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# MIRNA POLYMORPHISMS ARE ASSOCIATED WITH RISK OF DEVELOPING CHRONIC LYMPHOCYTIC LEUKEMIA

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# miRNA polymorphisms are associated with risk of developing chronic lymphocytic leukemia

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# ABSTRACT

Chronic Lymphocytic Leukemia (CLL) is the most frequent leukemia of adults in Western countries and shows a ~8.5-fold increased relative risk in first-degree relatives. Up to date several studies have identified low-penetrance susceptibility alleles in CLL. Nevertheless, these studies scarcely study regions that do not encode proteins such as microRNAs (miRNAs). Abnormalities in miRNAs, as altered expression patterns and mutations, have been described in CLL, suggesting their implication in the development of the disease. Polymorphisms in these miRNAs may deregulate miRNAs expression levels and affect to the miRNA function.

However, despite accumulating evidence that inherited genetic variation in miRNA genes can contribute to the predisposition for CLL, the role of these in the risk of CLL has not been extensively studied. Therefore, the aim of this study was to find new genetic markers of risk to CLL. To that end, we made a systematic search for SNPs in miRNAs and miRNAs deregulated in CLL and genotyped 213 polymorphisms in 401 samples of Spanish individuals.

The literature search resulted in more than 100 miRNAs deregulated in CLL and 43 polymorphisms studied in the disease.

Out of 213 genotyped SNPs, 13 showed to be significantly associated with CLL risk. rs2682818 in pre-mature miR618 was the most significant result, with 0.49 fold decreased risk to CLL. Interestingly, a previous study associated this SNP with an increased risk of developing follicular lymphoma. Secondly, rs10173558 SNP in mir-1302-4 showed the highest risk association, with a 5.24 fold increased risk, but there were no previous works studying it. Finally, rs61992671 in miR412, previously associated with CLL risk, showed also association in our sample.

In conclusion, we find 13 alleles which could contribute to the risk of CLL. However, new large-scale studies including functional analyses will be needed to validate our findings.

# RESUMEN

La Leucemia Linfática Crónica (LLC) es el tipo más frecuente de leucemia en adultos de países occidentales, y muestra un riesgo ~8,5 veces superior a padecer la enfermedad en parientes de primer orden de los enfermos. Hasta la fecha, muchos estudios han identificado alelos de baja penetrancia asociados a la susceptibilidad a LLC. Sin embargo, estos estudios apenas han estudiado regiones que no codifican proteínas, como los microARNs (miARNs). Patrones de expresión alterados, y mutaciones, han sido descritas en LLC sugiriendo su implicación en el desarrollo de la enfermedad. Polimorfismos en estos miARNs podrían desregular los niveles de expresión de los mismos y afectar a su función.

Sin embargo, a pesar de del cada vez mayor numero de evidencias apoyando que la herencia de variantes genéticas en miARNs puede contribuir a la predisposición a LLC, el papel de estos en el riesgo a LLC no ha sido extensamente estudiado. Por tanto, el objetivo de este estudio fue encontrar marcadores genéticos de riesgo a LLC. Con ese fin, realizamos una búsqueda sistemática de SNPs en miARNs y miARNs desregulados en LLC, y genotipamos 213 polimorfismos en 401 muestras de individuos españoles.

La búsqueda bibliográfica resultó en más de 100 miARNs desregulados en LLC y 43 polimorfismos estudiados en la misma.

De los 213 SNPs genotipados, 13 mostraron una asociación significativa con el riesgo a LLC. rs2682818 en el miR618 pre-maduro fue el resultado más significativo, con un riesgo 0.49 veces menor a padecer la enfermedad. Interesantemente, un estudio previo asoció este SNP con un incremento en el riesgo a padecer LLC. En segundo lugar, el SNP rs10173558 in mir-1302-4 mostró el mayor incremento del riesgo a padecer LLC de todos los SNPs analizados, 5,24 veces mayor, pero no fue estudiado en estudios previos. Finalmente, el SNP rs61992671 en miR412, previamente asociado al riesgo a LLC, mostro asociación en nuestra muestra.

Por tanto, encontramos 13 alelos que podrían contribuir al riesgo a LLC. Sin embargo, serán necesarios nuevos estudios de gran envergadura incluyendo análisis funcionales para validar nuestros resultados.

# 1. INTRODUCTION

## 1.1- Genetic susceptibility

Susceptibility is an increased likelihood of developing a particular disease based on individual genetic variations. These changes contribute to the development of a disease, but do not directly cause it since factors such as environment and lifestyle may also influence on it (Genetics Home Reference). One of the most common variations used as indicative of susceptibility studies are Single Nucleotide Polimorphisms (SNPs).

SNPs are single base substitutions of one nucleotide with another (Figure 1), observed in the general population at a frequency greater than 1%. SNPs are the simplest form of DNA variation among individuals occurring throughout the genome, in coding and non-coding regions, at a frequency of about one in 200-300 bp (1).

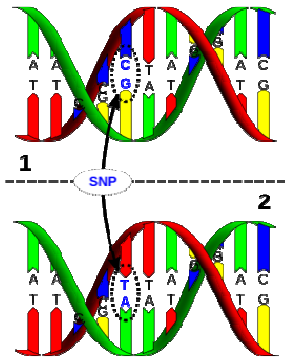


**Figure 1.** Example of a SNP. Two chromosomes in an individual with a C/T SNP.

In the last years, several SNPs have been associated with risk for different types of cancer (2, 3), showing a role for the genetic predisposition in these malignancies. Among them, chronic lymphocytic leukemia (CLL) has been reported to have the highest genetic predisposition of all hematologic neoplasias (4).

CLL is the most frequent leukemia of adults in Western countries, accounting for approximately 30% of all mature B-cell malignancies. There is increasing evidence for the role of inherited factors in the development of CLL. Indeed, CLL shows a ~8.5-fold increased relative risk in first-degree relatives (5), but no high-penetrance mutations for this increased risk have been detected. Recent genome-wide association studies

(GWAS) have identified several low-penetrance alleles associated with CLL (6-10), associations that have been thoroughly validated in multiple independent series (11-14).

For instance, rs872071 in *IRF4* (interferon regulatory factor 4), a transcription factor also found to be implicated in acute leukemias of childhood (15), was found to be associated with CLL (P= 1.91x $10^{-20}$) (6). Other polymorphisms in protein coding genes, such us, SCN3B, RPLP1, SP140 and SP110 have also been associated with CLL in 6 different GWAS, clearly demonstrating the existence polymorphisms associated with risk to CLL (6-10, 16).

Nevertheless, previous GWAs and association studies have focused their effort on identifying SNPs in genes, which represent only ~1.5% of the entire genome (17), and scarcely study regions that do not encode proteins. After the "Encyclopedia of DNA Elements" (ENCODE) project in 2007 it was shown that a large part of the genome is transcribed as non-coding RNAs (ncRNAs) (Figure 2).

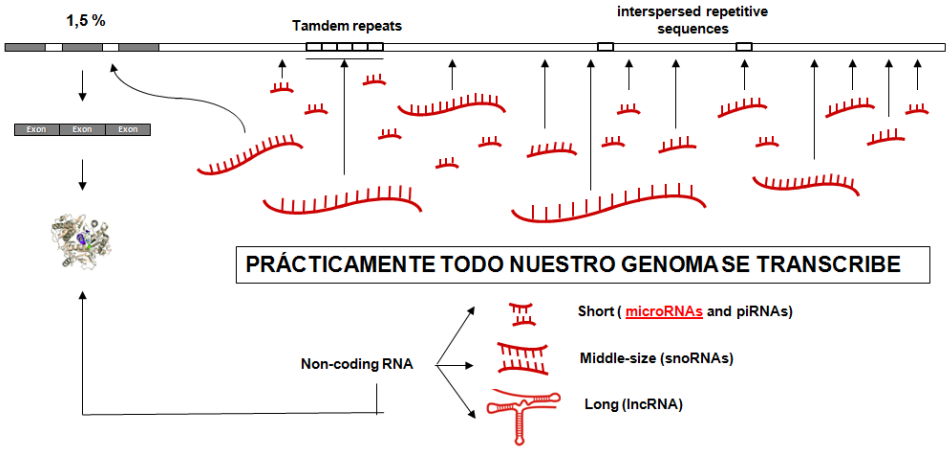


**Figure 2.** Coding vs non-coding transcribed regions of the genome.

### 1.2- Non-coding RNAs

ncRNAs are a class of RNAs with regulatory function that are widely expressed in organisms. They play critical roles in regulation processes, such as, differentiation, development, post-transcriptional regulation of gene expression and epigenetic regulation (18) and can be classified by size as (19):

1. Short ncRNAs: miRNAs, piRNAs and tiRNAs (19-31bp)
2. Mid-size ncRNAs: snoRNAs, PASRs, TSSa-RNAs and PROMPTs (22-200bp)

3. Long non-coding RNAs: lincRNAs, T-UCRs and other lncRNAs (>200bp).

Among these, one of the most studied ones are micro RNAs (miRNAs).

miRNAs are a group of small, functional, non-coding RNAs of approximately 20 nucleotides long. They function as regulators in processes such as proliferation, differentiation, apoptosis and development. Each miRNA can have hundreds of different targets, and it is estimated that they regulate more than 60% of protein coding genes (19).

They are transcribed in the nucleus by the RNA polymerase II as double stranded pri-miRNAs. Pri-miRNAs are processed by a multi-protein complex including DROSHA to form the pre-miRNAs. The pre-miRNAs are exported to the cytoplasm by RAN GTPase and exportin 5 (20) and cleaved by DICER1 to produce two strands of miRNA (21). Single stranded miRNAs after incorporating to RISC complex recognize their target mRNAs by binding to the 3´UTR of the target gene (22), which leads to an inhibition of translation or facilitated degradation of the target messenger RNAs (mRNAs)(Figure 3).

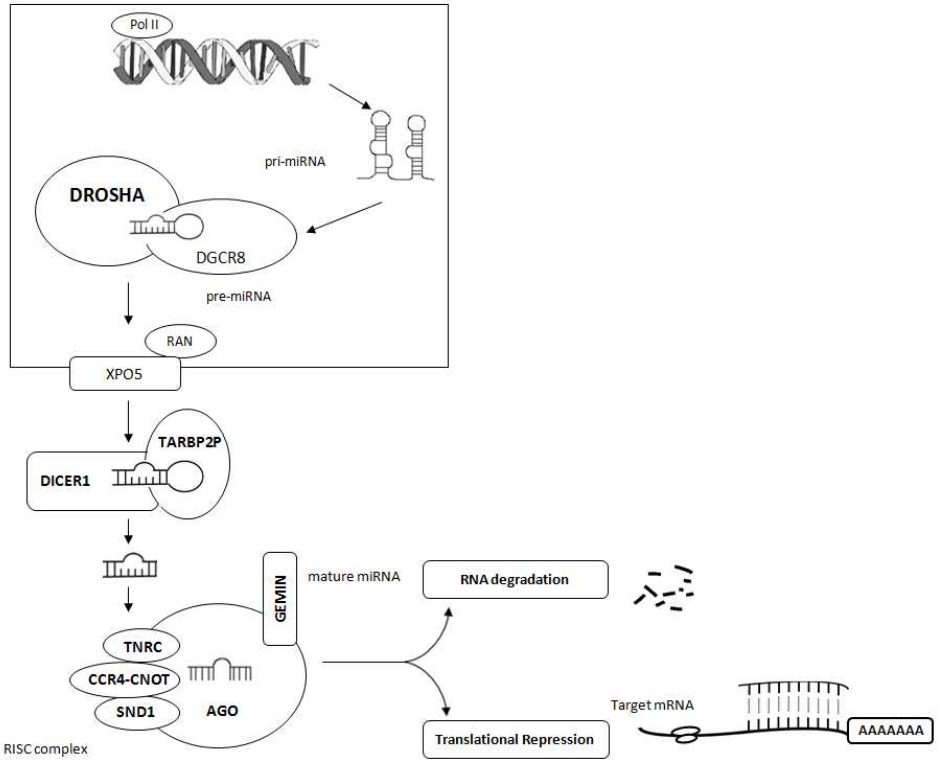


**Figure 3.** miRNA biogenesis and regulation processes.

### 1.3- miRNAs expression and CLL

Altered expression of miRNAs has been largely studied in CLL (23-25), and to date more than a hundred miRNA expression profiles have been analyzed For example, miR15a/16-1 cluster expression has been widely described to be downregulated in CLL (26-29). On the other hand, miR21 (30-31, 23) and miR150 (32-33, 23) are frequently found upregulated. Moreover, deregulation of some miRNAs has been associated with disease progression and aggressiveness (34-35).

### 1.4- miRNA expression deregulation by SNPs

Polymorphisims may deregulate miRNAs expression levels by affecting to the miRNA primary transcript production as well as to pri-miRNA and pre-miRNA processing (Figure 4A). Additionally, genetic polymorphisms in mature miRNAs and miRNA binding sites altering the miRNA-mRNA binding affect to the miRNA function (Figure 4B) (22).

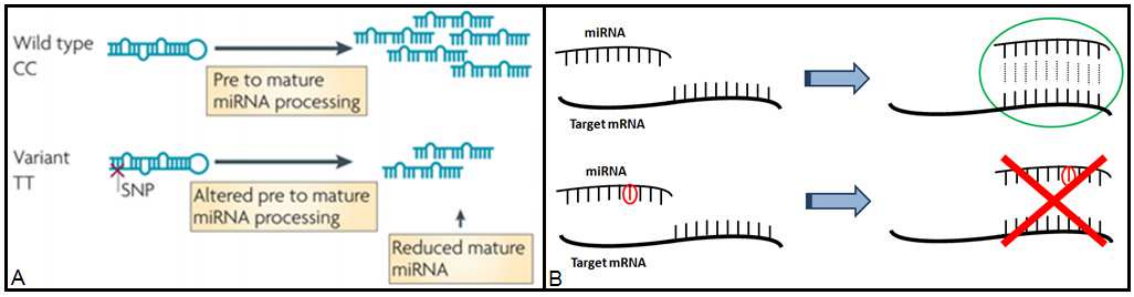


**Figure 4.** miRNA deregulation (22) (A) and function alteration (B) through SNPs.

### 1.5- miRNA polymorphisms in CLL

Several mutations in miRNAs have been seen to be common in CLL (25, 36), suggesting that CLL predisposition involves genetic alterations in miRNAs. Supporting this idea, a recent study found that certain miRNA SNPs show different frequencies in CLL patients compared with a control population (37). However, despite accumulating evidence that inherited genetic variation in miRNA genes can contribute to the predisposition for CLL, the role of these in the risk of CLL has not been extensively studied.

In the light of the above and taking into account that:

1. Most association studies in CLL have focused in coding regions.
2. miRNA expression levels are deregulated in CLL and associated with the pathogenesis of the disease.
3. SNPs in miRNAs are linked with deregulation of these molecules.
4. Only a few studies have sought SNPs in miRNAs associated with the risk to CLL.

We have decided to evaluate the effect of SNPs in miRNAs with the risk to CLL.

# 2. HIPOTHESIS AND AIM OF THE PROYECT

Our hypothesis is that polymorphisms in miRNAs could lead to the development of the disease by function and/or expression alteration.
Therefore, the aim of this study is to find new genetic markers of risk to CLL.

To that end, the following specific objectives will be made:

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

# 3. MATERIAL AND METHODS

### 3.1- Systematic search

We performed an exhaustive search to identify studies that examined (a) miRNA deregulation in CLL and (b) the association between miRNA SNPs and susceptibility in CLL. We used the keywords and subject terms “(CLL OR chronic lymphocytic leukemia) AND (micro RNA OR miRNA)” for Pubmed (http://www.ncbi.nlm.nih.gov/pubmed) searches for articles published until June 2014. Studies that investigated miRNA expression and the association between miRNA genetic variations and CLL risk were included. Studies in other diseases, analyzing animals and/or not available in English were excluded.

### 3.2- miRNA and miRNA $SNP_S$ selection

All pre-miRNAs described in miRBase database until March 2013 were selected for the study. All the SNPs described in the miRNASNiPer database (http://www.integratomics-time.com/) with a Minor Allele Frequency (MAF) greater than 1% in Caucasian population and present in the previous pre-miRNAs were selected.

### 3.3- Selection of patients

The study population included a total of 401 DNA samples from Spanish individuals, 164 CLL patients and 237 controls. 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 at the Spanish National Genotyping Center (CEGEN-ISCIII) using the GoldenGate® Genotyping Assay, form VeraCode Technology®, Illumina (Figure 5). This platform allows a multiplex PCR assay, abling to process a large number of SNPs simultaneously.

The GoldenGate® Assay consists briefly in (a) DNA activation with Biotin or Streptavidin for posterior binding to paramagnetic particles (250ng DNA at 50ng/µl). (b) hybridization between the DNA sample and specific oligonucleotides, which need to be designed for each SNP locus. Specifically, three oligonucleotides are designed: two of them are specific to each allele of the SNP site (Allele-Specific Oligos or ASOs and the third oligo is the Locus-Specific Oligo (LSO) that 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 (c) PCR amplification (d) the PCR products are hybridized

onto an Array Matrix allowing individual SNP genotype readout by analyzing the fluorescence signal.

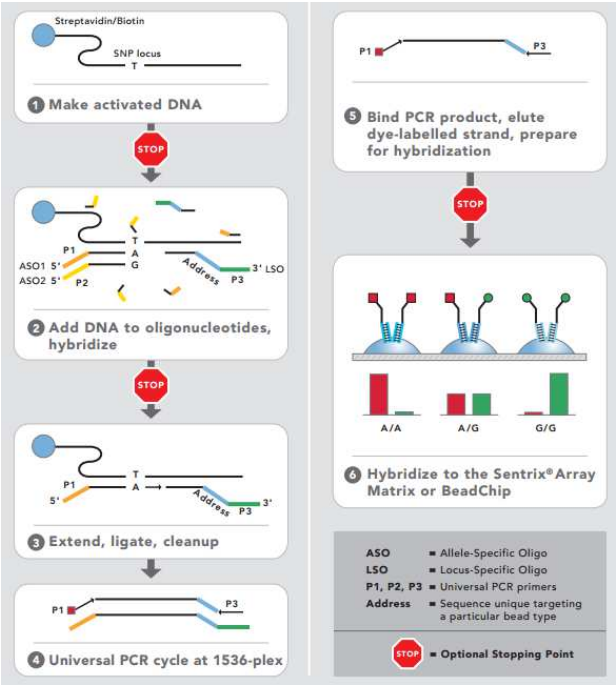


**Figure 5.** GoldenGate® Assay overview (http://www.illumina.com/technology.ilmn Retrieved: June 12th, 2014)

### 3.5- Statistical analyses

Hardy-Weinberg Equilibrium (HWE) between expected and observed genotype distributions in the control sample was evaluated using a $\chi 2$ test. The effect sizes of the associations were estimated by the odds ratios from univariate logistic regression. The most significant test among the different genetic models was used to determine the statistical significance of each SNP. The results were adjusted for multiple comparisons by the False Discovery Rate (FDR) (38). In all cases the significance level was set at 5%. Analyses were performed by using R v2.11 software.

Individuals with 80% of missing genotypes were excluded from the statistical analysis. The Haploview software (version 4.2) was used to calculate the success of the genotyping.

### 3.6- miRNAs secondary structures and expression prediction

The "Bioguo web toll" (http://www.bioguo.org retrieved: May 20th, 2014) (Release 2.0: July 2013) (39) was used to predict the most stable secondary structure of the miRNAs showing significant SNPs.

# 4. RESULTS

### 4.1- Literature search of deregulated miRNAs in CLL

#### 4.1.1- Flow chart

The search provided 234 records. Of these, 168 were discarded because they did not meet the required criteria for inclusion, and 66 which investigated miRNA expression deregulation in CLL and miRNA SNPs in CLL were identified and included. The flow chart below (Figure 6) represents the process of literature review.

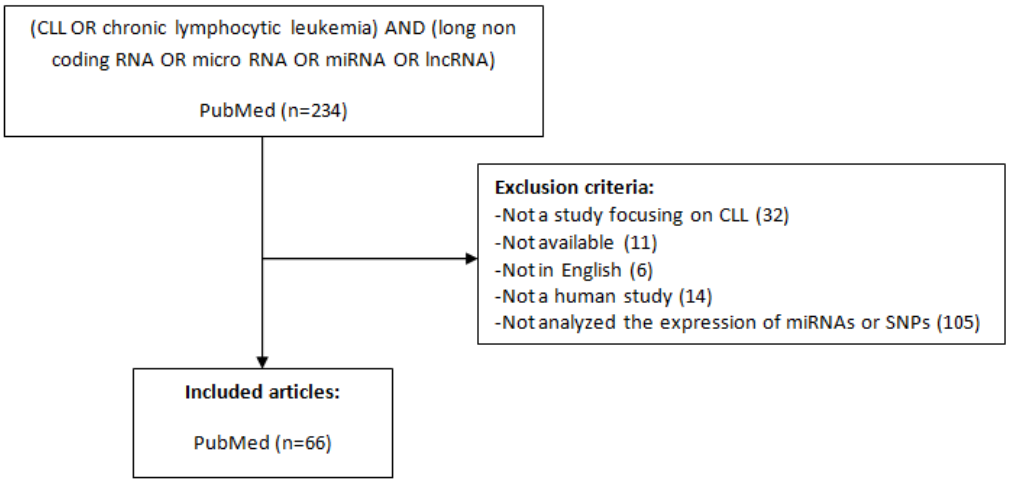


**Figure 6.** Flow chart of literature review process

#### 4.1.2- miRNA expression deregulation in CLL

We found 66 articles studying miRNA expression deregulation ($p<0.05$) in CLL. Overall, more than 100 different miRNAs belonging to 86 miRNA families were studied (Supplementary Table 1). We also found in this search 43 polymorphisms that had been described in 29 miRNAs in CLL. 8 miRNAs from those 29 had been analyzed in the expression deregulation studies, and 3 have SNPs associated with the risk to CLL (Supplementary table 2).

### 4.2- Association study

#### 4.2.1- Characterization of the sample

The study included DNA samples from 401 Spanish individuals, 164 CLL patients and 237 cancer-free controls. The average age was 70.21 in CLL patients and 75.46 in controls with a percentage of females of 39% and 58.65% (Table 1) and a percentage of males of 61.0 and 41.4, respectively.

**Table 1. Characteristics of the sample of study**

| | | CLL patients | Controls |
|---|---|---|---|
| Number | | 164 | 237 |
| Average age | | 70.21 | 75.46 |
| Sex | Females (%) | 64 (39.0) | 139 (58.7) |
| | Males (%) | 100 (61.0) | 98 (41.4) |

#### 4.2.2- Genotyping results

A successful genotyping was obtained in 392 DNA samples (97.7%); 164 of 164 CLL patients (100%) and 228 of 237 controls (96.2%). Of 213 SNPs located in 206 miRNA sequences, 159 (74.6%) were genotyped satisfactorily. Twelve SNPs were removed from the association study due to genotyping errors or deviations from HWE, being the average genotyping rate of 99.01%.

### 4.3- Association

A total of 13 SNPs showed statistically significant association with CLL risk ($P<0.05$) (Table 2). rs2682818, in pre-mature miR618, showed the most significant result under the dominant genetic model (CC + AC vs AA). The AA genotype showed a 0.49-fold decreased risk of CLL (95% CI: 0.29-0.81; P=0.0047). Other 4 SNPs located in pre-mature miRNAs; rs4822739 in miR548j, rs75715827 in miR944, rs61938575 in miR3922, rs12355840 in miR202, showed the following highest significant values. Interestingly, the CC genotype from rs10173558 SNP in mir-1302-4 showed a 5.24 fold increased risk of developing CLL under the recessive model, the highest OR value among the significant SNPs. Finally, rs61992671 in hsa-mir-412 was the only polymorphism in which the dominant model (GG+AG vs AA) was associated with an increased risk for CLL, OR=1.67 (95% CI: 1.06-2.62; P=0.02464).

**Table 2.** SNPs associated with risk to CLL and bibliographic data from cancer related studies for either the SNP or the miRNA.

| SNP ID | Gene | Genotype | N (%)control | N (%)case | OR (IC 95%) | P | Literature | Ref |
|---|---|---|---|---|---|---|---|---|
| **rs2682818** | hsa-mir-618 | CC | 172 (72.9) | 138 (84.7) | 1 | **0.005** | -No significant association of rs2682818 polymorphism and breast cancer risk | 2 |
| **In pre-mature** | | AC | 59 (25.0) | 22 (13.5) | 0.49 (0.29-0.81) | **Dominant** | | |
| | | AA | 5 (2.1) | 3 (1.8) | | | -rs2682818 G>T (in the stem-loop sequence )associated with follicular lymphoma | 3 |
| | | Total | 236(100) | 163(100) | | | -T allele resulted in reduced levels of mature miR-618 | |
| **rs4822739** | hsa-mir-548j | CC | 211 (89.0) | 156 (95.7) | 1 | **0.013** | -May potentially regulate *PTPN12* (involve in oncogenic transformation) gene expression | 6 |
| **pre-mature miR** | | CG | 26 (11.0) | 7 (4.3) | 0.36 (0.15-0.86) | **Codominant** | | |
| | | Total | 237(100) | 163(100) | | | | |
| **rs75715827** | hsa-mir-944 | TT | 194 (81.9) | 147 (90.2) | 1 | **0.018** | -Upregulation of miR-944 is related to the progression and metastasis of squamous cell carcinoma | 7 |
| **pre-mature miR** | | CT | 41 (17.3) | 16 (9.8) | 0.49 (0.27-0.91) | **Dominant** | | |
| | | CC | 2 (0.8) | 0 0.0 | | | -hsa-miR-944 is a candidate driver miRNAs (a gene whose dysfunction will cause tumorigenesis) | 9 |
| | | Total | 237(100) | 163(100) | | | | |
| **rs61938575** | hsa-mir-3922 | GG | 114 (49.4) | 80 (49.4) | 1 | **0.019** | Not found | |
| **pre-mature miR** | | AG | 101 (43.7) | 59 (36.4) | 2.22 (1.13-4.36) | **Recessive** | | |
| | | AA | 16 (6.9) | 23 (14.2) | | | | |
| | | Total | 231(100) | 162(100) | | | | |
| **rs12355840** | hsa-mir-202 | TT | 148 (64.6) | 102 (62.2) | 1 | **0.020** | -G allele in rs12355840 associated with follicular lymphoma and diminished pre-miR-202 processing capacity | 1 |
| **pre-mature miR** | | CT | 66 (28.8) | 59 (36.0) | 0.27 (0.08-0.93) | **Recessive** | | |
| | | CC | 15 (6.6) | 3 (1.8) | | | | |
| | | Total | 229(100) | 164(100) | | | -miR-202-3p significantly downregulated in 46.7% colorectal cancer samples | 17 |
| | | | | | | | -Upregulated in breast cancer cases | 20 |
| **rs10173558** | mir-1302-4 | TT | 182 (76.8) | 125 (76.2) | 1 | **0.022** | Not found | |
| **pri-miR** | | CT | 53 (22.4) | 32 (19.5) | 5.24 (1.07-25.55) | **Recessive** | | |
| | | CC | 2 (0.8) | 7 (4.3) | | | | |
| | | Total | 237(100) | 164(100) | | | | |
| **rs61992671** | hsa-mir-412 | GG | 79 (33.5) | 38 (23.2) | 1 | **0.024** | -rs61992671 associated with CLL ($p<0.0001$) in CLL vs 1000 genomes project | 4 |
| **mature miR** | | AG | 104 (44.1) | 87 (53.0) | 1.67 (1.06-2.62) | **Dominant** | | |
| | | AA | 53 (22.5) | 39 (23.8) | | | | |
| | | Total | 236(100) | 164(100) | | | | |
| **seq_rs117723462** | MIR3649 | TT | 237 (100.0) | 160 (97.6) | 1 | **0.027** | Not found | |
| **In pre-mature** | | GT | 0 0.0 | 4 (2.4) | | **Codominant** | | |
| | | Total | 237(100) | 164(100) | | | | |
| **rs77639117** | hsa-mir-576 | AA | 226 (96.2) | 149 (90.9) | 1 | **0.030** | -miR-576-3p upregulated in Early T-cell precursor acute lymphoblastic leukemia | 23 |
| **pre-mature miR** | | AT | 9 (3.8) | 15 (9.1) | 2.53 (1.08-5.93) | **Codominant** | | |
| | | Total | 235(100) | 164(100) | | | | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **rs266435**<br>**seed region** | hsa-mir-4804 | CC<br>CG<br>GG<br>Total | 182 (77.1)<br>51 (21.6)<br>3 (1.3)<br>236(100) | 109 (68.6)<br>44 (27.7)<br>6 (3.8)<br>159(100) | 1.55 (1.04-2.31) | **0.031**<br>**Log additive** | Not found |
| **rs2368392**<br>**In pre-mature** | hsa-mir-604 | CC<br>CT<br>TT<br>Total | 132 (56.2)<br>82 (34.9)<br>21 (8.9)<br>235(100) | 97 (59.9)<br>59 (36.4)<br>6 (3.7)<br>162(100) | 1<br>0.39 (0.15-0.99) | **0.03479**<br>**Recessive** | Not Found |
| **rs80128580**<br>**pre-mature miR** | hsa-mir-5707 | GG<br>AG<br>Total | 225 (94.9)<br>12 (5.1)<br>237(100) | 147 (89.6)<br>17 (10.4)<br>164(100) | 1<br>2.17 (1.01-4.67) | **0.04606**<br>**Codominant** | Not Found |
| **rs243080**<br>**pre-mature miR** | hsa-mir-4432 | CC<br>CT<br>TT<br>Total | 69 (29.2)<br>125 (53.0)<br>42 (17.8)<br>236(100) | 50 (30.7)<br>70 (42.9)<br>43 (26.4)<br>163(100) | 1<br>1.66 (1.02-2.68) | **0.04078**<br>**Recessive** | Not Found |
| ***None of the SNPs remained significantly associated with CLL after FDR correction** | | | | | | | |

**Table 3. Predicted efecto of the SNP in miRNA expression level**

| SNP | Gene | Predicted expression: |
|---|---|---|
| rs2682818 | hsa-mir-618 | UP |
| rs4822739 | hsa-mir-548j | DOWN |
| rs75715827 | hsa-mir-944 | DOWN |
| rs61938575 | hsa-mir-3922 | DOWN |
| rs12355840 | hsa-mir-202 | MILD |
| rs10173558 | hsa-mir-1302-4 | No data |
| rs61992671 | hsa-mir-412 | MILD |
| rs117723462 | MIR3649 | UP |
| rs77639117 | hsa-mir-576 | MILD |
| rs266435 | hsa-mir-4804 | UP |
| rs2368392 | hsa-mir-604 | MILD |
| rs243080 | hsa-mir-4432 | DOWN |
| rs80128580 | hsa-mir-5707 | DOWN |

Adjustment of data for age and sex revealed that 4 of 13 significant SNPs were influenced by sex showing different association results (Supplemental Table 3).After FDR correction, none of the significant 13 SNPs showed statistically significant association with CLL risk (Supplemental Table 4).

### 4.4- Prediction of miRNA expression deregulation by SNPs

Eight out of 13 significant SNPs were predicted to change the miRNA expression leading to the deregulation of these miRNAs. The eight SNPs were located in pre-mature miRNA sequence, excepting rs266435 in hsa-mir-4804, which was located in the seed region (Table 3).

## 5. DISCUSSION

miRNAs play a major role in the normal development of B cells (40). Conversely, miRNAs expression deregulation has been reported in several studies (23-25) and it is thought to be a key factor to the pathogenesis of CLL (40). Taking this into account, we performed an exhaustive search to identify studies that examined miRNA deregulation in CLL. Comparing the different studies we observed that there is no consensus at the time to show expression levels in CLL, while some divide CLL cells according to IgVH or TP53 mutational status, deleted regions etc., others make no distinction. This led to confusion at the time to validate expression levels registered by different groups. Comparing only those studies in which these alterations were described we were able to find inconsistencies in 6 miRNAs.

miR15a, miR16-1 and miR16-2 upregulation was found to be associated with mutated IgVH in (25), whereas (32) and (41) found miR15a expression downregulated in mutated IgVH cases and no association between miR16 expression and IgVH mutational status respectively. These discrepancies may be related to the small sample size used, 94 CLL patients in (25) and, 33 and 79 in (41) and (32) respectively.

Mature miRNA function or expression levels have been suggested to be affected by SNPs in pre-mature or mature miRNA regions (42). Since miRNA SNPs could be associated with risk to CLL few studies have focused their efforts in conducting association studies. Calin et al. (25) described a series of polymorphisms associated to CLL, but they only found mutations and yet not validated SNPs. Two other groups have continued this search, but only one has been able until now to find a SNP associated with risk to CLL in European populations (37).

Hence, driven by the low number of association studies in miRNA SNPs in CLL, and the large amount of miRNAs described as deregulated, we decided to carry out a case-control study and evaluate the association of a wide range of miRNA SNPs. With that goal, we looked for all the SNPs experimentally validated (MAF greater than 1% in Caucasian population) located in miRNAs. To our knowledge this is the first study to conduct association analyses for all miRNA SNPs in relation to CLL susceptibility.

We examined a total of 213 SNPs, finding 13 significantly associated, eleven in pre-miRNAs and one in mature miRNA, one in seed region. As we did not find expression data for these miRNAs in CLL, another search was conducted for the SNP ID and miRNA type in other cancers.

Interestingly, among the significant SNPs in miRNAs, AA genotype of rs2682818 in pre-miR618 showed to be protective (0.49 fold) versus CC+AC genotypes. This SNP had previously been studied in breast cancer (43) and follicular lymphoma (44). Whereas there was no significant association with the risk for breast cancer, the SNP was found to be associated with the risk for follicular lymphoma. Surprisingly, the A allele was associated with 1.65 increased fold risk of developing follicular lymphoma. The SNP was described to downregulate miR618 expression in the same work. So, miR618 controlled pathways deregulation may be beneath the development of CLL.

rs12355840 variant homozygote CC in pre-miR202 showed a 0.27 decreased fold risk of developing CLL. This miRNA has been predicted to target TET2, an oncogene found to be overexpressed in CLL (45). C allele has already been associated with diminished

miR202 processing capacity in an association study with follicular lymphoma (46), suggesting a possible role for this SNP in the pathogenesis of CLL. However, this study also revealed a 1.77 increased fold risk of developing the disease with a single G allele, revealing as in rs2682818 in miR618 (44) a discordance between the effects of this SNPs in CLL and follicular lymphoma.

In addition, miR202 has been widely studied in cancer, revealing to be upregulated in breast cancer cases (47). Also, miR202-3p strand was found to be downregulated in 46.7% of colorectal cancer samples in a human colorectal carcinoma study (48) and in gastric cancer tissues (49). This, with the fact that is a strong negative regulator of MYCN oncogene expression (50), suggests an important role in the development of CLL.

Another significant SNP, rs75715827, was located in miR944. CC genotype conferred a 0.49 fold increased protection against the development of CLL in our study ($p=0.0184$). This SNP was predicted to downregulate miR944 product. miR944 upregulation has been associated with progression and metastasis of squamous cell carcinoma (51). Additionally, reduced expression in the complete responses group compared to the progression of disease group was measured in advanced bladder cancer (52). Taking this into account, it seems that miR944 downregulation could be associated with a better outcome in different types of cancer, and that C allele confers a protective effect through downregulating it.

Surprisingly, C allele from rs10173558 SNP in mir-1302-4, which showed a 5.24 fold increased risk of developing CLL, has not yet been studied in relation to any disease. Given the high risk conferred by this allele, future studies should attempt to validate this result and analyze how an imbalance in the expression of this miRNA could affect susceptibility to CLL.

rs61992671 AA+AG genotype in miR412 our study was found to be associated with a 1.67 increased fold probability of developing CLL. Interestingly, a recent study showed that rs61992671 was associated with the risk for CLL ($p<0.0001$) when comparing with controls from the 1000 Genomes Projects (37). However, this association was not present when they used only European data from the 1000 Genomes Project. This

discrepancy highlights the need to analyze these polymorphisms in studies with larger sample sizes in order to obtain clear results, so that the effect of rs61992671 in CLL can be revealed.

It remains to discuss the effect of sex on the SNPs of mir-1302-4, hsa-mir-202 hsa-mir-604 and hsa-mir-4432. Since the incidence ratio of the disease is 1.98 times higher in men compared to women (54) is not unreasonable to think that variations in these miRNAs can affect differentially males and females. However, given this high sex effect, the amount of miRNAs differentially associated would be expected to be greater.

In conclusion, the findings of the present study indicated that SNPs located in hsa-mir-618, hsa-mir-548j, hsa-mir-944, hsa-mir-3922, hsa-mir-202, mir-1302-4, hsa-mir-412, hsa-mir-3649, hsa-mir-576, hsa-mir-4804, hsa-mir-604, hsa-mir-5707and hsa-mir-4432 may contribute to the risk of CLL. To our knowledge, this is one of the few studies analyzing specifically miRNA SNPs in CLL, which opens a promising approach to search for new susceptibility markers in CLL. New large-scale studies including functional analyses will help to validate our findings.

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# 7. ANNEXES

## ANNEX BIBLIOGRAPHY

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Supplementary Table 1. Deregulated miRNAs in CLL

| **miRNA** | **Effect** | **Description** | **Ref.** |
|---|---|---|---|
| miR7-1 | UP | with 13q14.3 deletions | 24 |
| miR9-3 | DW, meth. | silenced in CLL by metilation | 55 |
| miR10b | UP | in CLL vs CD5+ cells | 24 |
| miR15a/16-1 | DOWN | low miR15/16 expression associated with monoallelic 13q14del | 26 |
| | DOWN | miR-15 and 16 expression lower in Biallelic 13q14del than monoallelic. | 26 |
| | DOWN | miR15/16 downregulated in CLL with 13q14del | 27 |
| | DOWN | MiR-15a/16 expression downregulated only in patients with the biallelic loss of the miRNA cluster | 28 |
| | DOWN | 15% of del13q14 CLL cases have very low miR16/miR15a levels | 59 |
| | DOWN | A subset of showed CLL very low miR16-1/15a cluster expression | 29 |
| | DOWN | MicroRNA-15a/16-1 expression was down-regulated in the majority of patients | 69 |
| | Expression | in PB no significant correlation between expression of miR15a/16-1 cluster with presence of 13qDel. | 88 |
| miR15a | UP | pri-miRNA-15a in CLL | 87 |
| | UP | High miR-15a associated with no expression of ZAP-70 and mutated IgVH | 25 |
| | UP | miR-15a was high in CLL with 17pDEL | 72 |
| | DOWN | miR15a downregulated in patients with biallelic del (13q) | 84 |
| | DOWN | reduction in the expression of the MUT alleles of miR-15a in del 13q. | 36 |
| | DOWN | Downregulation of miR15a strongly correlated to biallelic but not monallelic 13q14.3 del CLL | 70 |
| | DOWN | miR-15a downregulated in M-CLL against U-CLL | 84 |
| | DOWN | pre- and mature miRNAs-15a downregulated in CLL | 87 |
| | DOWN | down-expression of miR-15a in CLL | 74 |
| | DOWN | miR-15a decreased in about 11% of CLL cases | 66 |
| | DOWN | miR-15a showed very low expression lymph node and blood CLL | 41 |
| | DOWN, PROG | miR-15a expressed at low levels in patients with good prognosis | 58 |
| miR15b | UP | precursor upregulated in CLL vs CD5+ cells | 24 |
| | DOWN | miR-15b is downregulated in CLL | 87 |
| | DOWN | showed very low expression in lymph node and blood CLL | 41 |
| miR16 | DOWN | miR-16 downregulated in B-CLL with a normal karyotype | 67 |
| | DOWN | miR-16 downregulation associated with the 13q14 deletion | 63 |

| | | | |
|---|---|---|---|
| | Expression | No correlation of expression levels of miR-16 with IgH mutation or ZAP-70 status | 41 |
| miR16-1 | UP | High miR-16-1 associated with no expression of ZAP-70 and mutated IgVH | 25 |
| | UP | pri-miRNA-16-1 in CLL | 87 |
| | DOWN | pre- and mature miRNAs-16-1 downregulated in CLL | 87 |
| | DOWN | miR-16-1 extremely reduced in 13q14del patients | 74 |
| | DOWN | miR-16-1 downregulated with 13q14.3 deletions | 24 |
| | DOWN | Downregulation miR16-1 strongly correlated to biallelic but not monallelic 13q14.3 del CLL | 70 |
| | DOWN | reduction in the expression of the MUT alleles of miR-16-1 in del 13q. | 36 |
| | DOWN | down-expression of miR-16-1 in CLL | 74 |
| | DOWN | miR-16-1 decreased in about 11% of CLL cases | 66 |
| | DOWN | miR-16-1 (83G>C) downregulates the expression of mature miR-16-1. | 37 |
| | DOWN | miR16-1 (C-to-T) 7bp + in 3' resulsts in downregulation | 25 |
| | DOWN, PROG | miR16-1 expressed at low levels in patients with good prognosis | 58 |
| miR-16-2 | UP | pri-miRNA-16-2 in CLL | 87 |
| | UP | High miR-16-2 associated with no expression of ZAP-70 and mutated IgVH | 25 |
| | DOWN | pre- and mature miRNAs-16-2 downregulated in CLL | 87 |
| miR17 | UP | significantly up-regulated in CLL cells | 31 |
| | UP | in unmutated CLL | 78 |
| | UP | in ZAP-70 positive cases | 78 |
| miR17-3p | DOWN | showed very low expression in lymph node and blood CLL | 41 |
| miR17-5p | DOWN | down regulation in TP53 mutated cells | 64 |
| | DOWN | downregulation in both TP53 (MUT and not) cases in CLL cells | 63 |
| | DOWN | showed very low expression in lymph node and blood CLL | 41 |
| miR18a | DOWN | showed very low expression in lymph node and blood CLL | 41 |
| miR19 | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 |
| miR19a | UP | in CLL vs CD5+ cells | 24 |
| | UP | with 13q14.3 deletions | 24 |
| | DOWN | very low expression lymph node and blood CLL | 41 |
| miR19b | UP | higher expression in blood samples than in lymph node | 41 |
| miR20a | UP | significantly up-regulated in CLL cells | 31 |
| | UP | higher expression in blood samples than in lymph node | 41 |
| | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 |
| miR21 | UP | Upregulated in CLL | 85 |
| | UP | significantly up-regulated in CLL cells | 31 |
| | UP | was high in CLL with 17pDEL | 72 |
| | UP | overexpressed in CLL patients | 66 |
| | UP | overexpressed in CLL | 23 |
| | DOWN | in B-CLL with a normal karyotype | 67 |
| | PROGNOSIS | shorter OS in CLL with high miR-21 | 86 |

| | | | | |
|---|---|---|---|---|
| | PROGNOSIS | High expression =significantly unfavorable prognostic factor | 72 | |
| | Expression | No correlation of expression levels with IgH mutation or ZAP-70 status | 41 | |
| | Expression | not difference in expression | 67 | |
| miR22 | UP | in CLL vs CD5+ cells | 24 | |
| miR23 | UP | upregulated in unmutated IgVH | 63 | |
| miR23a | UP | significantly up-regulated in CLL cells | 31 | |
| | UP | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN | In CLL | 33 | |
| | DOWN | related with long interval from diagnosis to initial therapy. | 25 | |
| miR23b | UP | High miR-23b associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | UP, PROG | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN | downregulated in unmutated IgVH and wt p53 | 63 | |
| | DOWN, PROG | related with long interval from diagnosis to initial therapy. | 25 | |
| miR24 | DOWN | In CLL | 33 | |
| miR24-1 | UP | High miR-24-1 associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | UP | precursor upregulated in CLL vs CD5+ cells | 24 | |
| miR24-2 | UP | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN | related with long interval from diagnosis to initial therapy. | 25 | |
| | DOWN | in CLL vs MNC | 24 | |
| | DOWN | with 13q14.3 deletions | 24 | |
| miR25 | DOWN | in CLL vs CD5+ cells | 24 | |
| miR26a | UP | In CLL | 33 | |
| | UP | significantly up-regulated in CLL cells | 31 | |
| miR27b | DOWN | In CLL | 33 | |
| miR29 | UP | Upregulated in CLL | 85 | |
| | UP | results in development of indolent CLL | 27 | |
| | UP, PROG | over-expression in B-cells results in development of indolent CLL | 27 | |
| | DOWN | down regulation in TP53 mutated cells | 64 | |
| miR29a | UP | In CLL | 33 | |
| | UP | significantly up-regulated in CLL cells | 31 | |
| | UP | higher expression in samples with unmutated IgVH | 63 | |
| miR29a-2 | DOWN | Low miR-29a-2 associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| miR29b | UP | In CLL | 33 | |
| | UP | higher expression in samples with unmutated IgVH | 63 | |
| | UP | up-regulated on average in mutated IgVH vs not mutated | 23 | |
| | DOWN | down-expression in CLL | 74 | |
| | DOWN, PROG | Low expression in patients with unmutated IGHV | 74 | |
| | DOWN, PROG | in 17p-aggressive cases | 34 | |

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|---|---|---|---|---|
| miR29b-2 | DOWN | Low miR-29b-2 associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | DOWN | by a polymorphic insertion rs141961287 in patients with unmutated IGHV but not with mut IGHV | 37 | |
| miR29c | UP | upregulated in M-CLL against U-CLL | 84 | |
| | UP | In CLL | 33 | |
| | UP | in B-CLL | 67 | |
| | UP | in IGHV mutated B-CLL cases | 67 | |
| | UP | up-regulated on average in mutated IgVH vs not mutated | 23 | |
| | UP, PROG | related with long interval from diagnosis to initial therapy. | 25 | |
| | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| | DOWN | in CLL with 17p del and IGHV unmutated | 34 | |
| | DOWN | in 17p del-aggressive cases | 34 | |
| | DOWN | downregulation in both TP53 (MUT and not) cases in CLL cells | 63 | |
| | DOWN | Low miR-29c associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | DOWN, PROG | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN, PROG | Low expression associated with disease progression in 17p del CLL | 34 | |
| | DOWN, PROG | down-regulation in malignant B cells is an adverse prognostic indicator | 65 | |
| | DOWN, PROG | down-regulation associated with higher tumor burden, disease aggressiveness, and poor prognostic factors. | 35 | |
| | DOWN, PROG | associated with ZAP70+ and IgVH unmutated patients and better clinical outomes | 33 | |
| miR30b | UP | significantly up-regulated in CLL cells | 31 | |
| miR30d | DOWN | downregulated in CLL cells | 60 | |
| miR30e | UP | significantly up-regulated in CLL cells | 31 | |
| miR33 | UP | in CLL vs CD5+ cells | 24 | |
| miR34 | UP | in CLL vs CD5+ cells | 24 | |
| miR34a | UP | In CLL compared to untransformed B cells | 33 | |
| | UP | significantly up-regulated in CLL cells | 31 | |
| | UP | expression increased in the leukemic phase and wild TP53 | 71 | |
| | UP | heterogeneous up-regulation | 68 | |
| | DOWN | Deletion of 17p is associated with low basal expression of miR-34a | 62 | |
| | DOWN | Low expression of miR-34a associated with TP53 mutations in the absence of 17p deletion | 62 | |
| | DOWN | downregulated in CLL patients carrying 17p13/TP53 deletions | 61 | |
| | DOWN | was down in CLL with 17pDEL | 72 | |
| | DOWN | in CLL with 17p del and IGHV unmutated | 34 | |
| | DOWN | down regulation in TP53 mutated cells | 64 | |
| | DOWN | downregulation in both TP53 (MUT and not) cases in CLL cells | 63 | |
| | DOWN | In CLL with TP53 mutations or 17p deletions | 71 | |
| | DOWN | downregulation is not associated with an (IGHV) mutation status | 61 | |

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|---|---|---|---|---|
| | DOWN, PROG | low miR-34a levels predicted shorter time to treatment | 71 | |
| | DOWN, PROG | More aggressive disease in the miR-34a low group | 73 | |
| | PROGNOSIS | IgVH mutational status, CD38 expression, or presence of del13q14 and trisomy 12 had no impact on miR-34a expression | 71 | |
| miR-34b/c cluster | Expression | lack of expression of miR-34b/c in CLL | 62 | |
| | Expression | expression was not detectable | 61 | |
| miR92 | DOWN | downregulated in CLL | 23 | |
| | Expression | No correlation of expression levels with IgH mutation or ZAP-70 status | 41 | |
| miR92a | UP | Upregulated in in ZAP70− patients | 33 | |
| miR92-1 | UP | in CLL vs CD5+ cells | 24 | |
| miR92-2 | DOWN | in CLL vs MNC | 24 | |
| miR96 | UP | in CLL vs CD5+ cells | 24 | |
| miR101 | UP | In CLL | 33 | |
| | UP | significantly up-regulated in CLL cells | 31 | |
| | UP | overexpressed in CLL | 23 | |
| | UP | in CLL vs CD5+ cells | 24 | |
| | DOWN | showed very low expression lymph node and blood CLL | 41 | |
| miR103 | DOWN | In CLL compared to untransformed B cells | 33 | |
| miR105-1 | UP | in CLL vs CD5+ cells | 24 | |
| miR106b | UP | significantly up-regulated in CLL cells | 31 | |
| miR107 | DOWN | down-regulated in CLL | 68 | |
| miR123 | UP | in CLL vs CD5+ cells | 24 | |
| miR124a-2 | UP | in CLL vs CD5+ cells | 24 | |
| miR125a | DOWN | down-regulated in CLL | 68 | |
| miR125b | DOWN | miR-125b expressed at low levels in both indolent and aggressive CLL | 89 | |
| | DOWN | MiR-125b is down-regulated in CLL | 81 | |
| miR126 | DOWN | downregulated in CLL cells | 80 | |
| | DOWN | down-regulated in CLL | 68 | |
| miR126+ | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR128b | DOWN | in CLL vs MNC | 24 | |
| miR-129-3p | UP | upregulated in 17p-aggressive cases | 34 | |
| miR130a | DOWN | significantly down-regulated in CLL cells | 31 | |
| | DOWN | downregulated in CLL cells | 80 | |
| miR-130b | UP | upregulated in 17p-aggressive cases | 34 | |
| miR132 | UP | precursor upregulated in CLL vs CD5+ cells | 24 | |
| | UP | in CLL vs CD5+ cells | 24 | |
| miR134 | UP | in CLL vs CD5+ cells | 24 | |
| miR135a | DOWN, PROG | low levels relationed with poorer prognostic outcome | 82 | |
| miR136 | UP | precursor upregulated in CLL vs CD5+ cells | 24 | |
| | UP | with 13q14.3 deletions | 24 | |

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|---|---|---|---|
| miR138-1 | UP | upregulated in CLL vs MNC | 24 |
| miR139 | DOWN | promoters found to be hypermethylated | 68 |
| miR140 | UP | in CLL vs CD5+ cells | 24 |
| miR141 | UP | precursor upregulated in CLL vs CD5+ cells | 24 |
| | UP | in CLL vs CD5+ cells | 24 |
| miR142-3p | UP | significantly up-regulated in CLL cells | 31 |
| | DOWN | downregulated in unmutated IgVH and wt p53 | 63 |
| miR142-5p | UP | significantly up-regulated in CLL cells | 31 |
| miR143 | UP | upregulated in M-CLL against U-CLL | 84 |
| | DOWN | downregulated in CLL cells | 80 |
| | DOWN | significantly decreased in B-cell malignancies | 57 |
| miR145 | DOWN | significantly decreased in B-cell malignancies | 57 |
| miR146 | UP | High miR-146 associated with no expression of ZAP-70 and mutated IgVH | 25 |
| | UP, PROG | related with short interval from diagnosis to initial therapy. | 25 |
| | DOWN | in CLL vs MNC | 24 |
| | DOWN, PROG | related with long interval from diagnosis to initial therapy. | 25 |
| miR146b | DOWN | In IgVH unmutated cases | 68 |
| miR146b-5p | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 |
| miR148 | UP | in CLL vs CD5+ cells | 24 |
| miR148b | UP | significantly up-regulated in CLL cells | 31 |
| miR150 | UP | upregulated in M-CLL against U-CLL | 84 |
| | UP | In CLL | 33 |
| | UP | upregulated in B-CLL | 67 |
| | UP | overexpressed in CLL | 23 |
| | UP | up-regulated on average in mutated IgVH vs not mutated | 23 |
| | Expression | No correlation of expression levels with IgH mutation or ZAP-70 status | 41 |
| miR151-3p | DOWN | in CLL with 17p del and IGHV unmutated | 34 |
| miR152 | UP | precursor upregulated in CLL vs CD5+ cells | 24 |
| miR153 | UP | precursor upregulated in CLL vs CD5+ cells | 24 |
| miR154 | UP | in CLL vs CD5+ cells | 24 |
| | UP | with 13q14.3 deletions | 24 |
| miR155 | UP | miR-155 significantly overexpressed | 86 |
| | UP | Upregulated in CLL | 85 |
| | UP | In CLL | 33 |
| | UP | In CLL compared to untransformed B cells | 33 |
| | UP | Elevated levels in B-CLL | 75 |
| | UP | significantly up-regulated in CLL cells | 31 |
| | UP | was high in CLL with 17pDEL | 72 |
| | UP | in CLL | 68 |
| | UP | upregulated in B-CLL | 67 |
| | UP | overexpressed in CLL patients | 66 |

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|---|---|---|---|
| | UP | overexpressed in CLL | 60 |
| | UP | overexpressed in CLL | 23 |
| | UP | High miR-155 associated with no expression of ZAP-70 and mutated IgVH | 25 |
| | UP, PROG | expression is higher in aggressive CLL | 60 |
| | UP, PROG | expression is higher in aggressive CLL | 25 |
| | UP, PROG | related with short interval from diagnosis to initial therapy. | 25 |
| | UP, PROG | expression increasing with disease progression | 67 |
| | DOWN | B-CLL with a normal karyotype | 67 |
| | DOWN, PROG | related with long interval from diagnosis to initial therapy. | 25 |
| | DOWN, PROG | expression lower (P = .0303) in CLL with complete response than with other responses | 86 |
| miR181 | DOWN | in 17p-aggressive cases | 34 |
| miR181a | UP, PROG | related with short interval from diagnosis to initial therapy. | 25 |
| | UP, PROG | trisomy 12 abnormality and high expressiona associated with more aggressive CLL | 34 |
| | DOWN | In CLL | 33 |
| | DOWN | In CLL compared to untransformed B cells | 33 |
| | DOWN | down-expression in CLL | 74 |
| | DOWN | Low expression in patients with unmutated IGHV | 74 |
| | DOWN | significantly down-regulated in CLL cells | 31 |
| | DOWN | downregulated in CLL cells | 80 |
| | DOWN | showed very low expression lymph node and blood CLL | 41 |
| | DOWN | downregulated in CLL cells | 60 |
| | DOWN, PROG | downregulation associated with adverse outcomes in CLL | 31 |
| | DOWN, PROG | related with long interval from diagnosis to initial therapy. | 25 |
| miR181b | UP | precursor upregulated in CLL vs CD5+ cells | 24 |
| | DOWN | In CLL | 33 |
| | DOWN | In CLL compared to untransformed B cells | 33 |
| | DOWN | down-expression in CLL | 74 |
| | DOWN | Low expression in patients with unmutated IGHV | 74 |
| | DOWN | significantly down-regulated in CLL cells | 31 |
| | DOWN | down in CLL with 17pDEL | 72 |
| | DOWN | differentially downregulated in the treated CLL patients samples | 72 |
| | DOWN | down-regulated in CLL | 68 |
| | DOWN, PROG | Decreased expression in progressive disease | 79 |
| | DOWN, PROG | low expression=significantly unfavorable prognostic factor | 72 |
| | DOWN, PROG | downregulation associated with adverse outcomes in CLL | 31 |
| miR183 | UP | precursor upregulated in CLL vs CD5+ cells | 24 |

| | | | | |
|---|---|---|---|---|
| miR186 | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| miR188 | UP | in CLL vs CD5+ cells | 24 | |
| miR190 | UP | in CLL vs CD5+ cells | 24 | |
| miR191 | DOWN | in CLL vs MNC | 24 | |
| miR192 | UP | significantly up-regulated in CLL cells | 31 | |
| | DOWN | in CLL vs CD5+ cells | 24 | |
| miR193 | UP | precursor upregulated in CLL vs MNC | 24 | |
| miR195 | UP | High miR-195 associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | DOWN | with 13q14.3 deletions | 24 | |
| miR196-2 | UP | in CLL vs CD5+ cells | 24 | |
| miR200a | UP | in CLL vs MNC | 24 | |
| miR201 | UP | precursor upregulated in CLL vs MNC | 24 | |
| miR203 | DOWN | with 13q14.3 deletions | 24 | |
| miR213 | DOWN | in CLL vs CD5+ cells | 24 | |
| miR217 | UP | in CLL vs CD5+ cells | 24 | |
| | UP | with 13q14.3 deletions | 24 | |
| miR218-2 | UP | precursor expresed at higher levels in samples with 13q14.3 deletions | 24 | |
| miR220 | DOWN | in CLL vs MNC | 24 | |
| | DOWN | in CLL vs CD5+ cells | 24 | |
| | DOWN | with 13q14.3 deletions | 24 | |
| miR221 | UP | High miR-221 associated with no expression of ZAP-70 and mutated IgVH | 25 | |
| | UP, PROG | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN | in CLL vs MNC | 24 | |
| | DOWN | with 13q14.3 deletions | 24 | |
| | DOWN, PROG | related with long interval from diagnosis to initial therapy. | 25 | |
| miR222 | UP | related with short interval from diagnosis to initial therapy. | 25 | |
| | DOWN | downregulated in CLL | 23 | |
| | DOWN | related with long interval from diagnosis to initial therapy. | 25 | |
| miR223 | UP | upregulated in M-CLL against U-CLL | 84 | |
| | UP | in IGHV mutated B-CLL cases | 67 | |
| | UP | up-regulated on average in mutated IgVH vs not mutated | 23 | |
| | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| | DOWN | expression significantly decreased in CLL | 76 | |
| | DOWN | expression lower in patients with elevated β(2)-microglobulin and unmutated IgVH | 76 | |
| | DOWN | In CLL | 33 | |
| | DOWN | in B-CLL | 67 | |
| | DOWN | in CLL vs MNC | 24 | |
| | DOWN | associated with ZAP70+ and IgVH unmutated patients and better clinical outomes | 33 | |
| | DOWN | Low miR-223 associated with no expression of ZAP-70 and mutated IgVH | 25 | |

| | | | | |
|---|---|---|---|---|
| | DOWN, PROG | Low expression associated with disease progression in 17p del CLL | 34 | |
| | DOWN, PROG | down-regulation in malignant B cells is an adverse prognostic indicator | 65 | |
| | DOWN, PROG | down-regulation associated with higher tumor burden, disease aggressiveness, and poor prognostic factors. | 35 | |
| | DOWN, PROG | expression decreased with progression from early to advanced clinical stages | 76 | |
| | PROGNOSTIC | Absence predicts shorter PFS | 76 | |
| | PROGNOSTIC | decrease in levels of MIR223 with increasing Binet stage | 67 | |
| miR324-3p | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| miR326 | DOWN | downregulated in CLL cells | 80 | |
| | DOWN | down-regulated in CLL | 68 | |
| miR342-3p | UP | In CLL cells compared to untransformed B cells | 33 | |
| | DOWN | significantly down-regulated in CLL cells | 31 | |
| | DOWN | down-regulated in 17p-aggressive cases | 34 | |
| miR342-5p | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR367 | DOWN | down-regulated in 17p-aggressive cases | 34 | |
| miR424 | DOWN | down-regulated in CLL | 68 | |
| miR-453 | UP | upregulated in 17p-aggressive cases | 34 | |
| miR483-5p | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR484 | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| miR494 | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR497 | DOWN | was down in CLL with 17pDEL | 72 | |
| miR572 | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR574-5p | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR582 | DOWN | promoters found to be hypermethylated | 68 | |
| miR-632 | UP | upregulated in 17p-aggressive cases | 34 | |
| miR638 | DOWN | significantly down-regulated in CLL cells | 31 | |
| | UP | upregulated in 17p-aggressive cases | 34 | |
| miR640 | DOWN | in CLL with trisomy 12 | 34 | |
| miR650 | UP | In CLL cells using subgenes from V2 family for IgLλ | 77 | |
| | UP, PROG | High expression related with longer OS and time to first treatment | 77 | |
| miR660 | UP | significantly up-regulated in CLL cells | 31 | |
| | DOWN | downregulated in CLL, AIHA developing, malignat B-Cells. | 83 | |
| | DOWN | in CD38+ versus CD38− CLL cells | 68 | |
| miR768-5p | DOWN | significantly down-regulated in CLL cells | 31 | |
| | UP | upregulated in 17p-aggressive cases | 34 | |
| miR801 | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR923 | DOWN | significantly down-regulated in CLL cells | 31 | |
| miR1225-5p | DOWN | significantly down-regulated in CLL cells | 31 | |
| miRLET7a | DOWN | downregulated in CLL cells | 60 | |

| | | | | |
|---|---|---|---|---|
| miRLET-7f | UP | significantly up-regulated in CLL cells | 31 | |
| | DOWN | in CLL vs MNC | 24 | |
| Let7g | UP | In CLL | 33 | |
| | UP | upregulated in IgVH mutated patients | 33 | |
| Let-7d-v2 | DOWN | precursor downregulated in CLL vs CD5+ cells | 24 | |
| UP= miRNA expression upregulated | | DOWN=miRNA expression downregulated | | |
| PROGNOSIS= Prognostic value | | Meth= Expression affected by methylation | | |

Supplementary table 2. miRNA SNPs studied in CLL

| SNP | miRNA | Asociación with CLL | Allele | Ref. |
|---|---|---|---|---|
| **rs9989532** | pri-miR-1-2 | Not described | (C-to-T) 15bp + in 5' | 36 |
| **rs78641532** | pri-miR-1-2 | Not described | (A-to-G) 74bp + in 5' | 36 |
| **rs41276928** | pri-miR-7-2 | not described | (C-to-T) 42bp + in 5' | 36 |
| rs146020563 | pre-miR-15b | No | 46bp (A-to-G) | 37 |
| novel variation | pri-miR 16-1 | Yes | (C-to-T) 7bp + in 3' | 25 |
| novel variation | pri-miR-15a/16-1cluster | extremely rare (<0.5%) in CLL | (C-to-T) 7bp + in 3' | 37 |
| novel variation | miR16-1 | not informative as a prognostic and diagnostic risk factor | (C-to-T) 7bp + in 3' | 90 |
| novel variation | pre-miR-16-1 | extremely rare (<0.5%) in CLL | 83 (G-to-C) | 37 |
| rs73528445 | pri-miR-24-1 | Not described | (G-to-A) 152bp + in 3' | 36 |
| novel variation | pri-miR-24-1 | involved in familial CLL | (C-to-T) + 40 bp in 3' | 36 |
| **rs41292017** | pri-miR-26a-2 | No | (C-to-T) 10bp in 3´ | 37 |
| **rs11671784** | pre-miR-27a | No | 36bp (C-to-T) | 37 |
| **rs895819** | pre-miR-27a | No | 40bp (A-to-C/G/T) | 37 |
| novel variation | pri-miR-27b | Yes | (G-to-A) +50bp in 3' | 25 |
| novel variation | pri-miR-29a | associated with CLL pathogenesis/prognosis | +22 (T-to-C) in 3´ | 37 |
| novel variation | pri-miR-29b-2 | Yes | (G-to-A) 212bp + in 3' | 25 |
| rs141961287 | pri-miR-29b-2 | Yes when samples from the 1000 genomes project versus CLL patients | (ins A) 107bp + in 3' | 37 |
| rs141961287 | pri-miR-29b-2 | Yes | (ins A) 107bp + in 3' | 25 |

| | | | | |
|---|---|---|---|---|
| novel variation | pri-miR-29b-2 | associated with CLL pathogenesis/prognosis | -256 (A-to-G) in 5´ | 37 |
| **rs114790693** | pri-miR-29b-2 | No | (G-to-A) 100bp in 5´ | 37 |
| **rs12401619** | pri-miR-29b-2 | Yes when samples from the 1000 genomes project versus CLL patients | (C-to-G) 408bp in 5´ | 37 |
| **rs12410786** | pri-miR-29b-2 | Yes when samples from the 1000 genomes project versus CLL patients | −337 (A-to-T) | 37 |
| rs145834945 | pri-miR-29b-2 | No | (C-to-G) 169bp in 5´ | 37 |
| **rs56075814** | pri-miR-29b-2 | No | (A-to-G) 81bp in 5´ | 37 |
| **rs78876157** | pri-miR-29b-2 | No | (G-to-A) 37bp in 5´ | 37 |
| rs150749580 | pri-miR-29c | No | (G-to-A) 31bp + in 5' | 25 |
| rs150749580 | pri-miR-29c | No | (G-to-A) 31bp + in 5' | 37 |
| rs147139948 | pri-miR-29c | No | (T-to-A) 137bp in 3' | 37 |
| **rs1358379** | pri-miR-30a | Not described | (A-to-G) 118 bp + in 3' | 25 |
| novel variation | pri-miR-30a | Not described | (C-to-T) 32bp + in 5' | 36 |
| novel variation | pri-miR-33a | Not described | (G-to-A) 242bp + in 5' | 36 |
| novel variation | pri-miR-93 | Not described | (A-to-G) 380bp + in 3' | 36 |
| **rs11939078** | pri-miR-95 | Not described | (G-to-A) 100bp + in 3' | 36 |
| **rs77249161** | pri-miR-95 | Not described | (A-to-G) 40bp + in 3' | 36 |
| **rs11939078** | pri-miR-95 | Not described | (C-to-T) 49bp + in 5' | 36 |
| **rs41274239** | pri-miR-96 | Not described | 36 (G-to-A) | 36 |
| rs73159662 | pre-miR-96 | No | 42bp (C-to-T) | 37 |
| **rs543412** | pri-miR-100 | Yes when samples from the 1000 genomes project versus CLL patients | (G-to-A) 10bp in 3´ | 37 |
| **rs455864** | pri-miR-101-2 | Not described | (G-to-C) 46bp + in 3' | 36 |
| **rs10974810** | pri-miR-101-2 | Not described | (A-to-G) 56bp + in 3' | 36 |
| **rs462480** | pri-miR-101-2 | Not described | (T-to-G) 61bp + in 3' | 36 |
| novel variation | pre-miR-106b | implicated in the biology of other tumors | 45 (C-to-T) | 37 |
| **rs41292412** | pre-miR-122a | No | 53bp (C-to-T) | 25 |
| **rs12976445** | pri-miR-125a | Not described | (T-to-C) 54bp + in 5' | 36 |
| novel variation | pri-miR-133a-1 | Not described | (C-to-T) 159bp + in 3' | 36 |

| | | | | |
|---|---|---|---|---|
| **rs2102066** | pri-miR-140 | Not described | (G-to-A) 10bp + in 3' | 36 |
| not described | mat-miR-142-3p | implicated in the biology of other tumors | 62bp (C-to-T) | 37 |
| **rs13158382** | pri-miR-143 | Not described | (C-to-T) 7bp + in 5' | 36 |
| **rs41291957** | pri-miR-143 | Not described | (G-to-A) 91bp + in 5' | 36 |
| **rs2910164** | pri-miR-146a | Not described | (G-to-C) + 18 bp in 3' | 25 |
| **rs2910164** | mat-miR-146a* | Yes when samples from the 1000 genomes project versus CLL patients | 60 (C-to-G) | 37 |
| **rs12940701** | pri-miR-152 | Not described | (C-to-T) 7bp + in 3' | 36 |
| **rs41280120** | pri-miR-152 | Not described | (C-to-G) 79bp + in 3' | 36 |
| **rs41286572** | pri-miR-154 | Yes when samples from the 1000 genomes project versus CLL patients | (G-to-A) 6bp in 3´ | 37 |
| rs113162007 | pri-miR-181b-1 | No | (G-to-C) 4bp in 3´ | 37 |
| **rs76481776** | pre-miR-182 | No | 106bp (G-to-A) | 37 |
| rs189301510 | pri-miR-185 | Not described | (C-to-T) 73bp + in 5' | 36 |
| novel variation | pri-miR186 | Not described | (A-to-T) 27bp + in 5' | 36 |
| rs74699517 | pri-miR-187 | Yes | (T-to-C) 73bp + in 3' | 25 |
| novel variation | pre-miR-187 | No | 34 (G-to-A) | 25 |
| rs57733751 | pri-miR-188 | Not described | (T-to-C) 32bp + in 3' | 25 |
| **rs1889470** | pri-miR-197 | Not described | (T-to-C) 94bp + in 5' T/C | 36 |
| **rs1700** | pri-miR-198 | Not described | (C-to-T) 61bp + in 3' | 36 |
| novel variation | pri-miR-206 | Not described | (A-to-G) 33bp + in 5' | 25 |
| novel variation | pre-miR-206 | Yes | (G-to-T) 49 nt before the pre- | 25 |
| novel variation | pri-miR-206 | Yes | (A-to-T) -116 in 5´ direction | 25 |
| **rs919001** | pri-miR-211 | Not described | (G-to-A) 87bp + in 5' | 36 |
| **rs3820455** | pri-miR-215 | Not described | (A-to-G) 173bp + in 3' | 36 |
| **rs10865292** | pri-miR-216a | Not described | (A-to-G) 33bp + in 5' | 36 |
| **rs11134527** | pri-miR-218-2 | Not described | (G-to-A) 96bp + in 3' | 36 |
| **rs213210** | pri-miR-219-1 | Not described | (T-to-C) 103bp + in 3' | 36 |
| **rs107822** | pri-miR-219-1 | Not described | (G-to-A) 37bp + | 36 |

| | | | | |
|---|---|---|---|---|
| | | | in 5' | |
| deleted from miRbase | pri-miR-220a | Not described | 33 (G-to-A) | 36 |
| rs186354597 | pre-miR-223 | No | 22bp (G-to-A) | 37 |
| **rs56103835** | pre-miR-323b | Yes when European samples from the 1000 genomes project VS CLL patients | 1bp (T-to-C) | 37 |
| rs75330474 | mat-miR-323b | No | 34bp (C-to-T) | 37 |
| novel variation | pre-miR-372 | implicated in the biology of other tumors | 13 (T-to-C) | 37 |
| **rs61992670** | pri-miR-409_miR-412 | No | (C-to-T) 51bp in 5´ | 37 |
| **rs61992671** | mat-miR-412 | Yes when samples from the 1000 genomes project versus CLL patients | 71bp (A-to-G) | 37 |
| rs76090066 | pre-miR-431 | No | 12bp (C-to-T) | 37 |
| rs139043404 | pri-miR-655 | No | (G-to-A) 7bp in 5´ | 37 |
| **rs58834075** | pre-miR-656 | Yes when samples from the 1000 genomes project versus CLL patients | 33bp (C-to-T) | 37 |
| | | | | |
| | Significative in our study | **rs00000** | MAF>0.01 | |
| | No frequency data/enough frequency in European population | | Significative in bibliography | |

| Supplementary table 4. P values after FDR | | |
|---|---|---|
| | p.obs | p.adj |
| [1] | 0.022550 | 0.05 |
| [2] | 0.032750 | 0.05 |
| [3] | 0.039100 | 0.05 |
| [4] | 0.045860 | 0.05 |
| [5] | 0.034790 | 0.05 |
| [6] | 0.040780 | 0.05 |
| [7] | 0.031980 | 0.05 |
| [8] | 0.004707 | 0.05 |
| [9] | 0.036270 | 0.05 |
| [10] | 0.011640 | 0.05 |
| [11] | 0.046750 | 0.05 |
| [12] | 0.013170 | 0.05 |
| [13] | 0.018860 | 0.05 |
| [14] | 0.018700 | 0.05 |
| [15] | 0.024640 | 0.05 |
| [16] | 0.027440 | 0.05 |

| | | |
|---|---|---|
| [17] | 0.018400 | 0.05 |
| [18] | 0.029500 | 0.05 |
| [19] | 0.046060 | 0.05 |
| [20] | 0.027370 | 0.05 |
| [21] | 0.050000 | 0.05 |
| [22] | 0.050000 | 0.05 |
| [23] | 0.050000 | 0.05 |
| [24] | 0.050000 | 0.05 |
| [25] | 0.050000 | 0.05 |
| [26] | 0.050000 | 0.05 |
| [27] | 0.050000 | 0.05 |
| [28] | 0.050000 | 0.05 |
| [29] | 0.050000 | 0.05 |
| [30] | 0.050000 | 0.05 |
| [31] | 0.050000 | 0.05 |
| [32] | 0.050000 | 0.05 |
| [33] | 0.050000 | 0.05 |
| [34] | 0.050000 | 0.05 |
| [35] | 0.050000 | 0.05 |
| [36] | 0.050000 | 0.05 |
| [37] | 0.050000 | 0.05 |
| [38] | 0.050000 | 0.05 |
| [39] | 0.050000 | 0.05 |
| [40] | 0.050000 | 0.05 |
| [41] | 0.050000 | 0.05 |
| [42] | 0.050000 | 0.05 |
| [43] | 0.050000 | 0.05 |
| [44] | 0.050000 | 0.05 |
| [45] | 0.050000 | 0.05 |
| [46] | 0.050000 | 0.05 |
| [47] | 0.050000 | 0.05 |
| [48] | 0.050000 | 0.05 |
| [49] | 0.050000 | 0.05 |
| [50] | 0.050000 | 0.05 |
| [51] | 0.050000 | 0.05 |
| [52] | 0.050000 | 0.05 |
| [53] | 0.050000 | 0.05 |
| [54] | 0.050000 | 0.05 |
| [55] | 0.050000 | 0.05 |
| [56] | 0.050000 | 0.05 |
| [57] | 0.050000 | 0.05 |
| [58] | 0.050000 | 0.05 |
| [59] | 0.050000 | 0.05 |
| [60] | 0.050000 | 0.05 |

| | | |
|---|---|---|
| [61] | 0.050000 | 0.05 |
| [62] | 0.050000 | 0.05 |
| [63] | 0.050000 | 0.05 |
| [64] | 0.050000 | 0.05 |
| [65] | 0.050000 | 0.05 |
| [66] | 0.050000 | 0.05 |
| [67] | 0.050000 | 0.05 |
| [68] | 0.050000 | 0.05 |
| [69] | 0.050000 | 0.05 |
| [70] | 0.050000 | 0.05 |
| [71] | 0.050000 | 0.05 |
| [72] | 0.050000 | 0.05 |
| [73] | 0.050000 | 0.05 |
| [74] | 0.050000 | 0.05 |
| [75] | 0.050000 | 0.05 |
| [76] | 0.050000 | 0.05 |
| [77] | 0.050000 | 0.05 |
| [78] | 0.050000 | 0.05 |
| [79] | 0.050000 | 0.05 |
| [80] | 0.050000 | 0.05 |
| [81] | 0.050000 | 0.05 |
| [82] | 0.050000 | 0.05 |
| [83] | 0.050000 | 0.05 |
| [84] | 0.050000 | 0.05 |
| [85] | 0.050000 | 0.05 |
| [86] | 0.050000 | 0.05 |
| [87] | 0.050000 | 0.05 |
| [88] | 0.050000 | 0.05 |
| [89] | 0.050000 | 0.05 |
| [90] | 0.050000 | 0.05 |
| [91] | 0.050000 | 0.05 |
| [92] | 0.050000 | 0.05 |
| [93] | 0.050000 | 0.05 |
| [94] | 0.050000 | 0.05 |
| [95] | 0.050000 | 0.05 |
| [96] | 0.050000 | 0.05 |
| [97] | 0.050000 | 0.05 |
| [98] | 0.050000 | 0.05 |
| [99] | 0.050000 | 0.05 |
| [100] | 0.050000 | 0.05 |
| [101] | 0.050000 | 0.05 |
| [102] | 0.050000 | 0.05 |
| [103] | 0.050000 | 0.05 |
| [104] | 0.050000 | 0.05 |

| | | |
|---|---|---|
| [105] | 0.050000 | 0.05 |
| [106] | 0.050000 | 0.05 |
| [107] | 0.050000 | 0.05 |
| [108] | 0.050000 | 0.05 |
| [109] | 0.050000 | 0.05 |
| [110] | 0.050000 | 0.05 |
| [111] | 0.050000 | 0.05 |
| [112] | 0.050000 | 0.05 |
| [113] | 0.050000 | 0.05 |
| [114] | 0.050000 | 0.05 |
| [115] | 0.050000 | 0.05 |
| [116] | 0.050000 | 0.05 |
| [117] | 0.050000 | 0.05 |
| [118] | 0.050000 | 0.05 |
| [119] | 0.050000 | 0.05 |
| [120] | 0.050000 | 0.05 |
| [121] | 0.050000 | 0.05 |
| [122] | 0.050000 | 0.05 |
| [123] | 0.050000 | 0.05 |
| [124] | 0.050000 | 0.05 |
| [125] | 0.050000 | 0.05 |
| [126] | 0.050000 | 0.05 |
| [127] | 0.050000 | 0.05 |
| [128] | 0.050000 | 0.05 |
| [129] | 0.050000 | 0.05 |
| [130] | 0.050000 | 0.05 |
| [131] | 0.050000 | 0.05 |
| [132] | 0.050000 | 0.05 |
| [133] | 0.050000 | 0.05 |
| [134] | 0.050000 | 0.05 |
| [135] | 0.050000 | 0.05 |
| [136] | 0.050000 | 0.05 |
| [137] | 0.050000 | 0.05 |
| [138] | 0.050000 | 0.05 |
| [139] | 0.050000 | 0.05 |
| [140] | 0.050000 | 0.05 |
| [141] | 0.050000 | 0.05 |
| [142] | 0.050000 | 0.05 |
| [143] | 0.050000 | 0.05 |
| [144] | 0.050000 | 0.05 |
| [145] | 0.050000 | 0.05 |
| [146] | 0.050000 | 0.05 |
| [147] | 0.050000 | 0.05 |
| [148] | 0.050000 | 0.05 |