doi: 10.7150/ijbs.20052.
123. Feiguin, F., Godena, V. K., Romano, G., D'Ambrogio, A., Klima, R. and Baralle, F. E. (2009) 'Depletion of TDP-43 affects Drosophila motoneurons terminal synapsis and locomotive behavior.', *FEBS letters*, 583(10), pp. 1586–92. doi: 10.1016/j.febslet.2009.04.019.
124. Fergani, A., Eschbach, J., Oudart, H., Larmet, Y., Schwalenstocker, B., Ludolph, A. C., Loeffler, J.-P. and Dupuis, L. (2011) 'A mutation in the dynein heavy chain gene compensates for energy deficit of mutant SOD1 mice and increases potentially neuroprotective IGF-1.', *Molecular neurodegeneration*, 6(1), p. 26. doi: 10.1186/1750-1326-6-26.
125. Flood, D. G., Reaume, A. G., Gruner, J. A., Hoffman, E. K., Hirsch, J. D., Lin, Y. G., Dorfman, K. S. and Scott, R. W. (1999) 'Hindlimb motor neurons require Cu/Zn superoxide dismutase for maintenance of neuromuscular junctions.', *The American journal of pathology*. American Society for Investigative Pathology Inc., 155(2), pp. 663–72. doi: 10.1016/S0002-

9440(10)65162-0.
126. Foster, D. W. and McGarry, J. D. (1983) 'The metabolic derangements and treatment of diabetic ketoacidosis.', *The New England journal of medicine*, 309(3), pp. 159–69. doi: 10.1056/NEJM198307213090307.
127. Fournier, C. N., Bedlack, R., Quinn, C., Russell, J., Beckwith, D., Kaminski, K. H., Tyor, W., Hertzberg, V., James, V., Polak, M. and Glass, J. D. (2019) 'Development and Validation of the Rasch-Built Overall Amyotrophic Lateral Sclerosis Disability Scale (ROADS)', *JAMA Neurology*. American Medical Association. doi: 10.1001/jamaneurol.2019.4490.
128. Freibaum, B. D., Lu, Y., Lopez-Gonzalez, R., Kim, N. C., Almeida, S., Lee, K.-H., Badders, N., Valentine, M., Miller, B. L., Wong, P. C., Petrucelli, L., Kim, H. J., Gao, F.-B. and Taylor, J. P. (2015) 'GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport', *Nature*, 525(7567), pp. 129–133. doi: 10.1038/nature14974.
129. French, P. W., Ludowyke, R. and Guillemin, G. J. (2019) 'Fungal Neurotoxins and Sporadic Amyotrophic Lateral Sclerosis', *Neurotoxicity Research*. Springer New York LLC, pp. 969–980. doi: 10.1007/s12640-018-9980-5.
130. Frontera, W. R. and Ochala, J. (2015) 'Skeletal Muscle: A Brief Review of Structure and Function', *Behavior Genetics*. Springer New York LLC, pp. 183–195. doi: 10.1007/s00223-014-9915-y.
131. Funalot, B., Desport, J.-C., Sturtz, F., Camu, W. and Couratier, P. (2009) 'High metabolic level in patients with familial amyotrophic lateral sclerosis', *Amyotrophic Lateral Sclerosis*, 10(2), pp. 113–117. doi: 10.1080/17482960802295192.
132. *Fundamentals of Human Physiology - Lauralee Sherwood - Google Libros* (no date). Available at: https://books.google.es/books?hl=es&lr=&id=qXwIAAAAQBAJ&oi=fnd&pg=PR7&dq=Sherwood+L+(2010)+Human+physiology.+Brooks/Cole-Cengage+Learning,+Belmont,+CA&ots=omQ__x9tUj&sig=IcJBBPXKdk_TDvaSo5hzG-5A_oo#v=onepage&q&f=false (Accessed: 16 January 2020).
133. Gallo, V., Vanacore, N., Bueno-de-Mesquita, H. B., Vermeulen, R., Brayne, C., Pearce, N., Wark, P. A., Ward, H. A., Ferrari, P., Jenab, M., Andersen, P. M., Wennberg, P., Wareham, N., Katzke, V., Kaaks, R., Weiderpass, E., Peeters, P. H., Mattiello, A., Pala, V., Barricante, A., Chirlaque, M.-D., Travier, N., Travis, R. C., Sanchez, M.-J., Pessah-Rasmussen, H., Petersson, J., Tjønneland, A., Tumino, R., Quiros, J. R., Trichopoulou, A., Kyrozis, A., Oikonomidou, D., Masala, G., Sacerdote, C., Arriola, L., Boeing, H., Vigl, M., Claver-Chapelon, F., Middleton, L., Riboli, E. and Vineis, P. (2016) 'Physical activity and risk of Amyotrophic Lateral Sclerosis in a prospective cohort study', *European Journal of Epidemiology*, 31(3), pp. 255–266. doi: 10.1007/s10654-016-0119-9.
134. Galpin, A. J., Raue, U., Jemiolo, B., Trappe, T. A., Harber, M. P., Minchev, K. and Trappe, S. (2012) 'Human skeletal muscle fiber type specific protein content.', *Analytical biochemistry*, 425(2), pp. 175–82. doi: 10.1016/j.ab.2012.03.018.
135. Ganassi, M., Badodi, S., Ortuste Quiroga, H. P., Zammit, P. S., Hinits, Y. and Hughes, S. M. (2018) 'Myogenin promotes myocyte fusion to balance fibre number and size', *Nature Communications*, 9(1), p. 4232. doi: 10.1038/s41467-018-06583-6.
136. Gao, J., Wang, L., Huntley, M. L., Perry, G. and Wang, X. (2018) 'Pathomechanisms of TDP-43 in neurodegeneration.', *Journal of*

neurochemistry. doi: 10.1111/jnc.14327.

137. Gao, J., Wang, L., Yan, T., Perry, G. and Wang, X. (2019) 'TDP-43 proteinopathy and mitochondrial abnormalities in neurodegeneration.', *Molecular and cellular neurosciences*, 100, p. 103396. doi: 10.1016/j.mcn.2019.103396.
138. Garbugino, L., Golini, E., Giuliani, A. and Mandillo, S. (2018) 'Prolonged Voluntary Running Negatively Affects Survival and Disease Prognosis of Male SOD1G93A Low-Copy Transgenic Mice.', *Frontiers in behavioral neuroscience*. Frontiers Media S.A., 12, p. 275. doi: 10.3389/fnbeh.2018.00275.
139. Garcia-Simon, M., Morales, J. M., Modesto-Alapont, V., Gonzalez-Marrachelli, V., Vento-Rehues, R., Jorda-Miñana, A., Blanquer-Olivas, J. and Monleon, D. (2015) 'Prognosis Biomarkers of Severe Sepsis and Septic Shock by 1H NMR Urine Metabolomics in the Intensive Care Unit', *PLOS ONE*. Edited by T. F. Bathen, 10(11), p. e0140993. doi: 10.1371/journal.pone.0140993.
140. García-Villaescusa, A., Morales-Tatay, J. M., Monleón-Salvadó, D., González-Darder, J. M., Bellot-Arcis, C., Montiel-Company, J. M. and Almerich-Silla, J. M. (2018) 'Using NMR in saliva to identify possible biomarkers of glioblastoma and chronic periodontitis', *PLOS ONE*. Edited by A. G. Passi, 13(2), p. e0188710. doi: 10.1371/journal.pone.0188710.
141. Gasco, S., Zaragoza, P., García-Redondo, A., Calvo, A. C. and Osta, R. (2017) 'Inflammatory and non-inflammatory monocytes as novel prognostic biomarkers of survival in SOD1G93A mouse model of Amyotrophic Lateral Sclerosis', *PLoS ONE*. Public Library of Science, 12(9). doi: 10.1371/journal.pone.0184626.
142. Geetha, T., Zheng, C., Vishwaprakash, N., Broderick, T. L. and Babu, J. R. (2012) 'Sequestosome 1/p62, a Scaffolding Protein, Is a Newly Identified Partner of IRS-1 Protein', *Journal of Biological Chemistry*, 287(35), pp. 29672–29678. doi: 10.1074/jbc.M111.322404.
143. Gibbons, C., Pagnini, F., Friede, T. and Young, C. A. (2018) 'Treatment of fatigue in amyotrophic lateral sclerosis/motor neuron disease', *Cochrane Database of Systematic Reviews*. John Wiley and Sons Ltd. doi: 10.1002/14651858.CD011005.pub2.
144. Gijselinck, I., Van Mossevelde, S., Van Der Zee, J., Sieben, A., Engelborghs, S., De Bleecker, J., Ivanoiu, A., Deryck, O., Edbauer, D., Zhang, M., Heeman, B., Bäumer, V., Van Den Broeck, M., Mattheijssens, M., Peeters, K., Rogaeva, E., De Jonghe, P., Cras, P., Martin, J. J., De Deyn, P. P., Cruts, M. and Van Broeckhoven, C. (2016) 'The C9orf72 repeat size correlates with onset age of disease, DNA methylation and transcriptional downregulation of the promoter', *Molecular Psychiatry*. Nature Publishing Group, 21(8), pp. 1112–1124. doi: 10.1038/mp.2015.159.
145. Giménez-Roldán, S., Morales-Asín, F., Ferrer, I. and Spencer, P. S. (2019) 'Historical setting and neuropathology of lathyrism: Insights from the neglected 1944 report by Oliveras de la Riva', *Journal of the History of the Neurosciences*. Routledge, 28(4), pp. 361–386. doi: 10.1080/0964704X.2019.1600357.
146. Glaser, J. and Suzuki, M. (2018) 'Chapter 5 Skeletal Muscle Fiber Types in Neuromuscular Diseases'. Available at: https://www.semanticscholar.org/paper/Chapter-5-Skeletal-Muscle-Fiber-Types-in-Diseases-Glaser-

Suzuki/b5bc787c5c9352cc0cc94914cc2d84262ad9c8f6 (Accessed: 12 August 2019).
147. Goetz, C. G. (2000) 'Amyotrophic lateral sclerosis: early contributions of Jean-Martin Charcot.', *Muscle & nerve*, 23(3), pp. 336–43. Available at: http://www.ncbi.nlm.nih.gov/pubmed/10679709 (Accessed: 5 March 2019).
148. Gomes, M. D., Lecker, S. H., Jagoe, R. T., Navon, A. and Goldberg, A. L. (2001) 'Atrogin-1, a muscle-specific F-box protein highly expressed during muscle atrophy', *Proceedings of the National Academy of Sciences*, 98(25), pp. 14440–14445. doi: 10.1073/pnas.251541198.
149. Goodall, E. F., Greenway, M. J., van Marion, I., Carroll, C. B., Hardiman, O. and Morrison, K. E. (2005) 'Association of the H63D polymorphism in the hemochromatosis gene with sporadic ALS.', *Neurology*, 65(6), pp. 934–7. doi: 10.1212/01.wnl.0000176032.94434.d4.
150. Gordon, P. H., Miller, R. G. and Moore, D. H. (2004) 'ALSFRS-R', *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*, 5(SUPPL. 1), pp. 90–93. doi: 10.1080/17434470410019906.
151. Gotkine, M., Friedlander, Y. and Hochner, H. (2014) 'Triathletes are over-represented in a population of patients with ALS', *Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration*, 15(7–8), pp. 534–536. doi: 10.3109/21678421.2014.932383.
152. Gotoh, F., Kitamura, A., Koto, A., Kataoka, K. and Atsuji, H. (1972) 'Abnormal insulin secretion in amyotrophic lateral sclerosis', *Journal of the Neurological Sciences*, 16(2), pp. 201–207. doi: 10.1016/0022-510X(72)90089-5.
153. Gunnarsson, L. G., Dahlbom, K. and Strandman, E. (1991) 'Motor neuron disease and dementia reported among 13 members of a single family.', *Acta neurologica Scandinavica*, 84(5), pp. 429–33. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1776392 (Accessed: 19 July 2019).
154. Gurel, A., Coskun, O., Armutcu, F., Kanter, M. and Ozen, O. A. (2005) 'Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies.', *Journal of chemical neuroanatomy*, 29(3), pp. 173–8. doi: 10.1016/j.jchemneu.2005.01.001.
155. Guskiewicz, K. M., Marshall, S. W., Bailes, J., McCrea, M., Cantu, R. C., Randolph, C. and Jordan, B. D. (2005) 'Association between recurrent concussion and late-life cognitive impairment in retired professional football players.', *Neurosurgery*, 57(4). doi: 10.1093/neurosurgery/57.4.719.
156. Gutiérrez-Rivas, E. (2017) *Manual de enfermedades neuromusculares*. Ergón. Available at: http://ergon.es/producto/manual-enfermedades-neuromusculares/ (Accessed: 24 June 2019).
157. Haley, R. W. (2003) 'Excess incidence of ALS in young Gulf War veterans.', *Neurology*, 61(6), pp. 750–6. doi: 10.1212/wnl.61.6.750.
158. Hallauer, P. L. and Hastings, K. E. M. (2002) 'Coregulation of fast contractile protein transgene and glycolytic enzyme expression in mouse skeletal muscle.', *American journal of physiology. Cell physiology*, 282(1), pp. C113-24. doi: 10.1152/ajpcell.00294.2001.
159. Ben Hamida, C., Soussi-Yanicostas, N., Butler-Browne, G. S., Bejaoui, K., Hentati, F. and Ben Hamida, M. (1994) 'Biochemical and immunocytochemical analysis in chronic proximal spinal muscular atrophy.', *Muscle & nerve*, 17(4), pp. 400–10. doi: 10.1002/mus.880170407.

160. Hegedus, J., Putman, C. T. and Gordon, T. (2009) 'Progressive motor unit loss in the G93A mouse model of amyotrophic lateral sclerosis is unaffected by gender', *Muscle and Nerve*, 39(3), pp. 318–327. doi: 10.1002/mus.21160.
161. HERS, H. G. (1984) 'The discovery and the biological role of fructose 2,6-bisphosphate', *Biochemical Society Transactions*, 12(5), pp. 729–735. doi: 10.1042/bst0120729.
162. Homsher, E. (1987) 'Muscle Enthalpy Production and ITS Relationship to Actomyosin ATPase', *Annual Review of Physiology*, 49(1), pp. 673–690. doi: 10.1146/annurev.ph.49.030187.003325.
163. Honda, D., Ishigaki, S., Iguchi, Y., Fujioka, Y., Udagawa, T., Masuda, A., Ohno, K., Katsuno, M. and Sobue, G. (2014) 'The ALS/FTLD-related RNA-binding proteins TDP-43 and FUS have common downstream RNA targets in cortical neurons', *FEBS Open Bio*, 4(1), pp. 1–10. doi: 10.1016/j.fob.2013.11.001.
164. Horner, R. D., Kamins, K. G., Feussner, J. R., Grambow, S. C., Hoff-Lindquist, J., Harati, Y., Mitsumoto, H., Pascuzzi, R., Spencer, P. S., Tim, R., Howard, D., Smith, T. C., Ryan, M. A. K., Coffman, C. J. and Kasarskis, E. J. (2003) 'Occurrence of amyotrophic lateral sclerosis among Gulf War veterans.', *Neurology*, 61(6), pp. 742–9. doi: 10.1212/01.wnl.0000069922.32557.ca.
165. Hosler, B. A., Siddique, T., Sapp, P. C., Sailor, W., Huang, M. C., Hossain, A., Daube, J. R., Nance, M., Fan, C., Kaplan, J., Hung, W. Y., McKenna-Yasek, D., Haines, J. L., Pericak-Vance, M. A., Horvitz, H. R. and Brown, R. H. (2000) 'Linkage of familial amyotrophic lateral sclerosis with frontotemporal dementia to chromosome 9q21-q22', *Journal of the American Medical Association*. American Medical Association, 284(13), pp. 1664–1669. doi: 10.1001/jama.284.13.1664.
166. Huang, Y.-H., Shih, C.-M., Huang, C.-J., Lin, C.-M., Chou, C.-M., Tsai, M.-L., Liu, T. P., Chiu, J.-F. and Chen, C.-T. (2006) 'Effects of cadmium on structure and enzymatic activity of Cu,Zn-SOD and oxidative status in neural cells', *Journal of Cellular Biochemistry*, 98(3), pp. 577–589. doi: 10.1002/jcb.20772.
167. Hukema, R. K., Riemslagh, F. W., Melhem, S., van der Linde, H. C., Severijnen, L.-A. W., Edbauer, D., Maas, A., Charlet-Berguerand, N., Willemsen, R. and van Swieten, J. C. (2014) 'A new inducible transgenic mouse model for C9orf72-associated GGGGCC repeat expansion supports a gain-of-function mechanism in C9orf72-associated ALS and FTD.', *Acta neuropathologica communications*, 2(1), p. 166. doi: 10.1186/s40478-014-0166-y.
168. Imai, S. and Yoshino, J. (2013) 'The importance of NAMPT/NAD/SIRT1 in the systemic regulation of metabolism and ageing', *Diabetes, Obesity and Metabolism*. Blackwell Publishing Ltd, pp. 26–33. doi: 10.1111/dom.12171.
169. Ivanova, M. I., Sievers, S. A., Guenther, E. L., Johnson, L. M., Winkler, D. D., Galaleldeen, A., Sawaya, M. R., Hart, P. J. and Eisenberg, D. S. (2014) 'Aggregation-triggering segments of SOD1 fibril formation support a common pathway for familial and sporadic ALS.', *Proceedings of the National Academy of Sciences of the United States of America*, 111(1), pp. 197–201. doi: 10.1073/pnas.1320786110.
170. Jensen, L., Jørgensen, L. H., Bech, R. D., Frandsen, U. and Schrøder, H. D. (2016) 'Skeletal Muscle Remodelling as a Function of Disease Progression in Amyotrophic Lateral Sclerosis', 2016. doi: 10.1155/2016/5930621.
171. Jez, P., Garlid, K. D. and Jabu, M. (2018) 'Mitochondrial Uncoupling

Proteins : Subtle Regulators of Cellular Redox Signaling', 00(00). doi: 10.1089/ars.2017.7225.

172. Ježek, P., Modrianský, M. and Garlid, K. D. (1997) 'Inactive fatty acids are unable to flip-flop across the lipid bilayer', *FEBS Letters*, 408(2), pp. 161–165. doi: 10.1016/S0014-5793(97)00334-7.
173. Joensen, P. (2012) 'Incidence of amyotrophic lateral sclerosis in the Faroe Islands', *Acta Neurologica Scandinavica*, 126(1), pp. 62–66. doi: 10.1111/j.1600-0404.2011.01611.x.
174. Johnson, J. O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V. M., Trojanowski, J. Q., Gibbs, J. R., Brunetti, M., Gronka, S., Wuu, J., Ding, J., McCluskey, L., Martinez-Lage, M., Falcone, D., Hernandez, D. G., Arepalli, S., Chong, S., Schymick, J. C., Rothstein, J., Landi, F., Wang, Y. D., Calvo, A., Mora, G., Sabatelli, M., Monsurrò, M. R., Battistini, S., Salvi, F., Spataro, R., Sola, P., Borghero, G., Galassi, G., Scholz, S. W., Taylor, J. P., Restagno, G., Chiò, A. and Traynor, B. J. (2010) 'Exome Sequencing Reveals VCP Mutations as a Cause of Familial ALS', *Neuron*, 68(5), pp. 857–864. doi: 10.1016/j.neuron.2010.11.036.
175. Johnson, J. O., Pioro, E. P., Boehringer, A., Chia, R., Feit, H., Renton, A. E., Pliner, H. A., Abramzon, Y., Marangi, G., Winborn, B. J., Gibbs, J. R., Nalls, M. A., Morgan, S., Shoai, M., Hardy, J., Pittman, A., Orrell, R. W., Malaspina, A., Sidle, K. C., Fratta, P., Harms, M. B., Baloh, R. H., Pestronk, A., Weihl, C. C., Rogaeva, E., Zinman, L., Drory, V. E., Borghero, G., Mora, G., Calvo, A., Rothstein, J. D., Drepper, C., Sendtner, M., Singleton, A. B., Taylor, J. P., Cookson, M. R., Restagno, G., Sabatelli, M., Bowser, R., Chio`, A., Traynor, B. J., Moglia, C., Cammarosano, S., Canosa, A., Gallo, S., Brunetti, M., Ossola, I., Marinou, K., Papetti, L., Pisano, F., Pinter, G. L., Conte, A., Luigetti, M., Zollino, M., Lattante, S., Marangi, G., la Bella, V., Spataro, R., Colletti, T., Battistini, S., Ricci, C., Caponnetto, C., Mancardi, G., Mandich, P., Salvi, F., Bartolomei, I., Mandrioli, J., Sola, P., Lunetta, C., Penco, S., Monsurrò, M. R., Conforti, F. L., Tedeschi, G., Gambardella, A., Quattrone, A., Volanti, P., Floris, G., Cannas, A., Piras, V., Marrosu, F., Marrosu, M. G., Murru, M. R., Pugliatti, M., Parish, L. D., Sotgiu, A., Solinas, G., Ulgheri, L., Ticca, A., Simone, I., Logroscino, G. and Pirisi, A. (2014) 'Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis', *Nature Neuroscience*. Nature Publishing Group, 17(5), pp. 664–666. doi: 10.1038/nn.3688.
176. Jucker, M. and Walker, L. C. (2013) 'Self-propagation of pathogenic protein aggregates in neurodegenerative diseases', *Nature*, pp. 45–51. doi: 10.1038/nature12481.
177. Jucker, M. and Walker, L. C. (2018) 'Propagation and spread of pathogenic protein assemblies in neurodegenerative diseases', *Nature Neuroscience*. Nature Publishing Group, pp. 1341–1349. doi: 10.1038/s41593-018-0238-6.
178. Juurlink, B. H., Munoz, D. G. and Devon, R. M. (1991) 'Muscle derived motoneuron trophic factors promote the survival of motoneurons in vitro only when serum is present in the growth medium.', *The International journal of neuroscience*, 58(3–4), pp. 249–54. Available at: http://www.ncbi.nlm.nih.gov/pubmed/1365045 (Accessed: 6 August 2019).
179. Kabashi, E., Bercier, V., Lissouba, A., Liao, M., Brustein, E., Rouleau, G. A. and Drapeau, P. (2011) 'FUS and TARDBP but Not SOD1 Interact in Genetic Models of Amyotrophic Lateral Sclerosis', *PLoS Genetics*. Edited by H. T.

Orr, 7(8), p. e1002214. doi: 10.1371/journal.pgen.1002214.
180. Kabashi, E., Lin, L., Tradewell, M. L., Dion, P. A., Bercier, V., Bourgouin, P., Rochefort, D., Bel Hadj, S., Durham, H. D., Velde, C. Vande, Rouleau, G. A. and Drapeau, P. (2010) 'Gain and loss of function of ALS-related mutations of TARDBP (TDP-43) cause motor deficits in vivo', *Human Molecular Genetics*, 19(4), pp. 671–683. doi: 10.1093/hmg/ddp534.
181. Kabashi, E., Valdmanis, P. N., Dion, P., Spiegelman, D., McConkey, B. J., Velde, C. Vande, Bouchard, J. P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P. F., Camu, W., Meininger, V., Dupre, N. and Rouleau, G. A. (2008) 'TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis', *Nature Genetics*, 40(5), pp. 572–574. doi: 10.1038/ng.132.
182. Kaivola, K., Kiviharju, A., Jansson, L., Rantalainen, V., Eriksson, J. G., Strandberg, T. E., Laaksovirta, H., Renton, A. E., Traynor, B. J., Myllykangas, L. and Tienari, P. J. (2019) 'C9orf72 hexanucleotide repeat length in older population: normal variation and effects on cognition', *Neurobiology of Aging*. Elsevier Inc., 84, p. 242.e7-242.e12. doi: 10.1016/j.neurobiolaging.2019.02.026.
183. Kamel, F., Umbach, D. M., Hu, H., Munsat, T. L., Shefner, J. M., Taylor, J. A. and Sandler, D. P. (2005) 'Lead Exposure as a Risk Factor for Amyotrophic Lateral Sclerosis', *Neurodegenerative Diseases*, 2(3–4), pp. 195–201. doi: 10.1159/000089625.
184. Kasarskis, E. J., Lindquist, J. H., Coffman, C. J., Grambow, S. C., Feussner, J. R., Allen, K. D., Oddone, E. Z., Kamins, K. A., Horner, R. D. and Als Gulf War Clinical Review Team (2009) 'Clinical aspects of ALS in Gulf War veterans.', *Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on Motor Neuron Diseases*, 10(1), pp. 35–41. doi: 10.1080/17482960802351029.
185. Katoh, M., Igarashi, M., Fukuda, H., Nakagama, H. and Katoh, M. (2013) 'Cancer genetics and genomics of human FOX family genes', *Cancer Letters*, pp. 198–206. doi: 10.1016/j.canlet.2012.09.017.
186. Kim, H. J., Kim, N. C., Wang, Y. D., Scarborough, E. A., Moore, J., Diaz, Z., MacLea, K. S., Freibaum, B., Li, S., Molliex, A., Kanagaraj, A. P., Carter, R., Boylan, K. B., Wojtas, A. M., Rademakers, R., Pinkus, J. L., Greenberg, S. A., Trojanowski, J. Q., Traynor, B. J., Smith, B. N., Topp, S., Gkazi, A. S., Miller, J., Shaw, C. E., Kottlors, M., Kirschner, J., Pestronk, A., Li, Y. R., Ford, A. F., Gitler, A. D., Benatar, M., King, O. D., Kimonis, V. E., Ross, E. D., Weihl, C. C., Shorter, J. and Taylor, J. P. (2013) 'Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS', *Nature*, 495(7442), pp. 467–473. doi: 10.1038/nature11922.
187. Kitamura, T., Kitamura, Y. I., Funahashi, Y., Shawber, C. J., Castrillon, D. H., Kollipara, R., DePinho, R. A., Kitajewski, J. and Accili, D. (2007) 'A Foxo/Notch pathway controls myogenic differentiation and fiber type specification', *Journal of Clinical Investigation*, 117(9), pp. 2477–2485. doi: 10.1172/JCI32054.
188. Kjellgren, D., Thornell, L.-E., Andersen, J. and Pedrosa-Domello¨f, F. (2003) 'Myosin Heavy Chain Isoforms in Human Extraocular Muscles', *Investigative Opthalmology & Visual Science*, 44(4), p. 1419. doi: 10.1167/iovs.02-0638.
189. Kohler, R. (1971) 'The background to Eduard Buchner's discovery of cell-free fermentation.', *Journal of the history of biology*, 4, pp. 35–61. doi:

10.1007/bf00356976.

190. Koppers, M., Blokhuis, A. M., Westeneng, H.-J., Terpstra, M. L., Zundel, C. A. C., Vieira de Sá, R., Schellevis, R. D., Waite, A. J., Blake, D. J., Veldink, J. H., van den Berg, L. H. and Pasterkamp, R. J. (2015a) 'C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits.', *Annals of neurology*, 78(3), pp. 426–38. doi: 10.1002/ana.24453.
191. Koppers, M., Blokhuis, A. M., Westeneng, H.-J., Terpstra, M. L., Zundel, C. A. C., Vieira de Sá, R., Schellevis, R. D., Waite, A. J., Blake, D. J., Veldink, J. H., van den Berg, L. H. and Pasterkamp, R. J. (2015b) 'C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits.', *Annals of neurology*. John Wiley and Sons Inc., 78(3), pp. 426–38. doi: 10.1002/ana.24453.
192. Kraemer, B. C., Schuck, T., Wheeler, J. M., Robinson, L. C., Trojanowski, J. Q., Lee, V. M. Y. and Schellenberg, G. D. (2010) 'Loss of murine TDP-43 disrupts motor function and plays an essential role in embryogenesis.', *Acta neuropathologica*, 119(4), pp. 409–19. doi: 10.1007/s00401-010-0659-0.
193. Kresge, N., Simoni, R. D. and Hill, R. L. (2005) 'Otto Fritz Meyerhof and the elucidation of the glycolytic pathway.', *The Journal of biological chemistry*, 280(4), p. e3. Available at: http://www.ncbi.nlm.nih.gov/pubmed/15665335 (Accessed: 6 February 2020).
194. Kumar, D. R., Aslinia, F., Yale, S. H. and Mazza, J. J. (2011) 'Jean-martin charcot: The father of neurology', *Clinical Medicine and Research*, 9(1), pp. 46–49. doi: 10.3121/cmr.2009.883.
195. Kuroda, Y. and Sugihara, H. (1991) 'Autopsy report of HTLV-I-associated myelopathy presenting with ALS-like manifestations.', *Journal of the neurological sciences*, 106(2), pp. 199–205. doi: 10.1016/0022-510x(91)90258-9.
196. Kwiatkowski, T. J., Bosco, D. A., LeClerc, A. L., Tamrazian, E., Vanderburg, C. R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E. J., Munsat, T., Valdmanis, P., Rouleau, G. A., Hosler, B. A., Cortelli, P., de Jong, P. J., Yoshinaga, Y., Haines, J. L., Pericak-Vance, M. A., Yan, J., Ticozzi, N., Siddique, T., McKenna-Yasek, D., Sapp, P. C., Horvitz, H. R., Landers, J. E. and Brown, R. H. (2009) 'Mutations in the FUS/TLS Gene on Chromosome 16 Cause Familial Amyotrophic Lateral Sclerosis', *Science*, 323(5918), pp. 1205–1208. doi: 10.1126/science.1166066.
197. Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., Kim, J., Yun, J., Xie, Y. and McKnight, S. L. (2014) 'Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells', *Science*, 345(6201), pp. 1139–1145. doi: 10.1126/science.1254917.
198. Lagier-Tourenne, C., Polymenidou, M., Hutt, K. R., Vu, A. Q., Baughn, M., Huelga, S. C., Clutario, K. M., Ling, S.-C., Liang, T. Y., Mazur, C., Wancewicz, E., Kim, A. S., Watt, A., Freier, S., Hicks, G. G., Donohue, J. P., Shiue, L., Bennett, C. F., Ravits, J., Cleveland, D. W. and Yeo, G. W. (2012) 'Divergent roles of ALS-linked proteins FUS/TLS and TDP-43 intersect in processing long pre-mRNAs', *Nature Neuroscience*, 15(11), pp. 1488–1497. doi: 10.1038/nn.3230.
199. Lang, L., Zetterström, P., Brännström, T., Marklund, S. L., Danielsson, J., Oliveberg, M. and Horwich, A. L. (2015) 'SOD1 aggregation in ALS mice shows simplistic test tube behavior', *Proceedings of the National*

Academy of Sciences of the United States of America. National Academy of Sciences, 112(32), pp. 9878–9883. doi: 10.1073/pnas.1503328112.

200. LaNoue, K. F. and Williamson, J. R. (1971) 'Interrelationships between malate-aspartate shuttle and citric acid cycle in rat heart mitochondria.', *Metabolism: clinical and experimental*, 20(2), pp. 119–40. doi: 10.1016/0026-0495(71)90087-4.
201. Larsson, L. and Ansved, T. (1995) 'Effects of ageing on the motor unit', *Progress in Neurobiology*. doi: 10.1016/0301-0082(95)98601-Z.
202. Laurin, N., Brown, J. P., Morissette, J. and Raymond, V. (2002) 'Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in paget disease of bone', *American Journal of Human Genetics*, 70(6), pp. 1582–1588. doi: 10.1086/340731.
203. Leal, S. S. and Gomes, C. M. (2015) 'Calcium dysregulation links ALS defective proteins and motor neuron selective vulnerability', 9(June), pp. 1–6. doi: 10.3389/fncel.2015.00225.
204. Lecker, S. H., Goldberg, A. L. and Mitch, W. E. (2006) 'Protein Degradation by the Ubiquitin–Proteasome Pathway in Normal and Disease States', *Journal of the American Society of Nephrology*, 17(7), pp. 1807–1819. doi: 10.1681/ASN.2006010083.
205. Lee, E. B., Lee, V. M.-Y. and Trojanowski, J. Q. (2011a) 'Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration.', *Nature reviews. Neuroscience*, 13(1), pp. 38–50. doi: 10.1038/nrn3121.
206. Lee, Y., Jonson, P. H., Sarparanta, J., Palmio, J., Sarkar, M., Vihola, A., Evilä, A., Suominen, T., Penttilä, S., Savarese, M., Johari, M., Minot, M. C., Hilton-Jones, D., Maddison, P., Chinnery, P., Reimann, J., Kornblum, C., Kraya, T., Zierz, S., Sue, C., Goebel, H., Azfer, A., Ralston, S. H., Hackman, P., Bucelli, R. C., Taylor, J. P., Weihl, C. C. and Udd, B. (2018) 'TIA1 variant drives myodegeneration in multisystem proteinopathy with SQSTM1 mutations', *Journal of Clinical Investigation*. American Society for Clinical Investigation, 128(3), pp. 1164–1177. doi: 10.1172/JCI97103.
207. Lehman, E. J., Hein, M. J., Baron, S. L. and Gersic, C. M. (2012) 'Neurodegenerative causes of death among retired national football league players', *Neurology*. Lippincott Williams and Wilkins, 79(19), pp. 1970–1974. doi: 10.1212/WNL.0b013e31826daf50.
208. Lehmann, S., Esch, E., Hartmann, P., Goswami, A., Nikolin, S., Weis, J., Beyer, C. and Johann, S. (2018) 'Expression profile of pattern recognition receptors in skeletal muscle of SOD1(G93A) amyotrophic lateral sclerosis (ALS) mice and sporadic ALS patients', *Neuropathology and Applied Neurobiology*. Blackwell Publishing Ltd, 44(6), pp. 606–627. doi: 10.1111/nan.12483.
209. Lehninger, A. L. (1993) *Lehninger principles of biochemistry, Second Edition*, *W H Freeman*. doi: 10.1021/ed070pA223.1.
210. Lekoubou, A., Matsha, T. E., Sobngwi, E. and Kengne, A. P. (2014) 'Effects of diabetes mellitus on amyotrophic lateral sclerosis: a systematic review', *BMC Research Notes*, 7(1), p. 171. doi: 10.1186/1756-0500-7-171.
211. Lim, M. A., Selak, M. A., Xiang, Z., Krainc, D., Neve, R. L., Kraemer, B. C., Watts, J. L. and Kalb, R. G. (2012) 'Reduced Activity of AMP-Activated Protein Kinase Protects against Genetic Models of Motor Neuron Disease', 32(3), pp. 1123–1141. doi: 10.1523/JNEUROSCI.6554-10.2012.
212. Link, W. and Fernandez-Marcos, P. J. (2017) 'FOXO transcription factors at the interface of metabolism and cancer.', *International journal of cancer*.

Wiley-Liss Inc., 141(12), pp. 2379–2391. doi: 10.1002/ijc.30840.
213. Liu-Yesucevitz, L., Bilgutay, A., Zhang, Y.-J., Vanderweyde, T., Vanderwyde, T., Citro, A., Mehta, T., Zaarur, N., McKee, A., Bowser, R., Sherman, M., Petrucelli, L. and Wolozin, B. (2010) 'Tar DNA binding protein-43 (TDP-43) associates with stress granules: analysis of cultured cells and pathological brain tissue.', *PloS one*. Edited by A. I. Bush, 5(10), p. e13250. doi: 10.1371/journal.pone.0013250.
214. Liu, G., Mac Gabhann, F. and Popel, A. S. (2012) 'Effects of fiber type and size on the heterogeneity of oxygen distribution in exercising skeletal muscle.', *PloS one*, 7(9), p. e44375. doi: 10.1371/journal.pone.0044375.
215. Liu, W. and Chakkalakal, J. V. (2018) 'The Composition, Development, and Regeneration of Neuromuscular Junctions', in *Current Topics in Developmental Biology*. Academic Press Inc., pp. 99–124. doi: 10.1016/bs.ctdb.2017.08.005.
216. Logroscino, G., Traynor, B. J., Hardiman, O., Chio, A., Mitchell, D., Swingler, R. J., Millul, A., Benn, E., Beghi, E. and EURALS (2010) 'Incidence of amyotrophic lateral sclerosis in Europe', *Journal of Neurology, Neurosurgery & Psychiatry*, 81(4), pp. 385–390. doi: 10.1136/jnnp.2009.183525.
217. Lou, J., Wang, Y., Wang, X. and Jiang, Y. (2014) 'Uncoupling Protein 2 Regulates Palmitic Acid-Induced Hepatoma Cell Autophagy', *BioMed Research International*. Hindawi Publishing Corporation, 2014. doi: 10.1155/2014/810401.
218. Lowe, J., Lennox, G., Jefferson, D., Morrell, K., McQuire, D., Gray, T., Landon, M., Doherty, F. J. and Mayer, R. J. (1988) 'A filamentous inclusion body within anterior horn neurones in motor neurone disease defined by immunocytochemical localisation of ubiquitin.', *Neuroscience letters*, 94(1–2), pp. 203–10. Available at: http://www.ncbi.nlm.nih.gov/pubmed/2853853 (Accessed: 27 June 2019).
219. Luo, F., Gui, X., Zhou, H., Gu, J., Li, Y., Liu, X., Zhao, M., Li, D., Li, X. and Liu, C. (2018) 'Atomic structures of FUS LC domain segments reveal bases for reversible amyloid fibril formation.', *Nature structural & molecular biology*, 25(4), pp. 341–346. doi: 10.1038/s41594-018-0050-8.
220. Mackay, D. F., Russell, E. R., Stewart, K., MacLean, J. A., Pell, J. P. and Stewart, W. (2019) 'Neurodegenerative disease mortality among former professional soccer players', *New England Journal of Medicine*. Massachussetts Medical Society, 381(19), pp. 1801–1808. doi: 10.1056/NEJMoa1908483.
221. Mailloux, R. J., Adjeitey, C. N. K. and Harper, M. E. (2010) 'Genipin-induced inhibition of uncoupling protein-2 sensitizes drug-resistant cancer cells to cytotoxic agents', *PLoS ONE*. Public Library of Science, 5(10), p. e13289. doi: 10.1371/journal.pone.0013289.
222. Malek, A. M., Barchowsky, A., Bowser, R., Youk, A. and Talbott, E. O. (2012) 'Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS.', *Environmental research*, 117, pp. 112–9. doi: 10.1016/j.envres.2012.06.007.
223. Mancuso, R. and Navarro, X. (2015) 'Amyotrophic lateral sclerosis: Current perspectives from basic research to the clinic.', *Progress in neurobiology*, 133, pp. 1–26. doi: 10.1016/j.pneurobio.2015.07.004.

224. Manzano, R., Toivonen, J. M., Calvo, A. C., Oliván, S., Zaragoza, P., Rodellar, C., Montarras, D. and Osta, R. (2013) 'Altered in vitro proliferation of mouse SOD1-G93A skeletal muscle satellite cells.', *Neuro-degenerative diseases*, 11(3), pp. 153–64. doi: 10.1159/000338061.
225. Manzo, E., Lorenzini, I., Barrameda, D., O'Conner, A. G., Barrows, J. M., Starr, A., Kovalik, T., Rabichow, B. E., Lehmkuhl, E. M., Shreiner, D. D., Joardar, A., Liévens, J. C., Bowser, R., Sattler, R. and Zarnescu, D. C. (2019) 'Glycolysis upregulation is neuroprotective as a compensatory mechanism in ALS', *eLife*. eLife Sciences Publications Ltd, 8. doi: 10.7554/eLife.45114.
226. Maraldi, T., Riccio, M., Zambonin, L., Vinceti, M., De Pol, A. and Hakim, G. (2011) 'Low levels of selenium compounds are selectively toxic for a human neuron cell line through ROS/RNS increase and apoptotic process activation.', *Neurotoxicology*, 32(2), pp. 180–7. doi: 10.1016/j.neuro.2010.10.008.
227. Marcuzzo, S., Zucca, I., Mastropietro, A., de Rosbo, N. K., Cavalcante, P., Tartari, S., Bonanno, S., Preite, L., Mantegazza, R. and Bernasconi, P. (2011) 'Hind limb muscle atrophy precedes cerebral neuronal degeneration in G93A-SOD1 mouse model of amyotrophic lateral sclerosis: A longitudinal MRI study', *Experimental Neurology*, 231(1), pp. 30–37. doi: 10.1016/j.expneurol.2011.05.007.
228. Mariosa, D., Kamel, F., Bellocco, R., Ye, W. and Fang, F. (2015) 'Association between diabetes and amyotrophic lateral sclerosis in Sweden.', *European journal of neurology*, 22(11), pp. 1436–42. doi: 10.1111/ene.12632.
229. Markert, C. L., Shaklee, J. B. and Whitt, G. S. (1975) 'Evolution of a gene. Multiple genes for LDH isozymes provide a model of the evolution of gene structure, function and regulation.', *Science (New York, N.Y.)*, 189(4197), pp. 102–14. doi: 10.1126/science.1138367.
230. Marrachelli, V. G., Rentero, P., Mansego, M. L., Morales, J. M., Galan, I., Pardo-Tendero, M., Martinez, F., Martin-Escudero, J. C., Briongos, L., Chaves, F. J., Redon, J. and Monleon, D. (2016) 'Genomic and Metabolomic Profile Associated to Clustering of Cardio-Metabolic Risk Factors', *PLOS ONE*. Edited by P. K. Singh, 11(9), p. e0160656. doi: 10.1371/journal.pone.0160656.
231. Martinez, H. R., Gonzalez-Garza, M. T., Moreno-Cuevas, J. E., Caro, E., Gutierrez-Jimenez, E. and Segura, J. J. (2009) 'Stem-cell transplantation into the frontal motor cortex in amyotrophic lateral sclerosis patients', *Cytotherapy*. Elsevier Inc., 11(1), pp. 26–34. doi: 10.1080/14653240802644651.
232. Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H., Komure, O., Matsuura, S., Kobatake, K., Morimoto, N., Abe, K., Suzuki, N., Aoki, M., Kawata, A., Hirai, T., Kato, T., Ogasawara, K., Hirano, A., Takumi, T., Kusaka, H., Hagiwara, K., Kaji, R. and Kawakami, H. (2010) 'Mutations of optineurin in amyotrophic lateral sclerosis', *Nature*, 465(7295), pp. 223–226. doi: 10.1038/nature08971.
233. MAURO, A. (1961) 'Satellite cell of skeletal muscle fibers.', *The Journal of biophysical and biochemical cytology*, 9, pp. 493–495. doi: 10.1083/jcb.9.2.493.
234. Mazzini, L., Fagioli, F., Boccaletti, R., Mareschi, K., Oliveri, G., Olivieri, C., Pastore, Il., Marasso, R. and Madon, E. (2003) 'Stem cell therapy in

amyotrophic lateral sclerosis: A methodological approach in humans', *Amyotrophic Lateral Sclerosis and Other Motor Neuron Disorders*, 4(3), pp. 158–161. doi: 10.1080/14660820310014653.

235. Mazzini, L., Vescovi, A., Cantello, R., Gelati, M. and Vercelli, A. (2016) 'Stem cells therapy for ALS', *Expert Opinion on Biological Therapy*. Taylor and Francis Ltd, 16(2), pp. 187–199. doi: 10.1517/14712598.2016.1116516.
236. McAlary, L., Aquilina, J. A. and Yerbury, J. J. (2016) 'Susceptibility of Mutant SOD1 to Form a Destabilized Monomer Predicts Cellular Aggregation and Toxicity but Not In vitro Aggregation Propensity.', *Frontiers in neuroscience*, 10, p. 499. doi: 10.3389/fnins.2016.00499.
237. McAlary, L., Plotkin, S. S., Yerbury, J. J. and Cashman, N. (2019) 'Prion-like Propagation of Protein Misfolding and Aggregation in Amyotrophic Lateral Sclerosis', *Frontiers in Molecular Neuroscience*, 12(November), p. 262. doi: 10.3389/FNMOL.2019.00262.
238. MCARDLE, B. and VEREL, D. (1956) 'Responses to ischaemic work in the human forearm.', *Clinical science*, 15(2), pp. 305–18. Available at: http://www.ncbi.nlm.nih.gov/pubmed/13356561 (Accessed: 20 February 2020).
239. McCauley, M. E. and Baloh, R. H. (2019) 'Inflammation in ALS/FTD pathogenesis.', *Acta neuropathologica*, 137(5), pp. 715–730. doi: 10.1007/s00401-018-1933-9.
240. McFarlane, C., Plummer, E., Thomas, M., Hennebry, A., Ashby, M., Ling, N., Smith, H., Sharma, M. and Kambadur, R. (2006) 'Myostatin induces cachexia by activating the ubiquitin proteolytic system through an NF-κB-independent, FoxO1-dependent mechanism', *Journal of Cellular Physiology*, 209(2), pp. 501–514. doi: 10.1002/jcp.20757.
241. McGarry, J. D. and Foster, D. W. (1980) 'Regulation of Hepatic Fatty Acid Oxidation and Ketone Body Production', *Annual Review of Biochemistry*. Annual Reviews, 49(1), pp. 395–420. doi: 10.1146/annurev.bi.49.070180.002143.
242. McGeer, P. L. and McGeer, E. G. (2002) 'Inflammatory processes in amyotrophic lateral sclerosis.', *Muscle & nerve*, 26(4), pp. 459–70. doi: 10.1002/mus.10191.
243. McKenna, C. F. and Fry, C. S. (2017) 'Altered satellite cell dynamics accompany skeletal muscle atrophy during chronic illness, disuse, and aging', *Current Opinion in Clinical Nutrition and Metabolic Care*, 20(6), p. 1. doi: 10.1097/MCO.0000000000000409.
244. Mcloughlin, T. J., Smith, S. M., Delong, A. D., Wang, H., Unterman, T. G., Esser, K. A., Tj, M., Sm, S., Ad, D., Wang, H. and Tg, U. (2019) 'FoxO1 induces apoptosis in skeletal myotubes in a DNA-binding-dependent manner', 43606, pp. 548–555. doi: 10.1152/ajpcell.00502.2008.
245. Mimuro, M., Yoshida, M., Kuzuhara, S. and Kokubo, Y. (2018) 'Amyotrophic lateral sclerosis and parkinsonism-dementia complex of the Hohara focus of the Kii Peninsula: A multiple proteinopathy?', *Neuropathology : official journal of the Japanese Society of Neuropathology*, 38(1), pp. 98–107. doi: 10.1111/neup.12434.
246. Mitsumoto, H., Santella, R. M., Liu, X., Bogdanov, M., Zipprich, J., Wu, H.-C., Mahata, J., Kilty, M., Bednarz, K., Bell, D., Gordon, P. H., Hornig, M., Mehrazin, M., Naini, A., Flint Beal, M. and Factor-Litvak, P. (2008) 'Oxidative stress biomarkers in sporadic ALS.', *Amyotrophic lateral sclerosis : official publication of the World Federation of Neurology Research Group on*

Motor Neuron Diseases, 9(3), pp. 177–83. doi: 10.1080/17482960801933942.
247. Mizielinska, S. and Isaacs, A. M. (2014) 'C9orf72 amyotrophic lateral sclerosis and frontotemporal dementia', *Current Opinion in Neurology*, 27(5), pp. 515–523. doi: 10.1097/WCO.0000000000000130.
248. Mompeán, M., Buratti, E., Guarnaccia, C., Brito, R. M. M., Chakrabartty, A., Baralle, F. E. and Laurents, D. V (2014) '"Structural characterization of the minimal segment of TDP-43 competent for aggregation".', *Archives of biochemistry and biophysics*, 545, pp. 53–62. doi: 10.1016/j.abb.2014.01.007.
249. Monroy, J. A., Powers, K. L., Gilmore, L. A., Uyeno, T. A., Lindstedt, S. L. and Nishikawa, K. C. (2012) 'What is the role of titin in active muscle?', *Exercise and sport sciences reviews*, 40(2), pp. 73–8. doi: 10.1097/JES.0b013e31824580c6.
250. Moraleda, J. M., Blanquer, M., Bleda, P., Iniesta, P., Ruiz, F., Bonilla, S., Cabanes, C., Tabares, L. and Martinez, S. (2006) 'Adult stem cell therapy: Dream or reality?', *Transplant Immunology*, 17(1), pp. 74–77. doi: 10.1016/j.trim.2006.09.030.
251. Moresi, V., Williams, A. H., Meadows, E., Flynn, J. M., Potthoff, M. J., McAnally, J., Shelton, J. M., Backs, J., Klein, W. H., Richardson, J. A., Bassel-Duby, R. and Olson, E. N. (2010) 'Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases.', *Cell*, 143(1), pp. 35–45. doi: 10.1016/j.cell.2010.09.004.
252. Mueller, P. S. and Quick, D. T. (1970) 'Studies of glucose, insulin, and lipid metabolism in amyotrophic lateral sclerosis and other neuromuscular disorders.', *The Journal of laboratory and clinical medicine*, 76(2), pp. 190–201. Available at: http://www.ncbi.nlm.nih.gov/pubmed/5434000 (Accessed: 3 February 2020).
253. Münch, C. and Bertolotti, A. (2010) 'Exposure of hydrophobic surfaces initiates aggregation of diverse ALS-causing superoxide dismutase-1 mutants.', *Journal of molecular biology*, 399(3), pp. 512–25. doi: 10.1016/j.jmb.2010.04.019.
254. Munsat, T. L. (1970) 'A standardized forearm ischemic exercise test', *Neurology*, 20(12), pp. 1171–1178. doi: 10.1212/wnl.20.12.1171.
255. Murakami, T., Qamar, S., Lin, J. Q., Schierle, G. S. K., Rees, E., Miyashita, A., Costa, A. R., Dodd, R. B., Chan, F. T. S., Michel, C. H., Kronenberg-Versteeg, D., Li, Y., Yang, S.-P., Wakutani, Y., Meadows, W., Ferry, R. R., Dong, L., Tartaglia, G. G., Favrin, G., Lin, W.-L., Dickson, D. W., Zhen, M., Ron, D., Schmitt-Ulms, G., Fraser, P. E., Shneider, N. A., Holt, C., Vendruscolo, M., Kaminski, C. F. and St George-Hyslop, P. (2015) 'ALS/FTD Mutation-Induced Phase Transition of FUS Liquid Droplets and Reversible Hydrogels into Irreversible Hydrogels Impairs RNP Granule Function.', *Neuron*, 88(4), pp. 678–90. doi: 10.1016/j.neuron.2015.10.030.
256. Murch, S. J., Cox, P. A., Banack, S. A., Steele, J. C. and Sacks, O. W. (2004) 'Occurrence of beta-methylamino-l-alanine (BMAA) in ALS/PDC patients from Guam', *Acta Neurologica Scandinavica*, 110(4), pp. 267–269. doi: 10.1111/j.1600-0404.2004.00320.x.
257. Murray, D. T., Kato, M., Lin, Y., Thurber, K. R., Hung, I., McKnight, S. L. and Tycko, R. (2017) 'Structure of FUS Protein Fibrils and Its Relevance to Self-Assembly and Phase Separation of Low-Complexity Domains.', *Cell*, 171(3), p. 615–627.e16. doi: 10.1016/j.cell.2017.08.048.
258. Myatt, S. S. and Lam, E. W. F. (2007) 'The emerging roles of forkhead box

(Fox) proteins in cancer', *Nature Reviews Cancer*, pp. 847–859. doi: 10.1038/nrc2223.

259. Nagano, Y., Tsubaki, T. and Chase, T. N. (1979) 'Endocrinologic regulation of carbohydrate metabolism. Amyotrophic lateral sclerosis and Parkinsonism-dementia on Guam.', *Archives of neurology*, 36(4), pp. 217–20. doi: 10.1001/archneur.1979.00500400071011.
260. Nandedkar, S. D. and Mulot, A. (2019) 'Instrumentation for electrodiagnostic studies', in *Handbook of Clinical Neurology*. Elsevier B.V., pp. 39–50. doi: 10.1016/B978-0-444-64032-1.00003-5.
261. Nedergaard, J. and Cannon, B. (2003) 'The "novel" "uncoupling" proteins UCP2 and UCP3: what do they really do? Pros and cons for suggested functions.', *Experimental physiology*, 88(1), pp. 65–84. Available at: http://www.ncbi.nlm.nih.gov/pubmed/12525856 (Accessed: 8 August 2019).
262. Nelson, D. L. and Cox, M. M. (2008) 'Lehninger 5th ed.', in *Principles of Biochemistry*, p. 275. doi: 10.2307/1309148.
263. Ng, A. S. L. and Tan, E. K. (2017) 'Intermediate C9orf72 alleles in neurological disorders: Does size really matter', *Journal of Medical Genetics*. BMJ Publishing Group, 54(9), pp. 591–597. doi: 10.1136/jmedgenet-2017-104752.
264. Nicolson, G. L., Nasralla, M. Y., Haier, J. and Pomfret, J. (2002) 'High frequency of systemic mycoplasmal infections in Gulf War veterans and civilians with Amyotrophic Lateral Sclerosis (ALS).', *Journal of clinical neuroscience : official journal of the Neurosurgical Society of Australasia*, 9(5), pp. 525–9. doi: 10.1054/jocn.2001.1075.
265. Nishimura, A. L., Mitne-Neto, M., Silva, H. C. A., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J. R. M., Gillingwater, T., Webb, J., Skehel, P. and Zatz, M. (2004) 'A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis', *American Journal of Human Genetics*. Cell Press, 75(5), pp. 822–831. doi: 10.1086/425287.
266. Nodera, H., Takamatsu, N., Muguruma, N., Ukimoto, K., Nishio, S., Oda, M., Izumi, Y. and Kaji, R. (2015) 'Frequent hepatic steatosis in amyotrophic lateral sclerosis : Implication for systemic involvement', 3, pp. 58–62. doi: 10.1111/ncn3.143.
267. Norenberg, M. D., Rao, K. V. R. and Jayakumar, A. R. (2009) 'Signaling factors in the mechanism of ammonia neurotoxicity', pp. 103–117. doi: 10.1007/s11011-008-9113-6.
268. Nutini, M., Frazzini, V., Marini, C., Spalloni, A., Sensi, S. L. and Longone, P. (2011) 'Zinc pre-treatment enhances NMDAR-mediated excitotoxicity in cultured cortical neurons from SOD1G93A mouse, a model of amyotrophic lateral sclerosis', *Neuropharmacology*, 60(7–8), pp. 1200–1208. doi: 10.1016/j.neuropharm.2010.11.001.
269. 'Œuvre scientifique de J. Babinski' (1934) *JAMA: The Journal of the American Medical Association*. American Medical Association (AMA), 103(18), p. 1403. doi: 10.1001/jama.1934.02750440063037.
270. Ogata, T. and Yamasaki, Y. (1993) 'Ultra-high resolution scanning electron microscopic studies on the sarcoplasmic reticulum and mitochondria in various muscles: a review.', *Scanning microscopy*, 7(1), pp. 145–56. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8316788 (Accessed: 8 February 2020).

271. Ohlendieck, K. (2010) 'Proteomics of skeletal muscle glycolysis', *Biochimica et Biophysica Acta - Proteins and Proteomics*, pp. 2089–2101. doi: 10.1016/j.bbapap.2010.08.001.
272. Oldstone, M. B., Wilson, C. B., Perrin, L. H. and Norris, F. H. (1976) 'Evidence for immune-complex formation in patients with amyotrophic lateral sclerosis.', *Lancet (London, England)*, 2(7978), pp. 169–72. doi: 10.1016/s0140-6736(76)92345-x.
273. Ottenheijm, C. A. C. and Granzier, H. (2010) 'Lifting the nebula: novel insights into skeletal muscle contractility.', *Physiology (Bethesda, Md.)*, 25(5), pp. 304–10. doi: 10.1152/physiol.00016.2010.
274. Palamiuc, L., Schlagowski, A., Ngo, S. T., Vernay, A., Dirrig-Grosch, S., Henriques, A., Boutillier, A., Zoll, J., Echaniz-Laguna, A., Loeffler, J. and René, F. (2015) 'A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis', *EMBO Molecular Medicine*. EMBO, 7(5), pp. 526–546. doi: 10.15252/emmm.201404433.
275. Pan, K. M., Baldwin, M., Nguyen, J., Gasset, M., Serban, A., Groth, D., Mehlhorn, I., Huang, Z., Fletterick, R. J., Cohen, F. E. and Prusiner, S. B. (1993) 'Conversion of α-helices into β-sheets features in the formation of the scrapie prion proteins', *Proceedings of the National Academy of Sciences of the United States of America*, 90(23), pp. 10962–10966. doi: 10.1073/pnas.90.23.10962.
276. Parekh, B. K. (2015) 'A(α)LS: Ammonia-induced amyotrophic lateral sclerosis'. doi: 10.7287/peerj.preprints.935v1.
277. Parker, S. E., Hanton, A. M., Stefanou, S. N., Noakes, P. G., Woodruff, T. M. and Lee, J. D. (2019) 'Revisiting the role of the innate immune complement system in ALS', *Neurobiology of Disease*, 127, pp. 223–232. doi: 10.1016/j.nbd.2019.03.003.
278. Pastor, D., Viso-León, M. C., Jones, J., Jaramillo-Merchán, J., Toledo-Aral, J. J., Moraleda, J. M. and Martínez, S. (2012) 'Comparative Effects between Bone Marrow and Mesenchymal Stem Cell Transplantation in GDNF Expression and Motor Function Recovery in a Motorneuron Degenerative Mouse Model', *Stem Cell Reviews and Reports*, 8(2), pp. 445–458. doi: 10.1007/s12015-011-9295-x.
279. Patten, B. M., Kurlander, H. M. and Evans, B. (1982) 'Free amino acid concentrations in spinal tissue from patients dying of motor neuron disease.', *Acta neurologica Scandinavica*, 66(5), pp. 594–9. Available at: http://www.ncbi.nlm.nih.gov/pubmed/7148401 (Accessed: 23 July 2019).
280. Pedersen, W. A., Fu, W., Keller, J. N., Markesbery, W. R., Appel, S., Smith, R. G., Kasarskis, E. and Mattson, M. P. (1998) 'Protein modification by the lipid peroxidation product 4-hydroxynonenal in the spinal cords of amyotrophic lateral sclerosis patients.', *Annals of neurology*, 44(5), pp. 819–24. doi: 10.1002/ana.410440518.
281. Peggion, C., Massimino, M. L., Biancotto, G., Angeletti, R., Reggiani, C., Sorgato, M. C., Bertoli, A. and Stella, R. (2017) 'Absolute quantification of myosin heavy chain isoforms by selected reaction monitoring can underscore skeletal muscle changes in a mouse model of amyotrophic lateral sclerosis', *Analytical and Bioanalytical Chemistry*, 409(8), pp. 2143–2153. doi: 10.1007/s00216-016-0160-2.
282. Pellman, E. J. (2003) 'Background on the National Football League's research on concussion in professional football', *Neurosurgery*. Lippincott

Williams and Wilkins, pp. 797–798. doi: 10.1093/neurosurgery/53.3.797.
283. Perera, N. D., Turner, B. J. and Turner, B. J. (2015) 'AMPK Signalling and Defective Energy Metabolism in Amyotrophic Lateral Sclerosis', *Neurochemical Research*. Springer US. doi: 10.1007/s11064-015-1665-3.
284. Peter, J. B., Barnard, R. J., Edgerton, V. R., Gillespie, C. A. and Stempel, K. E. (1972) 'Metabolic profiles of three fiber types of skeletal muscle in guinea pigs and rabbits.', *Biochemistry*, 11(14), pp. 2627–33. doi: 10.1021/bi00764a013.
285. Plato, C. C., Garruto, R. M., Galasko, D., Craig, U.-K., Plato, M., Gamst, A., Torres, J. M. and Wiederholt, W. (2003) 'Amyotrophic lateral sclerosis and parkinsonism-dementia complex of Guam: changing incidence rates during the past 60 years.', *American journal of epidemiology*, 157(2), pp. 149–57. doi: 10.1093/aje/kwf175.
286. Plomgaard, P., Penkowa, M., Leick, L., Pedersen, B. K., Saltin, B. and Pilegaard, H. (2006) 'The mRNA expression profile of metabolic genes relative to MHC isoform pattern in human skeletal muscles', *J Appl Physiol*, 101, pp. 817–825. doi: 10.1152/japplphysiol.00183.2006.-The.
287. Poesen, K., De Schaepdryver, M., Stubendorff, B., Gille, B., Muckova, P., Wendler, S., Prell, T., Ringer, T. M., Rhode, H., Stevens, O., Claeys, K. G., Couwelier, G., D'hondt, A., Lamaire, N., Tilkin, P., Van Reijen, D., Gourmaud, S., Fedtke, N., Heiling, B., Rumpel, M., Rödiger, A., Gunkel, A., Witte, O. W., Paquet, C., Vandenberghe, R., Grosskreutz, J. and Van Damme, P. (2017) 'Neurofilament markers for ALS correlate with extent of upper and lower motor neuron disease', *Neurology*. Lippincott Williams and Wilkins, 88(24), pp. 2302–2309. doi: 10.1212/WNL.0000000000004029.
288. Portegies, P. and Goudsmit, J. (1991) '[Human T-cell lymphotropic virus Type I (HTLV-I) as a cause of progressive myelopathy]', *Ned Tijdschr Geneeskd*, 135(29), pp. 1302–1306. Available at: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=1865933 (Accessed: 8 February 2020).
289. Porter, J. D., Khanna, S., Kaminski, H. J., Rao, J. S., Merriam, A. P., Richmonds, C. R., Leahy, P., Li, J. and Andrade, F. H. (2001) 'Extraocular muscle is defined by a fundamentally distinct gene expression profile', *Proceedings of the National Academy of Sciences of the United States of America*, 98(21), pp. 12062–12067. doi: 10.1073/pnas.211257298.
290. Pradas, J., Puig, T., Rojas-García, R., Viguera, M. L., Gich, I., Logroscino, G. and ALS-CAT Group (2013) 'Amyotrophic lateral sclerosis in Catalonia: a population based study.', *Amyotrophic lateral sclerosis & frontotemporal degeneration*, 14(4), pp. 278–83. doi: 10.3109/21678421.2012.749915.
291. Pradat, P., Bruneteau, G., Gordon, P. H., Dupuis, L. U. C., Bonnefont-rousselot, D., Simon, D., Salachas, F., Corcia, P., Frochot, V., Lacorte, J., Jardel, C., Coussieu, C., Forestier, N. L. E., Lacomblez, L., Loeffler, J. and Meininger, V. (2010) 'Impaired glucose tolerance in patients with amyotrophic lateral sclerosis', (February 2009). doi: 10.3109/17482960902822960.
292. Pradat, P. Ç. O. I. S., Barani, A., Wanschitz, J., Dubourg, O., S, A. L. È., Bigot, A., Mouly, V., Bruneteau, G., Salachas, F. Ç. O. I. S., Ée, T., Nglet, L. E., Meininger, V. and Butler-browne, G. (2011) 'Abnormalities of satellite cells function in amyotrophic lateral sclerosis', 33(February), pp. 264–271. doi: 10.3109/17482968.2011.566618.
293. Pradat, P. F., Barani, A., Wanschitz, J., Dubourg, O., Lombès, A., Bigot, A.,

Mouly, V., Bruneteau, G., Salachas, F., Lenglet, T., Meininger, V. and Butler-Browne, G. (2011) 'Abnormalities of satellite cells function in amyotrophic lateral sclerosis', *Amyotrophic Lateral Sclerosis*, 12(4), pp. 264–271. doi: 10.3109/17482968.2011.566618.

294. Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A. and Patel, B. K. (2019) 'Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis', *Frontiers in Molecular Neuroscience*. Frontiers Media S.A. doi: 10.3389/fnmol.2019.00025.
295. Prusiner, S. B. (2001) 'Shattuck lecture - Neurodegenerative diseases and prions', *New England Journal of Medicine*, 344(20), pp. 1516–1526. doi: 10.1056/NEJM200105173442006.
296. Pun, S., Santos, A. F., Saxena, S., Xu, L. and Caroni, P. (2006) 'Selective vulnerability and pruning of phasic motoneuron axons in motoneuron disease alleviated by CNTF', *Nature Neuroscience*, 9(3), pp. 408–419. doi: 10.1038/nn1653.
297. Pupillo, E., Messina, P., Logroscino, G., Zoccolella, S., Chiò, A., Calvo, A., Corbo, M., Lunetta, C., Micheli, A., Millul, A., Vitelli, E., Beghi, E., Bissolati, M., Formaglio, F., Gerardi, F., Giussani, G., Maestri, E., Millul, A., Prelle, A., Protti, A., Riva, N., Santoro, D., Al-Chalabi, A., Berro, A. C., De Mercanti, S., Giacone, S., Ilardi, A., Hardiman, O., Logroscino, G., Capozzo, R., Zoccolella, S., Ludolph, A., Mitchell, D., Preux, P. M., Swingler, R., van den Berg, L., Alvisi, E., Cereda, C., Ceroni, M., Citterio, A., di Poggio, M. B., Caponnetto, C., Buzzi, P., Colombo, I., Corbo, M., Cotelli, M. S., Filosto, M., Micheli, A., Padovani, A., De Lodovici, L., Ferrarese, C., Tremolizzo, L., Fetoni, V., Palazzini, E., Guaita, M. C., Perrone, P., Secchi, P., Lorusso, L., Sidoti, V., Perini, M., Tavernelli, F., Rigamonti, A. and Vitelli, E. (2012) 'Trauma and amyotrophic lateral sclerosis: A case-control study from a population-based registry', *European Journal of Neurology*. Blackwell Publishing Ltd, 19(12), pp. 1509–1517. doi: 10.1111/j.1468-1331.2012.03723.x.
298. PURVIS, J. L. and LOWENSTEIN, J. M. (1961) 'The relation between intra- and extramitochondrial pyridine nucleotides.', *The Journal of biological chemistry*, 236, pp. 2794–803. Available at: http://www.ncbi.nlm.nih.gov/pubmed/14489268 (Accessed: 7 August 2019).
299. Qaisar, R., Bhaskaran, S. and Van Remmen, H. (2016) 'Muscle fiber type diversification during exercise and regeneration', *Free Radical Biology and Medicine*. Elsevier Inc., 98, pp. 56–67. doi: 10.1016/j.freeradbiomed.2016.03.025.
300. Quick, D. T. and Greer, M. (1967) 'Pancreatic dysfunction in patients with amyotrophic lateral sclerosis', *Neurology*, 17(2), pp. 112–116. doi: 10.1212/wnl.17.2.112.
301. Ralli, M., Lambiase, A., Artico, M., de Vincentiis, M. and Greco, A. (2019) 'Amyotrophic lateral sclerosis: Autoimmune pathogenic mechanisms, clinical features, and therapeutic perspectives', *Israel Medical Association Journal*. Israel Medical Association, 21(7), pp. 438–443.
302. Rando, A., Pastor, D., Viso-León, M. C., Martínez, A., Manzano, R., Navarro, X., Osta, R. and Martínez, S. (2018) 'Intramuscular transplantation of bone marrow cells prolongs the lifespan of SOD1 G93A mice and modulates expression of prognosis biomarkers of the disease', *Stem Cell Research and Therapy*. BioMed Central Ltd., 9(1). doi: 10.1186/s13287-018-0843-z.

303. Ravits, J. M. and La Spada, A. R. (2009) 'ALS motor phenotype heterogeneity, focality, and spread: Deconstructing motor neuron degeneration', *Neurology*, 73(10), pp. 805–811. doi: 10.1212/WNL.0b013e3181b6bbbd.
304. Reaume, A. G., Elliott, J. L., Hoffman, E. K., Kowall, N. W., Ferrante, R. J., Siwek, D. F., Wilcox, H. M., Flood, D. G., Beal, M. F., Brown, R. H., Scott, R. W. and Snider, W. D. (1996) 'Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury', *Nature Genetics*, 13(1), pp. 43–47. doi: 10.1038/ng0596-43.
305. Relaix, F. and Zammit, P. S. (2012) 'Satellite cells are essential for skeletal muscle regeneration: The cell on the edge returns centre stage', *Development (Cambridge)*, pp. 2845–2856. doi: 10.1242/dev.069088.
306. Renton, A. E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J. R., Schymick, J. C., Laaksovirta, H., van Swieten, J. C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A. M., Kaganovich, A., Scholz, S. W., Duckworth, J., Ding, J., Harmer, D. W., Hernandez, D. G., Johnson, J. O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R. J., Orrell, R. W., Neal, J., Murray, A., Pearson, J., Jansen, I. E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J. B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M. A., Peuralinna, T., Jansson, L., Isoviita, V. M., Kaivorinne, A. L., Hölttä-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J. D., Sendtner, M., Drepper, C., Eichler, E. E., Alkan, C., Abdullaev, Z., Pack, S. D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N. M., Heutink, P., Pickering-Brown, S., Morris, H. R., Tienari, P. J. and Traynor, B. J. (2011) 'A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD', *Neuron*, 72(2), pp. 257–268. doi: 10.1016/j.neuron.2011.09.010.
307. Reyes, E. T., Perurena, O. H., Festoff, B. W., Jorgensen, R. and Moore, W. V. (1984) 'Insulin resistance in amyotrophic lateral sclerosis', *Journal of the Neurological Sciences*, 63(3), pp. 317–324. doi: 10.1016/0022-510X(84)90154-0.
308. Reyes, E. T., Perurena, O. H., Festoff, B. W., Jorgensen, R. and Moore, W. V (1984) 'Insulin resistance in amyotrophic lateral sclerosis.', *Journal of the neurological sciences*, 63(3), pp. 317–24. Available at: http://www.ncbi.nlm.nih.gov/pubmed/6374040 (Accessed: 23 July 2019).
309. Riancho, J., Gil-Bea, F., Santurtun, A. and López De Munaín, A. (2019) 'Amyotrophic lateral sclerosis: A complex syndrome that needs an integrated research approach', *Neural Regeneration Research*. Wolters Kluwer Medknow Publications, pp. 193–196. doi: 10.4103/1673-5374.244783.
310. Riancho, J., Ruiz-soto, M., Villagrá, N. T., Berciano, J., Berciano, M. T. and Lafarga, M. (2014) 'Compensatory motor neuron response to chromatolysi s in the murine hSOD1 G93A model of amyotrophic lateral sclerosis', 8(October), pp. 1–13. doi: 10.3389/fncel.2014.00346.
311. Ringholz, G. M., Appel, S. H., Bradshaw, M., Cooke, N. A., Mosnik, D. M. and Schulz, P. E. (2005) 'Prevalence and patterns of cognitive impairment in sporadic ALS.', *Neurology*, 65(4), pp. 586–90. doi: 10.1212/01.wnl.0000172911.39167.b6.
312. Rivadeneyra-Domínguez, E. and Rodríguez-Landa, J. F. (2014) 'Las

cícadas y su relación con algunas enfermedades neurodegenerativas', *Neurologia*. Spanish Society of Neurology, pp. 517–522. doi: 10.1016/j.nrl.2013.03.005.

313. Robberecht, W. and Philips, T. (2013) 'The changing scene of amyotrophic lateral sclerosis', *Nature Publishing Group*, 14. doi: 10.1038/nrn3430.
314. Robinson, A. M. and Williamson, D. H. (1980) 'Physiological roles of ketone bodies as substrates and signals in mammalian tissues', *Physiological Reviews*, pp. 143–187. doi: 10.1152/physrev.1980.60.1.143.
315. Rodriguez, A., Durán, A., Selloum, M., Champy, M.-F., Diez-Guerra, F. J., Flores, J. M., Serrano, M., Auwerx, J., Diaz-Meco, M. T. and Moscat, J. (2006) 'Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62.', *Cell metabolism*, 3(3), pp. 211–22. doi: 10.1016/j.cmet.2006.01.011.
316. Rojas-Garcia, R., Scott, K. M., Roche, J. C., Scotton, W., Martin, N., Janssen, A., Goldstein, L. H., Nigel Leigh, P., Ellis, C. M., Shaw, C. E. and Al-Chalabi, A. (2012) 'No evidence for a large difference in ALS frequency in populations of African and European origin: A population based study in inner city London', *Amyotrophic Lateral Sclerosis*, 13(1), pp. 66–68. doi: 10.3109/17482968.2011.636049.
317. Rolfe, D. F. and Brown, G. C. (1997) 'Cellular energy utilization and molecular origin of standard metabolic rate in mammals.', *Physiological reviews*, 77(3), pp. 731–58. doi: 10.1152/physrev.1997.77.3.731.
318. Rosen, D. R., Siddique, T., Patterson, D., Figlewicz, D. A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J. P., Deng, H. X., Rahmani, Z., Krizus, A., McKenna-Yasek, D., Cayabyab, A., Gaston, S. M., Berger, R., Tanzi, R. E., Halperin, J. J., Herzfeldt, B., Den Bergh, R. Van, Hung, W. Y., Bird, T., Deng, G., Mulder, D. W., Smyth, C., Laing, N. G., Soriano, E., Pericak-Vance, M. A., Haines, J., Rouleau, G. A., Gusella, J. S., Horvitz, H. R. and Brown, R. H. (1993) 'Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis', *Nature*, 362(6415), pp. 59–62. doi: 10.1038/362059a0.
319. Rothman, K. J. and Greenland, S. (2005) 'Causation and causal inference in epidemiology', *American Journal of Public Health*. doi: 10.2105/AJPH.2004.059204.
320. Ryall, J. G. (2013) 'Metabolic reprogramming as a novel regulator of skeletal muscle development and regeneration', *FEBS Journal*, pp. 4004–4013. doi: 10.1111/febs.12189.
321. Saberi, S., Stauffer, J. E., Schulte, D. J. and Ravits, J. (2015) 'Neuropathology of Amyotrophic Lateral Sclerosis and Its Variants', *Neurologic Clinics*. W.B. Saunders, pp. 855–876. doi: 10.1016/j.ncl.2015.07.012.
322. Saberi, S., Stauffer, J. E., Schulte, D. J. and Ravits, J. (no date) '"Neuropathology of amyotrophic lateral sclerosis and its variants&quot'; doi: 10.1016/j.ncl.2015.07.012.
323. Saccon, R. A., Bunton-Stasyshyn, R. K. A., Fisher, E. M. C. and Fratta, P. (2013) 'Is SOD1 loss of function involved in amyotrophic lateral sclerosis?', *Brain : a journal of neurology*, 136(Pt 8), pp. 2342–58. doi: 10.1093/brain/awt097.
324. Saffer, D., Morley, J. and Bill, P. L. A. (1977) 'Carbohydrate metabolism in motor neurone disease', *Journal of Neurology Neurosurgery and Psychiatry*, 40(6), pp. 533–537. doi: 10.1136/jnnp.40.6.533.
325. Sahlin, K., Söderlund, K., Tonkonogi, M. and Hirakoba, K. (1997)

'Phosphocreatine content in single fibers of human muscle after sustained submaximal exercise.', *The American journal of physiology*, 273(1 Pt 1), pp. C172-8. doi: 10.1152/ajpcell.1997.273.1.C172.

326. Saini, A. and Chauhan, V. S. (2011) 'Delineation of the core aggregation sequences of TDP-43 C-terminal fragment.', *Chembiochem : a European journal of chemical biology*, 12(16), pp. 2495–501. doi: 10.1002/cbic.201100427.
327. Salih, D. A. and Brunet, A. (2008) 'FoxO transcription factors in the maintenance of cellular homeostasis during aging', *Current Opinion in Cell Biology*, 20(2), pp. 126–136. doi: 10.1016/j.ceb.2008.02.005.
328. Samudio, I., Fiegl, M. and Andreeff, M. (2009) 'Mitochondrial Uncoupling and the Warburg Effect : Molecular Basis for the Reprogramming of Cancer Cell Metabolism', (6), pp. 2163–2167. doi: 10.1158/0008-5472.CAN-08-3722.
329. Samudio, I., Fiegl, M., McQueen, T., Clise-Dwyer, K. and Andreeff, M. (2008) 'The warburg effect in leukemia-stroma cocultures is mediated by mitochondrial uncoupling associated with uncoupling protein 2 activation.', *Cancer research*, 68(13), pp. 5198–205. doi: 10.1158/0008-5472.CAN-08-0555.
330. Sanes, J. R. and Lichtman, J. W. (1999) 'DEVELOPMENT OF THE VERTEBRATE NEUROMUSCULAR JUNCTION', *Annual Review of Neuroscience*. Annual Reviews, 22(1), pp. 389–442. doi: 10.1146/annurev.neuro.22.1.389.
331. Sanjak, M., Paulson, D., Sufit, R., Reddan, W., Beaulieu, D., Erickson, L., Shug, A. and Brooks, B. R. (1987) 'Physiologic and metabolic response to progressive and prolonged exercise in amyotrophic lateral sclerosis.', *Neurology*, 37(7), pp. 1217–20. doi: 10.1212/wnl.37.7.1217.
332. Sanjurjo, E., Laguno, M., Bedini, J. L., Miró, O. and Grau, J. M. (2004) '[Forearm ischemic exercise test. Standardization and diagnostic value in the identification of McArdle disease].', *Medicina clinica*, 122(20), pp. 761–6. doi: 10.1016/s0025-7753(04)74380-8.
333. Sargeant, A. J. (2007) 'Structural and functional determinants of human muscle power', *Experimental Physiology*, 92(2), pp. 323–331. doi: 10.1113/expphysiol.2006.034322.
334. Sartori, R., Milan, G., Patron, M., Mammucari, C., Blaauw, B., Abraham, R. and Sandri, M. (2009) 'Smad2 and 3 transcription factors control muscle mass in adulthood', *American Journal of Physiology-Cell Physiology*, 296(6), pp. C1248–C1257. doi: 10.1152/ajpcell.00104.2009.
335. Sau, D., De Biasi, S., Vitellaro-Zuccarello, L., Riso, P., Guarnieri, S., Porrini, M., Simeoni, S., Crippa, V., Onesto, E., Palazzolo, I., Rusmini, P., Bolzoni, E., Bendotti, C. and Poletti, A. (2007) 'Mutation of SOD1 in ALS: a gain of a loss of function.', *Human molecular genetics*, 16(13), pp. 1604–18. doi: 10.1093/hmg/ddm110.
336. Scaramozza, A., Marchese, V., Papa, V., Angelini, C., Salaroli, R., Soraru, G. and Cenacchi, G. (2014) 'Skeletal Muscle Satellite Cells in Amyotrophic Lateral Sclerosis', 38(June), pp. 295–302. doi: 10.3109/01913123.2014.937842.
337. SCHANTZ, P. G., SJÖBERG, B. and SVEDENHAG, J. (1986) 'Malate-aspartate and alpha-glycerophosphate shuttle enzyme levels in human skeletal muscle: methodological considerations and effect of endurance training', *Acta Physiologica Scandinavica*, 128(3), pp. 397–407. doi: 10.1111/j.1748-1716.1986.tb07993.x.

338. Schiaffino, S., Dyar, K. A., Ciciliot, S., Blaauw, B. and Sandri, M. (2013) 'Mechanisms regulating skeletal muscle growth and atrophy.', *The FEBS journal*, 280(17), pp. 4294–314. doi: 10.1111/febs.12253.
339. Schiaffino, S., Dyar, K. A., Ciciliot, S., Blaauw, B. and Sandri, M. (2013) 'Mechanisms regulating skeletal muscle growth and atrophy', *FEBS Journal*. John Wiley & Sons, Ltd (10.1111), 280(17), pp. 4294–4314. doi: 10.1111/febs.12253.
340. Schiaffino, S. and Reggiani, C. (2011) 'Fiber types in Mammalian skeletal muscles', *Physiological Reviews*, 91(4), pp. 1447–1531. doi: 10.1152/physrev.00031.2010.
341. Schmidt, S., Allen, K. D., Loiacono, V. T., Norman, B., Stanwyck, C. L., Nord, K. M., Williams, C. D., Kasarskis, E. J., Kamel, F., McGuire, V., Nelson, L. M. and Oddone, E. Z. (2008) 'Genes and Environmental Exposures in Veterans with Amyotrophic Lateral Sclerosis: the GENEVA study. Rationale, study design and demographic characteristics.', *Neuroepidemiology*, 30(3), pp. 191–204. doi: 10.1159/000126911.
342. Schoolwerth, A. C., LaNoue, K. F. and Hoover, W. J. (1984) 'Effect of pH on glutamate efflux from rat kidney mitochondria', *American Journal of Physiology-Renal Physiology*, 246(3), pp. F266–F271. doi: 10.1152/ajprenal.1984.246.3.F266.
343. Schwarz, S., Husstedt, I., Bertram, H. P. and Kuchelmeister, K. (1996) 'Amyotrophic lateral sclerosis after accidental injection of mercury.', *Journal of neurology, neurosurgery, and psychiatry*, 60(6), p. 698. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8648348 (Accessed: 11 March 2019).
344. Seals, R. M., Hansen, J., Gredal, O. and Weisskopf, M. G. (2016) 'Physical Trauma and Amyotrophic Lateral Sclerosis: A Population-Based Study Using Danish National Registries.', *American journal of epidemiology*, 183(4), pp. 294–301. doi: 10.1093/aje/kwv169.
345. Servián-Morilla, E., Cabrera-Serrano, M., Rivas-Infante, E., Carvajal, A., Lamont, P. J., Pelayo-Negro, A. L., Ravenscroft, G., Junckerstorff, R., Dyke, J. M., Fletcher, S., Adams, A. M., Mavillard, F., Fernández-García, M. A., Nieto-González, J. L., Laing, N. G. and Paradas, C. (2019) 'Altered myogenesis and premature senescence underlie human TRIM32-related myopathy', *Acta neuropathologica communications*. NLM (Medline), 7(1), p. 30. doi: 10.1186/s40478-019-0683-9.
346. Shan, X., Chiang, P.-M., Price, D. L. and Wong, P. C. (2010) 'Altered distributions of Gemini of coiled bodies and mitochondria in motor neurons of TDP-43 transgenic mice.', *Proceedings of the National Academy of Sciences of the United States of America*, 107(37), pp. 16325–30. doi: 10.1073/pnas.1003459107.
347. Shefner, J. M., Reaume, A. G., Flood, D. G., Scott, R. W., Kowall, N. W., Ferrante, R. J., Siwek, D. F., Upton-Rice, M. and Brown, R. H. (1999) 'Mice lacking cytosolic copper/zinc superoxide dismutase display a distinctive motor axonopathy', *Neurology*, 53(6), pp. 1239–1246. doi: 10.1212/wnl.53.6.1239.
348. Shi, J., Luo, L., Eash, J., Ibebunjo, C. and Glass, D. J. (2011) 'The SCF-Fbxo40 complex induces IRS1 ubiquitination in skeletal muscle, limiting IGF1 signaling.', *Developmental cell*, 21(5), pp. 835–47. doi: 10.1016/j.devcel.2011.09.011.
349. Silva, M. T. T., Leite, A. C. C., Alamy, A. H., Chimelli, L., Andrada-Serpa, M.

J. and Araújo, A. Q. C. (2005) 'ALS syndrome in HTLV-I infection.', *Neurology*, 65(8), pp. 1332–3. doi: 10.1212/01.wnl.0000180962.47653.5e.

350. Simpson, E. P., Henry, Y. K., Henkel, J. S., Smith, R. G. and Appel, S. H. (2004) 'Increased lipid peroxidation in sera of ALS patients: A potential biomarker of disease burden', *Neurology*. Lippincott Williams and Wilkins, 62(10), pp. 1758–1765. doi: 10.1212/WNL.62.10.1758.
351. Singer, M. A., Statland, J. M., Wolfe, G. I. and Barohn, R. J. (2007) 'Primary lateral sclerosis', *Muscle and Nerve*, pp. 291–302. doi: 10.1002/mus.20728.
352. Sioud, M. (2010) 'RNA therapeutics: function, design, and delivery. Preface.', *Methods in molecular biology (Clifton, N.J.)*, 629(2), pp. 1–15. doi: 10.1007/978-1-60761-657-3.
353. Smith, D. H., Johnson, V. E., Trojanowski, J. Q. and Stewart, W. (2019) 'Chronic traumatic encephalopathy - confusion and controversies.', *Nature reviews. Neurology*. Nature Publishing Group, 15(3), pp. 179–183. doi: 10.1038/s41582-018-0114-8.
354. Smith, Q. R., Nagura, H., Takada, Y. and Duncan, M. W. (1992) 'Facilitated Transport of the Neurotoxin, ?-N-Methylamino-l-Alanine, Across the Blood-Brain Barrier', *Journal of Neurochemistry*. John Wiley & Sons, Ltd (10.1111), 58(4), pp. 1330–1337. doi: 10.1111/j.1471-4159.1992.tb11346.x.
355. Smittkamp, S. E., Morris, J. K., Bomhoff, G. L., Chertoff, M. E., Geiger, P. C. and Stanford, J. A. (2013) 'SOD1-G93A Mice Exhibit Muscle-Fiber-Type-Specific Decreases in Glucose Uptake in the Absence of Whole-Body Changes in Metabolism', *Neurodegenerative Diseases*, 13(1), pp. 29–37. doi: 10.1159/000351606.
356. Sorrentino, V. and Gerli, R. (2003) 'Structure and molecular organisation of the sarcoplasmic reticulum of skeletal muscle fibers', *Italian Journal of Anatomy and Embryology*, 108(2), pp. 65–76.
357. Spencer, P. S., Palmer, V. S. and Ludolph, A. C. (2005) 'On the decline and etiology of high-incidence motor system disease in West Papua (southwest New Guinea).', *Movement disorders : official journal of the Movement Disorder Society*, 20 Suppl 12(S12), pp. S119-26. doi: 10.1002/mds.20552.
358. Sreedhar, A., Petruska, P., Miriyala, S., Panchatcharam, M. and Zhao, Y. (2017) 'UCP2 overexpression enhanced glycolysis via activation of PFKFB2 during skin cell transformation', *Oncotarget*. Impact Journals LLC, 8(56), pp. 95504–95515. doi: 10.18632/oncotarget.20762.
359. Sreedharan, J., Blair, I. P., Tripathi, V. B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J. C., Williams, K. L., Buratti, E., Baralle, F., De Belleroche, J., Mitchell, J. D., Leigh, P. N., Al-Chalabi, A., Miller, C. C., Nicholson, G. and Shaw, C. E. (2008) 'TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis', *Science*, 319(5870), pp. 1668–1672. doi: 10.1126/science.1154584.
360. Stallings, N. R., Puttaparthi, K., Dowling, K. J., Luther, C. M., Burns, D. K., Davis, K. and Elliott, J. L. (2013) 'TDP-43 , an ALS Linked Protein , Regulates Fat Deposition and Glucose Homeostasis', 8(8), pp. 1–14. doi: 10.1371/journal.pone.0071793.
361. Steele, J. C. (2005) 'Parkinsonism-dementia complex of Guam', *Movement Disorders*, 20(S12), pp. S99–S107. doi: 10.1002/mds.20547.
362. Stribl, C., Samara, A., Trümbach, D., Peis, R., Neumann, M., Fuchs, H., Gailus-durner, V., Rathkolb, B., Wolf, E., Beckers, J., Horsch, M., Neff, F., Kremmer, E., Koob, S., Reichert, A. S., Hans, W., Rozman, J., Klingenspor,

M., Aichler, M., Walch, K., Becker, L., Klopstock, T., Glasl, L., Hölter, S. M., Wurst, W. and Floss, T. (2014) 'Mitochondrial Dysfunction and Decrease in Body Weight of a Transgenic Knock-in Mouse Model for TDP-43 *', 289(15), pp. 10769–10784. doi: 10.1074/jbc.M113.515940.

363. Stromsdorfer, K. L., Yamaguchi, S., Yoon, M. J., Moseley, A. C., Franczyk, M. P., Kelly, S. C., Qi, N., Imai, S. ichiro and Yoshino, J. (2016) 'NAMPT-Mediated NAD+ Biosynthesis in Adipocytes Regulates Adipose Tissue Function and Multi-organ Insulin Sensitivity in Mice', *Cell Reports*. Elsevier B.V., 16(7), pp. 1851–1860. doi: 10.1016/j.celrep.2016.07.027.
364. Sun, S., Ling, S.-C., Qiu, J., Albuquerque, C. P., Zhou, Y., Tokunaga, S., Li, H., Qiu, H., Bui, A., Yeo, G. W., Huang, E. J., Eggan, K., Zhou, H., Fu, X.-D., Lagier-Tourenne, C. and Cleveland, D. W. (2015) 'ALS-causative mutations in FUS/TLS confer gain and loss of function by altered association with SMN and U1-snRNP.', *Nature communications*, 6, p. 6171. doi: 10.1038/ncomms7171.
365. Sun, Z., Diaz, Z., Fang, X., Hart, M. P., Chesi, A., Shorter, J. and Gitler, A. D. (2011) 'Molecular Determinants and Genetic Modifiers of Aggregation and Toxicity for the ALS Disease Protein FUS/TLS', *PLoS Biology*. Edited by J. S. Weissman, 9(4), p. e1000614. doi: 10.1371/journal.pbio.1000614.
366. Sutedja, N. A., Sinke, R. J., Van Vught, P. W. J., Van der Linden, M. W., Wokke, J. H. J., Van Duijn, C. M., Njajou, O. T., Van der Schouw, Y. T., Veldink, J. H. and Van den Berg, L. H. (2007) 'The association between H63D mutations in HFE and amyotrophic lateral sclerosis in a Dutch population.', *Archives of neurology*, 64(1), pp. 63–7. doi: 10.1001/archneur.64.1.63.
367. Suzuki, M. and Svendsen, C. N. (2016) 'Ex Vivo Gene Therapy Using Human Mesenchymal Stem Cells to Deliver Growth Factors in the Skeletal Muscle of a Familial ALS Rat Model', in *Methods in molecular biology (Clifton, N.J.)*, pp. 325–336. doi: 10.1007/978-1-4939-3271-9_24.
368. Şxahin, A., Held, A., Bredvik, K., Major, P., Achilli, T. M., Kerson, A. G., Wharton, K., Stilwell, G. and Reenan, R. (2017) 'Human SOD1 ALS mutations in a Drosophila knock-in model cause severe phenotypes and reveal dosage-sensitive gain- and loss-of-function components', *Genetics*. Genetics Society of America, 205(2), pp. 707–723. doi: 10.1534/genetics.116.190850.
369. Takahashi, Y., Fukuda, Y., Yoshimura, J., Toyoda, A., Kurppa, K., Moritoyo, H., Belzil, V. V., Dion, P. A., Higasa, K., Doi, K., Ishiura, H., Mitsui, J., Date, H., Ahsan, B., Matsukawa, T., Ichikawa, Y., Moritoyo, T., Ikoma, M., Hashimoto, T., Kimura, F., Murayama, S., Onodera, O., Nishizawa, M., Yoshida, M., Atsuta, N., Sobue, G., Fifita, J. A., Williams, K. L., Blair, I. P., Nicholson, G. A., Gonzalez-Perez, P., Brown, R. H., Nomoto, M., Elenius, K., Rouleau, G. A., Fujiyama, A., Morishita, S., Goto, J., Tsuji, S. and Tsuji, S. (2013) 'ERBB4 Mutations that Disrupt the Neuregulin-ErbB4 Pathway Cause Amyotrophic Lateral Sclerosis Type 19', *The American Journal of Human Genetics*, 93(5), pp. 900–905. doi: 10.1016/j.ajhg.2013.09.008.
370. *Talampanel for Amyotrophic Lateral Sclerosis (ALS) - Full Text View - ClinicalTrials.gov* (no date). Available at: https://clinicaltrials.gov/ct2/show/NCT00696332?cond=ALS&draw=2&rank=4 (Accessed: 5 February 2020).
371. Tefera, T. W., Ni, K., Tanya, T., Karin, S. M., Perera, N. D., Turner, B. J., Turner, B. J., Article, R., Vijayalakshmi, K., Anand, P., Sathyaprabha, T. N.,

Subramaniam, J. R., Nalini, A., Raju, T. R., Ruban, A., Malina, C., Cooper, I., Tang, Y., Liu, M., Zang, T., Zhang, C., Young, J. E., Vandoorne, T., Bock, K. De, Bosch, L. Van Den, Blacker, T. S., Duchen, M. R., Assary, E., Vincent, J. P., Keers, R., Pluess, M., Çalık, P., Akbay, A., Joardar, A., Manzo, E., Zarnescu, D. C., Madreiter-sokolowski, C. T., Klec, C., Parichatikanond, W., Stryeck, S., Gottschalk, B., Pulido, S., Rost, R., Eroglu, E., Hofmann, N. A., Bondarenko, A. I., Madl, T., Waldeck-weiermair, M., Malli, R., Graier, W. F., Jez, P., Garlid, K. D., Jabu, M., Allen, S. P., Duffy, L. M., Shaw, P. J., Grierson, A. J., Rajan, S., Duffy, L. M., Mortiboys, H., Higginbottom, A., Grierson, A. J., Shaw, P. J., Manuscript, A., Dienel, G. A., Du, Y., Wen, Y., Guo, X., Hao, J., Wang, W., Ma, P., Pardo, B., Wiederkehr, A., Arco, A., Wollheim, C. B., Satru, J., Mckenzie, M., Lim, S. C., Duchen, M. R., Quansah, E., Peelaerts, W., Langston, J. W., Simon, D. K., Colca, J. and Hardie, D. G. (2017) 'NIH Public Access', *Neurobiology of Aging*. Springer US, 35(4), pp. 1499–1509. doi: 10.1016/j.neurobiolaging.2013.11.025.

372. Tetsuka, S., Morita, M., Iida, A., Uehara, R., Ikegawa, S. and Nakano, I. (2013) 'ZNF512B gene is a prognostic factor in patients with amyotrophic lateral sclerosis', *Journal of the Neurological Sciences*, 324(1–2), pp. 163–166. doi: 10.1016/j.jns.2012.10.029.
373. *The Enzymes of glycolysis : structure, activity, and evolution : a Royal Society discussion held on 16 and 17 October 1980 / organized by Sir David Phillips, C.C.F. Blake, and H.C. Watson. - Version details - Trove* (no date). Available at: https://trove.nla.gov.au/work/17556012?selectedversion=NBD2106859 (Accessed: 6 February 2020).
374. *The physiology of excitable cells : Aidley, David J : Free Download, Borrow, and Streaming : Internet Archive* (no date). Available at: https://archive.org/details/physiologyofexci0000aidl_s5u8 (Accessed: 20 January 2020).
375. Tintignac, L. A., Lagirand, J., Batonnet, S., Sirri, V., Leibovitch, M. P. and Leibovitch, S. A. (2005) 'Degradation of MyoD Mediated by the SCF (MAFbx) Ubiquitin Ligase', *Journal of Biological Chemistry*, 280(4), pp. 2847–2856. doi: 10.1074/jbc.M411346200.
376. Tokutake, Y., Yamada, K., Ohata, M., Obayashi, Y. and Tsuchiya, M. (2015) 'ALS-Linked P56S-VAPB Mutation Impairs the Formation of Multinuclear Myotube in C2C12 Cells', pp. 18628–18641. doi: 10.3390/ijms160818628.
377. Tosolini, A. P. and Sleigh, J. N. (2017) 'Motor Neuron Gene Therapy : Lessons from Spinal Muscular Atrophy for Amyotrophic Lateral Sclerosis', 10(December). doi: 10.3389/fnmol.2017.00405.
378. *Trial of Oxaloacetate in ALS ClinicalTrials.gov*. Available at: https://clinicaltrials.gov/ct2/show/NCT04204889?cond=ALS&draw=2&rank=5 (Accessed: 5 February 2020).
379. Tseng, C., Sinha, K., Pan, H., Cui, Y., Guo, P., Lin, C. Y., Yang, F., Deng, Z., Eltzschig, H. K., Lu, A. and Huard, J. (2019) 'Markers of Accelerated Skeletal Muscle Regenerative Response in Murphy Roths Large Mice: Characteristics of Muscle Progenitor Cells and Circulating Factors.', *Stem cells (Dayton, Ohio)*. Wiley-Blackwell, 37(3), pp. 357–367. doi: 10.1002/stem.2957.
380. Tsitkanou, S., Della Gatta, P. A. and Russell, A. P. (2016) 'Skeletal Muscle Satellite Cells, Mitochondria, and MicroRNAs: Their Involvement in the Pathogenesis of ALS.', *Frontiers in physiology*, 7, p. 403. doi:

10.3389/fphys.2016.00403.

381. Turner, M. R. and Talbot, K. (2013) 'Mimics and chameleons in motor neurone disease', *Practical Neurology*. BMJ Publishing Group, pp. 153–164. doi: 10.1136/practneurol-2013-000557.
382. Umahara, T., Uchihara, T., Shibata, N., Nakamura, A. and Hanyu, H. (2016) '14-3-3 eta isoform colocalizes TDP-43 on the coarse granules in the anterior horn cells of patients with sporadic amyotrophic lateral sclerosis', *Brain Research*, 1646, pp. 132–138. doi: 10.1016/j.brainres.2016.05.051.
383. Uyan, Ö., Ömür, Ö., Ağım, Z. S., Özoğuz, A., Li, H., Parman, Y., Deymeer, F., Oflazer, P., Koç, F., Tan, E., Özçelik, H. and Başak, A. N. (2013) 'Genome-Wide Copy Number Variation in Sporadic Amyotrophic Lateral Sclerosis in the Turkish Population: Deletion of EPHA3 Is a Possible Protective Factor', *PLoS ONE*. Edited by M. R. Cookson, 8(8), p. e72381. doi: 10.1371/journal.pone.0072381.
384. Valavanis, A., Schwarz, U., Baumann, C. R., Weller, M. and Linnebank, M. (2014) 'Amyotrophic lateral sclerosis after embolization of cerebral arterioveneous malformations.', *Journal of neurology*, 261(4), pp. 732–7. doi: 10.1007/s00415-014-7260-8.
385. Valero, T. (2014) 'Editorial (Thematic Issue: Mitochondrial Biogenesis: Pharmacological Approaches)', *Current Pharmaceutical Design*, 20(35), pp. 5507–5509. doi: 10.2174/1381612820351409111421 18.
386. Ventura-Clapier, R., Kuznetsov, A., Veksler, V., Boehm, E. and Anflous, K. (1998) 'Functional coupling of creatine kinases in muscles: species and tissue specificity.', *Molecular and cellular biochemistry*, 184(1–2), pp. 231–47. Available at: http://www.ncbi.nlm.nih.gov/pubmed/9746324 (Accessed: 6 August 2019).
387. Vinceti, M., Guidetti, D., Pinotti, M., Rovesti, S., Merlin, M., Vescovi, L., Bergomi, M. and Vivoli, G. (1996) 'Amyotrophic lateral sclerosis after long-term exposure to drinking water with high selenium content.', *Epidemiology (Cambridge, Mass.)*, 7(5), pp. 529–32. Available at: http://www.ncbi.nlm.nih.gov/pubmed/8862986 (Accessed: 11 March 2019).
388. Visser, A. E., Rooney, J. P. K., D'ovidio, F., Westeneng, H. J., Vermeulen, R. C. H., Beghi, E., Chiò, A., Logroscino, G., Bru-No, A., Fanti, C., Fascendini, S., Tomasoni, A., Arnaboldi, M., Valsecchi, M., Moleri, M., Poloni, M., Alimonti, D., Tagliavini, F., Redaelli, V., Fede, B. D., Bizzozero, I., Prioni, S., Van der Kooi, A. J., Raaphorst, J., Calvo, A., Moglia, C., Casale, F., Canosa, A., Manera, U., Bertuzzo, D., Mazzini, L., Bersano, E., Heverin, M., Vadja, A., Pupillo, E., Comi, G., Lunetta, C., Gerardi, F., Filosto, M., Cotelli, M. S., Rinaldi, F., Chiveri, L., Guaita, M. C., Perrone, P., Mauro, C., Diamanti, L., Ferrarese, C., Tremolizzo, L., Delodovici, M. L., Bono, G., Zecca, C., Tortelli, R. and Riva, N. (2018) 'Multicentre, cross-cultural, population-based, case-control study of physical activity as risk factor for amyotrophic lateral sclerosis', *Journal of Neurology, Neurosurgery and Psychiatry*. BMJ Publishing Group, 89(8), pp. 797–803. doi: 10.1136/jnnp-2017-317724.
389. Vogler, T. O., Wheeler, J. R., Nguyen, E. D., Hughes, M. P., Britson, K. A., Lester, E., Rao, B., Betta, N. D., Whitney, O. N., Ewachiw, T. E., Gomes, E., Shorter, J., Lloyd, T. E., Eisenberg, D. S., Taylor, J. P., Johnson, A. M., Olwin, B. B. and Parker, R. (2018) 'TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle', *Nature*, 563(7732), pp. 508–513. doi: 10.1038/s41586-018-0665-2.

390. Voigt, A., Herholz, D., Fiesel, F. C., Kaur, K., Müller, D., Karsten, P., Weber, S. S., Kahle, P. J., Marquardt, T. and Schulz, J. B. (2010) 'TDP-43-Mediated Neuron Loss In Vivo Requires RNA-Binding Activity', *PLoS ONE*. Edited by M. B. Feany, 5(8), p. e12247. doi: 10.1371/journal.pone.0012247.
391. Vozza, A., Parisi, G., Leonardis, F. De, Lasorsa, F. M. and Castegna, A. (2014) 'regulating glucose and glutamine oxidation', 111(3), pp. 960–965. doi: 10.1073/pnas.1317400111.
392. Waibel, S., Neumann, M., Rabe, M., Meyer, T. and Ludolph, A. C. (2010) 'Novel missense and truncating mutations in FUS/TLS in familial ALS', *Neurology*, 75(9), pp. 815–817. doi: 10.1212/WNL.0b013e3181f07e26.
393. Walker, L. C. and Jucker, M. (2015) 'Neurodegenerative diseases: expanding the prion concept.', *Annual review of neuroscience*. Annual Reviews, 38(1), pp. 87–103. doi: 10.1146/annurev-neuro-071714-033828.
394. Wang, J.-W., Brent, J. R., Tomlinson, A., Shneider, N. A. and McCabe, B. D. (2011) 'The ALS-associated proteins FUS and TDP-43 function together to affect Drosophila locomotion and life span', *Journal of Clinical Investigation*, 121(10), pp. 4118–4126. doi: 10.1172/JCI57883.
395. Wang, L., Jiang, Z. and Lei, X. G. (2012a) 'Knockout of SOD1 alters murine hepatic glycolysis, gluconeogenesis, and lipogenesis.', *Free radical biology & medicine*, 53(9), pp. 1689–96. doi: 10.1016/j.freeradbiomed.2012.08.570.
396. Wang, L., Jiang, Z. and Lei, X. G. (2012b) 'Knockout of SOD1 alters murine hepatic glycolysis, gluconeogenesis, and lipogenesis', *Free Radical Biology and Medicine*, 53(9), pp. 1689–1696. doi: 10.1016/j.freeradbiomed.2012.08.570.
397. Wang, T., Jiang, X., Chen, G. and Xu, J. (2015) 'Interaction of amyotrophic lateral sclerosis/frontotemporal lobar degeneration–associated fused-in-sarcoma with proteins involved in metabolic and protein degradation pathways', *Neurobiology of Aging*, 36(1), pp. 527–535. doi: 10.1016/j.neurobiolaging.2014.07.044.
398. Wang, Y.-T., Kuo, P.-H., Chiang, C.-H., Liang, J.-R., Chen, Y.-R., Wang, S., Shen, J. C. K. and Yuan, H. S. (2013) 'The truncated C-terminal RNA recognition motif of TDP-43 protein plays a key role in forming proteinaceous aggregates.', *The Journal of biological chemistry*, 288(13), pp. 9049–57. doi: 10.1074/jbc.M112.438564.
399. Warburg, O., Wind, F. and Negelein, E. (1927) 'The metabolism of tumors in the body', *Journal of General Physiology*, 8(6), pp. 519–530. doi: 10.1085/jgp.8.6.519.
400. Wen, X., Tan, W., Westergard, T., Krishnamurthy, K., Markandaiah, S. S., Shi, Y., Lin, S., Shneider, N. A., Monaghan, J., Pandey, U. B., Pasinelli, P., Ichida, J. K. and Trotti, D. (2014) 'Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death.', *Neuron*, 84(6), pp. 1213–25. doi: 10.1016/j.neuron.2014.12.010.
401. Wong, M. and Martin, L. J. (2010) 'Skeletal muscle-restricted expression of human SOD1 causes motor neuron degeneration in transgenic mice', *Human Molecular Genetics*. Oxford University Press, 19(11), pp. 2284–2302. doi: 10.1093/hmg/ddq106.
402. Wu, L.-S., Cheng, W.-C., Hou, S.-C., Yan, Y.-T., Jiang, S.-T. and Shen, C.-K. J. (2010) 'TDP-43, a neuro-pathosignature factor, is essential for early mouse embryogenesis.', *Genesis (New York, N.Y. : 2000)*, 48(1), pp. 56–62. doi:

10.1002/dvg.20584.

403. Xu, M., Chen, X., Chen, D., Yu, B. and Huang, Z. (2017) 'FoxO1 : a novel insight into its molecular mechanisms in the regulation of skeletal muscle differentiation and fiber type specification', 8(6), pp. 10662–10674.
404. Yamakawa, M., Ito, D., Honda, T., Kubo, K., Noda, M., Nakajima, K. and Suzuki, N. (2015) 'Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS', *Human Molecular Genetics*, 24(6), pp. 1630–1645. doi: 10.1093/hmg/ddu576.
405. Yamanaka, K., Chun, S. J., Boillee, S., Fujimori-Tonou, N., Yamashita, H., Gutmann, D. H., Takahashi, R., Misawa, H. and Cleveland, D. W. (2008) 'Astrocytes as determinants of disease progression in inherited amyotrophic lateral sclerosis.', *Nature neuroscience*, 11(3), pp. 251–3. doi: 10.1038/nn2047.
406. Yedavalli, V. S., Patil, A. and Shah, P. (2018) 'Amyotrophic lateral sclerosis and its mimics/variants: A comprehensive review', *Journal of Clinical Imaging Science*. Wolters Kluwer Medknow Publications. doi: 10.4103/jcis.JCIS_40_18.
407. Yoshino, J., Baur, J. A. and Imai, S. ichiro (2018) 'NAD + Intermediates: The Biology and Therapeutic Potential of NMN and NR', *Cell Metabolism*. Cell Press, pp. 513–528. doi: 10.1016/j.cmet.2017.11.002.
408. Zalewska, E., Rowinska-Marcinska, K., Gawel, M. and Hausmanowa-Petrusewicz, I. (2012) 'Simulation studies on the motor unit potentials with satellite components in amyotrophic lateral sclerosis and spinal muscle atrophy.', *Muscle & nerve*, 45(4), pp. 514–21. doi: 10.1002/mus.22344.
409. van der Zee, J., Gijselinck, I., Dillen, L., Van Langenhove, T., Theuns, J., Engelborghs, S., Philtjens, S., Vandenbulcke, M., Sleegers, K., Sieben, A., Bäumer, V., Maes, G., Corsmit, E., Borroni, B., Padovani, A., Archetti, S., Perneczky, R., Diehl-Schmid, J., de Mendonça, A., Miltenberger-Miltenyi, G., Pereira, S., Pimentel, J., Nacmias, B., Bagnoli, S., Sorbi, S., Graff, C., Chiang, H. H., Westerlund, M., Sanchez-Valle, R., Llado, A., Gelpi, E., Santana, I., Almeida, M. R., Santiago, B., Frisoni, G., Zanetti, O., Bonvicini, C., Synofzik, M., Maetzler, W., vom Hagen, J. M., Schöls, L., Heneka, M. T., Jessen, F., Matej, R., Parobkova, E., Kovacs, G. G., Ströbel, T., Sarafov, S., Tournev, I., Jordanova, A., Danek, A., Arzberger, T., Fabrizi, G. M., Testi, S., Salmon, E., Santens, P., Martin, J. J., Cras, P., Vandenberghe, R., De Deyn, P. P., Cruts, M., Van Broeckhoven, C., Ramirez, A., Kurzwelly, D., Sachtleben, C., Mairer, W., Firmo, C., Antonell, A., Molinuevo, J., Forsell, C., Lillius, L., Kinhult Ståhlbom, A., Thonberg, H., Nennesmo, I., Börjesson-Hanson, A., Bessi, V., Piaceri, I., Helena Ribeiro, M., Oliveira, C., Massano, J., Garret, C., Pires, P., Danel, A., Ferrari, S. and Cavallaro, T. (2013) 'A Pan-European Study of the C9orf72 Repeat Associated with FTLD: Geographic Prevalence, Genomic Instability, and Intermediate Repeats', *Human Mutation*, 34(2), pp. 363–373. doi: 10.1002/humu.22244.
410. Zhang, T., Baldie, G., Periz, G. and Wang, J. (2014) 'RNA-Processing Protein TDP-43 Regulates FOXO-Dependent Protein Quality Control in Stress Response', *PLoS Genetics*, 10(10). doi: 10.1371/journal.pgen.1004693.
411. Zhang, Y.-J., Caulfield, T., Xu, Y.-F., Gendron, T. F., Hubbard, J., Stetler, C., Sasaguri, H., Whitelaw, E. C., Cai, S., Lee, W. C. and Petrucelli, L. (2013) 'The dual functions of the extreme N-terminus of TDP-43 in regulating its biological activity and inclusion formation.', *Human molecular genetics*, 22(15), pp. 3112–22. doi: 10.1093/hmg/ddt166.

412. Zhao, W., Varghese, M., Vempati, P., Dzhun, A., Cheng, A., Wang, J., Lange, D., Bilski, A., Faravelli, I. and Pasinetti, G. M. (2012) 'Caprylic Triglyceride as a Novel Therapeutic Approach to Effectively Improve the Performance and Attenuate the Symptoms Due to the Motor Neuron Loss in ALS Disease', *PLoS ONE*. Edited by U. Pandey, 7(11), p. e49191. doi: 10.1371/journal.pone.0049191.
413. Zhao, Z., Lange, D. J., Voustianiouk, A., MacGrogan, D., Ho, L., Suh, J., Humala, N., Thiyagarajan, M., Wang, J. and Pasinetti, G. M. (2006) 'A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis.', *BMC Neuroscience*, 7(1), p. 29. doi: 10.1186/1471-2202-7-29.
414. Zhong, H., Liu, M., Ji, Y., Ma, M., Chen, K., Liang, T. and Liu, C. (2018) 'Genipin Reverses HFD-Induced Liver Damage and Inhibits UCP2-Mediated Pyroptosis in Mice', *Cellular Physiology and Biochemistry*. S. Karger AG, 49(5), pp. 1885–1897. doi: 10.1159/000493651.
415. Zhu, C. H., Mouly, V., Cooper, R. N., Mamchaoui, K., Bigot, A., Shay, J. W., Di Santo, J. P., Butler-Browne, G. S. and Wright, W. E. (2007) 'Cellular senescence in human myoblasts is overcome by human telomerase reverse transcriptase and cyclin-dependent kinase 4: Consequences in aging muscle and therapeutic strategies for muscular dystrophies', *Aging Cell*, 6(4), pp. 515–523. doi: 10.1111/j.1474-9726.2007.00306.x.
416. Zufiría, M., Gil-Bea, F. J., Fernández-Torrón, R., Poza, J. J., Muñoz-Blanco, J. L., Rojas-García, R., Riancho, J. and de Munain, A. L. (2016) 'ALS: A bucket of genes, environment, metabolism and unknown ingredients.', *Progress in neurobiology*, 142, pp. 104–29. doi: 10.1016/j.pneurobio.2016.05.004.

Abreviaturas

A

Acetil- Coa: acetil coenzima a

AcHR: receptor de acetilcolina

ACTH: hormona adrenocorticotropa

ADN: ácido desoxirribonucleico

ADNc: ADN complemetario

ADP: adenosín difosfato

AGC1: *aspartae glutamate carrier 1* (proteína transportadora de aspartato y glutamato, aralar1)

AK: adenilato quinasa

ALS2: alsina-2

ALSFRS-R: *ALS Functional Rating Scale- revised* (Adaptación Española de la Escala revisada de Valoración Funcional de la Esclerosis Lateral Amiotrófica).

AMP: adenosín monofosfato

AMPA: receptor quiscualato

AMPD: adenosín monofosfato desaminasa

AMPK: adenosín monofosfato quinasa

ARN: ácido ribonucleico

ASP: aspartato

ATAXN2: ataxina-2

ATP: adenosín trifosfato

B

BMAA: beta-metilamino-L-alanina

BSA: seroalbúmina bovina

C

C9ORF72: *chromosome 9 open reading frame 72* (franja de lectura abierta 72 del cromosoma 9)

CD56: molécula de adhesión celular 56

CEIC: comités éticos de investigación clínica

CHIMP2B: *charged multivesicular body protein 2b* (proteína corporal multivesicular cargada 2b)

CK: creatina quinasa

CMA: Chaperone-mediated autophagy (autofagia mediada por chaperonas)

CX3CR1: *cx3c chemokine receptor 1* (receptor 1 de quimiocina cx3c)

CYP27A1: *cytochrome p450 family 27 subfamily a member 1* (esterol 27 hidroxilasa)

D

D_2O: agua pesada

DFT: demencia frontotemporal

DHAP: dihidroxiacetona fosfato

DHE: dihidroxietidio

DM: diabetes mellitus

DMEM: *dulbecco's modified eagle medium*

E

ECAR: *extracellular acidification rate* (tasa de acidificación extracelular)

ELA: esclerosis lateral amiotrófica

EMG: electromiografía

EPHA4: *ephrin type-a receptor 4* (receptor de ephrina a4)

ERBB4: proteína receptora tirosina quinasa erbb-4

ETC: encefalopatía traumática crónica

EWSR: *ewing sarcoma breakpoint region* (proteína de unión a ARN EWS)

F

FAD: flavín adenín dinucleótido oxidado

FADH2: flavín adenín dinucleótido reducido

FBS: suero fetal bovino

FBXO: *forkhead box protein o (drosophila)*

FCCP: carbonilcianuro-p-trifluorometoxifenilhidrazona

FIG4: polifosfoinositida fosfatasa

FOXO: *forkhead box protein o* (proteína o "caja de la cabeza del tenedor")

FUS: *fused in sarcoma* (proteína de unión al sarcoma)

G

G3P: gliceraldehído 3 fosfato

G3PD1/2: gliceraldehído 3 fosfato deshidrogenasa ½

GDNF: *glial derived neurotrophic factor* (factor neurotrófico derivado de la glía)

GFAP: *glial fibrilary acid protein* (proteína fibrilar ácida glial)

GLE1: nucleoporina GLE1

GLN: glutamina

GLU: glutamato

GLUT4: transportador de glucosa tipo 4

GO: ontología genética

GOT1/2: *glutamic-oxaloacetic transaminase 1/2* (aspartato aminotransferasa)

GSH: glutatión reducudo

GWAS: *genome wide association studies* (estudios de asociación genómica completa)

H

H3: histona 3

HFE: proteína reguladora de hierro

HKII: hexoquinasa 2

I

IGF-1: factor de crecimiento insulínico tipo 1

IL-1β: interlequina 1β

IL-6: interleuquina 6

INF-β1α: interferón β1-a

IRNA: ARN de interferencia

IRS-1: sustrato receptor de insulina 1

L

LCR: líquido cefalorraquídeo

LDHA/B: lactato deshidrogenasa A/B

M

MAFBX: atrogina-1

MATR3: matrina 3

MDH1/2: malato deshidrogenasa 1/2

MET: microscopio electrónico de transmisión

MET: *metabolic equivalent of task* (equivalentes metabólicos para realizar una tarea)

MN: motoneurona

MOE: músculo oculomotor extrínseco

MOI: *multiplicity of infections* (multiplicidad de infección)

MPC1: *malate piruvate carrier 1* (transportador de malato piruvato)

MS: espectrometría de masas

MTOR: *mammalian target of rapamycin* (diana de rapamicina en células de mamífero)

MURF-1: *muscle RING-finger protein-1* (proteina muscular dedo RING)

MYF5: factor miogénico 5

MYHC: *myosin heavy chain* (cadena pesada de miosina)

MYOD1: proteína 1 de diferenciación miogénica

MYOG: miogenina

N

NAD^+: nicotinamida adenina dinucleótido oxidado

NADH: nicotinamida adenina dinucleótido reducudo

NEFH: neurofilamento polipéptido pesado

NEK1: quinasa relacionada con NIMA 1

O

OCR: *oxygen consumption rate* (tasa de consumo de oxígeno)

OGC1: *oxolglutarate glutamate carrier 1* (proteína transportadora de oxoglutarato y glutamato)

OPTN: optineurina

P

p62: nucleoporina 62

PAX3: *paired box gene 3* (gen de la caja emparejada 3)

PAX7: *paired box gene 7* (gen de la caja emparejada 7)

PCr: fosfocreatina

PDE4: fosfodiesterasa 4

PFA: paraformaldehído

PFK2: fosfofructoquinasa 2

PFKFB3: 6-fosfofructo-2-quinasa / fructosa-2,6-bifosfatasa 3

PFKM: fosfofructuquinasa muscular

PFN1: profilina 1

PGC1α: *peroxisome proliferator-activated receptor gamma coactivator 1-alpha* (coactivador gamma del receptor activado por proliferador de peroxisomas 1-alfa)

P_i: fosfato inorgánico

PK: piruvato quinasa

PVDF: polifluoruro de vinilideno

R

RE: retículo endoplásmico

RMN: resonancia magnética nuclear

ROS: especies reactivas de oxígeno

RPM: revoluciones por minuto

RT-qPCR: *reverse transcription quantitative polymerase chain reaction* (reacción cuantitativa en cadena de la polimerasa con transcriptasa reversa)

RYR: receptor de rianodina

S

SDS: dodecilsulfato sódico

SERCA: bomba de calcio de retículo sarcoplásmico

SETX: senataxina

SGM: *skeletal muscle cell growth medium* (medio de cultivo para células musculares)

shRNA: *short hairpin RNA* (ARN de horquilla corta)

SIDA: síndrome de inmunodeficiencia adquirida

SIGMR1: receptor sigma tipo 1

SMN1: proteína de supervivencia de motoneuronas 1

SNC: sistema nervioso central

SOD1: superóxido dismutasa 1

SQSTM1: sequestosoma1

T

TDP-43: TAR DNA binbing protein (proteína 43 de union a DNA)

TREM2: *triggering receptor expressed on myeloid cells 2* (receptor desencadenante expresado en las células mieloides 2)

TAF15: *TATA binding protein associated factor* 15 (factor 15 asociado a la proteína de unión a TATA)

TGF-β: factor de crecimiento transformante beta

TSP: ácido trimetilsililpropanoico

TOMM20: homólogo de la subunidad del receptor de importación mitocondrial TOM20

TUBA4A: cadena alfa 4A de tubulina

U

UBQLN2: ubiquilina 2

UCP: proteína desacoplante mitocondrial

UPLC: *ultra performance liquid chromatography* (cromatrografía líquida de alta eficacia)

V

VAPB: *vesicle-associated membrane protein-associated protein B/C* (proteína B / C asociada a proteínas de membrana asociada a vesículas)

VCP: *valosin containing protein* (proteína contenedora de valosina)

VEGF: factor de crecimiento endotelial vascular

VitB12: vitamina B12

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Review article

ALS: A bucket of genes, environment, metabolism and unknown ingredients

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ABSTRACT

The scientific scenario of amyotrophic lateral sclerosis (ALS) has dramatically changed since TDP-43 aggregates were discovered in 2006 as the main component of the neuronal inclusions seen in the disease, and more recently, when the implication of *C9ORF72* expansion in familial and sporadic cases of ALS and frontotemporal dementia was confirmed. These discoveries have enlarged an extense list of genes implicated in different cellular processes such as RNA processing or autophagia among others and have broaden the putative molecular targets of the disease. Some of ALS-related genes such as *TARDBP* or *SOD1* among others have important roles in the regulation of glucose and fatty acids metabolism, so that an impairment of fatty acids (FA) consumption and ketogenic deficits during exercise in ALS patients would connect the physiopathology with some of the more intriguing epidemiological traits of the disease. The current understanding of ALS as part of a *continuum* with other neurodegenerative diseases and a crossroads between genetic, neurometabolic and environmental factors represent a fascinating model of interaction that could be translated to other neurodegenerative diseases. In this review we summarize the most relevant data obtained in the ten last years and the key lines for future research in ALS.

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Abbreviation: ALS, amyotrophic lateral sclerosis; ALSFRS, ALS functional rating scale; BMAA, beta-N-methylamino-L-alanine; CNS, central nervous system; CNTF, ciliary neurotrophic factor; CSF, cerebrospinal fluid; CX3CR1, CX3C chemokine receptor 1; EAAT2, excitatory amino acid transporter 2; ELP3, elongator complex protein 3; EPHA4, ephrin type-A receptor 4; ERBB4, erb-b2 receptor tyrosine kinase 4; EWSR, ewing sarcoma breakpoint region; FIG4, polyphosphoinositide phosphatase; FUS, fused in sarcoma; GWAS, genome-wide association studies; HFE, human hemochromatosis protein; IGF, insulin-like growth factor; IRAK4, interleukin-1 receptor-associated kinase 4; KIFAP3, kinesin-associated protein 3; NEFH, heavy chain of neurofilament; PLS, primary lateral sclerosis; OPTN, optineurin; PGRN, progranulin; PMA, progressive muscle atrophy; SCA2, dominant spinocerebellar ataxia; SOD1, superoxide dismutase 1; SQSTM1, sequestosome 1; TAF15, TATA-binding protein associated factor 15; TDP-43/TARDBP, transactive response DNA binding protein 43; TMEM106B, transmembrane protein 106B; TREM2, triggering receptor expressed in myeloid cells 2; TRPM7, transient receptor potential cation channel subfamily M member 7; UBQLN2, ubiquilin 2; UNC13A, unc-13 homolog A; VAPB, vesicle-associated membrane protein; VCP, valosin-containing protein; VEGF, vascular endothelial growth factor; ZNF512B, zinc finger protein 512B.

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Contents

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder that affects motor neurons in the brain, brainstem and spinal cord, resulting in progressive weakness and atrophy of voluntary skeletal muscles. As a result of the concurrent involvement of the upper and lower motor neurons, neurological examination reveals a combination of upper motor neurons signs (spasticity, hyperreflexia and extensor plantar response or Babinski sign) and lower motor neuron signs (muscle atrophy, fasciculations and cramps). The clinical phenotype is generally classified according to the site at which symptoms first emerge. *Classic* or *spinal* forms, with initial involvement of the limbs, are most common, comprising approximately 65% of cases. In *bulbar* forms, which accounts 30% of cases, the diseases starts with dysarthria, dysphagia or both. Five percent of cases begin aggressively with early respiratory failure (Gordon et al., 2006; Hardiman et al., 2011; Kiernan et al., 2011). The survival rates are variable; most of patients die within an average time that ranges from 2 to 5 years, usually due to respiratory failure (Shaw et al., 2001), but about 35% of patients will survive 5 years or more.

From the epidemiological point of view, ALS shows a stable incidence in Western European countries, with 2–3 new cases per 100,000 inhabitants/year and a prevalence of 4.6 per 100,000 (Imam et al., 2010; Logroscino et al., 2010; Joensen, 2012; Pradas et al., 2013). The peak incidence occurs between 50 and 75 years, decreasing thereafter (Chio et al., 2005; Chio et al., 2009; O'Toole et al., 2008; Joensen, 2012). It is still controversial if the incidence may be lower in non-Caucasian populations (Cronin et al., 2007; Zaldívar et al., 2009; Rojas-Garcia et al., 2012) or among American Indians and Eskimos, but most of the epidemiological studies concur of a slight male/women predominance of 1.2–1.5/1 (Logroscino et al., 2010).

Since its description by Charcot in 1874, the extensive corpus of accumulated knowledge about ALS has not been enough to enable successful therapeutic strategies against this devastating disease. Despite the extensive list of molecules tested, only riluzole, a glutamate antagonist, has been demonstrated to increase survival by few months (Bensimon et al., 1994; Lacomblez et al., 1996; Riviere et al., 1998). Trials with minocycline, lithium carbonate (Chio et al., 2010; Morrison et al., 2013) or pioglitazone (Dupuis et al., 2012) have recently failed to demonstrate efficacy and it has even been suggested that the neuroprotective effect of these molecules could be antagonized by the action of riluzole (Yañez et al., 2014).

However, this scenario could be changing due to a range of breakthroughs. First, the early discovery in 1993 that some inherited forms of ALS are caused by mutations in SOD1 have enabled the development of the transgenic mouse SOD1(G93A), which have largely stimulated basic and experimental research. Later, between 2006 and 2009, several groups discovered that TDP-43 and FUS proteins were the main components of the characteristic neuronal inclusions of TDP-43 and FUS proteins as the main components of the characteristic neuronal inclusions (Neumann et al., 2006, 2009; Vance et al., 2009). And few years later, in 2011, the genetic background of ALS forms linked to chromosome 9 was discovered (DeJesús-Hernández et al., 2011; Renton et al., 2011), bringing novel insigths into pathogenic mechanisms. These discoveries boosted the interest in disease, which is well evidenced by the number of entries in Pubmed under the term *"amyotrophic lateral sclerosis"* (5670 citations) during the last 4 years (between March 1st, 2012 and February 29th, 2016), accounting 29% of all historical entries for this disease (data reviewed on March 1st, 2016). Most people are also aware of the ice bucket challenge to obtain funding for ALS research, which has achieved global attention in 2014.

The current understanding of ALS is recognized to be part of a *continuum* that includes other nosological conditions of the central nervous system (CNS), such as frontotemporal dementia (FTD), ataxias or Parkinson's disease (PD) (Strong, 2008; Pradat et al., 2009; Mackenzie et al., 2010;). Epidemiological studies have identified genes involved not only in the inherited but also in the sporadic forms, which have contributed to this paradigm shift. Based on this scientific revolution, it has been possible to perceive a hope in the therapeutic trials that are recently ongoing or in those which are coming.

The aim of this review is to summarize the emerging information regarding genetic/environmental factors, pathogenic pathways, and metabolic alterations in ALS.

2. Etiology of ALS

In the majority of ALS cases, which are sporadic, the initial cause and the mechanisms leading to neuronal degeneration is still unknown. In the hereditary forms, the pathogenesis is linked to the function of defective genes. Despite that, in the last years the boundaries between inherited and sporadic forms are being blurred by the existence of sporadic cases associated with mutations in the same genes.

Numerous studies have attempted to establish genetic, environmental or lifestyle factors in the etiology of ALS. Data from the UK and Swedish Twin registries show that monozygotic twins have a higher risk than dizygotic twins, but both display a higher risk than the control population (Al-Chalabi et al., 2010) and the risk of ALS is increased in close relatives but not in wives, supporting a major role of genetic influences over unidentified environmental factors (Gibson et al., 2014). Linkage studies have provided a fairly extensive list of causative genes, while association studies have generated an even more extensive list of susceptibility genes, although in most cases of sporadic ALS the etiology remains unknown (see below).

Remarkably, a recent population-based study has estimated by using a mathematical model that ALS is a result of a multistep process, where the combination of 6 genetic and/or environmental exposures (steps) is needed to trigger disease (Al-Chalabi et al., 2014). This model is valid for both sporadic and familial types of ALS. And so, although familial mutations have high penetrance and therefore familial ALS could be interpreted as a one-step process, this model states that 5 subsequent steps (genetic and/or environmental) would determine the age of onset, severity of symptoms and the variability of clinical manifestations. Whatever the combination of steps in this multifactorial disease may be, there is no doubt that environmental exposures play an important role in this process. In contrast to the genetic load, which is expressed from birth and remains unmodified throughout life, environmental factors interact with genes to modify the etiopathogenic process of ALS. Thus, bringing new insights on the pathogenic mechanisms that lie behind the environmental factors and how they interact with genes may help the way to tailor novel therapeutic approaches for ALS.

2.1. Environmental factors

Regarding environmental conditions, epidemiological and experimental studies have highlighted a role of the environment in ALS pathogenesis. Among them, there are a wide list of studies implying factors, such as pesticide contamination, lead, smoking, alcohol, viral and fungal infections, physical exercise and electromagnetic radiation (Kamel et al., 2003; Armon, 2009; Sutedja et al., 2009a, 2009b; Alonso et al., 2010; Beghi et al., 2010; Yu et al., 2014; De Jong et al., 2012; Al-Chalabi and Hardiman, 2013; Trojsi et al., 2013; Ingre et al., 2015), as well as the exposure to toxins of unknown nature, as detected among combatants in the 1992 Persian Gulf war (Horner et al., 2008). We will discuss the most relevant ones in the following sections.

2.1.1. Cluster studies and the β-N-methylamino-L-alanine

Regarding environmental factors, the first epidemiological studies tried to find an explanation of the coexistence of regional clusters of patients. One of the first associations of ALS, PD and dementia was described in 1945 within the *Chamorros* population in Guam (Steele, 2005). In 1967, Vega and Bell discovered a neurotoxin, β-N-methylamino-L-alanine (BMAA), in the indigenous cycad (*Cycas micronesica)*, the seeds of which are used by Chamorros to make flour (Vega and Bell, 1967). It was later found that BMAA in cycads comes from symbiotic cyanobacteria resident in specialized coralloid roots of this plant, and that it gets concentrated in the process of flour production. These evidences, together with the postmortem observation that BMAA is concentrated in the brains of Guamanian patients with ALS but not in control brains, gave rise to the BMAA theory of motor neuron disease (MND) (Murch et al., 2004a, 2004b).

Another cluster of ALS patients with parkinsonism was described by Gadjusek between 1962 and 1982 in the Indonesian part of New Guinea, where *Cycas circinalis* seeds are also consumed (Gajdusek and Salazar, 1982; Okumiya et al., 2014 Okumiya et al., 2014). A third major cluster was detected in the Kii Peninsula of Honshu Island in Japan (Kuzuhara and Kokubo, 2005; Kuzuhara, 2011), where the type of drinking water was one possible link among patients. Interestingly, in some of these clusters of patients, mutations in *TRPM7* (Hermosura et al., 2005) or *C9ORF72* genes (Ishiura et al., 2012) were found, but not in all cases. Other clusters have been more recently reported (Field et al., 2013; Masseret et al., 2013, Caller et al., 2013; Torbick et al., 2014; Lannuzel et al., 2015). In the first two clusters, a link with the consumption of cyanobacteria in marine invertebrates have been postulated, although this association has not been confirmed yet. The third and fourth studies identified up to 12 areas of high incidence of ALS around Lake Mascoma in New Hampshire and in also northern New England, which are located in close proximity to waterbodies with frequent cyanobacterial blooms. These four studies add further support to the BMAA theory of motor neuron disease. The fifth and most recent study identified a new geographical cluster of ALS-Parkinsonism in the Caribbean island of Guadeloupe, but no specific genetic or environmental factor has been attributed to this frequent occurrence.

The mechanism explaining BMAA toxicity remains unclear, but it has been detected in brain tissues of Chamorro people affected by ALS/PD/dementia complex (Cox and Banack, 2003). It has been hypothesized that BMAA could behave as an endogenous neurotoxic reservoir, stored in a bound form, that would be slowly released within brain tissues inducing its neurotoxic effects over years (Murch et al., 2004a). Several studies from animal and cellular models have demonstrated that BMAA is incorporated into brain proteins, inducing neurotoxicity by several different mechanisms: glutamatergic toxicity; depletion of glutathione; synergism with other neurotoxins including methylmercury; and misincorporation into proteins via L-seryl tRNA synthetase, producing protein misfolding, intracellular protein aggregates and eventually neuronal cell death (Xie et al., 2011; Bradley, 2015). The potential role of genetic susceptibility to neurotoxicity of BMAA has been explored in several studies, without successfully identifying candidate genes yet (Sieh et al., 2009). Future genetic targets may be mutations or polymorphisms of BMAA transporters and the seryl-tRNA synthetase, which mediate the transport of BMAA across blood-brain barrier and the incorporation into neuronal proteins, respectively (Smith et al., 1992; Lee et al., 2006).

The progressive decrease in the incidence of ALS in most of these clusters concurs with lifestyle changes, which would suggest the existence of unknown environmental etiological factors (Spencer et al., 2005). Cluster studies are useful as hypothesis-generating tools, but their design usually presents a number of weaknesses, making those studies prone to biases. Therefore,

additional studies are usually required to confirm causal associations.

2.1.2. Heavy metals: lead, mercury and selenium

It is well known that heavy metals may be a cause of disease, both when they are present in insufficient amounts as well as when they are present in toxic concentrations. The potential role of these metals in motor neuron degeneration has been widely studied, but it is not completely understood yet (Johnson and Atchison 2009a, 2009b; Sutedja et al., 2009a,b). Among heavy metals, lead, mercury and selenium have been the most studied ones.

2.1.2.1. Lead. High lead levels have been reported in cerebrospinal fluid and blood samples of ALS (Fang et al., 2010). In keeping with this, some polymorphisms of the gene encoding aminolevulinic acid dehydratase (ALAD), which result positively associated with bone lead levels in ALS patients compared with controls, seem to increase the risk of ALS (Kamel et al., 2003).

However, there are some controversies regarding the role of lead in ALS. Remarkably, several studies in ALS patients and murine models of ALS showed that lead exposure (especially at low doses) was associated with greater survival (Kamel et al., 2005). A potential explanation for these paradoxical findings could be related to an increase expression of vascular endothelial growth factor (Barbeito et al., 2010). Also, it is conceivable that lead is just one part of the risk equation, while other mechanisms, such as epigenetic variations, which may be induced by lead, could be important in the pathogenesis and the individual susceptibility (Callaghan et al., 2011).

2.1.2.2. Mercury. Mercury exposure has been associated with an increased risk of ALS in different epidemiological studies, although there is no full concordance among them (Adams et al., 1983; Pamphlett and Waley, 1998; Praline et al., 2007). In this line, clinical manifestations in patients with long-term accidental exposure to mercury are very similar to those reported in classic ALS (Schwarz et al., 1996). Experimental studies in both animals and cell cultures also supported a role of mercury in ALS pathogenesis. Experimental murine models have demonstrated that a single dose of mercury is followed by its deposit in both upper a lower motor neurons (Arvidson 1992). Additionally, ALS mice exposed to mercury show a quicker and more abrupt disease course, suggesting that this metal may potentiate the development of the disease in those genetically predisposed individuals (Johnson et al., 2009a, 2009b). Some mechanistic experiments have shown that mercury impairs the interaction between microglia and astrocytes by influencing the secretion of proinflammatory cytokines as IL-6 (Bassett et al., 2012). More recently, Rooney et al. postulated that mercury can also interfere with normal DNA methylation (Rooney, 2011).

2.1.2.3. Selenium. First evidences relating selenium and ALS came from two epidemiological investigations that documented an increased risk of ALS in populations resident in seleniferous regions (Kilness and Hichberg 1977;Vinceti et al., 1996). Thus, a 11 year follow-up study including more than 5000 Italian people demonstrated that those who drank high-Selenium tap water had almost 7 times higher risk of ALS than those not exposed to selenium (Vinceti et al., 1996). Experimental and laboratory models have shown that selenium is specifically toxic for motor neurons in swine and cattle (Casteignau et al., 2006). In particular, selenium intoxication has been found to selectively damage motor neurons in pigs, with a bilateral focal affection of ventral horns at the spinal cord (Vinceti et al., 2001). More recently, it has been reported that selenium-induced oxidative stress leads to decreased cholinergic signaling and degeneration of cholinergic motor neurons (Estevez et al., 2012). Additionally, Maraldi et al. showed that selenium exposure influences SOD1 accumulation into mitochondria, an important feature in some models of ALS (Maraldi et al., 2011).

2.1.2.4. Zinc. Recently, it has been postulated that zinc exposure may have a neurotoxic effect in motor neurons. Some experiments have proven that zinc increases oxidative stress and enhances excitotoxicity, therefore promoting motor neuron death (Nutini et al., 2011). Interestingly, a paradoxical effect has been reported in the SOD1 murine model of ALS: high zinc doses were associated with earlier death, while low zinc supplementation extended mice survival (Groeneveld et al., 2003; Ermilova et al., 2005).

2.1.3. Other environmental factors: the role of tobacco, pesticides, electromagnetic fields and lifestyles

The relationship between tobacco and ALS has been suggested but not clearly confirmed. Although several epidemiological studies found a potential association (Alonso et al., 2010; De Jong et al., 2012), other studies did not confirm it (Fang et al., 2006). Among tobacco compounds, heavy metals, such as cadmium or lead (previously discussed), residues of pesticides (Djordjevic et al., 1995) and formaldehyde (Hoffmann et al., 2001) are some of the most toxic elements. Currently, there exist strong evidences supporting the role of cadmiun in neurodegeneration. Particularly, Huang et al. demonstrated that the enzymatic activity of Cu/Zn SOD1 may be strongly inhibited by this metal (Huang et al., 2006). Regarding formaldehyde, there are some studies revealing that exposed people have an increased risk of ALS (Weisskopf et al., 2009; Roberts et al., 2015). Additionally, experimental studies have shown that formaldehyde triggers oxidative stress because it reduces SOD1 activity (Nie et al., 2007) and induce mitochondrial damage (Gurel et al., 2005), two important cellular events in ALS pathogenesis (Robberecht and Philips, 2013). Among the susceptibility genes for formaldehyde toxicity, some allelic variants of the genes encoding paraoxanase and the cytochrome P450 cluster have been suggested (Boccia et al., 2007).

The association between ALS and exposure to pesticides has been studied in several studies (Weisskopf et al., 2009; Bonvicini et al., 2010). A few years ago, two *meta*-analysis were published revealing a positive association between ALS risk and the use of organochlorine insecticides, pyrethroids, herbicides, and fumigants (Malek et al., 2012; Kamel et al., 2012). Among pesticides, organophosphates are the most commonly used ones (Weiss et al., 2004). Interestingly, organophosphates irreversibly inhibit acetylcholinesterase, therefore inducing excitotoxicity and brain damage (Chen, 2012). Specifically, Morahan et al. showed that an impaired ability of sALS patients to detoxify pesticides could be associated with polymorphisms in the metallothionein family of genes (Morahan et al., 2007).

Regarding the role of electromagnetic fields (EMF), there is one *meta*-analysis reporting a slight but significant increase of the risk of developing ALS among extremely low frequencies-EMF-related occupations (Zhou et al., 2012). In this line, *in vitro* studies revealed that prolonged exposure to extremely low frequencies-EMF may induce oxidative stress, DNA damage and apoptosis (Falone et al., 2008). However, these results have not been replicated in ALS murine models (Poulletier et al., 2009). In addition, another *meta*-analysis did not confirm causal relationship between electric injury and the risk of ALS (Abhinav et al., 2007).

A disturbing and unexplained association has been recently reported in patients following embolization of cerebral arteriovenous malformations and subsequent radiation, a finding that authors attributed to reduced VEGF levels (Valavanis et al., 2014).

Regarding other lifestyle-related factors, it has been speculated that exercise and good physical condition, which are usually considered to have beneficial effects on health, could be risk factors for neurodegeneration later in life (Al-Chalabi and Hardiman, 2013; Eaglehouse et al., 2016). It is well known that repeated and strenuous exercise generates a stress situation that requires a good neuroprotective/recovery mechanism to ensure the integrity and function of neuromuscular system. This stress might overflow the possibilities of this system in a predisposed individual. Supporting this notion, there are several case-control studies reporting that intense and vigorous physical activity could be associated with the disease since sport proffesionals (football players, thriathletes, cross-country skiers) are usually over-represented in ALS cohorts (Beghi et al., 2010; Gotkine et al., 2014; Chio et al., 2005; Fang et al., 2015), while moderate exercise might be otherwise protective (Pupillo et al., 2014; Fang et al., 2015). These outcomes have been recently replicated and validated in a large prospective cohort from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. This work has found that total physical activity was weakly and inversely associated with ALS mortality; however, vigorous physical activity early in life was associated with a two-fold increased risk for ALS mortality (Gallo et al., 2016).

A curious fact is that the patients with hyperglycemia or hyperlipidemia seem to have a better prognosis, whereas individuals with athletic appearance and low body fat have an increased risk of developing the disease, suggesting that a negative energetic balance may be a critical factor in the etiology of ALS. This finding will merit further consideration (see later) (Dupuis et al., 2008; Dupuis et al., 2011)

In short, the heterogeneity of potential environmental factors capable to induce neurodegeneration in ALS highlights the multifactorial nature of this disease and the relevance of the gene-environment interactions. Some conflicting results might be related to the fact that there are not consistent studies considering the contribution of multiple susceptibility factors at the same time in ALS pathogenesis. Thus, additional epidemiological and experimental studies are needed to elucidate the actual role of each environmental factor in motor neuron degeneration and their interaction with genetic factors (Trojsi et al., 2013). In recent years, the field of epigenomics has highlighted the importance of epigenetic mechanisms mediating those gene-environment interactions. A better understanding of those mechanisms will surely help to discover new therapeutic targets in ALS.

2.2. Genetic factors

Over the past two decades evidence has accumulated regarding the contribution of genetics to the development of ALS. The involvement of genetics in ALS occurs at various levels. On one hand, there are causative genes that frequently lead to a classic autosomal dominant pattern of inheritance in a pure form or associated with other neurodegenerative syndromes (accounting 10% of cases) (Millecamps et al., 2012; Mok et al., 2012), whereas on the other hand, there are susceptibility genes that are associated with an increased the risk of developing a sporadic form of the disease. Furthermore, an increasing amount of data suggests that epigenetics can play a role in the development of ALS. Interestingly, most of causative and susceptibility genes are implicated in few particular cellular functions, mainly DNA/RNA processing, autophagy, vesicle transport, oxidative stress and metabolism (Fig. 1).

2.2.1. Causative genes

2.2.1.1. Major genes. The first gene implicated in a familial form of ALS was *SOD1*, although it only accounts for a very small percentage of familial forms of the disease (Rosen et al., 1993). Mutations in the genes for *FUS* and *TARDBP* (encoding for TDP-43 protein) were the following discoveries. Neuropathological analysis led to the identification of insoluble cytoplasmatic phosphorylated protein deposits in brain and spinal cord that are also present in other forms of brain neurodegeneration, such as certain types of FTD (Kabashi et al., 2008; Sreedharan et al., 2008; Van Deerlin et al., 2008; Corrado et al., 2009; Gitcho et al., 2009; Kwiatkowski et al., 2009; Vance et al., 2009). TDP-43 is a widely expressed and a highly conserved protein with diverse functions in RNA metabolism, including RNA translation, splicing and transport (Lee et al., 2015). In physiological conditions, TDP-43 is located in the nucleus to exert its function but a small amount can be found in the cytoplasm (Ayala et al., 2008). In 2011, hexanucleotide expansion mutations were discovered in a gene called *C9ORF72* located in a region of chromosome 9 that was previously identified in linkage studies in families with FTD, ALS or both. The form of ALS linked to this gene may represent up to one third of cases with European ancestors (DeJesús-Hernández et al., 2011; Renton et al., 2011). Since the publication of these two seminal works, many studies have been conducted to determine the presence of pathological expansions in different populations, ranging from 46% of familial forms and 21% of sporadic cases in Finland to 27.1% of familial forms and 3.2% of sporadic cases in Spain (García-Redondo et al., 2013) with intermediate rates in other European countries (Renton et al., 2011; Byrne et al., 2012; Cooper-Knock et al., 2012; Gijselinck et al., 2012; Majounie et al., 2012; Millecamps et al., 2012; Mok et al., 2012; Sabatelli et al., 2012). This mutation appears to have a European ancestry (Smith et al., 2013).

Together, the 4 genes described above (*SOD1, FUS, TARDBP* and *C9ORF72*) account 60–80% of familial ALS cases. *C9ORF72* expansions represents about 50% of the mutations found in the familial forms that appear after the age of 40, while mutations in *FUS* account for 35% of cases appearing before that age (Millecamps et al., 2012).

2.2.1.2. Minor genes. Although other genes have been linked to Mendelian forms of the disease, its epidemiological importance is nearly residual.

Genes involved in autophagy and vesicle transport. Mutations in genes such as *OPTN* have mainly been described in Japanese families with dominant or recessive inheritance (Maruyama et al., 2010; Iida et al., 2012a, 2012b), whereas others such as *VCP*, *VAPB*, *SQSTM1* (which encodes for the protein p62), *SORT1*, *UBQLN2* and *FIG4*, are responsible for a small number of the familial cases and have only a symbolic presence among sporadic cases (Hocking et al., 2002; Nishimura et al., 2004; Landers et al., 2008; Chow et al., 2009; Johnson et al., 2010; Del Bo et al., 2011; Deng et al., 2011; Abramzon et al., 2012; Belzil et al., 2012). Among these, *VCP* and *SQSTM1* are also related to early-onset Paget disease and inclusion body myopathy (Laurin et al., 2002; Haubenberger et al., 2005).

Genes involved in RNA metabolism. Based on the assumption that other proteins with RNA recognition motifs could play a role in the pathophysiology of the disease, six families were found to carry mutations in a gene coding for a protein with this function, *TAF15* (TATA-binding protein associated factor 15) (Couthouis et al., 2011; Ticozzi et al., 2011) and another three had variants in a different gene (*EWSR*) with putative functions similar to those of *TAF15* in sporadic ALS cases (Couthouis et al., 2012). EWSR and TAF15, together with FUS, constitute the FET protein family with highly conserved RNA-binding motifs, which participate in various cellular processes including transcription, pre-mRNA splicing and miRNA processing. These findings reinforce the idea that there may be more proteins with RNA-binding properties, particularly those that also act as a prion-like RNA recognition domain (a region rich in glutamine, asparagine, tyrosine and

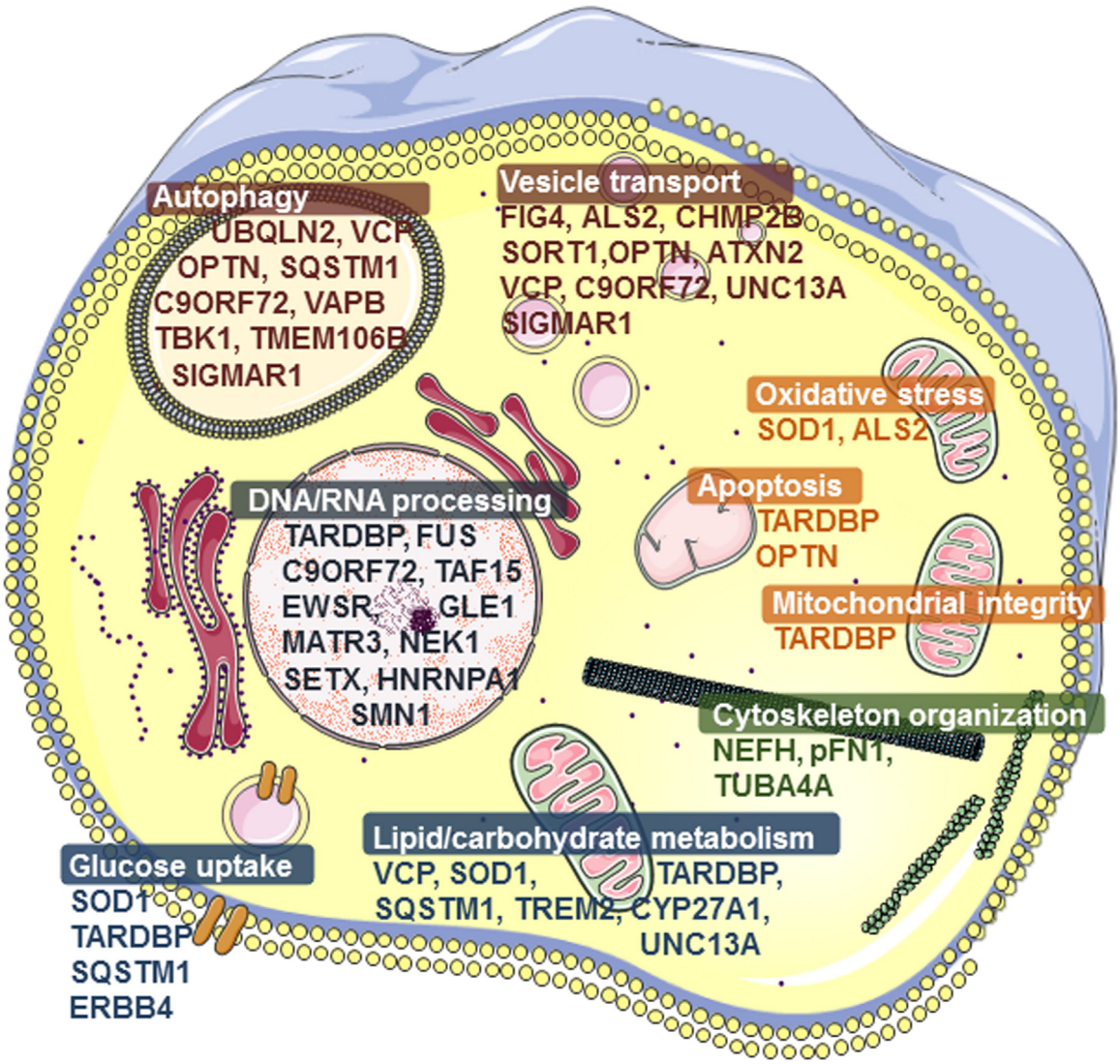

Fig. 1. Main cellular functions where ALS causative or susceptibility genes are involved. Most of the proteins encoded by causative and susceptibility genes for ALS are players of three major cellular functions: DNA/RNA processing, autophagy and vesicle transport. Several genes are also implicated in pathways regulating energy metabolism and in cytoskeletal dynamics. Apoptosis, oxidative stress scavenging and mithocondrial integrity are also common cellular functions in which ALS genes plays important roles.

glycine residues), with potential involvement in other neurodegenerative processes. *TARDBP*, *FUS*, *TAF-15* and *EWSR* are at the top of this hypothetical list, which confirms that the underlying assumption is valid (King et al., 2012).

Mutations in *MATR3*, which may play a role in transcription and is also involved in a form of distal myopathy associated with dysphonia and dysphagia (Senderek et al., 2009), have been identified in families with ALS and dementia. Interestingly, modifications in this protein in the spinal cord were found not only in affected, but also in unaffected individuals (Johnson et al., 2014). However, a large epidemiological study conducted on a sample of patients with ALS and FTD did not report any case with this mutation, so the contribution of *MATR3* to the familial forms of ALS appears to be residual (Millecamps et al., 2014). Mutations in the genes *HNRNPA2B1* and *HNRNPA1*, which encode for heterogeneous nuclear ribonucleoproteins involved in RNA transport and splicing, are reported to accelerate the formation of stress granules and cytoplasminc inclusions, and are a cause of inclusion body myopathy and ALS (Kim et al., 2013). Mutations in another gene (*ANG*, enconding angiogenin protein) involved in RNA metabolism and stress granule formation have been reported in familial and sporadic ALS patients (Greenway et al., 2006; van Es et al., 2011). Other gene, *GLE1*, with a putative role in RNA metabolism has been recently implicated in two sporadic and one familial ALS cases (Kaneb et al., 2015).

Seminal evidences of an altered gene transcript processing in motor neuron disease came from the identification of mutations in the *SMN1* gene, involved in RNA splicing and transport (Burghes and Beattie 2009; Fallini et al., 2012), as a cause of spinal muscular atrophy (SMA) (Lefebvre et al., 1995). A study in sporadic ALS patients found 'abnormal' number of SMN1 copies (one or three rather than two) occurring more frequently in cases than controls (Corcia et al., 2002). However, several studies have demonstrated that the typical deletions seen in SMA do not occur in ALS patients (Schymick et al., 2007) and the potential role of SMN in ALS has not been fully clarified yet.

Genes involved in cytoskeletal organization. A gene that encodes the heavy chain of neurofilament (*NEFH*) has also been implicated in some cases of ALS, although it needs to be confirmed (Al-Chalabi et al., 1999; García et al., 2006; Tomkins et al., 1998). Mutations in *PFN1* (encoding for Profilin-1) have been reported in some families with ALS (Wu et al., 2012; Chen et al., 2013). Some allelic variants in the *PFN1*, such as E117G, are considered to confer a low susceptibility to developing the disease (Fratta et al., 2014).

Other rare mutations. Mutations in *ALS2, SIGMAR1, SETX, SPG11 and CHMP2 B* genes (Yang et al., 2001; Hadano et al., 2001; Chen et al., 2004; Parkinson et al., 2006; Cox and Banack, 2010; Al-Saif et al., 2011; Daoud et al., 2012) are rare but most frequent in the juvenile forms of ALS (onset < 20 years-old). Mutations in the ALS2 gene, which encodes the protein alsin, have been also described in juvenile PLS (onset < 20 years-old) and infantile-onset asceding spastic paraparesis, which involves predominantly upper motor neuron starting at bulbar muscles and progressing to spastic paraparesis or, in some cases, to a generalized dystonia (Yang et al., 2001; Eymard-Pierre et al., 2002; Gros-Louis et al., 2003; Sheerin et al., 2014). Alsin contains three domains homologous to guanine nucleotide exchange factors (GEFs) (Hadano et al., 2001) that catalyze the exchange of GDP for GTP to activate GTPases of the RAS proteins superfamily (Bar-Sagi and Hall, 2000). In fact, it has been claimed that can activate Rho, Rac1 and Rab5 GTPases, which play important roles in endocytosis, cytoskeleton maintenance, protein transport, cell signaling and membrane trafficking (Yang et al., 2001). In addition, Alsin activates Rab 5 that mediates insulin-stimulated production of phosphatidylinositol 3-phosphate (PI(3)P) and regulates trafficking of Glut4 vesicles (Lodhi et al., 2008), so that it could generate the possible link to metabolic imbalance observed in ALS patients mentioned above. Beside the causative role of *SIGMAR1* mutations in juvenile ALS, an altered function of its encoded protein, the sigma non-opiod intracellular receptor 1 (SigmaR1), appears to contribute to ALS pathogenesis as well. In this sense, SigmaR1 have been found abnormally accumulated within enlarged C-terminals and endoplasmic reticulum (ER) structures of alpha motor neurons in lumbar ALS spinal cords (Prause et al., 2013), although is function is generally deregulated, leading to ER and mitochondrial abnormalities, impaired autophagic degradation, endosomal trafficking and intracellular calcium homeostasis (Prause et al., 2013; Vollrath et al., 2014). Importantly, a recent study has reported significant improvements of motor function and motor neuron survival by a SigmaR1 agonist in the SOD1(G93A) mouse model of ALS (Mancuso et al., 2012).

Mutations in the *ERBB4* gene have also been described in a Japanese family with late-onset ALS with no cognitive impairment and a slow progression. The causative mutation interferes with the interaction between the ErbB4 receptor and neuroregulin (NRG), identifying another metabolic pathway capable of generating motor neuron involvement (Takahashi et al., 2013). NRGs are a large family of proteins found in many tissues, which are functional ligands for the EGF receptor-related receptors erbB2, erbB3, and erbB4, regulating cell proliferation, differentiation and survival (Syroid et al., 1996). MNs have different types of synaptic inputs in their membranes, being important among them C-type cholinergic boutons (Bernstein and Bernstein, 1976), where ErbB4 is localized (Gallart-Palau et al., 2014). This type of boutons, which is characteristic of somatic α-MN, arises from local cholinergic interneurons that specifically contact spinal α-MN and regulate their activity (Witts et al., 2014; Frank 2009; Nagy et al., 1993). It was shown that NRGs increase the rate of AChR synthesis in skeletal muscle at neuromuscular junctions (Jo et al., 1995; Zhu et al., 1995), where cholinergic synapses occur through C-type bouton.

Analysis of whole exome sequence data in a cohort of mostly sporadic patients and in an European familial ALS index have confirmed most of the genes previously linked to ALS and discovered some new that are implicated in innate immune response such as *TBK1* (Cirulli et al., 2015; Williams et al., 2015). Indeed, haploinsufficiency of *TBK1* has found to cause ALS and FTD (Freischmidt et al., 2015). A subset of these exome sequence data has recently revealed mutations in the gene NEK1, which encodes for a serine/threonine kinase involved in DNA damage repair and previously associated with short-rib thoracic dysplasia 6 (El Hokayem et al., 2012), as a cause for ALS (Brenner et al., 2016).

In summary, altough the list of causative genes is extense (Table 1) and new genes will be added in the future, there are some clinical indications that can guide molecular studies in familial cases of ALS. In cases associated with FTD/ALS spectrum, the genes involved tend to be *C9ORF72*, *TARDBP*, *FUS* or *VCP*, and if there is no evidence of transmission from male to male, one might consider the *UBQLN2* gene, located on chromosome X (Deng et al., 2011; Williams et al., 2012).

2.2.2. Susceptibility genes

In addition to the genes that are presumably causative of ALS, there are other genes that seem to be involved in disease susceptibility. In 2010, some overlap was found between ALS and dominant spinocerebellar ataxia (SCA2), caused by an expansion of more than 34 CAG triplet repeats in the *ATX2* gene (Elden et al., 2010). This gene encodes for a protein (ataxin 2) involved in RNA metabolism, which was found mislocalized and aggregated in 27% of ALS cases (Elden et al., 2010). These dynamic expansions give rise to a tract of polyglutamine, which causes a large number of ataxias and other neurodegenerative diseases, such as Huntington's disease. The analysis of this expansion in ALS patients cohorts has led some authors to report that the disease risk is associated with alleles larger than 27 until 34 or even 39 repeats (which often causes ataxia) (Chen et al., 2011; Corrado et al., 2011; Daoud et al., 2011; Lee et al., 2011a, 2011b; Ross et al., 2011; Soraru et al., 2011; Van Damme et al., 2011). This association, which seems to occur only in sporadic cases (Gellera et al., 2012), has been extended to include intermediate expansions of ataxin 1 (*ATX1*) (Conforti et al., 2012).

Other susceptibility genes, related to metal metabolism or the processing of toxic genes, are involved in the putative pathophysiology of ALS. For instance, certain polymorphisms in the *HFE* gene implicated in iron metabolism have been repeatedly implicated in ALS (Wang et al., 2004; Yen et al., 2004; Goodall et al., 2005; Sutedja et al., 2007), although posterior studies have failed to replicate the initial results or to completely rule out this association (Praline et al., 2012; van Rheenen et al., 2013).

Another gene implicated in ALS susceptibility is *UNC13A*, which is involved in synaptic vesicle priming and insulin secretion. This gene was identified from a GWAS study (van Es et al., 2009) and was subsequently confirmed in further *meta*-analysis (Lill et al., 2011) and other studies (Diekstra et al., 2012).

ELP3 was also noted as an ALS risk factor in a GWAS study in several populations (Simpson et al., 2009), but although mutations in this gene cause neurodegeneration in an ALS fly model and are involved in RNA metabolism, no mutations have yet been found in any familial or sporadic case of ALS.

Other genes participating in a variety of putative functions, from neuromuscular development, cholesterol metabolism, transcriptional regulation, immune response, cytoskeletal dynamics and synaptic transmission, such as *EPHA4* (Uyan et al., 2013; Van Hoecke et al., 2012), *CYP27A1* (Diekstra et al., 2012), *ZNF512 B* (Tetsuka et al., 2013), *CX3CR1* (López-López et al., 2014), *KIFAP3* (Van Doormaal et al., 2014) and *HTR2 B* (El Oussini et al., 2016) are modulators of ALS disease progression and survival times.

The genes involved in monogenic forms of ALS have been progressively identified as both susceptibility factors for sporadic forms of the disease, modulators of the phenotype or for developing other neurodegenerative diseases. For instance, *SQSTM1* modulates the phenotype of FTDs (Van der Zee et al., 2014), and mutations in *FUS* (Deng et al., 2014) and *TREM2* (Cady et al., 2014) are risk factors for ALS. Recently, a case-control study found an excess of rare damaging variants in *TUBA4A* gene (encoding Tubulin Alpha 4a protein) in familial ALS index cases

Table 1
Description of genes related to Mendelian inheritance of ALS.

Gene	Chromosome localization	Heredity	Mean age at onset	Protein	Protein defective function/protein aggregates	Associated features	References
ALS1/ SOD1	21q22.11	Dominant/ Recessive	47	Superoxide dismutase 1 (mitochondrial protein)	Protein aggregation, possible gains of redox function, impaired axonal transport, metabolic alterations/ Citoplasmic inclusions of SOD1, ubiquitin and p62 but not TDP-43 aggregates	Lower an upper motor neuron involvement. Amyotrophy, autonomic and cognitive dysfunction, cerebellar ataxia, frontotemporal dementia (rarely)	Rosen et al. (1993)
ALS2	2q33.2	Recessive	1	Alsin	Endosome, membrane trafficking, oxidative stress	Childhood or juvenile ALS, spastic paraplegia, primary lateral sclerosis	Hadano et al. (2001)
ALS4/ SETX	9q34.13	Recessive	18	Senataxin	DNA and RNA processing	Oculomotor apraxia type 2, cerebellar ataxia, motor neuropathy	Chen et al. (2004)
ALS5	15q15.1-q21.1	Recessive	<10	Unknown	Unknown	ALS rapidly progressive but one case associated with slow progression and SPG mutation	Hentati et al. (1998)
ALS6/ FUS	16p11.2	Dominant/ Recessive	46	Fused in sarcoma (FUS)	Altered RNA processing, formation of inclusion bodies/FUS but not TDP-43 aggregates	Lower an upper motor neuron involvement and frontotemporal dementia	Vance et al. (2009) and Kwiatkowski et al. (2009)
ALS8/ VAPB	20q13.3	Dominant	44	Vesicle-associated membrane protein (VAMP)	Vesicle trafficking/TDP43 aggregates	Lower an upper motor neuron involvement.	Nishimura et al. (2004) and Landers et al. (2008)
ALS9/ ANG	14q11.1	Dominant	55	Angiogenin	rRNA transcription, stress granule formation/TDP-43 aggregates	Lower an upper motor neuron involvement and frontotemporal dementia. Parkinson disease	Greenway et al. (2006) and van Es et al. (2011)
ALS10/ TARDBP	1p36.22	Dominant/ Recessive (rare)	55	TDP-43	RNA splicing, formation of protein inclusion bodies, metabolic alterations, mitochondrial integrity/Ubiquitin, p62 and TDP-43 positive aggregates	Lower an upper motor neuron involvement and frontotemporal dementia	Gitcho et al. (2009), Sreedharan et al. (2008) and Kabashi et al. (2008)
ALS11/ FIG4	6q21	Dominant	55	Polyphosphoinositide phosphatase	Overexpression of SAC3 rendered cells prone to developing dilated intracellular membranes whereas depletion enhance endosome carrier vesicles, multivesicular body formation	Hereditary motor and sensory neuropathy (CMT4J). Typical familial and sporadic late-onset ALS and primary lateral sclerosis	Chow et al. (2007) and Chow et al. (2009)
ALS12/ OPTN	10p15-p14	Dominant/ Recessive	51	Optineurin	Golgi maintenance, membrane trafficking and exocytosis, formation of inclusion bodies. Also interaction with huntingtin, transcription factor IIIA and RAB8/Ubiquitin, p62, optineurin and TDP-43 positive aggregates	Lower an upper motor neuron involvement and frontotemporal dementia. Primary open-angle glaucoma, ataxia, Paget disease	Maruyama et al. (2010) and Iida et al. (2012a, 2012b)
ALS13/ ATX2	12q23q24.1	Dominant	57	Ataxin-2	The rate of cleavage of the protein increases with the length of the polyglutamine repeat and truncated protein products may be toxic to neuronal cells. ATXN2 and TDP-43 associate in a complex that depends on RNA; ATXN2 is abnormally localized in spinal cord neurons of ALS patients/ Ubiquitin and p62 positive aggregates.	High-length ATXN2 repeat alleles are associated to high risk of ALS. Alleles with more than 24 repeats cause Cerebellar ataxia type 2 (SCA2)	Elden et al. (2010)
ALS14/ VCP	9p13.3	Dominant	49	Valosin-containing protein (VCP)	Proteasomal degradation, endosomal trafficking, vesicle sorting/TDP-43 aggregates.	Frontotemporal dementia, parkinsonism, Paget disease	Johnson et al. (2010) and Haubenberger et al. (2005)
ALS15/ UBQLN2	Xp11.21	Dominant X-linked	41	Ubiquilin-2	Proteasomal protein degradation, inclusion body formation/Ubiquilin-2 aggregates, positive for ubiquiting, p62, TDP-43 and FUS inclusions.	Lower an upper motor neuron involvement and frontotemporal dementia	Deng et al. (2011) and Williams et al. (2012)
ALS16/ SIGMAR1	9p13.3	Recessive	1–2	Sigma1R	Modulation of Potassium channels activity (Kv1.4)	Early onset motor neuron involvement	Al-Saif et al. (2011)
ALS17/ CHMP2B	3p11.2	Dominant	50–75	CHMP2b	Multivesicular bodies (MVBs) formation and sorting of endosomal cargo proteins/Intraneuronal inclusions inmunopositive for ubiquitin	Lower motor neuron predominantly involved. Bulbar involvement. Some mutations in this gene could cause frontotemporal dementia	Skibinski et al. (2005), Parkinson et al. (2006) and Cox et al. (2010)
PFN1/ ALS18	17p13.3	Dominant	44	Profilin 1	Disruption of cytoskeletal pathways	ALS with limb onset. No bulbar forms	Wu et al., (2012) and Chen et al. (2013)

Table 1 (*Continued*)

Gene	Chromosome localization	Heredity	Mean age at onset	Protein	Protein defective function/protein aggregates	Associated features	References
ERBB4/ ALS19	2q34	Dominant/ Sporadic	60–70	ERBB4	Tyrosin-kinase receptor for neurregulins that suppres induction of long-term potentiation in the hippocampal CA1 region without affecting basal synaptic transmission; glucose uptake.	Classic upper and lower motor neuron degeneration	Takahashi et al. (2013)
HNRNPA1/ A2B1/ ALS20	12q13.13	Dominant	Late onset	hnRNPA1 hnRNPA2B1	Pathogenic mutations strengthen a 'steric zipper' motif in the PrLD, which accelerates formation of self-seeding fibrils that cross-seed polymerization of WT hnRNP	Motor neuron disease associated to Paget disease, cognitive disturbance and myopathy	Kim et al. (2013)
MATR3/ ALS21	5q31.2	Dominant	60	Matrin 3	Appers in the nuclei of motor neurons interacting with TDP-43 and regulating transcription.	Lower and upper motor neuron involvement. Dementia.	Johnson et al. (2014)
C9ORF72/ FTDALS1	9p21.2	Dominant	57	C9orf72 Uncharacterized	Endosomal trafficking and autophagy; altered C9ORF72 RNA splicing, formation of nuclear RNA foci/TDP-43 positive aggregates and TDP-43 negative aggregates localized in hippocampus and cerebellum positive for ubiquitin and p62.	Lower an upper motor neuron involvement and frontotemporal dementia	Renton et al. (2011) and DeJesus-Hernandez et al. (2011)
SQSTM1	5q35.3	Dominant		P62/Sequestosome-1	Association with the NF-kappaB pathway, regulation of glucose metabolism.	Paget disease associated	Hocking et al. (2002) and Laurin et al. (2002)
NEFH	22q12.2	Dominant/ Sporadic	60–70	NF-H	Neurofilament accumulation and axonal degeneration	Typical ALS	Al-Chalabi et al. (1999), Tomkins et al. (1998)
GLE1	9q3411	Dominant?	50–65	hGLE1	RNA metabolism	Lethal congenital syndrome, lethal artrogryposis with motor neuron disease	Kaneb et al. (2015)
TAF15	17q12	Unknown	Unknown	TAFII68	RNA-binding protein similar to FUS and ESW3 (FET family proteins).	Neuron and glial FUS inclusions in frontotemporal dementia but not in ALS are stained with TAFII68.	Ticozzi et al., (2011) and Couthouis et al. (2012)
TBK1	12q14.2	Dominant		TANK-binding kinase	Regulates inflammatory responses to foreing agents; TBK1 phosphorilates OPTN and SQSTM1 to enhance autophagy	Involved in frontotemporal dementia and a form of primary open angle glaucoma	Cirulli et al. (2015), Williams et al. (2015) and Freischmidt et al. (2015)

using exome-sequencing data (Smith et al., 2014). Importantly, among the seven ALS patients carrying rare variants in *TUBA4A*, two of them developed FTD and one had a first-degree relative affected by FTD. The involvement of *TUBA4A* in ALS has been recently replicated in a cohort of sporadic ALS patients (Pensato et al., 2015) but it is not present in a large Spanish cohort of FTD patients (Dols-Icardo et al., 2016).

An additional gene, *TMEM106B*, which is related with lysosomal transport (Schwenk et al., 2014), has been found involved not only in the phenotypic modulation of FTDs caused by mutations in *PGRN* (Van Deerlin et al., 2010) or *C9ORF72* (Gallagher et al., 2014), but also in ALS (Vass et al., 2011).

2.2.3. Epigenetics

Epigenetic studies as a potencial convergence point between genetic predisposition and environmental exposures in ALS are still at an early stage. These epigenetic studies include DNA gene expression modifiers through histone changes and miRNAs that modulate functions involved in the pathophysiology of the disease that could be therapeutic targets (Lazo-Gomez et al., 2013; Lu et al., 2013). It has been described that the percentage of DNA methylation in blood and neural tissue is increased in ALS patients regardless the age of onset (Tremolizzo et al., 2014) and there are changes in the methylation pattern of *C9ORF72* gene (Xi et al., 2013, 2014; Belzil et al., 2014). Otherway, modifications in FUS dynamics induce changes in the histone configuration due to a loss of function of protein Arginine-N-methyltransferase 1 (PRMT1) (Tibshirani et al., 2014).

Postranslational modifications of histones tails are made by acetylation or methylation, and histone deacetylases (HDACs) promote the removal of acetylation repressing the gene expression. Overexpression of several HDACs has been reported in spinal cord and brain tissues from ALS patients and ALS animal models (Janssen et al., 2010; Valle et al., 2014).

Taking into account miRNAs studies, overexpression of miRNA-206 has been reported in an experimental model of denervation and there is an accelerated disease progression in mice that are genetically deficient in this miRNA (Williams et al., 2009). Beside this, miRNA-206 is elevated in the plasma of SOD1-deficient mice and sporadic ALS patients, which makes it a potential biomarker for the disease (Toivonen et al., 2014).

The contribution of miRNA-365 and miRNA-125b could modulate the immune response mediated by TNF-alpha and thereby exacerbate the disease, as reviewed by Parisi et al. (Parisi et al., 2013). It has also been found that other miRNAs, such as miRNA-155, are overexpressed in the spinal cord of patients who died with sporadic or familial ALS (Koval et al., 2013). Furthermore, miRNome analysis has revealed that some miRNAs are associated

with early stages of the disease and miRNA-338-3p could be deregulated in the brain of ALS patients (Shioya et al., 2010; De Felice et al., 2012).

Interestingly, some of mutations that causes protein aggregates such as TDP-43 or FUS could be implicated in the sequestration of some miRNAs whitin protein aggregates or indirectly across dysregulation of HDAC4 (Freischmidt et al., 2015; Gascon and Gao, 2014; King et al., 2014; Takanashi and Yamaguchi, 2014). Regarding to therapies, the reversible nature of epigenetic troubles detected in ALS marks theses changes as potential pharmacological target for ALS therapy development.

During the last 6 years, the discovery of *TARDBP*, *C9ORF72* and several other genes associated to the Mendelian inheritance of ALS has offered a valuable tool to postulate about putative cellular malfunctions that underlie the neurodegenerative process of motor neurons, from the dysregulation of energetic homeostasis (eg.: mitochondria) to the alterations of RNA metabolism, inability to cope with cellular waste (autophagy) or susceptibility to exogenous toxics. Most of these genetic variants determine the cellular vulnerability to certain types of stressors, and in particular the vulnerability of those cellular populations with high metabolic demands such as motor neurons. In some instances, other environmental or genetic (still unknown) factors will be needed to reach the vulnerability threshold that is necessary to initiate the neurodegenerative cascade (sporadic ALS), while in others (familial ALS) this combination of factors will determine the penetrance of a Mendelian inheritance, the clinical onset and the rate of disease progression.

3. Pathogenesis of ALS

Various cellular disturbances have been described in both forms of ALS, such as alterations in RNA proccessing, protein metabolism abnormalities, increased oxidative stress, defects in axonal transport, synaptic disorders and altered motor neuron environment (Robberecht and Philips 2013; Riancho et al., 2016), which are all associated to motor neuron degeneration. Among them, defects in protein metabolism (proteostasis), appear as particularly important. The chronic stress response at the endoplasmic reticulum (ER) represents one of the most proteostatic-disturbing conditions. The ER is essential for protein synthesis, including secreted, membrane-bound and lysosomal proteins, as well as for their subsequent folding and quality control (Hetz, 2012). Under ER stress conditions, cells activate the unfold protein response, which may have neurodegenerative effects depending on the intensity and nature of the stressor (Hetz and Mollereau, 2014). The main manifestations of the chronic ER stress are the fragmentation and loss of the ER cisterns (chromatolysis) and the accumulation of misfolded and aberrant proteins in cytoplasmic inclusions. These two events have been described in murine models as well as in ALS patients (Oyanagi et al., 2008; Sasaki 2010). Interestingly, under stress conditions, it has been reported that motor neurons reorganize their ER remnants at the perinuclear region and maintain their nucleolar activity as a transient mechanism to preserve the synthesis of the proteins critical for cell survival (Riancho et al., 2014). As it has been discussed above, the ubiquitylated cytoplasmic inclusions are a hallmark of the pathogenesis of ALS and highlight the failure of the proteolytic systems (Robberecht and Philips 2013). Within the cell, protein clearance is carried out by the ubiquitin proteasome system (UPS) and the autophagy-lysosome pathway (activated in situations in which the UPS is collapsed or for the degradation of very large and/or unfolded proteins). Autophagy is a physiological pathway in protein degradation that is tightly regulated, but an uncontrolled exaggerated autophagy may result extremely harmful for the cell. In fact, proteasomal collapse and uncontrolled autophagic responses have been described in some ALS murine models and in ALS patients (Bendotti et al., 2012; Robberecht and Philips, 2013). Remarkably, treatment with proteostatic modulators exerts neuroprotective effects in ALS experimental models (Riancho et al., 2015). It remains unclear whether the UPS or the autophagic machinery failure is at the heart of the protein degradation impairment, because there is an intense crosstalk between them. Some investigations in murine models suggested that neurons may have a greater sensitivity to UPS dysfunction than to authophagic failure, but this issue has not been fully elucidated yet (Kraft et al., 2010; Tashiro et al., 2012).

Intracellular inclusions in ALS have a heterogeneous composition. In fact, mutated or affected proteins, such as SOD1, TDP 43, and FUS, may have a prion-like behaviour, recruiting other proteins, RNAs and other cell essential components (Robberecht and Philips 2013). Interestingly, under stress conditions even wild-type (WT) SOD1 misfolds and tends to aggregate and display some prion-like properties (Rakhit et al., 2002; Ezzi et al., 2007). Thus, the prion-like hypothesis in ALS is gaining more importance in recent years. Several studies have demonstrated that some proteins, including SOD1, may be secreted to contiguous territories, thus spreading the disease to adjacent areas (Ayers et al., 2016; Pokrishevsky et al., 2016). In this line, Sabado et al. speculated that this prion-like mechanism could be responsible for the transsynaptic propagation of missfolded SOD1 from the motor neurons to other structures of the nervous system, such as to the dorsal root sensory neurons (Sabado et al., 2014). Overall, there is experimental evidence strongly suggesting that impaired proteostasis plays a role in the pathogenesis of ALS. The involved mechanisms are still incompletely understood, but appear to be complex and include both gain of function and loss of function mechanisms.

The involvement of the superoxide dismutase (*SOD1*) gene in some familial forms of ALS (Rosen et al., 1993) led to the hypothesis that ALS was due to an increase in the production of free radicals as a result of a loss of function in the activity of the antioxidant SOD1 enzyme. Reaume et al. (Reaume et al., 1996) demonstrated that motor neurons develop normally in SOD1–deficient mice but become more vulnerable in physiological-stress situations, for instance following an axonal injury. However as the neuropathology of the SOD1 model is different from that observed in both sporadic cases and familial cases due to mutations in *FUS*, *TARDP43* or *C9ORF72*, the hypothesis of a disturbance in oxidative metabolism was becoming less attractive until recently. In fact, despite the controversy about common mechanisms in *SOD1*-related familiar and sporadic ALS, several SOD1 mutations related to familial ALS showed typical inclusions (Sumi et al., 2009; Okamoto et al., 2011) and even SOD1 mice model showed also TDP-43 inclusions if the pathological study is performed at the end-stages. Moreover, the observations that WT SOD1 is missfolded and exibits prion-like properties in sporadic ALS (Forsberg et al., 2010) have been used by some authors to argue in favor of SOD1 as common pathogenic mechanism in both forms of ALS, claiming that WT SOD1 could acquire a toxic conformation of mutated SOD1 through oxidative damage (Ezzi et al., 2007; Bosco et al., 2010; (Rotunno and Bosco, 2013).

Neuroinflammation is a common pathological trait in most of neurodegenerative diseases which is currently viewed more as a trigger than a consequence of neuronal damage (Hooten et al., 2015). Both microglia, the CNS-resident macrophages, and astrocytes are activated in the presence of misfolded protein aggregates and secrete reactive oxygen species and pro-inflammatory cytokines; TNF-α, IL1β and IL-6 (Puentes et al., 2016). In 1993, microglial activation was associated with infiltration of helper Th cells and cytotoxic Tc cells in spinal cord and motor cortex of ALS patients (Appel et al., 1993). These Th helper cells reactivate astrocytes and microlgia towards a M1 phenotype and thereby

turning them from neuroprotective to neurotoxic (Philips and Robberecht, 2011). Furthermore, low levels of Treg cells, which are associated with priming the neuroprotective microglial M2 phenotype, have been found in blood samples from ALS patients and mouse models correlating with disease progression (Henkel et al., 2013; Hooten et al., 2015), suggesting that physiological immune response against pathogens may have been likely deregulated and become pathological in ALS.

A defect in glutamate signaling has been attributed to a deficit in the glial transporter of this amino acid (EAAT2/GLT1) (Rothstein et al., 1995), which leads to an increase of glutamate levels in the cerebrospinal fluid (CSF). Importantly, overexpression of EAAT2/GLT-1 in ALS mice have delayed disease onset and prolonged survival times. Althogh preclinal studies suggested that pharmacological targets that upregulate EAAT2 may be neuroprotective in ALS, a multi-stage, randomised, double-blind, placebo-controlled trial at stage 3 reported that ceftriaxone, a FDA–approved beta-lactam antibiotic that significantly increases EAAT2 activity and GLT-1 expression in rodent brains (Rothstein et al., 2005), failed to show any positive clinical outcome in ALS patients (Cudkowicz et al., 2014). Some authors have claimed that deficits in EAAT2/GLT-1 may be secondary to other primary disorders related to neuronal stress (Redler et al., 2011)

3.1. TDP-43: common pathological endpoint in ALS

Currently, ALS is considered as a TDP-43 proteinopathy and the accumulation of this protein is thought to be critical in the neurodegenerative process, rather than being simply markers of the disease (Al-Sarraj et al., 2011; Ince et al., 2011). Taking this into account, there is a common pathological link between sporadic and inherited forms. Except from familiar *SOD1* and *FUS* forms, the rest of ALS forms share a characteristic protein deposition made of TDP-43 with p62 and ubiquitin colocalization (Blokhuis et al., 2013), which may be found mainly in spinal cord but also in hippocampal and cerebellar regions, although in different locations.

The typical neuropathological features of ALS are the degeneration and loss of motor neurons with increased astrocytic proliferation and the formation of intraglial and intraneuronal inclusions. Protein aggregates and intracytoplasmic inclusions are the hallmark of many neurodegenerative diseases. In ALS, the inclusions are ubiquitin-positive and are preferentially formed in the spinal motor neurons. The discovery of genes involved in familial forms of ALS has revealed that proteins encoded by these genes are an essential component of these inclusions (Shibata et al., 1996; Neumann et al., 2006; Deng et al., 2010; Mackenzie et al., 2010, 2007). However, these proteins aggregates may be developed despite any pathogenic mutations. TDP-43 inclusions are present until 80% of ALS patients with different severity range (Coand and Mitchell, 2015). Some patients suffering from clinical pictures combining FTD, progressive supranuclear palsy, corticobasal syndrome and motor neuron disease present globular oligodendroglial and astroglial inclusions (GOI and GAI) positive for 4R Tauand predominantly negative for Gallyas silver staining (Piao et al., 2005; Josephs et al., 2006; Fu et al., 2010; Ahmed et al., 2011). Depending on the severity and distribution of these globular inclusions, subtypes II and III are associated with motor neuron disease (Ahmed et al., 2013).

The mechanisms that lead to TDP-43 mislocalization and accumulation into the cytoplasm of motor neurons in ALS are not fully understood yet. It is important to know that this protein autoregulates itself through a negative feedback loop mechanism in which TDP 43 binds to the 3′ UTR region of its own mRNA in order to induce its degradation (Ayala et al., 2011, Igaz et al., 2011). Indeed, it has been observed that ALS and FTLD/ALS patients with mutations in this 3′ UTR region show overexpressed levels of TDP-43. The dysregulated production of TDP-43 might be the cause for its accumulation in the cytoplasm and loss of transport to the nucleus (Neumann et al., 2006; Gitcho et al., 2009). Yet, elevated levels of WT or mutated TDP-43 do not always give rise to insoluble cytoplasmatic inclusions, as has been found in several organs other than brain and spinal cord, suggesting that other altered mechanisms are necessary. For example, TDP-43 aggregates could be promoted by defective function of cell autophagic system, in which the proteasome fails to degrade ubiquitinated TDP-43.

Involvement of *UBQLN2* and *SQSTM1* in familiar forms of ALS suggest that autophagy may also be implicated in the pathogenesis of ALS and FTD (Fecto et al., 2011; Fecto and Siddique, 2012; Rubino et al., 2012). SQSTM1 encodes a protein, p62, which is present in ubiquitin-positive inclusions in various neurodegenerative diseases, co-localizing with TPD-43 and FUS in the motor neurons of patients with sporadic ALS.

Other causative and susceptibility genes associated to ALS codify for proteins that have important functions in lysosomal transport (*PGRN, TMEM106B*) or unfolded protein response (*VAPB*), all of them components of the autophagy machinery. Other alterations may be related with oxidative stress that results in TDP-43 lysine acetylation (Cohen et al., 2015) or TDP-43 phosphorylation by casein kinase Ie (CKIe) (Choksi et al., 2014), which imped its nuclear transport.

It still remains a debate whether the harmful effects of TDP-43 are due to the cytoplasmatic inclusions, the loss of its nuclear localization, or both. Although it is difficult to distinguish the particular contribution of each phenomena, a recent study has proved that removal of cytoplasmic TDP-43 and the concomitant return of nuclear TDP-43 leads to neuron preservation, muscle reinnervation and functional recovery in new mouse model that reflect accumulation of insoluble, phosphorylated cytoplasmic TDP-43 in brain and spinal cord (Walker et al., 2015), suggesting that ALS pathology could be caused by loss of nuclear functional TDP-43. The loss of nuclear TDP-43 as the degenerative event in motor neurons has been supported by studies with heterozigotic ablation or post-natal deletion of TDP-43, where deficient levels of TDP-43 in the nuclei of neurons despite no accumulation in the cytoplasm is sufficient to induce loss of motor neurons and motor symptoms (Iguchi et al., 2013).

3.2. C9ORF72: frequent pathogenic mechanism in ALS

The hexanucleotide GGGGCC repeat expansion (HRE) in *C9ORF72* is the most common cause of familial ALS and also contributes to the majority of sporadic forms of ALS. This HRE is not restricted to ALS since it is as well a common cause of FTLD and ALS-FTLD, and has been identified in other non-motor diseases as Alzheimer's disease and Lewy body dementia as modifiers of clinical phenotype (Cooper-Knock et al., 2014). The bulk of works during the last 2 years have made such an enormous contribution to the understanding of the toxicity mechanisms of *C9ORF72* mutation to motor neurons that the involvement of *C9ORF72* in the pathogenesis of ALS merits special attention in this review.

Although the repeat size among patients, or even among different tissues within the same individual, shows a heterogeneous pattern, and the correlation between the number of repeats and the severity of clinical phenotypes is not clear, it has been demonstrated that carriers of *C9ORF72* expansions have a higher incidence of ALS, in particular the bulbar onset ALS (Debray et al., 2013; Ratti et al., 2012 Ratti et al., 2012). It was first reported that expansions cause haploinsufficiency of *C9ORF72*. Despite the function of the C9orf72 protein transcript is not known, some evidence pointed out that C9orf72 plays a role in endosomal trafficking, and therefore its depletion resulted in impaired endocytosis and autophagy-mediated trafficking, which suggested

C9orf72 downregulation as a putative pathogenic mechanism (Farg et al., 2014). Two further studies in *C9ORF72*-deficient mouse lines have unveiled that C9orf72 expression is crucial for the regulation of immune and autoimmune responses in macrophages and microglia (Atanasio et al., 2016; O'Rourke et al., 2016). Although one of the studies reported age-related neuroinflammatory processes that are similar to those observed in *C9ORF72* familial ALS (O'Rourke et al., 2016), none of these studies found motor neuron disease, which is in accordance with previous works that did not observe motor neuron degeneration or motor symptoms in primary neurons or mice lacking *C9ORF72* (Wen et al., 2014; Koppers et al., 2015). Further studies are needed to confirm whether C9orf72 haploinsufficiency is a pathogenic factor in *C9ORF72* ALS. Instead, the own nucleotid repeat structure has emerged as the entity that triggers the molecular cascade of disease. In this sense, Haeusler et al. (Haeusler et al., 2014) found for the first time in cells from ALS patients that *C9ORF72* HRE form DNA and RNA G-quadruplexes with distinct structures promoting RNA·DNA hybrids, which are able to bind ribonucleoproteins and induce nuecleolar stress. Rossi et al. (2015) implemented these findings by showing that accumulation of these repeat expansions into nuclear RNA foci affected the efficiency of cytoplasmic translation machinery as a result of impaired nuclear RNA export. Further studies demonstrated that these HREs, beyong forming G-quadruplexes, induced the expression of dipeptide repeat (DPR) proteins generated by an unconventional repeat-associated non-ATG translation. Indeed, DPR proteins of glycine-alanine (poly-GA), glycine-proline (poly-GP), glycine-arginine (poly-GR), proline-arginine (poly-PR) and proline-alanine (poly-PA) have been found deposited in ALS brains and have arised as the principal neurotoxic component of HRE. In particular, experiments in mammalian primary neurons and in Drosophila have evidenced that the expression of poly-GA proteins form soluble and insoluble p62-positive cytoplasmic inclusions, bind nucleoli, impair RNA biogenesis, inhibit proteasome activity and induce endoplasmic reticulum stress and cause neurodegeneration (Yamakawa et al., 2015; Zhang et al., 2014; May et al., 2014; Mizielinska et al., 2014; Kwon et al., 2014). Other studies have also reported that poly-GR and poly-PR are cytotoxic by aggregating in nucleoli and inducing global translational dysregulation, nucleolar stress and, eventually, cell death (Wen et al., 2014; Tao et al., 2015; Kanekura et al., 2016). Recent findings by three independent and parallel works have provided deeper insights into the mechanistic action of *C9ORF72* toxicity by showing that *C9ORF72*-associated HRE and DPR proteins disrupt nucleocytoplasmic transport: both nuclear protein import and RNA export (Freibaum et al., 2015; Jovicic et al., 2015; Zhang et al., 2015). These and posterior works (Boeynaems et al., 2016) identified several members of the nuclear transport machinery, including nuclear pore components and importins/exportins such as karyopherins and Ran-GTP cycle regulators, as key player mediating the nucleocytoplasmic transport defects associated to the pathogenic *C9ORF72* mutation.

The fact that DPR proteins are abundant in cerebellum and frontotemporal lobe of ALS patients, but are rare in spinal cord, might explain the reason why *C9ORF72* expansions do not contribute to lower motor degeneration but are instead associated with the incidence of the bulbar onset ALS (Gómez-Deza et al., 2015; Schludi et al., 2015).

The link between *C9ORF72* and TDP-43 pathology has also been studied. The presence of the antisense foci for the repeat expansion of *C9ORF72* is correlated with the mislocalization and accumulation of TDP-43 in cerebellar neurons of ALS patients (Cooper-Knock et al., 2015), and cytoplasmatic TDP-43 inclusions were found in skin cells derived from pre-symptomatic *C9ORF72*-linked ALS patients carrying the HRE (Pare et al., 2015). On the other hand, the expression of *C9ORF72* HRE in mouse brains caused TDP-43 pathology, as well as DPR protein inclusions, neuronal loss and behavioural dificits (Chew et al., 2015). Contrarily, a previous transgenic mouse for *C9ORF72*-associated HRE showed ubiquitin-positive inclusions but failed to recapitulate TDP-43 and DPR pathology (Hukema et al., 2014).

Although the pathogenic molecular pathways of *C9ORF72* are being deciphered in detail, there is still a lot of work to do in order to uncover the mechanisms that control the expansion length and to understand how environmental factors could regulate the pathogenic nature of this *C9ORF72* expansions. In this sense, it is known that the pathological contribution of *C9ORF72* repeat expansions are strongly regulated by epigenetic methylation (Xi et al., 2014, 2015; Russ et al., 2015), in such a way that hyper-methylated HRE is associated with reduced accumulation of RNA foci and DPR proteins (Liu et al., 2014). In addition, it is important to note that two different studies have failed to find neurodegeneration and motor/behavioural abnormalities in transgenic mice carrying disease *C9ORF72* HRE although reporting accumulation of DPRs and nuclear RNA foci (O'Rourke et al., 2015; Peters et al., 2015), suggesting that *C9ORF72*-associated pathology occur presimptomatically and need a second pathological hit to induce the neurodegenerative process.

4. ALS as a metabolic disease

Metabolic dysfunction in ALS was reported many years ago and since this early reports (Reyes et al., 1984; Van den Bergh et al., 1977), numerous studies suggest that ALS patients may have an impairment in the energy homeostasis and this imbalance has a negatively influence in the rate of progression of disease and survival expectative (Dupuis et al., 2004, 2011; Desport et al., 1999, 2001, 2005; Limousin et al., 2010; Gallo et al., 2013; O'Reilly et al., 2013; Shimizu et al., 2012). Other evidences come from mice expressing SOD1(G86R) or SOD1(G93A) mutations, which present a hypermetabolic state and defects in glucose metabolism (Dupuis et al., 2004; Palamiuc et al., 2015).

4.1. Evidences of impaired metabolic homeostasis in ALS

It is known that ALS patients exhibit a metabolic dysfunction that leads to a loss of lean weight and subcutaneous fat mass (Lim et al., 2012).

In 2001, a clinical study proposed that ALS is a metabolic disorder after the observation that ALS patients are hypercatabolic (Desport et al., 2001), a condition that was further demonstrated to be the result of increased systemic energy expenditure at rest (Funalot et al., 2009). However, the origin of the failure in the energy homeostasis that drives to this increased systemic energy expenditure observed in ALS patients is unknown, and might represent a key etiopathogenic mechanism. In fact, there are evidences of impaired glucose utilization and deficits in the β-oxidation of fatty acids (FAβO) in ALS patients (Pradat et al., 2010; Sanjak et al., 1987). However, impaired glucose tolerance could be explained as a secondary event to the disease due to muscle atrophy (Reyes et al., 1984). Although longitudinal studies have associated type 2 diabetes with lower risk for ALS (Lekoubou et al., 2014), possibly due to the neuroprotective effects of antidiabetic drugs (eg., pioglitazone) and higher fat mass in those patients, type 1 diabetes have found instead to triple the risk for ALS (Mariosa et al., 2015), suggesting that impaired glucose utilization is an etiopathogenic factor of ALS by contributing to the impaired energy homeostasis. The impairment of fatty acids (FA) utilization and its involvement in ALS pathogeny has been documented as well by one study that demonstrated ketogenic deficits (FAβO is an important source for ketone bodies production) in ALS patients during exercise (Sanjak et al., 1987), and one

recent clinical trial with L-carnitine (necessary for mitochondrial FAβO) that could improve survival for ALS (Beghi, 2013). When FA metabolism fails, FAs tend to accumulate into lipid droplets in the liver causing fatty liver. In agreement with this, clinical studies suggest that hepatic steatosis (non-alcoholic fatty liver degeneration) is a common and unique phenomenon in ALS compared with other neurodegenerative diseases (Dupuis et al., 2008), and, indeed, up to 76% of ALS patients were diagnosed with fatty liver by abdominal sonography in a small Japanese cohort (Nodera et al., 2015). Hepatic steatosis is linked to metabolic syndrome, characterized by insulin resistance and abnormal levels of plasma glucose, glucagon and triglycerides, which are also altered in ALS (Moriwaka et al., 1993; Dodge et al., 2013; Goto et al., 1972; Den Boer et al., 2004). Glucagon secretion is a potent inductor of amino acid release from muscular proteins that are used for energy production, a process that generates high levels of ammonia. Plasma levels of glucagon have been associated with ALS progression (Ngo et al., 2015) and high levels of amino acids and ammonia have been found in patients with motor neuron disease (Patten et al., 1982).

Recently an extensive populational prospective survey conducted in Netherlands over 674 incident ALS cases included during a period of 5 years, showed that ALS appeared associated with a higher recalled rate of dietary fat intake, and lower presymptom body mass index compared to controls, suggesting a basal hypermetabolic state (Huisman et al., 2015). Taken together these all data there is growing evidence that at least a fraction of ALS cases have some type of underlying defect in metabolism that generate or a defect in the energy supply and/or an increased production of metabolic rubbish that some individuals are unable to process properly.

4.2. TDP-43 and other genes linked to ALS are regulators of energetic metabolism

The fact that some of the most pathogenic ALS genes have important roles in the regulation of metabolism supports the notion that metabolic dysfunction occurs early in the pathophysiology of ALS. In particular, multiple studies have evidenced the participation *TARDBP*, but also *SOD1* and other genes, in glucose and fatty acid metabolism.

TARDBP has a pivotal role in glucose and lipid metabolism (Stallings et al., 2013). In this sense, it has been observed that postnatal depletion of *TARDBP* in mice leads to dramatic loss of body fat followed by rapid death and results in reduced levels of protein Tbc1d1 (Chiang et al., 2010). Tbc1d1 is a Rab GTPase-activating protein that regulates glucose uptake, lipolysis and insulin sensibity (Hargett et al., 2015). Conversely, the over-expression of WT or A315T mutated TDP-43 in the CNS results in weigth gain, increased fat mass and obesity-induced insulin resistance (Stallings et al., 2013), whereas when mutant A315T TDP-43 is expressed under the control of its own promoter, and therefore its expression is not confined to CNS but other peripheral tissues, mice undergo a 10% loss of body fat mass, deficits in FA metabolism and mithocondrial dysfunction (Stribl et al., 2014). Little is known about the molecular mechanisms behind the metabolic regulation exerted by TDP-43. Recent studies have demonstrated that TDP-43 is particularly related with AMP-activated protein kinase (AMPK) activation and therefore might play an important role in muscle glucose uptake during exercise. It has been shown that mutated TDP-43 A315T mice present reduced AMPK activity in motorneural cells (Perera et al., 2014) while over-expression of wild type TDP-43 increased AMPK activity in spinal cord (Liu et al., 2015). Other studies have demonstrated that TDP-43 interacts with the long non-coding RNA lncLSTR, which is a liver-specific triglyceride regulator involved in lipid clearance by liver (Li et al., 2015), and also appears to participate in the mRNA splicing of ADIPOR2 gene, a receptor that mediates fatty acid oxidation and glucose uptake by adiponectin (Ling et al., 2015).

Multiple experimental works in mutated or SOD1 knockout mice have evidenced that SOD1 is also essential to maintain glucose and lipid homeostasis. One study found that the deletion of SOD1 led to a decreased liver glucose production and glycogen storage, and elevated hepatic lipid profiles in adult mice (Wang et al., 2012). The absence of SOD1 was further associated with the development of impaired glucose tolerance and in an impaired β-cell function and volume (Muscogiuri et al., 2013). The deletion of SOD1 also inhibits triglyceride secretion to the blood and leads to dysfunctional lipid accumulation in mouse enterocytes upon a high-fat diet (Kurahashi et al., 2012) and in hepatocytes (Lee et al., 2015). The absence of SOD1 also leads to reduced levels of plasma triglycerides, increased levels of the lipolytic hormone glucagon, and decreased fat mass (Dodge et al., 2013). In addition, the overexpression of mutated SOD1(G93A) in mice showed impaired insulin-mediated glucose uptake in skeletal muscle (Smittkamp et al., 2014), reduced fat mass and impaired lipid metabolism (Fergani et al., 2011). It is important to note that antidiabetic drugs have shown potent effects on pathological and clinical progression of motor neuron disease in the SOD1(G93A) mouse (reviewed below). Moreover, *FUS* mutations in culture cells are linked to a decreased in levels of adenosine triphosphate (ATP) (Wang et al., 2015) and patients with *C9ORF72* repeat expansion present reduced used of glucose in several brain regions (Cistaro et al., 2014).

Besides the relationship between *SOD1* and *TDP-43* with energetic metabolism, other genes related with ALS, such as *ERBB4* and *SQSTM1*, are invoved in glucose uptake by muscle cells. In particular, neuregulin-ERBB4 mediates the glucose uptake during muscle contraction induced by calcium (Gumà et al., 2010; Takahashi et al., 2013). In addition, *p62/SQSTM1* regulates the insulin signaling pathway by interacting with insulin receptor substrate-1 (IRS-1). Over-expression of p62 improves insulin signaling and favors GLUT4-mediated glucose uptake (Geetha et al., 2012), while deletion of p62 impairs insulin sensivity (Rodriguez et al., 2006).

All this findings provide evidence that defects in different ALS genes might converge in a common metabolic dysfunction, but still further investigations are necesary to unveil this metabolic paradigm in ALS keeping in mind environmental risk factors and genetic susceptibility.

Based on the whole aforementioned evidence about metabolic dysfunction in ALS, an hypothetical scenario linking energetic metabolism with motor neuron degeneration can be proposed, where particular interactions between genetic and environmental factors drive to an impaired systemic energy homeostasis that eventually leads to a maladaptative hypercatabolic remodeling and the production of metabotoxic intermediates, which could be eventually detrimental to motor neurons (Fig. 2).

4.3. Therapeutic approaches for ALS based on metabolic modulation

4.3.1. Nutritional interventions

The benefits of nutritional interventions consisting of specific diets containing metabolic substitutes of glucose have been under study. For instance, experimental study in the SOD1(G93A) mouse model showed that ketone bodies- or medium chain triglycerides (such as caprylic acid)-enriched diets, which serve as an alternate energy substrate for neuronal metabolism, slowed symptomatic progression (weakness) and lower the mortality rate (Zhao et al., 2012; Zhao et al., 2006). In addition, dietary supplements including arginine, GABA, alpha-ketoglutarate and ketone bodies have also demonstrated significantly extended survival time and increased

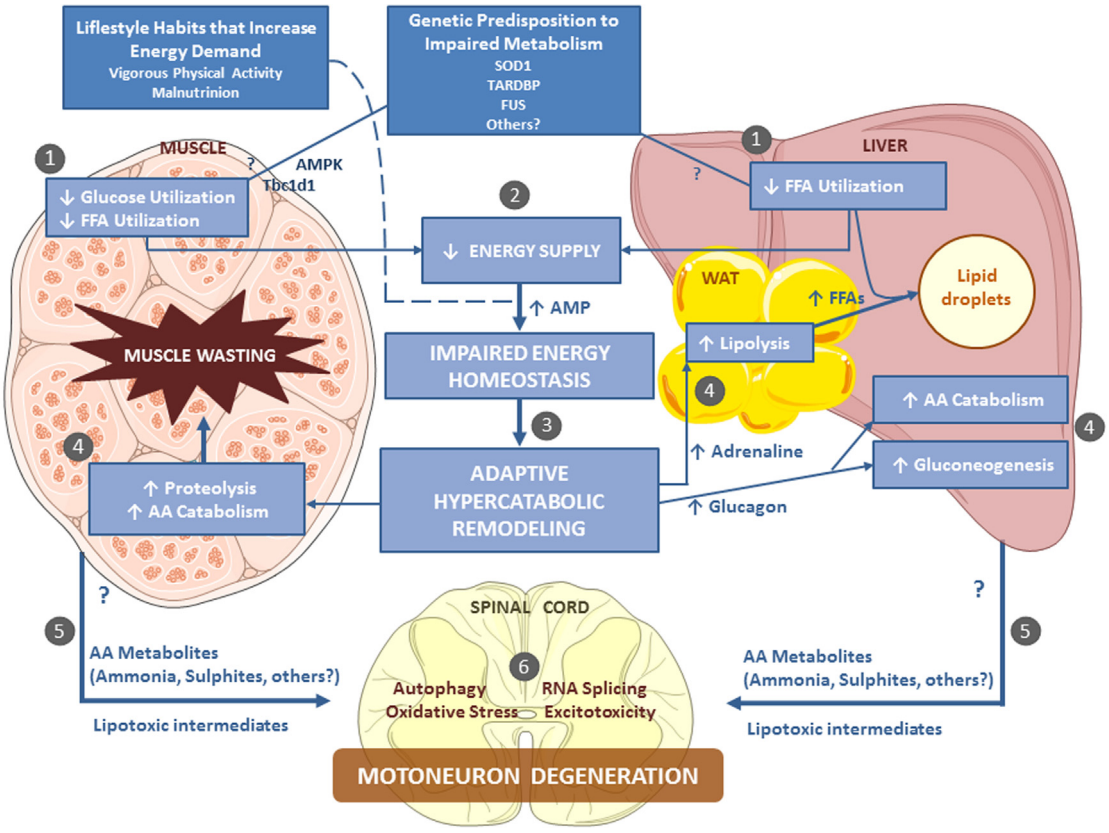

Fig. 2. Hypothetical scenario linking systemic failure of energy homeostasis with motor neuron degeneration in ALS. Mutations in such ALS genes as *TARDBP*, *SOD1* and *FUS* have been associated with decreased glucose and FFA utilization by muscle cells through a mechanism that is likely dependent on Tbc1d1 and AMPK (1). Decreased FFA utilization by liver has also been associated with these mutations, although the mechanistic link is unknown (1). As glucose and FFA are the major fuels to the muscle, one the most energy-consuming organs, the chronification of this situation of low energy supply, together or not with high energy-demand life-style habits (2), will eventually end up in a systemic state of impaired energy homeostasis. In order to cope with this energetic deficit the organism suffers an adaptation towards an hypercatabolic remodeling (3). Signs of hypercatabolism have been observed in ALS patients, as discussed in this review. Some of the mediators of the hipercatabolic remodeling are specific hormones as glucagon, released by the pancreas, and adrenaline, secreted by adrenal glands. Glucagon and adrenaline has been found increased in blood from ALS patients. Adrenaline switchs on pathways in the muscle to break proteins (proteolysis) and use resulting amino acids as energy fuels (4). Adrenaline also activates proteolysis in liver in order to utilize carbons derived from amino acids to build up glucose (gluconeogenesis) (4). Glucagon induces lipolysis in adipocytes in order to movilize triglicerydes from lipid stores to liver and muscle (4). As lipid utilization might be compromised in ALS, triglicerydes may accumulate in the liver into droplets and develop fatty liver. It has been evidenced that hepatic steatosis is a common feature in ALS patients. Perhaps, the impaired utilization of FFAs by liver might drive to the activation of compensatory routes to utilize FFAs, as peroxisomal or microsomal oxidation. As a result of this hypercatabolic shift, which in a complex disease as ALS might mean overuse of amino acids and alternative routes to burn FFAs by liver and muscles, might cause the accumulation of metabolic intermediates (ammonia, sulphites, ketoacids, lipotoxic intermediates. . .). Eventually, the chronic exposure to this metabotoxic intermediates to the CNS might be detrimental to motor neurons and eventually trigger ALS (5). The lattest step is unexplored but its study deserves attention as it can bring new insigths into the etiology of ALS. AA, aminoacid; FFA, free fatty acid; WAT, white adipose tissue.

motor function in the same animal model (Ari et al., 2014). In another study, oral administration of L-carnitine prior to disease onset has also showed therapeutic potential in the SOD1(G93A) mouse, in terms of delayed onset of symptoms, less deterioration of motor activity and extended life span (Kira et al., 2006).

Iron is a redox-active transition metal involved in a variety of biological events, but most particularly in oxygen transport and energy metabolism. Defects in its homeostasis can lead to increased reactive free radicals and neurodegeneration (Singh et al., 2014). The observations of iron accumulation in CSF and spinal cords of ALS patients and ALS mouse model have suggested a putative toxic effect of iron in motor neurons (Kasarskis et al., 1995; Winkler et al., 2014). In this sense, a treatment with a monoamine oxidase (MAO) inhibitor/iron-chelating compound in combination with a caloric supplemented diet induced the expression of genes involved in mitochondrial biogenesis and metabolic regulation and showed potent therapeutic effects in the SOD1(G93A) transgenic mouse in terms of neuroprotection and increased survival (Golko-Perez et al., 2016).

Nutritional interventions have been also trialed in patients. For instance, the effects of acetyl L-carnityne as diet supplement on disability and mortality of ALS patients was studied in a in a double- blind, placebo controlled, pilot trial (Beghi, 2013). This study reported that acetyl L-carnityne was well tolerated and safe, and resulted in an increased median survival. A more recent double-blind, placebo-controlled, randomised phase 2 clinical trial have compared the effects of two hypercaloric enteral nutrition interventions, a high-carbohydrate tube-fed and high-fat tube-fed diet, with respect to a control isocarolic tube-fed diet on safety, tolerability, survival and disease progression in ALS patients with no history of metabolic disease (Wills et al., 2014). Patients under high-carbohydrate diet presented less serious adverse events and incrased survival rate (no death) after a 5 months follow-up when compared to those that received high-fat or isocaloric diets. These studies provide promising data that support the importance of modulating the sources of energetic metabolism by nutritional interventions in ALS patients to slow the disease progression; however larger randomised controlled trials with longer follow-up times at

different stages of the disease are needed to strengthten these prelimary outcomes.

4.3.2. Antidiabetic drugs

Thiazolidinediones (TZDs) are a class of antidiabetic medications that improve the metabolic control by decreasing plasma free fatty acid concentrations and fasting hyperglycemia via PPAR gamma activation and insulin sensitivity (Desvergne et al., 2006). Oral treatment with the TZD pioglitazone showed potent beneficial effects in the SOD1(G93A) transgenic mice at both the pathological level (attenuated motor neuron loss, reduced gliosis and preserved muscle fiber diameter) and the clinical manifestations (improved motor performance, delayed weight loss and disease onset, and extended survival) (Kiaei et al., 2005; Schutz et al., 2005a, 2005b). The positive outcomes of pioglitazone in experimental models stimulated the initiation of trials in humans. Nevertheless, neither a pilot trial nor a randomised, double blind, placebo-controlled trial of pioglitazone in combination with riluzole in ALS patients demonstrated benefits on disease progression and survival rate (Dupuis et al., 2012; Levine et al., 2012). A more recent work have revealed that pioglitazone is effective in treating motor neuron degeneration and locomotor dysfunction in TDP-43 and FUS but not in SOD1 models of ALS in Drosophila, but does not increase lifespan in none of this models, which is consistent with clinical trials (Joardar et al., 2015). It was argued that positive results achieved in the SOD1(G93A) mouse were due to the anti-inflammatory properties of pioglitazone (Shibata et al., 2008; Shibata et al., 2010), while this anti-inflammatory effects could have been partly counteracted by the resulting decrease in energy substrates for motor neurons and aggravation of ALS-related metabolic dysfunction in patients (Jawaid et al., 2014). It is also plausible to speculate that pioglitazone could target metabolic mechanisms that are specific of SOD1 models and perhaps SOD1 patients, but are different to those present in other forms of familial and sporadic ALS. A pilot trial of pioglitazone in familial SOD1 patients, although hard to design due to the low frequency of SOD1 mutations, would respond the latter question.

The antidiabetic drug metmorfin, a small molecule activator of the metabolic regulator AMPK that exerts its therapeutical action by reducing hepatic glucose production, improving insulin sensitivity and fighting both inflammatory and oxidative damage, was tested in SOD1(G93A) mice. However, oral administration of metformin did not only show lack of effects on disease onset, progression or survival in male transgenic mice but also negative effects on females (Kaneb et al., 2011) Again, the fact that metmorfin might target a specific metabolic mechanism that is not related to SOD1 mutation is a possible explanation for the reported lack of effect. Contrarily, preconditioning of SOD1(G93A) mice with latreperidin (another AMPK activator, although not used as antidiabetic drug) did show delayed onset of symptoms and significant increase in life span (Coughlan et al., 2015). However, whether latreperidim positive effects are dependent on the activation of AMPK or through other reported mechanisms of action, such as antihistaminic, inhibition of L-type calcium channels or modulation of glutamate and serotonin receptors, is unknown.

Mimetics of the incretin hormone glucagon-like peptide-1 (GLP-1) are currently being used as antidiabetic drugs for their effects in facilitating insulin signaling and decreasing glucagon secretion. The GLP-1 receptor agonist exendin-4 demonstrated improved glucose tolerance, increased locomotor activity and attenuated cell death when administered subcutaneously in SOD1(G93A) mice, and also showed neuroprotection against several insults when used in a motor neuron cell line over-expressing SOD1 mutants (Li et al., 2012).

A much deeper understanding on the specific cellular and molecular mechanisms that drive to the systemic dysregulation of metabolic homeostasis observed in different forms of ALS is needed in order to unveil potential targets that might be adequately treated by some of the existing classes of antidiabetic drugs, such as biguanides, thiazolidinediones, sulfonylureas, meglitinides, alpha-glucosidase inhibitors, incretin mimetics, amylin analogues and glycosurics. In addition, the use of newly generated animals models of ALS based in *TARDBP*, *FUS* or *C9ORF72* mutations should be seriously considered when designing preclinical trials as a way to scape from promising overestimated outcomes that could only be relevant to a rare pathogenic cascade of ALS, as the one initiated by SOD1 mutations.

5. The future

The findings accumulated over the last 10 years have revolutionized our understanding of ALS, positioning it between the central and peripheral neurodegeneration. After seeing the large list of implicated genes, it seems clear that the maintenance of the motor neuron depends on many and different molecular structures. Almost all of them orientate to a basic underlying problem in the processing of RNAs, linking ALS with other neurodegenerative proteinopathies. However, we have to consider that after repeated and extensive genetic studies in different populations, most ALS cases remain sporadic and most of them present TDP-43 proteinopathy. It is important to keep in mind that more than two decades of fruitless efforts to find a cure in neurodegenerative proteinopathies such as Alzheimer's or Parkinson's diseases have been focused on unraveling the mechanisms of protein accumulation and designing therapies against these protein aggregates. Recent clinical observation and experimental discoveries have instead pointed out that proteinopathy in these disorders is just one entity of a whole pathological process, which has its origin from complex interactions of multiple pathogenic hits occurring within the CNS or even outside it, and resulting from genetic, epigenetic and environmental factors. Lessons learnt from other neurodegenerative disorders must be applied to the field of ALS research in order to design integrative studies that analyze multiple factors from different perspectives.

Two promising lines of research emerge from different epidemiological studies: First, the one that tries to identify ethiological agents and metabolic pathways involved in the geographical cluster of ALS cases presumably linked to the consumption of animal toxins or exposure to environmental toxics; Second, the associations of low-caloric diets and body mass index profile as well as the intensive exercise on the risk of suffering from ALS or their influence on disease progression. Future experimental designs that integrate both lines together would merit consideration.

Given that one of the main difficulties in developing new therapies lies on the multiple events that contribute to motor neuron death in ALS, any intervention aimed at solely reducing any particular pathological condition might not be sufficient to treat the disease and its symptoms. Instead, studies aimed to integrating the current/future knowledge on ALS pathogenesis and tailor interventions that target multiple pathogenic mechanisms will bring future therapeutic success for this multi-factorial and heterogeneous disease.

Author contributions

Zufiría M, Gil-Bea FJ and Fernández-Torrón R equally contributed as first and main authors, making special contributions in the design of the review, bibliographic data collection and interpretation, and drafting most of the content of the article; Poza JJ, Muñoz-

Blanco JL and Rojas-García R made critical revisions related to the content of the manuscript; Riancho J made critical revisions related to the content of the manuscript and reviewed some sections of the manuscript; López de Munain A made the conception of the article, reviewed the structure, made critical revisions of the whole content of the manuscript and gave the final approval before submission.

Conflict of interest

None.

Search criteria

We searched PubMed with the terms "motor neuron disease" and "amyotrophic lateral sclerosis". The search was limited to full-length articles (original, reviews and reports) in peer-reviewed journals published in English or Spanish. We searched the reference lists of retrieved papers to identify further articles. Some identified papers were not included in the reference list owing to restrictions on space and focus.

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References

Abhinav, K., Al Chalabi, A., Hortobagyi, T., Leigh, P.N., 2007. Electrical injury and amyotrophic lateral sclerosis: a systematic review of the literature. J. Neurol. Neurosurg. Psychiatry 78, 450–453.

Abramzon, Y., Johnson, J.O., Scholz, S.W., Taylor, J.P., Brunetti, M., Calvo, A., Mandrioli, J., Benatar, M., Mora, G., Restagno, G., Chio, A., Traynor, B.J., 2012. Valosin-containing protein (VCP) mutations in sporadic amyotrophic lateral sclerosis. Neurobiol. Aging 33 (9), 2231.

Adams, C.R., Ziegler, D.K., Lin, J.T., 1983. Mercury intoxication simulating amyotrophic lateral sclerosis. JAMA 250, 642–643.

Ahmed, Z., Doherty, K.M., Silveira-Moriyama, L., Bandopadhyay, R., Lashley, T., Mamais, A., Hondhamuni, G., Wray, S., Newcombe, J., O'Sullivan, S.S., Wroe, S., de Silva, R., Holton, J.L., Lees, A.J., Revesz, T., 2011. Globular glial tauopathies (GGT) presenting with motor neuron disease or frontotemporal dementia: an emerging group of 4-repeat tauopathies. Acta Neuropathol. 122, 415–428.

Ahmed, Z., Bigio, E.H., Budka, H., Dickson, D.W., Ferrer, I., Ghetti, B., Giaccone, G., Hatanpaa, K.J., Holton, J.L., Josephs, K.A., Powers, J., Spina, S., Takahashi, H., White 3rd, C.L., Revesz, T., Kovacs, G.G., 2013. Globular glial tauopathies (GGT): consensus recommendations. Acta Neuropathol. 126 (4), 537–544.

Al-Chalabi, A., Hardiman, O., 2013. The epidemiology of ALS: a conspiracy of genes, environment and time. Nat. Rev. Neurol. 9, 617–628.

Al-Chalabi, A., Andersen, P.M., Nilsson, P., Chioza, B., Andersson, J.L., Russ, C., Shaw, C.E., Powell, J.F., Leigh, P.N., 1999. Deletions of the heavy neurofilament subunit tail in amyotrophic lateral sclerosis. Hum. Mol. Genet. 8, 157–164.

Al-Chalabi, A., Fang, F., Hanby, M.F., Leigh, P.N., Shaw, C.E., Ye, W., Rijsdijk, F., 2010. An estimate of amyotrophic lateral sclerosis heritability using twin data. J. Neurol. Neurosurg. Psychiatry 81, 1324–1326.

Al-Chalabi, A., Calvo, A., Chio, A., Colville, S., Ellis, C.M., Hardiman, O., Heverin, M., Howard, R.S., Huisman, M.H.B., Keren, N., Leigh, P.N., Mazzini, L., Mora, G., Orrell, R.W., Rooney, J., Scott, K.M., Scotton, W.J., Seelen, M., Shaw, C.E., Sidle, K.S., Swingler, R., Tsuda, M., Veldink, J.H., Visser, A.E., van den Berg, L.H., Pearce, N., 2014. Analysis of amyotrophic lateral sclerosis as a multistep process: a population-based modelling study. Lancet Neurol. 13, 1108–1113.

Al-Saif, A., Al-Mohanna, F., Bohlega, S., 2011. A mutation in sigma-1 receptor causes juvenile amyotrophic lateral sclerosis. Ann. Neurol. 70, 913–919.

Al-Sarraj, S., King, A., Troakes, C., Smith, B., Maekawa, S., Bodi, I., Rogelj, B., Al-Chalabi, A., Hortobagyi, T., Shaw, C.E., 2011. p62 positive, TDP-43 negative, neuronal cytoplasmic and intranuclear inclusions in the cerebellum and hippocampus define the pathology of C9orf72-linked FTLD and MND/ALS. Acta Neuropathol. (Berl.) 122 (6), 691–702.

Alonso, A., Logroscino, G., Hernan, M.A., 2010. Smoking and the risk of amyotrophic lateral sclerosis: a systematic review and meta-analysis. J. Neurol. Neurosurg. Psychiatry 81, 1249–1252.

Appel, S.H., Smith, R.G., Engelhardt, J.I., Stefani, E., 1993. Evidence for autoimmunity in amyotrophic lateral sclerosis. J. Neurol. Sci. 118, 169–174.

Ari, C., Poff, A.M., Held, H.E., Landon, C.S., Goldhagen, C.R., Mavromates, N., D'Agostino, D.P., 2014. Metabolic therapy with Deanna Protocol supplementation delays disease progression and extends survival in amyotrophic lateral sclerosis (ALS) mouse model. PLoS One 9, e103526.

Armon, C., 2009. Smoking may be considered an established risk factor for sporadic ALS. Neurology 73, 1693–1698.

Arvidson, B., 1992. Inorganic mercury is transported from muscular nerve terminals to spinal and brainstem motoneurons. Muscle Nerve 15, 1089–1094.

Atanasio, A., Decman, V., White, D., Ramos, M., Ikiz, B., Lee, H.C., Siao, C.J., Brydges, S., LaRosa, E., Bai, Y., Fury, W., Burfeind, P., Zamfirova, R., Warshaw, G., Orengo, J., Oyejide, A., Fralish, M., Auerbach, W., Poueymirou, W., Freudenberg, J., Gong, G., Zambrowicz, B., Valenzuela, D., Yancopoulos, G., Murphy, A., Thurston, G., Lai, K.M., 2016. C9orf72 ablation causes immune dysregulation characterized by leukocyte expansion, autoantibody production, and glomerulonephropathy in mice. Sci. Rep. 6, 23204.

Ayala, Y.M., Zago, P., D'Ambrogio, A., Xu, Y.F., Petrucelli, L., Buratti, E., Baralle, F.E., 2008. Structural determinants of the cellular localization and shuttling of TDP-43. J. Cell. Sci. 121, 3778–3785.

Ayala, Y.M., De Conti, L., Avendano-Vazquez, S.E., Dhir, A., Romano, M., D'Ambrogio, A., Tollervey, J., Ule, J., Baralle, M., Buratti, E., Baralle, F.E., 2011. TDP-43 regulates its mRNA levels through a negative feedback loop. EMBO J. 30, 277–288.

Ayers, J.I., Fromholt, S.E., O'Neal, V.M., Diamond, J.H., Borchelt, D.R., 2016. Prion-like propagation of mutant SOD1 misfolding and motor neuron disease spread along neuroanatomical pathways. Acta Neuropathol. 131, 103–114.

Bar-Sagi, D., Hall, A., 2000. Ras and Rho GTPases: a family reunion. Cell 103, 227–238.

Barbeito, A.G., Martinez-Palma, L., Vargas, M.R., Pehar, M., Manay, N., Beckman, J.S., Barbeito, L., Cassina, P., 2010. Lead exposure stimulates VEGF expression in the spinal cord and extends survival in a mouse model of ALS. Neurobiol. Dis. 37, 574–580.

Bassett, T., Bach, P., Chan, H.M., 2012. Effects of methylmercury on the secretion of pro-inflammatory cytokines from primary microglial cells and astrocytes. Neurotoxicology 33, 229–234.

Beghi, E., Logroscino, G., Chio, A., Hardiman, O., Millul, A., Mitchell, D., Swingler, R., Traynor, B.J., 2010. Amyotrophic lateral sclerosis, physical exercise, trauma and sports: results of a population-based pilot case-control study. Amyotroph. Lateral Scler. 11, 289–292.

Beghi, E., 2013. Are professional soccer players at higher risk for ALS? Amyotroph Lateral Scler. Frontotemporal Degener 14, 501–506.

Belzil, V.V., Andre-Guimont, C., Atallah, M.-R., Daoud, H., Dupre, N., Bouchard, J.-P., Camu, W., Dion, P.A., Rouleau, G.A., 2012. Analysis of the SORT1 gene in familial amyotrophic lateral sclerosis. Neurobiol. Aging 33 (8) (1845. e7-9).

Belzil, V.V., Bauer, P.O., Gendron, T.F., Murray, M.E., Dickson, D., Petrucelli, L., 2014. Characterization of DNA hypermethylation in the cerebellum of c9FTD/ALS patients. Brain Res. 1584, 15–21.

Bendotti, C., Marino, M., Cheroni, C., Fontana, E., Crippa, V., Poletti, A., De Biasi, S., 2012. Dysfunction of constitutive and inducible ubiquitin-proteasome system in amyotrophic lateral sclerosis: implication for protein aggregation and immune response. Prog. Neurobiol. 97, 101–126.

Bensimon, G., Lacomblez, L., Meininger, V., 1994. A controlled trial of riluzole in amyotrophic lateral sclerosis: ALS/Riluzole Study Group. N. Engl. J. Med. 330, 585–591.

Bernstein, J.J., Bernstein, M.E., 1976. Ventral horn synaptology in the rat. J. Neurocytol. 5, 109–123.

Blokhuis, A.M., Groen, E.J., Koppers, M., van den Berg, L.H., Pasterkamp, R.J., 2013. Protein aggregation in amyotrophic lateral sclerosis. Acta Neuropathol. 125, 777–794.

Boccia, S., Sayed-Tabatabaei, F.A., Persiani, R., Gianfagna, F., Rausei, S., Arzani, D., La Greca, A., D'Ugo, D., La Torre, G., van Duijn, C.M., Ricciardi, G., 2007. Polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population, BMC. Cancer 7, 206.

Boeynaems, S., Bogaert, E., Michiels, E., Gijselinck, I., Sieben, A., Jovicic, A., De Baets, G., Scheveneels, W., Steyaert, J., Cuijt, I., Verstrepen, K.J., Callaerts, P., Rousseau, F., Schymkowitz, J., Cruts, M., Van Broeckhoven, C., Van Damme, P., Gitler, A.D., Robberecht, W., Van Den Bosch, L., 2016. Drosophila screen connects nuclear transport genes to DPR pathology in c9ALS/FTD. Sci. Rep. 6, 20877.

Bonvicini, F., Marcello, N., Mandrioli, J., Pietrini, V., Vinceti, M., 2010. Exposure to pesticides and risk of amyotrophic lateral sclerosis: a population-based case-control study. Ann. Ist. Super. Sanita 46, 284–287.

Bosco, D.A., Morfini, G., Karabacak, N.M., Song, Y., Gros-Louis, F., Pasinelli, P., Goolsby, H., Fontaine, B.A., Lemay, N., McKenna-Yasek, D., Frosch, M.P., Agar, J.N., Julien, J.P., Brady, S.T., Brown Jr., R.H., 2010. Wild-type and mutant SOD1 share an aberrant conformation and a common pathogenic pathway in ALS. Nat. Neurosci. 13, 1396–1403.

Bradley, W., 2015. The john walton muscular dystrophy research centre in the university of newcastle and the BMAA theory of motor neuron disease. J. Neuromuscular Dis. 2, S77–S81.

Brenner, D., Muller, K., Wieland, T., Weydt, P., Bohm, S., Lule, D., Hubers, A., Neuwirth, C., Weber, M., Borck, G., Wahlqvist, M., Danzer, K.M., Volk, A.E., Meitinger, T., Strom, T.M., Otto, M., Kassubek, J., Ludolph, A.C., Andersen, P.M., Weishaupt, J.H., 2016. NEK1 mutations in familial amyotrophic lateral sclerosis. Brain.

Burghes, A.H., Beattie, C.E., 2009. Spinal muscular atrophy: why do low levels of survival motor neuron protein make motor neurons sick? Nat. Rev. Neurosci. 10, 597–609.

Byrne, S., Elamin, M., Bede, P., Shatunov, A., Walsh, C., Corr, B., Heverin, M., Jordan, N., Kenna, K., Lynch, C., McLaughlin, R.L., Iyer, P.M., O'Brien, C., Phukan, J., Wynne, B., Bokde, A.L., Bradley, D.G., Pender, N., Al-Chalabi, A., Hardiman, O., 2012. Cognitive and clinical characteristics of patients with amyotrophic lateral sclerosis carrying a C9orf72 repeat expansion: a population-based cohort study. Lancet Neurol. 11, 232–240.

Cady, J., Koval, E.D., Benitez, B.A., Zaidman, C., Jockel-Balsarotti, J., Allred, P., Baloh, R.H., Ravits, J., Simpson, E., Appel, S.H., Pestronk, A., Goate, A.M., Miller, T.M., Cruchaga, C., Harms, M.B., 2014. TREM2 variant p: r47H as a risk factor for sporadic amyotrophic lateral sclerosis. JAMA Neurol. 71, 449–453.

Callaghan, B., Feldman, D., Gruis, K., Feldman, E., 2011. The association of exposure to lead, mercury, and selenium and the development of amyotrophic lateral sclerosis and the epigenetic implications. Neurodegener. Dis. 8, 1–8.

Caller, T.A., Chipman, J.W., Field, N.C., Stommel, E.W., 2013. Spatial analysis of amyotrophic lateral sclerosis in northern new england, USA, 1997–2009. Muscle Nerve 48, 235–241.

Casteignau, A., Fontan, A., Morillo, A., Oliveros, J.A., Segales, J., 2006. Clinical, pathological and toxicological findings of a iatrogenic selenium toxicosis case in feeder pigs. J. Vet. Med. A Physiol Pathol. Clin. Med. 53, 323–326.

Chen, Y.Z., Bennett, C.L., Huynh, H.M., Blair, I.P., Puls, I., Irobi, J., Dierick, I., Abel, A., Kennerson, M.L., Rabin, B.A., Nicholson, G.A., Auer-Grumbach, M., Wagner, K., De Jonghe, P., Griffin, J.W., Fischbeck, K.H., Timmerman, V., Cornblath, D.R., Chance, P.F., 2004. DNA/RNA helicase gene mutations in a form of juvenile amyotrophic lateral sclerosis (ALS4). Am. J. Hum. Genet. 74, 1128–1135.

Chen, Y., Huang, R., Yang, Y., Chen, K., Song, W., Pan, P., Li, J., Shang, H.-F., 2011. Ataxin-2 intermediate-length polyglutamine: a possible risk factor for Chinese patients with amyotrophic lateral sclerosis. Neurobiol. Aging 32 (10) (1925. e1-5.).

Chen, Y., Zheng, Z.-Z., Huang, R., Chen, K., Song, W., Zhao, B., Chen, X., Yang, Y., Yuan, L., Shang, H.-F., 2013. PFN1 mutations are rare in Han Chinese populations with amyotrophic lateral sclerosis. Neurobiol. Aging 34 (7) (1922. e1-5).

Chen, Y., 2012. Organophosphate-induced brain damage: mechanisms, neuropsychiatric and neurological consequences, and potential therapeutic strategies. Neurotoxicology 33, 391–400.

Chew, J., Gendron, T.F., Prudencio, M., Sasaguri, H., Zhang, Y.J., Castanedes-Casey, M., Lee, C.W., Jansen-West, K., Kurti, A., Murray, M.E., Bieniek, K.F., Bauer, P.O., Whitelaw, E.C., Rousseau, L., Stankowski, J.N., Stetler, C., Daughrity, L.M., Perkerson, E.A., Desaro, P., Johnston, A., Overstreet, K., Edbauer, D., Rademakers, R., Boylan, K.B., Dickson, D.W., Fryer, J.D., Petrucelli, L., 2015. Neurodegeneration. C9ORF72 repeat expansions in mice cause TDP-43 pathology, neuronal loss, and behavioral deficits. Science 348 (6239), 1151–1154.

Chiang, P.M., Ling, J., Jeong, Y.H., Price, D.L., Aja, S.M., Wong, P.C., 2010. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc.Natl.Acad.Sci. U.S.A 107, 16320–16324.

Chio, A., Benzi, G., Dossena, M., Mutani, R., Mora, G., 2005. Severely increased risk of amyotrophic lateral sclerosis among Italian professional football players. Brain J. Neurol. 128, 472–476.

Chio, A., Mora, G., Calvo, A., Mazzini, L., Bottacchi, E., Mutani, R., 2009. Epidemiology of ALS in Italy: a 10-year prospective population-based study. Neurology 72, 725–731.

Chio, A., Borghero, G., Calvo, A., Capasso, M., Caponnetto, C., Corbo, M., Giannini, F., Logroscino, G., Mandrioli, J., Marcello, N., Mazzini, L., Moglia, C., Monsurro, M.R., Mora, G., Patti, F., Perini, M., Pietrini, V., Pisano, F., Pupillo, E., Sabatelli, M., Salvi, F., Silani, V., Simone, I.L., Soraru, G., Tola, M.R., Volanti, P., Beghi, E., 2010. Lithium carbonate in amyotrophic lateral sclerosis: lack of efficacy in a dose-finding trial. Neurology 75, 619–625.

Choksi, D.K., Roy, B., Chatterjee, S., Yusuff, T., Bakhoum, M.F., Sengupta, U., Ambegaokar, S., Kayed, R., Jackson, G.R., 2014. TDP-43 Phosphorylation by casein kinase Iepsilon promotes oligomerization and enhances toxicity in vivo. Hum. Mol. Genet. 23, 1025–1035.

Chow, C.Y., Zhang, Y., Dowling, J.J., Jin, N., Adamska, M., Shiga, K., Szigeti, K., Shy, M.E., Li, J., Zhang, X., Lupski, J.R., Weisman, L.S., Meisler, M.H., 2007. Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4. J. Nature 448, 68–72.

Chow, C.Y., Landers, J.E., Bergren, S.K., Sapp, P.C., Grant, A.E., Jones, J.M., Everett, L., Lenk, G.M., McKenna-Yasek, D.M., Weisman, L.S., Figlewicz, D., Brown, R.H., Meisler, M.H., 2009. Deleterious variants of FIG4, a phosphoinositide phosphatase, in patients with ALS. Am. J. Hum. Genet. 84, 85–88.

Cirulli, E.T., Lasseigne, B.N., Petrovski, S., Sapp, P.C., Dion, P.A., Leblond, C.S., Couthouis, J., Lu, Y.F., Wang, Q., Krueger, B.J., Ren, Z., Keebler, J., Han, Y., Levy, S.E., Boone, B.E., Wimbish, J.R., Waite, L.L., Jones, A.L., Carulli, J.P., Day-Williams, A.G., Staropoli, J.F., Xin, W.W., Chesi, A., Raphael, A.R., McKenna-Yasek, D., Cady, J., Vianney de Jong, J.M., Kenna, K.P., Smith, B.N., Topp, S., Miller, J., Gkazi, A., Sequencing Consortium, F.A.L.S., Al-Chalabi, A., van den Berg, L.H., Veldink, J., Silani, V., Ticozzi, N., Shaw, C.E., Baloh, R.H., Appel, S., Simpson, E., Lagier-Tourenne, C., Pulst, S.M., Gibson, S., Trojanowski, J.Q., Elman, L., McCluskey, L., Grossman, M., Shneider, N.A., Chung, W.K., Ravits, J.M., Glass, J.D., Sims, K.B., Van Deerlin, V.M., Maniatis, T., Hayes, S.D., Ordureau, A., Swarup, S., Landers, J., Baas, F., Allen, A.S., Bedlack, R.S., Harper, J.W., Gitler, A.D., Rouleau, G.A., Brown, R., Harms, M.B., Cooper, G.M., Harris, T., Myers, R.M., Goldstein, D.B., 2015. Exome sequencing in amyotrophic lateral sclerosis identifies risk genes and pathways. Science 347 (6229), 1436–1441.

Cistaro, A., Pagani, M., Montuschi, A., Calvo, A., Moglia, C., Canosa, A., Restagno, G., Brunetti, M., Traynor, B.J., Nobili, F., Carrara, G., Fania, P., Lopiano, L., Valentini, M.C., Chio, A., 2014. The metabolic signature of C9ORF72-related ALS: FDG PET comparison with nonmutated patients. Eur. J. Nucl. Med. Mol. Imaging. 41, 844–852.

Coand, G., Mitchell, C.S., 2015. An assessment of possible neuropathology and clinical relationships in 46 sporadic amyotrophic lateral sclerosis patient autopsies. Neurodegen. Dis. 15, 301–312.

Cohen, T.J., Hwang, A.W., Restrepo, C.R., Yuan, C.X., Trojanowski, J.Q., Lee, V.M., 2015. An acetylation switch controls TDP-43 function and aggregation propensity. Nat. Commun. 6, 5845.

Conforti, F.L., Spataro, R., Sproviero, W., Mazzei, R., Cavalcanti, F., Condino, F., Simone, I.L., Logroscino, G., Patitucci, A., Magariello, A., Muglia, M., Rodolico, C., Valentino, P., Bono, F., Colletti, T., Monsurro, M.R., Gambardella, A., La Bella, V., 2012. Ataxin-1 and ataxin-2 intermediate-length PolyQ expansions in amyotrophic lateral sclerosis. Neurology 79, 2315–2320.

Cooper-Knock, J., Hewitt, C., Highley, J.R., Brockington, A., Milano, A., Man, S., Martindale, J., Hartley, J., Walsh, T., Gelsthorpe, C., Baxter, L., Forster, G., Fox, M., Bury, J., Mok, K., McDermott, C.J., Traynor, B.J., Kirby, J., Wharton, S.B., Ince, P.G., Hardy, J., Shaw, P.J., 2012. Clinico-pathological features in amyotrophic lateral sclerosis with expansions in C9ORF72. Brain J. Neurol. 135, 751–764.

Cooper-Knock, J., Shaw, P.J., Kirby, J., 2014. The widening spectrum of C9ORF72-related disease; genotype/phenotype correlations and potential modifiers of clinical phenotype. Acta Neuropathol. 127, 333–345.

Cooper-Knock, J., Higginbottom, A., Stopford, M.J., Highley, J.R., Ince, P.G., Wharton, S.B., Pickering-Brown, S., Kirby, J., Hautbergue, G.M., Shaw, P.J., 2015. Antisense RNA foci in the motor neurons of C9ORF72-ALS patients are associated with TDP-43 proteinopathy. Acta Neuropathol. 130, 63–75.

Corcia, P., Khoris, J., Couratier, P., Mayeux-Portas, V., Bieth, E., De Toffol, B., Autret, A., Muh, J.P., Andres, C., Camu, W., 2002. SMN1 gene study in three families in which ALS and spinal muscular atrophy co-exist. Neurology 59, 1464–1466.

Corrado, L., Ratti, A., Gellera, C., Buratti, E., Castellotti, B., Carlomagno, Y., Ticozzi, N., Mazzini, L., Testa, L., Taroni, F., Baralle, F.E., Silani, V., D'Alfonso, S., 2009. High frequency of TARDBP gene mutations in Italian patients with amyotrophic lateral sclerosis. Hum. Mutat. 30, 688–694.

Corrado, L., Mazzini, L., Oggioni, G.D., Luciano, B., Godi, M., Brusco, A., D'Alfonso, S., 2011. ATXN-2 CAG repeat expansions are interrupted in ALS patients. Hum. Genet. 130, 575–580.

Coughlan, K.S., Mitchem, M.R., Hogg, M.C., Prehn, J.H., 2015. Preconditioning with latrepirdine, an adenosine 5'-monophosphate-activated protein kinase activator, delays amyotrophic lateral sclerosis progression in SOD1(G93A) mice. Neurobiol. Aging 36, 1140–1150.

Couthouis, J., Hart, M.P., Shorter, J., DeJesus-Hernandez, M., Erion, R., Oristano, R., Liu, A.X., Ramos, D., Jethava, N., Hosangadi, D., Epstein, J., Chiang, A., Diaz, Z., Nakaya, T., Ibrahim, F., Kim, H.-J., Solski, J.A., Williams, K.L., Mojsilovic-Petrovic, J., Ingre, C., Boylan, K., Graff-Radford, N.R., Dickson, D.W., Clay-Falcone, D., Elman, L., McCluskey, L., Greene, R., Kalb, R.G., Lee, V.M.-Y., Trojanowski, J.Q., Ludolph, A., Robberecht, W., Andersen, P.M., Nicholson, G.A., Blair, I.P., King, O.D., Bonini, N.M., Van Deerlin, V., Rademakers, R., Mourelatos, Z., Gitler, A.D., 2011. A yeast functional screen predicts new candidate ALS disease genes. Proc. Natl. Acad. Sci. U. S. A. 108 108, 20881–20890.

Couthouis, J., Hart, M.P., Erion, R., King, O.D., Diaz, Z., Nakaya, T., Ibrahim, F., Kim, H.-J., Mojsilovic-Petrovic, J., Panossian, S., Kim, C.E., Frackelton, E.C., Solski, J.A., Williams, K.L., Clay-Falcone, D., Elman, L., McCluskey, L., Greene, R., Hakonarson, H., Kalb, R.G., Lee, V.M.Y., Trojanowski, J.Q., Nicholson, G.A., Blair, I.P., Bonini, N.M., Van Deerlin, V.M., Mourelatos, Z., Shorter, J., Gitler, A.D., 2012. Evaluating the role of the FUS/TLS-related gene EWSR1 in amyotrophic lateral sclerosis. Hum. Mol. Genet. 21, 2899–2911.

Cox, P.A., Banack, S.A., Murch, S.J., 2003. Biomagnification of cyanobacterial neurotoxins and neurodegenerative disease among the Chamorro people of Guam. Proc. Natl. Acad. Sci. U. S. A. 100, 13380–13383.

Cox, L.E., Ferraiuolo, L., Goodall, E.F., Heath, P.R., Higginbottom, A., Mortiboys, H., Hollinger, H.C., Hartley, J.A., Brockington, A., Burness, C.E., Morrison, K.E., Wharton, S.B., Grierson, A.J., Ince, P.G., Kirby, J., Shaw, P.J., 2010. Mutations in CHMP2B in lower motor neuron predominant amyotrophic lateral sclerosis (ALS). PLoS One 5, e9872.

Cronin, S., Hardiman, O., Traynor, B.J., 2007. Ethnic variation in the incidence of ALS: a systematic review. Neurology 68, 1002–1007.

Cudkowicz, M.E., Titus, S., Kearney, M., Yu, H., Sherman, A., Schoenfeld, D., Hayden, D., Shui, A., Brooks, B., Conwit, R., Felsenstein, D., Greenblatt, D.J., Keroack, M., Kissel, J.T., Miller, R., Rosenfeld, J., Rothstein, J.D., Simpson, E., Tolkoff-Rubin, N., Zinman, L., Shefner, J.M., Ceftriaxone Study Investigators, 2014. Safety and efficacy of ceftriaxone for amyotrophic lateral sclerosis: a multi-stage, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 13, 1083–1091.

Daoud, H., Belzil, V., Martins, S., Sabbagh, M., Provencher, P., Lacomblez, L., Meininger, V., Camu, W., Dupre, N., Dion, P.A., Rouleau, G.A., 2011. Association of long ATXN2 CAG repeat sizes with increased risk of amyotrophic lateral sclerosis. Arch. Neurol. 68, 739–742.

Daoud, H., Zhou, S., Noreau, A., Sabbagh, M., Belzil, V., Dionne-Laporte, A., Tranchant, C., Dion, P., Rouleau, G.A., 2012. Exome sequencing reveals SPG11 mutations causing juvenile ALS. Neurobiol. Aging 33 (839) (e5-839. e9).

De Felice, B., Guida, M., Guida, M., Coppola, C., De Mieri, G., Cotrufo, R., 2012. A miRNA signature in leukocytes from sporadic amyotrophic lateral sclerosis. Gene 508 (1), 35–40.

DeJesús-Hernández, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.-Y.R., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman,

D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. Neuron 72, 245–256.

De Jong, S.W., Huisman, M.H.B., Sutedja, N.A., van der Kooi, A.J., de Visser, M., Schelhaas, H.J., Fischer, K., Veldink, J.H., van den Berg, L.H., 2012. Smoking, alcohol consumption, and the risk of amyotrophic lateral sclerosis: a population-based study. Am. J. Epidemiol. 176, 233–239.

Debray, S., Race, V., Crabbe, V., Herdewyn, S., Matthijs, G., Goris, A., Dubois, B., Thijs, V., Robberecht, W., Van Damme, P., 2013. Frequency of C9orf72 repeat expansions in amyotrophic lateral sclerosis: a Belgian cohort study. Neurobiol. Aging 34 (2890) (e7-2890. e12).

Del Bo, R., Tiloca, C., Pensato, V., Corrado, L., Ratti, A., Ticozzi, N., Corti, S., Castellotti, B., Mazzini, L., Soraru, G., Cereda, C., D'Alfonso, S., Gellera, C., Comi, G.P., Silani, V., 2011. Novel optineurin mutations in patients with familial and sporadic amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 82, 1239–1243.

Den Boer, M., Voshol, P.J., Kuipers, F., Havekes, L.M., Romijn, J.A., 2004. Hepatic steatosis: a mediator of the metabolic syndrome: lessons from animal models. Arterioscler. Thromb. Vasc. Biol. 24, 644–649.

Deng, H.-X., Zhai, H., Bigio, E.H., Yan, J., Fecto, F., Ajroud, K., Mishra, M., Ajroud-Driss, S., Heller, S., Sufit, R., Siddique, N., Mugnaini, E., Siddique, T., 2010. FUS-immunoreactive inclusions are a common feature in sporadic and non-SOD1 familial amyotrophic lateral sclerosis. Ann. Neurol. 67, 739–748.

Deng, H.-X., Chen, W., Hong, S.-T., Boycott, K.M., Gorrie, G.H., Siddique, N., Yang, Y., Fecto, F., Shi, Y., Zhai, H., Jiang, H., Hirano, M., Rampersaud, E., Jansen, G.H., Donkervoort, S., Bigio, E.H., Brooks, B.R., Ajroud, K., Sufit, R.L., Haines, J.L., Mugnaini, E., Pericak-Vance, M.A., Siddique, T., 2011. Mutations in UBQLN2 cause dominant X-linked juvenile and adult-onset ALS and ALS/dementia. Nature 477, 211–215.

Deng, H., Gao, K., Jankovic, J., 2014. The role of FUS gene variants in neurodegenerative diseases. Nat. Rev. Neurol. 10, 337–348.

Desport, J.C., Preuxm, P.M., Truong, T.C., Vallat, J.M., Sautereau, D., Couratier, P., 1999. Nutritional status is a prognostic factor for survival in ALS patients. Neurology 53, 1059–1063.

Desport, J.C., Preux, P.M., Magy, L., Boirie, Y., Vallat, J.M., Beaufrere, B., Couratier, P., 2001. Factors correlated with hypermetabolism in patients with amyotrophic lateral sclerosis. Am. J. Clin. Nutr. 74, 328–334.

Desport, J.C., Torny, F., Lacoste, M., Preux, P.M., Couratier, P., 2005. Hypermetabolism in ALS: correlations with clinical and paraclinical parameters. Neurodegener Dis. 2, 202–207.

Desvergne, B., Michalik, L., Wahli, W., 2006. Transcriptional regulation of metabolism. Physiol. Rev. 86, 465–514.

Diekstra, F.P., van Vught, P.W.J., van Rheenen, W., Koppers, M., Pasterkamp, R.J., van Es, M.A., Schelhaas, H.J., de Visser, M., Robberecht, W., Van Damme, P., Andersen, P.M., van den Berg, L.H., Veldink, J.H., 2012. UNC13A is a modifier of survival in amyotrophic lateral sclerosis. Neurobiol. Aging 33 (3) (630. e3-8).

Djordjevic, M.V., Fan, J., Hoffmann, D., 1995. Assessment of chlorinated pesticide residues in cigarette tobacco based on supercritical fluid extraction and GC-ECD. Carcinogenesis 16, 2627–2632.

Dodge, J.C., Treleaven, C.M., Fidler, J.A., Tamsett, T.J., Bao, C., Searles, M., Taksir, T.V., Misra, K., Sidman, R.L., Cheng, S.H., Shihabuddin, L.S., 2013. Metabolic signatures of amyotrophic lateral sclerosis reveal insights into disease pathogenesis. Proc. Natl. Acad. Sci. U.S.A. 110, 10812–10817.

Dols-Icardo, O., Iborra, O., Valdivia, J., Pastor, P., Ruiz, A., López de Munain, A., Sánchez-Valle, R., Álvarez, V., Sánchez-Juan, P., Lleó, A., Blesa, R., Cardona-Serrate, F., Baquero, M., Alonso, M.D., Ortega-Cubero, S., Pastor, M.A., Razquin, C., Boada, M, Hernández, I, Gorostidi, A., Moreno, F., Zulaica, M., Lladó, A., Coto, E., Combarros, O., Pérez-Tur, J., Clarimón, J., 2016. Assessing the role of TUBA4A gene in frontotemporal dementia. on behalf of The dementia genetic Spanish consortium (DEGESCO) Neurobiol. Aging (submitted).

Dupuis, L., Oudart, H., Rene, F., Gonzalez de Aguilar, J.L., Loeffler, J.P., 2004. Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. Proc. Natl. Acad. Sci. U.S.A. 101, 11159–11164.

Dupuis, L., Corcia, P., Fergani, A., Gonzalez De Aguilar, J.-L., Bonnefont-Rousselot, D., Bittar, R., Seilhean, D., Hauw, J.-J., Lacomblez, L., Loeffler, J.-P., Meininger, V., 2008. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology 70 (13), 1004–1009.

Dupuis, L., Pradat, P.-F., Ludolph, A.C., Loeffler, J.-P., 2011. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol. 10 (1), 75–82.

Dupuis, L., Dengler, R., Heneka, M.T., Meyer, T., Zierz, S., Kassubek, J., Fischer, W., Steiner, F., Lindauer, E., Otto, M., Dreyhaupt, J., Grehl, T., Hermann, A., Winkler, A.S., Bogdahn, U., Benecke, R., Schrank, B., Wessig, C., Grosskreutz, J., Ludolph, A.C., 2012. A randomized, double blind, placebo-controlled trial of pioglitazone in combination with riluzole in amyotrophic lateral sclerosis. PLoS One 7 (6), e37885.

Eaglehouse, Y.L., Talbott, E.O., Chang, Y., Kuller, L.H., 2016. Participation in physical activity and risk for amyotrophic lateral sclerosis mortality among postmenopausal women. JAMA Neurol. 1–9.

El Hokayem, J., Huber, C., Couve, A., Aziza, J., Baujat, G., Bouvier, R., Cavalcanti, D.P., Collins, F.A., Cordier, M.P., Delezoide, A.L., Gonzales, M., Johnson, D., Le Merrer, M., Levy-Mozziconacci, A., Loget, P., Martin-Coignard, D., Martinovic, J., Mortier, G.R., Perez, M.J., Roume, J., Scarano, G., Munnich, A., Cormier-Daire, V., 2012. NEK1 and DYNC2H1 are both involved in short rib polydactyly Majewski type but not in Beemer Langer cases. J. Med. Genet. 49, 227–233.

El Oussini, H., Bayer, H., Scekic-Zahirovic, J., Vercruysse, P., Sinniger, J., Dirrig-Grosch, S., Dieterle, S., Echaniz-Laguna, A., Larmet, Y., Muller, K., Weishaupt, J.H., Thal, D.R., van Rheenen, W., van Eijk, K., Lawson, R., Monassier, L., Maroteaux, L., Roumier, A., Wong, P.C., van den Berg, L.H., Ludolph, A.C., Veldink, J.H., Witting, A., Dupuis, L., 2016. Serotonin 2B receptor slows disease progression and prevents degeneration of spinal cord mononuclear phagocytes in amyotrophic lateral sclerosis. Acta Neuropathol. 131, 465–480.

Elden, A.C., Kim, H.-J., Hart, M.P., Chen-Plotkin, A.S., Johnson, B.S., Fang, X., Armakola, M., Geser, F., Greene, R., Lu, M.M., Padmanabhan, A., Clay-Falcone, D., McCluskey, L., Elman, L., Juhr, D., Gruber, P.J., Rub, U., Auburger, G., Trojanowski, J.Q., Lee, V.M.-Y., Van Deerlin, V.M., Bonini, N.M., Gitler, A.D., 2010. Ataxin-2 intermediate-length polyglutamine expansions are associated with increased risk for ALS. Nature 466, 1069–1075, http://dx.doi.org/10.1038/nature09320.

Ermilova, I.P., Ermilov, V.B., Levy, M., Ho, E., Pereira, C., Beckman, J.S., 2005. Protection by dietary zinc in ALS mutant G93A SOD transgenic mice. Neurosci. Lett. 379, 42–46.

Estevez, A.O., Mueller, C.L., Morgan, K.L., Szewczyk, N.J., Teece, L., Miranda-Vizuete, A., Estevez, M., 2012. Selenium induces cholinergic motor neuron degeneration in Caenorhabditis elegans. Neurotoxicology 33, 1021–1032.

Eymard-Pierre, E., Lesca, G., Dollet, S., Santorelli, F.M., di Capua, M., Bertini, E., Boespflug-Tanguy, O., 2002. Infantile-onset ascending hereditary spastic paralysis is associated with mutations in the alsin gene. Am. J. Hum. Genet. 71, 518–527.

Ezzi, S.A., Urushitani, M., Julien, J.P., 2007. Wild-type superoxide dismutase acquires binding and toxic properties of ALS-linked mutant forms through oxidation. J. Neurochem. 102, 170–178.

Fallini, C., Bassell, G.J., Rossoll, W., 2012. Spinal muscular atrophy: the role of SMN in axonal mRNA regulation. Brain Res. 1462, 81–92.

Falone, S., Mirabilio, A., Carbone, M.C., Zimmitti, V., Di Loreto, S., Mariggio, M.A., Mancinelli, R., Di Ilio, C., Amicarelli, F., 2008. Chronic exposure to 50 Hz magnetic fields causes a significant weakening of antioxidant defence systems in aged rat brain. Int. J. Biochem. Cell Biol. 40, 2762–2770.

Fang, F., Bellocco, R., Hernan, M.A., Ye, W., 2006. Smoking, snuff dipping and the risk of amyotrophic lateral sclerosis–a prospective cohort study. Neuroepidemiology 27, 217–221.

Fang, F., Kwee, L.C., Allen, K.D., Umbach, D.M., Ye, W., Watson, M., Keller, J., Oddone, E.Z., Sandler, D.P., Schmidt, S., Kamel, F., 2010. Association between blood lead and the risk of amyotrophic lateral sclerosis. Am. J. Epidemiol. 171, 1126–1133.

Fang, F., Hallmarker, U., James, S., Ingre, C., Michaelsson, K., Ahlbom, A., Feychting, M., 2015. Amyotrophic lateral sclerosis among cross-country skiers in Sweden. Eur. J. Epidemiol..

Farg, M.A., Sundaramoorthy, V., Sultana, J.M., Yang, S., Atkinson, R.A., Levina, V., Halloran, M.A., Gleeson, P.A., Blair, I.P., Soo, K.Y., King, A.E., Atkin, J.D., 2014. C9ORF72, implicated in amytrophic lateral sclerosis and frontotemporal dementia: regulates endosomal trafficking. Hum. Mol. Genet. 23, 3579–3595.

Fecto, F., Siddique, T., 2012. UBQLN2/P62 cellular recycling pathways in amyotrophic lateral sclerosis and frontotemporal dementia. Muscle Nerve 45, 157–162.

Fecto, F., Yan, J., Vemula, S.P., Liu, E., Yang, Y., Chen, W., Zheng, J.G., Shi, Y., Siddique, N., Arrat, H., Donkervoort, S., Ajroud-Driss, S., Sufit, R.L., Heller, S.L., Deng, H.-X., Siddique, T., 2011. SQSTM1 mutations in familial and sporadic amyotrophic lateral sclerosis. Arch. Neurol. 68, 1440–1446.

Fergani, A., Eschbach, J., Oudart, H., Larmet, Y., Schwalenstocker, B., Ludolph, A.C., Loeffler, J.P., Dupuis, L., 2011. A mutation in the dynein heavy chain gene compensates for energy deficit of mutant SOD1 mice and increases potentially neuroprotective IGF-1. Mol. Neurodegener. 6 (1), 26.

Field, N.C., Metcalf, J.S., Caller, T.A., Banack, S.A., Cox, P.A., Stommel, E.W., 2013. Linking beta-methylamino-L-alanine exposure to sporadic amyotrophic lateral sclerosis in Annapolis, MD. Toxicon Off. J. Int. Soc. Toxinol. 70, 179–183.

Forsberg, K., Jonsson, P.A., Andersen, P.M., Bergemalm, D., Graffmo, K.S., Hultdin, M., Jacobsson, J., Rosquist, R., Marklund, S.L., Brannstrom, T., 2010. Novel antibodies reveal inclusions containing non-native SOD1 in sporadic ALS patients. PLoS One 5, e11552.

Frank, E., 2009. A new class of spinal interneurons: the origin and function of C boutons is solved. Neuron 64, 593–595.

Fratta, P., Charnock, J., Collins, T., Devoy, A., Howard, R., Malaspina, A., Orrell, R., Sidle, K., Clarke, J., Shoai, M., Lu, C.-H., Hardy, J., Plagnol, V., Fisher, E.M.C., 2014. Profilin1 E117G is a moderate risk factor for amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 85, 506–508.

Freibaum, B.D., Lu, Y., Lopez-Gonzalez, R., Kim, N.C., Almeida, S., Lee, K.H., Badders, N., Valentine, M., Miller, B.L., Wong, P.C., Petrucelli, L., Kim, H.J., Gao, F.B., Taylor, J.P., 2015. GGGGCC repeat expansion in C9orf72 compromises nucleocytoplasmic transport. Nature 525, 129–133.

Freischmidt, A., Wieland, T., Richter, B., Ruf, W., Schaeffer, V., Müller, K., Marroquin, N., Nordin, F., Hübers, A., Weydt, P., Pinto, S., Press, R., Millecamps, S., Molko, N., Bernard, E., Desnuelle, C., Soriani, M.H., Dorst, J., Graf, E., Nordström, U., Feiler, M.S., Putz, S., Boeckers, T.M., Meyer, T., Winkler, A.S., Winkelman, J., de Carvalho, M., Thal, D.R., Otto, M., Brännström, T., Volk, A.E., Kursula, P., Danzer, K.M., Lichtner, P., Dikic, I., Meitinger, T., Ludolph, A.C., Strom, T.M., Andersen, P.M., Weishaupt, J.H., 2015. Haploinsufficiency of TBK1 causes familial ALS and fronto-temporal dementia. Nat. Neurosci. 18 (5), 631–636.

Fu, Y.J., Nishihira, Y., Kuroda, S., Toyoshima, Y., Ishihara, T., Shinozaki, M., Miyashita, A., Piao, Y.S., Tan, C.F., Tani, T., Koike, R., Iwanaga, K., Tsujihata, M., Onodera, O., Kuwano, R., Nishizawa, M., Kakita, A., Ikeuchi, T., Takahashi, H., 2010. Sporadic four-repeat tauopathy with frontotemporal lobar degeneration, Parkinsonism,

and motor neuron disease: a distinct clinicopathological and biochemical disease entity. Acta Neuropathol. 120, 21–32.
Funalot, B., Desport, J.C., Sturtz, F., Camu, W., Couratier, P., 2009. High metabolic level in patients with familial amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 10, 113–117.
Gómez-Deza, J., Lee, Y.B., Troakes, C., Nolan, M., Al-Sarraj, S., Gallo, J.M., Shaw, C.E., 2015. Dipeptide repeat protein inclusions are rare in the spinal cord and almost absent from motor neurons in C9ORF72 mutant amyotrophic lateral sclerosis and are unlikely to cause their degeneration. Acta Neuropathol. Commun. 3 (1), 38.
Gajdusek, D.C., Salazar, A.M., 1982. Amyotrophic lateral sclerosis and parkinsonian syndromes in high incidence among the Auyu and Jakai people of West New Guinea. Neurology 32, 107–126.
Gallagher, M.D., Suh, E., Grossman, M., Elman, L., McCluskey, L., Van Swieten, J.C., Al-Sarraj, S., Neumann, M., Gelpi, E., Ghetti, B., Rohrer, J.D., Halliday, G., Van Broeckhoven, C., Seilhean, D., Shaw, P.J., Frosch, M.P., Alafuzoff, I., Antonell, A., Bogdanovic, N., Brooks, W., Cairns, N.J., Cooper-Knock, J., Cotman, C., Cras, P., Cruts, M., De Deyn, P.P., DeCarli, C., Dobson-Stone, C., Engelborghs, S., Fox, N., Galasko, D., Gearing, M., Gijselinck, I., Grafman, J., Hartikainen, P., Hatanpaa, K.J., Highley, J.R., Hodges, J., Hulette, C., Ince, P.G., Jin, L.-W., Kirby, J., Kofler, J., Kril, J., Kwok, J.B.J., Levey, A., Lieberman, A., Llado, A., Martin, J.-J., Masliah, E., McDermott, C.J., McKee, A., McLean, C., Mead, S., Miller, C.A., Miller, J., Munoz, D.G., Murrell, J., Paulson, H., Piguet, O., Rossor, M., Sanchez-Valle, R., Sano, M., Schneider, J., Silbert, L.C., Spina, S., van der Zee, J., Van Langenhove, T., Warren, J., Wharton, S.B., White 3rd, C.L., Woltjer, R.L., Trojanowski, J.Q., Lee, V.M.Y., Van Deerlin, V., Chen-Plotkin, A.S., 2014. TMEM106B is a genetic modifier of frontotemporal lobar degeneration with C9ORF72 hexanucleotide repeat expansions. Acta Neuropathol. (Berl.) 127, 407–418.
Gallart-Palau, X., Tarabal, O., Casanovas, A., Sabado, J., Correa, F.J., Hereu, M., Piedrafita, L., Caldero, J., Esquerda, J.E., 2014. Neuregulin-1 is concentrated in the postsynaptic subsurface cistern of C-bouton inputs to alpha-motoneurons and altered during motoneuron diseases. FASEB J. 28, 3618–3632.
Gallo, V., Wark, P.A., Jenab, M., Pearce, N., Brayne, C., Vermeulen, R., Andersen, P.M., Hallmans, G., Kyrozis, A., Vanacore, N., Vahdaninia, M., Grote, V., Kaaks, R., Mattiello, A., Bueno-de-Mesquita, H.B., Peeters, P.H., Travis, R.C., Petersson, J., Hansson, O., Arriola, L., Jimenez-Martin, J.M., Tjønneland, A., Halkjær, J., Agnoli, C., Sacerdote, C., Bonet, C., Trichopoulou, A., Gavrila, D., Overvad, K., Weiderpass, E., Palli, D., Quirós, J.R., Tumino, R., Khaw, K.T., Wareham, N., Barricante-Gurrea, A., Fedirko, V., Ferrari, P., Clavel-Chapelon, F., Boutron-Ruault, M.C., Boeing, H., Vigl, M., Middleton, L., Riboli, E., Vineis, P., 2013. Prediagnostic body fat and risk of death from amyotrophic lateral sclerosis: the EPIC cohort. Neurology 80, 829–838.
Gallo, V., Vanacore, N., Bueno-de-Mesquita, H.B., Vermeulen, R., Brayne, C., Pearce, N., Wark, P.A., Ward, H.A., Ferrari, P., Jenab, M., Andersen, P.M., Wennberg, P., Wareham, N., Katzke, V., Kaaks, R., Weiderpass, E., Peeters, P.H., Mattiello, A., Pala, V., Barricante, A., Chirlaque, M.D., Travier, N., Travis, R.C., Sanchez, M.J., Pessah-Rasmussen, H., Petersson, J., Tjonneland, A., Tumino, R., Quiros, J.R., Trichopoulou, A., Kyrozis, A., Oikonomidou, D., Masala, G., Sacerdote, C., Arriola, L., Boeing, H., Vigl, M., Claver-Chapelon, F., Middleton, L., Riboli, E., Vineis, P., 2016. Physical activity and risk of Amyotrophic Lateral Sclerosis in a prospective cohort study. Eur. J. Epidemiol. 3, 255–266.
García, M.L., Singleton, A.B., Hernandez, D., Ward, C.M., Evey, C., Sapp, P.A., Hardy, J., Brown, R.H.J., Cleveland, D.W., 2006. Mutations in neurofilament genes are not a significant primary cause of non-SOD1-mediated amyotrophic lateral sclerosis. Neurobiol. Dis. 21, 102–109.
García-Redondo, A., Dols-Icardo, O., Rojas-Garcia, R., Esteban-Perez, J., Cordero-Vazquez, P., Munoz-Blanco, J.L., Catalina, I., Gonzalez-Munoz, M., Varona, L., Sarasola, E., Povedano, M., Sevilla, T., Guerrero, A., Pardo, J., Lopez de Munain, A., Marquez-Infante, C., de Rivera, F.J.R., Pastor, P., Jerico, I., de Arcaya, A.A., Mora, J.S., Clarimon, J., Gonzalo-Martinez, J.F., Juarez-Rufian, A., Atencia, G., Jimenez-Bautista, R., Moran, Y., Mascias, J., Hernandez-Barral, M., Kapetanovic, S., Garcia-Barcina, M., Alcala, C., Vela, A., Ramirez-Ramos, C., Galan, L., Perez-Tur, J., Quintans, B., Sobrido, M.J., Fernandez-Torron, R., Poza, J.J., Gorostidi, A., Paradas, C., Villoslada, P., Larrode, P., Capablo, J.L., Pascual-Calvet, J., Goni, M., Morgado, Y., Guitart, M., Moreno-Laguna, S., Rueda, A., Martin-Estefania, C., Cemillan, C., Blesa, R., Lleo, A., 2013. Analysis of the C9orf72 gene in patients with amyotrophic lateral sclerosis in Spain and different populations worldwide. Hum. Mutat. 34 (1), 79–82.
Gascon, E., Gao, F.B., 2014. The emerging roles of microRNAs in the pathogenesis of frontotemporal dementia-amyotrophic lateral sclerosis (FTD-ALS) spectrum disorders. J. Neurogenet. 28, 30–40.
Geetha, T., Zheng, C., Vishwaprakash, N., Broderick, T.L., Babu, J.R., 2012. Sequestosome 1/p62, a scaffolding protein, is a newly identified partner of IRS-1 protein. J. Biol. Chem. 287, 29672–29678.
Gellera, C., Ticozzi, N., Pensato, V., Nanetti, L., Castucci, A., Castellotti, B., Lauria, G., Taroni, F., Silani, V., Mariotti, C., 2012. ATAXIN2 CAG-repeat length in Italian patients with amyotrophic lateral sclerosis: risk factor or variant phenotype? Implication for genetic testing and counseling. Neurobiol. Aging 33 (8) (1847. e15-21.).
Gibson, S.B., Figueroa, K.P., Bromberg, M.B., Pulst, S.-M., Cannon-Albright, L., 2014. Familial clustering of ALS in a population-based resource. Neurology 82 (1), 17–22.
Gijselinck, I., Van Langenhove, T., van der Zee, J., Sleegers, K., Philtjens, S., Kleinberger, G., Janssens, J., Bettens, K., Van Cauwenberghe, C., Pereson, S., Engelborghs, S., Sieben, A., De Jonghe, P., Vandenberghe, R., Santens, P., De Bleecker, J., Maes, G., Baumer, V., Dillen, L., Joris, G., Cuijt, I., Corsmit, E., Elinck, E., Van Dongen, J., Vermeulen, S., Van den Broeck, M., Vaerenberg, C., Mattheijssens, M., Peeters, K., Robberecht, W., Cras, P., Martin, J.-J., De Deyn, P.P., Cruts, M., Van Broeckhoven, C., 2012. A C9ORF72 promoter repeat expansion in a Flanders-Belgian cohort with disorders of the frontotemporal lobar degeneration-amyotrophic lateral sclerosis spectrum: a gene identification study. Lancet Neurol. 11 (1), 54–65.
Gitcho, M.A., Bigio, E.H., Mishra, M., Johnson, N., Weintraub, S., Mesulam, M., Rademakers, R., Chakraverty, S., Cruchaga, C., Morris, J.C., Goate, A.M., Cairns, N.J., 2009. TARDBP 3¢-UTR variant in autopsy-confirmed frontotemporal lobar degeneration with. Acta Neuropathol. (Berl.) 118, 633–645.
Golko-Perez, S., Mandel, S., Amit, T., Kupershmidt, L., Youdim, M.B., Weinreb, O., 2016. Additive neuroprotective effects of the multifunctional iron chelator M30 with enriched diet in a mouse model of amyotrophic lateral sclerosis. Neurotox. Res. 29, 208–217.
Goodall, E.F., Greenway, M.J., van Marion, I., Carroll, C.B., Hardiman, O., Morrison, K.E., 2005. Association of the H63D polymorphism in the hemochromatosis gene with sporadic ALS. Neurology 65, 934–937.
Gordon, P.H., Cheng, B., Katz, I.B., Pinto, M., Hays, A.P., Mitsumoto, H., Rowland, L.P., 2006. The natural history of primary lateral sclerosis. Neurology 66, 647–653.
Gotkine, M., Friedlander, Y., Hochner, H., 2014. Triathletes are over-represented in a population of patients with ALS, Amyotroph Lateral Scler, Frontotemporal Degener 15, 534–536
Goto, F., Kitamura, A., Koto, A., Kataoka, K., Atsuji, H., 1972. Abnormal insulin secretion in amyotrophic lateral sclerosis. J. Neurol. Sci. 16, 201–207.
Greenway, M.J., Andersen, P.M., Russ, C., Ennis, S., Cashman, S., Donaghy, C., Patterson, V., Swingler, R., Kieran, D., Prehn, J., Morrison, K.E., Green, A., Acharya, K.R., Brown, R.H.J., Hardiman, O., 2006. ANG mutations segregate with familial and sporadic amyotrophic lateral sclerosis. Nat. Genet. 38, 411–413.
Groeneveld, G.J., de Leeuw, v. W., van Muiswinkel, F.L., Veldman, H., Veldink, J.H., Wokke, J.H., Bar, P.R., van den Berg, L.H., 2003. Zinc amplifies mSOD1-mediated toxicity in a transgenic mouse model of amyotrophic lateral sclerosis. Neurosci. Lett. 352, 175–178.
Gros-Louis, F., Meijer, I.A., Hand, C.K., Dube, M.-P., MacGregor, D.L., Seni, M.-H., Devon, R.S., Hayden, M.R., Andermann, F., Andermann, E., Rouleau, G.A., 2003. An ALS2 gene mutation causes hereditary spastic paraplegia in a Pakistani kindred. Ann. Neurol. 53, 144–145.
Gumà, A., Martínez-Redondo, V., López-Soldado, I., Canto, C., Zorzano, A., 2010. Emerging role of neuregulin as a modulator of muscle metabolism. Am. J. Physiol. Endocrinol. Metab. 298, E742–50.
Gurel, A., Coskun, O., Armutcu, F., Kanter, M., Ozen, O.A., 2005. Vitamin E against oxidative damage caused by formaldehyde in frontal cortex and hippocampus: biochemical and histological studies. J. Chem. Neuroanat. 29, 173–178.
Hadano, S., Hand, C.K., Osuga, H., Yanagisawa, Y., Otomo, A., Devon, R.S., Miyamoto, N., Showguchi-Miyata, J., Okada, Y., Singaraja, R., Figlewicz, D.A., Kwiatkowski, T., Hosler, B.A., Sagie, T., Skaug, J., Nasir, J., Brown, R.H.J., Scherer, S.W., Rouleau, G.A., Hayden, M.R., Ikeda, J.E., 2001. A gene encoding a putative GTPase regulator is mutated in familial amyotrophic lateral sclerosis 2. Nat. Genet. 29, 166–173.
Haeusler, A.R., Donnelly, C.J., Periz, G., Simko, E.A., Shaw, P.G., Kim, M.S., Maragakis, N.J., Troncoso, J.C., Pandey, A., Sattler, R., Rothstein, J.D., Wang, J., 2014. C9orf72 nucleotide repeat structures initiate molecular cascades of disease. Nature 507, 195–200.
Hardiman, O., van den Berg, L.H., Kiernan, M.C., 2011. Clinical diagnosis and management of amyotrophic lateral sclerosis. Nat. Rev. Neurol. 7, 639–649.
Hargett, S.R., Walker, N.N., Hussain, S.S., Hoehn, K.L., Keller, S.R., 2015. Deletion of the Rab GAP Tbc1d1 modifies glucose, lipid, and energy homeostasis in mice. Am. J. Physiol. Endocrinol. Metab. 309, E233–45.
Haubenberger, D., Bittner, R.E., Rauch-Shorny, S., Zimprich, F., Mannhalter, C., Wagner, L., Mineva, I., Vass, K., Auff, E., Zimprich, A., 2005. Inclusion body myopathy and Paget disease is linked to a novel mutation in the VCP gene. Neurology 65, 1304–1305.
Henkel, J.S., Beers, D.R., Wen, S., Rivera, A.L., Toennis, K.M., Appel, J.E., Zhao, W., Moore, D.H., Powell, S.Z., Appel, S.H., 2013. Regulatory T-lymphocytes mediate amyotrophic lateral sclerosis progression and survival. EMBO Mol. Med. 5 (1), 64–79.
Hentati, A., Ouahchi, K., Pericak-Vance, M.A., Nijhawan, D., Ahmad, A., Yang, Y., Rimmler, J., Hung, W., Schlotter, B., Ahmed, A., Ben Hamida, M., Hentati, F., Siddique, T., 1998. Linkage of a commoner form of recessive amyotrophic lateral sclerosis to chromosome 15q15-q22 markers. Neurogenetics 2, 55–60.
Hermosura, M.C., Nayakanti, H., Dorovkov, M.V., Calderon, F.R., Ryazanov, A.G., Haymer, D.S., Garruto, R.M., 2005. A TRPM7 variant shows altered sensitivity to magnesium that may contribute to the pathogenesis of two Guamanian neurodegenerative disorders. Proc. Natl. Acad. Sci. U. S. A. 102, 11510–11515.
Hetz, C., Mollereau, B., 2014. Disturbance of endoplasmic reticulum proteostasis in neurodegenerative diseases. Nat. Rev. Neurosci. 15, 233–249.
Hetz, C., 2012. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. Nat. Rev. Mol. Cell Biol. 13, 89–102.
Hocking, L.J., Lucas, G.J.A., Daroszewska, A., Mangion, J., Olavesen, M., Cundy, T., Nicholson, G.C., Ward, L., Bennett, S.T., Wuyts, W., Van Hul, W., Ralston, S.H., 2002. Domain-specific mutations in sequestosome 1 (SQSTM1) cause familial and sporadic Paget's disease. Hum. Mol. Genet. 11, 2735–2739.
Hoffmann, D., Hoffmann, I., El Bayoumy, K., 2001. The less harmful cigarette: a controversial issue: a tribute to Ernst L. Wynder. Chem. Res. Toxicol. 14, 767–790.

Hooten, K.G., Beers, D.R., Zhao, W., Appel, S.H., 2015. Protective and toxic neuroinflammation in amyotrophic lateral sclerosis. Neurotherapeutics 12, 364–375.
Horner, R.D., Grambow, S.C., Coffman, C.J., Lindquist, J.H., Oddone, E.Z., Allen, K.D., Kasarskis, E.J., 2008. Amyotrophic lateral sclerosis among 1991 Gulf War veterans: evidence for a time-limited outbreak. Neuroepidemiology 31 (1), 28–32.
Huang, Y.H., Shih, C.M., Huang, C.J., Lin, C.M., Chou, C.M., Tsai, M.L., Liu, T.P., Chiu, J.F., Chen, C.T., 2006. Effects of cadmium on structure and enzymatic activity of Cu,Zn-SOD and oxidative status in neural cells. J. Cell Biochem. 98, 577–589.
Huisman, M.H.B., Seelen, M., van Doormaal, P.T.C., de Jong, S.W., De Vries, J.H.M., Van der Kooi, A.J., De Visser, M., Schelhaas, J., Van den Berg, L.H., Veldink, J.H., 2015. Effect of presymptomatic body mass index and consumption of fat and alcohol on myotrophic lateral sclerosis. JAMA Neurol. 72 (10), 1155–1162.
Hukema, R.K., Riemslagh, F.W., Melhem, S., van der Linde, H.C., Severijnen, L.A., Edbauer, D., Maas, A., Charlet-Berguerand, N., Willemsen, R., van Swieten, J.C., 2014. A new inducible transgenic mouse model for C9orf72-associated GGGGCC repeat expansion supports a gain-of-function mechanism in C9orf72-associated ALS and FTD. Acta Neuropathol. Commun. 2, 166.
Igaz, L.M., Kwong, L.K., Lee, E.B., Chen-Plotkin, A., Swanson, E., Unger, T., Malunda, J., Xu, Y., Winton, M.J., Trojanowski, J.Q., Lee, V.M., 2011. Dysregulation of the ALS-associated gene TDP-43 leads to neuronal death and degeneration in mice. J. Clin. Invest. 121, 726–738.
Iguchi, Y., Katsuno, M., Ikenaka, K., Ishigaki, S., Sobue, G., 2013. Amyotrophic lateral sclerosis: an update on recent genetic insights. J. Neurol. 260, 2917–2927.
Iida, A., Hosono, N., Sano, M., Kamei, T., Oshima, S., Tokuda, T., Kubo, M., Nakamura, Y., Ikegawa, S., 2012a. Optineurin mutations in Japanese amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 83, 233–235.
Iida, A., Hosono, N., Sano, M., Kamei, T., Oshima, S., Tokuda, T., Nakajima, M., Kubo, M., Nakamura, Y., Ikegawa, S., 2012b. Novel deletion mutations of OPTN in amyotrophic lateral sclerosis in Japanese. Neurobiol. Aging 33 (8) (1843. e19-24).
Imam, I., Ball, S., Wright, D., Hanemann, C.O., Zajicek, J., 2010. The epidemiology of motor neurone disease in two counties in the southwest of England. J. Neurol. 257, 977–981.
Ince, P.G., Highley, J.R., Kirby, J., Wharton, S.B., Takahashi, H., Strong, M.J., Shaw, P.J., 2011. Molecular pathology and genetic advances in amyotrophic lateral sclerosis: an emerging molecular pathway and the significance of glial pathology. Acta Neuropathol. (Berl.) 122, 657–671.
Ingre, C., Roos, P.M., Piehl, F., Kamel, F., Fang, F., 2015. Risk factors for amyotrophic lateral sclerosis. Clin. Epidemiol. 7, 181–193.
Ishiura, H., Takahashi, Y., Mitsui, J., Yoshida, S., Kihira, T., Kokubo, Y., Kuzuhara, S., Ranum, L.P.W., Tamaoki, T., Ichikawa, Y., Date, H., Goto, J., Tsuji, S., 2012. C9ORF72 repeat expansion in amyotrophic lateral sclerosis in the Kii peninsula of Japan. Arch. Neurol. 69, 1154–1158.
Janssen, C., Schmalbach, S., Boeselt, S., Sarlette, A., Dengler, R., Petri, S., 2010. Differential histone deacetylase mRNA expression patterns in amyotrophic lateral sclerosis. J. Neuropathol Exp. Neurol. 69, 573–581.
Jawaid, A., Paganoni, S., Hauser, C., Schulz, P.E., 2014. Trials of antidiabetic drugs in amyotrophic lateral sclerosis: proceed with caution? Neurodegener Dis. 13, 205–208.
Jo, S.A., Zhu, X., Marchionni, M.A., Burden, S.J., 1995. Neuregulins are concentrated at nerve-muscle synapses and activate ACh-receptor gene expression. Nature 373, 158–161.
Joardar, A., Menzl, J., Podolsky, T.C., Manzo, E., Estes, P.S., Ashford, S., Zarnescu, D.C., 2015. PPAR gamma activation is neuroprotective in a Drosophila model of ALS based on TDP-43. Hum. Mol. Genet. 24, 1741–1754.
Joensen, P., 2012. Incidence of amyotrophic lateral sclerosis in the Faroe Islands. Acta Neurol. Scand. 126, 62–66.
Johnson, F., Atchison, W., 2009a. Postnatal exposure to methylmercury enhances development of paralytic phenotype in SOD1-G93A female mice. In: The Toxicologist, Proceedings of the 48th Annual Meeting and ToxExpoÔ, Baltimore, MD, USA, 15–19 March 2009Oxford University Press, Society of Toxicology, Reston, VA, USA, pp. 2009.
Johnson, F.O., Atchison, W.D., 2009b. The role of environmental mercury, lead and pesticide exposure in development of amyotrophic lateral sclerosis. Neurotoxicology 30, 761–765.
Johnson, J.O., Mandrioli, J., Benatar, M., Abramzon, Y., Van Deerlin, V.M., Trojanowski, J.Q., Gibbs, J.R., Brunetti, M., Gronka, S., Wuu, J., Ding, J., McCluskey, L., Martinez-Lage, M., Falcone, D., Hernandez, D.G., Arepalli, S., Chong, S., Schymick, J.C., Rothstein, J., Landi, F., Wang, Y.-D., Calvo, A., Mora, G., Sabatelli, M., Monsurro, M.R., Battistini, S., Salvi, F., Spataro, R., Sola, P., Borghero, G., Galassi, G., Scholz, S.W., Taylor, J.P., Restagno, G., Chio, A., Traynor, B.J., 2010. Exome sequencing reveals VCP mutations as a cause of familial ALS. Neuron 68, 857–864.
Johnson, J.O., Pioro, E.P., Boehringer, A., Chia, R., Feit, H., Renton, A.E., Pliner, H.A., Abramzon, Y., Marangi, G., Winborn, B.J., Gibbs, J.R., Nalls, M.A., Morgan, S., Shoai, M., Hardy, J., Pittman, A., Orrell, R.W., Malaspina, A., Sidle, K.C., Fratta, P., Harms, M.B., Baloh, R.H., Pestronk, A., Weihl, C.C., Rogaeva, E., Zinman, L., Drory, V.E., Borghero, G., Mora, G., Calvo, A., Rothstein, J.D., Drepper, C., Sendtner, M., Singleton, A.B., Taylor, J.P., Cookson, M.R., Restagno, G., Sabatelli, M., Bowser, R., Chio, A., Traynor, B.J., 2014. Mutations in the Matrin 3 gene cause familial amyotrophic lateral sclerosis. Nat. Neurosci. 17, 664–666.
Josephs, K.A., Katsuse, O., Beccano-Kelly, D.A., Lin, W.L., Uitti, R.J., Fujino, Y., Boeve, B.F., Hutton, M.L., Baker, M.C., Dickson, D.W., 2006. Atypical progressive supranuclear palsy with corticospinal tract degeneration. J Neuropathol. Exp. Neurol. 65, 396–405.
Jovicic, A., Mertens, J., Boeynaems, S., Bogaert, E., Chai, N., Yamada, S.B., Paul 3rd, J.W., Sun, S., Herdy, J.R., Bieri, G., Kramer, N.J., Gage, F.H., Van Den Bosch, L., Robberecht, W., Gitler, A.D., 2015. Modifiers of C9orf72 dipeptide repeat toxicity connect nucleocytoplasmic transport defects to FTD/ALS. Nat. Neurosci. 18, 1226–1229.
Kabashi, E., Valdmanis, P.N., Dion, P., Spiegelman, D., McConkey, B.J., Vande Velde, C., Bouchard, J.-P., Lacomblez, L., Pochigaeva, K., Salachas, F., Pradat, P.-F., Camu, W., Meininger, V., Dupre, N., Rouleau, G.A., 2008. TARDBP mutations in individuals with sporadic and familial amyotrophic lateral sclerosis. Nat. Genet. 40, 572–574.
Kamel, F., Umbach, D.M., Lehman, T.A., Park, L.P., Munsat, T.L., Shefner, J.M., Sandler, D.P., Hu, H., Taylor, J.A., 2003. Amyotrophic lateral sclerosis, lead, and genetic susceptibility: polymorphisms in the delta-aminolevulinic acid dehydratase and vitamin D receptor genes. Environ. Health Perspect. 111, 1335–1339.
Kamel, F., Umbach, D.M., Hu, H., Munsat, T.L., Shefner, J.M., Taylor, J.A., Sandler, D.P., 2005. Lead exposure as a risk factor for amyotrophic lateral sclerosis. Neurodegener. Dis. 2, 195–201.
Kamel, F., Umbach, D.M., Bedlack, R.S., Richards, M., Watson, M., Alavanja, M.C., Blair, A., Hoppin, J.A., Schmidt, S., Sandler, D.P., 2012. Pesticide exposure and amyotrophic lateral sclerosis. Neurotoxicology 33, 457–462.
Kaneb, H.M., Sharp, P.S., Rahmani-Kondori, N., Wells, D.J., 2011. Metformin treatment has no beneficial effect in a dose-response survival study in the SOD1(G93A) mouse model of ALS and is harmful in female mice. PLoS One 6, e24189.
Kaneb, H.M., Folkmann, A.W., Belzil, V.V., Jao, L.-E., Leblond, C.S., Girard, S.L., Daoud, H., Noreau, A., Rochefort, D., Hince, P., Szuto, A., Levert, A., Vidal, S., Andre-Guimont, C., Camu, W., Bouchard, J.-P., Dupre, N., Rouleau, G.A., Wente, S.R., Dion, P.A., 2015. Deleterious mutations in the essential mRNA metabolism factor, hGle1, in amyotrophic lateral sclerosis. Hum. Mol. Genet. 24, 1363–1373.
Kanekura, K., Yagi, T., Cammack, A.J., Mahadevan, J., Kuroda, M., Harms, M.B., Miller, T.M., Urano, F., 2016. Poly-dipeptides encoded by the C9ORF72 repeats block global protein translation. Hum. Mol. Genet..
Kasarskis, E.J., Tandon, L., Lovell, M.A., Ehmann, W.D., 1995. Aluminum, calcium, and iron in the spinal cord of patients with sporadic amyotrophic lateral sclerosis using laser microprobe mass spectroscopy: a preliminary study. J. Neurol. Sci. 130, 203–208.
Kiaei, M., Kipiani, K., Chen, J., Calingasan, N.Y., Beal, M.F., 2005. Peroxisome proliferator-activated receptor-gamma agonist extends survival in transgenic mouse model of amyotrophic lateral sclerosis. Exp. Neurol. 191, 331–336.
Kiernan, M.C., Vucic, S., Cheah, B.C., Turner, M.R., Eisen, A., Hardiman, O., Burrell, J.R., Zoing, M.C., 2011. Amyotrophic lateral sclerosis. Lancet 377, 942–955.
Kilness, A.W., Hichberg, F.H., 1977. Amyotrophic lateral sclerosis in a high selenium environment. JAMA 237, 2843–2844.
Kim, H.J., Kim, N.C., Wang, Y.D., Scarborough, E.A., Moore, J., Diaz, Z., MacLea, K.S., Freibaum, B., Li, S., Molliex, A., Kanagaraj, A.P., Carter, R., Boylan, K.B., Wojtas, A.M., Rademakers, R., Pinkus, J.L., Greenberg, S.A., Trojanowski, J.Q., Traynor, B.J., Smith, B.N., Topp, S., Gkazi, A.S., Miller, J., Shaw, C.E., Kottlors, M., Kirschner, J., Pestronk, A., Li, Y.R., Ford, A.F., Gitler, A.D., Benatar, M., King, O.D., Kimonis, V.E., Ross, E.D., Weihl, C.C., Shorter, J., Taylor, J.P., 2013. Mutations in prion-like domains in hnRNPA2B1 and hnRNPA1 cause multisystem proteinopathy and ALS. Nature 495, 467–473.
King, O.D., Gitler, A.D., Shorter, J., 2012. The tip of the iceberg: RNA-binding proteins with prion-like domains in neurodegenerative disease. Brain Res. 1462, 61–80.
King, I.N., Yartseva, V., Salas, D., Kumar, A., Heidersbach, A., Ando, D.M., Stallings, N.R., Elliott, J.L., Srivastava, D., Ivey, K.N., 2014. The RNA-binding protein TDP-43 selectively disrupts microRNA-1/206 incorporation into the RNA-induced silencing complex. J. Biol. Chem. 289, 14263–14271.
Kira, Y., Nishikawa, M., Ochi, A., Sato, E., Inoue, M., 2006. L-carnitine suppresses the onset of neuromuscular degeneration and increases the life span of mice with familial amyotrophic lateral sclerosis. Brain Res. 1070, 206–214.
Koppers, M., Blokhuis, A.M., Westeneng, H.J., Terpstra, M.L., Zundel, C.A., Vieira de Sa, R., Schellevis, R.D., Waite, A.J., Blake, D.J., Veldink, J.H., van den Berg, L.H., Pasterkamp, R.J., 2015. C9orf72 ablation in mice does not cause motor neuron degeneration or motor deficits. Ann. Neurol. 78, 426–438.
Koval, E.D., Shaner, C., Zhang, P., du Maine, X., Fischer, K., Tay, J., Chau, B.N., Wu, G.F., Miller, T.M., 2013. Method for widespread microRNA-155 inhibition prolongs survival in ALS-model mice. Hum. Mol. Genet. 22, 4127–4135.
Kraft, C., Peter, M., Hofmann, K., 2010. Selective autophagy: ubiquitin-mediated recognition and beyond. Nat. Cell Biol. 12, 836–841.
Kurahashi, T., Konno, T., Otsuki, N., Kwon, M., Tsunoda, S., Ito, J., Fujii, J., 2012. A malfunction in triglyceride transfer from the intracellular lipid pool to apoB in enterocytes of SOD1-deficient mice. FEBS Lett. 586, 4289–4295.
Kuzuhara, S., Kokubo, Y., 2005. Atypical parkinsonism of Japan: amyotrophic lateral sclerosis-parkinsonism-dementia complex of the Kii peninsula of Japan (Muro disease): an update. Mov. Disord. Off. J. Mov. Disord. Soc. 20 (Suppl. 12), S108–S113.
Kuzuhara, S., 2011. Muro disease: amyotrophic lateral sclerosis/parkinsonism-dementia complex in Kii peninsula of Japan. Brain Nerve Shinkei Kenkyu No Shinpo 63, 119–129.
Kwiatkowski, T.J.J., Bosco, D.A., Leclerc, A.L., Tamrazian, E., Vanderburg, C.R., Russ, C., Davis, A., Gilchrist, J., Kasarskis, E.J., Munsat, T., Valdmanis, P.,

Rouleau, G.A., Hosler, B.A., Cortelli, P., de Jong, P.J., Yoshinaga, Y., Haines, J.L., Pericak-Vance, M.A., Yan, J., Ticozzi, N., Siddique, T., McKenna-Yasek, D., Sapp, P.C., Horvitz, H.R., Landers, J.E., Brown, R.H.J., 2009. Mutations in the FUS/TLS gene on chromosome 16 cause familial amyotrophic lateral sclerosis. Science 323, 1205–1208.

Kwon, I., Xiang, S., Kato, M., Wu, L., Theodoropoulos, P., Wang, T., Kim, J., Yun, J., Xie, Y., McKnight, S.L., 2014. Poly-dipeptides encoded by the C9orf72 repeats bind nucleoli, impede RNA biogenesis, and kill cells. Science 345, 1139–1145.

López-López, A., Gámez, J., Syriani, E., Morales, M., Salvado, M., Rodriguez, M.J., Mahy, N., Vidal-Taboada, J.M., 2014. CX3CR1 is a modifying gene of survival and progression in amyotrophic lateral sclerosis. PLoS One 9 (5), e96528.

Lacomblez, L., Bensimon, G., Leigh, P.N., Guillet, P., Powe, L., Durrleman, S., Delumeau, J.C., Meininger, V., 1996. A confirmatory dose-ranging study of riluzole in ALS. ALS/Riluzole Study Group-II. Neurology 47, S242–S250.

Landers, J.E., Leclerc, A.L., Shi, L., Virkud, A., Cho, T., Maxwell, M.M., Henry, A.F., Polak, M., Glass, J.D., Kwiatkowski, T.J., Al-Chalabi, A., Shaw, C.E., Leigh, P.N., Rodriguez-Leyza, I., McKenna-Yasek, D., Sapp, P.C., Brown Jr., R.H., 2008. New VAPB deletion variant and exclusion of VAPB mutations in familial ALS. Neurology 70, 1179–1185.

Lannuzel, A., Mecharles, S., Tressières, B., Demoly, A., Alhendi, R., Hédreville-Tablon, M.A., Alecu, C., 2015. Clinical varieties and epidemiological aspects of amyotrophic lateral sclerosis in the Caribbean island of Guadeloupe: a new focus of ALS associated with Parkinsonism. Amyotroph Lateral Scler Frontotemporal Degener. 16 (3-4), 216–223.

Laurin, N., Brown, J.P., Morissette, J., Raymond, V., 2002. Recurrent mutation of the gene encoding sequestosome 1 (SQSTM1/p62) in Paget disease of bone. Am. J. Hum. Genet. 70, 1582–1588.

Lazo-Gomez, R., Ramirez-Jarquin, U.N., Tovar-Y-Romo, L.B., Tapia, R., 2013. Histone deacetylases and their role in motor neuron degeneration. Front. Cell. Neurosci. 7, 243.

Lee, J.W., Beebe, K., Nangle, L.A., Jang, J., Longo-Guess, C.M., Cook, S.A., Davisson, M.T., Sundberg, J.P., Schimmel, P., Ackerman, S.L., 2006. Editing-defective tRNA synthetase causes protein misfolding and neurodegeneration. Nature 443, 50–55.

Lee, T., Li, Y.R., Ingre, C., Weber, M., Grehl, T., Gredal, O., de Carvalho, M., Meyer, T., Tysnes, O.-B., Auburger, G., Gispert, S., Bonini, N.M., Andersen, P.M., Gitler, A.D., 2011a. Ataxin-2 intermediate-length polyglutamine expansions in European ALS patients. Hum. Mol. Genet. 20, 1697–1700.

Lee, E.B., Lee, V.M., Trojanowski, J.Q., 2011b. Gains or losses: molecular mechanisms of TDP43-mediated neurodegeneration. Nat. Rev. Neurosci. 13, 38–50.

Lee, D.Y., Jeon, G.S., Shim, Y.M., Seong, S.Y., Lee, K.W., Sung, J.J., 2015. Modulation of SOD1 subcellular localization by transfection with wild- or mutant-type SOD1 in primary neuron and astrocyte cultures from ALS mice. Exp. Neurobiol. 24, 226–234.

Lefebvre, S., Burglen, L., Reboullet, S., Clermont, O., Burlet, P., Viollet, L., Benichou, B., Cruaud, C., Millasseau, P., Zeviani, M., 1995. Identification and characterization of a spinal muscular atrophy-determining gene. Cell 80, 155–165.

Lekoubou, A., Matsha, T.E., Sobngwi, E., Kengne, A.P., 2014. Effects of diabetes mellitus on amyotrophic lateral sclerosis: a systematic review. BMC Res. Notes 7, 171.

Levine, T.D., Bowser, R., Hank, N.C., Gately, S., Stephan, D., Saperstein, D.S., Van Keuren-Jensen, K., 2012. A pilot trial of pioglitazone HCl and tretinoin in ALS: cerebrospinal fluid biomarkers to monitor drug efficacy and predict rate of disease progression. Neurol. Res. Int. 2012, 582075.

Li, Y., Chigurupati, S., Holloway, H.W., Mughal, M., Tweedie, D., Bruestle, D.A., Mattson, M.P., Wang, Y., Harvey, B.K., Ray, B., Lahiri, D.K., Greig, N.H., 2012. Exendin-4 ameliorates motor neuron degeneration in cellular and animal models of amyotrophic lateral sclerosis. PLoS One 7, e32008.

Li, P., Ruan, X., Yang, L., Kiesewetter, K., Zhao, Y., Luo, H., Chen, Y., Gucek, M., Zhu, J., Cao, H., 2015. A liver-enriched long non-coding RNA, lncLSTR, regulates systemic lipid metabolism in mice. Cell. Metab. 21, 455–467.

Lill, C.M., Abel, O., Bertram, L., Al-Chalabi, A., 2011. Keeping up with genetic discoveries in amyotrophic lateral sclerosis: the ALSoD and ALSGene databases. Amyotroph. Lateral Scler. Off. Publ. World Fed. Neurol. Res. Group Mot. Neuron Dis. 12, 238–249.

Lim, M.A., Selak, M.A., Xiang, Z., Krainc, D., Neve, R.L., Kraemer, B.C., Watts, J.L., Kalb, R.G., 2012. Reduced activity of AMP-activated protein kinase protects against genetic models of motor neuron disease. J. Neurosci. 32, 1123–1141.

Limousin, N., Blasco, H., Corcia, P., Gordon, P.H., De Toffol, B., Andres, C., Praline, J., 2010. Malnutrition at the time of diagnosis is associated with a shorter disease duration in ALS. J. Neurol. Sci. 297, 36–39.

Ling, J.P., Pletnikova, O., Troncoso, J.C., Wong, P.C., 2015. NEURODEGENERATION: TDP-43 repression of nonconserved cryptic exons is compromised in ALS-FTD. Science 349, 650–655.

Liu, E.Y., Russ, J., Wu, K., Neal, D., Suh, E., McNally, A.G., Irwin, D.J., Van Deerlin, V.M., Lee, E.B., 2014. C9orf72 hypermethylation protects against repeat expansion-associated pathology in ALS/FTD. Acta Neuropathol. 128, 525–541.

Liu, Y.J., Ju, T.C., Chen, H.M., Jang, Y.S., Lee, L.M., Lai, H.L., Tai, H.C., Fang, J.M., Lin, Y.L., Tu, P.H., Chern, Y., 2015. Activation of AMP-activated protein kinase alpha1 mediates mislocalization of TDP-43 in amyotrophic lateral sclerosis. Hum. Mol. Genet. 24, 787–801.

Lodhi, I.J., Bridges, D., Chiang, S.H., Zhang, Y., Cheng, A., Geletka, L.M., Weisman, L.S., Saltiel, A.R., 2008. Insulin stimulates phosphatidylinositol 3-phosphate production via the activation of Rab5. Mol. Biol. Cell 19, 2718–2728.

Logroscino, G., Traynor, B.J., Hardiman, O., Chio, A., Mitchell, D., Swingler, R.J., Millul, A., Benn, E., Beghi, E., 2010. Incidence of amyotrophic lateral sclerosis in Europe. J. Neurol. Neurosurg. Psychiatry 81, 385–390.

Lu, H., Liu, X., Deng, Y., Qing, H., 2013. DNA methylation, a hand behind neurodegenerative diseases. Front. Aging Neurosci. 5, 85.

Mackenzie, I.R.A., Bigio, E.H., Ince, P.G., Geser, F., Neumann, M., Cairns, N.J., Kwong, L.K., Forman, M.S., Ravits, J., Stewart, H., Eisen, A., McClusky, L., Kretzschmar, H.A., Monoranu, C.M., Highley, J.R., Kirby, J., Siddique, T., Shaw, P.J., Lee, V.M.-Y., Trojanowski, J.Q., 2007. Pathological TDP-43 distinguishes sporadic amyotrophic lateral sclerosis from amyotrophic lateral sclerosis with SOD1 mutations. Ann. Neurol. 61, 427–434.

Mackenzie, I.R., Rademakers, R., Neumann, M., 2010. TDP-43 and FUS in amyotrophic lateral sclerosis and frontotemporal dementia. Lancet Neurol. 9 (10), 995–1007.

Majounie, E., Renton, A.E., Mok, K., Dopper, E.G.P., Waite, A., Rollinson, S., Chio, A., Restagno, G., Nicolaou, N., Simon-Sanchez, J., van Swieten, J.C., Abramzon, Y., Johnson, J.O., Sendtner, M., Pamphlett, R., Orrell, R.W., Mead, S., Sidle, K.C., Houlden, H., Rohrer, J.D., Morrison, K.E., Pall, H., Talbot, K., Ansorge, O., Hernandez, D.G., Arepalli, S., Sabatelli, M., Mora, G., Corbo, M., Giannini, F., Calvo, A., Englund, E., Borghero, G., Floris, G.L., Remes, A.M., Laaksovirta, H., McCluskey, L., Trojanowski, J.Q., Van Deerlin, V.M., Schellenberg, G.D., Nalls, M.A., Drory, V.E., Lu, C.-S., Yeh, T.-H., Ishiura, H., Takahashi, Y., Tsuji, S., Le Ber, I., Brice, A., Drepper, C., Williams, N., Kirby, J., Shaw, P., Hardy, J., Tienari, P.J., Heutink, P., Morris, H.R., Pickering-Brown, S., Traynor, B.J., 2012. Frequency of the C9orf72 hexanucleotide repeat expansion in patients with amyotrophic lateral sclerosis and frontotemporal dementia: a cross-sectional study. Lancet Neurol. 11, 323–330.

Malek, A.M., Barchowsky, A., Bowser, R., Youk, A., Talbott, E.O., 2012. Pesticide exposure as a risk factor for amyotrophic lateral sclerosis: a meta-analysis of epidemiological studies: pesticide exposure as a risk factor for ALS. Environ. Res. 117, 112–119.

Mancuso, R., Olivan, S., Rando, A., Casas, C., Osta, R., Navarro, X., 2012. Sigma-1R agonist improves motor function and motoneuron survival in ALS mice. Neurotherapeutics 9, 814–826.

Maraldi, T., Riccio, M., Zambonin, L., Vinceti, M., De Pol, A., Hakim, G., 2011. Low levels of selenium compounds are selectively toxic for a human neuron cell line through ROS/RNS increase and apoptotic process activation. Neurotoxicology 32, 180–187.

Mariosa, D., Kamel, F., Bellocco, R., Ye, W., Fang, F., 2015. Association between diabetes and amyotrophic lateral sclerosis in Sweden. Eur. J. Neurol. 22 (11), 1436–1442.

Maruyama, H., Morino, H., Ito, H., Izumi, Y., Kato, H., Watanabe, Y., Kinoshita, Y., Kamada, M., Nodera, H., Suzuki, H., Komure, O., Matsuura, S., Kobatake, K., Morimoto, N., Abe, K., Suzuki, N., Aoki, M., Kawata, A., Hirai, T., Kato, T., Ogasawara, K., Hirano, A., Takumi, T., Kusaka, H., Hagiwara, K., Kaji, R., Kawakami, H., 2010. Mutations of optineurin in amyotrophic lateral sclerosis. Nature 465, 223–226.

Masseret, E., Banack, S., Boumédiène, F., Abadie, E., Brient, L., Pernet, F., Juntas-Morales, R., Pageot, N., Metcalf, J., Cox, P., Camu, W., 2013. Dietary BMAA exposure in an amyotrophic lateral sclerosis cluster from southern France. French Network on ALS Clusters Detection and Investigation PLoS One 8 (12), e83406.

May, S., Hornburg, D., Schludi, M.H., Arzberger, T., Rentzsch, K., Schwenk, B.M., Grasser, F.A., Mori, K., Kremmer, E., Banzhaf-Strathmann, J., Mann, M., Meissner, F., Edbauer, D., 2014. C9orf72 FTLD/ALS-associated Gly-Ala dipeptide repeat proteins cause neuronal toxicity and Unc119 sequestration. Acta Neuropathol. 128, 485–503.

Millecamps, S., Boillee, S., Le Ber, I., Seilhean, D., Teyssou, E., Giraudeau, M., Moigneu, C., Vandenberghe, N., Danel-Brunaud, V., Corcia, P., Pradat, P.F., Le Forestier, N., Lacomblez, L., Bruneteau, G., Camu, W., Brice, A., Cazeneuve, C., Leguern, E., Meininger, V., Salachas, F., 2012. Phenotype difference between ALS patients with expanded repeats in C9ORF72 and patients with mutations in other ALS-related genes. J. Med. Genet. 49, 258–263.

Millecamps, S., De Septenville, A., Teyssou, E., Daniau, M., Camuzat, A., Albert, M., LeGuern, E., Galimberti, D., 2014. Genetic analysis of matrin 3 gene in French amyotrophic lateral sclerosis patients and frontotemporal lobar degeneration with amyotrophic lateral sclerosis patients. Neurobiol. Aging 35 (12) (2882. e13-5).

Mizielinska, S., Gronke, S., Niccoli, T., Ridler, C.E., Clayton, E.L., Devoy, A., Moens, T., Norona, F.E., Woollacott, I.O., Pietrzyk, J., Cleverley, K., Nicoll, A.J., Pickering-Brown, S., Dols, J., Cabecinha, M., Hendrich, O., Fratta, P., Fisher, E.M., Partridge, L., Isaacs, A.M., 2014. C9orf72 repeat expansions cause neurodegeneration in Drosophila through arginine-rich proteins. Science 345, 1192–1194.

Mok, K.Y., Koutsis, G., Schottlaender, L.V., Polke, J., Panas, M., Houlden, H., 2012. High frequency of the expanded C9ORF72 hexanucleotide repeat in familial and sporadic Greek ALS patients. Neurobiol. Aging 33 (8) (1851. e1-5).

Morahan, J.M., Yu, B., Trent, R.J., Pamphlett, R., 2007. Genetic susceptibility to environmental toxicants in ALS. Am. J. Med. Genet. B Neuropsychiatr. Genet. 144B, 885–890.

Moriwaka, F., Tashiro, K., Shima, K., Hamada, T., 1993. Glucagon and ALS. Neurology 43, 1061.

Morrison, K.E., Dhariwal, S., Hornabrook, R., Savage, L., Burn, D.J., Khoo, T.K., Kelly, J., Murphy, C.L., Al-Chalabi, A., Dougherty, A., Leigh, P.N., Wijesekera, L., Thornhill, M., Ellis, C.M., O'Hanlon, K., Panicker, J., Pate, L., Ray, P., Wyatt, L., Young, C.A., Copeland, L., Ealing, J., Hamdalla, H., Leroi, I., Murphy, C.,

O'Keeffe, F., Oughton, E., Partington, L., Paterson, P., Rog, D., Sathish, A., Sexton, D., Smith, J., Vanek, H., Dodds, S., Williams, T.L., Steen, I.N., Clarke, J., Eziefula, C., Howard, R., Orrell, R., Sidle, K., Sylvester, R., Barrett, W., Merritt, C., Talbot, K., Turner, M.R., Whatley, C., Williams, C., Williams, J., Cosby, C., Hanemann, C.O., Iman, I., Philips, C., Timings, L., Crawford, S.E., Hewamadduma, C., Hibberd, R., Hollinger, H., McDermott, C., Mils, G., Rafiq, M., Shaw, P.J., Taylor, A., Waines, E., Walsh, T., Addison-Jones, R., Birt, J., Hare, M., Majid, T., 2013. Lithium in patients with amyotrophic lateral sclerosis (LiCALS): a phase 3 multicentre, randomised, double-blind, placebo-controlled trial. Lancet Neurol. 12, 339–345.

Murch, S.J., Cox, P.A., Banack, S.A., Steele, J.C., Sacks, O.W., 2004a. Occurrence of beta-methylamino-l-alanine (BMAA) in ALS/PDC patients from Guam. Acta Neurol. Scand. 110, 267–269.

Murch, S.J., Cox, P.A., Banack, S.A., 2004b. A mechanism for slow release of biomagnified cyanobacterial neurotoxins and neurodegenerative disease in Guam. Proc. Natl. Acad. Sci. U. S. A 101, 12228–12231.

Muscogiuri, G., Salmon, A.B., Aguayo-Mazzucato, C., Li, M., Balas, B., Guardado-Mendoza, R., Giaccari, A., Reddick, R.L., Reyna, S.M., Weir, G., Defronzo, R.A., Van Remmen, H., Musi, N., 2013. Genetic disruption of SOD1 gene causes glucose intolerance and impairs beta-cell function. Diabetes 62, 4201–4207.

Nagy, J.I., Yamamoto, T., Jordan, L.M., 1993. Evidence for the cholinergic nature of C-terminals associated with subsurface cisterns in alpha-motoneurons of rat. Synapse 15, 17–32.

Neumann, M., Sampathu, D.M., Kwong, L.K., Truax, A.C., Micsenyi, M.C., Chou, T.T., Bruce, J., Schuck, T., Grossman, M., Clark, C.M., McCluskey, L.F., Miller, B.L., Masliah, E., Mackenzie, I.R., Feldman, H., Feiden, W., Kretzschmar, H.A., Trojanowski, J.Q., Lee, V.M.-Y., 2006. Ubiquitinated TDP-43 in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Science 314, 130–133.

Neumann, M., Rademakers, R., Roeber, S., Baker, M., Kretzschmar, H.A., Mackenzie, I.R.A., 2009. A new subtype of frontotemporal lobar degeneration with FUS pathology. Brain J. Neurol. 132, 2922–2931.

Ngo, S.T., Steyn, F.J., Huang, L., Mantovani, S., Pfluger, C.M., Woodruff, T.M., O'Sullivan, J.D., Henderson, R.D., McCombe, P.A., 2015. Altered expression of metabolic proteins and adipokines in patients with amyotrophic lateral sclerosis. J. Neurol. Sci. 357 (1–2), 22–27.

Nie, C.L., Wang, X.S., Liu, Y., Perrett, S., He, R.Q., 2007. Amyloid-like aggregates of neuronal tau induced by formaldehyde promote apoptosis of neuronal cells. BMC Neurosci. 8, 9.

Nishimura, A.L., Mitne-Neto, M., Silva, H.C., Richieri-Costa, A., Middleton, S., Cascio, D., Kok, F., Oliveira, J.R., Gillingwater, T., Webb, J., Skehel, P., Zatz, M., 2004. A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am. J. Hum. Genet. 75, 822–831.

Nodera, H., Takamatsu, N., Muguruma, N., Ukimoto, K., Nishio, S., 2015. Frequent hepatic steatosis in amyotrophic lateral sclerosis: implication for systemic involvement. Neurol. Clin. Neurosci. 3, 58–62.

Nutini, M., Frazzini, V., Marini, C., Spalloni, A., Sensi, S.L., Longone, P., 2011. Zinc pre-treatment enhances NMDAR-mediated excitotoxicity in cultured cortical neurons from SOD1(G93A) mouse, a model of amyotrophic lateral sclerosis. Neuropharmacology 60, 1200–1208.

O'Reilly, E.J., Wang, H., Weisskopf, M.G., Fitzgerald, K.C., Falcone, G., McCullough, M.L., Thun, M., Park, Y., Kolonel, L.N., Ascherio, A., 2013. Premorbid body mass index and risk of amyotrophic lateral sclerosis. Amyotroph Lateral Scler Frontotemporal Degener. 14, 205–211.

O'Rourke, J.G., Bogdanik, L., Muhammad, A.K., Gendron, T.F., Kim, K.J., Austin, A., Cady, J., Liu, E.Y., Zarrow, J., Grant, S., Ho, R., Bell, S., Carmona, S., Simpkinson, M., Lall, D., Wu, K., Daughrity, L., Dickson, D.W., Harms, M.B., Petrucelli, L., Lee, E.B., Lutz, C.M., Baloh, R.H., 2015. C9orf72 BAC transgenic mice display typical pathologic features of ALS/FTD. Neuron 88, 892–901.

O'Rourke, J.G., Bogdanik, L., Yanez, A., Lall, D., Wolf, A.J., Muhammad, A.K., Ho, R., Carmona, S., Vit, J.P., Zarrow, J., Kim, K.J., Bell, S., Harms, M.B., Miller, T.M., Dangler, C.A., Underhill, D.M., Goodridge, H.S., Lutz, C.M., Baloh, R.H., 2016. C9orf72 is required for proper macrophage and microglial function in mice. Science 351, 1324–1329.

O'Toole, O., Traynor, B.J., Brennan, P., Sheehan, C., Frost, E., Corr, B., Hardiman, O., 2008. Epidemiology and clinical features of amyotrophic lateral sclerosis in Ireland between 1995 and 2004. J. Neurol. Neurosurg. Psychiatry 79, 30–32.

Okamoto, Y., Ihara, M., Urushitani, M., Yamashita, H., Kondo, T., Tanigaki, A., Oono, M., Kawamata, J., Ikemoto, A., Kawamoto, Y., Takahashi, R., Ito, H., 2011. An autopsy case of SOD1-related ALS with TDP-43 positive inclusions. Neurology 77 (22), 1993–1995.

Okumiya, K., Wada, T., Fujisawa, M., Ishine, M., Garcia Del Saz, E., Hirata, Y., Kuzuhara, S., Kokubo, Y., Seguchi, H., Sakamoto, R., Manuaba, I., Watofa, P., Rantetampang, A.L., Matsubayashi, K., 2014. Amyotrophic lateral sclerosis and parkinsonism in Papua, Indonesia: 2001–2012 survey results. BMJ Open 4 (4), e004353.

Oyanagi, K., Yamazaki, M., Takahashi, H., Watabe, K., Wada, M., Komori, T., Morita, T., Mizutani, T., 2008. Spinal anterior horn cells in sporadic amyotrophic lateral sclerosis show ribosomal detachment from, and cisternal distention of the rough endoplasmic reticulum. Neuropathol. Appl. Neurobiol. 34, 650–658.

Palamiuc, L., Schlagowski, A., Ngo, S.T., Vernay, A., Dirrig-Grosch, S., Henriques, A., Boutillier, A.L., Zoll, J., Echaniz-Laguna, A., Loeffler, J.P., René, F., 2015. A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis. EMBO Mol Med. 7 (5), 526–546.

Pamphlett, R., Waley, P., 1998. Mercury in human spinal motor neurons. Acta Neuropathol. 96, 515–519.

Pare, B., Touzel-Deschenes, L., Lamontagne, R., Lamarre, M.S., Scott, F.D., Khuong, H.T., Dion, P.A., Bouchard, J.P., Gould, P., Rouleau, G.A., Dupre, N., Berthod, F., Gros-Louis, F., 2015. Early detection of structural abnormalities and cytoplasmic accumulation of TDP-43 in tissue-engineered skins derived from ALS patients. Acta Neuropathol. Commun. 3, (5-014-0181-z).

Parisi, C., Arisi, I., D'Ambrosi, N., Storti, A.E., Brandi, R., D'Onofrio, M., Volonte, C., 2013. Dysregulated microRNAs in amyotrophic lateral sclerosis microglia modulate genes linked to neuroinflammation. Cell Death Dis 4, e959.

Parkinson, N., Ince, P.G., Smith, M.O., Highley, R., Skibinski, G., Andersen, P.M., Morrison, K.E., Pall, H.S., Hardiman, O., Collinge, J., Shaw, P.J., Fisher, E.M., MRC Proteomics in ALS Study, FReJA Consortium, 2006. ALS phenotypes with mutations in CHMP2 B (charged multivesicular body protein 2B). Neurology 67, 1074–1077.

Patten, B.M., Kurlander, H.M., Evans, B., 1982. Free amino acid concentrations in spinal tissue from patients dying of motor neuron disease. Acta Neurol. Scand. 66, 594–599.

Pensato, V., Tiloca, C., Corrado, L., Bertolin, C., Sardone, V., Del Bo, R., Calini, D., Mandrioli, J., Lauria, G., Mazzini, L., Querin, G., Ceroni, M., Cantell, o, R., Corti, S., Castellotti, B., Soldà, G., Duga, S., Comi, G.P., Cereda, C., Sorarù, G., D'Alfonso, S., Taroni, F., Shaw, C.E., Landers, J.E., Ticozzi, N., Ratti, A., Gellera, C., Silani, V., the SLAGEN Consortium, 2015. TUBA4A gene analysis in sporadic amyotrophic lateral sclerosis: identification of novel mutations. J. Neurol. 262, 1376–1378.

Perera, N.D., Sheean, R.K., Scott, J.W., Kemp, B.E., Horne, M.K., Turner, B.J., 2014. Mutant TDP-43 deregulates AMPK activation by PP2A in ALS models. PLoS One 9, e95549.

Peters, O.M., Cabrera, G.T., Tran, H., Gendron, T.F., McKeon, J.E., Metterville, J., Weiss, A., Wightman, N., Salameh, J., Kim, J., Sun, H., Boylan, K.B., Dickson, D., Kennedy, Z., Lin, Z., Zhang, Y.J., Daughrity, L., Jung, C., Gao, F.B., Sapp, P.C., Horvitz, H.R., Bosco, D.A., Brown, S.P., de Jong, P., Petrucelli, L., Mueller, C., Brown Jr., R.H., 2015. Human C9ORF72 hexanucleotide expansion reproduces RNA foci and dipeptide repeat proteins but not neurodegeneration in BAC transgenic mice. Neuron 88, 902–909.

Philips, T., Robberecht, W., 2011. Neuroinflammation in amyotrophic lateral sclerosis: role of glial activation in motor neuron disease. Lancet Neurol. 10 (3), 253–263.

Piao, Y.S., Tan, C.F., Iwanaga, K., Kakita, A., Takano, H., Nishizawa, M., Lashley, T., Revesz, T., Lees, A., de Silva, R., Tsujihata, M., Takahashi, H., 2005. Sporadic four-repeat tauopathy with frontotemporal degeneration, parkinsonism and motor neuron disease. Acta Neuropathol. 110, 600–609.

Pokrishevsky, E., Grad, L.I., Cashman, N.R., 2016. TDP-43 or FUS-induced misfolded human wild-type SOD1 can propagate intercellularly in a prion-like fashion. Sci. Rep. 6, 22155.

Poulletier, D.G., Ruffie, G., Taxile, M., Ladeveze, E., Hurtier, A., Haro, E., Duleu, S., Charlet d, S., Billaudel, B., Geffard, M., Veyret, B., Lagroye, I., 2009. Amyotrophic lateral sclerosis (ALS) and extremely-low frequency (ELF) magnetic fields: a study in the SOD-1 transgenic mouse model. Amyotroph. Lateral. Scler. 10, 370–373.

Pradas, J., Puig, T., Rojas-Garcia, R., Viguera, M.L., Gich, I., Logroscino, G., 2013. Amyotrophic lateral sclerosis in Catalonia: a population based study: amyotroph. Lateral Scler. Front. Degener. 14, 278–283.

Pradat, P.F., Bruneteau, G., Munerati, E., Salachas, F., Le Forestier, N., Lacomblez, L., Lenglet, T., Meininger, V., 2009. Extrapyramidal stiffness in patients with amyotrophic lateral sclerosis. Mov. Disord. 24 (14), 2143–2148.

Pradat, P.F., Bruneteau, G., Gordon, P.H., Dupuis, L., Bonnefont-Rousselot, D., Simon, D., Salachas, F., Corcia, P., Frochot, V., Lacorte, J.M., Jardel, C., Coussieu, C., Le Forestier, N., Lacomblez, L., Loeffler, J.P., Meininger, V., 2010. Impaired glucose tolerance in patients with amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 11 (1-2), 166–171.

Praline, J., Guennoc, A.M., Limousin, N., Hallak, H., de Toffol, B., Corcia, P., 2007. ALS and mercury intoxication: a relationship? Clin. Neurol. Neurosurg. 109, 880–883.

Praline, J., Blasco, H., Vourc'h, P., Rat, V., Gendrot, C., Camu, W., Andres, C.R., 2012. Study of the HFE gene common polymorphisms in French patients with sporadic amyotrophic lateral sclerosis. J. Neurol. Sci. 317 (1–2), 58–61.

Prause, J., Goswami, A., Katona, I., Roos, A., Schnizler, M., Bushuven, E., Dreier, A., Buchkremer, S., Johann, S., Beyer, C., Deschauer, M., Troost, D., Weis, J., 2013. Altered localization, abnormal modification and loss of function of Sigma receptor-1 in amyotrophic lateral sclerosis. Hum. Mol. Genet. 22, 1581–1600.

Puentes, F., Malaspina, A., van Noort, J.M., Amor, S., 2016. Non-neuronal cells in ALS: Role of glial, immune cells and blood-CNS barriers. Brain Pathol..

Pupillo, E., Messina, P., Giussani, G., Logroscino, G., Zoccolella, S., Chio, A., Calvo, A., Corbo, M., Lunetta, C., Marin, B., Mitchell, D., Hardiman, O., Rooney, J., Stevic, Z., Bandettini di Poggio, M., Filosto, M., Cotelli, M.S., Perini, M., Riva, N., Tremolizzo, L., Vitelli, E., Damiani, D., Beghi, E., 2014. Physical activity and amyotrophic lateral sclerosis: a European population-based case-control study. Ann. Neurol. 75, 708–716.

Rakhit, R., Cunningham, P., Furtos-Matei, A., Dahan, S., Qi, X.F., Crow, J.P., Cashman, N.R., Kondejewski, L.H., Chakrabartty, A., 2002. Oxidation-induced misfolding and aggregation of superoxide dismutase and its implications for amyotrophic lateral sclerosis. J. Biol. Chem. 277, 47551–47556.

Ratti, A., Corrado, L., Castellotti, B., Del Bo, R., Fogh, I., Cereda, C., Tiloca, C., D'Ascenzo, C., Bagarotti, A., Pensato, V., Ranieri, M., Gagliardi, S., Calini, D., Mazzini, L., Taroni, F., Corti, S., Ceroni, M., Oggioni, G.D., Lin, K., Powell, J.F.,

Soraru, G., Ticozzi, N., Comi, G.P., D'Alfonso, S., Gellera, C., Silani, V., SLAGEN Consortium, 2012. C9ORF72 repeat expansion in a large Italian ALS cohort: evidence of a founder effect. Neurobiol. Aging 33, (2528. e7-2528.14).
Reaume, A.G., Elliott, J.L., Hoffman, E.K., Kowall, N.W., Ferrante, R.J., Siwek, D.F., Wilcox, H.M., Flood, D.G., Beal, M.F., Brown, R.H.J., Scott, R.W., Snider, W.D., 1996. Motor neurons in Cu/Zn superoxide dismutase-deficient mice develop normally but exhibit enhanced cell death after axonal injury. Nat. Genet. 13, 43–47.
Redler, R.L., Wilcox, K.C., Proctor, E.A., Fee, L., Caplow, M., Dokholyan, N.V., 2011. Glutathionylation at Cys-111 induces dissociation of wild type and FALS mutant SOD1 dimers. Biochem. (Mosc.) 50, 7057–7066.
Renton, A.E., Majounie, E., Waite, A., Simon-Sanchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peuralinna, T., Jansson, L., Isoviita, V.-M., Kaivorinne, A.-L., Holtta-Vuori, M., Ikonen, E., Sulkava, R., Benatar, M., Wuu, J., Chio, A., Restagno, G., Borghero, G., Sabatelli, M., Heckerman, D., Rogaeva, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. Neuron 72, 257–268.
Reyes, E.T., Perurena, O.H., Festoff, B.W., Jorgensen, R., Moore, W.V., 1984. Insulin resistance in amyotrophic lateral sclerosis. J. Neurol. Sci. 63, 317–324.
Riancho, J., Ruiz-Soto, M., Villagrá, N.T., Berciano, J., Berciano, M.T., Lafarga, M., 2014. Compensatory motor neuron response to chromatolysis in the murine hSOD1 G93A model of amyotrophic lateral sclerosis. Fron. Cell. Neurosci. 8 .
Riancho, J., Ruiz-Soto, M., Berciano, M.T., Berciano, J., Lafarga, M., 2015. Neuroprotective effect of bexarotene in the SOD1(G93A) mouse model of amyotrophic lateral sclerosis. Front. Cell. Neurosci. 9, 250.
Riancho, J., Gonzalo, I., Ruiz-Soto, M., Berciano, J., 2016. Why do motor neurons degenerate? Actualization in the pathogenesis of amyotrophic lateral sclerosis. Neurologia.
Riviere, M., Meininger, V., Zeisser, P., Munsat, T., 1998. An analysis of extended survival in patients with amyotrophic lateral sclerosis treated with riluzole. Arch. Neurol. 55, 526–528.
Robberecht, W., Philips, T., 2013. The changing scene of amyotrophic lateral sclerosis. Nat. Rev. Neurosci. 14, 248–264.
Roberts, A.L., Johnson, N.J., Cudkowicz, M.E., Eum, K.D., Weisskopf, M.G., 2015. Job-related formaldehyde exposure and ALS mortality in the USA. J. Neurol. Neurosurg. Psychiatry.
Rodriguez, A., Duran, A., Selloum, M., Champy, M.F., Diez-Guerra, F.J., Flores, J.M., Serrano, M., Auwerx, J., Diaz-Meco, M.T., Moscat, J., 2006. Mature-onset obesity and insulin resistance in mice deficient in the signaling adapter p62. Cell. Metab. 3 (3), 211–222.
Rojas-Garcia, R., Scott, K.M., Roche, J.C., Scotton, W., Martin, N., Janssen, A., Goldstein, L.H., Leigh, P.N., Ellis, C.M., Shaw, C.E., Al-Chalabi, A., 2012. No evidence for a large difference in ALS frequency in populations of African and European origin: a population based study in inner city London. Amyotroph. Lateral Scler. 13 (1), 66–68.
Rooney, J., 2011. Further thoughts on mercury, epigenetics, genetics and amyotrophic lateral sclerosis. Neurodegener. Dis. 8, 523–524.
Rosen, D.R., Siddique, T., Patterson, D., Figlewicz, D.A., Sapp, P., Hentati, A., Donaldson, D., Goto, J., O'Regan, J.P., Deng, H.X., 1993. Mutations in Cu/Zn superoxide dismutase gene are associated with familial amyotrophic lateral sclerosis. Nature 364 (6435), 362.
Ross, O.A., Rutherford, N.J., Baker, M., Soto-Ortolaza, A.I., Carrasquillo, M.M., DeJesus-Hernandez, M., Adamson, J., Li, M., Volkening, K., Finger, E., Seeley, W.W., Hatanpaa, K.J., Lomen-Hoerth, C., Kertesz, A., Bigio, E.H., Lippa, C., Woodruff, B.K., Knopman, D.S., White 3rd, C.L., Van Gerpen, J.A., Meschia, J.F., Mackenzie, I.R., Boylan, K., Boeve, B.F., Miller, B.L., Strong, M.J., Uitti, R.J., Younkin, S.G., Graff-Radford, N.R., Petersen, R.C., Wszolek, Z.K., Dickson, D.W., Rademakers, R., 2011. Ataxin-2 repeat-length variation and neurodegeneration. Hum. Mol. Genet. 20, 3207–3212.
Rossi, S., Serrano, A., Gerbino, V., Giorgi, A., Di Francesco, L., Nencini, M., Bozzo, F., Schinina, M.E., Bagni, C., Cestra, G., Carri, M.T., Achsel, T., Cozzolino, M., 2015. Nuclear accumulation of mRNAs underlies G4C2-repeat-induced translational repression in a cellular model of C9orf72 ALS. J. Cell. Sci. 128, 1787–1799.
Rothstein, J.D., Van Kammen, M., Levey, A.I., Martin, L.J., Kuncl, R.W., 1995. Selective loss of glial glutamate transporter GLT-1 in amyotrophic lateral sclerosis. Ann. Neurol. 38, 73–84.
Rothstein, J.D., Patel, S., Regan, M.R., Haenggeli, C., Huang, Y.H., Bergles, D.E., Jin, L., Dykes Hoberg, M., Vidensky, S., Chung, D.S., Toan, S.V., Bruijn, L.I., Su, Z.Z., Gupta, P., Fisher, P.B., 2005. Beta-lactam antibiotics offer neuroprotection by increasing glutamate transporter expression. Nature 433, 73–77.
Rotunno, M.S., Bosco, D.A., 2013. An emerging role for misfolded wild-type SOD1 in sporadic ALS pathogenesis. Front. Cell. Neurosci. 7, 253.
Rubino, E., Rainero, I., Chio, A., Rogaeva, E., Galimberti, D., Fenoglio, P., Grinberg, Y., Isaia, G., Calvo, A., Gentile, S., Bruni, A.C., St George-Hyslop, P.H., Scarpini, E., Gallone, S., Pinessi, L., 2012. SQSTM1 mutations in frontotemporal lobar degeneration and amyotrophic lateral sclerosis. Neurology 79, 1556–1562.
Russ, J., Liu, E.Y., Wu, K., Neal, D., Suh, E., Irwin, D.J., McMillan, C.T., Harms, M.B., Cairns, N.J., Wood, E.M., Xie, S.X., Elman, L., McCluskey, L., Grossman, M., Van Deerlin, V.M., Lee, E.B., 2015. Hypermethylation of repeat expanded C9orf72 is a clinical and molecular disease modifier. Acta Neuropathol. 129, 39–52.
Sabado, J., Casanovas, A., Tarabal, O., Hereu, M., Piedrafita, L., Caldero, J., Esquerda, J.E., 2014. Accumulation of misfolded SOD1 in dorsal root ganglion degenerating proprioceptive sensory neurons of transgenic mice with amyotrophic lateral sclerosis. Biomed. Res. Int. 2014, 852163.
Sabatelli, M., Conforti, F.L., Zollino, M., Mora, G., Monsurro, M.R., Volanti, P., Marinou, K., Salvi, F., Corbo, M., Giannini, F., Battistini, S., Penco, S., Lunetta, C., Quattrone, A., Gambardella, A., Logroscino, G., Simone, I., Bartolomei, I., Pisano, F., Tedeschi, G., Conte, A., Spataro, R., La Bella, V., Caponnetto, C., Mancardi, G., Mandich, P., Sola, P., Mandrioli, J., Renton, A.E., Majounie, E., Abramzon, Y., Marrosu, F., Marrosu, M.G., Murru, M.R., Sotgiu, M.A., Pugliatti, M., Rodolico, C., Moglia, C., Calvo, A., Ossola, I., Brunetti, M., Traynor, B.J., Borghero, G., Restagno, G., Chio, A., 2012. C9ORF72 hexanucleotide repeat expansions in the Italian sporadic ALS population. Neurobiol. Aging 33 (8) (1848. e15-20).
Sanjak, M., Paulson, D., Sufit, R., Reddan, W., Beaulieu, D., Erickson, L., Shug, A., Brooks, B.R., 1987. Physiologic and metabolic response to progressive and prolonged exercise in amyotrophic lateral sclerosis. Neurology 37, 1217–1220.
Sasaki, S., 2010. Endoplasmic reticulum stress in motor neurons of the spinal cord in sporadic amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 69, 346–355.
Schludi, M.H., May, S., Grasser, F.A., Rentzsch, K., Kremmer, E., Kupper, C., Klopstock, T., German Consortium for Frontotemporal Lobar Degeneration Bavarian Brain Banking Alliance, Arzberger, T., Edbauer, D., 2015. Distribution of dipeptide repeat proteins in cellular models and C9orf72 mutation cases suggests link to transcriptional silencing. Acta Neuropathol. 130 (4), 537–555.
Schutz, B., Reimann, J., Dumitrescu-Ozimek, L., Kappes-Horn, K., Landreth, G.E., Schurmann, B., Zimmer, A., Heneka, M.T., 2005a. The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice. J. Neurosci. 25, 7805–7812.
Schutz, B., Reimann, J., Dumitrescu-Ozimek, L., Kappes-Horn, K., Landreth, G.E., Schurmann, B., Zimmer, A., Heneka, M.T., 2005b. The oral antidiabetic pioglitazone protects from neurodegeneration and amyotrophic lateral sclerosis-like symptoms in superoxide dismutase-G93A transgenic mice. J. Neurosci. 25, 7805–7812.
Schwarz, S., Husstedt, I., Bertram, H.P., Kuchelmeister, K., 1996. Amyotrophic lateral sclerosis after accidental injection of mercury. J. Neurol. Neurosurg. Psychiatry 60, 698.
Schwenk, B.M., Lang, C.M., Hogl, S., Tahirovic, S., Orozco, D., Rentzsch, K., Lichtenthaler, S.F., Hoogenraad, C.C., Capell, A., Haass, C., Edbauer, D., 2014. The FTLD risk factor TMEM106 B and MAP6 control dendritic trafficking of lysosomes. EMBO J. 33, 450–467.
Schymick, J.C., Talbot, K., Traynor, B.J., 2007. Genetics of sporadic amyotrophic lateral sclerosis. Hum. Mol. Genet. (16 Spec No. 2, R233-42).
Senderek, J., Garvey, S.M., Krieger, M., Guergueltcheva, V., Urtizberea, A., Roos, A., Elbracht, M., Stendel, C., Tournev, I., Mihailova, V., Feit, H., Tramonte, J., Hedera, P., Crooks, K., Bergmann, C., Rudnik-Schoneborn, S., Zerres, K., Lochmuller, H., Seboun, E., Weis, J., Beckmann, J.S., Hauser, M.A., Jackson, C.E., 2009. Autosomal-dominant distal myopathy associated with a recurrent missense mutation in the gene encoding the nuclear matrix protein, matrin 3. Am. J. Hum. Genet. 84, 511–518.
Shaw, C.E., al-Chalabi, A., Leigh, N., 2001. Progress in the pathogenesis of amyotrophic lateral sclerosis. Curr. Neurol. Neurosci. 1 (1), 69–76.
Sheerin, U.-M., Schneider, S.A., Carr, L., Deuschl, G., Hopfner, F., Stamelou, M., Wood, N.W., Bhatia, K.P., 2014. ALS2 mutations: juvenile amyotrophic lateral sclerosis and generalized dystonia. Neurology 82, 1065–1067.
Shibata, N., Hirano, A., Kobayashi, M., Siddique, T., Deng, H.X., Hung, W.Y., Kato, T., Asayama, K., 1996. Intense superoxide dismutase-1 immunoreactivity in intracytoplasmic hyaline inclusions of familial amyotrophic lateral sclerosis with posterior column involvement. J. Neuropathol. Exp. Neurol. 55, 481–490.
Shibata, N., Kawaguchi-Niida, M., Yamamoto, T., Toi, S., Hirano, A., Kobayashi, M., 2008. Effects of the PPARgamma activator pioglitazone on p38 MAP kinase and IkappaBalpha in the spinal cord of a transgenic mouse model of amyotrophic lateral sclerosis. Neuropathology 28, 387–398.
Shibata, N., Yamamoto, T., Hiroi, A., Omi, Y., Kato, Y., Kobayashi, M., 2010. Activation of STAT3 and inhibitory effects of pioglitazone on STAT3 activity in a mouse model of SOD1-mutated amyotrophic lateral sclerosis. Neuropathology 30, 353–360.
Shimizu, T., Nagaoka, U., Nakayama, Y., Kawata, A., Kugimoto, C., Kuroiwa, Y., Kawai, M., Shimohata, T., Nishizawa, M., Mihara, B., Arahata, H., Fujii, N., Namba, R., Ito, H., Imai, T., Nobukuni, K., Kondo, K., Ogino, M., Nakajima, T., Komori, T., 2012. Reduction rate of body mass index predicts prognosis for survival in amyotrophic lateral sclerosis: a multicenter study in Japan. Amyotroph. Lateral Scler. 13, 363–366.
Shioya, M., Obayashi, S., Tabunoki, H., Arima, K., Saito, Y., Ishida, T., Satoh, J., 2010. Aberrant microRNA expression in the brains of neurodegenerative diseases: miR-29a decreased in Alzheimer disease brains targets neurone navigator 3. Neuropathol. Appl. Neurobiol. 36, 320–330.
Sieh, W., Choi, Y., Chapman, N.H., Craig, U.K., Steinbart, E.J., Rothstein, J.H., Oyanagi, K., Garruto, R.M., Bird, T.D., Galasko, D.R., Schellenberg, G.D., Wijsman, E.M., 2009. Identification of novel susceptibility loci for Guam neurodegenerative disease: challenges of genome scans in genetic isolates. Hum. Mol. Genet. 18, 3725–3738.

Simpson, C.L., Lemmens, R., Miskiewicz, K., Broom, W.J., Hansen, V.K., van Vught, P.W.J., Landers, J.E., Sapp, P., Van Den Bosch, L., Knight, J., Neale, B.M., Turner, M.R., Veldink, J.H., Ophoff, R.A., Tripathi, V.B., Beleza, A., Shah, M.N., Proitsi, P., Van Hoecke, A., Carmeliet, P., Horvitz, H.R., Leigh, P.N., Shaw, C.E., van den Berg, L.H., Sham, P.C., Powell, J.F., Verstreken, P., Brown, R.H.J., Robberecht, W., Al-Chalabi, A., 2009. Variants of the elongator protein 3 (ELP3) gene are associated with motor neuron degeneration. Hum. Mol. Genet. 18, 472–481.

Singh, N., Haldar, S., Tripathi, A.K., Horback, K., Wong, J., Sharma, D., Beserra, A., Suda, S., Anbalagan, C., Dev, S., Mukhopadhyay, C.K., Singh, A., 2014. Brain iron homeostasis: from molecular mechanisms to clinical significance and therapeutic opportunities. Antioxid. Redox Signal. 20, 1324–1363.

Skibinski, G., Parkinson, N.J., Brown, J.M., Chakrabarti, L., Lloyd, S.L., Hummerich, H., Nielsen, J.E., Hodges, J.R., Spillantini, M.G., Thusgaard, T., Brandner, S., Brun, A., Rossor, M.N., Gade, A., Johannsen, P., Sorensen, S.A., Gydesen, S., Fisher, E.M., Collinge, J., 2005. Mutations in the endosomal ESCRTIII-complex subunit CHMP2B in frontotemporal dementia. Nat. Genet. 37, 806–808.

Smith, Q.R., Nagura, H., Takada, Y., Duncan, M.W., 1992. Facilitated transport of the neurotoxin, beta-N-methylamino-L-alanine, across the blood-brain barrier. J. Neurochem. 58, 1330–1337.

Smith, B.N., Newhouse, S., Shatunov, A., Vance, C., Topp, S., Johnson, L., Miller, J., Lee, Y., Troakes, C., Scott, K.M., Jones, A., Gray, I., Wright, J., Hortobagyi, T., Al-Sarraj, S., Rogelj, B., Powell, J., Lupton, M., Lovestone, S., Sapp, P.C., Weber, M., Nestor, P.J., Schelhaas, H.J., Asbroek, A.A.T., Silani, V., Gellera, C., Taroni, F., Ticozzi, N., Van den Berg, L., Veldink, J., Van Damme, P., Robberecht, W., Shaw, P.J., Kirby, J., Pall, H., Morrison, K.E., Morris, A., de Belleroche, J., Vianney de Jong, J.M.B., Baas, F., Andersen, P.M., Landers, J., Brown, R.H.J., Weale, M.E., Al-Chalabi, A., Shaw, C.E., 2013. The C9ORF72 expansion mutation is a common cause of ALS+/−FTD in Europe and has a single founder. Eur. J. Hum. Genet. 21, 102–108.

Smith, B.N., Ticozzi, N., Fallini, C., Gkazi, A.S., Topp, S., Kenna, K.P., Scotter, E.L., Kost, J., Keagle, P., Miller, J.W., Calini, D., Vance, C., Danielson, E.W., Troakes, C., Tiloca, C., Al-Sarraj, S., Lewis, E.A., King, A., Colombrita, C., Pensato, V., Castellotti, B., de Belleroche, J., Baas, F., ten Asbroek, A.L., Sapp, P.C., McKenna-Yasek, D., McLaughlin, R.L., Polak, M., Asress, S., Esteban-Pérez, J., Muñoz-Blanco, J.L., Simpson, M., Consortium, S.L.A.G.E.N., van Rheenen, W., Diekstra, F.P., Lauria, G., Duga, S., Corti, S., Cereda, C., Corrado, L., Sorarù, G., Morrison, K.E., Williams, K.L., Nicholson, G.A., Blair, I.P., Dion, P.A., Leblond, C.S., Rouleau, G.A., Hardiman, O., Veldink, J.H., van den Berg, L.H., Al-Chalabi, A., Pall, H., Shaw, P.J., Turner, M.R., Talbot, K., Taroni, F., García-Redondo, A., Wu, Z., Glass, J.D., Gellera, C., Ratti, A., Brown Jr., R.H., Silani, V., Shaw, C.E., Landers, J.E., 2014. Exome-wide rare variant analysis identifies TUBA4A mutations associated with familial ALS. Neuron 84, 324–331.

Smittkamp, S.E., Morris, J.K., Bomhoff, G.L., Chertoff, M.E., Geiger, P.C., Stanford, J.A., 2014. SOD1-G93A mice exhibit muscle-fiber-type-specific decreases in glucose uptake in the absence of whole-body changes in metabolism. Neurodegener Dis. 13, 29–37.

Soraru, G., Clementi, M., Forzan, M., Orsetti, V., D'Ascenzo, C., Querin, G., Palmieri, A., Ermani, M., Angelini, C., Pegoraro, E., 2011. ALS risk but not phenotype is affected by ataxin-2 intermediate length polyglutamine expansion. Neurology 76, 2030–2031.

Spencer, P.S., Palmer, V.S., Ludolph, A.C., 2005. On the decline and etiology of high-incidence motor system disease in West Papua (southwest New Guinea). Mov. Disord. 20 (Suppl. 12), S119–126.

Sreedharan, J., Blair, I.P., Tripathi, V.B., Hu, X., Vance, C., Rogelj, B., Ackerley, S., Durnall, J.C., Williams, K.L., Buratti, E., Baralle, F., de Belleroche, J., Mitchell, J.D., Leigh, P.N., Al-Chalabi, A., Miller, C.C., Nicholson, G., Shaw, C.E., 2008. TDP-43 mutations in familial and sporadic amyotrophic lateral sclerosis. Science 319, 1668–1672.

Stallings, N.R., Puttaparthi, K., Dowling, K.J., Luther, C.M., Burns, D.K., Davis, K., Elliott, J.L., 2013. TDP-43, an ALS linked protein, regulates fat deposition and glucose homeostasis. PLoS One 8 (8), e71793.

Steele, J.C., 2005. Parkinsonism-dementia complex of Guam. Mov. Disord. 20 (Suppl. 12), S99–S107.

Stribl, C., Samara, A., Trumbach, D., Peis, R., Neumann, M., Fuchs, H., Gailus-Durner, V., Hrabe de Angelis, M., Rathkolb, B., Wolf, E., Beckers, J., Horsch, M., Neff, F., Kremmer, E., Koob, S., Reichert, A.S., Hans, W., Rozman, J., Klingenspor, M., Aichler, M., Walch, A.K., Becker, L., Klopstock, T., Glasl, L., Holter, S.M., Wurst, W., Floss, T., 2014. Mitochondrial dysfunction and decrease in body weight of a transgenic knock-in mouse model for TDP-43. J. Biol. Chem. 289, 10769–10784.

Strong, M.J., 2008. The syndromes of frontotemporal dysfunction in amyotrophic lateral sclerosis. Amyotroph. Lateral Scler. 9, 323–338.

Sumi, H., Kato, S., Mochimaru, Y., Fujimura, H., Etoh, M., Sakoda, S., 2009. Nuclear TAR DNA binding protein 43 expression in spinal cord neurons correlates with the clinical course in amyotrophic lateral sclerosis. J. Neuropathol. Exp. Neurol. 68, 37–47.

Sutedja, N.A., Sinke, R.J., Van Vught, P.W.J., Van der Linden, M.W., Wokke, J.H.J., Van Duijn, C.M., Njajou, O.T., Van der Schouw, Y.T., Veldink, J.H., Van den Berg, L.H., 2007. The association between H63D mutations in HFE and amyotrophic lateral sclerosis in a Dutch population. Arch. Neurol. 64, 63–67.

Sutedja, N.A., Fischer, K., Veldink, J.H., van der Heijden, G.J.M.G., Kromhout, H., Heederik, D., Huisman, M.H.B., Wokke, J.J.H., van den Berg, L.H., 2009a. What we truly know about occupation as a risk factor for ALS: a critical and systematic review. Amyotroph. Lateral Scler. 10 (5–6), 295–301.

Sutedja, N.A., Veldink, J.H., Fischer, K., Kromhout, H., Heederik, D., Huisman, M.H.B., Wokke, J.H.J., van den Berg, L.H., 2009b. Exposure to chemicals and metals and risk of amyotrophic lateral sclerosis: a systematic review. Amyotroph. Lateral Scler. 10, 302–309.

Syroid, D.E., Maycox, P.R., Burrola, P.G., Liu, N., Wen, D., Lee, K.F., Lemke, G., Kilpatrick, T.J., 1996. Cell death in the Schwann cell lineage and its regulation by neuregulin. Proc. Natl. Acad. Sci. U. S. A. 93, 9229–9234.

Takahashi, Y., Fukuda, Y., Yoshimura, J., Toyoda, A., Kurppa, K., Moritoyo, H., Belzil, V.V., Dion, P.A., Higasa, K., Doi, K., Ishiura, H., Mitsui, J., Date, H., Ahsan, B., Matsukawa, T., Ichikawa, Y., Moritoyo, T., Ikoma, M., Hashimoto, T., Kimura, F., Murayama, S., Onodera, O., Nishizawa, M., Yoshida, M., Atsuta, N., Sobue, G., JaC.A.L.S. Fifita, J.A., Williams, K.L., Blair, I.P., Nicholson, G.A., Gonzalez-Perez, P., Brown Jr., R.H., Nomoto, M., Elenius, K., Rouleau, G.A., Fujiyama, A., Morishita, S., Goto, J., Tsuji, S., 2013. ERBB4 mutations that disrupt the neuregulin-ErbB4 pathway cause amyotrophic lateral sclerosis type 19. Am. J. Hum. Genet. 93, 900–905.

Takanashi, K., Yamaguchi, A., 2014. Aggregation of ALS-linked FUS mutant sequesters RNA binding proteins and impairs RNA granules formation. Biochem. Biophys. Res. Commun. 452, 600–607.

Tao, Z., Wang, H., Xia, Q., Li, K., Li, K., Jiang, X., Xu, G., Wang, G., Ying, Z., 2015. Nucleolar stress and impaired stress granule formation contribute to C9orf72 RAN translation-induced cytotoxicity. Hum. Mol. Genet. 24, 2426–2441.

Tashiro, Y., Urushitani, M., Inoue, H., Koike, M., Uchiyama, Y., Komatsu, M., Tanaka, K., Yamazaki, M., Abe, M., Misawa, H., Sakimura, K., Ito, H., Takahashi, R., 2012. Motor neuron-specific disruption of proteasomes, but not autophagy, replicates amyotrophic lateral sclerosis. J. Biol. Chem. 287, 42984–42994.

Tetsuka, S., Morita, M., Iida, A., Uehara, R., Ikegawa, S., Nakano, I., 2013. ZNF512B gene is a prognostic factor in patients with amyotrophic lateral sclerosis. J. Neurol. Sci. 324, 163–166.

Tibshirani, M., Tradewell, M.L., Mattina, K.R., Minotti, S., Yang, W., Zhou, H., Strong, M.J., Hayward, L.J., Durham, H.D., 2014. Cytoplasmic sequestration of FUS/TLS associated with ALS alters histone marks through loss of nuclear protein arginine methyltransferase 1. Hum. Mol. Genet. 24, 773–786.

Ticozzi, N., Vance, C., Leclerc, A.L., Keagle, P., Glass, J.D., McKenna-Yasek, D., Sapp, P.C., Silani, V., Bosco, D.A., Shaw, C.E., Brown, R.H.J., Landers, J.E., 2011. Mutational analysis reveals the FUS homolog TAF15 as a candidate gene for familial amyotrophic lateral sclerosis. Am. J. Med. Genet. B. Neuropsychiatr. Genet. 156B (3), 285–290.

Toivonen, J.M., Manzano, R., Olivan, S., Zaragoza, P., Garcia-Redondo, A., Osta, R., 2014. MicroRNA-206: a potential circulating biomarker candidate for amyotrophic lateral sclerosis. PLoS One 9 (2), e89065.

Tomkins, J., Usher, P., Slade, J.Y., Ince, P.G., Curtis, A., Bushby, K., Shaw, P.J., 1998. Novel insertion in the KSP region of the neurofilament heavy gene in amyotrophic lateral sclerosis (ALS). Neuroreport 9, 3967–3970.

Torbick, N., Hession, S., Stommel, E., Caller, T., 2014. Mapping amyotrophic lateral sclerosis lake risk factors across northern New England. Int. J. Health. Geogr. 13, (1-072X-13-1).

Tremolizzo, L., Messina, P., Conti, E., Sala, G., Cecchi, M., Airoldi, L., Pastorelli, R., Pupillo, E., Bandettini Di Poggio, M., Filosto, M., Lunetta, C., Agliardi, C., Guerini, F., Mandrioli, J., Calvo, A., Beghi, E., Ferrarese, C., 2014. Whole-blood global DNA methylation is increased in amyotrophic lateral sclerosis independently of age of onset. Amyotroph Lateral Scler Frontotemporal Degener. 15 (1-2), 98–105.

Trojsi, F., Monsurro, M.R., Tedeschi, G., 2013. Exposure to environmental toxicants and pathogenesis of amyotrophic lateral sclerosis: state of the art and research perspectives. Int. J. Mol. Sci. 14, 15286–15311.

Uyan, O., Omur, O., Agim, Z.S., Ozoguz, A., Li, H., Parman, Y., Deymeer, F., Oflazer, P., Koc, F., Tan, E., Ozcelik, H., Basak, A.N., 2013. Genome-wide copy number variation in sporadic amyotrophic lateral sclerosis in the Turkish population: deletion of EPHA3 is a possible protective factor. PLoS One 8 (8), e72381.

Valavanis, A., Schwarz, U., Baumann, C.R., Weller, M., Linnebank, M., 2014. Amyotrophic lateral sclerosis after embolization of cerebral arterioveneous malformations. J. Neurol. 261, 732–737.

Valle, C., Salvatori, I., Gerbino, V., Rossi, S., Palamiuc, L., René, F., Carrì, M.T., 2014. Tissue-specific deregulation of selected HDACs characterizes ALS progression in mouse models: pharmacological characterization of SIRT1 and SIRT2 pathways. Cell Death Dis. 5, e1296.

Van Damme, P., Veldink, J.H., van Blitterswijk, M., Corveleyn, A., van Vught, P.W.J., Thijs, V., Dubois, B., Matthijs, G., van den Berg, L.H., Robberecht, W., 2011. Expanded ATXN2 CAG repeat size in ALS identifies genetic overlap between ALS and SCA2. Neurology 76, 2066–2072.

Van Deerlin, V.M., Leverenz, J.B., Bekris, L.M., Bird, T.D., Yuan, W., Elman, L.B., Clay, D., Wood, E.M., Chen-Plotkin, A.S., Martinez-Lage, M., Steinbart, E., McCluskey, L., Grossman, M., Neumann, M., Wu, I.-L., Yang, W.-S., Kalb, R., Galasko, D.R., Montine, T.J., Trojanowski, J.Q., Lee, V.M.-Y., Schellenberg, G.D., Yu, C.-E., 2008. TARDBP mutations in amyotrophic lateral sclerosis with TDP-43 neuropathology: a genetic and histopathological analysis. Lancet Neurol. 7, 409–416.

Van Deerlin, V.M., Sleiman, P.M.A., Martinez-Lage, M., Chen-Plotkin, A., Wang, L.-S., Graff-Radford, N.R., Dickson, D.W., Rademakers, R., Boeve, B.F., Grossman, M., Arnold, S.E., Mann, D.M.A., Pickering-Brown, S.M., Seelaar, H., Heutink, P., van Swieten, J.C., Murrell, J.R., Ghetti, B., Spina, S., Grafman, J., Hodges, J., Spillantini, M.G., Gilman, S., Lieberman, A.P., Kaye, J.A., Woltjer, R.L., Bigio, E.H., Mesulam, M., Al-Sarraj, S., Troakes, C., Rosenberg, R.N., White 3rd, C.L., Ferrer, I., Llado, A., Neumann, M., Kretzschmar, H.A., Hulette, C.M., Welsh-

Bohmer, K.A., Miller, B.L., Alzualde, A., Lopez de Munain, A., McKee, A.C., Gearing, M., Levey, A.I., Lah, J.J., Hardy, J., Rohrer, J.D., Lashley, T., Mackenzie, I.R.A., Feldman, H.H., Hamilton, R.L., Dekosky, S.T., van der Zee, J., Kumar-Singh, S., Van Broeckhoven, C., Mayeux, R., Vonsattel, J.P.G., Troncoso, J.C., Kril, J.J., Kwok, J.B.J., Halliday, G.M., Bird, T.D., Ince, P.G., Shaw, P.J., Cairns, N.J., Morris, J.C., McLean, C.A., DeCarli, C., Ellis, W.G., Freeman, S.H., Frosch, M.P., Growdon, J.H., Perl, D.P., Sano, M., Bennett, D.A., Schneider, J.A., Beach, T.G., Reiman, E.M., Woodruff, B.K., Cummings, J., Vinters, H.V., Miller, C.A., Chui, H.C., Alafuzoff, I., Hartikainen, P., Seilhean, D., Galasko, D., Masliah, E., Cotman, C.W., Tunon, M.T., Martinez, M.C.C., Munoz, D.G., Carroll, S.L., Marson, D., Riederer, P.F., Bogdanovic, N., Schellenberg, G.D., Hakonarson, H., Trojanowski, J.Q., Lee, V.M.-Y., et al., 2010. Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. Nat. Genet. 42, 234–239.

Van Doormaal, P.T.C., Ticozzi, N., Gellera, C., Ratti, A., Taroni, F., Chio, A., Calvo, A., Mora, G., Restagno, G., Traynor, B.J., Birve, A., Lemmens, R., van Es, M.A., Saris, C.G.J., Blauw, H.M., van Vught, P.W.J., Groen, E.J.N., Corrado, L., Mazzini, L., Del Bo, R., Corti, S., Waibel, S., Meyer, T., Ludolph, A.C., Goris, A., van Damme, P., Robberecht, W., Shatunov, A., Fogh, I., Andersen, P.M., D'Alfonso, S., Hardiman, O., Cronin, S., Rujescu, D., Al-Chalabi, A., Landers, J.E., Silani, V., van den Berg, L.H., Veldink, J.H., 2014. Analysis of the KIFAP3 gene in amyotrophic lateral sclerosis: a multicenter survival study. Neurobiol. Aging 35 (2420), e13–14.

Van Es, M.A., Veldink, J.H., Saris, C.G.J., Blauw, H.M., van Vught, P.W.J., Birve, A., Lemmens, R., Schelhaas, H.J., Groen, E.J.N., Huisman, M.H.B., van der Kooi, A.J., de Visser, M., Dahlberg, C., Estrada, K., Rivadeneira, F., Hofman, A., Zwarts, M.J., van Doormaal, P.T.C., Rujescu, D., Strengman, E., Giegling, I., Muglia, P., Tomik, B., Slowik, A., Uitterlinden, A.G., Hendrich, C., Waibel, S., Meyer, T., Ludolph, A.C., Glass, J.D., Purcell, S., Cichon, S., Nothen, M.M., Wichmann, H.-E., Schreiber, S., Vermeulen, S.H.H.M., Kiemeney, L.A., Wokke, J.H.J., Cronin, S., McLaughlin, R.L., Hardiman, O., Fumoto, K., Pasterkamp, R.J., Meininger, V., Melki, J., Leigh, P.N., Shaw, C.E., Landers, J.E., Al-Chalabi, A., Brown, R.H.J., Robberecht, W., Andersen, P.M., Ophoff, R.A., van den Berg, L.H., 2009. Genome-wide association study identifies 19p13: 3 (UNC13A) and 9p21.2 as susceptibility loci for sporadic amyotrophic lateral sclerosis. Nat. Genet. 41, 1083–1087.

Van Es, M.A., Schelhaas, H.J., van Vught, P.W.J., Ticozzi, N., Andersen, P.M., Groen, E.J.N., Schulte, C., Blauw, H.M., Koppers, M., Diekstra, F.P., Fumoto, K., LeClerc, A.L., Keagle, P., Bloem, B.R., Scheffer, H., van Nuenen, B.F.L., van Blitterswijk, M., van Rheenen, W., Wills, A.-M., Lowe, P.P., Hu, G., Yu, W., Kishikawa, H., Wu, D., Folkerth, R.D., Mariani, C., Goldwurm, S., Pezzoli, G., P.Van DammeLemmens, R., Dahlberg, C., Birve, A., Fernandez-Santiago, R., S.WaibelKlein, C., Weber, M., van der Kooi, A.J., de Visser, M., Verbaan, D., van Hilten, J.J., Heutink, P., Hennekam, E.A.M., Cuppen, E., Berg, D., Brown, R.H.J., Silani, V., Gasser, T., Ludolph, A.C., Robberecht, W., Ophoff, R.A., Veldink, J.H., Pasterkamp, R.J., de Bakker, P.I.W., Landers, J.E., van de Warrenburg, B.P., van den Berg, L.H., 2011. Angiogenin variants in Parkinson disease and amyotrophic lateral sclerosis. Ann. Neurol. 70, 964–973.

Van Rheenen, W., Diekstra, F.P., van Doormaal, P.T.C., Seelen, M., Kenna, K., McLaughlin, R., Shatunov, A., Czell, D., van Es, M.A., van Vught, P.W.J., van Damme, P., Smith, B.N., Waibel, S., Schelhaas, H.J., van der Kooi, A.J., de Visser, M., Weber, M., Robberecht, W., Hardiman, O., Shaw, P.J., Shaw, C.E., Morrison, K.E., Al-Chalabi, A., Andersen, P.M., Ludolph, A.C., Veldink, J.H., van den Berg, L.H., 2013. H63D polymorphism in HFE is not associated with amyotrophic lateral sclerosis. Neurobiol. Aging 34 (5) (1517. e5-7).

Van Hoecke, A., Schoonaert, L., Lemmens, R., Timmers, M., Staats, K.A., Laird, A.S., Peeters, E., Philips, T., Goris, A., Dubois, B., Andersen, P.M., Al-Chalabi, A., Thijs, V., Turnley, A.M., van Vught, P.W., Veldink, J.H., Hardiman, O., Van Den Bosch, L., Gonzalez-Perez, P., Van Damme, P., Brown, R.H.J., van den Berg, L.H., Robberecht, W., 2012. EPHA4 is a disease modifier of amyotrophic lateral sclerosis in animal models and in humans. Nat. Med. 18, 1418–1422.

Van den Bergh, R., Swerts, L., Hendrikx, A., Boni, L., Meulepas, E., 1977. Adipose tissue cellularity in patients with amyotrophic lateral sclerosis. Clin. Neurol. Neurosurg. 80, 226–239.

Van der Zee, J., Van Langenhove, T., Kovacs, G.G., Dillen, L., Deschamps, W., Engelborghs, S., Matej, R., Vandenbulcke, M., Sieben, A., Dermaut, B., Smets, K., Van Damme, P., Merlin, C., Laureys, A., Van Den Broeck, M., Mattheijssens, M., Peeters, K., Benussi, L., Binetti, G., Ghidoni, R., Borroni, B., Padovani, A., Archetti, S., Pastor, P., Razquin, C., Ortega-Cubero, S., Hernandez, I., Boada, M., Ruiz, A., de Mendonca, A., Miltenberger-Miltenyi, G., do Couto, F.S., Sorbi, S., Nacmias, B., Bagnoli, S., Graff, C., Chiang, H.-H., Thonberg, H., Perneczky, R., Diehl-Schmid, J., Alexopoulos, P., Frisoni, G.B., Bonvicini, C., Synofzik, M., Maetzler, W., vom Hagen, J.M., Schols, L., Haack, T.B., Strom, T.M., Prokisch, H., Dols-Icardo, O., Clarimon, J., Lleo, A., Santana, I., Almeida, M.R., Santiago, B., Heneka, M.T., Jessen, F., Ramirez, A., Sanchez-Valle, R., Llado, A., Gelpi, E., Sarafov, S., Tournev, I., Jordanova, A., Parobkova, E., Fabrizi, G.M., Testi, S., Salmon, E., Strobel, T., Santens, P., Robberecht, W., De Jonghe, P., Martin, J.-J., Cras, P., Vandenberghe, R., De Deyn, P.P., Cruts, M., Sleegers, K., Van Broeckhoven, C., 2014. Rare mutations in SQSTM1 modify susceptibility to frontotemporal lobar degeneration. Acta Neuropathol. 128 (3), 397–410.

Vance, C., Rogelj, B., Hortobagyi, T., De Vos, K.J., Nishimura, A.L., Sreedharan, J., Hu, X., Smith, B., Ruddy, D., Wright, P., Ganesalingam, J., Williams, K.L., Tripathi, V., Al-Saraj, S., Al-Chalabi, A., Leigh, P.N., Blair, I.P., Nicholson, G., de Belleroche, J., Gallo, J.-M., Miller, C.C., Shaw, C.E., 2009. Mutations in FUS, an RNA processing protein, cause familial amyotrophic lateral sclerosis type 6. Science 323, 1208–1211.

Vass, R., Ashbridge, E., Geser, F., Hu, W.T., Grossman, M., Clay-Falcone, D., Elman, L., McCluskey, L., Lee, V.M., Van Deerlin, V.M., Trojanowski, J.Q., Chen-Plotkin, A.S., 2011. Risk genotypes at TMEM106B are associated with cognitive impairment in amyotrophic lateral sclerosis. Acta Neuropathol. 121, 373–380.

Vega, A., Bell, E.A., 1967. α-amino-β-methylaminopropionic acid, a new amino acid from seeds of Cycas circinalis. Phytochemistry 6, 759–762.

Vinceti, M., Guidetti, D., Pinotti, M., Rovesti, S., Merlin, M., Vescovi, L., Bergomi, M., Vivoli, G., 1996. Amyotrophic lateral sclerosis after long-term exposure to drinking water with high selenium content. Epidemiology 7, 529–532.

Vinceti, M., Wei, E.T., Malagoli, C., Bergomi, M., Vivoli, G., 2001. Adverse health effects of selenium in humans. Rev. Environ. Health 16, 233–251.

Vollrath, J.T., Sechi, A., Dreser, A., Katona, I., Wiemuth, D., Vervoorts, J., Dohmen, M., Chandrasekar, A., Prause, J., Brauers, E., Jesse, C.M., Weis, J., Goswami, A., 2014. Loss of function of the ALS protein SigR1 leads to ER pathology associated with defective autophagy and lipid raft disturbances. Cell. Death Dis. 5, e1290.

Walker, A.K., Spiller, K.J., Ge, G., Zheng, A., Xu, Y., Zhou, M., Tripathy, K., Kwong, L.K., Trojanowski, J.Q., Lee, V.M., 2015. Functional recovery in new mouse models of ALS/FTLD after clearance of pathological cytoplasmic TDP-43. Acta Neuropathol. 130 (5), 643–660.

Wang, X.S., Lee, S., Simmons, Z., Boyer, P., Scott, K., Liu, W., Connor, J., 2004. Increased incidence of the Hfe mutation in amyotrophic lateral sclerosis and related cellular consequences. J. Neurol. Sci. 227 (1), 27–33.

Wang, L., Jiang, Z., Lei, X.G., 2012. Knockout of SOD1 alters murine hepatic glycolysis, gluconeogenesis, and lipogenesis. Free Radic. Biol. Med. 53, 1689–1696.

Wang, T., Jiang, X., Chen, G., Xu, J., 2015. Interaction of amyotrophic lateral sclerosis/frontotemporal lobar degeneration-associated fused-in-sarcoma with proteins involved in metabolic and protein degradation pathways. Neurobiol. Aging 36, 527–535.

Weiss, B., Amler, S., Amler, R.W., 2004. Pesticides. Pediatrics 113, 1030–1036.

Weisskopf, M.G., Morozova, N., O'Reilly, E.J., McCullough, M.L., Calle, E.E., Thun, M.J., Ascherio, A., 2009. Prospective study of chemical exposures and amyotrophic lateral sclerosis. J. Neurol. Neurosurg. Psychiatry 80, 558–561.

Wen, X., Tan, W., Westergard, T., Krishnamurthy, K., Markandaiah, S.S., Shi, Y., Lin, S., Shneider, N.A., Monaghan, J., Pandey, U.B., Pasinelli, P., Ichida, J.K., Trotti, D., 2014. Antisense proline-arginine RAN dipeptides linked to C9ORF72-ALS/FTD form toxic nuclear aggregates that initiate in vitro and in vivo neuronal death. Neuron 84, 1213–1225.

Williams, A.H., Valdez, G., Moresi, V., Qi, X., McAnally, J., Elliott, J.L., Bassel-Duby, R., Sanes, J.R., Olson, E.N., 2009. MicroRNA-206 delays ALS progression and promotes regeneration of neuromuscular synapses in mice. Science 326, 1549–1554.

Williams, K.L., Warraich, S.T., Yang, S., Solski, J.A., Fernando, R., Rouleau, G.A., Nicholson, G.A., Blair, I.P., 2012. UBQLN2/ubiquilin 2 mutation and pathology in familial amyotrophic lateral sclerosis. Neurobiol. Aging 33 (10) (2527. e3-10).

Williams, K.L., McCann, E.P., Fifita, J.A., Zhang, K., Duncan, E.L., Leo, P.J., Marshall, M., Rowe, D.B., Nicholson, G.A., Blair, I.P., 2015. Novel TBK1 truncating mutation in a familial amyotrophic lateral sclerosis patient of Chinese origin. Neurobiol. Aging.

Wills, A.M., Hubbard, J., Macklin, E.A., Glass, J., Tandan, R., Simpson, E.P., Brooks, B., Gelinas, D., Mitsumoto, H., Mozaffar, T., Hanes, G.P., Ladha, S.S., Heiman-Patterson, T., Katz, J., Lou, J.-S., Mahoney, K., Grasso, D., Lawson, R., Yu, H., Cudkowicz, M., 2014. Hypercaloric enteral nutrition in patients with amyotrophic lateral sclerosis: a randomised, double-blind, placebo-controlled phase 2 trial. Lancet 383, 2065–2072.

Winkler, E.A., Sengillo, J.D., Sagare, A.P., Zhao, Z., Ma, Q., Zuniga, E., Wang, Y., Zhong, Z., Sullivan, J.S., Griffin, J.H., Cleveland, D.W., Zlokovic, B.V., 2014. Blood-spinal cord barrier disruption contributes to early motor-neuron degeneration in ALS-model mice. Proc. Natl. Acad. Sci. U. S. A. 111, E1035–42.

Witts, E.C., Zagoraiou, L., Miles, G.B., 2014. Anatomy and function of cholinergic C bouton inputs to motor neurons. J. Anat. 224, 52–60.

Wu, C.H., Fallini, C., Ticozzi, N., Keagle, P.J., Sapp, P.C., Piotrowska, K., Lowe, P., Koppers, M., McKenna-Yasek, D., Baron, D.M., Kost, J.E., Gonzalez-Perez, P., Fox, A.D., Adams, J., Taroni, F., Tiloca, C., Leclerc, A.L., Chafe, S.C., Mangroo, D., Moore, M.J., Zitzewitz, J.A., Xu, Z.S., van den Berg, L.H., Glass, J.D., Siciliano, G., Cirulli, E.T., Goldstein, D.B., Salachas, F., Meininger, V., Rossoll, W., Ratti, A., Gellera, C., Bosco, D.A., Bassell, G.J., Silani, V., Drory, V.E., Brown Jr., R.H., Landers, J.E., 2012. Mutations in the profilin 1 gene cause familial amyotrophic lateral sclerosis. Nature 488, 499–503.

Xi, Z., Zinman, L., Moreno, D., Schymick, J., Liang, Y., Sato, C., Zheng, Y., Ghani, M., Dib, S., Keith, J., Robertson, J., Rogaeva, E., 2013. Hypermethylation of the CpG Island near the G4C2 repeat in ALS with a C9orf72 expansion. Am. J. Hum. Genet. 92, 981–989.

Xi, Z., Rainero, I., Rubino, E., Pinessi, L., Bruni, A.C., Maletta, R.G., Nacmias, B., Sorbi, S., Galimberti, D., Surace, E.I., Zheng, Y., Moreno, D., Sato, C., Liang, Y., Zhou, Y., Robertson, J., Zinman, L., Tartaglia, M.C., St George-Hyslop, P., Rogaeva, E., 2014. Hypermethylation of the CpG-island near the C9orf72 G(4)C(2)-repeat expansion in FTLD patients. Hum. Mol. Genet. 23, 5630–5637.

Xi, Z., Zhang, M., Bruni, A.C., Maletta, R.G., Colao, R., Fratta, P., Polke, J.M., Sweeney, M.G., Mudanohwo, E., Nacmias, B., Sorbi, S., Tartaglia, M.C., Rainero, I., Rubino, E., Pinessi, L., Galimberti, D., Surace, E.I., McGoldrick, P., McKeever, P., Moreno, D., Sato, C., Liang, Y., Keith, J., Zinman, L., Robertson,

J., Rogaeva, E., 2015. The C9orf72 repeat expansion itself is methylated in ALS and FTLD patients. Acta Neuropathol. 129, 715–727.
Xie, X., Mondo, K., Basile, M.J., Bradley, W.G., Mash, D.C., 2011. Tracking brain uptake and protein incorporation of cyanobacterial toxin BMAA. In: Proceedings of the 22nd International Symposium on ALS/MND, Sydney, Australia, 30 November–2 December 2011; Informa Healthcare: Stockholm, Sweden.
Yañez, M., Matías-Guiu, J., Arranz-Tagarro, J.-A., Galán, L., Vina, D., Gómez-Pinedo, U., Vela, A., Guerrero, A., Martinez-Vila, E., Garcia, A.G., 2014. The neuroprotection exerted by memantine, minocycline and lithium against neurotoxicity of CSF from patients with amyotrophic lateral sclerosis, is antagonized by riluzole. Neurodegener. Dis. 13, 171–179.
Yamakawa, M., Ito, D., Honda, T., Kubo, K., Noda, M., Nakajima, K., Suzuki, N., 2015. Characterization of the dipeptide repeat protein in the molecular pathogenesis of c9FTD/ALS. Hum. Mol. Genet. 24, 1630–1645.
Yang, Y., Hentati, A., Deng, H.X., Dabbagh, O., Sasaki, T., Hirano, M., Hung, W.Y., Ouahchi, K., Yan, J., Azim, A.C., Cole, N., Gascon, G., Yagmour, A., Ben-Hamida, M., Pericak-Vance, M., Hentati, F., Siddique, T., 2001. The gene encoding alsin, a protein with three guanine-nucleotide exchange factor domains, is mutated in a form of recessive amyotrophic lateral sclerosis. Nat. Genet. 29, 160–165.
Yen, A.A., Simpson, E.P., Henkel, J.S., Beers, D.R., Appel, S.H., 2004. HFE mutations are not strongly associated with sporadic ALS. Neurology 62, 1611–1612.
Yu, Y., Su, F.-C., Callaghan, B.C., Goutman, S.A., Batterman, S.A., Feldman, E.L., 2014. Environmental risk factors and amyotrophic lateral sclerosis (ALS): a case-control study of ALS in Michigan. PLoS One 9 (6), e101186.
Zaldívar, T., Gutierrez, J., Lara, G., Carbonara, M., Logroscino, G., Hardiman, O., 2009. Reduced frequency of ALS in an ethnically mixed population: a population-based mortality study. Neurology 72, 1640–1645.
Zhang, Y.J., Jansen-West, K., Xu, Y.F., Gendron, T.F., Bieniek, K.F., Lin, W.L., Sasaguri, H., Caulfield, T., Hubbard, J., Daughrity, L., Chew, J., Belzil, V.V., Prudencio, M., Stankowski, J.N., Castanedes-Casey, M., Whitelaw, E., Ash, P.E., DeTure, M., Rademakers, R., Boylan, K.B., Dickson, D.W., Petrucelli, L., 2014. Aggregation-prone c9FTD/ALS poly (GA) RAN-translated proteins cause neurotoxicity by inducing ER stress. Acta Neuropathol. 128, 505–524.
Zhang, K., Donnelly, C.J., Haeusler, A.R., Grima, J.C., Machamer, J.B., Steinwald, P., Daley, E.L., Miller, S.J., Cunningham, K.M., Vidensky, S., Gupta, S., Thomas, M.A., Hong, I., Chiu, S.L., Huganir, R.L., Ostrow, L.W., Matunis, M.J., J.WangSattler, R., Lloyd, T.E., Rothstein, J.D., 2015. The C9orf72 repeat expansion disrupts nucleocytoplasmic transport. Nature 525, 56–61.
Zhao, Z., Lange, D.J., Voustianiouk, A., MacGrogan, D., Ho, L., Suh, J., Humala, N., Thiyagarajan, M., Wang, J., Pasinetti, G.M., 2006. A ketogenic diet as a potential novel therapeutic intervention in amyotrophic lateral sclerosis. BMC Neurosci. 7, 29.
Zhao, W., Varghese, M., Vempati, P., Dzhun, A., Cheng, A., Wang, J., Lange, D., Bilski, A., Faravelli, I., Pasinetti, G.M., 2012. Caprylic triglyceride as a novel therapeutic approach to effectively improve the performance and attenuate the symptoms due to the motor neuron loss in ALS disease. PLoS One 7, e49191.
Zhou, H., Chen, G., Chen, C., Yu, Y., Xu, Z., 2012. Association between extremely low-frequency electromagnetic fields occupations and amyotrophic lateral sclerosis: a meta-analysis. PLoS One 7, e48354.
Zhu, X., Lai, C., Thomas, S., Burden, S.J., 1995. Neuregulin receptors, erbB3 and erbB4, are localized at neuromuscular synapses. EMBO J. 14, 5842–5848.

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A comprehensive serum lipidome profiling of amyotrophic lateral sclerosis

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RESEARCH ARTICLE

A comprehensive serum lipidome profiling of amyotrophic lateral sclerosis

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Abstract

Objective: To perform a comprehensive lipid profiling to evaluate potential lipid metabolic differences between patients with amyotrophic lateral sclerosis (ALS) and controls, and to provide a more profound understanding of the metabolic abnormalities in ALS. *Methods*: Twenty patients with ALS and 20 healthy controls were enrolled in a cross-sectional study. Untargeted lipidomics profiling in fasting serum samples were performed by optimized UPLC–MS platforms for broad lipidome coverage. Datasets were analyzed by univariate and a variety of multivariate procedures. *Results*: We provide the most comprehensive blood lipid profiling of ALS to date, with a total of 416 lipids measured. Univariate analysis showed that 28 individual lipid features and two lipid classes, triacylglycerides and oxidized fatty acids (FAs), were altered in patients with ALS, although none of these changes remained significant after multiple comparison adjustment. Most of these changes remained constant after removing from the analysis individuals treated with lipid-lowering drugs. The non-supervised principal component analysis did not identify any lipid clustering of patients with ALS and controls. Despite this, we performed a variety of linear and non-linear supervised multivariate models to select the most reliable features that discriminate the lipid profile of patients with ALS from controls. These were the monounsaturated FAs C24:1n-9 and C14:1, the triglyceride TG(51:4) and the sphingomyelin SM(36:2). *Conclusions*: Peripheral alterations of lipid metabolism are poorly defined in ALS, triacylglycerides and certain types of FAs could contribute to the different lipid profile of patients with ALS. These findings should be validated in an independent cohort.

Keywords: ALS, serum, lipidomics, lipidome, triacylglycerides

Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal neurological disorder characterized by the degeneration of motor neurons. Most ALS cases are sporadic, and diagnosis remains based upon clinical and electrophysiological examination (1). Early diagnosis and accurate measuring of disease progression can be difficult, making the search for diagnostic and prognostic biomarkers a high priority in ALS (1). Over the last decade, several candidate biomarkers, including levels of neurofilament light and heavy chain (NfL and NfH) (2–4) and functional measures (e.g. MUNIX) (5), have proven to be potentially able to differentiate ALS

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from other related disorders and have been used as outcome measures in clinical trials. However, there is no accurate biomarker hitherto introduced in clinical practice since heterogeneity in the aetiopathogenesis of ALS has complicated the interpretation of their diagnostic and prognostic value.

Novel biomarkers of disease may reasonably arise from the study of the different metabolic pathways -in particular those related to lipid metabolism—that appear to be involved in motor neuron degeneration. A bulk of clinical and experimental studies in patients and disease models has established clear evidence of a status of systemic hypermetabolism and increased basal energy expenditure in ALS (6,7). This metabolic feature is accompanied by increased fatty acid (FA) oxidation and decreased subcutaneous fat stores (8–10), which might occur even prior to MN degeneration (11,12). Furthermore, some studies have reported increased blood levels of triacylglycerides (TAGs) and cholesterol in patients with ALS, which are associated with a better prognosis (13–15), or high-fat diets that are associated with extended survival in animal models (16) and patients (17,18); while others have reported that lower levels of these lipids are associated with a decreased forced vital capacity (19). Altogether, adjustments in lipid metabolism probably respond to the high energy demands of the disease and modulate ALS phenotypes, making an association between the lipid profile and disease progression plausible.

Lipids are the most abundant metabolites in peripheral blood, and fasting blood lipids have been reported to be easily accessible indicators of metabolic dysfunction (20). The reported blood lipid profiles of ALS are generally incomplete, lack associations with clinical variables or have not been controlled for dietary habits; therefore, further investigation is needed.

To better characterize the nature and extent of metabolic changes in ALS and presumably aid in its diagnosis, we have performed a comprehensive analysis of the serum lipid profile in a cohort of patients with ALS and healthy controls.

Materials and methods

Study design, sample, and data collection

Patients with suspected motor neuron disease seen at the Donostia University Hospital ALS clinic and fulfilling El Escorial criteria for definite or probable ALS (either spinal or bulbar) were selected in a consecutive manner ($n = 13$). Araba University Hospital and Marques de Valdecilla University Hospital contributed with the inclusion of $n = 2$ and $n = 5$ patients, respectively, following the same clinical criteria ($n = 20$ in total). Twenty controls were included with the following criteria: they suffered no chronic illness and shared a living space with anyone of the selected patients with ALS. All patients remained ambulant and had not received noninvasive mechanical ventilation or undergone percutaneous endoscopic gastrostomy at time of sampling.

This is a cross-sectional study to characterize the nature and extent of lipidomic changes in the serum of patients with ALS when compared to healthy controls. To minimize potential factors influencing blood lipid profiling that could confound group differences (21), these criteria were followed: (i) controls lived with the recruited ALS patient and therefore came from the same community, increasing the likelihood of a similar diet on a day-to-day basis; (ii) both patients and controls underwent overnight fasting and had a similar meal the day before; (iii) blood was collected at the same time (between 09:00 and 10:00) in every participant; (iv) a standard operating procedure was followed by all groups and hospitals: Venous blood samples were collected in BD Vacutainer™ SST™ II Advance tubes; serum extraction was performed in the Basque Biobank unit at Donostia University Hospital following standardized procedures; variations in the interval time between blood draw and serum extraction were limited to 1–2 h.

The study was approved by the local Ethics Committees of the participant institutions, and samples were collected after participants gave written informed consent according to the Declaration of Helsinki.

Ultrahigh-performance liquid chromatography–mass spectrometry (UPLC–MS) lipidomics

UPLC–MS lipidomic profiles of the serum samples were carried out by OWL Metabolomics (Derio, Spain; www.owlmetabolomics.com/) as described previously (22), summarized in Supplementary Table S1 and Figure S1, and detailed in Supplementary Methods.

Statistical analysis

UPLC–MS datasets were analyzed by means of multivariate and univariate methods as described in Supplementary Methods.

Results

Demographic characteristics of patients and controls

Twenty patients with ALS and 20 healthy controls, with average ages at time of sample collection of 62.7 and 54.3 years, respectively, were enrolled in the study. Due to the priority criterion to include patients' partners as controls in order to match controls and patients for dietary habits and same meal before blood sampling, a gender and age bias was introduced. However, in doing so, the

Table 1. Demographic and clinical characteristics of patients with ALS and control subjects at study.

	ALS ($n = 20$)	Control ($n = 20$)
n	20	20
Age (years)[a]	62.7 ± 10.6	54.7 ± 12.8
Sex, m/f	14/6	5/15
Sporadic/familial	17/3	NA
Spinal/bulbar	18/2	NA
Cognitive symptoms	1	0
Lipid-lowering treatment[b]	3	0
Dyslipidemia[c]	14	1
Diabetes mellitus type 2	3	0
Time from onset to sampling (days)[a]	745.1 ± 494.9	NA

ALS: amyotrophic lateral sclerosis; UPLC: ultrahigh-performance liquid chromatography.
People with ALS from Donostia, Araba and Marqués de Valdecilla University Hospitals were recruited after diagnosis according to the revised El Escorial criteria. Controls were patients' partners who were healthy at the time of recruitment.
[a]Values given as mean values ± standard deviation.
[b]Patients on statins treatment upon blood collection.
[c]Dyslipidemia condition includes people with hypercholesterolemia and/or hypertriglyceridemia upon blood collection.

participants were matched for diet, one of the most significant lifestyle factors together with smoking and physical activity that influence the blood lipid profile. Three index cases had a family history of ALS, which are negative for mutations in the C9orf72, TDP43, SOD1, and FUS genes. Three patients were under treatment with lipid-lowering drugs. Average time from onset to sampling is 741.2 d. Demographics and clinical characteristics are summarized in Table 1.

Multivariate analysis

Baseline serum samples were subjected to a comprehensive lipidomic profiling by using two different analytical platforms of UPLC–MS. A total of 416 individual lipid features were detected (Supplementary Table S2). From these, 20 were found to be out of the linear detection range, and although they were not included in the subsequent multivariate data analysis, they were taken into consideration for the univariate data analysis.

After validating the quality of the experiment (Supplementary Figure S2) and removing two outliers (Figure 1(A)), the score scatter plot of the unsupervised PCA model generated for serum samples showed a random distribution of control subjects and patients with ALS (Figure 1(B)). The supervised orthogonal partial least squares-discriminant analysis (OPLS-DA) model to achieve the maximum separation between the groups illustrated a clustering of samples according to the presence or absence of the disease (Figure 2(A)); however, Q2Y was negative (Q2Y = −0.136) and some models built after random permutation of the *Y* values displayed higher Q2Y values, indicating that the model is not valid to separate both groups (Figure S3(A)). We removed from the dataset three patients that were receiving lipid-lowering drugs at time of sampling, since these treatments may influence the lipid profile. Again, the OPLS-DA model failed to separate ALS from controls (Q2Y = −0.143, Figure S3(B)). Despite not being able to provide a significant separation between ALS and controls, the loadings scatter plot showed that the lipid features contributing most to the separation between groups are the phospholipids PC(38:5), PC(0:0/20:5), PC(20:5/0:0), PC(18:2/20:4), PE(P-20:1/0:0), PC(20:0/18:2), and PE(O-16:0/0:0), the sphingolipids SM(33:1) and SM(39:1), the steroid sulfates androsterone and etiocholanolone, and the free FAs 14:1n-x and 14:2n-x (Figure 2(B)).

As the dimension reduction models PCA and OPLS-DA were overfitted and did not provide a significant separation between groups, we next analyzed the dataset by shrinkage/regularization methods, which are particularly indicated for situations in which the number of variables exceeds the number of observations. We developed the LASSO model with a cross-validation to select the optimal value of λ for which minimum MSE + 1SE was obtained. Using this method, we selected a subset of lipid metabolites that contributed the most into classification between groups. These are: 24:1n-9, SM(36:2), AC(12:0), PC(P-16:0/18:2), TG(51:4), PC(16:1/18:2), PC(0:0/16:1), TG(54:6), CMH(d18:1/24:1), x-HODE, PC(40:1), glycodeoxycholic acid, deoxycholic acid, and 14:1n-x. Similarly, with the optimal value of λ we developed a Ridge regression model, which introduces a shrinkage penalty and is able to reduce the variance without increasing the bias of the variables' coefficients. The top 10 lipid features selected by this model in order of coefficient rank are: 13-HODE, 8-HEPE, PC(16:0/0:0), PC(P-18:1/0:0), PC(17:0/0:0), PC(18:0/0:0), NAE(18:2n-6), AC(18:0), 22:5n-6, and PE(18:0/0:0).

Finally, we performed the non-parametric random forest method in an attempt to find non-linear

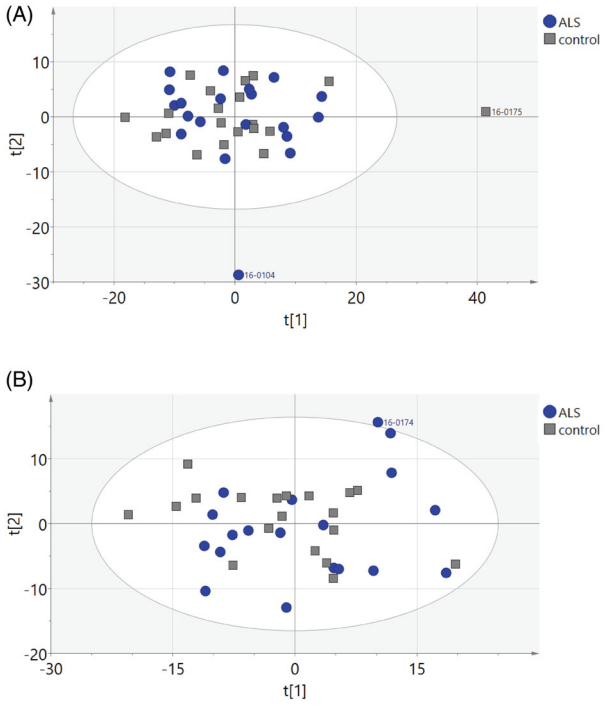

Figure 1. Principal component analysis (PCA) of serum lipid profilings from ALS and controls. (A) Score scatter plot of the PCA model of all serum samples shows a random distribution of samples and identifies two samples as potential outliers (16-0104 and 16-0175) since they appear far outside Hotelling's T2 ellipse. Model diagnostics (A = 7, R2X = 0.679, Q2X = 0.344). Following Chauvenet's criterion, further inspection of the data relating to those samples revealed that one sample from the ALS group had elevated levels of bile acids (BA), lysophosphatidylcholines (LPC), lysophosphatidylethanolamines (LPE), and steroids (ST), and another sample from the control group had increased levels of several phosphatidylcholines (PC), phophatidylethanolamines (PE), diacylglycerols (DG), triacylglycerols (TG), and sphingomyelins (SM). These outliers were excluded from the multivariate and univariate analyses performed in this study. (B) After removing two outlier samples and 20 metabolites out of the linear detection range, the score scatter plot of the unsupervised PCA model of serum samples shows a random distribution of healthy controls and patients with ALS. Although sample 16-0174 appears outside the Hotelling's T2 ellipse, it presented similar levels of individual lipids when compared to the rest of the samples, and so that it was not considered an outlier. Model diagnostics (A = 7, R2X = 0.647, Q2X = 0.316).

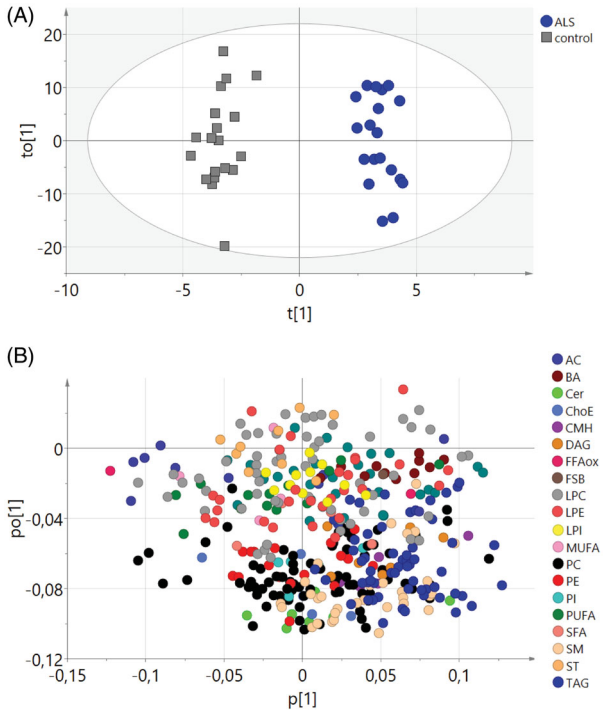

Figure 2. Orthogonal partial least square discriminant analysis (OPLS-DA) of serum lipid profilings from ALS and controls. (A) Score scatter plot of the OPLS-DA model shows a clear clustering of samples according to the presence or absence of the disease. Model diagnostics (A = 6; R2X = 0.299 R2Y = 0.633, Q2Y=–0.136). (B) After achieving the maximum separation between groups, the loadings scatter plot of the OPLS-DA multivariate model shows that metabolites responsible for the differences observed between patients with ALS and controls are the phospholipids PC(38:5), PC(0:0/20:5), PC(20:5/0:0), PC(18:2/20:4), PE(P-20:1/0:0), PC(20:0/18:2), and PE(O-16:0/0:0), the sphingolipids SM(33:1) and SM(39:1), the steroid sulfates androsterone and etiocholanolone, and the free fatty acids 14:1n-x and 14:2n-x. All are increased in patients with ALS. In this plot, metabolites lying away from the plot origin have stronger impact on the model; besides, variables positively correlated are grouped together, while variables negatively correlated are positioned in the opposite sides of the plot origin. AC: acyl carnitines; BA: bile acids; Cer: ceramides; ChoE: cholesteryl esters: CMH: monohexoxylceramides (cerebrosides); DAG: diacylglycerides; FFAox: oxidized fatty acids; FSB: free sphingoid bases; LPC: lysophosphatidylcholines; LPE: lysophosphatidylethanolamines; LPI: lysophosphatidylinositols; MUFA: monounsaturated fatty acids; PC: phosphatidylcholines; PCA: principal component analysis; PE: phosphatidylethanolamines; PI: phosphatidylinositols; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; SM: sphingomyelins; ST: steroid sulfates; TAG: triacylglycerides.

patterns in lipid metabolites that can explain variation between groups. This model provided a rank list with the most important lipid metabolites, which are: 24:1n-9, TG(51:4), PC(P-20:2/0:0), LPC(22:0), DG(38:6), PE(22:5/0:0), and PC(0:0/22:4).

Univariate analysis

Univariate data analysis showed that 28 individual lipid features out of 416 displayed *p*-values below 0.05 when comparing patients with ALS against healthy controls (Figures 3(A) and 4(A)). The free FAs C24:1n-9, the TGs (51:4) and (54:6), and the phosphatidylcholine PC(40:1) had the lowest *p*-values, and showed a positive $\log_2$-(fold-change) (Figure 3(A)). After removing from the analysis the three patients under treatment with lipid-lowering drugs, most of the changes remained constant (Figures 3(B) and 4(B)). In addition, most of the changes remained constant after removing from the analysis either patients with type 2 diabetes mellitus ($n = 3$) or bulbar-onset cases ($n = 2$) (Supplementary Table S3). However, in none of the comparisons any of the potential changes displayed significant *p*-values after accounting for multiple comparisons.

Furthermore, lipid classes were also calculated as the sum of the normalized areas of all the individual lipids features with the same chemical characteristics. Patients with ALS showed lower serum levels of oxidized FAs (hydroxyl-octadecadienoic acids; HODE) (log_2-(fold-change) = −0.24, p= 0.039) and increased levels of TAGs (log_2-(fold-change)=0.53, p= 0.022) when compared to control subjects (Figure 3(C)). Levels of TAGs, but not those of HODE, were also found altered in the patients who are free of any lipid-lowering drug, with p-values below 0.05 (Figure 3(D)). Again, none of the changes remained significant after adjusting the p-values for multiple comparisons.

It is interesting to note that there was an increased trend toward those TAGs containing long or very long-chain FAs moieties and 3–10 double bonds (Figure 5).

Summary of lipid features consistently selected by the different statistical procedures

Figure 4 shows a heatmap of the lipid features selected or prioritized by the different tested approaches, in either the comparisons between the whole cohort of ALS and controls, or after excluding the cases under lipid-lowering treatment. Four lipid metabolites were found to be selected or prioritized by at least three of the five tested approaches in each of the comparisons. These are: C24:1n-9, TG(51:4), SM(36:2), and 14:1n-x. None of these lipid features were significantly associated with age (Figure S4).

Discussion

In the present study, we have performed a novel and extensive scan of the serum lipidome profile in ALS in an attempt to identify specific lipid features that could be associated to the disease and provide new insights into the pathophysiology of ALS. The high sensitivity, high-throughput capacity of the different UPLC–MS platforms used in this study is optimized to give one of the broadest coverage across various lipid classes reported in literature (22), making this multi-technological approach ideally suited for taking a comprehensive picture of lipid metabolism in a single experiment. Owing to the aforementioned characteristics of this technology, we were able to detect 416 individual lipid ion features across 20 families in serum samples of 20 patients with ALS and 20 healthy controls. To our knowledge, this present study is the most comprehensive lipid profiling that has been performed in ALS.

Although reports recognizing the role of lipid metabolism in ALS are growing, most are limited to TAGs and cholesterol metabolites, and lack standardized sample collection protocols to avoid major influential factors, such as fed states (14,15,19,23–26). Two studies have made a broad analysis of the ALS lipidome profiling by means of HPLC–MS, one was carried out in CSF samples of patients with ALS and controls (27), and the other in plasma of patients with different treatment regimes (28). These studies were able to respectively analyze a considerable amount of lipid features: 200 and 105, respectively; but still far from the quantification of 416 individual lipids across 20 families that are analyzed in the present study. In disagreement with the latter study, in which patients with ALS were discriminated from controls on the basis of a group of lipid metabolites in the CSF compartment (27), our study did not find an specific serum lipid signature of ALS. Although CSF exchanges metabolites with blood, this overlap may not be substantial (29,30), and therefore any comparison should be taken with caution.

More recently, a metabolomics study was carried out in fasting prediagnostic ALS cases where 404 plasma metabolites were quantified (31). In connection with our study, Bjornevik et al. found a similar proportion of altered metabolites (around 7% of the total metabolites), and also failed to reliably distinguish patients with ALS from controls in the preclinical phase. Levels of various lipid features were oppositely distributed in comparison with our results, such as the levels of TAGs. However, the patient populations are different (symptomatic ALS patients in our study vs. presymptomatic in the latter) and, as mentioned previously, none of these changes retained their significance after correcting for multiple comparisons in neither of the studies. Nevertheless, the fact that these two independent studies with the most comprehensive scan of the blood metabolome in ALS to date fail to reliably distinguish patients with ALS from controls strengthens the idea that the metabolic alterations of ALS are broad and poorly defined both in preclinical (31) and clinical stages of ALS. This differs from other neurodegenerative disorders where blood metabolic signatures have been reported and specific lipid classes appeared to be altered, such as higher GM3 glangliosides in Parkinson's disease (32) or phosphatidylcholines in Alzheimer's dementia (33,34).

Notwithstanding that the hypermetabolic disease state of ALS (7,8) may likely induce complex and extensive metabolic reconfigurations that are not specific for any class of lipids, we observed a particular trend of change in the levels of TAGs and HODE in the serum of patients with ALS. An increase in TAGs in patients with ALS is among the most consistent data reported in literature (14,15,24) and could be explained by an increased lipolysis and lipid release from fat stores in response to the high metabolic rate associated to ALS (6,7), as it is the case for other hypermetabolic conditions such as burns, cancers, or

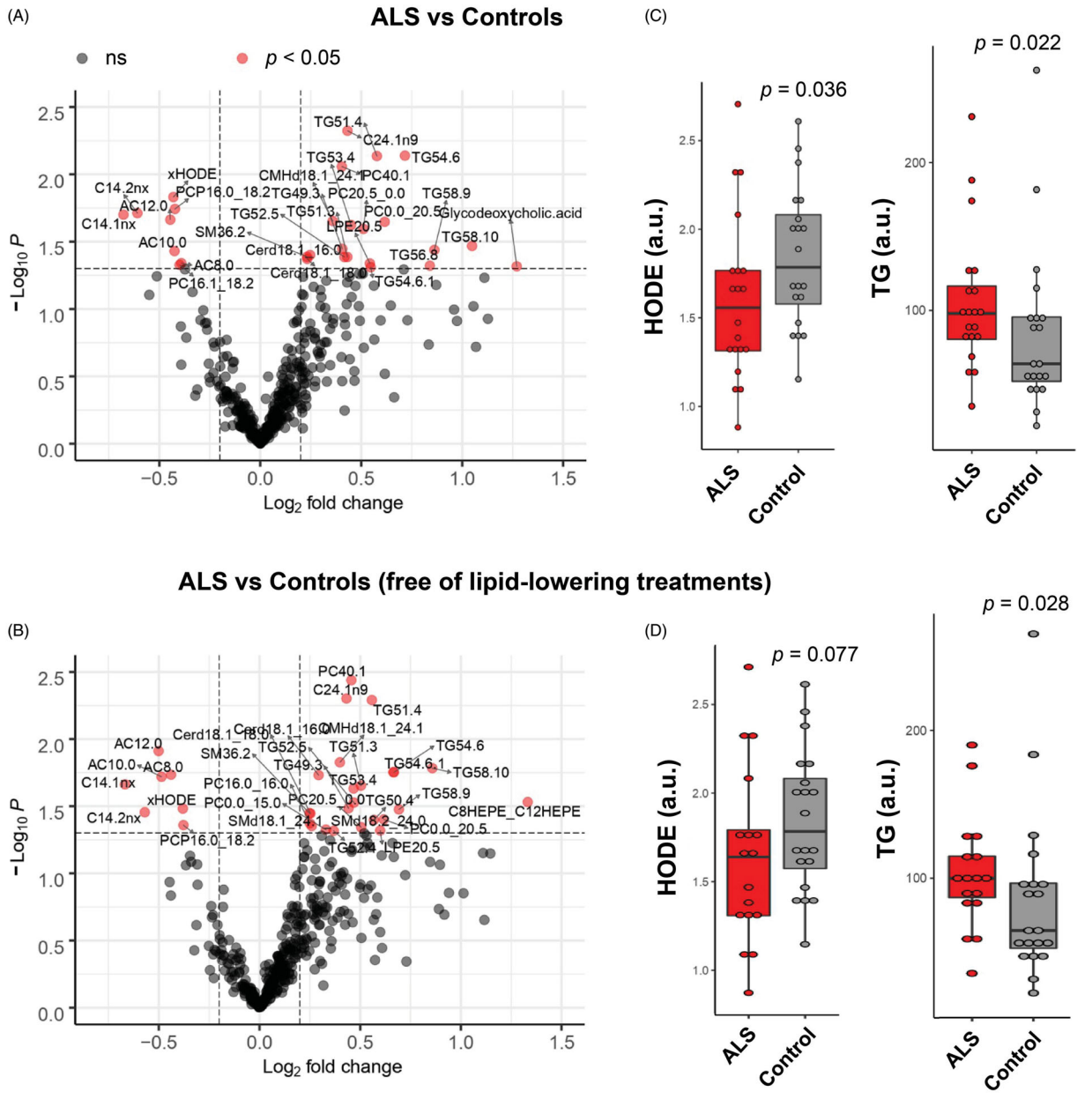

Figure 3. Univariate data analysis of the comprehensive UPLC–MS-based lipidomics reveals trends of various individual lipid signatures and certain lipid classes in the serum of ALS. Group percentage changes and paired Student's *t*-test *p*-value (or Welch's *t*-test where unequal variances were found) were calculated for the comparison shown above. (A) Volcano plot representation indicating the $\log_{10}$-(*p*-value) and the $\log_2$(fold-change) of the 416 individual serum lipidomic ion features of ALS. The non-esterified free fatty acid (FFA) 24:1-n9, the phosphatidylcholine (PC) 40:1 and the triacylglycerides (TAGs) 51:4 and 54:6 are the most prominent changes ($p< 0.01$), which are all increased in ALS patients when compared to healthy controls. (B) Volcano plot of the 416 individual serum lipidomic ion features of ALS after excluding patients under lipid-lowering treatment ($n=3$). The non-esterified free fatty acid (FFA) 24:1-n9, the phosphatidylcholine (PC) 40:1 and the TAG 51:4 are the most prominent changes ($p< 0.01$), which are all increased in ALS patients when compared to healthy controls. (C) Box and whisker plot illustrating the lipid classes that displayed *p*-values lower than 0.05 in the univariate analysis: a decrease of serum of oxidized fatty acids—in particular hydroxy-octadecadienoic acids (HODE)—($\log_2$-(fold-change)$=-0.24$, $p=0.036$) and an increase of serum TAGs ($\log_2$-(fold-change)$=0.53$, $p= 0.022$) in patients with ALS when compared to controls. (D) Box and whisker plot illustrating increased serum TAGs in ALS after excluding patients being treated with lipid-lowering drugs from the analysis ($\log_2$-(fold-change)$=0.48$, $p= 0.028$); however levels of HODE displayed *p*-values above 0.05. Medians, interquartile ranges (boxes), minimal and maximal values (whiskers) and missing values (black dots) are represented. However, none of the changes in the individual lipid features or lipid classes remained significant after correcting for multiple comparisons.

uncontrolled diabetes mellitus (35,36). Moreover, this excess of circulating TAGs can result in fat deposition and increased incidence of fatty liver, a frequent finding in patients with ALS (13,37). The extensive utilization of FAs from TAGs as energy source by mitochondria can lead to oxidative stress (38), which is extremely harmful to motor neurons and a claimed mechanism of ALS (1). However, various studies have shown that higher levels of TAG tend to decrease the risk of developing ALS

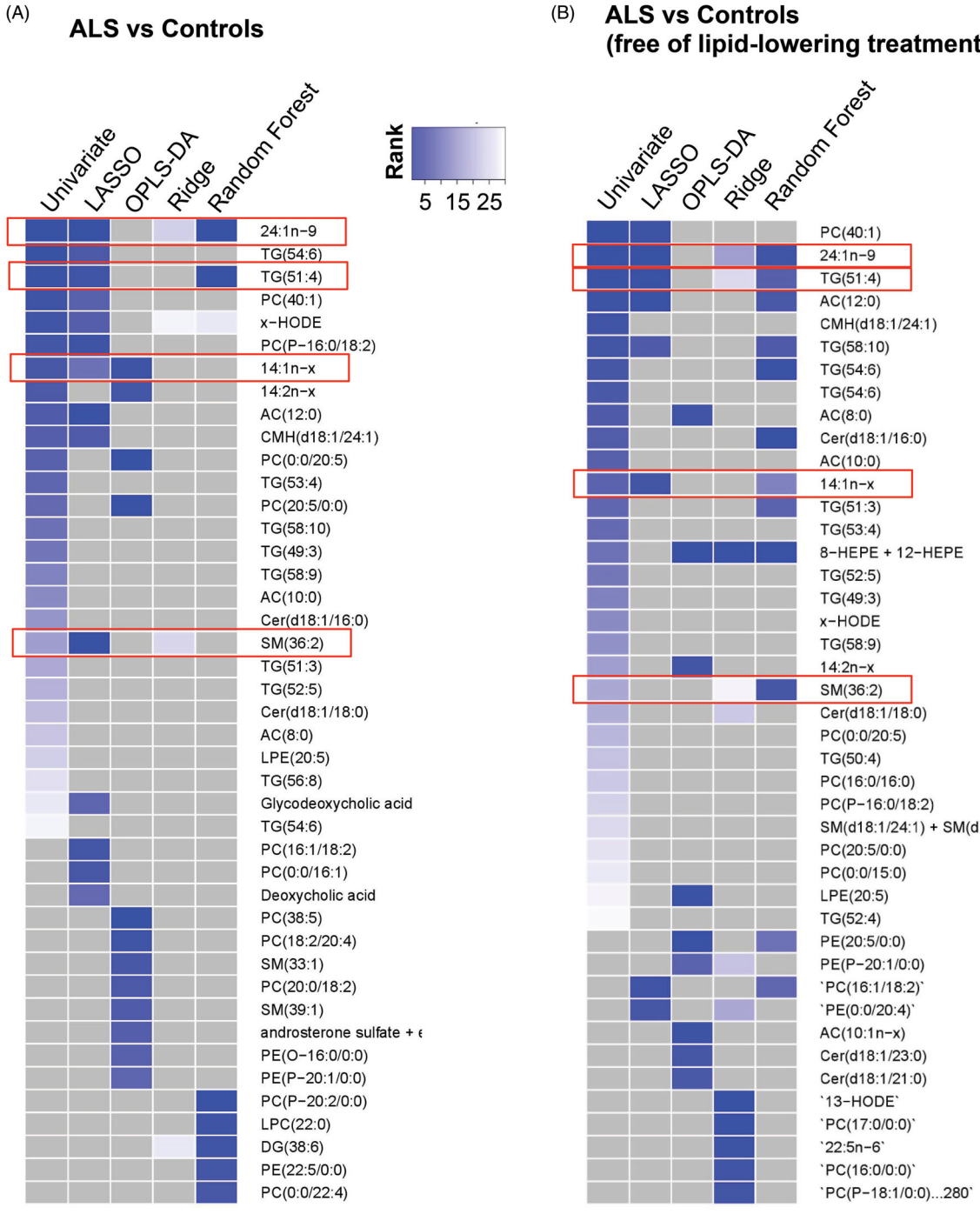

Figure 4. Lipid features selected and prioritized by different models. Heatmap of the lipid features selected or prioritized by the different tested approaches in rank order, in either the comparisons between the whole cohort of ALS and controls (A), or after excluding the cases under lipid-lowering treatment (B). Four lipid metabolites were found to be selected or prioritized by at least three of the five tested approaches in each of the comparisons: C24:1n-9, TG(51:4), SM(36:2), and 14:1n-x, highlighted in red.

(39) or correlate with an increased life expectancy in patients with ALS (15). In total, ALS is a fast-progressing disease with a marked energy metabolism disruption (40), and thus it is likely to speculate that energy reconfiguration toward the use of lipids, a phenomenon that has been already reported to occur in peripheral and central tissues in models of ALS (11,41), is an adaptive response to cope with the lack of nutrients and energy in ALS. This is a simplistic interpretation when considering TAGs as a whole class of lipids. However, the pool of TAGs in blood is complex and diverse. Thanks to the chromatographic detection settings utilized in the present study, we have been able to identify 60 different types of TAGs, which provides a more detailed overview of this lipid class. Indeed, we have observed that not all types of TAGs are equally altered in the serum of patients with ALS, and those TAGs built with FAs containing bigger number of carbons and double

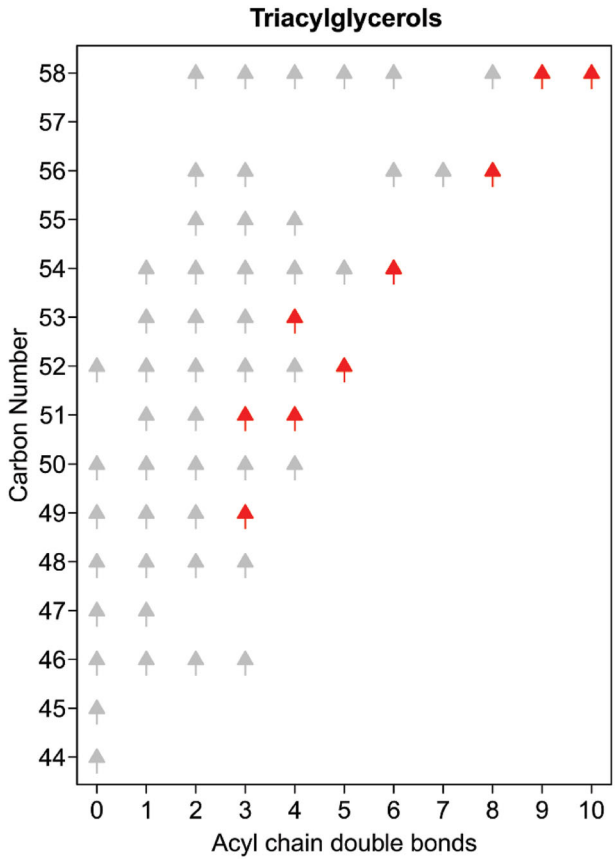

Figure 5. Influence of the total acyl chain carbon number and double bond content in the increment of triacylglycerides (TAGs) in patients with ALS compared to controls. Upregulated changes with *p*-values lower than 0.05 are indicated by darker arrows, while lighter arrows indicate upregulated changes with *p*-values higher than 0.05. However, none of these changes remained significant after correcting for multiple comparisons.

bonds (polyunsaturated) appeared to be particularly increased in ALS. An alternative explanation to the mobilization of TAGs to deal with energy deficits in ALS, we suggest that the specific increase of TAGs with long-chain and polyunsaturated FAs might be a consequence of an impaired capacity to specifically oxidize these TAG species. Long and very long-chain polyunsaturated FAs are predominantly oxidized in peroxisomes, and indeed defects of peroxisomes are increasingly being recognized as an aethiopathogenic factor of ALS (42,43). Therefore, our data may provide compelling evidence to support peroxisomal alterations in ALS.

HODE are the dominant components of atheromatous plaques in vessels (44), and statins are widely used to prevent atherosclerosis and atherosclerosis-related diseases. The decreased levels of HODE that we observed might be due to a confounding effect of lipid-lowering statins, as the trend is lost when removing patients under these treatments. Thus, from the present study's results, HODE in ALS disease do not appear to be relevant.

One of the most consistent changes achieved through different statistical processing of the current data is the monounsaturated and very long-chain FA C24:1n.9, also known as nervonic acid. Changes in the relative content of nervonic acid were observed both for C24:1 sphingolipids (in particular one CMH cerebroside) and the non-esterified C24:1n-9 FA. Levels of this FA have not been previously found altered in the CSF or spinal cord of patients with ALS (27,45). Despite nervonic acid is one of the major FAs in brain sphingolipids and the most abundant component of the myelin sheath in the CNS, it is also one of the most abundant FAs in the myelin sheath of peripheral nerves and skeletal muscle (46). In contrast, the presence of nervonic acid in TAGs is scarce; therefore their increased levels are likely to occur independently of the rise in TAGs with long-chain FAs. In this sense, previous work identified nervonic acid as one of the specific lipids whose circulating levels display an increase during the period of distal demyelination but not after remission in an induced experimental model of autoimmune encephalomyelitis (47). Taking together, the rise of nervonic acid in serum of patients with ALS is more likely to derive from the muscle denervation process and peripheral nervous damage than from the central neuron loss, and further studies should assess the relationship of nervonic acid with the levels of NfL, a marker of axonal degeneration (48). In addition, the relative amount of C24 sphingolipids, in particular those containing nervonic acid, with respect to the amount of the FA C16, induces important changes in receptor signaling and specialized cell membranes properties, such as the contact sites between mitochondria and endoplasmic reticulum, known as MAMs (49,50). Thus, it is also possible to argue that the increased levels of nervonic acid are related to alterations in MAMs, which indeed are widely recognized to be involved in the pathology of ALS and other neurodegenerative conditions (51).

Finally, the fact that the C14:1n-x FA is the other lipid feature selected by at least three of the five tested approaches indicates the probable relevance of an altered metabolism of FAs in ALS disease. It is likely that the FA represented by the lipid feature C14:1n-x is C14:1n-5, since there is no other FA of 14 carbons desaturated at any other position in mammals. The desaturation at the position n-5 is catalyzed by the stearoyl-CoA desaturase (SCD1), which also catalyzes a rate-limiting step in the synthesis of n-7 and n-9 monounsaturated FAs (52) and has a critical role in the homeostasis of lipid metabolism and energy expenditure in different tissues (53,54). Furthermore, a decreased desaturation of C14 and C16 FAs by SCD1 has been previously shown in

ALS, and this correlated with decreased survival of patients (55). Thus, the alterations in C14:1n-x may be connected to the observed changes in nervonic acid through a dysregulated synthetic pathway that compromise the desaturation of C14 and C16 FAs in the face of promoting the desaturation of C18-derived n-9 FAs. The over-production of nervonic acid as a result of a dysregulated FA synthetic pathway would support the structural and signaling effect of nervonic acid on MAMs in ALS. Further research is needed to provide compelling evidence on the role of nervonic acid in ALS.

As already mentioned, dietary habits and fasting state have a profound influence on blood lipid profiles, since a major change in the lipid trafficking occurs in the fed state as tissues and organs deal with the large influx of meal fat. In prioritizing a strict control for homogeneous nutrition habits and fasting states between groups, a significant difference in age and gender distribution between groups was raised, given the inclusion of patient's partners as controls. This is why all results were adjusted for age and gender. In addition, given the extensive comprehensive nature of the analysis, our sample size was also moderate, which could have limited our ability to detect smaller lipid changes or confirm our findings.

Overall, we report one of the most comprehensive lipid profiling of ALS, which did not reveal any serum lipid signature discriminating patients with ALS from controls. However, certain types of TAGs and FAs could contribute to the different lipid profile of patients with ALS. Despite the fact that peripheral metabolic derangements have been consistently observed in patients with ALS, such as hypermetabolism, systemic lipid abnormalities in patients with ALS seem to be poorly defined.

Acknowledgments

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Declaration of interest

The authors report no conflict of interest.

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References

1. van Es MA, Hardiman O, Chio A, Al-Chalabi A, Pasterkamp RJ, Veldink JH, et al. Amyotrophic lateral sclerosis. Lancet. 2017;390:2084–98.
2. Gaiani A, Martinelli I, Bello L, Querin G, Puthenparampil M, Ruggero S, et al. Diagnostic and prognostic biomarkers in amyotrophic lateral sclerosis: neurofilament light chain levels in definite subtypes of disease. JAMA Neurol. 2017; 74:525–32.
3. Lu C-H, Macdonald-Wallis C, Gray E, Pearce N, Petzold A, Norgren N, et al. Neurofilament light chain: a prognostic biomarker in amyotrophic lateral sclerosis. Neurology. 2015;84:2247–57.
4. Feneberg E, Oeckl P, Steinacker P, Verde F, Barro C, Van Damme P, et al. Multicenter evaluation of neurofilaments in early symptom onset amyotrophic lateral sclerosis. Neurology. 2018;90:e22–30.
5. Paganoni S, Cudkowicz M, Berry JD. Outcome measures in amyotrophic lateral sclerosis clinical trials. Clin Investig. 2014;4:605–18.
6. Steyn FJ, Ioannides ZA, van Eijk RPA, Heggie S, Thorpe KA, Ceslis A, et al. Hypermetabolism in ALS is associated with greater functional decline and shorter survival. J Neurol Neurosurg Psychiatry. 2018;89:1016–23.
7. Jésus P, Fayemendy P, Nicol M, Lautrette G, Sourisseau H, Preux P-M, et al. Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. Eur J Neurol. 2018;25:97–104.
8. Zufiría M, Gil-Bea FJ, Fernández-Torrón R, Poza JJ, Muñoz-Blanco JL, Rojas-García R, et al. ALS: a bucket of genes, environment, metabolism and unknown ingredients. Prog Neurobiol. 2016;142:104–29.
9. Chiang P-M, Ling J, Jeong YH, Price DL, Aja SM, Wong PC. Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proc Natl Acad Sci USA. 2010;107:16320–4.
10. Fergani A, Oudart H, Gonzalez De Aguilar J-L, Fricker B, René F, Hocquette J-F, et al. Increased peripheral lipid clearance in an animal model of amyotrophic lateral sclerosis. J Lipid Res. 2007;48:1571–80.
11. Palamiuc L, Schlagowski A, Ngo ST, Vernay A, Dirrig-Grosch S, Henriques A, et al. A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis. EMBO Mol Med. 2015;7:526–46.
12. Dobrowolny G, Lepore E, Martini M, Barberi L, Nunn A, Scicchitano BM, et al. Metabolic changes associated with muscle expression of SOD1G93A. Front Physiol. 2018;9: 831.
13. Dupuis L, Corcia P, Fergani A, Gonzalez De Aguilar J-L, Bonnefont-Rousselot D, Bittar R, et al. Dyslipidemia is a protective factor in amyotrophic lateral sclerosis. Neurology. 2008;70:1004–9.
14. Dorst J, Kühnlein P, Hendrich C, Kassubek J, Sperfeld AD, Ludolph AC. Patients with elevated triglyceride and cholesterol serum levels have a prolonged survival in amyotrophic lateral sclerosis. J Neurol. 2011;258:613–7.
15. Huang R, Guo X, Chen X, Zheng Z, Wei Q, Cao B, et al. The serum lipid profiles of amyotrophic lateral sclerosis patients: a study from south-west China and a meta-analysis. Amyotroph Lateral Scler Front Degener. 2015; 16:359–65.
16. Zhao Z, Sui Y, Gao W, Cai B, Fan D. Effects of diet on adenosine monophosphate-activated protein kinase activity

and disease progression in an amyotrophic lateral sclerosis model. J Int Med Res. 2015;43:67–79.
17. Kim B, Jin Y, Kim SH, Park Y. Association between macronutrient intake and amyotrophic lateral sclerosis prognosis. Nutr Neurosci. 2018;23:8–15.
18. Fitzgerald KC, O'Reilly ÉJ, Falcone GJ, McCullough ML, Park Y, Kolonel LN, et al. Dietary ω-3 polyunsaturated fatty acid intake and risk for amyotrophic lateral sclerosis. JAMA Neurol. 2014;71:1102.
19. Chiò A, Calvo A, Ilardi A, Cavallo E, Moglia C, Mutani R, et al. Lower serum lipid levels are related to respiratory impairment in patients with ALS. Neurology. 2009;73: 1681–5.
20. Lydic TA, Goo Y-H. Lipidomics unveils the complexity of the lipidome in metabolic diseases. Clin Transl Med. 2018;7:26.
21. Burla B, Arita M, Arita M, Bendt AK, Cazenave-Gassiot A, Dennis EA, et al. MS-based lipidomics of human blood plasma: a community-initiated position paper to develop accepted guidelines. J Lipid Res. 2018;59:2001–17.
22. Alonso C, Fernández-Ramos D, Varela-Rey M, Martínez-Arranz I, Navasa N, Van Liempd SM, et al. Metabolomic identification of subtypes of nonalcoholic steatohepatitis. Gastroenterology. 2017;152:1449.e7–61.e7.
23. Paganoni S, Deng J, Jaffa M, Cudkowicz ME, Wills A-M. Body mass index, not dyslipidemia, is an independent predictor of survival in amyotrophic lateral sclerosis. Muscle Nerve. 2011;44:20–4.
24. Ikeda K, Hirayama T, Takazawa T, Kawabe K, Iwasaki Y. Relationships between disease progression and serum levels of lipid, urate, creatinine and ferritin in Japanese patients with amyotrophic lateral sclerosis: a cross-sectional study. Intern Med. 2012;51:1501–8.
25. Yang JW, Kim S-M, Kim H-J, Kim J-E, Park KS, Kim S-H, et al. Hypolipidemia in patients with amyotrophic lateral sclerosis: a possible gender difference? J Clin Neurol. 2013;9:125–9.
26. Delaye JB, Patin F, Piver E, Bruno C, Vasse M, Vourc'h P, et al. Low IDL-B and high LDL-1 subfraction levels in serum of ALS patients. J Neurol Sci. 2017;380:124–7.
27. Blasco H, Veyrat-Durebex C, Bocca C, Patin F, Vourc'h P, Kouassi Nzoughet J, et al. Lipidomics reveals cerebrospinal-fluid signatures of ALS. Sci Rep. 2017;7: 17652.
28. Blasco H, Patin F, Descat A, Garçon G, Corcia P, Gelé P, et al. A pharmaco-metabolomics approach in a clinical trial of ALS: identification of predictive markers of progression. PLoS One. 2018;13:e0198116.
29. Wuolikainen A, Jonsson P, Ahnlund M, Antti H, Marklund SL, Moritz T, et al. Multi-platform mass spectrometry analysis of the CSF and plasma metabolomes of rigorously matched amyotrophic lateral sclerosis, Parkinson's disease and control subjects. Mol Biosyst. 2016;12:1287–98.
30. Trupp M, Jonsson P, Ohrfelt A, Zetterberg H, Obudulu O, Malm L, et al. Metabolite and peptide levels in plasma and CSF differentiating healthy controls from patients with newly diagnosed Parkinson's disease. J Parkinsons Dis. 2014;4:549–60.
31. Bjornevik K, Zhang Z, O'Reilly ÉJ, Berry JD, Clish CB, Deik A, et al. Prediagnostic plasma metabolomics and the risk of amyotrophic lateral sclerosis. Neurology. 2019;92: e2089–100.
32. Chan RB, Perotte AJ, Zhou B, Liong C, Shorr EJ, Marder KS, et al. Elevated GM3 plasma concentration in idiopathic Parkinson's disease: a lipidomic analysis. PLoS One. 2017;12:e0172348.
33. Proitsi P, Kim M, Whiley L, Simmons A, Sattlecker M, Velayudhan L, et al. Association of blood lipids with Alzheimer's disease: a comprehensive lipidomics analysis. Alzheimer's Dement. 2017;13:140–51.
34. Simpson BN, Kim M, Chuang Y-F, Beason-Held L, Kitner-Triolo M, Kraut M, et al. Blood metabolite markers of cognitive performance and brain function in aging. J Cereb Blood Flow Metab. 2016;36:1212–23.
35. Abdullahi A, Jeschke MG. White adipose tissue browning: a double-edged sword. Trends Endocrinol Metab. 2016; 27:542–52.
36. Nair KS, Halliday D, Garrow JS. Increased energy expenditure in poorly controlled Type 1 (insulin-dependent) diabetic patients. Diabetologia. 1984;27:13–6.
37. Nodera H, Takamatsu N, Muguruma N, Ukimoto K, Nishio S, Oda M, et al. Frequent hepatic steatosis in amyotrophic lateral sclerosis: implication for systemic involvement. Neurol Clin Neurosci. 2015;3(2):58–62.
38. Schönfeld P, Reiser G. Why does brain metabolism not favor burning of fatty acids to provide energy-reflections on disadvantages of the use of free fatty acids as fuel for brain. J Cereb Blood Flow Metab. 2013;33:1493–9.
39. O'Reilly ÉJ, Bjornevik K, Furtado JD, Kolonel LN, Le Marchand L, McCullough ML, et al. Prediagnostic plasma polyunsaturated fatty acids and the risk of amyotrophic lateral sclerosis. Neurology. 2019. doi:10.1212/WNL.0000000000008676
40. Dupuis L, Pradat P-F, Ludolph AC, Loeffler J-P. Energy metabolism in amyotrophic lateral sclerosis. Lancet Neurol. 2011;10:75–82.
41. Szelechowski M, Amoedo N, Obre E, Léger C, Allard L, Bonneu M, et al. Metabolic reprogramming in amyotrophic lateral sclerosis. Sci Rep. 2018;8:3953.
42. Du Y, Wen Y, Guo X, Hao J, Wang W, He A, et al. A genome-wide expression association analysis identifies genes and pathways associated with amyotrophic lateral sclerosis. Cell Mol Neurobiol. 2018;38:635–9.
43. Mitchell J, Paul P, Chen H-J, Morris A, Payling M, Falchi M, et al. Familial amyotrophic lateral sclerosis is associated with a mutation in D-amino acid oxidase. Proc Natl Acad Sci USA. 2010;107:7556–61.
44. Staprans I, Pan XM, Rapp JH, Feingold KR. The role of dietary oxidized cholesterol and oxidized fatty acids in the development of atherosclerosis. Mol Nutr Food Res. 2005; 49:1075–82.
45. Ilieva EV, Ayala V, Jove M, Dalfo E, Cacabelos D, Povedano M, et al. Oxidative and endoplasmic reticulum stress interplay in sporadic amyotrophic lateral sclerosis. Brain. 2007;130:3111–23.
46. Sandhir R, Khan M, Chahal A, Singh I. Localization of nervonic acid beta-oxidation in human and rodent peroxisomes: impaired oxidation in Zellweger syndrome and X-linked adrenoleukodystrophy. J Lipid Res. 1998;39: 2161–71.
47. Lee G, Hasan M, Kwon O-S, Jung BH. Identification of altered metabolic pathways during disease progression in EAE mice via metabolomics and lipidomics. Neuroscience. 2019;416:74–87.
48. Menke RAL, Gray E, Lu C-H, Kuhle J, Talbot K, Malaspina A, et al. CSF neurofilament light chain reflects corticospinal tract degeneration in ALS. Ann Clin Transl Neurol. 2015;2:748–55.
49. Iwabuchi K, Nakayama H, Iwahara C, Takamori K. Significance of glycosphingolipid fatty acid chain length on membrane microdomain-mediated signal transduction. FEBS Lett. 2010;584:1642–52.
50. Courtney KC, Pezeshkian W, Raghupathy R, Zhang C, Darbyson A, Ipsen JH, et al. C24 sphingolipids govern the transbilayer asymmetry of cholesterol and lateral organization of model and live-cell plasma membranes. Cell Rep. 2018;24:1037–2018.
51. Bernard-Marissal N, Chrast R, Schneider BL. Endoplasmic reticulum and mitochondria in diseases of motor and sensory neurons: a broken relationship? Cell Death Dis. 2018;9:333.

52. Flowers MT, Ntambi JM. Role of stearoyl-coenzyme A desaturase in regulating lipid metabolism. Curr Opin Lipidol. 2008;19:248–56.
53. Hussain G, Schmitt F, Henriques A, Lequeu T, Rene F, Bindler F, et al. Systemic down-regulation of delta-9 desaturase promotes muscle oxidative metabolism and accelerates muscle function recovery following nerve injury. PLoS One. 2013;8:e64525.
54. ALJohani AM, Syed DN, Ntambi JM. Insights into stearoyl-CoA desaturase-1 regulation of systemic metabolism. Trends Endocrinol Metab. 2017;28: 831–42.
55. Henriques A, Blasco H, Fleury M-C, Corcia P, Echaniz-Laguna A, Robelin L, et al. Blood cell palmitoleate–palmitate ratio is an independent prognostic factor for amyotrophic lateral sclerosis. PLoS One. 2015;10:e0131512.

Defective TDP-43 and FUS affect muscle function through FoxO-mediated dysregulation of energy metabolism

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SUMMARY

Beyond the extensive motor neuron loss that characterizes amyotrophic lateral sclerosis (ALS), abnormalities of skeletal muscle regeneration due to cell-autonomous dysfunction of muscle progenitor cells are being recognized as early features of the disease. However, whether genes found mutated in familiar ALS contribute to these phenotypes and the molecular mechanisms behind are unknown. In this study, we find that human myoblasts with induced TARDBP or FUS loss-of-function displayed aberrant myogenic differentiation capacity, a phenotype that was concomitantly expressed with a dramatic repression of energy production from anaerobic glycolysis and the induction of pro-atrophic pathways. Gene expression profiling and biochemical surveys identified FoxO transcription factors as key regulators of the transcriptional reprogramming of TARDBP-silenced myoblasts, with a marked nuclear over-activation of FoxO1 and FoxO3 factors in proliferating myoblasts. Interestingly, either pharmacological or genetic inhibition of FoxO1/3 alleviated the metabolic disturbances related to glycolysis and boosted the myogenic program in FUS and TARDBP-deficient myoblasts. In additon, inhibition of *foxo* improved neuromuscular function in Drosophila models with muscle-specific deficiency of TARDBP and FUS orthologs. Finally, correction of metabolic and myogenic abnormalities with FoxO1/3 inhibitor in primary myoblasts derived from patients with ALS bolstered the argument for the existence of muscle intrinsic phenotypes in ALS that can be managed pharmacologically. We conclude that FoxO factors are important functional drivers of FUS or TARDBP loss-of-function in skeletal muscle, and thus these factors may be targets to improve muscular fitness in patients with ALS.

INTRODUCTION

Amyotrophic lateral sclerosis (ALS) is a devastating disorder characterized by a progressive degeneration of upper and lower motor neurons that inevitably leads to the death of the patient within an average of 36 months from onset (Chiò et al., 2009; Taylor et al., 2016). Major symptoms, including weakness, motor disability, paralysis and ultimately death arise from the progressive wasting of skeletal muscle. Although motor neuron loss and subsequent dismantlement of neuromuscular junctions are considered the primary cause of muscle atrophy, recent studies have challenged this view by showing clear evidence of early muscle metabolic derangements in relation to disease progression (Loeffler et al., 2016), occurring even prior to muscle denervation in SOD1 mouse models (Dobrowolny et al., 2018a, 2018b; Palamiuc et al., 2015).

Maintenance of muscle fiber homeostasis in adulthood is fundamentally supported by intrinsic repairing mechanisms that involve a sequence of metabolically fine-tuned steps; starting from the activation and proliferation of skeletal muscle resident stem cells (known as satellite cells) until the differentiation of myogenic myoblasts into new myofibers (Relaix and Zammit, 2012). Several lines of evidence have brought into light that muscle turnover is affected in ALS, and that impaired myogenic processes would aggravate the denervation-induced muscle wasting. In this sense, satellite cells isolated from patients with ALS have shown changes in various myogenic markers (Jensen et al., 2016) and impaired ability to form mature myotubes in *in vitro* conditions (Pradat et al., 2011; Scaramozza et al., 2014). Likewise, myogenic defects have been also reported in cultured myoblasts derived from mouse models expressing mutations in either SOD1 (Manzano et al., 2013) or VAPB (Tokutake et al., 2015). Although these myogenic defects may be legitimately considered secondary to changes in the denervated stem cell niche, recent discovery of the moonlighting activity of TARDBP (a major ALS-causative gene) in muscle regeneration have bolstered the argument for the implication of myogenesis in the pathophysiology of ALS. In that study, the product of TARDBP gene known as TDP-43 was proved essential for normal skeletal muscle formation by recruiting into cytoplasmic amyloid-like granules specific mRNAs that encode sarcomeric proteins (Vogler et al., 2018).

We postulate that muscle regeneration and wasting associated to ALS might stem from cell-autonomous defects of metabolic stasis in muscle stem cells, and seek for pathological mechanisms underlying these abnormalities. Thus, we have studied the contribution of two major ALS genes, including TARDBP and FUS, in the metabolic regulation of myogenesis and muscle regeneration, by using human immortalized myoblasts with induced loss-of-function of these genes and primary myoblasts from patients with ALS, as well as Drosophila models. Metabolic flux analyses enabled the discovery of repressed glycolysis as a consistent metabolic

feature linking energy defects with the impaired myogenic capacity induced by TDP-43 and FUS knockdown. In addition, gene expression surveys led to the identification of FoxO transcription factors as potential regulators of the differentially expressed genes in TARDBP and FUS knockdown myoblasts. Interestingly, pharmacological and genetic inhibition of FoxO alleviated the metabolic defects and corrected the myogenic differentiation ability in the immortalized myoblasts with induced deficiency of TARDBP and FUS. The metabolic and myogenic abnormalities of silenced myoblasts were reproduced in primary myoblasts derived from two patients with ALS, and consequently alleviated by FoxO inhibition. Finally, in drosophila models that bear a conditional knockdown of the fly gene orthologs for TARDBP and FUS, (*Tbph* and *Caz)* in muscle progenitor cells, we found impaired motor function and decreased lifespan, both of them alleviated by pharmacological inhibition of *foxo*. Thus, we have identified FoxO factors as mediators of the effects of TDP-43 or FUS loss-of-function on metabolic and functional stasis in skeletal muscle.

MATERIAL AND METHODS

Cell models

Immortalized human myoblasts line (8220)

The immortalized human myoblast cell line 8220 was provided by Dr. Vincent Mouly and generated on the Platform for the Immortalization of Human Cells, at the Myology Institute in Paris (France). The proliferation medium consisted of Promocell Skeletal Growth Medium (SGM) supplemented with 10% FBS, 1% Glutamax and 1% gentamicin (both from Gibco-Invitrogen). Myogenesis have not been induced with any specific differentiation media, but in natural way using DMEM (Gibco-Invitrogen) supplemented with insulin 10 mg / ml, human transferrin 100 was used mg / ml and gentamicin 50 mg / ml.

Gene silencing was carried out by infection with lentiviral particles containing short hairpin RNA (shRNA). The plasmids used to create these particles were: SHC001 (shRNA Empty Vector Control Plasmid DNA), TRCN000016038 (human, TARDBP MISSION shRNA), TRCN0000288639 (human, FUS MISSION shRNA), TRCN00, 33 FOXO MISSION shRNA) (MISSION® pLKO.1-Pure, Sigma-Aldrich). Viral particles were produced and titled by the Viral Vector Unit of CNIC (Madrid). When the 8220 myoblasts were found at 50% confluence, viral particles at a MOI of 10 were added. After 24 hours the medium was removed and replaced by the fresh SGM proliferation medium. To verify the degree of gene silencing, TDP-43 and FUS proteins were measured by Western Blot.

Primary myoblasts from patients' muscle biopsies

Three muscle biopsies were performed: 1) "Control", 45 year old male, left gastrocnemius; 2) "Sporadic ALS", 66-year-old woman, right biceps; 3) "Familial ALS", 45 year old male, left anterior tibial. Muscle biopsies were taken using the open sky technique, transported in conditioning medium and washed in 1% HBSS-Ca / Mg-PSF medium, removing capillaries and adipose tissue. The washed biopsies were cut into fragments and incubated at 37 ° C with 5% CO2 overnight. The next day, the muscle fragments were frozen in liquid nitrogen submerged in freezing medium. After thawing, muscle fragments were washed in 1% HBSS-Ca / Mg-PSF medium. They were cut into smaller fragments in conditioning medium. These were immersed in conditioning medium mixed with 25% human serum for 15-30 minutes to coagulate (this is called clot). At that time they were grown in an incubator at 37 ° C, with 5% CO2 for 4-5 days. After this long incubation the muscle fragments were washed in 1% HBSSCa / Mg-PSF medium and cut into even smaller fragments (approximately 1 mm). After coating with a drop of the gelatin / plasma mixture the surface of a p35 plate, the muscle fragments or explants were placed on top (5 fragments / p35). A few drops of proliferation medium were added between the

muscle fragments to maintain moisture and incubated at 37 ° C for 30 minutes, so that the fragments adhered to the plate. Then more proliferation medium was added and the cells were grown in an incubator at 37 ° C, with 5% CO2 and a humidified atmosphere for 5-7 days (the medium was changed twice a week). After this time, once cells growing from the explants were observed, they were trypsinised and transferred to new plates to be cultured again. The trypsinized cells were centrifuged at 1,500 rpm for 10 minutes. After discarding the supernatant and resuspending the pellet in AutoMACS separation buffer, the cells were centrifuged again at 1,500 rpm for 10 minutes. After discarding the supernatant, the pellet was mixed with 80 µl of AutoMACS separation buffer and 20 µl of CD56 + magnetic beads were added. The mixture was incubated at 4 ° C for 15 minutes and subsequently washed with AutoMACS separation buffer and centrifuged at 1,500 rpm for 10 minutes. After discarding the supernatant, the pellet was washed again with AutoMACS separation buffer. At this point the selection of CD56 + cells was carried out. To do this, an LS column was placed on the magnet and washed with 3 ml of AutoMACS separation buffer. The sample was then added to the column and then the column was washed with another 3 ml of AutoMACS separating buffer 3 times. As the myoblasts express CD56, they remain attached to the column, therefore, as soon as the entire AutoMACS separation buffer passed through the column, it was released from the magnet and after adding 5 ml of AutoMACS separation buffer, the syringe was used to release the CD56 + cells (myoblasts) from the spine and collect them. The cells were centrifuged at 1,500 rpm for 10 minutes and after discarding the supernatant the pellet was resuspended in proliferation medium. The myoblasts were grown at the desired density in plates in an incubator at 37 ° C, with 5% CO2 and a humidified atmosphere. The proliferation medium used was the SGM.

Drosophila models

One Drosophila strain silencing Cabeza (Caz) gene in muscle cells (UAS-Dicer2-iCaz-Mef2-GAL4, iCaz) and two Drosophila strain silencing Tbph gene at different locus in muscle cells (UAS-iTbphattp40-Mef2-GAL4; UAS-iTbphp(GD6943)-Mef2-GAL4) have been generated. As control flies, the strain UAS-Dicer2-+-Mef2-GAL4 was used. Flies were housed at 24 °C, 70% humidity and 12 h/12 h light/darkness cycle.

For longevity assays, one hundred flies (five per tube) were selected from each strain. Dead flies were counted every 2 days. Kaplan–Meier method was used to plot the results. A log-rank test was used to analyse results and Bonferroni correction for multiple comparisons.

For locomotor activity, ten and fifteen day-old flies were analysed in groups of five. They were placed in a tube with an 8 cm mark from the bottom. The number of flies that passed the 8 cm mark in 10 s was counted (5 times/tube).

Transmission electron microscopy (TEM)

After fixing with 3.5% glutaraldehyde, the cell cultures were washed with 0.1M PBS (pH 7.4) and treated with 2% osmium tetraoxide in 0.1M PBS (pH7.4), for 2 hours at room temperature. The samples were rinsed and dehydrated with ethanol and stained with 2% uranyl acetate. Dehydrated cell cultures were embedded in araldite (Fluka). Semi-thin sections (1.5 µm thick) were made with a diamond blade and stained with a solution of 1% Toluidine Blue (Sigma-Aldrich). Finally, ultra-thin cuts (70nm thick) were made to be examined under Tecnai-Spirit transmission electron microscopy coupled to the Purple TEM CCD (Soft Imaging System) camera.

Western blotting

Protein extraction was done with sample buffer containing 62 mM Tris (pH 7.5), 5% glycerol, 2% SDS, 5mg / ml bromophenol blue and 5% β-mercaptoethanol. After heating the sample buffer at 100 ° for 5 minutes, 100 µl was added to 300,000 cells, resuspended and boiled for 5 min. Subsequently, it was stored at -20 ° C for lysis of plasma membranes. Cell fractionation was carried out using the NE-PER kit (Thermo Scientific, Catalog Code 78833). The Western blot technique was used to detect specific proteins in cell lysates by gel electrophoresis. We use the SDS-PAGE method that allows the separation of proteins by molecular weight in polyacrylamide gels. Total protein extracts were introduced in prefabricated SDS-PAGE gels in 4-20% polyacrylamide gradient (Mini-PROTEAN® TGXTM Bio-Rad). The proteins were transferred to PVDF membranes, previously activated with methanol, by electroblotting and blocked for 1 hour with a 5% skim milk solution in TBS-tween (tris buffered saline). After that, the membranes were incubated overnight at 4 ° C with primary antibodies (table S1) diluted in TBS-tween with 2% horse serum, 5% BSA and 0.02% sodium azide. The next day, the membranes were incubated with donkey secondary antibodies conjugated with Alexa fluor plus fluorophores (488 donkey anti-rabbit IgG (H + L) (A32790), 488 donkey anti-mouse IgG (H + L) (A32766) , 647 donkey anti-rabbit IgG (H + L) (A32795), 647donkey anti-mouse IgG (H + L) (A32787) from Invitrogen. The development was performed by fluorescence analysis emitted by the iBright FL1000 Imaging System and quantified with Image Studio Lite software.

Immunofluorescence

Immunofluorescence was used to analyse the intracellular location and distribution of different proteins in primary myoblasts and in silenced myoblasts. Myoblasts on coverslips were fixed after several washes in DPBS (Gibco), with 4% paraformaldehyde (PFA) for 10 min. After several washes, it was blocked with blocking solution (5% BSA, in TBS-tween with 2% horse serum, 0.02% sodium azide and 0.5% Triton X-100) for 30 min. Subsequently, the first

incubation with the primary antibody was performed and left overnight at 4th. After performing 3 washes with TBS-tween, the second incubation was carried out for 1 h at room temperature and in the dark. In this case, donor Alexa fluor Plus (Invitrogen) type secondary antibodies conjugated with Alexa fluor plus fluorophores (488 donkey anti-rabbit IgG (H + L) (A32790), 488 donkey anti-mouse IgG (H + L) (A32766), 647 donkey anti-rabbit IgG (H + L) (A32795), 647 donkey anti-mouse IgG (H + L) (A32787), 555 donkeyanti-mouse IgG (H + L) (A31570) and 555 donkey anti-mouse IgG (H + L) (A31572) were used. After 2 washes with TBS-tween, 1 wash with TBS and another with distilled H2O, the covers were mounted with Prolong Diamond Antifade Mountant with DAPI (Invitrogen). Cells were observed in a Fluorescent Nikon 80i microscope and in an inverted epifluorescent microscope (Nikon Ti-S). The images were obtained with a high resolution ORCA Flash 2.8 camera (Hamamatsu). The quantification of nuclei, to calculate the index Fusion and analysis of protein, nuclear and cytoplasmic expression was carried out using the Image J program.

RNA microarrays

The RNA obtained was analysed using GeneChip complete human transcription expression microarrays of the type Clariom D (Affymetrix). These microarrays analyse more than 540,000 transcripts and allow analysis at two levels: the level of gene expression and the level of alternative splicing. The microarrays were hybridized following the recommended protocols, using a hybridization oven at 45 ° C at 60 rpm for 16 h. After hybridization was completed, a washing and staining step was performed in GeneChip Fluidics Station 450. Image capture was performed on the GeneChip 7G (Affymetrix) scanner. The analysis software used was Expression Console (Affymetrix), Transcriptome Analysis Console (TAC) 4.0 and Gene Set Enrichment Analysis (GSEA; Broad Institute).

Analysis of metabolic flux by SeaHorse

Metabolic pathways were studied using the Seahorse XF96 Extracellular Flux Analyzer (Agilent). This equipment allows simultaneous study of the two main energy pathways, mitochondrial respiration and glycolysis, in living cells and in real time. The analyzer measures the ratio of oxygen consumption (OCR, Oxygen Consumption Rate), as an indicator of mitochondrial respiration; and the ratio of extracellular acidification (ECAR, ExtraCellular Acidification Rate), as an indicator of glycolysis and lactate production.

Mitochondrial Function

The mitochondrial stress test allows obtaining information on different bioenergetic parameters, such as: baseline respiration, maximum respiration, ATP production, reserve respiratory capacity (coupling respiratory capacity), and coupling efficiency (coupling efficiency), all of

them depending on the changes in oxygen consumption by adding enzyme modulators. To obtain the parameters, 3 serial injections are performed at minute 20, 50 and 80, respectively, of the following modulators: oligomycin, carbonylcyanide-p-trifluoromethoxyphenylhydrazone (FCCP) and rotenone / antimycin. The final concentrations in the Seahorse culture plate of these inhibitors were the following: 1 μM oligomycin, 1 μM FCCP, 0.5 μM rotenone and 0.5 μM antimycin. Seeding of cells in the culture plate 24 hours before the test was performed with 13,000 myoblasts per well for both silenced myoblasts and primary myoblasts.

Glycolytic function

ECAR was measured in cells that were incubated in a medium without glucose or pyruvate. The first injection is a saturation of glucose (10 mM) that the cells catabolize by the metabolic pathway, generating ATP, NADH, H_2O and protons. The release of protons to the extracellular environment generates a rapid increase in ECAR. This response, generated by the injection of glucose, offers the information of the glycolysis of the samples under study. The injection of oligomycin (1 μM) inhibits the production of ATP in the mitochondria and energy production shifts to glycolysis, thus promoting an increase in ECAR, showing maximum glycolytic capacity. The last injection, 2-DG (50 mM), inhibits glycolysis by the competitive binding of 2-dg to hexokinase II, an enzyme that catalyzes the first reaction of glycolysis. Thus, there is a decrease in ECAR, confirming in turn that the increase in ECAR has been the cause of glycolysis. The difference between glycolytic capacity and glycolysis rate defines the glycolytic reserve. The final concentrations of the enzyme modulators were: 10mM glucose, 1μM oligomycin and 50mM 2-dg.

Study of energy phenotypes

Seahorse XF measures the cellular energy phenotype by measuring mitochondrial respiration and glycolysis in basal conditions and stressed conditions, to reveal the three key parameters of the cell's energy metabolism: basal phenotype, stressed phenotype and metabolic potential. The energy phenotype test measures the metabolic potential of living cells, using oligomycin and FCCP. With a simultaneous injection of these stressors, two events occur: oligomycin inhibits the production of ATP by mitochondria, and causes a compensatory increase in the glycolysis rate, as cells try to meet their energy demands through the glycolytic pathway. The FCCP depolarizes the mitochondrial membrane, activating the entry of protons from the inner membrane space into the mitochondrial matrix, and leads to higher OCR in the mitochondria, in order to restore the mitochondrial membrane potential. This test reports on the metabolic preference of oxidative or glycolytic cell types and the ability to metabolically respond to different cell insults.

Study of ATP production rate

This test allows the production of mitochondrial and glycolytic ATP to be measured simultaneously through a series of equations based on the measurement of OCR and the measurement of the increase in ECAR, after administration to the culture medium of enzymatic modulators. The concentrations used of these modulators were: 1 μM oligomcicin, 0.5 μM rotenone and 0.5 μM antimycin. All metabolic tests using the Seahorse technique were performed when myoblasts were at a confluence of 90%. For the study of metabolism throughout muscle differentiation, cell seeding was carried out at different times in order to obtain, in the same Seahorse plate, the different phases of the process. One hour before the test the cells were incubated at 37 ° C without CO2 and in the specific medium for the Seahorse assay at pH 7.4.

Statistical analysis

Data was analysed by SPSS for Windows, version 15.0. Normality was verified by the Shapiro-Wilks test ($p > 0.05$). Student's t or Mann-Whitney's U tests were used when normality criteria were met or not, respectively, for comparisons between control cells and silenced cells in any of the the different biochemical, metabolic or histological parameters. Unless otherwise indicated, the data are expressed as the mean ± the standard error of the mean.

RESULTS

Deficiency of TDP-43 or FUS halts the myogenic capacity of myoblasts

Various lines of evidence have suggested that the myogenic program appears to be altered in patients with ALS (Jensen et al., 2016; Pradat et al., 2011; Scaramozza et al., 2014), so we first sought to know whether these defects may be directly associated with the aberrant behavior of genes found mutated in ALS. A previous study has shown that TDP-43 is essential for normal skeletal muscle formation by forming cytoplasmic assemblies that regulate the expression of sarcomeric proteins (Vogler et al., 2018). Besides TDP-43, we also focused on the study of FUS as another causative gene of ALS. We performed cell fractionation and immunofluorescence experiments throughout different stages of myogenic differentiation in human immortalized myoblasts (cell line 8220), including 1) proliferative myoblasts, 2) myoblast at 100% confluence (myocytes), 3) fusing myocytes, and 4) myotubes at day 3 post-fusion (Fig. 1A). These assessments revealed changes in the subcellular localization of these proteins during early steps of myogenic differentiation despite no changes in their expression levels. In particular, higher levels of TDP-43 in nuclei and cytoplasmic puncta were found in early differentiating myotubes when compared to proliferative myoblasts. In contrast, FUS was notably decreased in nuclei but increased in cytoplasm during early steps of differentiation when compared to proliferative stages (Fig. 1B, C).

According to Vogler et al., the translocation of TDP-43 to cytoplasm is involved in myoblast differentiation and myotube generation. We have observed that not only TDP-43, but also FUS, suffers fluctuations of subcellular localization during myogenesis, suggesting that they both may be involved in the regulation of gene expression programs during muscle differentiation. Thus, given the potential implication of TDP-43 and FUS in myogenesis, we modelled the loss-of-function of these genes in the human immortalized myoblast cell line 8220 by treating these cells with shRNA-expressing lentiviral particles for TDP-43 or FUS, and further analyzed any change on cell morphology, growth and myogenic capacity (Fig. 1D).

Two days after treatment with lentiviral particles, myoblasts underwent severe reductions in TDP-43 or FUS relative to control scramble-infected cells (Fig. 1E). Transmission electron microscopy (TEM) imaging revealed ultrastructure abnormalities in silenced myoblasts. Compared to scramble-infected myoblasts, 8820 myoblasts treated with shTDP-43 exhibited a rounded and enlarged shape with a cluster of organelles around nuclei, containing very short rough endoplasmic reticulum (RER) but lots of fragmented mitochondria. Similarly, the FUS-deficient myoblasts were found longer with abundant mitochondria although dilated RER (Fig. S1A). Providing further evidence for increased numbers of mitochondria associated to TDP-43

and FUS deficiency, TOMM20 immunostaining showed higher mitochondrial mass in these myoblasts lines compared to Scramble-infected (Fig. S1B). Despite these changes in cellular morphology and distribution of intracellular components, 8820 myoblasts treated with either shTDP-43 or shFUS were viable, although the proliferating capacity was faintly reduced (Fig. 1F).

We then studied the effect of gene silencing on the myogenic process. Myogenesis was first induced by an established protocol consisting on medium replacement, in which myoblasts were exposed to differentiation medium two days after infection with lentiviral particles. However, TDP-43 and FUS knockdown myoblasts were not able to survive to the differentiating challenge (data not shown). Then we switched to a more physiologically-relevant myogenic that consists on allowing spontaneous differentiation and fusion when myoblasts reach 100% confluence. Lentiviral particles of scramble shRNA, shTDP-43 or shFUS were added to the medium when 8820 myoblasts were at 30-40% confluence (Fig. 1D). Myoblasts treated with control shRNA differentiated and formed MyHC-positive myotubes 2 days after reaching 100% confluence (Fig. 1F, Fig. S2A). However TDP-43 and FUS knockdown affected the natural capacity of cultured myoblasts to fuse and form myotubes: shTDP-43-treated myoblasts remained undifferentiated for at least 2 days after reaching 100% confluence, and shFUS-treated myoblasts formed less MyHC-positive myotubes (Fig. 1F, Fig. S2A). At this time point, markers of myotube maturation, such as calstabin, troponin C, α-actinin and myosin, were found downregulated in shTDP-43 and shFUS treated myoblasts (Fig. S2B), corroborating defects of myogenic differentiation and fusion. We then analyzed levels of myoD, a transcription factor that is required for myoblast differentiation and fusion (Olguín and Pisconti, 2012). Indeed, shTDP-43 and shFUS-treated myoblasts displayed a dramatic decrease in the total levels of myoD (Fig. 1G) and in the number of myoD-positive nuclei (Fig. 1H) when compared to shRNA-treated myoblasts.

Deficiency of TDP-43 or FUS curtails anaerobic glucose metabolism and induces pro-atrophic pathways in myoblasts

Molecular signaling factors and reprogramming of energy metabolism are considered crucial regulators of myogenesis during adult muscle regeneration. Since previous evidence indicates that TDP-43 controls skeletal muscle glucose homeostasis (Chiang et al., 2010; Joardar et al., 2017; Stallings et al., 2013), we wanted to investigate whether myogenic defects induced by loss of TDP-43, and in extension of FUS, may be mediated by abnormalities of glucose metabolism. Regarding the latter factor, various studies conducted in Drosophila, zebrafish and mouse muscle precursor cells have highlighted glucose metabolism as part of the core myogenic program because of its ability to provide both the rapid source of energy and the building blocks

(amino acids and nucleotides) that are required for biomass production during the formation of syncytial muscles (Tixier et al., 2013; Yucel et al., 2019; Yun et al., 2005). Thus, we investigated the extent of the metabolic reconfigurations during the myogenic process in the human myoblast cell line used in our study and, if any, whether deficiency of TDP-43 or FUS in myoblasts may impinge on these metabolic phenotypes. Then, we performed metabolic flux analysis by SeaHorse technologies throughout the different stages of myogenesis. Glycolysis was found to be the predominant metabolic pathway to produce basal ATP during the proliferation stages of the 8220 myoblast cell line, as indicated by higher extracellular acidification rates (ECAR) and lower oxygen consumption rates (OCR) (Fig. 2A,B). However, differentiating and fusing myoblasts as well as early myotubes at day 3 underwent a switch in their metabolic potential towards a more energetic phenotype, which was due to an increase in the aerobic metabolism (Fig. 2C). As previous works have evidence the role of TDP-43 in controlling the skeletal muscle glucose homeostasis (Chiang et al., 2010; Joardar et al., 2017; Stallings et al., 2013), we expected that the myogenic defects induced by loss of TDP-43, and in extension of FUS, may be mediated by abnormalities of glucose metabolism.

Accordingly, we found that basal glycolysis and maximal glycolytic capacity parameters, as evidenced by ECAR, were consistently repressed after the silencing of TDP-43 or FUS in myoblasts (Fig. 2D). Decreased levels of lactate in homogenates from shTDP-43 and shFUS-treated myoblasts when compared to those from shRNA-treated myoblasts corroborated defects in the anaerobic oxidation of glucose (Fig. 2E). Regarding mitochondrial respiration, FUS silencing had stronger effects than TDP-43 silencing reducing basal oxygen consumption with respect to shRNA-treated myoblasts (Fig. 2F), although spare respiratory capacity was particularly increased in shTDP-43-treated myoblasts (Fig. 2F). The increased numbers of mitochondria, as observed by TEM (Fig. S1A,B), may provide a compelling explanation for the increased aerobic potential of shTDP-43 treated myoblasts. These data indicates that the loss of TDP-43 or FUS causes general abnormalities in energy metabolism during the myogenic differentiation process, with specific dowregulation of the glycolytic flux. A treatment with 2-deoxyglucose (2-DG), which was employed to reduce glycolysis-dependent ATP production during myoblast proliferation, reduced the activation of MyoD (Fig. S3A,B) and stalled the myogenic process in a similar way than the silencing of TDP-43 (Fig. S3C), providing further suggestions on the potential link between the metabolic and myogenic phenotypes in silenced myoblasts.

A poor metabolic fitness in muscle is often associated to the upregulation of protein degradation pathways and atrophy. Expression of the key muscle specific E3 ubiquitin ligases, muscle RING finger 1 (MuRF1) and muscle atrophy F-box (MAFbx)/atrogin-1, are markers of the activation of ubiquitin/proteasome system and atrophy in skeletal muscle. Interestingly, we observed that

these proteins were significantly reduced in silenced myoblasts, particularly in shTDP-treated myoblasts (Fig. 2G). Further, the treatment with 2-DG mimicked the effects of TDP-43 and FUS silencing on the expression levels of MurF1 and atrogin-1 (Fig. S3D).

Collectively, these data support the strong energetic reliance of proliferative and differentiating 8820 myoblasts on the anaerobic glycolysis, and therefore makes this model suitable to study whether the effects of TDP-43 and FUS on myogenesis are mediated by specific impairment of this metabolic pathway.

FoxO inhibition corrects metabolic derangements and myogenic defects induced by deficiency of TDP-43 or FUS

Because the TDP-43 and FUS loss-of-function are associated with extensive changes in gene expression and splicing events (Kapeli et al., 2016), we performed microarray analysis to define the global gene expression profiles of myoblasts with TDP-43 and FUS silencing, in an attempt to find hints of gene regulation that support the observed metabolic reprogramming. The analysis of shTDP-43-infected cells compared with control shRNA–infected cells revealed 1,329 upregulated genes (log_2 fold change ≥1, false discovery rate [FDR]≤ 0.05) while 673 downregulated genes (log_2 fold change ≤ -1, false discovery rate [FDR] ≤ 0.05). The analysis of shFUS-infected cells compared with control siRNA–infected cells revealed 702 upregulated genes (log2 fold change ≥1, false discovery rate [FDR] ≤ 0.05) while 579 downregulated genes (log2 fold change ≤ -1, false discovery rate [FDR]≤ 0.05). Using oPOSSUM-3 (http://opossum.cisreg.ca/oPOSSUM3/), a tool for the identification of over-represented transcription factor binding sites (TFBS) in co-expressed genes generated from high-throughput methods, we generated a list of upstream transcription factors (ordered by Z-scores) that were predicted to regulate the differential gene expression between scramble shRNA and shTDP-43-treated myoblasts (Fig. 3A). Among the top candidates predicting the differentially expressed genes (DEGs) in shTDP-43-treated myoblasts, the Forkhead Box O-3 (FoxO3) factor called our attention for mainly two reasons. First, the family of FoxO transcription factors are evolutionarily conserved mediators of insulin and growth factor signaling governing programs of gene expression to regulate apoptosis, cell-cycle, cell differentiation, metabolism and autophagy (Link and Fernandez-Marcos, 2017). Second, FoxO factors are involved in many types of muscle atrophy through the regulation of ligases MuRF1 and atrogin-1 (Sandri et al., 2004; Waddell et al., 2008), which we have found upregulated in shTDP-43 and shFUS-treated myoblasts. We performed an alternative TFBS analysis by using DAVID software (https://david.ncifcrf.gov/home.jsp), and found FoxO1 as well within the top ten list predicting TDP-43 knockdown DEGs (Table S2). FoxO1 have particular implications in the regulation of multiple genes that control muscle progenitor cell maintenance and differentiation (Kitamura et

al., 2007), including MyoD. Interestingly, gene ontology (GO) analysis in shTDP-43 knockdown 8820 myoblasts revealed a significant enrichment of KEGG pathways that are upstream or downstream of FoxO transactivation, such as insulin and PI3K-Akt signaling or cell cycle (Fig. 3A).

Taking these data together, we hypothesize that FoxO factors are underlying mediators connecting the metabolic and myogenic alterations of myoblasts with the loss of function of either TDP-43 or FUS. If this hypothesis is correct, deficiency of TDP-43 and FUS would lead to an increased localization of FoxO factors into the nucleus and activation, while the manipulation of FoxO activities might influence the cellular phenotypes of silenced myoblasts. Regarding the subcellular localization of FoxO proteins, we observed by means of immunofluorescence and western-blotting that expression level of FoxO1 was weak and predominantly cytoplasmic during myoblast proliferation, and little bit increased in cytoplasm as well during early fusion events in control myoblasts (Fig. S4A,C). This is expected since pro-atrophic actions of FoxO1 must be switch off during muscle formation. However, we found that FoxO1 protein levels became dramatically increased in the nuclear compartment of shTDP-43 and shFUS treated myoblasts when compared to shRNA-treated cells (Fig. 3B). FoxO3 was found instead highly expressed in nuclei in control myoblasts since it is needed for proliferation but decreases as differentiation starts (Lee et al., 2019), becoming almost exclusively cytoplasmatic in early myotubes (Fig. S4B,C). Levels of nuclear FoxO3 were found slightly increased in shTDP-43-treated myoblasts, although not in shFUS-treated myoblasts, when compared to control shRNA myoblasts (Fig. 3C). Despite TDP-43 and FUS silencing induced little or no changes on the levels of nuclear FoxO3 in myoblasts, where FoxO3 is constitutively active, we cannot rule out the idea that the lack of TDP-43 or FUS may affect the shutdown of FoxO3 that is needed for myoblast differentiation.

We next explored the consequences of pharmacological or genetic inhibition of FoxO on metabolism and myogenesis in TDP-43 and FUS-silenced myoblasts. We treated all the different models of myoblasts with the compound AS1842856 at 30 nM during proliferation and let them differentiate, adding the compound at the same concentration every 2 days. At the selected concentration, this compound binds selectively to the dephosphorylated FoxO1, and to less extent dephosphorylated FoxO3, interfering with DNA interaction and thereby resulting in inhibition of FoxO transactivation (Nagashima et al., 2010).

Interestingly, AS1842856 treatment induced a significant recovery of the repressed glycolytic traits in proliferating myoblasts caused by the silencing of either TDP-43 or FUS (Fig. 4A), as indicated by both glucose- and oligomycin-stimulated ECAR. This treatment also improved the

mitochondrial ATP production of silenced myoblasts (Fig. 4B), thus exerting thorough benefits in the pathways of energy production in shTDP-43 and shFUS-treated myoblasts.

Concomitantly with this, we observed that treatment with AS1842856 disinhibited the myogenic arrest of TDP-43 silenced myoblasts and improved the myogenic capacity of FUS-silenced myoblasts, as indicated by the presence of MHC1-positive myotubes and the differentiation index (Fig. 4C), while it had little effects on control myoblasts. In addition, treatment with the AS1842856 inhibitor modifies the expression of some of FoxO-regulated genes that are crucial for proper myogenic differentiation. Specifically, AS1842856 increased the expression of MyoD while decreased the expression of myostatin in shTDP-43 and sh-FUS-treated 8820 myoblasts (Fig. 4D). On top of this, the treatment of TDP-43 and FUS-knockdown myoblasts with a shRNA sequence that targets mRNA of both FoxO1 and FoxO3 was able to mitigate the abnormal nuclear expression of FoxO1 (Fig. S5) and confirmed the benefits of FoxO inhibiton on the myogenic capacity (Fig. 4E).

FoxO inhibition alleviates glycolytic and myogenic defects of ALS myoblasts

We looked at preliminary links between the findings in genetically-induced myoblasts and features of ALS myoblasts. To this aim, we cultured primary myoblasts obtained from muscle biopsies of two patients with ALS (patient 1 is sporadic [sALS] and patient 2 is familial [fALS]) and one disease-control. Surprisingly, when we looked at markers of ALS pathology in patients' primary myoblasts we found clear evidences of TDP-43 mislocalization. In particular, levels of nuclear TDP-43 were found decreased in the patient 2 (Fig. 5A), and although unchanged in the patient 1, TDP-43 showed noticeably perinuclear localization (Fig. 5A,B). Changes in TDP-43 localization were accompanied by an altered subcellular distribution of FoxO1, as reflected by increased levels of FoxO1 in nuclei (Fig. 5A,C) and enhanced nuclear-to-cytosolic ratio in both patients (Fig. 5C). Remarkably, nuclear localizations of TDP-43 and FoxO1 were inversely associated in the patient 2 (Fig. S6).

To study whether these pathological features were linked to functional outcomes of ALS muscle, we studied the myogenic capacity of ALS myoblasts and the effect of FoxO inhibition. Control primary myoblasts showed spontaneous myogenic potential, as reflected by normal fusion events and formation of mature myotubes (Fig. 5D). In contrast, and similar to TDP-43 or FUS-silenced myoblasts, primary myoblasts from patients with ALS displayed myogenic abnormalities that were alleviated after treatment with the FoxO1 inhibitor AS1842856 (Fig. 5D).

As expected, alterations of FoxO1 in ALS myoblasts were accompanied by aberrant glycolytic flux, as indicated by lower ECAR values after addition of glucose to the media (Fig. 5E). Yet

again, the treatment with the FoxO1 inhibitor AS1842856 improved the basal glycolysis rate in primary ALS myoblasts (Fig. 5E) concomitantly to the beneficial effects on myogenic process.

Foxo inhibition improves muscle function and lifespan in Drosophila flies with muscle-conditioned deficiency of TDP-43 or FUS

After knowing that FoxO inhibition provides benefits to muscle cells in vitro, we wanted to further test the effects of targeting FoxO factors on muscle function under the context of TDP-43 or FUS loss-of-function in vivo. To that purpose, we generated various Drosophila models with muscle-conditioned silencing of either Cabeza (Caz) or Tbph genes, which are orthologs of human FUS or TARDBP, respectively. Caz-silenced (iCaz) flies and two different lines of Tbph-silencing (iTbph^{attp40} and iTbph$^{p(GD6943)}$) were viable; a third line of Tbph-silencig (iTbph$^{pkk(108254)}$) did not instead reach adult stage despite proper development into the late pupal period. Adult iCaz and iTbph^{attp40} flies displayed the most prominent muscle phenotypes already at 10-day old, as indicated by a significant reduction in the percentage of flies able to climb in the locomotor activity test, when compared to control flies. Adult iTbph$^{p(GD6943)}$ flies showed similar degree of locomotor affection at 15-day old (Fig. 6A). Drosophila has only one orthologs for mammalian FoxO factors, which is called *foxo*. Although the affinity of AS1842856 for Drosophila *foxo* has never been tested, the fact that the binding sites of FoxO proteins for this compound are well conserved among species provides enough rationale to test its efficacy in Drosophila models. We first treated control flies with AS1842856 at a concentration of 30 μM and observed a lack of toxicity on motor function and lifespan (Fig. S7A,B). Interestingly, the same treatment given to silenced flies induced strong benefits on muscle function as observed by increased climbing activity, being more pronounced in Tbph- than in Caz-silenced lines (Fig. 6B).

We performed longevity assays to further characterize these fly models. Either of the Caz or Tbph-silenced flies manifested dramatic reductions of survival times when compared to control group, as indicated by Kaplan-Meier curves and median lifespan (Figure 6C). Interestingly, flies that were treated with AS1842856 had higher survival than flies that did not receive treatment. This effect was particularly noticeable in iCaz and iTbph^{attp40} fly lines (Fig. 6D).

DISCUSSION

In the present work we provide in vitro evidence to prove that muscular wasting may occur in ALS independently of MN loss, and that this phenomenon may be operated by the defective function of at least two ALS-causative factors, TDP-43 and FUS, through dysregulation of FoxO proteins.

First finding is related to the loss of myogenic capacity of isolated myoblasts from patients with ALS. Although only two patients have been studied, this phenotype seems to be very consistent and is in agreement with previous reports (Pradat et al., 2011; Scaramozza et al., 2014). Thus, there must be some intrinsic aberrant function in ALS muscle that predisposes it to impaired self-renewal. This raises the question that activation of defective muscle regenerative mechanisms in individuals at risk may aggravate or accelerate the development of ALS. Although being out of the scope of the present work, it would be interesting to study whether aberrant muscle regeneration provides a compelling link between physical lesions induced by strenuous exercise or by head concussions, which have being described in various cases to be the site of symptoms onset in a short time (Pupillo et al., 2018; Seals et al., 2016), with a "dying-back" neuronopathy and the risk to develop ALS. Beyond this hypothetical scenario, our work points that myogenic defects in ALS may be caused by intrinsic TDP-43 or FUS toxicity due to defective function of these proteins. In this sense, TDP-43 pathology in terms of abnormal deposition into protein inclusions is not restricted to nervous tissue; several studies have reported TDP-43 pathology in peripheral tissues of sporadic ALS cases, including skeletal muscle and skin (Cykowski et al., 2018; Mori et al., 2019; Suzuki et al., 2010). Cytosolic aggregation of aberrant TDP-43 is often associated with nuclear mislocalization, and that is believed to confer loss-of-function toxicity (Prasad et al., 2019). We have not found cytosolic TDP-43 aggregation in myoblasts from the two patients with ALS under study, however a defective function of TDP-43 in both may be deduced from the observed nuclear mislocalization: in one of the patients levels of nuclear TDP-43 were certainly decreased, while in the other patient TDP-43 localization was dramatically restricted to perinuclear area. TDP-43 is involved in myogenesis, as demonstrated by its role in recruiting RNAs coding for sarcomeric proteins into myogranules in early myotubes (Vogler et al., 2018), and that the deficiency of TDP-43 stalls the capacity of human myoblasts to differentiate, as demonstrated by us. Given that, the loss of TDP-43 function due to nuclear mislocalization appears to provide a legitimate explanation for the abnormal myogenic capacity of ALS myoblasts. The present work extends the potential role of other ALS-causative genes, in particular FUS, on the myogenic capacity of skeletal muscle. Little is known about the presence of FUS pathology in ALS muscle, and only few mislocalization events have been observed in animal models or patients with other types of

neuromuscular disorders, such as facioscapohumeral and myotonic dystrophies (DeSimone et al., 2019; Homma et al., 2016; Llamusi et al., 2013). Despite this, mutated FUS have been reported to induce defects in motor endplates and intrinsic toxicity in muscle (Picchiarelli et al., 2019), supporting our data that FUS may play a fundamental role in ALS muscle.

A important conclusion derived from our study is that by repressing ALS genes in cultured human myoblasts it is possible to achieve a functional phenotype of ALS, and therefore this cellular model can be reasonably used as an experimental approach to investigate ALS-related mechanisms of TDP-43 and FUS toxicity. Indeed, through this approach we have been able to identify a common mechanism of toxicity downstream either TDP-43 or FUS loss-of-function, consisting on an aberrant link between energy-producing pathways and FoxO transcriptional activity, as we further discuss. Alterations in energy metabolism are recognized as inherent traits of ALS since evidences from early studies reporting increased resting energy expenditure (also known as hypermetabolism) in patients with ALS (Bouteloup et al., 2009; Desport et al., 2001), and further works showing the association of metabolic perturbations with the severity of ALS (Jésus et al., 2018; Steyn et al., 2018). But recent studies have unveiled that disturbances of glucose metabolism are principal to the overall metabolic derangements of ALS, reinforcing our observations of a predominant repression of glycolysis over mitochondrial respiration after the silencing of two ALS genes in muscle cells. In this sense, the activation of glycolysis, but no other energy-producing pathways, has been demonstrated to exert neuroprotection in reprogrammed MNs derived from patients (Manzo et al., 2019), and forms part of an early adapting mechanism against ALS pathology in the SOD1-G93A mouse (Ravera et al., 2019; Tefera et al., 2019). In the central nervous system, glycolysis is a crucial energy source for astrocytes to maintain the nutritional homeostasis of neurons by producing lactate, without which neurons become vulnerable to excitotoxic injuries and other types of damage. As spinal cord MNs have huge energy demands, due to their bigger soma sizes and higher firing rates (LeMasson et al., 2014), boosting glycolysis offers a reasonable response to promote neuroprotection against ALS pathology. Beyond the potential role of glycolysis in nerve tissues, the anaerobic oxidation of glucose in muscle is fundamental not only for the contractibility of fast glycolytic fibres, but also for supporting the early steps of myogensis (Tixier et al., 2013; Yun et al., 2005), when myoblasts needs to differentiate into myocytes before fusing to form myotubes. Anaerobic glycolysis facilitates rapid sources of energy and carbon anabolism to support growth of myoblasts, and also acts as an epigenetic regulator of myoblast proliferation through the modulation of histone acetylation (Yucel et al., 2019). We have illustrated the almost pure reliance of human differentiating myoblasts on glycolysis, which is progressively switched down during myotube maturation, in a way that 2-DG treatment is able to completely halt the process. In consequence, myoblasts should be a type of cells with a particular

susceptibility to glycolytic defects, and in turn to the metabolic disturbances that are predominantly associated to ALS. According to this, we have observed a decreased glycolytic capacity concomitant with alterations in the myogenic potential in primary myoblasts from two patients with ALS, mimicking the phenotypes of TDP-43 and FUS-deficient myoblasts. Taking altogether, these evidences point to a chief role of glucose metabolism on ALS muscle phenotypes, and subsequently raise the common-sense question as to whether these muscle metabolic disturbances may cause non-cell-autonomous toxicity to MNs. The latter question has been somehow addressed by two previous studies, where a switch from glycolytic metabolism to the use of lipids in muscle were found to precede the onset of symptoms and the loss of MNs in the SOD1-G93A mouse (Dobrowolny et al., 2018a; Palamiuc et al., 2015). However, further studies need to be carried out to find out whether favouring glycolysis in muscle has benefits on neuromuscular function and MN degeneration in ALS.

In an attempt to devise a mechanistic link between impaired myogenesis and disturbances of glycolysis in TDP-43 and FUS-silenced myoblasts, we got to the discovery of dysregulated FoxO transcription factors downstream TDP-43 or FUS loss-of-function. Since aberrant over-activation of FoxO factors offers a reasonable explanation for the induction of muscle pro-atrophic mechanisms, atrogin-1 and MurF1 (Reed et al., 2012; Sandri et al., 2004), that we observed upon TDP-43 or FUS silencing, and because we collected evidences from prediction tools indicating FoxO transactivation as responsible for the differential gene expression profile of TDP-43 silenced cells, we focused on this family of transcription factors, and studied the effects of targeting them on muscle pathways and processes. Dysregulation of FoxO may occur secondary to a glycolysis repression in TDP-43 and FUS-silenced myoblasts, as a cellular homeostatic response to cope with a low-nutrient stress by inducing the ubiquitin-proteasome and autophagy-lysosome systems (Milan et al., 2015). However, FoxO transcription factors, and in particular FoxO1, are key regulators of PDK4 expression (Gross et al., 2009; Puthanveetil et al., 2010), an enzyme that control the activity of the major rate-limiting enzyme of glucose oxidation, PDH. Thus, any up-regulation of this transcriptional factor would inhibit glycolysis, suggesting that disturbances of glucose oxidations are secondary to FoxO dysregulation. The fact that TDP-43 has been demonstrated to directly repress nuclear FoxO1 transactivation (Zhang et al., 2014), together with our evidences showing recovery of glycolytic flux after either pharmacological or genetic inhibition of FoxO factors, provide sound arguments to sustain the latter assumption. In this context, it is legitimate to deduce that the inhibition of FoxO factors exerts benefits on myogenesis under pathological conditions of TDP-43 or FUS loss-of-function toxicity, through the regulation of glucose oxidation. Thus, the use of inhibitors of FoxO may have potential to treat muscle phenotypes that are induced under certain pathological circumstances, such as TDP-43 or FUS deficiency, as well as in ALS. The

treatment of muscle phenotypes is not restricted to myogenic defects but may be extended to neuromuscular function and muscle fitness in general, as observed by the overall locomotor and survival benefits induced by FoxO inhibition in Drosophila models of muscle-specific loss of TDP-43 and FUS orthologs.

Overall, the restoration of myogenic process being concomitant to glycolitic normalization reinforces the idea that metabolic defects are linked to the myogenic phenotypes of TDP-43 and FUS knockdown cells. Given that FoxO factors are in the interface between metabolism and disease since they orchestrate many of the physiological responses to metabolic stimuli (Link and Fernandez-Marcos, 2017), including those mediated by insulin signaling, our findings suggest that interventions that alleviate metabolic fitness through the inhibition of FoxO may counteract key functional consequences of TDP-43 or FUS loss of function. In consequence, this strategy may have potential benefits for treating neuromuscular disorders that are associated with pathological TDP-43 or FUS mislocalization in muscle, such as ALS. However, the potential of this approach should not be limited to treat ALS muscle, since abnormal nuclear TDP-43 and/or FUS localizations have been evidenced in animal models and patients from a range of muscle disorders, including facioscapulohumeral dystrophy, myotonic dystrophy and inclusion body myositis.

REFERENCES

Bouteloup, C., Desport, J.-C., Clavelou, P., Guy, N., Derumeaux-Burel, H., Ferrier, A., and Couratier, P. (2009). Hypermetabolism in ALS patients: an early and persistent phenomenon. Journal of Neurology *256*, 1236–1242.

Chiang, P.-M., Ling, J., Jeong, Y.H., Price, D.L., Aja, S.M., and Wong, P.C. (2010). Deletion of TDP-43 down-regulates Tbc1d1, a gene linked to obesity, and alters body fat metabolism. Proceedings of the National Academy of Sciences of the United States of America *107*, 16320–16324.

Chiò, A., Logroscino, G., Hardiman, O., Swingler, R., Mitchell, D., Beghi, E., Traynor, B.G., and On Behalf of the Eurals Consortium (2009). Prognostic factors in ALS: A critical review. Amyotrophic Lateral Sclerosis *10*, 310–323.

Cykowski, M.D., Powell, S.Z., Appel, J.W., Arumanayagam, A.S., Rivera, A.L., and Appel, S.H. (2018). Phosphorylated TDP-43 (pTDP-43) aggregates in the axial skeletal muscle of patients with sporadic and familial amyotrophic lateral sclerosis. Acta Neuropathologica Communications *6*, 28.

DeSimone, A.M., Leszyk, J., Wagner, K., and Emerson, C.P. (2019). Identification of the hyaluronic acid pathway as a therapeutic target for facioscapulohumeral muscular dystrophy. Science Advances *5*.

Desport, J.C., Preux, P.M., Magy, L., Boirie, Y., Vallat, J.M., and Couratier, P. (2001). Factors correlated with hypermetabolism in patients with ALS. American Journal of Clinical Nutrition 328–334.

Dobrowolny, G., Lepore, E., Martini, M., Barberi, L., Nunn, A., Scicchitano, B.M., and Musarò, A. (2018a). Metabolic Changes Associated With Muscle Expression of SOD1G93A. Frontiers in Physiology *9*, 831.

Dobrowolny, G., Martini, M., Scicchitano, B.M., Romanello, V., Boncompagni, S., Nicoletti, C., Pietrangelo, L., De Panfilis, S., Catizone, A., Bouchè, M., et al. (2018b). Muscle Expression of SOD1G93A Triggers the Dismantlement of Neuromuscular Junction via PKC-Theta. Antioxidants & Redox Signaling *28*, 1105–1119.

Gross, D.N., Wan, M., and Birnbaum, M.J. (2009). The role of FOXO in the regulation of metabolism. Current Diabetes Reports *9*, 208–214.

Homma, S., Beermann, M. Lou, Yu, B., Boyce, F.M., and Miller, J.B. (2016). Nuclear bodies reorganize during myogenesis in vitro and are differentially disrupted by expression of FSHD-associated DUX4. Skeletal Muscle *6*.

Jensen, L., Jørgensen, L.H., Bech, R.D., Frandsen, U., and Schrøder, H.D. (2016). Skeletal Muscle Remodelling as a Function of Disease Progression in Amyotrophic Lateral Sclerosis. BioMed Research International *2016*, 1–12.

Jésus, P., Fayemendy, P., Nicol, M., Lautrette, G., Sourisseau, H., Preux, P.-M., Desport, J.-C., Marin, B., and Couratier, P. (2018). Hypermetabolism is a deleterious prognostic factor in patients with amyotrophic lateral sclerosis. European Journal of Neurology *25*, 97–104.

Joardar, A., Manzo, E., and Zarnescu, D.C. (2017). Metabolic Dysregulation in Amyotrophic Lateral Sclerosis: Challenges and Opportunities. Current Genetic Medicine Reports *5*, 108–114.

Kapeli, K., Pratt, G.A., Vu, A.Q., Hutt, K.R., Martinez, F.J., Sundararaman, B., Batra, R., Freese, P., Lambert, N.J., Huelga, S.C., et al. (2016). Distinct and shared functions of ALS-associated proteins TDP-43, FUS and TAF15 revealed by multisystem analyses. Nature Communications *7*, 12143.

Kitamura, T., Kitamura, Y.I., Funahashi, Y., Shawber, C.J., Castrillon, D.H., Kollipara, R., DePinho, R.A., Kitajewski, J., and Accili, D. (2007). A Foxo/Notch pathway controls myogenic differentiation and fiber type specification. The Journal of Clinical Investigation *117*, 2477–2485.

Lee, J.H., Park, J.W., Kang, K.S., and Park, T.S. (2019). Forkhead box O3 promotes cell proliferation and inhibits myotube differentiation in chicken myoblast cells. British Poultry Science *60*, 23–30.

LeMasson, G., Przedborski, S., and Abbott, L.F. (2014). A Computational Model of Motor Neuron Degeneration. Neuron *83*, 975–988.

Link, W., and Fernandez-Marcos, P.J. (2017). FOXO transcription factors at the interface of metabolism and cancer. International Journal of Cancer *141*, 2379–2391.

Llamusi, B., Bargiela, A., Fernandez-Costa, J.M., Garcia-Lopez, A., Klima, R., Feiguin, F., and Artero, R. (2013). Muscleblind, BSF and TBPH are mislocalized in the muscle sarcomere of a Drosophila myotonic dystrophy model. DMM Disease Models and Mechanisms *6*, 184–196.

Loeffler, J.-P., Picchiarelli, G., Dupuis, L., and Gonzalez De Aguilar, J.-L. (2016). The Role of

Skeletal Muscle in Amyotrophic Lateral Sclerosis. Brain Pathology (Zurich, Switzerland) *26*, 227–236.

Manzano, R., Toivonen, J.M., Calvo, A.C., Oliván, S., Zaragoza, P., Rodellar, C., Montarras, D., and Osta, R. (2013). Altered in vitro Proliferation of Mouse SOD1-G93A Skeletal Muscle Satellite Cells. Neurodegenerative Diseases *11*, 153–164.

Manzo, E., Lorenzini, I., Barrameda, D., O'Conner, A.G., Barrows, J.M., Starr, A., Kovalik, T., Rabichow, B.E., Lehmkuhl, E.M., Shreiner, D.D., et al. (2019). Glycolysis upregulation is neuroprotective as a compensatory mechanism in ALS. eLife *8*.

Milan, G., Romanello, V., Pescatore, F., Armani, A., Paik, J.-H., Frasson, L., Seydel, A., Zhao, J., Abraham, R., Goldberg, A.L., et al. (2015). Regulation of autophagy and the ubiquitin-proteasome system by the FoxO transcriptional network during muscle atrophy. Nature Communications *6*, 6670.

Mori, F., Tada, M., Kon, T., Miki, Y., Tanji, K., Kurotaki, H., Tomiyama, M., Ishihara, T., Onodera, O., Kakita, A., et al. (2019). Phosphorylated TDP-43 aggregates in skeletal and cardiac muscle are a marker of myogenic degeneration in amyotrophic lateral sclerosis and various conditions. Acta Neuropathologica Communications *7*, 165.

Nagashima, T., Shigematsu, N., Maruki, R., Urano, Y., Tanaka, H., Shimaya, A., Shimokawa, T., and Shibasaki, M. (2010). Discovery of novel Foxo1 inhibitors for treating type 2 diabetes: Improvement of fasting glycemia in diabetic db/db mice. Molecular Pharmacology Fast Forward. Published on August *24*.

Olguín, H.C., and Pisconti, A. (2012). Marking the tempo for myogenesis: Pax7 and the regulation of muscle stem cell fate decisions. Journal of Cellular and Molecular Medicine *16*, 1013–1025.

Palamiuc, L., Schlagowski, A., Ngo, S.T., Vernay, A., Dirrig-Grosch, S., Henriques, A., Boutillier, A.-L., Zoll, J., Echaniz-Laguna, A., Loeffler, J.-P., et al. (2015). A metabolic switch toward lipid use in glycolytic muscle is an early pathologic event in a mouse model of amyotrophic lateral sclerosis. EMBO Molecular Medicine *7*, 526–546.

Picchiarelli, G., Demestre, M., Zuko, A., Been, M., Higelin, J., Dieterlé, S., Goy, M.-A., Mallik, M., Sellier, C., Scekic-Zahirovic, J., et al. (2019). FUS-mediated regulation of acetylcholine receptor transcription at neuromuscular junctions is compromised in amyotrophic lateral sclerosis. Nature Neuroscience *22*, 1793–1805.

Pradat, P.-F., Barani, A., Wanschitz, J., Dubourg, O., Lombès, A., Bigot, A., Mouly, V., Bruneteau, G., Salachas, F., Lenglet, T., et al. (2011). Abnormalities of satellite cells function in amyotrophic lateral sclerosis. Amyotrophic Lateral Sclerosis *12*, 264–271.

Prasad, A., Bharathi, V., Sivalingam, V., Girdhar, A., and Patel, B.K. (2019). Molecular mechanisms of TDP-43 misfolding and pathology in amyotrophic lateral sclerosis. Frontiers in Molecular Neuroscience *12*, 25.

Pupillo, E., Poloni, M., Bianchi, E., Giussani, G., Logroscino, G., Zoccolella, S., Chiò, A., Calvo, A., Corbo, M., Lunetta, C., et al. (2018). Trauma and amyotrophic lateral sclerosis: a european population-based case-control study from the EURALS consortium. Amyotrophic Lateral Sclerosis and Frontotemporal Degeneration *19*, 118–125.

Puthanveetil, P., Wang, Y., Wang, F., Kim, M.S., Abrahani, A., and Rodrigues, B. (2010). The increase in cardiac pyruvate dehydrogenase kinase-4 after short-term dexamethasone is controlled by an Akt-p38-forkhead box other factor-1 signaling axis. Endocrinology *151*, 2306–2318.

Ravera, S., Torazza, C., Bonifacino, T., Provenzano, F., Rebosio, C., Milanese, M., Usai, C., Panfoli, I., and Bonanno, G. (2019). Altered glucose catabolism in the presynaptic and perisynaptic compartments of SOD1G93A mouse spinal cord and motor cortex indicates that mitochondria are the site of bioenergetic imbalance in ALS. Journal of Neurochemistry jnc.14819.

Reed, S.A., Sandesara, P.B., Senf, S.M., and Judge, A.R. (2012). Inhibition of FoxO transcriptional activity prevents muscle fiber atrophy during cachexia and induces hypertrophy. FASEB Journal : Official Publication of the Federation of American Societies for Experimental Biology *26*, 987–1000.

Relaix, F., and Zammit, P.S. (2012). Satellite cells are essential for skeletal muscle regeneration: the cell on the edge returns centre stage. Development *139*, 2845–2856.

Sandri, M., Sandri, C., Gilbert, A., Skurk, C., Calabria, E., Picard, A., Walsh, K., Schiaffino, S., Lecker, S.H., and Goldberg, A.L. (2004). Foxo transcription factors induce the atrophy-related ubiquitin ligase atrogin-1 and cause skeletal muscle atrophy. Cell *117*, 399–412.

Scaramozza, A., Marchese, V., Papa, V., Salaroli, R., Sorarù, G., Angelini, C., and Cenacchi, G. (2014). Skeletal Muscle Satellite Cells in Amyotrophic Lateral Sclerosis. Ultrastructural Pathology *38*, 295–302.

Seals, R.M., Hansen, J., Gredal, O., and Weisskopf, M.G. (2016). Physical Trauma and Amyotrophic Lateral Sclerosis: A Population-Based Study Using Danish National Registries. American Journal of Epidemiology *183*, 294–301.

Stallings, N.R., Puttaparthi, K., Dowling, K.J., Luther, C.M., Burns, D.K., Davis, K., and Elliott, J.L. (2013). TDP-43, an ALS linked protein, regulates fat deposition and glucose homeostasis. PloS One *8*, e71793.

Steyn, F.J., Ioannides, Z.A., van Eijk, R.P.A., Heggie, S., Thorpe, K.A., Ceslis, A., Heshmat, S., Henders, A.K., Wray, N.R., van den Berg, L.H., et al. (2018). Hypermetabolism in ALS is associated with greater functional decline and shorter survival. Journal of Neurology, Neurosurgery & Psychiatry *89*, 1016–1023.

Suzuki, M., Mikami, H., Watanabe, T., Yamano, T., Yamazaki, T., Nomura, M., Yasui, K., Ishikawa, H., and Ono, S. (2010). Increased expression of TDP-43 in the skin of amyotrophic lateral sclerosis. Acta Neurologica Scandinavica *122*, 367–372.

Taylor, A.A., Fournier, C., Polak, M., Wang, L., Zach, N., Keymer, M., Glass, J.D., Ennist, D.L., and Pooled Resource Open-Access ALS Clinical Trials Consortium (2016). Predicting disease progression in amyotrophic lateral sclerosis. Annals of Clinical and Translational Neurology *3*, 866–875.

Tefera, T.W., Bartlett, K., Tran, S.S., Hodson, M.P., and Borges, K. (2019). Impaired Pentose Phosphate Pathway in the Spinal Cord of the hSOD1G93A Mouse Model of Amyotrophic Lateral Sclerosis. Molecular Neurobiology *56*, 5844–5855.

Tixier, V., Bataille, L., Etard, C., Jagla, T., Weger, M., DaPonte, J.P., Strahle, U., Dickmeis, T., and Jagla, K. (2013). Glycolysis supports embryonic muscle growth by promoting myoblast fusion. Proceedings of the National Academy of Sciences *110*, 18982–18987.

Tokutake, Y., Yamada, K., Ohata, M., Obayashi, Y., Tsuchiya, M., and Yonekura, S. (2015). ALS-Linked P56S-VAPB Mutation Impairs the Formation of Multinuclear Myotube in C2C12 Cells. International Journal of Molecular Sciences *16*, 18628–18641.

Vogler, T.O., Wheeler, J.R., Nguyen, E.D., Hughes, M.P., Britson, K.A., Lester, E., Rao, B., Betta, N.D., Whitney, O.N., Ewachiw, T.E., et al. (2018). TDP-43 and RNA form amyloid-like myo-granules in regenerating muscle. Nature *563*, 508–513.

Waddell, D.S., Baehr, L.M., Van Den Brandt, J., Johnsen, S.A., Reichardt, H.M., Furlow, J.D., and Bodine, S.C. (2008). The glucocorticoid receptor and FOXO1 synergistically activate the

skeletal muscle atrophy-associated MuRF1 gene. American Journal of Physiology - Endocrinology and Metabolism *295*.

Yucel, N., Wang, Y.X., Mai, T., Porpiglia, E., Lund, P.J., Markov, G., Garcia, B.A., Bendall, S.C., Angelo, M., and Blau, H.M. (2019). Glucose Metabolism Drives Histone Acetylation Landscape Transitions that Dictate Muscle Stem Cell Function. Cell Reports *27*, 3939–3955.e6.

Yun, Z., Lin, Q., and Giaccia, A.J. (2005). Adaptive Myogenesis under Hypoxia. Molecular and Cellular Biology *25*, 3040–3055.

Zhang, T., Baldie, G., Periz, G., and Wang, J. (2014). RNA-Processing Protein TDP-43 Regulates FOXO-Dependent Protein Quality Control in Stress Response. PLoS Genetics *10*, e1004693.

FIGURE LEGENDS

Figure 1. Deficiency of TDP-43 or FUS halts the myogenic capacity of 8220 human immortalized myoblasts. A, Schematic illustration of the myogenic process. B,C, Subcellular localization of TDP-43 and FUS throughout stages of myoblast differentiation. D, E, lentiviral transduction of shTDP-43 and shFUS induced silencing of either proteins, and affects growth rates (F). G, shTDP-43 and shFUS-treated myoblasts have abnormal myogenic capacity, as evidenced by lower MyHC-positive myotubes. H, I, western-blot and immunofluorescence analysis reveal that shTDP-43 and shFUS-treated myoblasts have reduced levels of the myogenic markers MyoD and myogenin. *$p<0.01$; **$p<0.01$.

Figure 2. Deficiency of TDP-43 or FUS curtails anaerobic glucose metabolism and induces pro-atrophic pathways in myoblasts. A,B,C, Metabolic switch during myogenesis. Myoblasts rely mainly on anaerobic glycolysis while differentiating myocytes and myotubes switch towards a more aerobic metabolism. D, shTDP-43 and shFUS-treated myoblasts display consistent reductions in basal glycolysis and glycolytic capacity. E, levels of lactate are decreased in shTDP-43 and shFUS-treated myoblasts. F, basal mitochondrial ATP production is sligthly affected by TDP-43 or FUS silencing, however effects on spare respiratory capacity are variable. G, pro-atrophic pathways of the UPS are dramatically augmented in shTDP-43 and shFUS-treated myoblasts. *$p<0.05$; **$p<0.01$.

Figure 3. Deficiency of TDP-43 or FUS induces dysregulation of FoxO transcription factors. A, microarray of gene expression shows 1,329 upregulated genes while 673 downregulated genes in TDP-43-silenced myoblasts. Using oPOSSUM-3 online tool, FoxO3 was found among the top candidates predicting the differentially expressed genes (DEGs) in shTDP-43-treated myoblasts. Gene ontology (GO) analysis in shTDP-43 knockdown myoblasts revealed a significant enrichment of KEGG pathways that are upstream or downstream of FoxO transactivation, such as insulin and PI3K-Akt signaling or cell cycle. B, FoxO1 protein levels became dramatically increased in the nuclear compartment of shTDP-43 and shFUS treated myoblasts when compared to shRNA-treated cells. C, Levels of nuclear FoxO3 were found slightly increased in shTDP-43-treated myoblasts, although not in shFUS-treated myoblasts. *$p<0.05$.

Figure 4. FoxO inhibition corrects metabolic derangements and myogenic defects induced by deficiency of TDP-43 or FUS. A, B, treatment with FoxO inhibitor AS1842856 at 30 nM induced a significant recovery of the repressed glycolytic traits in proliferating myoblasts caused by the silencing of either TDP-43 or FUS, and enhanced mitochondrial ATP production. C, AS1842856 was able to increase Glu/Asp ratio to levels of control myoblasts in TDP-43

silenced cells without affecting Glu/Gln ratio. D, AS1842856 treatment disinhibited the myogenic arrest of TDP-43 silenced myoblasts and improved the myogenic capacity of FUS-silenced myoblasts, as indicated by the presence of MHC1-positive myotubes and the differentiation index. E, AS1842856 treatment increased the expression of MyoD in shTDP-43 and shFUS-treated myoblasts. F, the treatment of TDP-43 and FUS-knockdown myoblasts with a shRNA sequence that targets mRNA of both FoxO1 and FoxO3 improved the myogenic capacity as well. *p<0.05; **p<0.01.

Figure 5. FoxO inhibition alleviates glycolytic and myogenic defects of ALS myoblasts. A,B, nuclear TDP-43 were found decreased in the patient 2 (fALS) and displayed an striking perinuclear localization in the patient 1 (sALS). C, altered subcellular distribution of FoxO1, as reflected by increased levels of FoxO1 in nuclei and enhanced nuclear-to-cytosolic ratio, in both patients with ALS. D, primary myoblasts from patients with ALS displayed myogenic abnormalities that were alleviated after treatment with the FoxO1 inhibitor AS1842856 at 30 nM. E, ALS myoblasts have reduced basal glycolytic flux, which was corrected b the treatment with the FoxO inhibitor AS1842856. *p<0.05; **p<0.01.

Figure 6. Foxo inhibition improves muscle function and lifespan in Drosophila flies with muscle-conditioned deficiency of TDP-43 or FUS. A, Adult iCaz and iTbph^{attp40} flies displayed the most prominent muscle phenotypes already at 10-day old, as indicated by a significant reduction in the percentage of flies able to climb in the locomotor activity test. Adult iTbph$^{p(GD6943)}$ flies showed similar degree of locomotor affection at 15-day old. B, Treatment with AS1842856 at a concentration of 30 μM given to silenced flies induced strong benefits on muscle function as observed by increased climbing activity. C, Caz or Tbph-silenced flies have reduced survival times, as indicated by Kaplan-Meier curves and median lifespan. D, flies that were treated with AS1842856 at 30 μM had higher survival than flies that did not receive treatment. This effect was particularly noticeable in iCaz and iTbph^{attp40} fly lines. *p<0.05; **p<0.01.

FIGURE 1

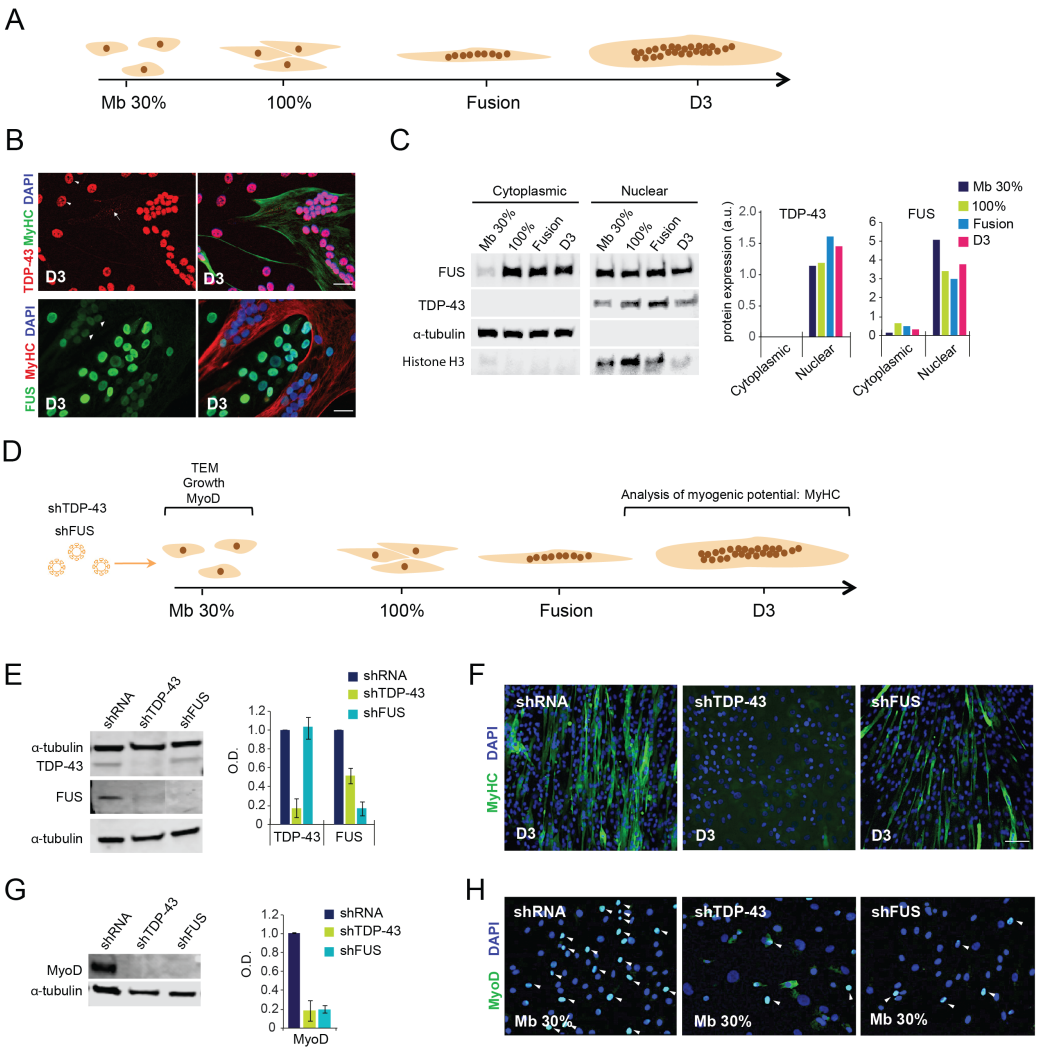

A
Mb 30%
100%
Fusion
D3
B
TDP-43 MyHC DAPI
FUS MyHC DAPI
D3
C
Cytoplasmic
Nuclear
Mb 30%
100%
Fusion
D3
FUS
TDP-43
α-tubulin
Histone H3
protein expression (a.u.)
TDP-43
FUS
Cytoplasmic
Nuclear
D
shTDP-43
shFUS
TEM
Growth
MyoD
Analysis of myogenic potential: MyHC
Mb 30%
100%
Fusion
D3
E
shRNA
shTDP-43
shFUS
α-tubulin
TDP-43
FUS
O.D.
TDP-43
FUS
F
MyHC DAPI
shRNA
shTDP-43
shFUS
D3
G
MyoD
α-tubulin
MyoD
H
MyoD DAPI
Mb 30%

FIGURE 2

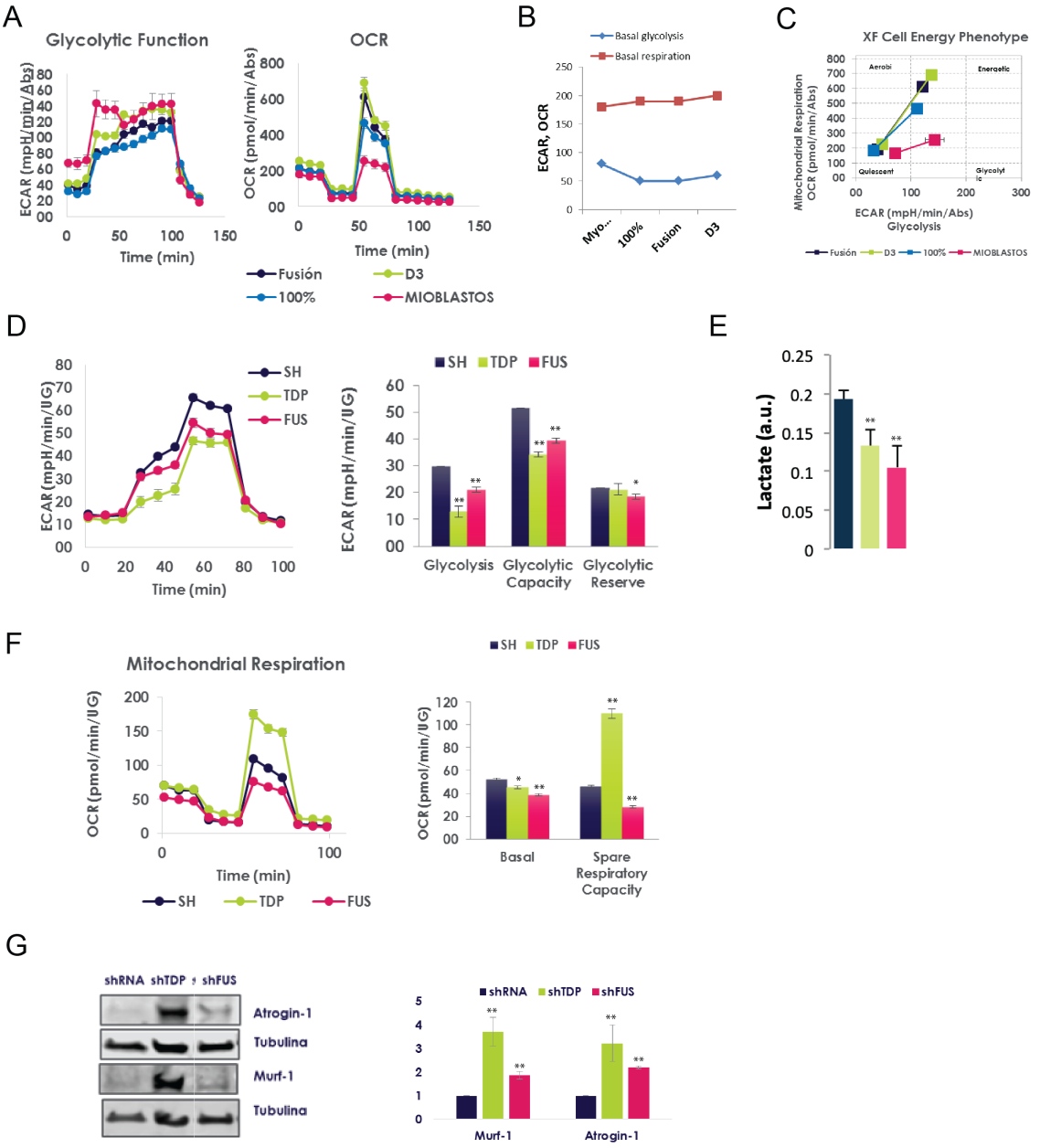

A
Glycolytic Function
ECAR (mpH/min/Abs)
Time (min)
OCR
OCR (pmol/min/Abs)
Time (min)
Fusión
D3
100%
MIOBLASTOS
B
Basal glycolysis
Basal respiration
ECAR, OCR
Myo...
100%
Fusion
D3
C
XF Cell Energy Phenotype
Aerobi
Energetic
Quiescent
Glycolyt ic
Mitochondrial Respiration OCR (pmol/min/Abs)
ECAR (mpH/min/Abs) Glycolysis
Fusión
D3
100%
MIOBLASTOS
D
SH
TDP
FUS
ECAR (mpH/min/UG)
Time (min)
Glycolysis
Glycolytic Capacity
Glycolytic Reserve
E
Lactate (a.u.)
F
Mitochondrial Respiration
OCR (pmol/min/UG)
Time (min)
Basal
Spare Respiratory Capacity
G
shRNA
shTDP
shFUS
Atrogin-1
Tubulina
Murf-1
Tubulina
Murf-1
Atrogin-1

FIGURE 3

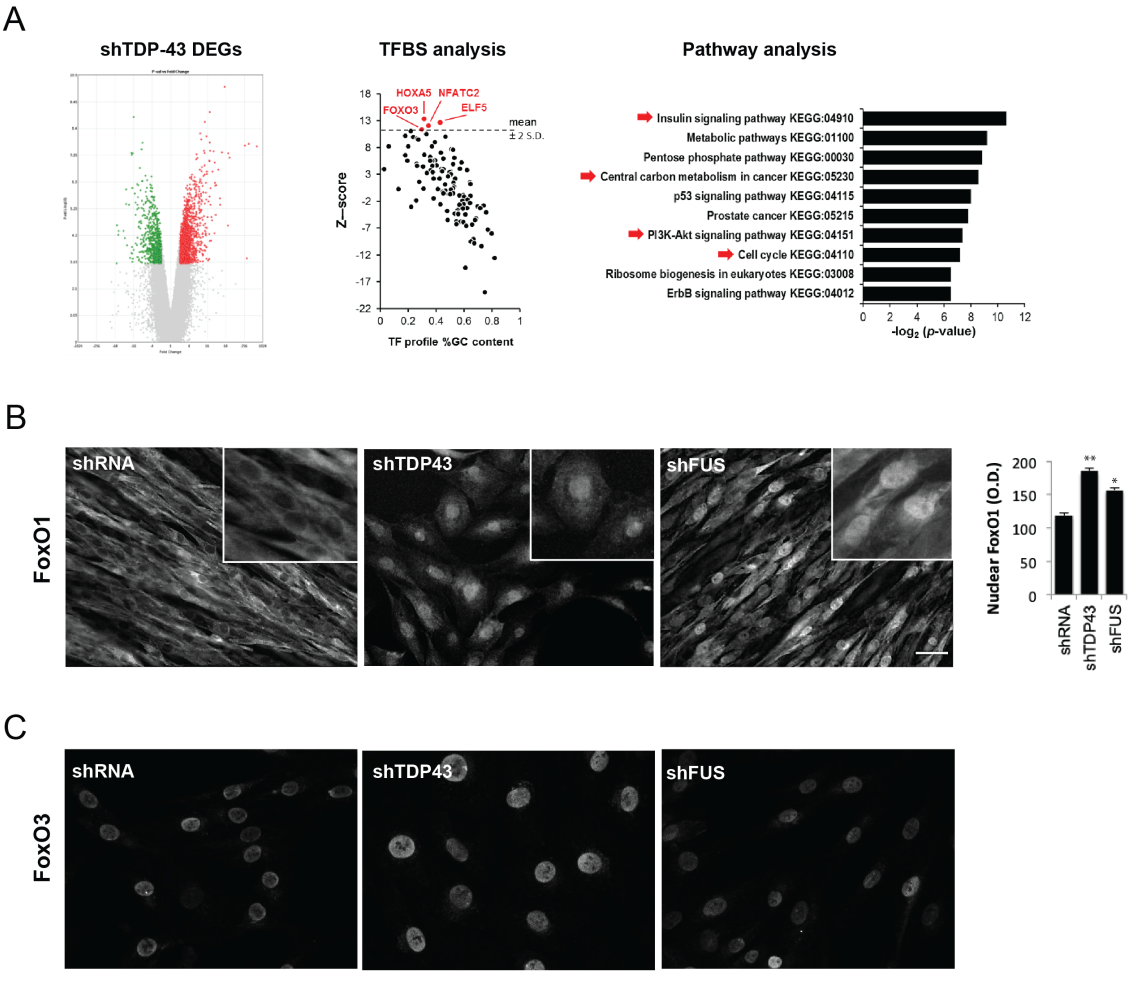

A
shTDP-43 DEGs
TFBS analysis
Pathway analysis
HOXA5
NFATC2
FOXO3
ELF5
mean
± 2 S.D.
Z–score
TF profile %GC content
Insulin signaling pathway KEGG:04910
Metabolic pathways KEGG:01100
Pentose phosphate pathway KEGG:00030
Central carbon metabolism in cancer KEGG:05230
p53 signaling pathway KEGG:04115
Prostate cancer KEGG:05215
PI3K-Akt signaling pathway KEGG:04151
Cell cycle KEGG:04110
Ribosome biogenesis in eukaryotes KEGG:03008
ErbB signaling pathway KEGG:04012
-log2 (p-value)
B
FoxO1
shRNA
shTDP43
shFUS
Nuclear FoxO1 (O.D.)
C
FoxO3
shRNA
shTDP43
shFUS

FIGURE 4

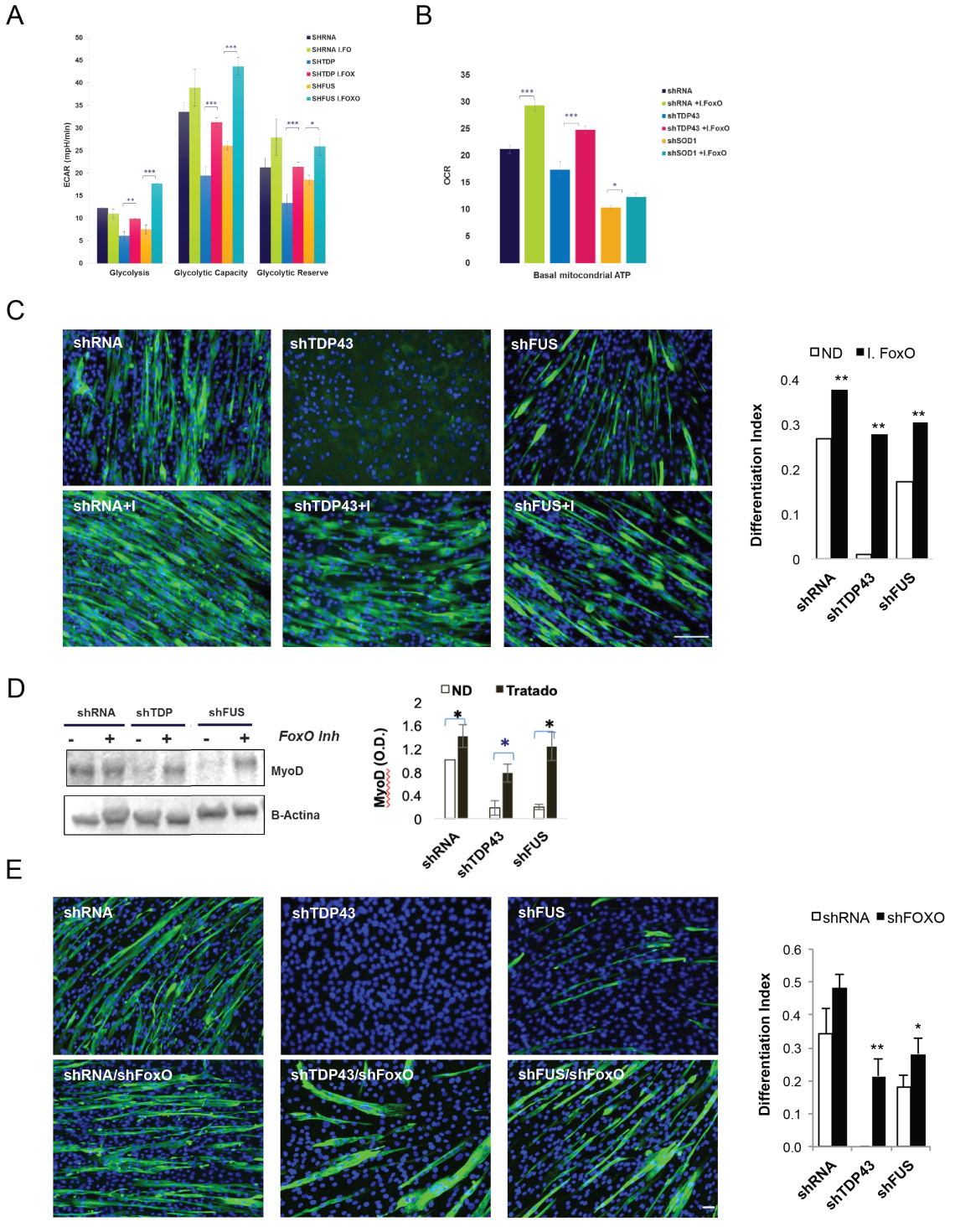

A
ECAR (mpH/min)
Glycolysis
Glycolytic Capacity
Glycolytic Reserve
SHRNA
SHRNA I.FO
SHTDP
SHTDP I.FOX
SHFUS
SHFUS I.FOXO
B
OCR
Basal mitocondrial ATP
shRNA
shRNA +I.FoxO
shTDP43
shTDP43 +I.FoxO
shSOD1
shSOD1 +I.FoxO
C
shRNA
shTDP43
shFUS
shRNA+I
shTDP43+I
shFUS+I
ND
I. FoxO
Differentiation Index
D
shRNA
shTDP
shFUS
FoxO Inh
MyoD
B-Actina
ND
Tratado
MyoD (O.D.)
E
shRNA
shTDP43
shFUS
shRNA/shFoxO
shTDP43/shFoxO
shFUS/shFoxO
shRNA
shFOXO
Differentiation Index

FIGURE 5

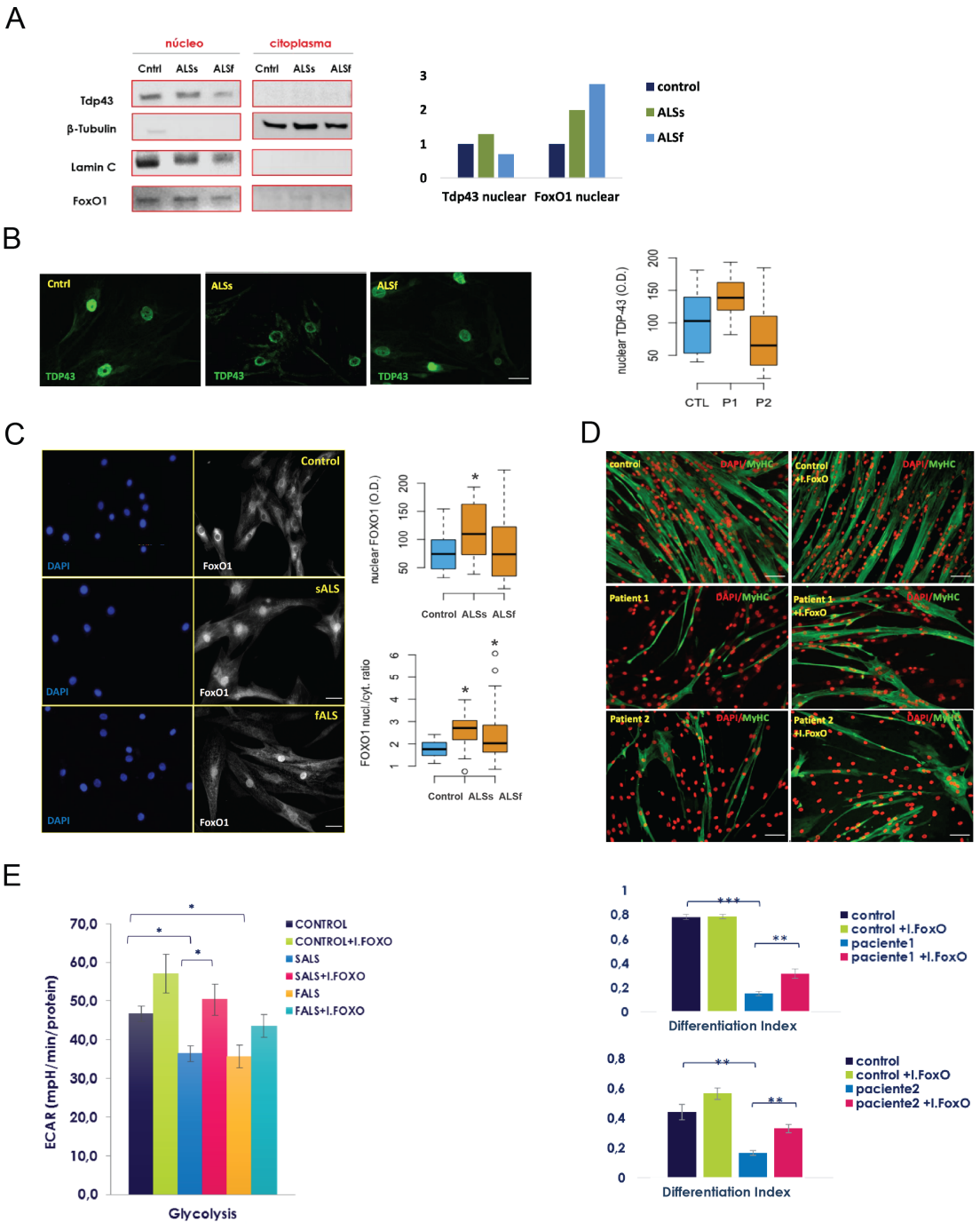
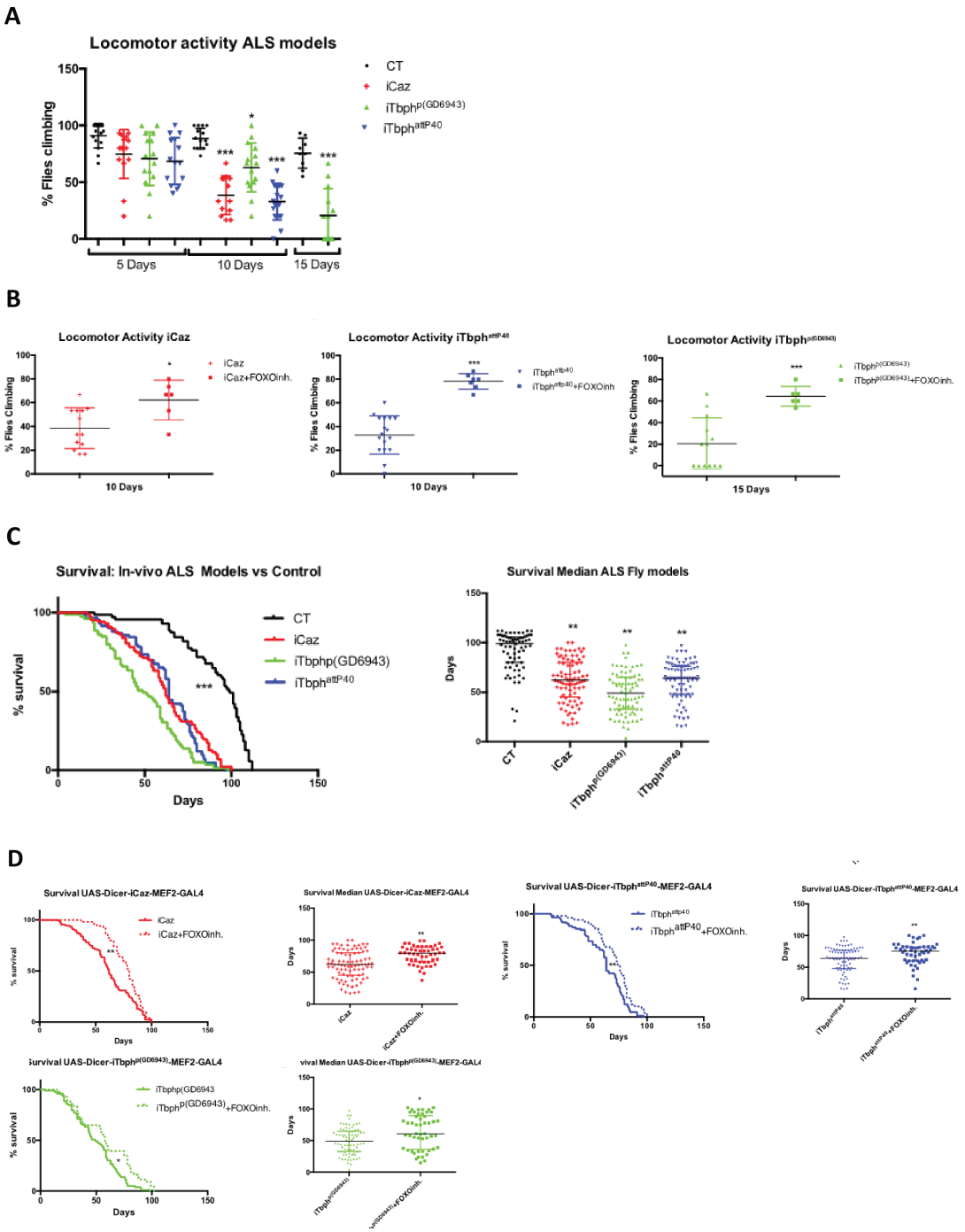

A
núcleo
citoplasma
Cntrl ALSs ALSf Cntrl ALSs ALSf
Tdp43
β-Tubulin
Lamin C
FoxO1
Tdp43 nuclear FoxO1 nuclear
control
ALSs
ALSf
B
Cntrl
ALSs
ALSf
TDP43
nuclear TDP-43 (O.D.)
CTL P1 P2
C
Control
DAPI
FoxO1
sALS
fALS
nuclear FOXO1 (O.D.)
Control ALSs ALSf
FOXO1 nucl./cyt. ratio
D
control
Control +I.FoxO
Patient 1
Patient 1 +I.FoxO
Patient 2
Patient 2 +I.FoxO
DAPI/MyHC
E
ECAR (mpH/min/protein)
CONTROL
CONTROL+I.FOXO
SALS
SALS+I.FOXO
FALS
FALS+I.FOXO
Glycolysis
Differentiation Index
control
control +I.FoxO
paciente1
paciente1 +I.FoxO
paciente2
paciente2 +I.FoxO

FIGURE 6


A
Locomotor activity ALS models
% Flies climbing
CT
iCaz
iTbphP(GD6943)
iTbphattP40
5 Days
10 Days
15 Days
B
Locomotor Activity iCaz
iCaz
iCaz+FOXOinh.
10 Days
Locomotor Activity iTbphattP40
Locomotor Activity iTbphP(GD6943)
15 Days
C
Survival: In-vivo ALS Models vs Control
% survival
Days
CT
iCaz
iTbphp(GD6943)
iTbphattP40
Survival Median ALS Fly models
D
Survival UAS-Dicer-iCaz-MEF2-GAL4
Survival Median UAS-Dicer-iCaz-MEF2-GAL4
Survival UAS-Dicer-iTbphattP40-MEF2-GAL4
iTbphattP40+FOXOinh.
Survival UAS-Dicer-iTbphP(GD6943)-MEF2-GAL4
iTbphp(GD6943)
iTbphP(GD6943)+FOXOinh.

SUPPLEMENTARY MATERIAL

FIGURE S1

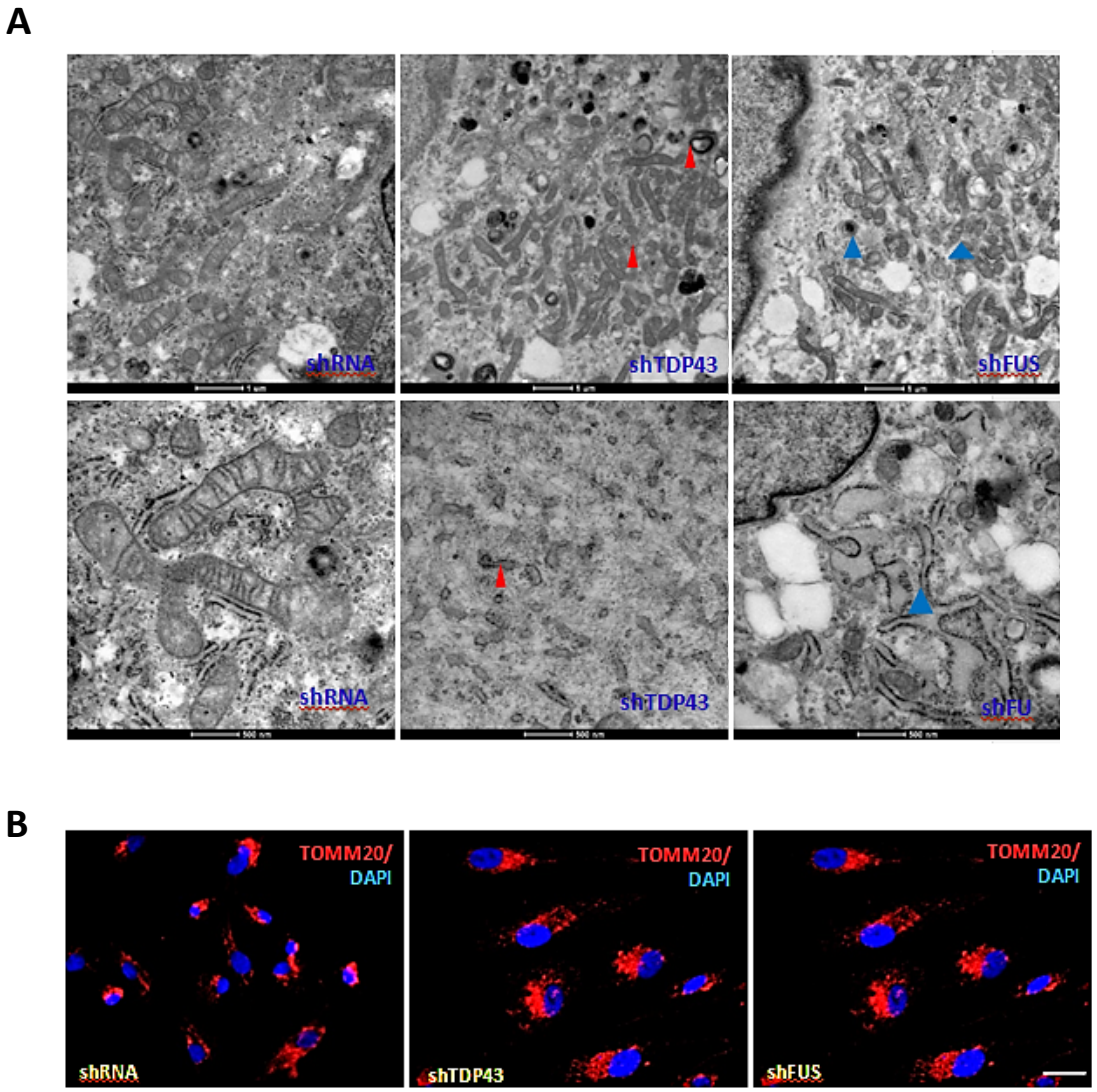


Figure S1. Morphological features of shTDP-43 and shFUS-treated myoblasts. A, Ultrastructure of silenced myoblasts observed by transmission electron microscopy. B, Mitochondrial network of silenced myoblasts as evidenced by immunostaining with mitochondria marker TOMM20.

FIGURE S2

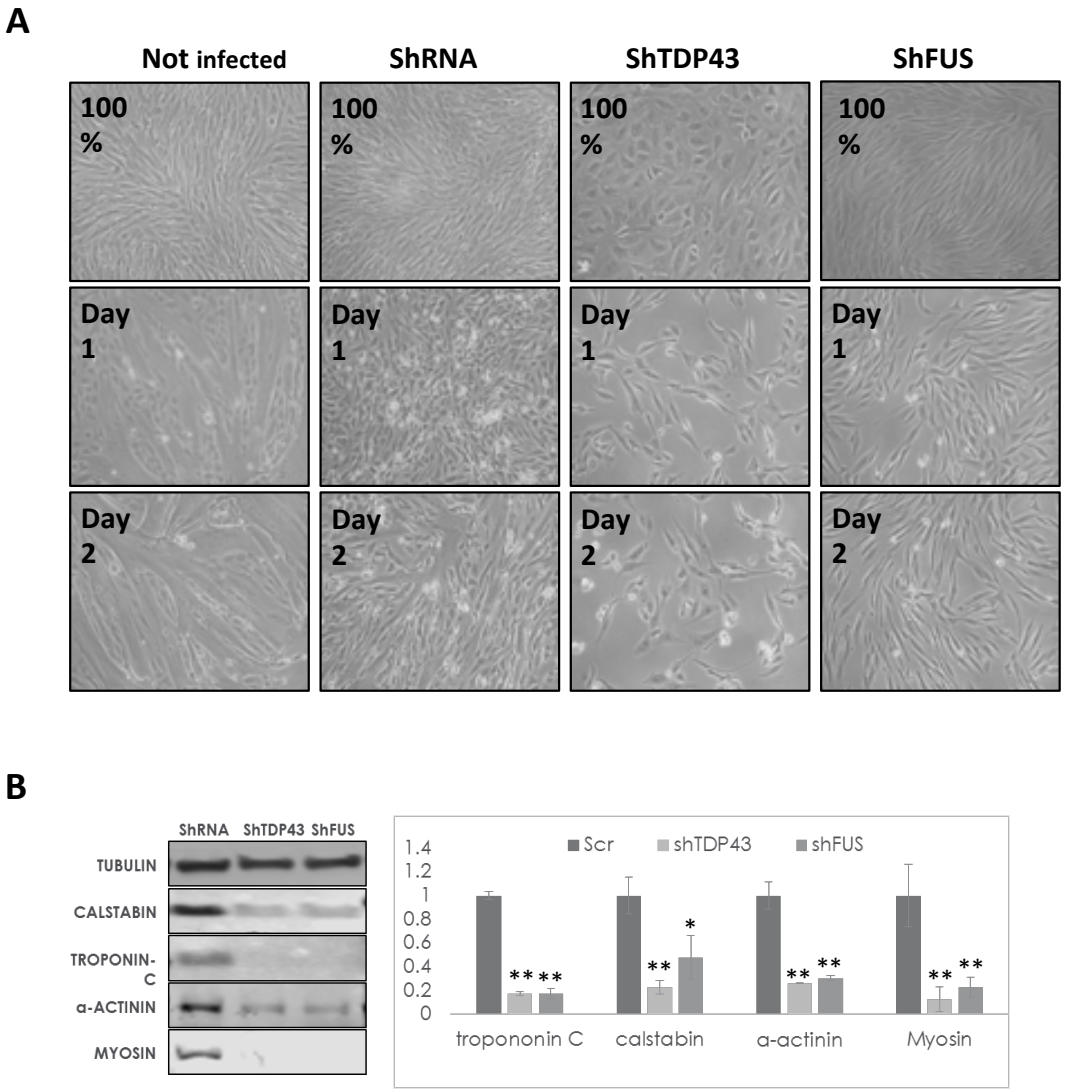


Figure S2. Effects of TDP-43 and FUS deficiency on myogenesis. A, Bright field microscope images of control (not infected) myoblasts, and shRNA, shTDP-43 and shFUS-treated myoblasts during different states of myoblast differentiation. B, Effects of shTDP-43 and shFUS treatment on markers of myogenesis.

FIGURE S3

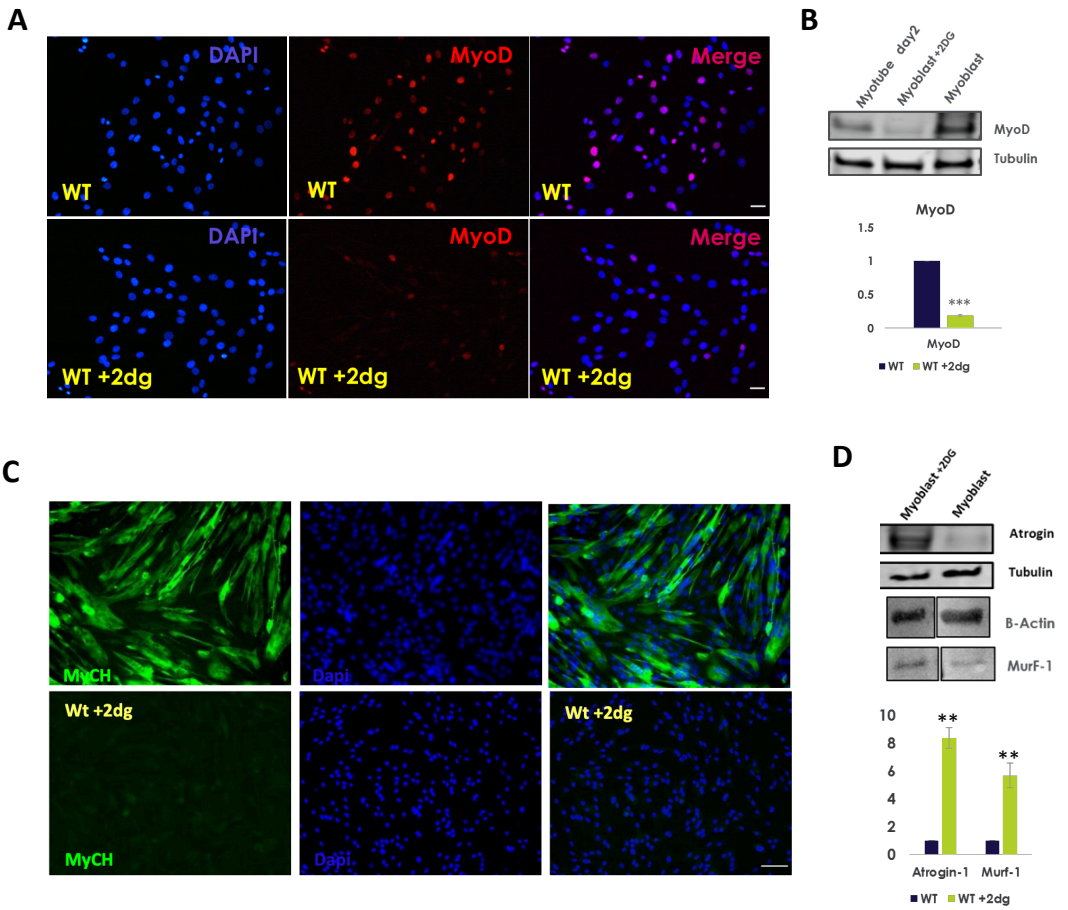


Figure S3. Effects of glycolysis inhibition on myogenic differentiation and pro-atrophic pathways. A, B, Immunofluorescence and immublot images of MyoD in 2-dexoyglucose (2-DG)-treated myoblasts. C, Immunostaining images showing the effect of 2-DG on the number of MyHC-postive myotubes. D, Expression of proteins of the ubiquitin-proteasome system after treatment with 2-DG.

FIGURE S4

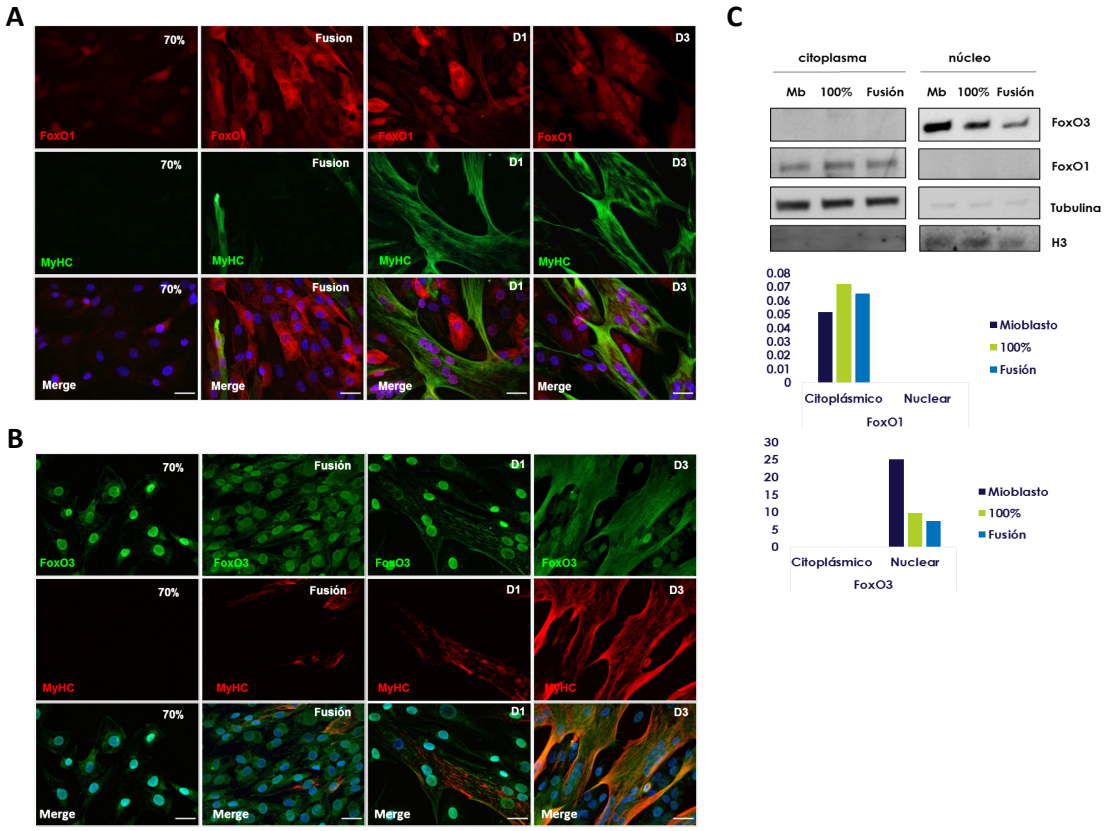


Figure S4. Levels of FoxO1 and FoxO3 factors throughout the myogenic process. A,B, Immunofluorescence images of FoxO1 (A) and FoxO3 staining (B) in wild-type human myoblast during different states of myoblast differentiation. C, Immunoblot of FoxO1 and FoxO3 proteins in nuclear and cytoplasmic cell fractions in different stages of myoblast differentiation.

FIGURE S6

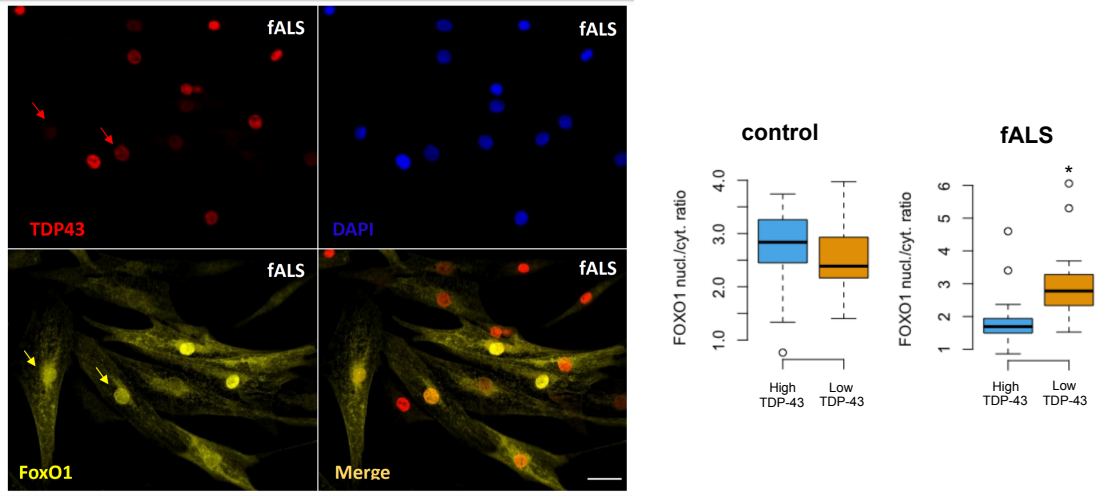


Figure S6. Inverse correlation of nuclear TDP-43 and FoxO1 levels in a familial case of ALS.

FIGURE S7

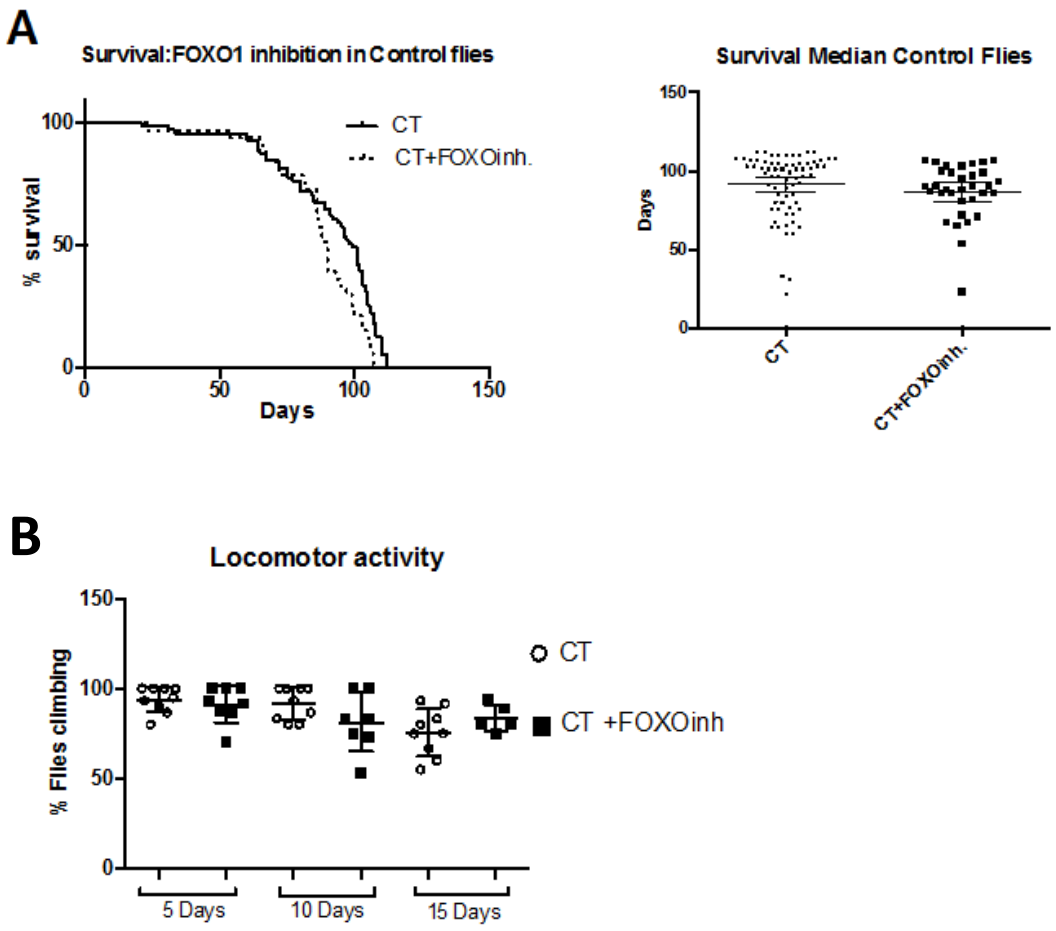


Figure S7. Effects of FoxO inhibition by compound AS1842856 in a control Drosophila melanogaster strain. A, Kaplan-Meyer curves (left panel) and median survival (right panel) of the control Drosophila strain UAS-Dicer2-+-Mef2-GAL4 with or without treatment with AS1842856. B, Climbing assay to report locomotor activity of the control Drosophila strain UAS-Dicer2-+-Mef2-GAL4 with or without treatment with AS1842856.

Table S1. List of antibodies.

Antibody	Dilution	Host	Company	Ref
Myogenin	1:250	Ms	Abcam	ab1835
Fbx32 (Atrogin1)	1-3ug/mL	Goat	Abcam	ab92281
MuRF1	1:1000	Rb	ECM Biosciences	# MP3401
TDP-43	1/5000	Ms	Abcam	ab104223
TDP-43 (G400)	1:1000	Rb	Cell Signaling	#3448
FUS/TLS	0.4 µg/mL	Rb	Invitrogen	PA5-52610
Fox03	1:100-1:1000	Rb	Thermo Fisher	PA5-27145
β-Tubulin	1:2000-1:5000	Ms	Invitrogen	MA5-16308
β-Actin	1:100.000	Ms	Sigma-Aldrich	A5441
MDH2	0.4 ug/mL	Rb	Sigma-Aldrich	HPA026720
GPD2	0.4 µg/mL	Rb	Invitrogen	PA5-61022
MDH2	0.4 µg/mL	Rb	Invitrogen	PA5-82692
FoxO3A	1:500-1:3000	Rb	Invitrogen	PA5-27145
FoxO1 (S.502.4)	1:1000	Rb	Invitrogen	MA5-14846
MyoD1(D8G3) XP	1:1000	Rb	Cell Signaling	#13812

Table S2. List of transcription factors predicting TDP-43 knockdown DEGs according to DAVID's TFBS analysis.

Pos.	*FACTOR*	*Count*	*%*	*P-Value*	*Fold Enr.*	*FDR*
1	CDP	695	54.3	5.20E-11	1.2	6.30E-08
2	NKX22	527	41.1	3.10E-10	1.2	3.80E-07
3	POU3F2	674	52.6	9.60E-09	1.2	1.20E-05
4	BRN2	576	45	1.30E-08	1.2	1.60E-05
5	FREAC3	494	38.6	2.10E-08	1.2	2.60E-05
6	PBX1	653	51	2.10E-08	1.2	2.60E-05
7	MRF2	584	45.6	2.50E-08	1.2	3.10E-05
8	CART1	546	42.6	3.80E-08	1.2	4.70E-05
9	E4BP4	504	39.3	4.90E-08	1.2	6.00E-05
10	**FOXO1**	**481**	**37.5**	**5.10E-08**	**1.2**	**6.20E-05**
11	CDC5	519	40.5	6.60E-08	1.2	8.20E-05
12	TATA	615	48	1.10E-07	1.2	1.40E-04
13	HFH1	513	40	1.20E-07	1.2	1.50E-04
14	CDPCR3	616	48.1	1.50E-07	1.2	1.90E-04
15	SOX5	545	42.5	2.40E-07	1.2	3.00E-04
16	ELK1	568	44.3	2.60E-07	1.2	3.20E-04
17	RSRFC4	536	41.8	3.00E-07	1.2	3.70E-04
18	**FOXO4**	**610**	**47.6**	**4.40E-07**	**1.2**	**5.40E-04**
19	NKX3A	525	41	4.80E-07	1.2	5.90E-04
20	IRF2	468	36.5	2.80E-06	1.2	3.50E-03
21	NKX61	487	38	4.00E-06	1.2	4.90E-03
22	HLF	457	35.7	5.00E-06	1.2	6.20E-03
23	HNF3B	462	36.1	6.60E-06	1.2	8.10E-03
24	FREAC2/ MEIS1AH	374	29.2	7.20E-06	1.2	8.80E-03
25	OXA9	397	31	8.60E-06	1.2	1.10E-02
26	CREBP1	530	41.4	8.70E-06	1.2	1.10E-02
27	ISRE	491	38.3	9.30E-06	1.2	1.10E-02
28	MYB	503	39.3	9.60E-06	1.2	1.20E-02
29	LHX3	414	32.3	2.20E-05	1.2	2.70E-02
30	FREAC4	507	39.6	2.40E-05	1.2	2.90E-02
31	POU6F1	470	36.7	3.60E-05	1.2	4.40E-02
32	MSX1	462	36.1	4.00E-05	1.2	5.00E-02
33	SP1	287	22.4	4.90E-05	1.2	6.00E-02
34	FOXD3	380	29.7	1.30E-04	1.2	1.60E-01
35	TBP	393	30.7	1.60E-04	1.2	1.90E-01
36	NRF2	333	26	3.80E-04	1.2	4.60E-01
37	IRF1	279	21.8	1.20E-03	1.2	1.50E+00
38	CEBPA	313	24.4	1.60E-03	1.2	1.90E+00
39	CETS1P54	272	21.2	3.80E-03	1.2	4.60E+00
40	ZIC1	155	12.1	1.70E-02	1.2	1.90E+01
41	EVI1	923	72.1	4.70E-12	1.1	5.80E-09
42	FOXJ2	738	57.6	2.50E-08	1.1	3.00E-05