# *IKZF1* INTRAGENIC DELETIONS IN PEDIATRIC B-CELL PRECURSOR ACUTE LYMPHOBLASTIC LEUKEMIA

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## INTRODUCTION

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is caused by accumulation of genetic alterations, such *IKZF1* deletion, which are considered a high risk biomarker. The detection of *IKZF1* deletions (Δ*IKZF1*) has a great clinical impact, and an efficient method was established for the detection of the main types of Δ*IKZF1*. Thus, the present study aims to investigate the clinical and laboratory profile of patients with other intragenic Δ*IKZF1*. RAG (Recombination Activating Gene)-mediated recombination has been associated with the generation of recurrent intragenic Δ*IKZF1*, after mapping the breakpoint sequences, we will also search for motifs related RAG recombination.

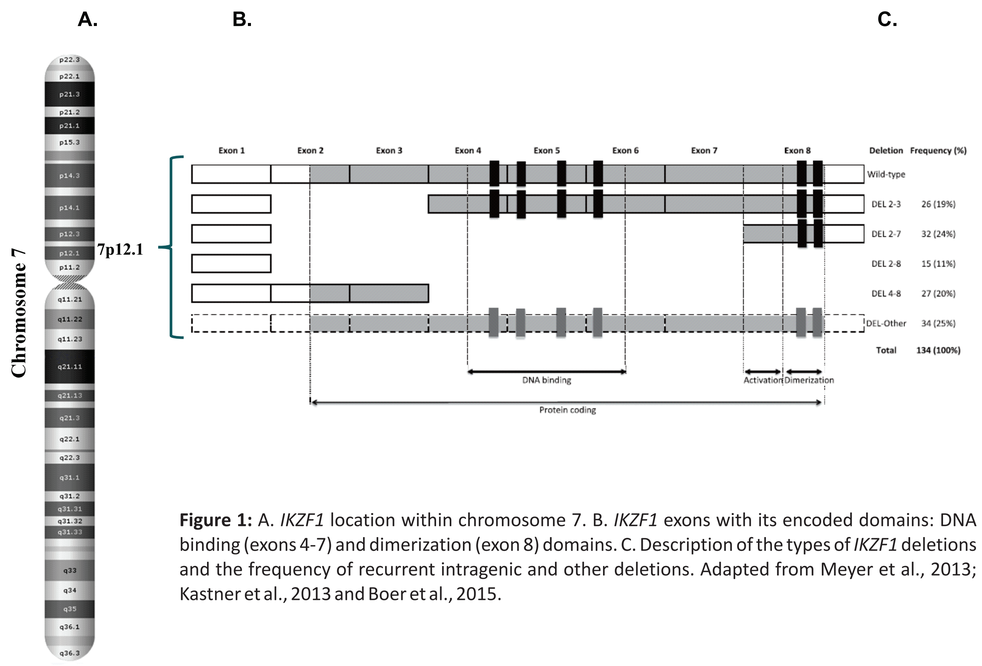


**Figure 1:** A. *IKZF1* location within chromosome 7. B. *IKZF1* exons with its encoded domains: DNA binding (exons 4-7) and dimerization (exon 8) domains. C. Description of the types of *IKZF1* deletions and the frequency of recurrent intragenic and other deletions. Adapted from Meyer et al., 2013; Kastner et al., 2013 and Boer et al., 2015.

## METHODS

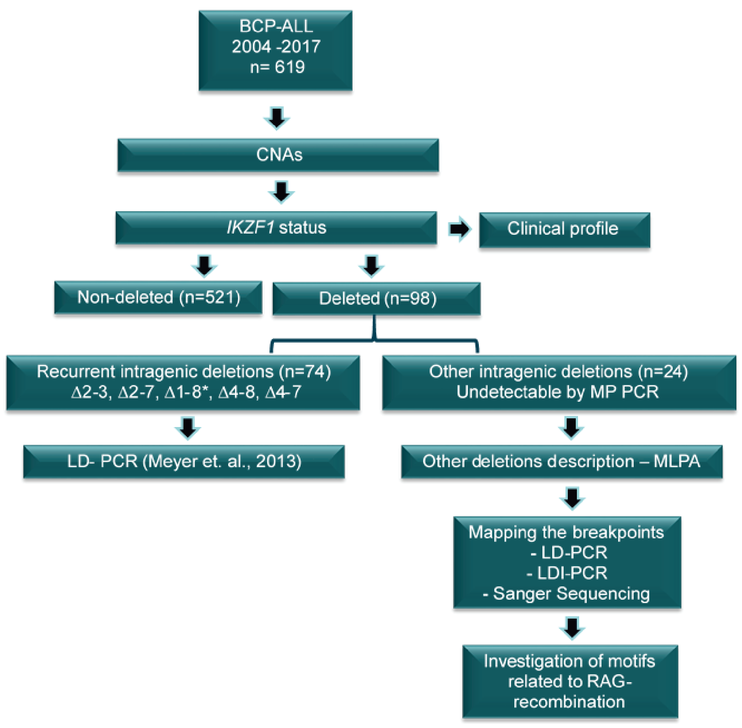


**Figure 2:** Study flowchart. Pediatric patients (n=619) diagnosed with BCP-ALL (2004-2017) are included in this study. DNA was obtained from mononuclear cells of the bone marrow (MO) of patients. The multiplex ligation-dependent probe amplification (MLPA) SALSA P335/P202 assays were performed to detect *IKZF1* deletions, and the results were analyzed on Coffalyser.Net. The relative copy numbers of patient samples were normalized to control samples, and the result allowed us to determinate the *IKZF1* status. Then, the clinical and laboratory characteristics of patients were compared according to *IKZF1* status using GraphPad Prism, and the qui-square test was used for analysis. Long-distance (LD)-PCR assays are currently being performed in order to confirm the *IKZF1* intragenic deletions. Long-distance inverse (LDI)-PCR will be applied for amplification of deletions involving the *IKZF1* exon 1. The PCR products were separated by 2% agarose gel electrophoresis, purified (PureLink, Invitrogen) and sequenced by the Sanger method, using the Big Dye Terminator v3.1 Cycle Sequencing Kit and the 3500 Genetic Analyzer Sequencer (Applied Biosystems). The electropherograms will be analyzed with the BioEdit Sequence Alignment Editor v7.0.9 program (Ibis BioSciences, USA), and the BLAST platform will be used for comparison between target sequences and the human genome sequence in order to identify the breakpoint regions. Soon after, the RSS database will be used for the identification of recombination signal sequences (RSS) nearby breakpoint sites.

## RESULTS

*IKZF1* deletions were found in 16% (98/619) of patients with BCP-ALL. Although most of the patients with *IKZF1* deletions displayed recurrent deletions (Δ1-8, Δ2-3, Δ2-7, Δ2-8, Δ4-7, Δ4-8), 24% (24/98) of them presented other types of deletion, which cannot be detected with the currently available multiplex PCR assay. Most of these patients were males (54.2%), diagnosed with leukemia between 13-120 months-old (79.2%), presented a white blood cell count lower than 50x10$^9$/L (54.2%), and were classified as high risk by NCI criteria (58.3%), similar to overall patients with *IKZF1* deletions. The MLPA analysis also showed that the majority (38%) of patients had *IKZF1* deletion within its promoter until exon 1. The remaining patients displayed miscellaneous deletions, such as Δ1-3 (17%), Δ1-7 (17%), both Δ1 and Δ8 (13%), both Δ1 and 4-8 (4%), Δ4-5 (4%), Δ5-7 (4%), and Δ8 (4%).

**Table 1.** Demographic and laboratory data of patients with BCP-ALL.

| | All cases | *IKZF1* non- deleted | All *IKZF1* deleted | *IKZF1* *recurrent deletions* | *IKZF1* *other deletions* |
|---|---|---|---|---|---|
| | n=619 | n=521 | n=98 | n=74 | n=24 |
| | n (%) | n (%) | n (%) | n (%) | n (%) |
| Gender | | | | | |
| Male | 342 (55.3) | 292 (44.0) | 50 (51.0) | 37 (50.0) | 13 (54.2) |
| Female | 277 (44.7) | 229 (56.0) | 48 (49.0) | 37 (50.0) | 11 (45.8) |
| *P*-value | 0.916 | 0.856 | 0.782 | 0.722 | reference |
| Age at diagnosis (months) | | | | | |
| <12 | 33 (5.30) | 31(6.0) | 2 (2.0) | 2 (2.7) | 0 (0.0) |
| 13-120 | 463 (74.8) | 405 (77.7) | 58(59.2) | 39 (52.7) | 19 (79.2) |
| >120 | 123 (19.9) | 85 (16.3) | 38 (38.8) | 33 (44.6) | 5 (20.8) |
| *P*-value | 0.509 | 0.423 | 0.176 | 0.066 | reference |
| WBC (x10$^9$/L) | | | | | |
| <50 | 436 (70.4) | 376 (72.2) | 60 (61.2) | 47 (63.5) | 13 (54.2) |
| >50 | 179 (28.9) | 141(27.3) | 38 (38.8) | 27 (36.5) | 11(45.2) |
| *P*-value | 0.078 | 0.048 | 0.527 | 0.414 | reference |
| NCI risk | | | | | |
| Standard | 355 (57.4) | 321 61.7) | 34 (34.7) | 24 (32.4) | 10 (41.7) |
| High | 263 (42.5) | 199 (38.3) | 64 (65.3) | 50 (67.6) | 14 (58.3) |
| *P*-value | 0.125 | 0.049 | 0.523 | 0.408 | reference |

Abbreviations: WBC, white blood cell count; NCI, National Cancer Institute of US; BCP-ALL, B-cell precursor acute lymphoblastic leukemia.

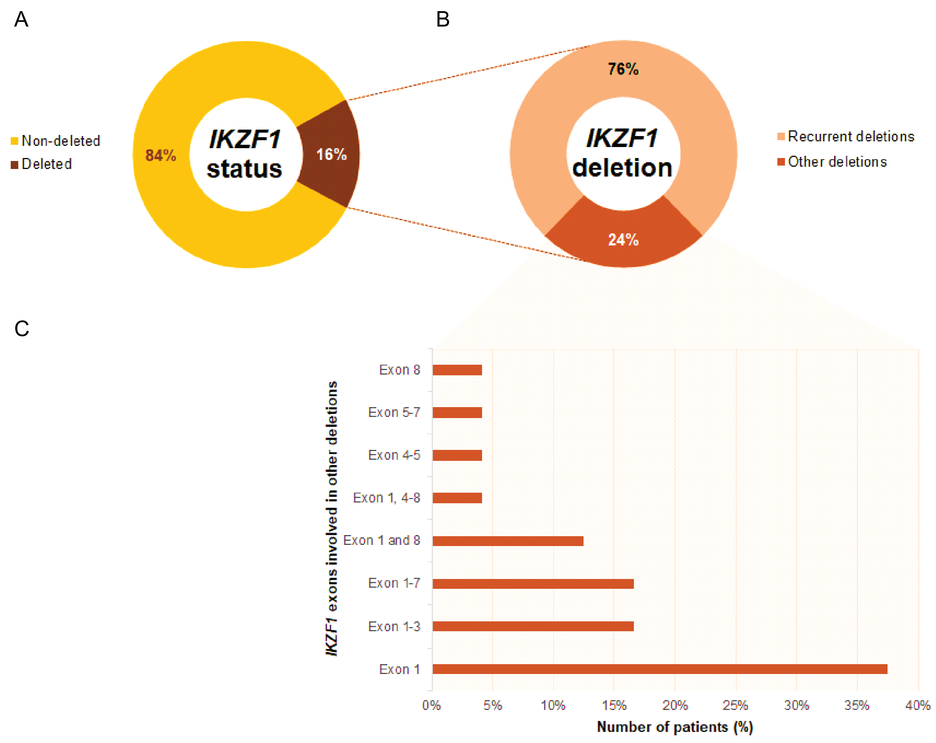


**Figure 3.** The status of *IKZF1* was determinated by MLPA SALSA P335 and P202 assays, and analyzed on Coffalyser. *IKZF1* status was evaluated for 619 patients with BCP-ALL, who were classified as *IKZF1* deleted or non-deleted (A). We also determined the frequency of recurrent and other deletions (24%) of *IKZF1* (B). The later is composed by miscellaneous deletions (C). The MLPA analysis also showed that the majority (38%) of patients had *IKZF1* deletion, which involved its promoter until exon 1. The remaining patients displayed miscellaneous deletions, such as Δ1-3 (17%), Δ1-7 (17%), both Δ1 and Δ8 (13%), both Δ1 and 4-8 (4%), Δ4-5 (4%), Δ5-7 (4%), and Δ8 (4%).




**Figure 4:** Electrophoresis of LD-PCR products from samples of the "other *IKZF1* deletions" cohort. We could amplify the regions flanking *IKZF1* deletions from samples (255/15, 533/11, 486/11,193/10, 428/14, 288/15, 060/11, 112/14, 124/14). The patient 061/14 presented a recurrent intragenic deletion. Samples 006/14, 369/13, 360/06, 173/10, and 144/14 were not amplified, thus we cannot confirm *IKZF1* deletions for these patients so far. All PCR products will be purified and sequenced for the search of breakpoint sites in order to confirm these alterations.

## CONCLUSIONS AND PERSPECTIVES

Although multiplex PCR has allowed a rapid detection of recurrent *IKZF1* deletions, 24% of these alterations are composed by miscellaneous deletions. Most of them are defined by loss of *IKZF1* promoter and its first exons, and might cause the haploinsufficiency of its coded protein. After mapping the breakpoint sequences of these *IKZF1* of deletions types, we will be able to search for RSSs close to the breakpoint sites and also to extend the coverage of the current multiplex PCR assay.

**Acknowledgement:** [2] List of co-authors included as part of BSGCALL: Noronha EP, Faro AJ, Gonçalves BAA, Pina ETG, Poubel CP, Blunck CB, Santoro JC, Maciel ALT, Mansur MB, Santini AV, Arancibia AM, Marinho AM, Nobrega AG, Curvello C, Alencar DMS, Fialho ECE, Carvalho EG, Júnior ER, Novaes FIR, Werneck FA, Araújo FNS, Costa GA, Filho GSG, Mamede GTL, Neves GR, Costa I, Quesado Magalhães IM, Agareno JMA, Costa JT, Carvalho LMB, Bonfim LN, Silva LN, Souza MS, Dorea MD, Ferreira M, Lankszner M, Brito NP, Brito PC, Souza RQ, Gurgel RSC, Melaragno R, Basegio RM, Agra SRC, Araújo SS, Jesus T, Pombo-de-Oliveira MS.

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