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SNPs in microRNAs and susceptibility to Late-Onset Alzheimer´s Disease

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1. INTRODUCTION

1.1- ALZHEIMER DISEASE (AD)

Alzheimer's disease (AD) is the most common form of age-related neurodegenerative disorder. It affects an estimated 24 to 35 million people worldwide (Querfurth and LaFerla, 2010; Brookmeyer et al., 2007; Ballard et al. 2011).

Alzheimer´s Disease is the most common cause of dementia. Patients with dementia begin having forgetfulness, then progress to having irreversible loss of memory and other previously well-learned skills (Querfurth and LaFerla, 2010).

1.1.1- Pathologic features of AD

AD is associated with atrophy and death of neurons in specific brain regions. The predominant characteristics of neuropathology associated with AD are neuritic senile plaques (NSP) and neurofibrillary tangles (NFT) (Armstrong, 2011). The former, are comprised of β-amyloid peptides (Aβ) which accumulate as extracellular deposits mainly in the frontal and temporal lobes, including the hippocampus. The later, although present as well in the same regions of the brain, are accumulated inside neuronal cells. In more advanced cases of AD the pathology extends to other regions of the cortex such as the parietal and occipital lobes. NSP and NFT do occur as well in normal ageing brains, but they are more numerous and more widely distributed in brains of patients with AD (Nowotny et al., 2001).

1.1.1.1- Neuritic Senile Plaques (NSPs)

The first evidence of the disease is the formation of NSPs, comprised of Aβ peptides. The Aβ peptides are derived from a larger protein, β-amyloid precursor protein (APP), by proteolitic processing. The APP gene is located on human chromosome 21 and its cleavage occurs via 2 enzymes: β-secretase and γ-secretase, leading to a secreted C-terminal fragment. This Aβ peptide obtained varies from 40 to 43 amino acids in length ($A\beta_{40}$, $A\beta_{42}$, $A\beta_{43}$). The NSP are formed by heterogeneous peptides

of all lengths. $A\beta_{40}$ is normally the most abundant form in healthy people and it is known to inhibit amyloid deposition (Kim et al., 2007). On the contrary, the proportions of $A\beta_{42}$ and $A\beta_{43}$ are increased in NSP of AD patients. In fact, it has been demonstrated that $A\beta_{42}$tends to self-aggregate and can grow into extracellular fibrils arranged onto β- pleated sheets which are the insoluble fibers of NSP (Querfurth and LaFerla, 2010). This is thought to be the first step in AD development (Armstrong, 2011).

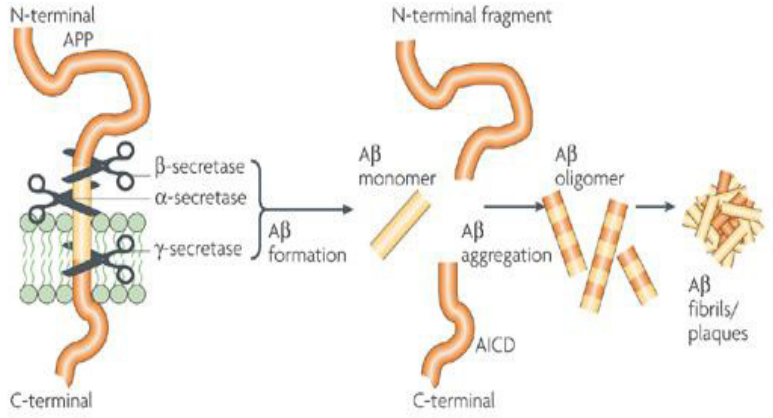

Figure 1 Schematic diagram of β-amyloid precursor protein (APP) and its cleavage process for the formation of Aβ plaques (Götz and Ittner, 2008).

1.1.1.2- Neurofibrillary tangles (NFT)

The other hallmark of the disease is the formation of NFTs, which are comprised of modified tau protein, a normal component of the cytoskeleton. According to the amyloid cascade hypothesis, the formation of NFTs seems to be driven by the accumulation of NSP. Tau is normally found in great abundance in neurons, where it binds tubulin monomers together to form stable polymers that are essential in cellular transport and axonal growth. In AD tangles, the tau becomes hyperphosphorilated leading to a less efficient binding to microtubules (Götz and Ittner, 2008). Therefore, the tau protein that is unbound tends to aggregate into insoluble filaments, the so called NFTs, which are seen as deposits in the neurons.

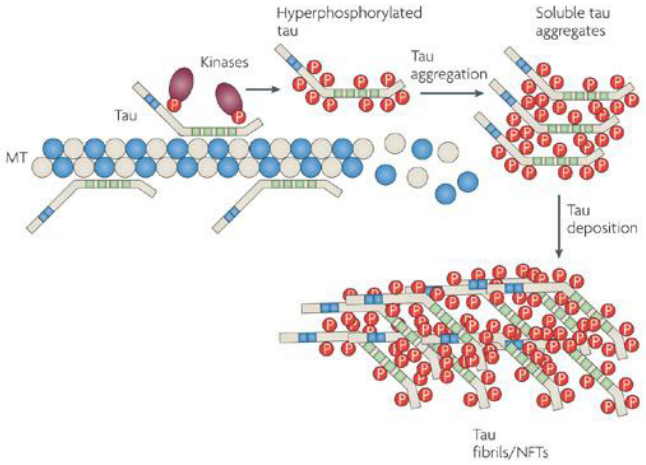

Figure 2 The NFTs contain aggregates of the microtubule- associated protein tau (Götz and Ittner, 2008).

1.1.2- Types of Alzheimer´s Disease

AD is commonly classified into two types based on the time of its onset (Blennow et al., 2006). In the majority of the cases, the disease is diagnosed after the age of 65 years, and it is referred to as late-onset Alzheimer´s Disease (LOAD) or sporadic AD. However, around 5-10% of all persons diagnosed with AD develop symptoms before the age of 65 years, and this is referred to as early-onset Alzheimer disease (EOAD). Compared with LOAD, EOAD has some distinctive features including early age at onset, a variety of non-cognitive neurological symptoms and signs, and a more aggressive course.

1.2- GENETICS OF AD

The extent to which genetics is involved in the development of AD depends on the 2 types of AD: EOAD and LOAD. In the former condition, genetic mutations in a small number of genes are known to cause AD. In contrast, for LOAD the genetic implication in the development of the disease is much more complex and it is not completely understood yet. In spite of the fact that various genetic and non-genetic factors have been associated with risk for LOAD, these variants are insufficient to cause the disease.

1.2.1- Early-Onset Alzheimer disease (EOAD)

Genetic analysis in EOAD patients have identified mutations in genes that result in increased production and deposition of Aβ leading to de development of the disease. The main three genes involved are: (1) the APP gene on chromosome 21 (Goate et al, 1991), (2) the presenilin-1 (PSEN1) gene on chromosome 14 (Sherrington et al., 1995) and (3) the presenilin-2 (PSEN2) gene on chromosome 1 (Levy-Lahad et al., 1995). The number of pathogenic mutations found within these genes are described in Table 1.

Table 1 Early-onset Alzheimer´s Disease (EOAD) causative mutations

Gene	Locus	Protein	Pathogenic mutations	Reference
APP	21q21.2	Amyloid beta (Aβ)	25	Thinakaran and Koo, 2008
PSEN1	14q24.3	Presenilin-1	185	Cruts and Van Broeckhoven, 1998
PSEN2	1q31–q42	Presenilin-2	12	Steiner et al., 2002

1.2.2- Late-Onset Alzheimer disease (LOAD) or sporadic AD

In contrary to early-onset patients, no causative gene has been identified for LOAD. It has been suggested that various non-genetic factors impact risk for AD—the greatest of which is age (Querfurth and LaFerla, 2010). Other risk factors include hypertension, estrogen supplements, smoking, stroke, heart disease, depression, arthritis, and diabetes (Lindsay et al., 2002). On the other hand, certain lifestyle choices appear to decrease the risk of AD: exercise (Podewils et al., 2005) and intellectual stimulation (Wang et al., 2002).

Although these non-genetic factors may affect LOAD risk, it is thought that the genetic factors also play a critical role. For this reason, extensive effort is being made in order to discover common genetic variants that confer susceptibility to LOAD.

1.2.2.1- Genetic variants and susceptibility to LOAD

Human genetic variation is the genetic differences within individuals. One of the most prevalent classes of genetic variation are Single Nucleotide Polymorphisms

(SNPs) (Kruglyak and Nickerson, 2001). SNPs consists in single base substitutions of one nucleotide with another (Figure 3), observed in the general population at a frequency greater than 1%. It has been estimated that the human genome contains at least 11 million SNPs (Kruglyak and Nickerson, 2001) which can be found all along the genome. Nowadays, extensive efforts are being made to catalogue SNP variation among individuals, and correlate it with risk to diseases.

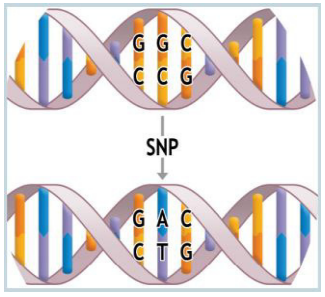

Figure 3 Example of a SNP showing a substitution at a single position in DNA.

In reference to LOAD, the main genetic variant associated to risk is located in the coding region of apolipoprotein E gene (ApoE) (Harold et al., 2009). Apart from the ApoE allele, recent Genomic Wide Association Studies (GWAS) have identified variations in over 20 loci that contribute to AD risk. These variants fall in the regulatory regions of the protein coding genes (regions 3´ and 5´ UTR) or in their introns. The top variants associated with risk to AD that were obtained in a meta-analysis (Bertram et al., 2007) are shown in Table 2.

Table 2 Top variants associated with late-onset Alzheimer's disease. From meta-analysis done by the Alzheimer Research Forum (Bertram et al., 2007)

Gene	Variant	Location	Risk/ Protective
APOE	rs7412	Coding region	Risk
	rs429358	Coding region	Protective
BIN1	rs744373	5´ UTR	Risk
CLU	rs11136000	Intron	Protective
ABCA7	rs3764650	Intron	Risk
CR1	rs3818361	Intron	Risk
PICALM	rs3851179	3´ UTR	Protective
MS4A6A	rs610932	3´ UTR	Protective
CD33	rs3865444	5´ UTR	Protective
MS4A4E	rs670139	Intergenic region	Risk
CD2AP	rs9349407	Intron	Risk

The majority of the association studies related to LOAD, have evaluated the risk of SNPs that are located in protein coding genes, which after the completion of the Human Genome Project, we know that barely represent ~ 1.5 % of the entire genome (Esteller, 2011). Surprisingly, scarcely study has been done in non-coding RNA regions of the genome.

The research named "Encyclopedia of DNA Elements" (ENCODE) shed light into the importance of non-coding RNA genes (ncRNAs). A number of 22,411 non-coding RNA molecules were identified and it was suggested that they are involved in the regulation numerous the protein-coding genes (Esteller, 2011).

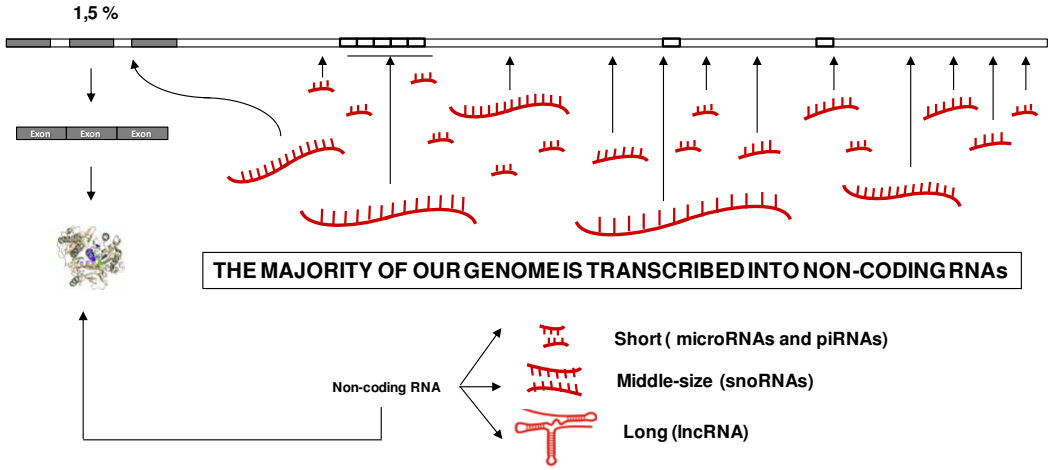

Figure 4 The ENCODE project has shown the importance of ncRNAs genes. The ncRNA molecules play a major role regulating the expression of protein coding genes, which only represent 1,5% of our entire genome.

Therefore, since the non-coding RNA molecules constitute an important part of the genome, and play a crucial role in the regulation of gene expression, it has been suggested that genetic variants within these molecules may be associated to risk for disease (Cai et al., 2009).

1.3- NON-CODING RNA GENES: miRNAs

There are three different categories of non-coding RNAs (ncRNAs) depending on the length of the RNA chain (Esteller, 2011): (1) short ncRNAs: like the miRNAs, piRNAs, or tiRNAs. (2) mid-size ncRNAs: snoRNAs and (3) long ncRNAs: lincRNAs.

Among the non-coding RNAs, miRNAs are the ones that have been studied the most. miRNAs are a subset of non-coding small RNAs (20-22 nucleotides) that play a key role in the post-transcriptional regulation of gene expression. Their function is to mediate the post-transcriptional gene silencing of target RNA transcripts. In mammals, miRNAs are predicted to control the activity of ~ 60% of all the protein genes (Esteller, 2011). Currently, 2578 mature human miRNAs are catalogued in the most recent version of miRBase (version 20, June 2013, http://www.mirbase.org/). Each miRNA is estimated to regulate around 200 targets, and mRNA transcripts may be regulated by multiple miRNAs (Esteller, 2011).

The brain, in particular, hosts a diverse collection of miRNAs (approximately 70% of experimentally detectable miRNAs) (Cao et al., 2006) and, in addition, it has been reported that the expression of many miRNAs are deregulated in AD (Lau et al., 2014). For this reason, we are going to focus on the miRNAs involved in AD pathogenesis.

1.3.1- miRNA biogenesis and mechanism of action

Biogenesis of miRNAs starts with the production of the primary miRNA transcript (pri-miRNA) by RNA polymerase II or III and cleavage of the pri-miRNA in the nucleus. The resulting precursor hairpin, the pre-miRNA, is exported from the nucleus. In the cytoplasm, the pre-miRNA hairpin is cleaved to its mature length obtaining a miRNA/miRNA* duplex, that is rapidly unwound. The functional strand from the duplex (miRNA) is loaded together with Argonaute proteins into the RNA-induced silencing complex (RISC), where it guides RISC to silence target mRNAs through mRNA cleavage or translational repression, whereas the passenger strand (miRNA*) is degraded (Winter et al. 2009) or, in some cases associated with different Ago proteins complexes to also become active (Czech and Hannon, 2011).

Therefore, the role of miRNAs in this regulatory process is to act as a guide and enable the specific recognition of the target to be silenced. This recognition is carried out via base-pairing interactions between the “seed region” of the miRNA – this is a 2-6 nucleotide region in the mature miRNA– and the 3´ UTR region of the mRNA (Bartel, 2009).

1.3.2- miRNA deregulation in AD

Lately, the dysfunction of miRNAs in neurodegenerative diseases such as AD, is increasingly being recognized and studied (Lau et al., 2014). In this regard, many studies have shown that miRNAs expression levels are upregulated or downregulated in the brain of AD patients in comparison to healthy controls. This suggests that miRNAs may be involved in the pathogenesis of the disease (Maciotta et al., 2013).

To explain the origin of the deregulation in miRNA expression levels it has been suggested that the presence of SNPs within the precursor miRNA or in the seed region of the mature miRNA, may be the cause of it (Sun et al., 2009).

1.3.3- SNPs in miRNAs (miR-SNPs)

SNPs in miRNA genes can affect their secondary structure, and thus compromise its biogenesis process or function. Indeed, some miR-SNPs have been associated to risk for disease (Cai et al., 2009).

If the SNPs are located within precursor miRNAs (pri-miRNA and pre-miRNA) they may affect miRNA processing and consequently, alter the mature miRNA levels, causing an increase or decrease in expression. For example, it has been reported that SNPs in pri-miR-16-1 or pri-let-7e lead to decrease mature miRNA levels (Wu et al., 2008). Moreover, several studies have shown an association between SNPs in pri and pre-miRNAs and risk for diseases such as schizophrenia (Hansen et al., 2007) or late insomnia (Saus et al., 2010). (Figure 5, A)

On the other hand, SNPs within the seed sequence of the mature miRNA, apart from being capable of altering the expression level of mature the mature miRNA, they can also affect the affinity for the target. This is, the SNP may strengthen or reduce the binding between the miRNA and its target, and thus alter the expression of the target or even lead to gain or lose target sites (Cai et al., 2009). An example of this is the SNP rs2910164, which is located in the seed region of miR-146a and is associated with lower expression of mature miRNA and susceptibility to papillary thyroid carcinoma (Jazdzewski et al., 2008). (Figure 5, B)

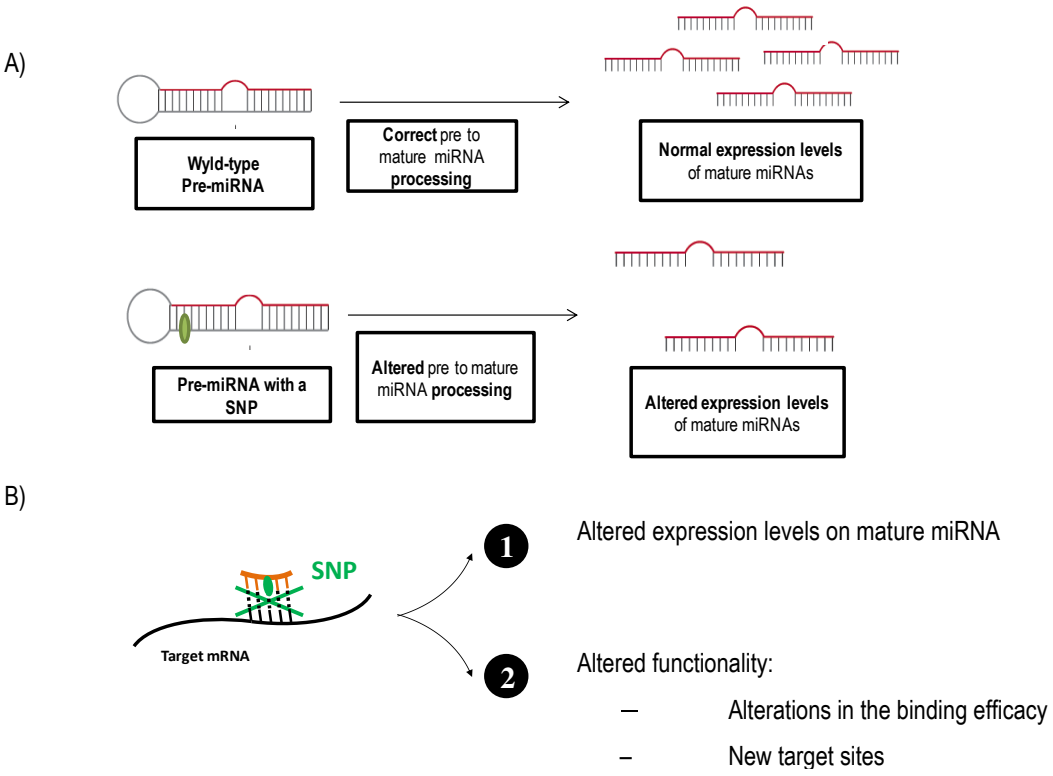

Figure 5 **A)** Effects of a SNP in a pri/pre mirRNA due to alterations in its biogenesis process. **B)** Possible effects of a SNP in the seed region of the mature miRNA.

The functionality of the miRNA can be also compromised when a SNP is present in its target site since it would also affect binding. In reference to AD, several studies have focus on evaluating the consequences of SNPs located in the 3'UTR region of genes, which can potentially be target sites. In fact, susceptibility variants to LOAD within this region have already been reported (Bertram et al., 2007).

To clarify possible confusions in terminology with respect to SNPs, we will make a distinction between the variation than occurs in the miRNA gene sequence: ''miR-SNP''; and the SNPs that occur in the miRNA target site (TS): ''miR-TS-SNP''

1.3.3.1- SNPs in miRNAs and AD

To date only a couple of studies have evaluated the implication of miR-SNPs in susceptibility to LOAD.

Qi et al, (2012) studied the association of SNP rs531564 located in pri-miR-124, a neuronal miRNA, to AD susceptibility. Although they proved that the SNP caused a

change in the miRNA expression, there was no association between rs531564 and risk for AD in Mongolian population.

Cui L. et al, (2014) carried out a case-control association study to check whether rs2910164 and rs57095329 SNPs increased the risk of AD in Chinese population. Of note was that the SNP rs57095329 located in the promoter region of miR-146a was significantly associated with AD. However, the SNP rs2910164 located in the pre-mature region did not show association.

Since only a couple of studies have evaluated the implication of miR-SNPs in susceptibility to LOAD and considering that miRNAs play important regulatory roles, this seems a promising field of research.

2. HIPOTHESIS AND AIM OF THE PROYECT

In the light of the above and considering that:

(1) No causative mutations have been found in Late-onset Alzheimer´s Disease and therefore the genetic research is focused on finding risk variants.

(2) The risk variants associated to LOAD are located in protein coding genes or in their 3´UTR or 5´UTR regulatory regions, which only represent ~ 1.5% of the genome.

(3) Non-coding RNAs represent a major part of the genome and are involved in the regulation of protein-coding gene expression.

(4) miRNAs expression levels are deregulated in AD and therefore, could be implicated in the pathogenesis of the disease

(5) The deregulation of miRNAs may be due to the presence of SNPs in the pre-miRNAs and only a couple of studies have evaluated their association to AD risk.

Our **hypothesis** is that polymorphisms in miRNAs may be markers of genetic susceptibility to LOAD.

The **aim of this project** is to identify polymorphisms in miRNAs that increase the risk for LOAD.

In order to do that, the following specific objectives will be aimed at:

1. Systematic search of deregulated miRNAs
2. Selection of miR-SNPs
3. Case-control association study

3. MATERIALS AND METHODS

3.1- SYSTEMATIC SEARCH

An exhaustive search to identify studies that reported deregulated miRNAs in AD was performed (articles included until April 7th, 2014). For Pubmed database (http://www.ncbi.nlm.nih.gov/pubmed/) we used the key words and subject terms: "(mir OR microrna OR miRNA)"AND alzheimer". The results of the search were assessed one by one to select all the articles that studied the deregulation of miRNA expression in AD patients in comparison to controls. The articles that did not meet this criterion were excluded. These excluded articles were classified according to the reason of exclusion: type of publication, not in English, other disease, do not study miRNA deregulation, not human sample, SNPs in miRNAs and no abstract. The information about the deregulated miRNAs in AD was ordered by gathering together all the results obtained for each individual miRNA in the different papers.

3.2- miRNA AND miR-SNP$_S$ SELECTION

All pre-miRNAs described in miRBase (http://www.mirbase.org/) database were selected for the study (retrieved: March 2013).

The miRNA SNipER database (http://www.integratomics-time.com/miRNA-SNiPer/) was used to select all the SNPs located in the pre-miRNAs with a minor allele frequency (MAF) greater than 1% in Caucasian population (retrieved: March 2013).

3.3- SELECTION OF PATIENTS

The study population included a total of 466 Spanish individuals, 229 AD patients and 237 controls, from which a DNA sample was obtained. The study was approved by the local Institutional Review Board. For both, cases and controls, written informed consent was obtained (before sample collection).

3.4- GENOTYPING OF POLYMORPHISMS

The polymorphisms were genotyped using the GoldenGate® Genotyping Assay, form VeraCode Technology®, Illumina (Figure 6) at the Spanish National Genotyping Center (CeGen-ISCIII). This platform allows a multiplex PCR assay, meaning that it is able to process a large number of SNPs simultaneously (up to 1,536 SNPs) thereby minimizing time, reagent volumes, and material requirements of the process. Therefore, it is very a useful technique when targeting specific genomic regions like SNPs.

The GoldenGate® Assay consists in several steps. First of all, the DNA sample needs to be activated (with Biotin or Streptavidin) for posterior binding to paramagnetic particles (250ng DNA at 50ng/µl). The following step is the hybridization between the sample DNA and specific oligonucleotides, which need to be designed for each SNP locus. More concretely, three oligonucleotides are designed: two of them are specific to each allele of the SNP site and therefore are called Allele-Specific Oligos (ASOs). The third oligo is the Locus-Specific Oligo (LSO) and hybridizes several bases downstream from the SNP site. After DNA hybridization with the LSO and with one of the ASOs, extension of the appropriate ASO and ligation of the extended product to the LSO occurs. These full-length products provide a template for PCR given that both, the ASOs and the LSO contain regions of complementarity to universal PCR primers. Moreover, the primers for each of the ASOs are dye-labeled differently (Cy3- and Cy5-) so that the specific allele of the SNP can be determined. Since this assay allows processing a large number of SNPs, the LSO designed for each of the SNPs contains a unique address sequence so that after PCR amplification, the products are hybridized onto an Array Matrix allowing individual SNP genotype readout by analyzing the fluorescence signal.

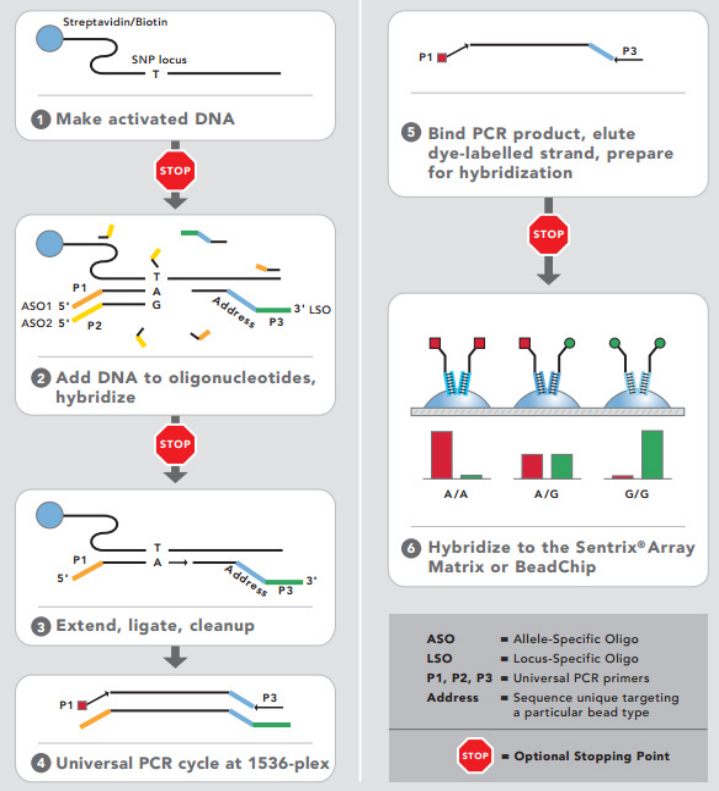

Figure 6 GoldenGate® Assay overview (http://www.illumina.com/technology.ilmn retrieved: June 12th, 2014)

3.5- STATISTICAL ANALYSES

All analyses were performed using R software (version 2.11.0). Hardy-Weinberg Equilibrium (HWE) between expected and observed genotype distributions in the control sample was assessed. The association between each miR-SNP and susceptibility to AD was evaluated comparing the genotypic frequencies of each group using a logistic regression model. Association was expressed as odds ratios (OR) with 95% confidence intervals (CI). P-values < 0.05 were considered to be statistically significant. The results were adjusted for multiple comparisons by the False Discovery Rate method (FDR). The p-value was adjusted by sex and age in the total sample. The Haploview software (version 4.2) was used to calculate the success of the genotyping. Individuals with 50% of missing genotypes were excluded from the statistical analysis.

3.6- miRNA SECONDARY STRUCTURE PREDICTION

The "Bioguo web tool" (http://bioguo.org retrieved: May 20th, 2014) (Release 2.0: July 2013) (Gong et al., 2012) was used to predict the most stable secondary structures of the miRNAs showing significant SNPs.

4. RESULTS

4.1- SYSTEMATIC SEARCH

4.1.1- Flow chart

A systematic search was carried out in order to gain insight into the deregulated expression of miRNAs in AD. The Pub Med search: "(mir OR microrna OR miRNA) AND alzheimer" provided 180 records (retrieved: April 7th, 2014). Of these 180 records, 143 were discarded after being examined in detail. A number of 37 records were included . Figure 7 represents the literature review process.

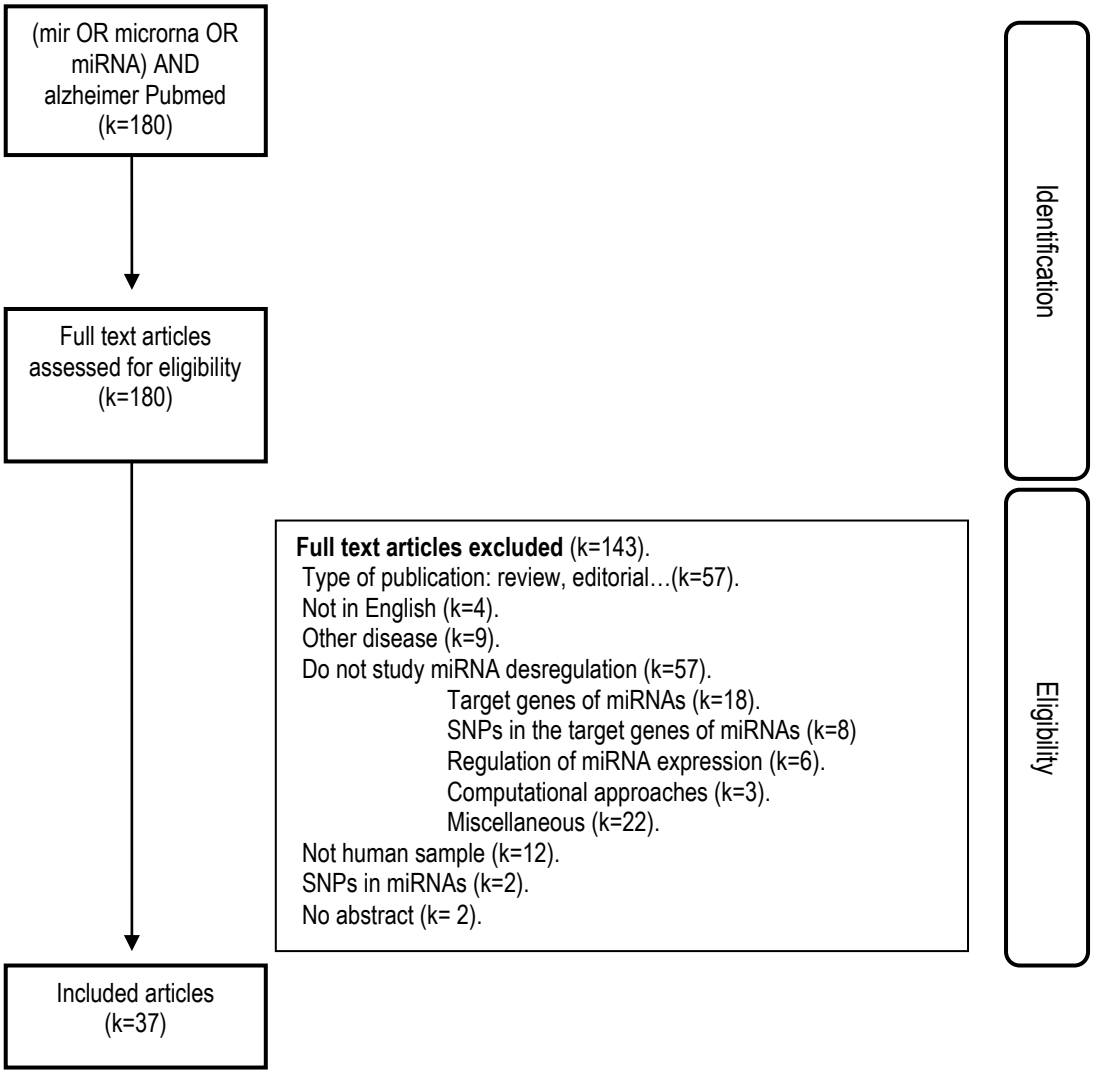

Figure 7 Flow chart of the systematic search. Out of the 180 results, 37 articles studied the deregulation of miRNAs in AD patients in comparison to healthy controls.

4.1.2- miRNA deregulation in AD

Finally, thirty seven articles were found to study the deregulation of miRNA in AD. In these studies, the methods used to detect and profile miRNA expression were quantitative real time PCR (qRT-PCR), microarrays, and more recently next generation technologies (NGS). Since the pathophisiology of the disease is found in the brain, the majority of the articles (n= 27) compared miRNA expression profiles in the postmortem brain of LOAD patients and controls. The rest (n= 10) analyzed the deregulation in other tissues such as blood, serum or cerebrospinal fluid. Finally, a total number of 316 miRNAs was found to be deregulated in AD (Table 1, Appendix 1). Of note is that for some specific miRNAs there are discrepant results among different studies.

4.2- miRNA AND miR-SNP$_S$ SELECTION

The total of deregulated miRNAs were launched in the miRNA SNipER database to search for SNPs in their sequence. Of them, only 10 showed a SNP validated experimentally and with a MAF greater than 1% in Caucasian population. However, considering that:

(1) SNPs located in the seed region of mature miRNAs may affect their functionality but produce no change in the mature miRNA expression level.

(2) SNPs present in pre-miRNAs may alter the expression level in the mature miRNA in such a subtle way that it is not detected by conventional microarray expression assays.

(3) miRNAs can regulate a wide variety of genes that are not completely defined. Therefore, any miRNA could be implicated in the regulation of the genes affecting LOAD risk.

(4) The genotyping of all miR-SNPs with a MAF >0.01 in Caucasic described in the miRNA SNipER database at the time of the selection was affordable.

We decided to analyze all the SNPs in pre-miRNAs that were described in miRNA SNipER database with a MAF greater than 1% in Caucasian population. This

selection provided a total number of 213 SNPs in 206 miRNAs. (Table 1, Appendix 2)

4.3- CASE-CONTROL ASOCIATION STUDY

4.3.1- Sample Characterization

In total, 229 AD patients and 237 gender and age-matched healthy controls were included in this case-control study.

Taking into account that our aim was to study the risk for AD in patients in which the disease develops at a late-onset, we selected the samples from AD patients older than 65 years. Hence, the sample used for the association study consisted in a total number of 212 AD patients and 220 controls. Table 3 summarizes the characteristic of the study population.

Table 3 Characteristics of the study population

	AD Patients> 65	Controls
Number, n	212	220
Average age	78,9 ∓ 6.40	78,2 ∓ 18.5
Sex, n (%)		
Female	145 (68.4)	132 (59.73)
Male	67 (31.5)	89 (40.27)

4.3.2- Genotyping Results

Out of the 213 miR-SNPs analyzed, 163 SNPs were successfully genotyped. All individuals showed a genotyping success higher than 50%.The overall genotyping success was 72.86%. Sixty-one SNPs were removed from the association study due to genotyping failures (n=50) or deviation from HWE (n=11). Details regarding the genotyping results are shown in Table 1, Appendix 2.

4.3.3- miR-SNPs association in susceptibility to AD

In order to study the association between miR-SNPs and susceptibility to LOAD, the genotypic frequencies from patients with AD older than 65 and controls were compared by estimating the odds ratios (OR) and 95% confidence interval (CI) for each SNP.

A total of 20 SNPs showed statistically significant association with LOAD risk ($p < 0.05$). After adjusting the p-value for age and sex, the 20 SNPs remained significant (Table 4). The information regarding all statistical results of the 20 significant SNPs is shown in Table 1, Appendix 3.

The most significant SNP was rs174561 under the dominant genetic model (TT vs CT+CC). rs174561 is located in the pre-mature region of hsa-miR-1908. The genotypes CT and CC rs174561 showed 0.49-fold decreased risk to LOAD (95% CI: 0.30-0.80, P= 0.004).

The second most significant SNP was rs74704964, under the codominant genetic model and located in pre-mature region of hsa-mir-518d. The CT genotype showed a 2.78- fold increased risk for LOAD (95% CI: 1.3-5.95, P= 0.006).

Remarkably, other 3 significant SNPs were located in miRNAs that showed putative or validated target genes related to AD. These SNPs were rs4809383, rs112328520, and rs61992671, located in pre-hsa-mir-941-1, pre-hsa-mir-520g and mature hsa-mir-412, respectively. Two of them, rs4809383 and rs112328520 were associated with a decreased risk to LOAD under a codominant genetic model, being the CT genotype protective in both cases. (rs4809383: 95% CI: 0.34-0.87, P=0.008; rs112328520: 95% CI: 0.28-0.95, P= 0.0311). The third SNP, rs61992671 was associated with an increased risk under the dominant genetic model (GG vs AG/AA). The genotypes AG and AA rs61992671 showed 1.58-fold increase risk to LOAD compared to GG genotype (95% CI: 1.03-2.41, P= 0.033).

After correcting with the False Discovery Rate correction method (FDR), none of the SNPs reached a significant value.

Table 4 20 SNPs significantly associated to LOAD risk. P-values adjusted by age and sex.

SNP/miRNA	Position	Genotype	N (%) Control	N (%) Case	p(Model)	p.Adj	Risk/Protection	miRNA targets with relevance to AD
rs174561 hsa-mir-1908	pre-miRNA	TT CT CC	53(42.7) 60 (48.4) 11 (8.9)	81 (60.4) 45 (33.6) 8 (6.0)	0.004357 Dominant	0.007478 Dominant	CT/CC Protection	APOE Validated target. ApoE expression levels are deregulated in AD and may be involved in the pathogenesis of the disesase (Pencheva et al., 2012)
rs74704964 hsa-mir-518d	pre-miRNA	CC CT TT	207 (95.4) 10 (4.6) 0 (0)	186 (88.2) 25 (11.8) 0 (0)	0.005584 Codominant	0.00627 Codominant	CT Risk	Not found
rs56292801 hsa-mir-5189	pre-miRNA	GG AG AA	82 (56.2) 44 (30.1) 20 (13.7)	123 (58.9) 75 (35.9) 11 (5.3)	0.006016 Recessive	0.00799 Recessive	AA Protection	Not found
rs71363366 hsa-mir-1283-2	pre-miRNA	CC CG GG	207 (95.0) 11 (5.0) 0 (0)	185 (87.7) 26 (12.3) 0 (0)	0.006592 Codominant	0.00772 Codominant	CG Risk	Not found
rs11983381 hsa-mir-4653	pre-miRNA	AA AG GG	163 (73.8) 56 (25.3) 2 (0.9)	132 (63.5) 67 (32.2) 9 (4.3)	0.006604 Log-additive	0.00724 Log-additive	G Risk	Not found
rs10934682 hsa-mir-544b	pre-miRNA	TT GT GG	162 (73.3) 56 (25.3) 3 (1.4)	130 (62.2) 71 (34.0) 8 (3.8)	0.007399 Log-additive	0.00466 Log-additive	G Risk	Not found
rs4809383 hsa-mir-941-1	pre-miRNA	CC CT TT	160 (73.4) 58 (26.6) 0 (0.0)	174 (82.9) 34 (16.2) 2 (1.0)	0.007930 Codominant	0.01048 Codominant	CT Protection	DNAJC5 Validated Target. Possible involvement in synapse lose in neurodegenerative diseases. (Hu et al., 2012)
rs6062431 hsa-mir-4326	pre-miRNA	GG CG CC	103 (47.2) 84 (38.5) 31 (14.2)	123 (58.0) 70 (33.0) 19 (9.0)	0.01551 Log-additive	0.01929 Log-additive	C Protection	Not found
rs243080 hsa-mir-4432	pre-miRNA	CC CT TT	65 (29.5) 116(52.7) 39 (17.7)	76 (36.5) 82 (39.4) 50 (24.0)	0.020723 Codominant	0.02664 Codominant	CT Protection	Not found
rs72996752 hsa-mir-4999	Mature	AA AG GG	136 (62.1) 73 (33.3) 10 (4.6)	108 (52.7) 79 (38.5) 18 (8.8)	0.02300 Log-additive	0.02723 Log-additive	G Risk	Not found
rs7522956 hsa-mir-4742	pre-miRNA	AA AC CC	135 (61.4) 75 (34.1) 10 (4.5)	115 (54.8) 74 (35.2) 21 (10.0)	0.02744 Recessive	0.01854 Recessive	CC Risk	Not found

(Continues)

SNP/miRNA	Position	Genotype	N (%) Control	N (%) Case	p(Model)	p.Adj	Risk/Protection	miRNA targets with relevance to AD
rs6726779 hsa-mir-4431	pre-miRNA	TT	86 (38.9)	75 (36.2)	0.02870	0.01878	CC Risk	Not found
		CT	110 (49.8)	93 (44.9)	Recessive	Recessive		
		CC	25 (11.3)	39 (18)				
rs112328520 hsa-mir-520g	pre-miRNA	CC	187 (85.4)	194 (91.9)	0.03112	0.04761	CT Protection	MMP2 Validated Target. Possible involvement in AD pathogenesis (Tsai et al., 2013).
		CT	32 (14.6)	17 (8.1)	Codominant	Codominant		
		TT	0 (0)	0 (0)				
rs1683709 hsa-mir-3612	pre-miRNA	CC	141 (63.8)	132 (62.6)	0.03138	0.02909	TT Risk	Not found
		CT	74 (33.5)	64 (30.3)	Recessive	Recessive		
		TT	6 (2.7)	15 (7.1)				
rs266435 hsa-mir-4804	In Seed	CC	174 (79.1)	152 (73.4)	0.03297	0.03909	GG Risk	Not found
		CG	43 (19.5)	45 (21.7)	Recessive	Recessive		
		GG	3 (1.4)	10 (4.8)				
rs61992671 hsa-mir-412	Mature	GG	73 (33.2)	50 (23.9)	0.03360	0.04565	AG/AA Risk	Several putative targets involved in neuronal cell-death processes (Melamed et al., 2013).
		AG	98 (44.5)	112 (53.6)	Dom	Dom		
		AA	49 (22.3)	47 (22.5)				
rs12894467 hsa-mir-300	pre-miRNA	CC	98 (44.5)	77 (37.4)	0.03601	0.04351	T Risk	Not found
		CT	102 (46.4)	97 (47.1)	Log-additive	Log-additive		
		TT	20 (9.1)	32 (15.5)				
rs13299349 hsa-mir-3152	Mature	GG	95 (43.4)	95 (45.7)	0.0428	0.04771	AA Protection	Not found
		AG	86 (39.3)	91 (43.8)	Recessive	Recessive		
		AA	38 (17.4)	22 (10.6)				
rs68035463 hsa-mir-3144	pre-miRNA	CC	148 (67.3)	124 (59.3)	0.04348	0.04043	A Risk	Not found
		AC	67 (30.5)	74 (35.4)	Log-additive	Log-additive		
		AA	5 (2.3)	11 (5.3)				
rs56103835 hsa-mir-323b	pre-miRNA	TT	157 (71.0)	133 (63.6)	0.04575	0.05043	C Risk	Not found
		CT	58 (26.2)	63 (30.1)	Log-additive	Log-additive		
		CC	6 (2.7)	13 (6.2)				

6. DISCUSSION

It has been demonstrated that neuronal-specific miRNAs are crucial to control neuronal differentiation, excitability and function (Maciotta et al., 2013). Several studies have reported deregulated levels of miRNA expression in AD, suggesting their involvement in the disease. Considering this, we conducted a systematic search to find all miRNAs that have been reported as deregulated. An interesting and intriguing observation resulting from comparing the different studies, was the identification of miRNAs that were found inversely altered. For example, miR-9 was reported as upregulated in the hipoccampus of AD patients in one study (Lukiw, 2007) whereas another study (Cogswell et al., 2008) found it downregulated in the same tissue. Such problematic discrepancy can be explained by differences in experimental technique, age, postmortem interval and specific part of the brain that is was sampled. In fact, the use of tissue homogenates, with diverse cell type composition, and various regions of the tissue at different stages of the disease, makes comparing the result of individual studies quite challenging. In spite of these discrepancies and at the light of the numerous deregulated miRNAs that have been reported, it thus appeared evident that they may be involved in AD pathogenesis.

Several studies have suggested that the expression level of the mature miRNA or its function can be compromised by the presence of SNPs within the pre-mature or mature region of the miRNA (Cai et al., 2009). Indeed, several miR-SNPs have been associated to risk for various diseases (Clop et al., 2006; Jiang et al., 2013). In reference to susceptibility for LOAD, most of the research has been focused on finding variants within protein coding regions or in their regulatory 3´UTR and 5´UTR regions; and only a couple of studies have evaluated the contribution of SNPs within the miRNAs to AD susceptibility. Moreover, these 2 studies are based on a candidate gene approach and therefore, they conducted the association study for only 3 SNPs located in 2 deregulated miRNAs: miR-124 (Qi et al., 2012) and miR-146a (Cui et al., 2014). Only 1 of the SNPs within the promoter region of miR-146a was associated to an increased risk for LOAD.

Therefore, at the light of the scarce number of association studies regarding miR-SNP and LOAD susceptibility, and the numerous miRNAs reported as deregulated in

AD, we decided to conduct a case-control study and evaluate the association of a wide range of miR-SNPs.

We looked for all the SNPs experimentally validated and with a MAF greater than 1% in Caucasian population, located in the miRNAs that were reported as deregulated in AD. We found that only 10 SNPs present in 10 miRNAs met these criterion. Considering that SNPs located in the seed region of mature miRNAs may affect their functionality but produce no change in the mature miRNA expression level and the fact that the expression of the mature miRNA can be altered in such a subtle way that it is not detected by conventional microarray expression assays, we decided to conduct an association study for all the SNPs in pre-miRNAs (MAF greater than 1% in Caucasian population), regardless if they had been reported as deregulated or not (213 SNPs in 206 miRNAs). To our knowledge this is the first study to conduct association analyses for all miR-SNPs (MAF greater that 1% in Caucasian population) in relation to AD susceptibility.

We found 20 significant SNPs associated to risk for LOAD using a sample of 212 patients and 220 healthy controls. Despite the fact that after FDR correction none of them reached a significant level, this result could be explained by the fact that a larger sample size is needed to obtain lower p-values.

We also found that among the 20 significant SNPs, three of them (miR-1908, miR-941-1, and miR-520g) had a validated target that could be involved in AD pathogenesis.

The first of them, hsa-miR-1908, has the SNP rs174561 which is the most significant finding in our study. The genotypes CT and CC rs174561 showed 0.49-fold decreased risk for LOAD. We predicted in silico the secondary structure of the pre-hsa-miR-1908- rs174561 and observed that the presence of the C allele resulted in a subtle change in the structure. Therefore, we speculate that the SNP rs174561 might influence the stability of the pre-miR or the efficiency of processing it into the mature miRNA and as a consequence, it would cause alterations in the level of mature miRNA.

In regard to miR-1908 deregulation, it has been already reported that it is over expressed in human melanoma metastasis and that it targets protein coding gene ApoE (Pencheva et al., 2012). Of interest in this study is the ApoE target validation through a luciferase assay, since one of the alleles of this protein coding gene constitutes the strongest genetic risk factor for LOAD that has been described to date (Bertram et al., 2007). There are three common alleles of ApoE: Apoε2 (rs42958), Apoε3 (wyld-type), and Apoε4 (rs7412), being the Apoε4 the risk allele and Apoε2 the protective allele (Corder et al., 1993). Apoε4 cannot be associated to the cause of the disease since some individuals homozygous for Apoε4 never develop AD. Evidence suggests that ApoE protein plays a key role in the pathogenesis of AD.

In spite of the fact that the majority of the risk at the locus has been associated with the different isoforms of ApoE, there has been ongoing research about whether other factors, such as the variability in ApoE expression, may also contribute to AD risk. In fact, ApoE mRNA and protein levels have been shown to be elevated in brains of Alzheimer's subjects (Yamagata et al., 2001). Of note is that ApoE mRNA in an AD group with ApoE ε4 alleles was significantly higher than that in the control group with ApoE ε4 alleles. This suggests that a higher level of expression in carriers of ApoE ε4 allele may play an important role in the development of LOAD. Other expression analysis suggest that ApoE ε4 /ApoE ε3 heterozygotes with a high ApoE ε4 /ApoE ε3 expression ratio are at higher risk than those with a low ratio (Lambert et al., 1997). Therefore, there is evidence to suggest that the deregulation of ApoE levels may be involved in the risk to LOAD. Interestingly, some recent studies suggest that decreasing human APOE levels regardless of isoform or age at treatment may be a good therapeutic approach (Bien-Ly et al., 2012).

Our study showed an association between SNP rs174561 in mir-1908 and LOAD decrease in risk. We suggest that upregulation in the expression level of this miRNA because of the SNP, could cause the reduction of ApoE expression leading to a decreased risk to LOAD.

Nevertheless, some potential limitations of our study should be addressed. First of all, mir-1908 has not been reported as deregulated in AD. This could be due to the fact that the SNP produces a very slight alteration in the expression and that the actual technologies are unable to detect. For this reason, further studies thoroughly

evaluating the expression levels of miR-1908 in the brain are required. Moreover, despite we predicted that the SNP produces a change in the secondary structure of miR-1908, it should be validated that it causes a variation in the miRNA expression levels. Lastly, the target of miR-1908, APOE, has only been validated in human melanoma cell lines (Pencheva et al., 2012) and therefore it is necessary to do likewise for brain cell lines.

A second miRNA with a significant SNP and a validated target involved in AD pathogenesis was miR-941-1, which is highly expressed in human brain (Hu et al., 2012). In our study we associated the SNP rs4809383 located in pre-miR-941 to a decreased risk for LOAD. It has been demonstrated that one of the miR-941 target genes is DNAJC5 (Hu et al., 2012) which codes for Cysteine String Protein (CSPα). This protein has been shown to have an essential anti-neurodegenerative role, by maintaining the synapse mechanism between neurons (Johnson et al., 2010). Interestingly, synapse loss is a common feature of neurodegenerative diseases such as Alzheimer's disease (Selkoe, 2002) and CSPα expression in humans with Alzheimer's disease is decreased (Zhang et al., 2012). In view of this, we speculate that a decreased in level in mir-941, as a consequence of carrying the protective genotype (CT) could augment the expression of CSPα, thus enhancing its anti-neurodegenerative functions in human brain. Nevertheless, further functional studies are necessary to validate the correlation between the presence of the protective SNP, the decreased mir-941 levels in AD brain and the improvement in the synapse mechanism.

The third and last miRNA with a validated target involved in AD pathogenesis is hsa-miR-520g. We detected a significant association between SNP rs112328520 located in its pre-mature region and decreased in risk to LOAD. A recent study has shown that gene MMP2 presents a target site for miR-520g (Tsai et al., 2013). Furthermore, growing evidence shows the involvement of matrix metalloproteinases (MMPs) in neurodegeneration processes. In this regard, it has been demonstrated that MMPs can degrade Aβ peptides and play important roles in the extracellular Aβ catabolism and clearance (Lim et al., 2011). The expression of MMP2 levels in plasma are decreased in AD patients compared to controls (Lim et al., 2011). For this reason, we speculate that high expression levels of MMP2 may contribute to

protection for the disease. Given that hsa-miR-520g is capable of regulating the expression of its target gene MMP2, we suggest that the protective T allele in hsa-miR-520g may cause a decrease in miR-520g expression levels and in turn, the MMP2 levels would be increased. We speculate that this could lead to a higher efficiency at Aβ peptides degradation.

Another relevant result was the association of rs61992671 in mature has-miR-412 to augmented risk for LOAD. It has been demonstrated that miR-412 is active in neuronal cells and that four putative miR-412 targets are integrated in a molecular network of proteins that are involved in neuronal cell death processes (Melamed et al., 2013). In fact, it has been demonstrated that in embryonic tissue the levels of these targets are significantly reduced in comparison to adult tissues. This suggests that the dysfunction of miR-412 may cause an increase of its cell-death related target genes, leading to neuronal degeneration, which is a pathological characteristic of AD. Therefore, we suggest that the presence of the SNP rs61992671 in mature miR-412 may be a risk factor of AD by causing the acceleration of neuron degeneration.

Of interest was the evaluation of the SNP rs2910164 in miR-146a. It has been experimentally proven that the presence of the SNP affects the expression level of miR-146a. Furthermore this variant has been associated with risk to several immune and inflammatory-related diseases such as papillary thyroid carcinoma (Jazdezewski et al., 2008), Behcet´s disease (Zhou et al., 2014), ulcerative colitis (Okubo et al., 2011), adult glioma (Permuth-Wey et al., 2011) and prostate cancer (Xu et al., 2010). Nevertheless, in our study we did not find association between this variant and susceptibility to LOAD. Our results are consistent with that of Cui et al. (2014) who also studied this variant in Chinese population. Therefore, it can be suggested that miR-146a- rs2910164 may not represent a risk factor in AD susceptibility.

In summary, this is the first study to explore the relationship between all SNPs within pre-miRNAs and LOAD susceptibility. We identified significant association between 20 miR-SNPs and risk for LOAD.

6. CONCLUSION

Polymorphisms within pre- and mature miRNAs may be markers of risk for LOAD and could be useful to determine genetic predisposition to develop the disease. In this study we have identified 20 significant SNPs associated with risk to LOAD. Remarkably, 4 of the miRNAs with significant SNPs (miR-1908, miR-941-1, miR-520g, miR-412and) had a validated or putative target that can be involved in AD pathogenesis. Further research is needed to reproduce the case-control study in a larger cohort and to experimentally validate our findings.

7. APPENDICES

APPENDIX 1

Table 1. List of miRNA deregulated in LOAD tissues reported in the 37 studies. Abbreviations for alterations (Alt): Upregulated (UP), Downregulated (DW), Not significant (NS). Abbreviations for tissue sample: Hippocapus (HC); In the cerebral cortex: frontal cortex (FC), temporal cortex (TC), and parietal cortex (PC); within the FC some studies focus on prefrontal cortex (PFC), within the TC some studies focus on the anterior temporal cortex (ATC) and superior temporal cortex (STC); in the neocortical brain (NB), some focus on the temporal neocortex (TN) or the superior temporal neocortex (STN); cerebellum (CB); Medial frontal gyrus (MFG); Blood serum sample (BSS); Blood (BL) ; plasma (PM) ; white blood cell (WBC); Cerebrospinal fluid (CSF). Abbreviations for method: Quantitative Real Time Polimerase Chain Reaction (q-RT-PCR), MicroArray, (MA), Northern Blot (N-blot), Next Generation Sequencing, (NGS), Illumina, (IL). Abbreviations for sample, P= Patients, C= Controls; B= Braak Stage of the sample, this is common method used to classify the degree of pathology in AD.

miRNA family	miRNA	Alt.	Tissue	Sample	Method	Ref.
miR-29	**miR-29a**					
		UP/NS	CSF/PM	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			FC	P=7 ; C=4	q-RT-PCR	[Shioya et al., 2010]
			NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
			BSS	P=7 ; C=7	q-RT-PCR	[Geekiyanage et al., 2011]
		NS	BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
			TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-29a-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	mir-29b-1					
		DW	NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
			ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
	miR-29b					
		UP/NS	CSF/ PM	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
			BL	P= 287 ; C=344	q-RT-PCR	[Villa et al., 2013]
			BSS	P=7 ; C=7	q-RT-PCR	[Geekiyanage et al., 2011]
		NS	BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
			TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-29b-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-29c					
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
	miR-29c-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-9	**miR-9**					
		UP	HC	P= 5 ; C=5	N-Blot	[Lukiw, 2007]
			TC	P=6 ; C=13	MA,N-blot	[Sethi ad Lukiw, 2009]
			BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			HC and MFG	P=10 (B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
			BSS	P=7 ; C=7	q-RT-PCR	[Geekiyanage et al., 2011]
		NS	PM	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
			NB or HC	P=23 ; C=23	MA,N-blot	[Lukiw et al., 2008]
			TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
			HC	P=20 (B3 + B4; B6) ; C=11	q-RT-PCR	[Müller et al., 2013]
miR-128	**miR-128**					
		UP	HC	P= 5 ; C=5	N-Blot	[Lukiw, 2007]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
			BL	P= 34 ; C=37	q-RT-PCR	[Tiribuzi et al., 2013]
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	miR-128a					
		DW	HC	P=20 (B6) ; C=11	q-RT-PCR	[Müller et al., 2013]
			TN	P= 12 ; C= 6	q-RT-PCR	[Culpan et al., 2011]
	miR-128b					
		DW	TN, FC	P= 12 ; C= 6	q-RT-PCR	[Culpan et al., 2011]
miR-124	**miR-124**					
		DW	ATC	P=11 ; C=11	q-RT-PCR	[Smith et al., 2011]
			NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
		NS	FC	P= 14/15 ; C=5	q-RT-PCR	[Long et al., 2014]

	miR-124-3p					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-137	**miR-137**					
		DW	NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
			BSS	P=7 ; C=7	q-RT-PCR	[Geekiyanage et al., 2011]
miR-146	**miR-146a**					
		UP	NB and HC	P=23 ; C=23	MA,N-blot	[Lukiw et al., 2008]
			HC, STC	P=36 ; C=30	MA,N-blot	[Cui et al., 2010]
			TC	P=6 ; C=13	MA,N-blot	[Sethi ad Lukiw, 2009]
			STN and HC	P= 6 ; C=6	N-Blot	[Li et al., 2011]
			HC	P= 20 (B.3 + B.4) ; C=11	q-RT-PCR	[Müller et al., 2013]
		DW	PM and CSF	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
			CSF	P= 15 ; C=20	q-RT-PCR	[Müller et al., 2013]
			HC	P= 20 (B.6) ; C=11	q-RT-PCR	[Müller et al., 2013]
	miR-146b					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			HC and MFG	P=20 (10 B.3 +B.4; 10 B5 +B6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-107/103	**miR-107**					
		DW	TC	P= 6 ; C= 11	MA,N-blot, ISH	[Wang et al., 2008]
			HC	P=20 (B.6) ; C=11	q-RT-PCR	[Müller et al., 2013]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
		NS	TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-103					
		NS	TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-103a-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-15	**miR-15**					
miR16		DW	NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
miR-195	**miR-15b**					
miR-497		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-15b-5p					
		DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
	miR-15a					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
			ATC	P= 8 ; C=10	q-RT-PCR	[Hérbert et al., 2010]
	miR-15a-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-16					
		DW	HC	P= 20 (B.6) ; C=11	q-RT-PCR	[Müller et al., 2013]
		UP	HC	P= 20 (B.3 + B.4) ; C=11	q-RT-PCR	[Müller et al., 2013]
		NS	CSF	P= 18 ; C=20	q-RT-PCR	[Müller et al., 2013]
	miR-16-2-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-195					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		NS	ATC	P= 8 ; C=10	q-RT-PCR	[Hérbert et al., 2010]
	miR-195-5p					
		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	miR-497					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-19	**miR-19b**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
miR-22	**miR-22**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
miR-26	**miR-26b**					
		UP	TC	P=6 ; C=6	q-RT-PCR	[Absalon et al., 2013]
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
	miR-26b-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-26b-5p					
		DW	BL	P= 48 ; C= 22	NGS, q-RT-PCR	[Leidinger et al., 2013]
miR 93	**miR-93**					
miR-105		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
miR-106	**miR-105**					
miR-302		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-520	**miR-106b**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			ATC	P= 19 ; C=11	q-RT-PCR	[Hérbert et al., 2009]
	miR-106b-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-106b-5p					

		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-302b					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-520a*					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-101	**miR-101**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
		NS	BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
	miR-101-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-181	**miR-181c**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
			CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			NB	P=7 ; C=7	q-RT-PCR	[Geekiyanage and Chan, 2011]
			BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
			BSS	P=7 ; C=7	q-RT-PCR	[Geekiyanage et al., 2011]
		NS	TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-181a					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-181a-2-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-181b					
		UP	WBC	P=16 ; C=16	MA. q-RT-PCR	[Schipper et al., 2007]
miR-210	**miR-210**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			HC	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-363	**miR-363**					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
	miR-363-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
			HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-197	**miR-197**					
		UP	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-320	**miR-320**					
		UP	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
			PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR132 miR-212	**miR-132**					
		DW	HC	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			TC	P=30(14 B.3 / B.4) + (16 B.6) ; C=16	q-RT-PCR	[Wong et al., 2013]
		NS	HC, STC	P=36 ; C=30	MA,N-blot	[Cui et al., 2010]
			NB or HC	P=23 ; C=23	MA,N-blot	[Lukiw et al., 2008]
	miR-132-3p					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
			TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-212					
		DW	HC and MFG	P=10 (B.5 +B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			TC	P=30(14 B.3 / B.4) + (16 B.6) ; C=16	q-RT-PCR	[Wong et al., 2013]
miR-425	**miR-425**					
		DW	HC	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-425-5p					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
let-7/98	**miR-98**					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7c					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7i					
		DW	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
	let-7i-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]

		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	let-7d-5p					
		DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7d-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7g-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
			PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
	let-7f					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		NS	HC	P=20 (B3 + B4; B6) ; C=11	q-RT-PCR	[Müller et al., 2013]
	let-7f-1-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7f-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	let-7a/b					
		NS	TN or FC	P= 12 ; C= 6	q-RT-PCR	[Culpan et al., 2011]
	let-7b-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7b-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7a-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	let-7e-5p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-206	**miR-206**					
		UP	TC	P= 4 ; C=4	RT-PCR	[Lee et al., 2012]
miR-26	**miR-26a**					
		UP	HC	P=10 (B.3+B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		DW	MFG	P=10 (B.5 +B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		NS	TC	P=6 ; C=6	q-RT-PCR	[Absalon et al., 2013]
	miR-26a-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-27	**miR-27a**					
		UP	HC and MFG	P=10 (B.3 +B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-27a/b					
		NS	TN or FNC	P= 12 ; C= 6	q-RT-PCR	[Culpan et al., 2011]
	miR-27b					
		UP	HC	P= 20(10 B.3+B.4; 10 B.5 + B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-27a-3p					
		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
		DW	CSF	P=20 ; C=19 and P= 15 ; C=18	q-RT-PCR	[Sala Frigerio et al., 2013]
miR-30	**miR-30e-5p**					
		UP	HC	P= 10(B.3+B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		UP	MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
	miR-30e-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-30c					
		DW / UP	HC / MFG	P=10 (B.3 +B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-30c-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-30b					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-30b-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-30d					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-30d-5p					
	miR-30a-3p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
			CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-30a-5p					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-30c					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]

miR-34	**miR-34a**					
		UP	HC and MFG	P= 10(B.3+B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			WBC	P=16 ; C=16	MA. q-RT-PCR	[Schipper et al., 2007]
			PM and CSF	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
		NS	HC	P=20 (B3 + B4; B6) ; C=11	q-RT-PCR	[Müller et al., 2013]
	miR-34c					
		UP	HC	P=6 ; C=8	q-RT-PCR	[Zovoilis et al., 2011]
			HC	P=20 (B3 + B4) ; C=11	q-RT-PCR	[Müller et al., 2013]
miR-125	**miR-125b**					
		UP	HC	P= 10(B.3+B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 20(10 B.3+B.4; 10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			TC	P=6 ; C=13	MA,N-blot	[Sethi ad Lukiw, 2009]
			HC	P= 5 ; C=5	N-Blot	[Lukiw, 2007]
		DW	BBS	P=105 ; C=150	q-RT-PCR	[Tan et al., 2014b]
		DW/NS	CSF/PM	P= 10 ; C=10	q-RT-PCR	[Kiko et al., 2014]
	miR-125a					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-125a-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-145	**miR-145**					
		UP	HC	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-381	**miR-381**					
		UP	HC	P= 10(B.3+B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-100 miR-99	**miR-100**					
		UP	MFG	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		DW	TNC	P= 8 ; C= 8	q-RT-PCR	[Hérbert et al., 2013]
	miR-99a					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-99b-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-20	**miR-20b**					
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-374	**miR-374**					
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
		UP	CSF	P=1O ; C=1O	q-RT-PCR	[Cogswell et al., 2008]
miR-582	**miR-582**					
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-95	**miR-95**					
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-148	**miR-148b**					
		UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
	miR-148b-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-148b-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-148a					
		UP	MFG	P= 20(10 B.3+B.4;10 B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-148a-5p					
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-148a-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-216	**miR-216**					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-129	**miR-129-5p**					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	miR-129-2-3p					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-143	**miR-143**					
		DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-143-3p					
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-138	**miR-138**					
		UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
			HC	P= 5 ; C=5	N-Blot	[Lukiw, 2007]
	miR-138-5p					
		DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]

miR-135	**miR-135a**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-126	**miR-126**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-126*	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-126-3p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-126-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-221	**miR-221**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-221-3p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-487	**miR-487a**		HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	miR-487b	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-214	**miR-214**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-451	**miR-451**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-422	**miR-422a**	UP	HC and MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-422b	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-320	**miR-32**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-194	**miR-194**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-339	**miR-339-5p**	DW	FC	P= 14/15 ; C=5	q-RT-PCR	[Long et al., 2014]
	miR-339-3p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-142	**miR-142-5p**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-142-3p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
		DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
miR-186	**miR-186**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-186-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-412	**miR-412**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-191	**miR-191**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-191-5p	DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
miR-154	**miR-154**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-155	**miR-155**	NS	TN or FNC	P= 12 ; C= 6	q-RT-PCR	[Culpan et al., 2011]
miR-511	**miR-511**	UP	ATC	P= 5 ; C=5	MA, q-RT-PCR	[Hérbert et al., 2008]
miR-10	**miR-10a**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-10a-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-10b	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-10b-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-127	**miR-127**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-127-3p	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-141	**miR-141**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-151	**miR-151**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-151a-3p					

		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-199	**miR-199a***	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-199a-3p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
	miR-199b-3p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
		UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-204	**miR-204**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miIR-205	**miR-205**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-338	**miR-338**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-338-3p	DW	FC	P=7 ; C=4	q-RT-PCR	[Shioya et al., 2010]
miR-345	**miR-345**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-345-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-362	**miR-362**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-362-3p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-371	**miR-371**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-371b-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-375	**miR-375**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-380	**miR-380-3p**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-429	**miR-429**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-448	**miR-448**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-445	**miR-455**	DW	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-455-5p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-494	**miR-494**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
		DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-501	**miR-501**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-517	**miR-517a**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-517b	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-518	**miR-518b**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-518f	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-526	**miR-526a**	UP	CSF	P=10 ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-200	**miR-200c**	UP / DW	HC / MFG	P=10 (B.3 +B.4) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-200a-3p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-423	**miR-423**	UP	HC and MFG	P= 10(B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
miR-92	**miR-92**	UP	HC and MFG	P=10 (B.5+B.6) ; C=10	q-RT-PCR	[Cogswell et al., 2008]
	miR-92b-3p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-30184	**miR-30184**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-617	**miR-617**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-188	**miR-188**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]

			PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-06383	**miR-06383**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-10912	**miR-10912**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-20546	**miR-20546**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-601	**miR-601**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-23974	**miR-23974**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-08570	**miR-08570**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-10939	**miR-10939**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-42448	**miR-42448**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-19790	**miR-19790**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-44608	**miR-44608**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-02532	**miR-02532**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-35456	**miR-35456**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-12497	**miR-12497**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-134	**miR-134**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-671	**miR-671**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-575	**miR-575**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-05109	**miR-05109**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-572	**miR-572**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-45605	**miR-45605**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-130a	**miR-130a**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-765	**miR-765**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-598	**miR-598**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-376	**miR-376a**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-12504	**miR-12504**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-18895	**miR-18895**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-432	**miR-432**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-382	**miR-382**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-185	**miR-185**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
	miR-185-3p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-486	**miR-486**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-28648	**miR-28648**	UP	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-368	**miR-368**	DW	PLC	P=4 ; C=4	MA	[Nunez-Iglesias et al., 2010]
miR-485	**miR-485-5p**	DW	EC and HC	P= 35 ; C=35	q-RT-PCR	[Faghihi et al., 2010]
		NS	CB or SFG	P= 35 ; C=35	q-RT-PCR	[Faghihi et al., 2010]
miR-153	**miR-153**	DW	FC	P=5-10 ; C=5-10	q-RT-PCR	[Long et al., 2012]
miR-301	**miR-301a-3p**	DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
			BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]

miR-545	**miR-545-3p**	DW	PM	P=11; C=20.	q-RT-PCR	[Kumar et al., 2013]
miR-590	**miR-590-3p**	NS	BL	P=287 ; C=344	q-RT-PCR	[Villa et al., 2011]
miR-23	**miR-23**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-23a-3p	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-370	**miR-370**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-223	**miR-223-3p**	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
		DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-433	**miR-433**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-150-5p	**miR-150-5p**	UP	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-136	**miR-136-5p**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-329	**miR-329**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-495	**miR-495-3p**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-409	**miR-409-5p**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-410	**miR-410**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-543	**miR-543**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-769	**miR-769-5p**	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
mir-219	**mir-219**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-219-2-3p	DW	HC	P=41 ; C=23	RT-PCR and IL	[Lau et al., 2013]
miR-4781	**miR-4781-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
mir-112	**mir-112**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3157	**miR-3157-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-5001	**miR-5001-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
mir-431	**mir-431**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-550	**miR-550a-3-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-550a-5p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-1285	**miR-1285-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-361	**miR-361-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-53	**miR-53**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-625	**miR-625-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-4659a	**miR-4659a-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3127	**miR-3127-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-5010	**miR-5010-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-589	**miR-589-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-330	**miR-330-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-330-3p	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3909	**miR-3909**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-340	**miR-340-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]

	miR-340-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-5690	**miR-5690**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-28	**miR-28-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-378	**miR-378a-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-378d	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-378g	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-378f	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-2110	**miR-2110**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3074	**miR-3074-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
mir-293	**mir-293**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-1468	**miR-1468**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-161	**miR-161**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-505	**miR-505-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-189	**miR-189**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-190	**miR-190**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-190a	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-576	**miR-576-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-4746	**miR-4746-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-641	**miR-641**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-484	**miR-484**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-403	**miR-403**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-4435	**miR-4435**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-654	**miR-654-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-4742	**miR-4742-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-182	**miR-182**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-328	**miR-328**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-1303	**miR-1303**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3158	**miR-3158-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-323	**miR-323b-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-4755	**miR-4755-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-5094	**miR-5094**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-644	**miR-644b-3p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-421	**miR-421**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-413	**miR-413**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-139	**miR-139-5p**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-942	**miR-942**	UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-3605	**miR-3605-3p**					

		UP	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-144	**miR-144-3p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-144-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-17	**miR-17-3p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-660	**miR-660-5p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-1294	**miR-1294**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-548	**miR-548h-5p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548aj-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548ar-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548g-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548x-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548av-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548k	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548am-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548au-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548c-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
	miR-548o-5p	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-152	**miR-152**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-532	**miR-532-5p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-628	**miR-628-3p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-21	**miR-21-5p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]
miR-33	**miR-33b-5p**	DW	BL	P= 48 ; C= 22	NGS	[Leidinger et al., 2013]

APPENDIX 2

Table 1. List of the SNPs genotyped in each miRNA and the genotyping success for each SNP. 213 SNPs in 206 miRNAs were genotyped in a sample of 212 AD patients and 220 controls. 61 SNPs were removed from the association study due to genotyping failures (50) or deviation from HWE (11). Minor Allele Frequency (MAF).

	SNP	Alleles	miRNA	Genotyping Sucess (%)	MAF	Reason of exclusion
1	rs10061133	A:G	hsa-mir-449b	98.8	0.085	
2	rs10173558	T:C	mir-1302-4	100.0	0.128	
3	rs10406069	G:A	hsa-mir-5196	98.6	0.204	
4	rs10422347	C:T	hsa-mir-4745	97.0	0.077	
5	rs10461441	T:T	hsa-mir-548ae-2	0.0	0.0	Genotyping failure
6	rs10505168	A:G	hsa-mir-2053	98.4	0.282	
7	rs1055070	T:G	hsa-mir-4700	99.5	0.068	
8	rs1077020	T:T	hsa-mir-943	0.0	0.0	Genotyping failure
9	rs10878362	T:T	hsa-mir-6074	0.0	0.0	Genotyping failure
10	rs10934682	T:G	hsa-mir-544b	99.3	0.173	
11	rs11014002	T:T	hsa-mir-603	0.0	0.0	Genotyping failure
12	rs11032942	T:T	hsa-mir-1343	0.0	0.0	
13	rs11156654	T:A	hsa-mir-624	99.1	0.224	
14	rs11237828	T:T	hsa-mir-5579	0.0	0.0	Genotyping failure
15	rs11259096	T:C	hsa-mir-1265	99.3	0.059	
16	rs11614913	C:T	hsa-mir-196a-2	99.5	0.37	
17	rs11651671	T:T	hsa-mir-548at	0.0	0.0	Genotyping failure
18	rs11713052	C:G	hsa-mir-5092	100.0	0.036	
19	rs11714172	T:G	hsa-mir-4792	99.3	0.374	
20	rs11907020	T:C	hsa-mir-3192	99.3	0.019	
21	rs11983381	A:G	hsa-mir-4653	99.1	0.169	
22	rs12197631	T:T	hsa-mir-548a-1	0.0	0.0	Genotyping failure
23	rs12355840	T:C	hsa-mir-202	99.3	0.205	Not in HWE
24	rs12402181	G:A	hsa-mir-3117	99.8	0.13	
25	rs12451747	T:T	hsa-mir-1269b	0.0	0.0	Genotyping failure
26	rs12456845	T:C	hsa-mir-4744	99.1	0.024	
27	rs12473206	T:T	hsa-mir-4433	0.0	0.0	Genotyping failure
28	rs12512664	A:G	hsa-mir-4274	98.8	0.461	
29	rs12523324	T:T	hsa-mir-4277	0.0	0.0	Genotyping failure
30	rs12780876	T:A	hsa-mir-4293	98.6	0.256	
31	rs12803915	G:A	hsa-mir-612	98.8	0.18	
32	rs12879262	G:C	hsa-mir-4308	99.3	0.157	
33	rs12894467	C:T	hsa-mir-300	98.4	0.356	
34	rs13186787	T:T	hsa-mir-1294	0.0	0.0	Genotyping failure
35	rs13299349	G:A	hsa-mir-3152	98.6	0.348	Not in HWE
36	rs1414273	T:T	hsa-mir-548ac	0.0	0.0	Genotyping failure
37	rs1439619	C:A	hsa-mir-3175	98.6	0.482	
38	rs1572687	C:T	hsa-mir-5007	98.6	0.446	
39	rs1683709	C:T	hsa-mir-3612	99.8	0.208	
40	rs17022749	T:T	hsa-mir-5700	0.0	0.0	Genotyping failure
41	rs17091403	C:T	hsa-mir-2110	98.8	0.088	
42	rs17111728	T:C	hsa-mir-4422	99.3	0.064	
43	rs174561	T:C	mir-1908	59.6	0.277	
44	rs17737028	A:G	hsa-mir-3143	99.8	0.0070	
45	rs17759989	A:G	hsa-mir-633	99.5	0.013	

46	rs17797090	G:A	hsa-mir-3652	99.8	0.105	
47	rs17885221	C:T	hsa-mir-4733	99.1	0.061	
48	rs2042253	A:G	hsa-mir-5197	99.1	0.247	
49	rs2043556	A:G	hsa-mir-605	98.2	0.236	
50	rs2060455	T:T	hsa-mir-4511	0.0	0.0	Genotyping failure
51	rs2070960	C:T	hsa-mir-3620	99.3	0.047	
52	rs2114358	T:C	hsa-mir-1206	99.3	0.438	
53	rs215383	G:A	hsa-mir-1206	98.2	0.182	
54	rs2241347	T:T	hsa-mir-3130-1	0.0	0.0	Genotyping failure
55	rs2273626	A:C	hsa-mir-4707	81.8	0.479	
56	rs2289030	C:G	hsa-mir-492	100.0	0.075	
57	rs2291418	C:T	hsa-mir-1229	99.5	0.035	
58	rs2292181	G:C	hsa-mir-564	98.6	0.059	
59	rs2292832	T:T	hsa-mir-149	0.0	0.0	Genotyping failure
60	rs2368392	C:T	hsa-mir-604	99.3	0.252	
61	rs243080	C:T	hsa-mir-4432	98.8	0.439	
62	rs257095	A:G	hsa-mir-4636	99.3	0.133	
63	rs2648841	C:A	hsa-mir-1208	99.3	0.127	
64	rs2663345	T:T	hsa-mir-3183	0.0	0.0	Genotyping failure
65	rs266435	C:G	hsa-mir-4804	98.6	0.133	
66	rs2682818	C:A	hsa-mir-6128	99.1	0.142	
67	rs28477407	C:T	hsa-mir-4308	98.6	0.103	
68	rs28645567	G:A	hsa-mir-378d-1	99.5	0.019	
69	rs28655823	G:C	hsa-mir-4472-1	76.7	0.108	
70	rs28664200	T:C	hsa-mir-1255a	96.5	0.237	
71	rs2910164	G:C	hsa-mir-146a	100.0	0.251	
72	rs2967897	G:G	hsa-mir-5695	99.8	0.0	
73	rs3112399	T:A	hsa-mir-4803	99.1	0.477	Not in HWE
74	rs34115976	C:G	hsa-mir-577	99.1	0.186	Not in HWE
75	rs35196866	T:T	hsa-mir-4669	0.0	0.0	Genotyping failure
76	rs356125	G:A	hsa-mir-2278	100.0	0.04	
77	rs35613341	C:G	hsa-mir-5189	99.1	0.318	Not in HWE
78	rs35650931	G:C	hsa-mir-6076	100.0	0.087	
79	rs35770269	A:T	hsa-mir-449c	99.1	0.334	
80	rs35854553	A:T	hsa-mir-3166	98.6	0.078	
81	rs367805	G:A	hsa-mir-3936	99.3	0.307	
82	rs3734050	C:T	hsa-mir-6499	99.3	0.047	
83	rs3746444	T:C	hsa-mir-499a	98.4	0.207	
84	rs3823658	G:A	hsa-mir-5090	99.8	0.141	Not in HWE
85	rs4112253	C:G	hsa-mir-4751	99.1	0.352	
86	rs41274239	A:G	hsa-mir-96	99.5	0.0020	
87	rs41274312	G:A	hsa-mir-187	99.3	0.0090	
88	rs41286570	G:G	hsa-mir-154	100.0	0.0	
89	rs41291179	A:T	hsa-mir-216a	100.0	0.057	
90	rs41292412	C:T	hsa-mir-122	100.0	0.0030	
91	rs4285314	T:T	hsa-mir-3135b	0.0	0.0	Genotyping failure
92	rs4414449	T:C	hsa-mir-548ap	79.2	0.388	
93	rs45530340	C:C	hsa-mir-6084	99.8	0.0	
94	rs4577031	A:T	hsa-mir-548ap	99.3	0.376	
95	rs4674470	T:C	hsa-mir-548at	99.8	0.212	
96	rs4809383	C:T	hsa-mir-941-1	98.8	0.112	
97	rs4822739	C:G	hsa-mir-548j	100.0	0.066	
98	rs487571	T:T	hsa-mir-5680	0.0	0.0	Genotyping failure
99	rs4909237	C:T	hsa-mir-595	99.3	0.169	
100	rs4919510	C:G	hsa-mir-608	99.3	0.199	

101	rs515924	A:G	hsa-mir-548al	98.2	0.079	
102	rs521188	A:G	hsa-mir-3671	99.5	0.041	
103	rs56088671	T:T	hsa-mir-4424	0.0	0.0	Genotyping failure
104	rs56103835	T:C	hsa-mir-323b	99.3	0.185	
105	rs56195815	T:T	hsa-mir-548aw	0.0	0.0	Genotyping failure
106	rs56292801	G:A	hsa-mir-5189	82.0	0.255	Not in HWE
107	rs57111412	T:T	hsa-mir-1283-1	0.0	0.0	Genotyping failure
108	rs58450758	T:T	hsa-mir-559	0.0	0.0	Genotyping failure
109	rs58834075	C:T	hsa-mir-656	99.5	0.015	
110	rs5965660	T:G	hsa-mir-888	98.8	0.178	Not in HWE
111	rs5997893	G:A	hsa-mir-3928	98.8	0.275	Not in HWE
112	rs60308683	T:T	hsa-mir-4762	0.0	0.0	Genotyping failure
113	rs6062431	G:C	hsa-mir-4326	99.3	0.295	Not in HWE
114	rs60871950	A:G	hsa-mir-4467	98.2	0.488	
115	rs61388742	T:C	hsa-mir-596	99.8	0.104	
116	rs61938575	G:A	hsa-mir-3922	98.8	0.266	
117	rs61992671	G:A	hsa-mir-412	99.1	0.469	
118	rs62154973	C:T	hsa-mir-4772	99.1	0.094	
119	rs62376935	C:T	hsa-mir-585	99.1	0.051	
120	rs641071	T:T	hsa-mir-4482	0.0	0.0	Genotyping failure
121	rs6430498	G:A	hsa-mir-3679	98.8	0.343	
122	rs6505162	T:T	hsa-mir-423	0.0	0.0	Genotyping failure
123	rs6513496	T:C	hsa-mir-646	99.8	0.193	
124	rs66507245	T:T	hsa-mir-4731	0.0	0.0	Genotyping failure
125	rs66683138	T:T	hsa-mir-3622a	0.0	0.0	Genotyping failure
126	rs67042258	G:A	hsa-mir-6128	98.6	0.248	
127	rs670637	T:T	hsa-mir-3167	91.9	0.0	
128	rs67182313	A:G	hsa-mir-4642	99.8	0.171	
129	rs6726779	T:C	hsa-mir-4431	98.8	0.387	
130	rs67339585	T:T	hsa-mir-3908	0.0	0.0	Genotyping failure
131	rs6787734	T:T	hsa-mir-3135a	0.0	0.0	Genotyping failure
132	rs67976778	T:T	hsa-mir-4305	0.0	0.0	Genotyping failure
133	rs68035463	C:A	hsa-mir-3144	99.1	0.202	
134	rs6841938	T:T	hsa-mir-1255b-1	0.0	0.0	Genotyping failure
135	rs6977967	A:G	hsa-mir-3683	99.5	0.216	
136	rs6997249	T:T	hsa-mir-3686	0.0	0.0	Genotyping failure
137	rs701213	T:T	hsa-mir-4427	0.0	0.0	Genotyping failure
138	rs702742	A:G	hsa-mir-378h	99.1	0.129	
139	rs7070684	T:T	hsa-mir-548aj-2	0.0	0.0	Genotyping failure
140	rs71363366	C:G	hsa-mir-1283-2	99.1	0.043	
141	rs7205289	C:C	hsa-mir-140	79.7	0.0	
142	rs7207008	T:A	hsa-mir-2117	98.4	0.46	
143	rs7227168	C:T	hsa-mir-4741	99.5	0.096	
144	rs7247237	C:T	hsa-mir-3188	98.8	0.286	
145	rs72502717	T:T	hsa-mir-3689f	0.0	0.0	Genotyping failure
146	rs72631816	T:T	hsa-mir-105-2	99.5	0.0	
147	rs72631825	G:G	hsa-mir-222	100.0	0.0	
148	rs72631826	T:T	hsa-mir-16-1	99.8	0.0	
149	rs72631827	G:T	hsa-mir-106b	99.8	0.0010	
150	rs72631831	G:G	hsa-mir-323b	100.0	0.0	
151	rs72631833	G:G	hsa-mir-183	100.0	0.0	
152	rs72646786	C:T	hsa-mir-3972	99.5	0.119	
153	rs72855836	G:A	hsa-mir-3976	98.8	0.051	
154	rs72996752	A:G	hsa-mir-4999	97.9	0.245	
155	rs73112689	T:T	hsa-mir-4459	0.0	0.0	Genotyping failure

156	rs7311975	T:C	hsa-mir-1178	99.1	0.033	
157	rs73147065	T:T	hsa-mir-647	0.0	0.0	Genotyping failure
158	rs73177830	T:T	hsa-mir-4532	0.0	0.0	Genotyping failure
159	rs73235381	T:T	hsa-mir-548h-4	0.0	0.0	Genotyping failure
160	rs73239138	G:A	hsa-mir-1269a	98.6	0.245	
161	rs73410309	T:T	hsa-mir-4739	0.0	0.0	Genotyping failure
162	rs74428911	G:T	hsa-mir-4474	100.0	0.013	Not in HWE
163	rs74469188	T:C	hsa-mir-6504	97.5	0.133	
164	rs745666	C:G	hsa-mir-3615	99.1	0.396	
165	rs74704964	C:T	hsa-mir-518d	98.8	0.041	
166	rs74904371	C:T	hsa-mir-2682	99.8	0.029	
167	rs74949342	C:G	hsa-mir-5702	99.5	0.0050	
168	rs7500280	T:T	hsa-mir-4719	0.0	0.0	Genotyping failure
169	rs75019967	A:A	hsa-mir-4477a	99.5	0.0	
170	rs7522956	A:C	hsa-mir-4742	99.3	0.245	
171	rs75598818	G:A	hsa-mir-520f	99.1	0.017	
172	rs75715827	T:C	hsa-mir-944	99.3	0.085	
173	rs75966923	C:A	hsa-mir-4298	99.8	0.035	
174	rs76481776	C:T	hsa-mir-182	100.0	0.08	
175	rs76800617	A:G	hsa-mir-4521	99.8	0.022	
176	rs77055126	T:T	hsa-mir-1303	0.0	0.0	Genotyping failure
177	rs7709117	A:G	hsa-mir-4634	98.4	0.457	
178	rs77639117	A:T	hsa-mir-576	99.3	0.033	
179	rs78396863	G:C	hsa-mir-4743	98.8	0.011	
180	rs78541299	G:A	hsa-mir-6075	100.0	0.0030	
181	rs78790512	G:A	hsa-mir-6083	99.5	0.2	
182	rs78831152	C:T	hsa-mir-4789	100.0	0.076	
183	rs78832554	G:A	hsa-mir-4786	99.8	0.028	
184	rs7896283	A:G	hsa-mir-4481	63.7	0.375	
185	rs7911488	A:A	hsa-mir-1307	0.2	0.5	
186	rs79397096	G:A	hsa-mir-597	100.0	0.015	
187	rs79512808	T:G	hsa-mir-3976	100.0	0.018	
188	rs80128580	G:A	hsa-mir-5707	100.0	0.032	
189	rs8054514	T:G	hsa-mir-3176	99.1	0.145	
190	rs8078913	C:T	hsa-mir-4520a	98.4	0.44	
191	rs832733	T:T	hsa-mir-4698	0.0	0.0	Genotyping failure
192	rs850108	T:T	hsa-mir-550a-3	0.0	0.0	Genotyping failure
193	rs8667	G:A	hsa-mir-4751	79.7	0.371	
194	rs877722	A:T	hsa-mir-4671	99.8	0.13	
195	rs895819	T:C	mir-27a	15.2	0.235	
196	rs897984	T:T	hsa-mir-4519	0.0	0.0	Genotyping failure
197	rs9295535	T:T	hsa-mir-5689	0.0	0.0	Genotyping failure
198	rs936581	G:A	hsa-mir-3141	99.5	0.135	
199	rs9842591	C:A	hsa-mir-5186	98.6	0.468	
200	rs9877402	A:G	hsa-mir-5680	98.4	0.042	
201	rs9913045	T:T	hsa-mir-548h-3	0.0	0.0	Genotyping failure
202	rs11048315	G:A	hsa-mir-4302	99.3	0.13	
203	rs111803974	T:T	hsa-mir-3908	0.0	0.0	Genotyping failure
204	rs111906529	T:C	hsa-mir-411	99.5	0.01	
205	rs112328520	C:T	hsa-mir-520g	99.3	0.057	
206	rs11269	G:G	mir-1282	99.8	0.0	
207	rs113808830	C:T	hsa-mir-4532	99.8	0.094	
208	rs116932476	G:A	hsa-mir-4479	98.8	0.0060	
209	rs117258475	G:A	hsa-mir-296	99.5	0.015	
210	rs117650137	G:A	hsa-mir-6717	100.0	0.038	

211	rs117723462	T:G	hsa-mir-3649	99.5	0.0070	
212	rs163642	T:T	hsa-mir-4436b-1	0.0	0.0	Genotyping failure
213	rs62571442	A:G	hsa-mir-3689d-2	97.9	0.43	

APPENDIX 3

Table 1. SNPs significantly associated (p < 0.05) to LOAD. Odds Ratios (OR), Confidence Intervales IC 95% : 95%, Codominant model (Cod), Dominant model (Dom), Recessive model (Rec), Log-additive model (Log).

SNP/miRNA	Genotype	N (%) Control	N (%) Case	OR (IC 95%). Cod	p. Cod	OR (IC 95%). Dom	p. Dom	OR (IC 95%). Rec	p. Rec	OR (IC 95%). Log	p. Log
rs174561 hsa-mir-1908	TT	53(42.7)	81 (60.4)	1	0.017144	1	0.004357				
	CT	60 (48.4)	45 (33.6)	0.49 (0.29 0.82)		0.49 (0.30 0.80)				0.59 (0.39 0.88)	0.008183
	CC	11 (8.9)	8 (6.0)	0.48 (0.18 1.26)							
rs74704964 hsa-mir-518d	CC	207 (95.4)	186 (88.2)	1	0.005584						
	CT	10 (4.6)	25 (11.8)	2.78 (1.3 5.95)							
	TT	0 (0)	0 (0)	0 (0 0)							
rs56292801 hsa-mir-5189	GG	82 (56.2)	123 (58.9)					1	0.006016		
	AG	44 (30.1)	75 (35.9)					0.35 (0.16 0.76)			
	AA	20 (13.7)	11 (5.3)								
rs71363366 hsa-mir-1283-2	CC	207 (95.0)	185 (87.7)	1	0.006592						
	CG	11 (5.0)	26 (12.3)	2.64 (1.27 5.5)							
	GG	0 (0)	0 (0)	0 (0 0)							
rs11983381 hsa-mir-4653	AA	163 (73.8)	132 (63.5)	1	0.013083	1	0.021388	1	0.020352		
	AG	56 (25.3)	67 (32.2)	1.48 (0.97 2.25)		1.62 (1.07 2.44)		4.95 (1.06 23.19)		1.66 (1.15 2.40)	0.006604
	GG	2 (0.9)	9 (4.3)	5.56 (1.18 26.15)							
rs10934682 hsa-mir-544b	TT	162 (73.3)	130 (62.2)	1	0.025822	1	0.013612				
	GT	56 (25.3)	71 (34.0)	1.58 (1.04 2.40)		1.67 (1.11 2.51)				1.64 (1.14 2.37)	0.007399
	GG	3 (1.4)	8 (3.8)	3.32 (0.86 12.78)							
rs4809383 hsa-mir-941-1	CC	160 (73.4)	174 (82.9)	1	0.007930	1	0.017611				
	CT	58 (26.6)	34 (16.2)	0.54 (0.34 0.87)		0.57 (0.36 0.91)				0.62 (0.40 0.98)	0.007930
	TT	0 (0.0)	2 (1.0)	0 (0 0)							
rs6062431 hsa-mir-4326	GG	103 (47.2)	123 (58.0)			1	0.02518				
	CG	84 (38.5)	70 (33.0)			0.65 (0.44 0.95)				0.71 (0.54 0.94)	0.01551
	CC	31 (14.2)	19 (9.0)								
rs243080 hsa-mir-4432	CC	65 (29.5)	76 (36.5)	1	0.020723						
	CT	116(52.7)	82 (39.4)	0.60 (0.39 0.93)							
	TT	39 (17.7)	50 (24.0)	1.10 (0.64 1.87)							

(Continues)

SNP/miRNA	Genotype	N (%) Control	N (%) Case	OR (IC 95%). Cod	p. Cod	OR (IC 95%). Dom	p. Dom	OR (IC 95%). Rec	p. Rec	OR (IC 95%). Log	p. Log
rs72996752 hsa-mir-4999	AA	136 (62.1)	108 (52.7)			1	0.04981				
	AG	73 (33.3)	79 (38.5)			1.47 (1.00 2.17)				1.43 (1.05 1.96)	0.02300
	GG	10 (4.6)	18 (8.8)								
rs7522956 hsa-mir-4742	AA	135 (61.4)	115 (54.8)					1	0.02744		
	AC	75 (34.1)	74 (35.2)					2.33 (1.07 5.08)		1.36 (1.00 1.84)	0.04620
	CC	10 (4.5)	21 (10.0)								
rs6726779 hsa-mir-4431	TT	86 (38.9)	75 (36.2)					1	0.02870		
	CT	110 (49.8)	93 (44.9)					1.82 (1.06 3.13)			
	CC	25 (11.3)	39 (18)								
rs112328520 hsa-mir-520g	CC	187 (85.4)	194 (91.9)	1	0.03112						
	CT	32 (14.6)	17 (8.1)	0.51 (0.28 0.95)							
	TT	0 (0)	0 (0)	0 (0 0)							
rs1683709 hsa-mir-3612	CC	141 (63.8)	132 (62.6)					1	0.03138		
	CT	74 (33.5)	64 (30.3)					2.74 (1.04 7.21)			
	TT	6 (2.7)	15 (7.1)								
rs266435 hsa-mir-4804	CC	174 (79.1)	152 (73.4)					1	0.03297		
	CG	43 (19.5)	45 (21.7)					3.67 (1.00 13.53)			
	GG	3 (1.4)	10 (4.8)								
rs61992671 hsa-mir-412	GG	73 (33.2)	50 (23.9)			1	0.03360				
	AG	98 (44.5)	112 (53.6)			1.58 (1.03 2.41)					
	AA	49 (22.3)	47 (22.5)								
rs12894467 hsa-mir-300	CC	98 (44.5)	77 (37.4)					1	0.04180		
	CT	102 (46.4)	97 (47.1)					1.84 (1.01 3.33)		1.36 (1.02 1.81)	0.03601
	TT	20 (9.1)	32 (15.5)								
rs13299349 hsa-mir-3152	GG	95 (43.4)	95 (45.7)					1	0.0428		
	AG	86 (39.3)	91 (43.8)					0.56 (0.32 0.99)			
	AA	38 (17.4)	22 (10.6)								
rs68035463 hsa-mir-3144	CC	148 (67.3)	124 (59.3)								
	AC	67 (30.5)	74 (35.4)							1.42 (1.01 1.99)	0.04348
	AA	5 (2.3)	11 (5.3)								
rs56103835 hsa-mir-323b	TT	157 (71.0)	133 (63.6)								
	CT	58 (26.2)	63 (30.1)							1.41 (1.00 1.97)	0.04575
	CC	6 (2.7)	13 (6.2)								

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