Chronic lymphocytic leukemias with trisomy 12 show a distinct DNA methylation profile linked to altered chromatin activation

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Supplemental Methods

Patient samples

The study group included samples of 232 CLL patients from the Hospital Clinic of Barcelona that belong to the International Cancer Genome Consortium. In addition, we included a series of 23 CLL patients from the Greek cohort in Thessaloniki, Greece. All cases were diagnosed with CLL according to the guidelines of the International Workshop Chronic Lymphocytic Leukemia/National Cancer Institute (iwCLL/NCI). All patients were untreated and did not carry a complex karyotype. The main clinico-biological features of the two cohorts are summarized in **Supplementary Table 1**. The study was approved by the local Ethics Review Committee of the participating institutions.

Illumina 450k DNA methylation arrays and data analysis

Methylation estimates of 255 CLL cases, analyzed by the 450k Human Methylation Array (Illumina), were mined. We excluded the CpGs that overlapped with the chromosomal abnormalities of the study group. The above filtering resulted in a total number of 426627 CpG sites. CpG sites were considered as differentially methylated by applying criteria such as: i) a minimum absolute difference between mean beta-values (beta difference) of the two subgroups and ii) the statistical significance was evaluated with Benjamini& Hochberg. The 232 microarray data are available at ICGC and the 23 were available at: https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-7575.

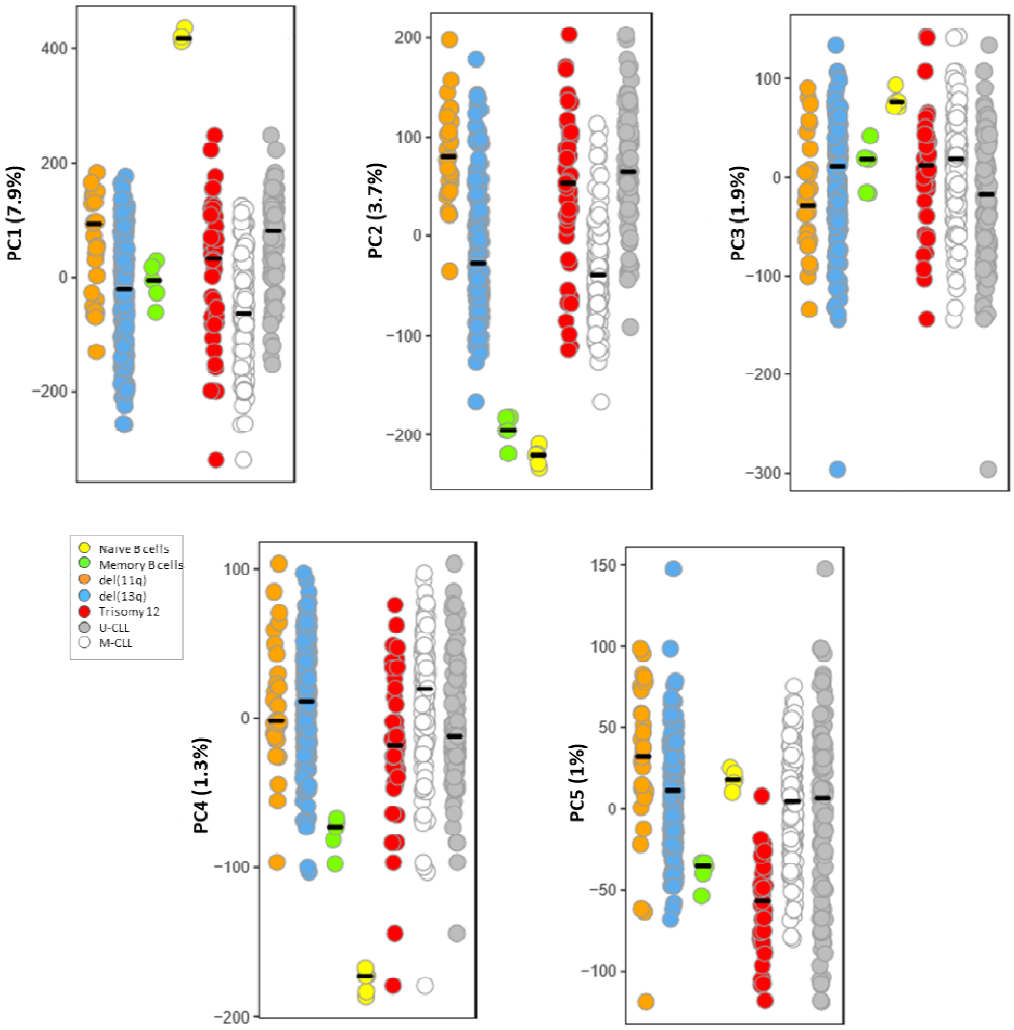
Genomic location, chromatin states and TFBS enrichment analysis

The DMCpGs were characterized according to their genomic locations and chromatin states for memory B cells from healthy donors, recently published (18). The p-value was calculated using the hypergeometric distribution, when enrichment analysis was performed.We performed TFBS analysis using the MEME suite and AME motif enrichment based on the criterions of average odds score andRanksum test.

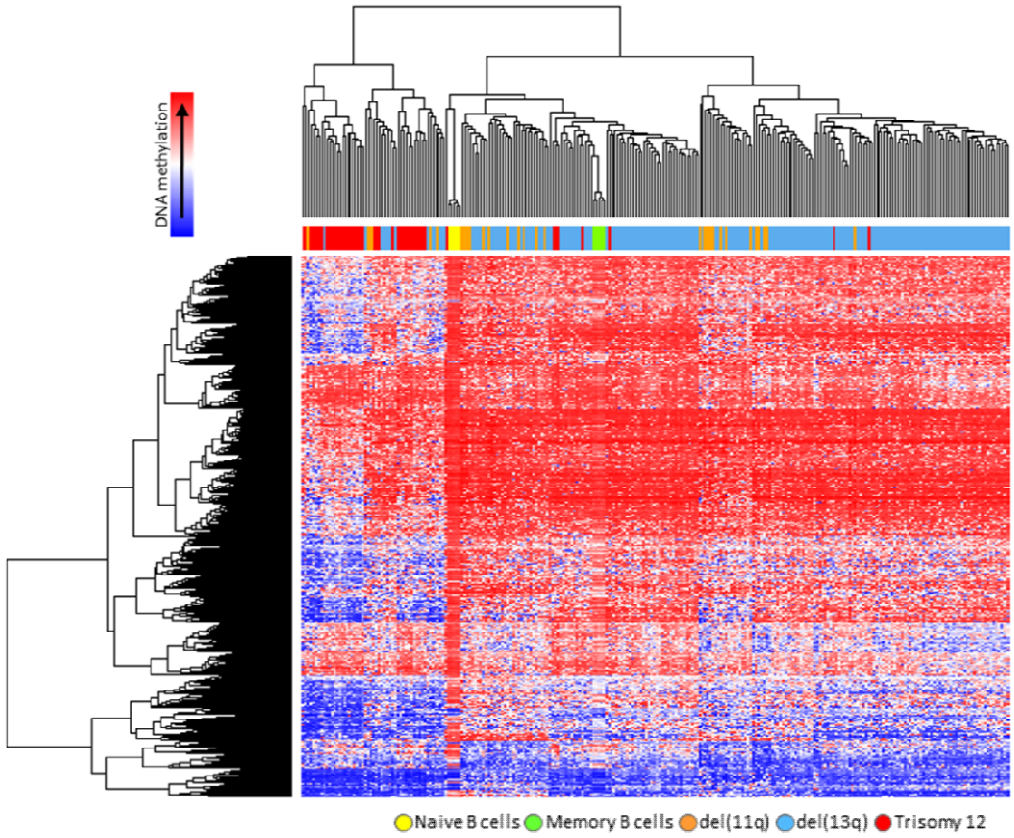
Linking DNA methylation signature with active regions, chromatin accessibility and gene expression

The interpretation of the CpGs were performed by previously published data from expression arrays, ChiP-seq for H3K27ac antibody and ATAC-seq of the same samples and they are part of the BLUEPRINT epigenome project, at the European Genome-Phenome Archive (EGA) which is hosted at the European Bioinformatics Institute. We investigated the overlap of the genomic ranges between the CpGs of interest with the peaks of H3K27ac and ATAC which were detected in a previous study of our group(1). The differential expression and peak analysis were examined using limma and DeSeq2, respectively.

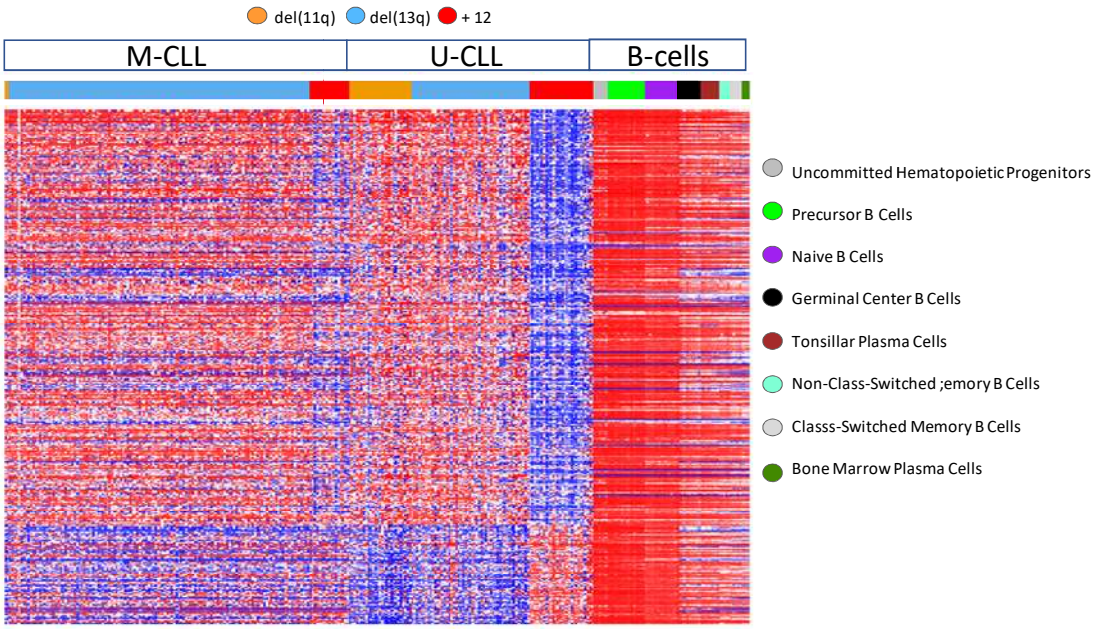
Supplemental Figures



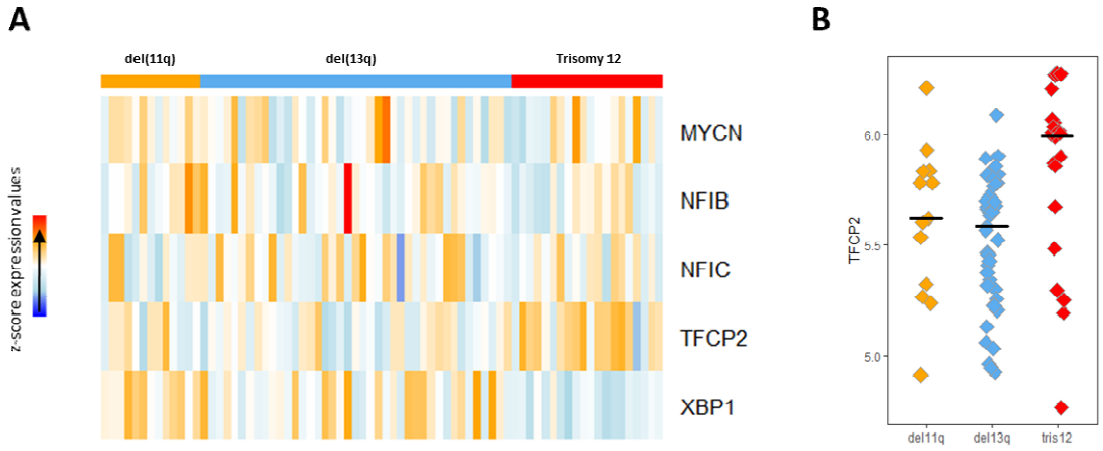
Supplemental Figure 1. The boxplots represent the PC values in each of the 5 principal components in relation to cytogenetic markers aberrations, somatic hypermutation status of the IGHV genes and normal B cells. Each color points to a particular subgroup: orange for del(11q), blue for del(13q), red for tris12, green for memory B cells, yellow for naïve B cells, white for M-CLL, grey for U-CLL



Supplemental Figure 2. Hierarchical clustering based on 1760 CpGs whose PC5 values correlated with the b-values (|r|=0.3)



Supplemental Figure 3. DNA methylation levels of 646 DMCpGs in +12 U-CLLs in the context of M-CLLs and the entire B cell differentiation program.



Supplemental Figure 4. A. Heatmap of expression levels in U-CLL casesof the 5 TFs highlighted from the TF binding site analysis. The expression levels are indicated as row z-scores **B.** Dot plot with median showing the expression levels of the *TFCP2* gene in each CLL cytogenetic subgroup.

Supplemental Tables

- Supplemental Table 1: Demographic, clinical and biological data for the patient cohort and B cell subpopulations from healthy donors.

- Supplemental Table 2: 646 DMCpGs revealed after differential methylation analysis between trisomy 12 vs non-trisomy 12 U-CLL.

- Supplemental Table 3: 312 CpG sites which showed overlap with H327ac-associated regions and the results of DeSeq2 for the differences on H3K27ac.

- Supplemental Table 4: 52 accessible and acetylation-associated regions which showed hypomethylation between tris12 and non-tris12 U-CLL.

- Supplemental Table 5: List of the differentially expressed genes (FDR<0.05) associated with the TADs on analysis of the tris12 vs non-tris12 U-CLL.

References

1. Beekman R, Chapaprieta V, Russinol N, Vilarrasa-Blasi R, Verdaguer-Dot N, Martens JHA, et al. The reference epigenome and regulatory chromatin landscape of chronic lymphocytic leukemia. Nat Med. 2018;24(6):868-80.