CHARACTERIZATION OF *IKZF1* COMPLETE DELETIONS IN CHILDHOOD ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

IKZF1 deletion (Δ*IKZF1*) is an important predictor of relapse in childhood B-cell precursor acute lymphoblastic leukemia (BCP-ALL). Therefore, PCR systems to generate a rapid diagnostic to identify Δ*IKZF1* are of clinical importance. We previously mapped the breakpoints of intragenic deletions and developed a multiplex PCR (MP-PCR) assay to detect recurrent intragenic Δ*IKZF1*. Since MP-PCR was not able to detect complete deletions (*IKZF1* Δ1-8), which accounts for ~30% of all Δ*IKZF1*, we aimed at investigating the genomic scenery of *IKZF1* Δ1-8.

METHODS

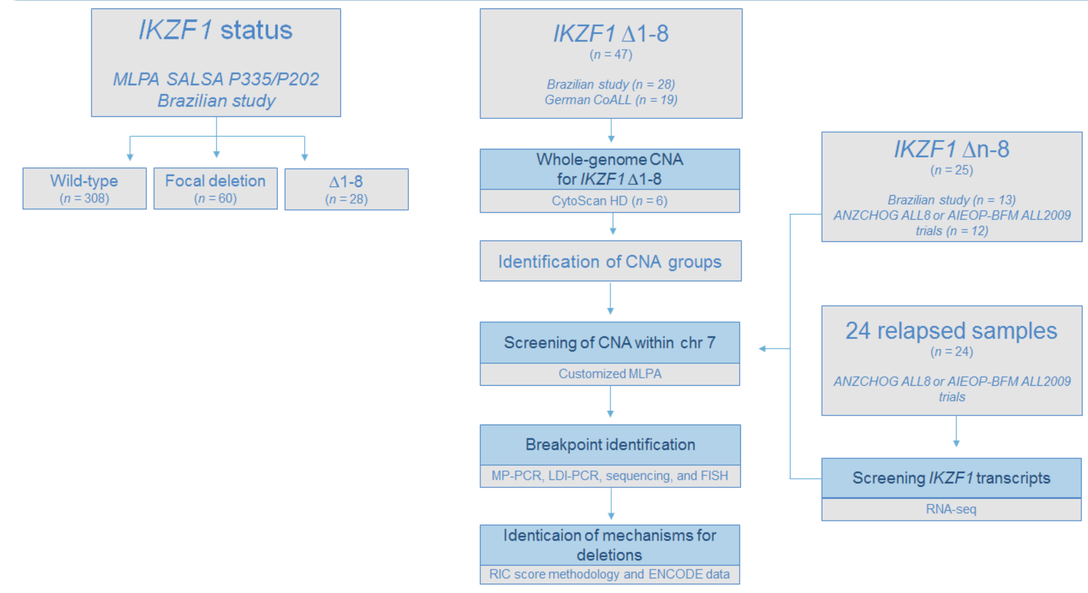

Figure 1. Study flowchart

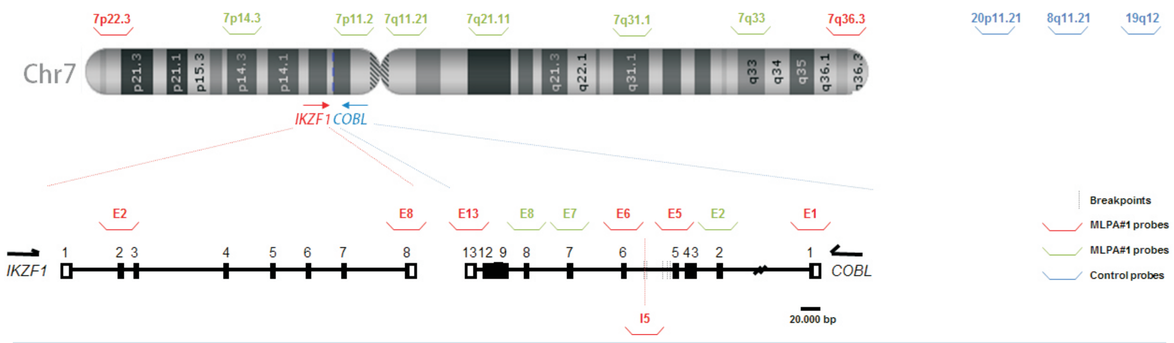

Figure 2. Customized MLPA for screening of CNA within chromosome 7

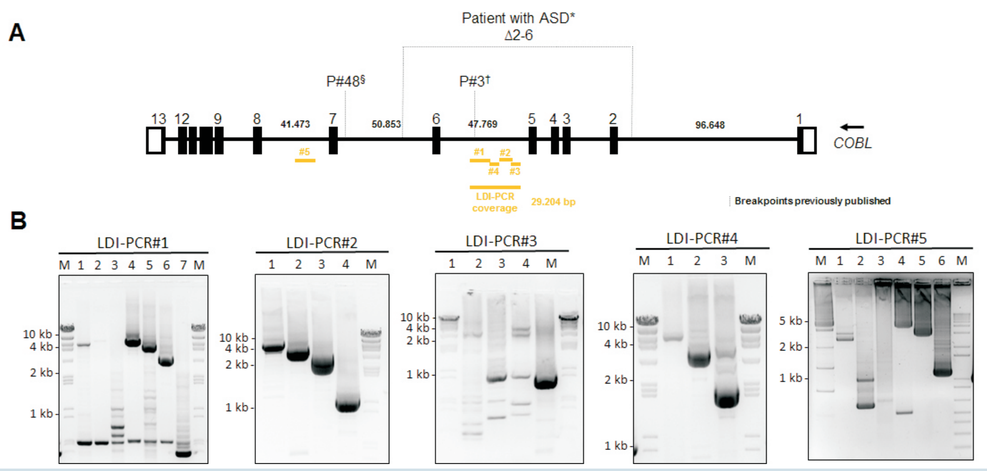

Figure 3. Design of the LDI-PCR for the identification of breakpoints within *COBL*.

RESULTS

Table 1. Clinical and laboratorial characteristics of patients

Characteristics	*IKZF1* complete deletion n (%)
Gender	
Male	27 (60.0)
Female	18 (40.0)
Age at diagnosis (yrs)	
≤ 1	3 (6.7)
1-9	32 (71.1)
10-18	10 (22.2)
WBC (x10⁶/l)	
< 50,000	32 (71.1)
≥ 50,000	13 (28.9)
ALL subtype	
Pro-B	3 (6.7)
c-ALL	29 (64.4)
Pre-B	13 (28.9)

ALL, acute lymphoblastic leukemia; c-ALL, common ALL; WBC, white blood cell count.

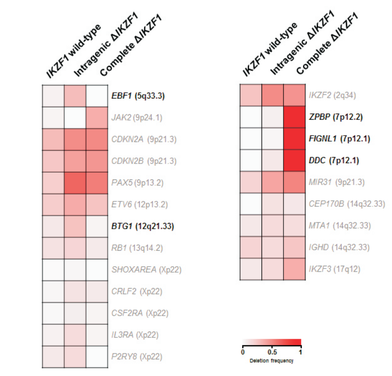

Figure 4. Frequency of CNAs in pediatric BCP-ALL according to *IKZF1* status. Genes with differential CNA frequency among complete and intragenic *IKZF1* deletions were highlighted in bold.

RESULTS

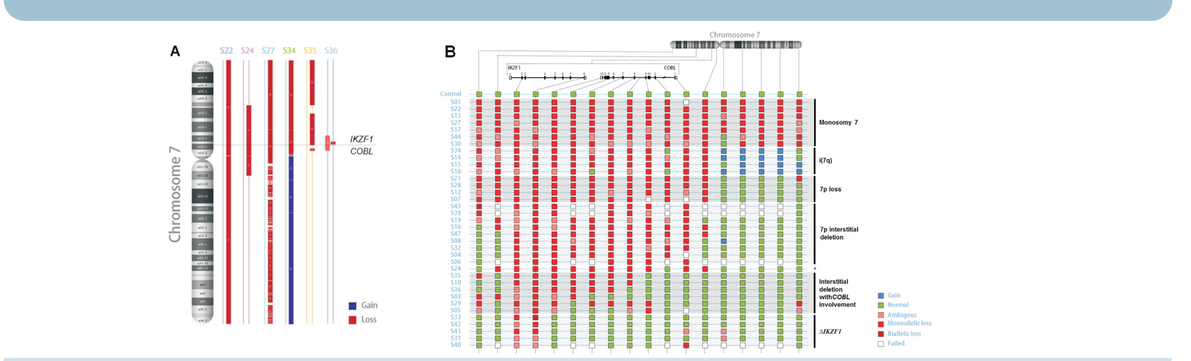

Figure 5. Copy number alterations in the chromosome 7 of samples with complete deletion of *IKZF1*. (A) The array analysis identified different CNA whithin chromosome 7; interestingly two out of six samples with *IKZF1* Δ1-8 had breakpoints within *COBL* intron 5. (B) A custom MLPA analysis identified six groups of CNA within chromosome 7 for patients with *IKZF1* Δ1-8. Notably, most of them presented monosomy 7, large interstitial deletions, which also presented breakpoints within *COBL*.

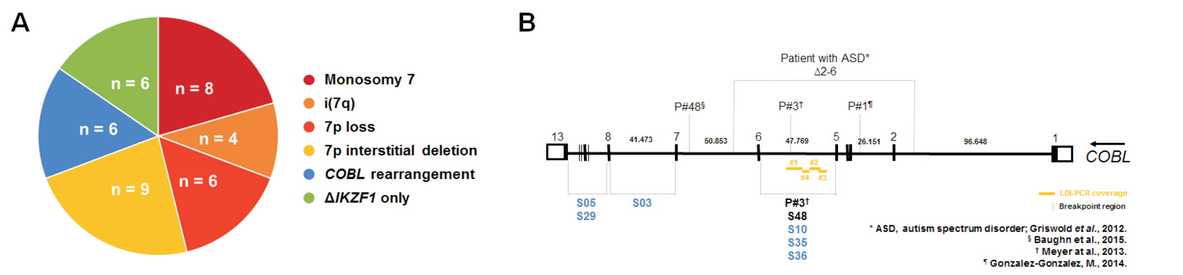

Figure 6. (A) Description of chromosomal alterations associated to *IKZF1* complete deletions, including *COBL* rearrangements. (B) Map of breakpoints within *COBL*, as detected by MLPA and/or LDI-PCR analysis.

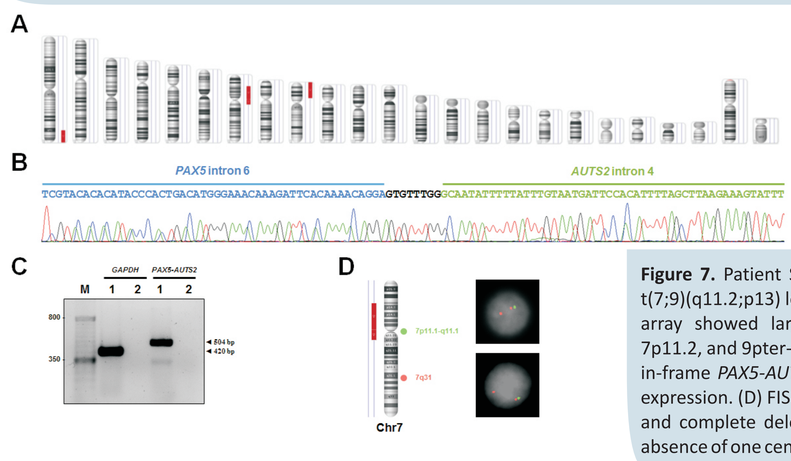

Figure 7. Patient S24 presented na unbaleced translocation t(7;9)(q11.2;p13) leading to *IKZF1* complete deletion. (A) SNP-array showed large deletions within 1q41-1qter, 7p15.2-7p11.2, and 9pter-p13.2; (B) Sequencing analysis identified an in-frame *PAX5-AUTS2* fusion with (C) the chimeric transcript expression. (D) FISH analysis demonstrates loss of derivative 7 and complete deletion of *IKZF1* (7p12), as observed by the absence of one centromeric probe (green).

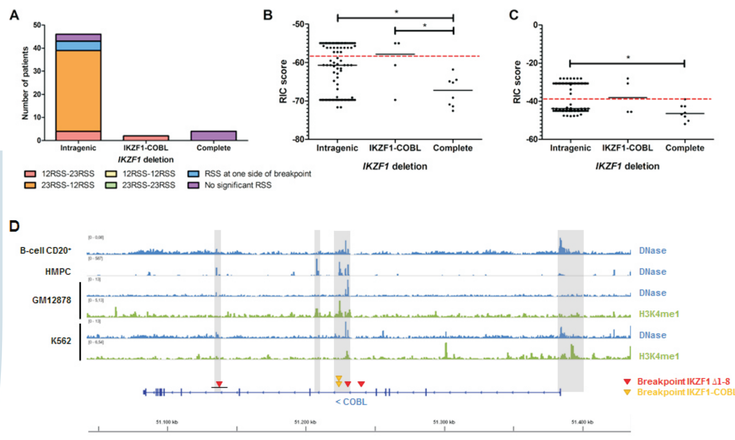

Figure 8. Comparisson of RAG-consensus sequences at breakpoint sites of intragenic and complete deletions of *IKZF1*. (A) Intragenic deletions, which includes *IKZF1-COBL* fusions, are marked by 12RSS and 23RSS recombination, while complete deletions do not present significant RAG-consensus sequences. Analysis of (B) 12RSS and (C) 23RSS at the breakpoint regions showing absence of RAG-analogous sequences for complete deletions. * *p*-value < 0.05

CONCLUSION

IKZF1 Δ1-8 are defined by several numeric and structural changes on chromosome 7. They are mainly represented by monosomy and large interstitial deletions, which recurrently have breakpoints within *COBL*, a novel hotspot located ~611 Kb downstream of *IKZF1*. We also described *IKZF1-COBL* fusions, establishing that there is a breakpoint cluster within *COBL* intron 5 for both complete and 3' end deletions of *IKZF1*. Although intragenic deletions of *IKZF1* are associated to a RAG-type mechanism, this work demonstrates that complete deletion are not generated by this process and such breakpoints overlap open chromatin sites. Finally, we designed MLPA probes on *COBL*, and suggest its addition to current diagnostic MLPA assays. (Lopes et al. *COBL* is a novel hotspot for *IKZF1* deletions in childhood acute lymphoblastic leukemia. Oncotarget, 2016).

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