Genetic and Epigenetic disorders in one cohort of Acute Lymphoblastic Leukemia

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INTRODUCTION

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is a heterogeneous disease. Approximately 60% of BCP-ALL present aberrations involving chromosome 21 (chr 21), including high hyperdiploid, *ETV6-RUNX1* fusion and intrachromosomal amplification of the chromosome 21 (iAMP21). Contributing with this process, epigenetic mechanisms could regulate the transcription and induce leukemogenesis. Polymorphisms in genes involved in folate metabolism could influence this aberrant methylation.

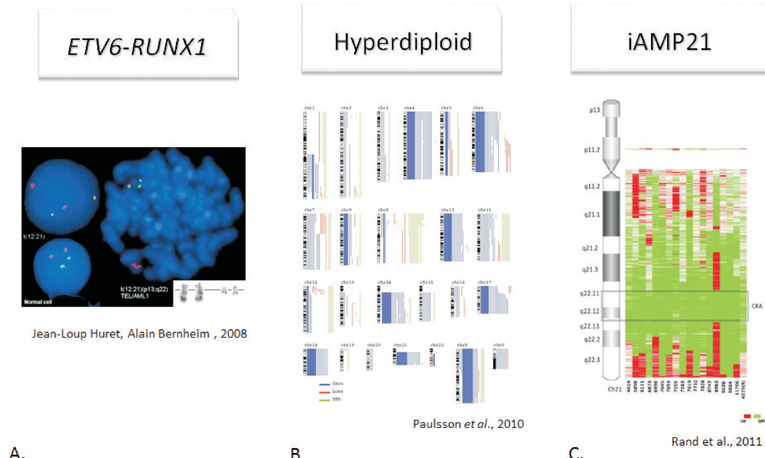

Figure 1: Recurrent chromosome 21 alteration in acute lymphoblastic leukemia. A. *ETV6-RUNX1* gene fusions. B. Hyperdiploid acute lymphoblastic leukemia. C. Intrachromosomal amplification of the chromosome 21.

AIMS

This study aims to characterize the genetic and epigenetic profile of BCP-ALL with chr 21 aberrations, as well as identify the epigenetic signatures of different ALL subgroups.

METHODS

A series of 373 BCP-ALL were selected for CNA analysis concerning the chromosome 21. Multiplex ligation probe amplification (MLPA, SALSA P327_A1 and P327_B1) was performed according to manufacturer instructions. FISH was performed using the "LPH012 *TEL/AML1* translocation, dual fusion probe and centromere probes to the Chr 4, 8, 10, 14, 17, 18, X and Y (Cytocell, Cambridge, UK). Additionally, to the DNA from cases of BCP-ALL, controls and remission samples was modified with EZ DNA Methylation™ Kit (Zymo Research, Irvine, CA) and analyzed by the HumanMethylation450 Infinium Assay (Illumina, San Diego, CA). For the array validation and global (LINE-1) methylation we analyzed not just the samples with chr 21 gains, but also ALL from different subtypes using pyrosequencing. The *MTHFR* rs1801133 was identified by PCR-RFLP. The statistical analysis was performed using RStudio software with Bioconductor packages.

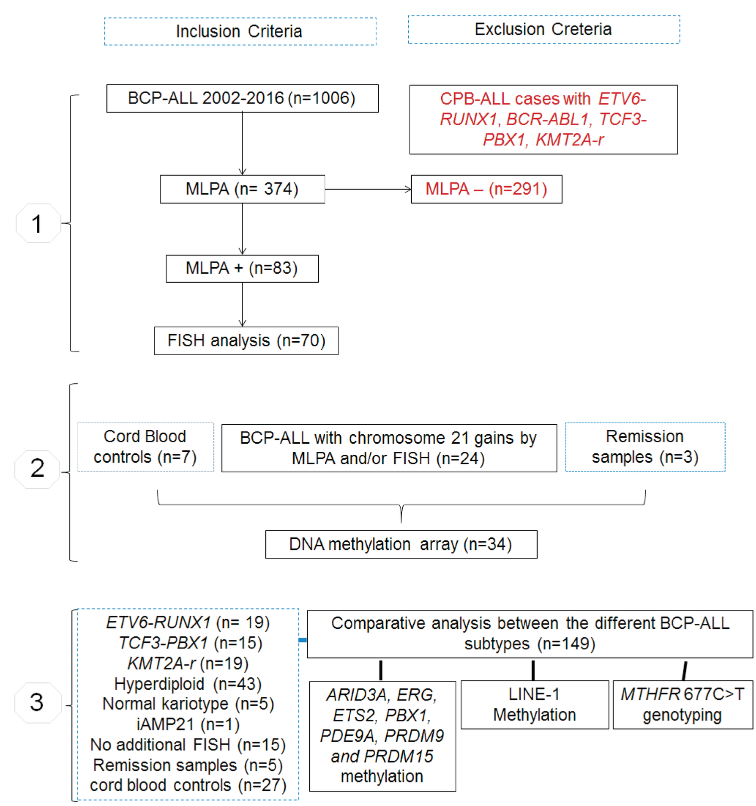

Figure 2: Study design. This study has been performed in 3 steps. 1. The identification of the chromosome 21 gains. For this step, cases with excluding gene fusions were removed. 2. Global DNA methylation and copy number alteration characterization. In this step, we included 24 BCP-ALL samples with evidence of chromosome 21 gains, cord blood controls (n=7) and remission samples (n=3). 3. Comparative analysis between the different ALL subtypes. In this step, we compared gene-specific methylation, LINE-1 methylation and *MTHFR* genotypes across the ALL subtypes.

RESULTS

We found evidence of gains in chr 21 in 83 samples analyzed by MLPA. Most gains were verified by FISH and 11 samples had ≥ 5 *RUNX1* signals. The centromere probes characterized 53 samples as hyperdiploid. The gain of an extra copy of chr 21 was confirmed in all cases in addition to other gains: chr 4 (58%), 10 (57%), 14 (84%), 17 (53%), 18 (60%), X (86%) and Y (46%). Losses of the chr 17 (2%) and Y (4%). Two BCP-ALL with ETV6/RUNX1 had an extra copy of the chr 21. One patient had iAMP21 identified by MLPA and confirmed by FISH with telomere probe targeted to chr 13 and 21. The case *vs* control analysis regarding the DNA methylation showed 30.046 differentially methylated CpG sites (adjusted p <0.001). We observed that the methylation profiles of cases and controls were distinct, while the controls and remission samples showing similar profiles. In the validation analysis, the *ARID3A* gene was found hypermethylated in controls when compared with BCP-ALL and remission samples. The hyperdiploid group presents lower methylation levels in *ARID3A*, *ERG*, *PDE9A*, *PRDM9* and *PRDM15* genes while BCP-ALL with *KTM2A-r* showed a higher methylation level for *ARID3A*, *PDE9A*, *PRDM9* and *PRDM15* genes. The *ETV6-RUNX1* cases had an intermediate *ARID3A* methylation level and, lastly, the *TCF3-PBX1* cases presented high *PRDM15* methylation when compared with the other BCP-ALL subtypes, but lower than the *KTM2A-r* group. The gene-specific methylation was capable of predicting ALL subtypes with good accuracy. For LINE-1, the hyperdiploid group presented a distinct profile of increased methylation level in comparison with the other BCP-ALL subtypes and heathy controls. *MTHFR* rs1801133 was not associated with methylation changes.

Table 1: Demography and clinical features of B-cell precursor acute lymphoblastic leukemia, Brazil, 2002-2015

Characteristics	Positive MLPA n (%)	Negative MLPA n (%)	P value
Total	83 (22)	291 (78)	
Sex			
Males	47 (57)	164 (56)	
Females	36 (43)	127 (44)	0.899
Age (ys)			
⩽1	4 (5)	34 (12)	
2-10	67 (81)	177 (61)	
>10	12 (14)	80 (27)	0.004**
Ethnicity			
Whites	42 (51)	117 (43)	
Non -Whites	39 (49)	157 (57)	0.146
WBC, $x10^9/l$			
<50	59 (73)	198 (69)	
>50	22 (27)	88 (31)	0.491
Immunophenotype			
CD10 -	0 (0)	29 (10)	
CD10 +	83 (100)	262 (90)	<0.001***
NCI Risk group			
Standard risk	49 (60)	139 (48)	
High risk	32 (40)	152 (52)	0.135
Status			
Alive	30 (71)	28 (65)	
Dead	12 (29)	15 (35)	0.214

Abbreviation: n, number; ys, years; WBC, white blood cell count $x10^9/l$; NCI, National Cancer Institute; **, p<0.05; ***, p<0,001. The number can vary due to missing data.

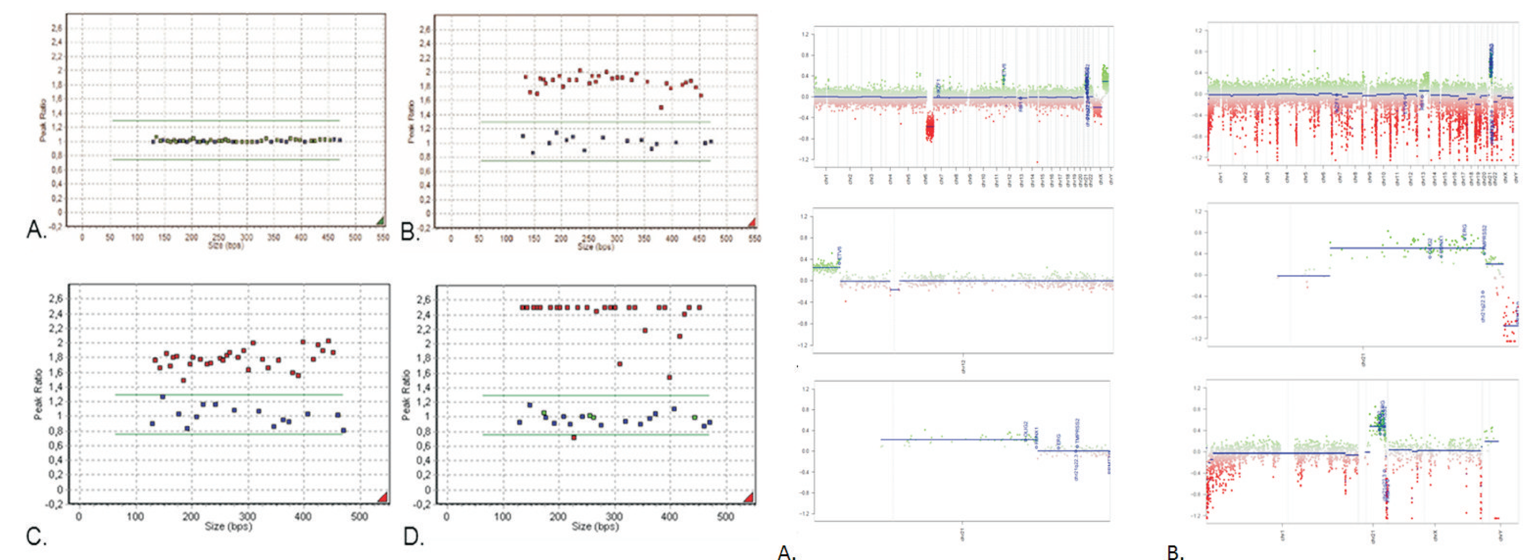

Figure 3: Screen shots of the graphics showing chromosome 21 by the MLPA using GeneMarker software. A. A normal sample with no gains in the chromosome 21. B, C and D are examples of samples with gains involving the chromosome 21.

Figure 5: Different profiles of chromosome 21 gains by array analysis. A. One sample *ETV6-RUNX1* +21. B. One iAMP21 sample.

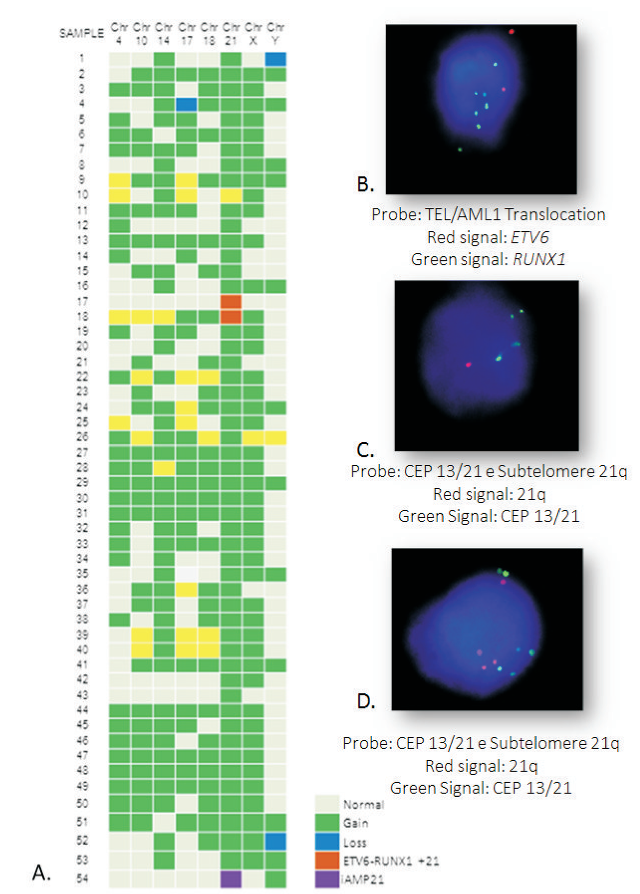

Figure 4: FISH results showing chromosome 21 gains. A. Heat map plotting the FISH results from the telomeric probes depicted by color-coded squares. B. Interphase FISH showing one cell with 7 *RUNX1* signals. C. Interphase FISH showing four centromere signals (2 chr 13 and 2 chr 21) and one telomere signal showing 21q deletion in the iAMP21 blasts. D. Interphase FISH showing six centromere signals (2 chr 13 and 4 chr 21) and four telomere signals (21q) in one hyperdiploid cell.

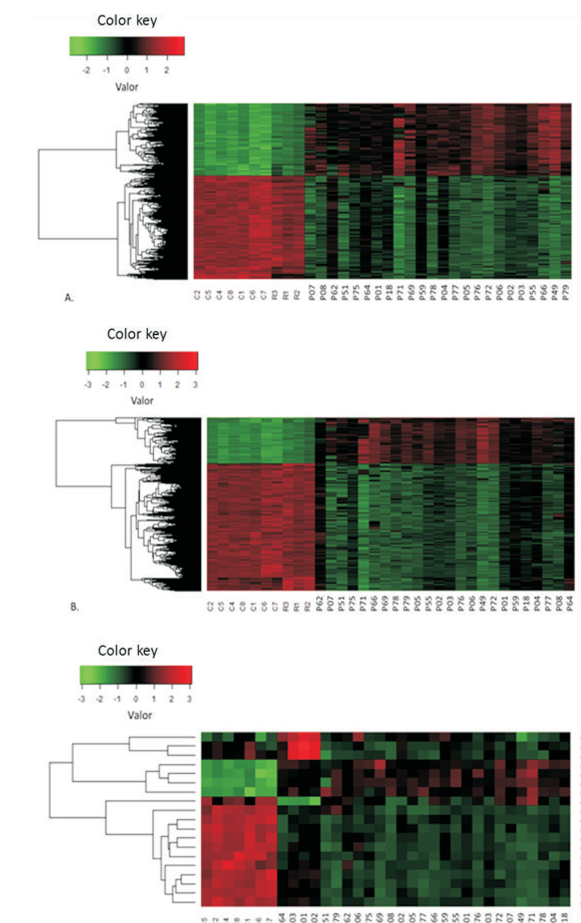

Figure 6: Heat map of the DNA methylation array. A. The 5.000 most significant different methylated sites in the case-control analysis. B. The 5.000 most significant different methylated sites in the case-remission analysis. C. Heat map showing 18 different methylated sites in the control-remission analysis.

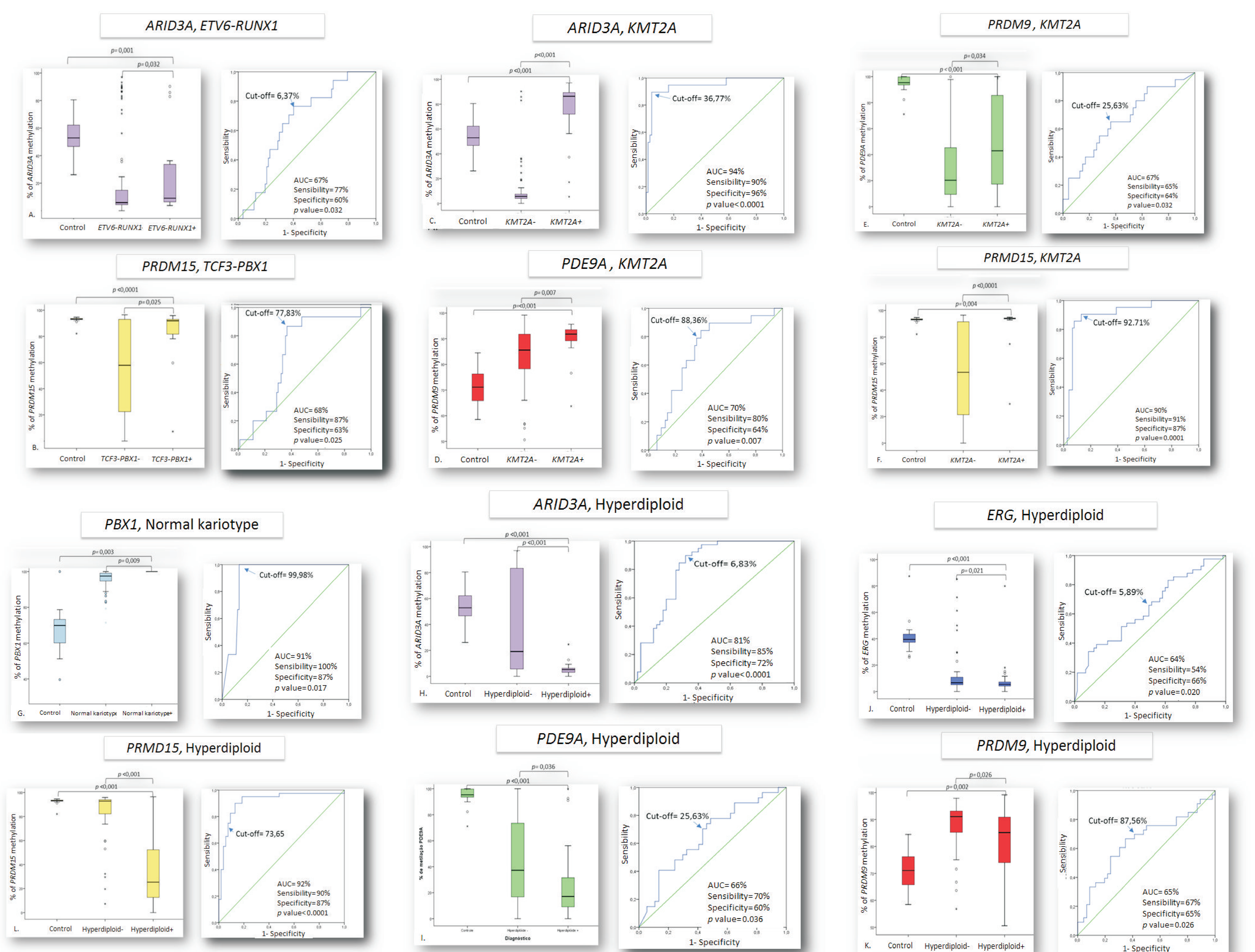

Figure 7: Box-plot graphs and ROC curve showing gene-specific DNA methylation profile differing between acute lymphoblastic leukemia subtypes. AUC, area under the curve.

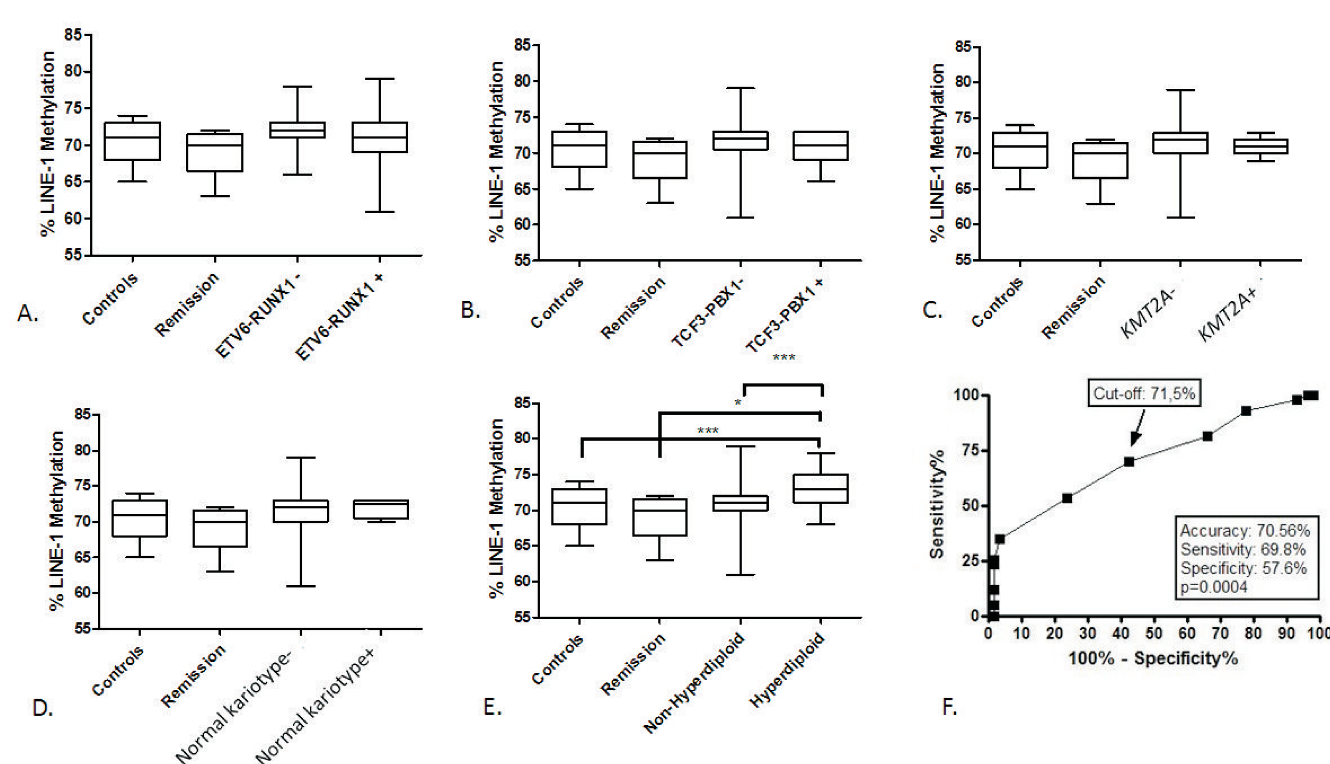

Figure 8: LINE-1 methylation in the *ETV6-RUNX1* (A), *TCF3-PBX1* (B), *KMT2A* (C), Normal kariotype, Hyperdiploid, controls and remission samples. Each ALL subgroup was compared against all other subtypes. F. The ROC curve showing the value of LINE-1 methylation test in the hyperdiploid identification.

CONCLUSION

To discriminate the iAMP21 from hyperdiploid subgroup, we recommend the use of centromeric and/or telomeric FISH probes. The BCP-ALL presents a distinct global and gene-specific DNA methylation signature, which are also subtype-specific.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

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