BTG1 DELETIONS IN ACUTE LYMPHOBLASTIC LEUKEMIA

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Introduction

B-cell precursor acute lymphoblastic leukemia (BCP-ALL) is the most common cancer among children and is caused by initiating lesions (e.g. translocations and aneuploidy), and secondary events, such as deletions in genes related to the regulation of lymphoid cell maturation, and progression through the cell cycle (e.g. *IKZF1*, *CDKN2A*, *JAK2* and *BTG1*) (MULLIGHAN *et al.*, 2007; WAANDERS *et al.*, 2012). Studies showed co-occurrence of additional lesions affect leukemia onset and can be related with drug resistance, such as in cases of *BTG1* and *IKZF1* deletions (SCHEIJEN et al., 2016). The present study aims to identify the most recurrent types of *BTG1* deletions and search for correlations with *IKZF1* status. We explore the clinical and laboratory characteristics of patients with *BTG1* deletions and will verify whether its gene expression is modified in the presence of different types of deletion. Based on the study will provide data that can support therapeutic risk stratification in patients with BCP-ALL.

Methods

We performed multiplex ligation-dependent probe amplification (SALSA MLPA P335) in 602 pediatric patients to identify copy-number alterations (CNAs) of genes frequently deleted in BCP-ALL and confirmed *IKZF1* deletion by MP-PCR. Patient characteristics and CNA results were compared according to *BTG1* status, and chi-square was used for analyses. P-values < 0.05 were interpreted as statistically significant. *BTG1* deletions and their extension is being confirmed by MP-PCR, followed by Sanger sequencing. Then, *BTG1* expression will be compared according to *BTG1* status using RT-qPCR, and GraphPad Prism 5 will be used for data analyses.

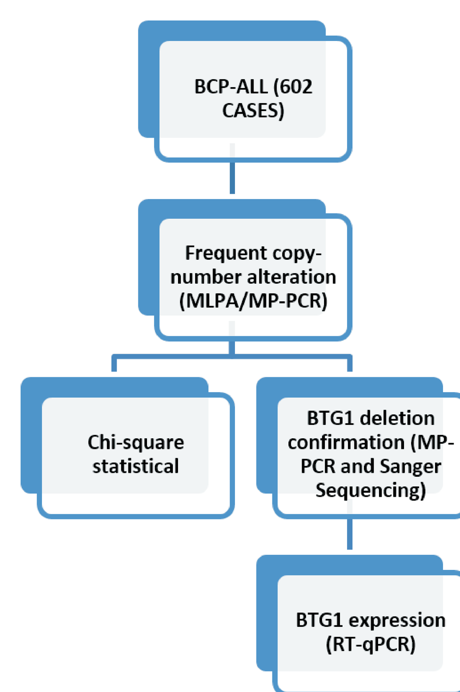

Fig 1: Flowchart describing the steps of this study, including their methodologies.

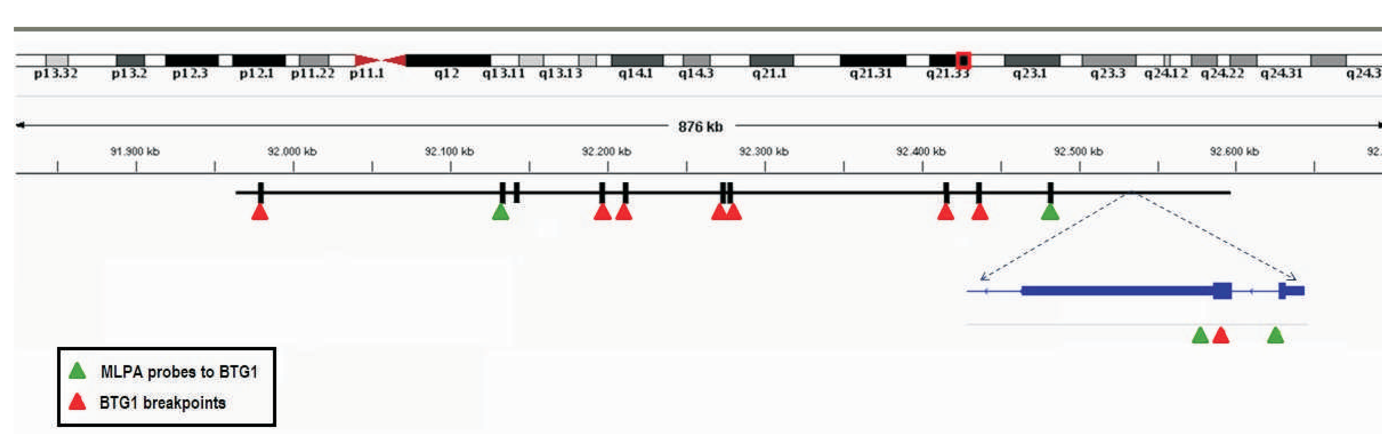

Fig 2: Representation of *BTG1* gene (blue line) and the breakpoint hotspots (red arrows). Additionally, the position of MLPA SALSA P335 probes were displayed within the long arm of chromosome 12 (green arrows).

Results

MLPA analysis revealed that *BTG1* deletions occur in 11% (68/602) of patients with BCP-ALL. The majority of cases were diagnosed with common-ALL subtype (69%) in children with 13-119 months-old (75%). MLPA results showed association between *BTG1* deletions and the follow genes: *IKZF1*, *CDKN2A*, *CDKN2B*, *JAK2*, *PAX5*, *CRLF2*, *EBF1*, *ETV6* or *RB1* deletions ($p < 0.05$). The MP-PCR assay for the confirmation of *BTG1* deletions has been standardized, and the method will be applied for screening the whole series of childhood BCP-ALL.

Table 1: Demographic and laboratory data of patients with BCP-ALL according to BTG1 status.

	All cases n=602	*BTG1* non-deleted	*BTG1* deleted	P-value
	Cases (%)	Cases (%)	Cases (%)	
Gender				.576
Male	335 (55.6)	295 (55.2)	40 (58.8)	
Female	267 (44.4)	239 (44.8)	28 (41.2)	
Age at diagnosis (months)				.588
<12	34 (5.6)	32 (6.0)	2 (2.9)	
13-119	454 (75.4)	401 (75.1)	53 (77.9)	
>120-216	114 (18.9)	534 (18.9)	13 (19.1)	
Down Syndrome				.144
No	590 (98.0)	525 (98.3)	65 (95.6)	
Yes	12 (2.0)	9 (1.7)	3 (4.4)	
WBC ($x10^9/L$)				.398
<50	425 (70.6)	374 (70.0)	51 (75.0)	
>50	177 (29.4)	160 (30.0)	17 (25.0)	
NCI risk				.465
Standard	347 (57.6)	305 (57.1)	42 (61.8)	
High	255 (42.4)	229 (42.9)	26 (38.2)	

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

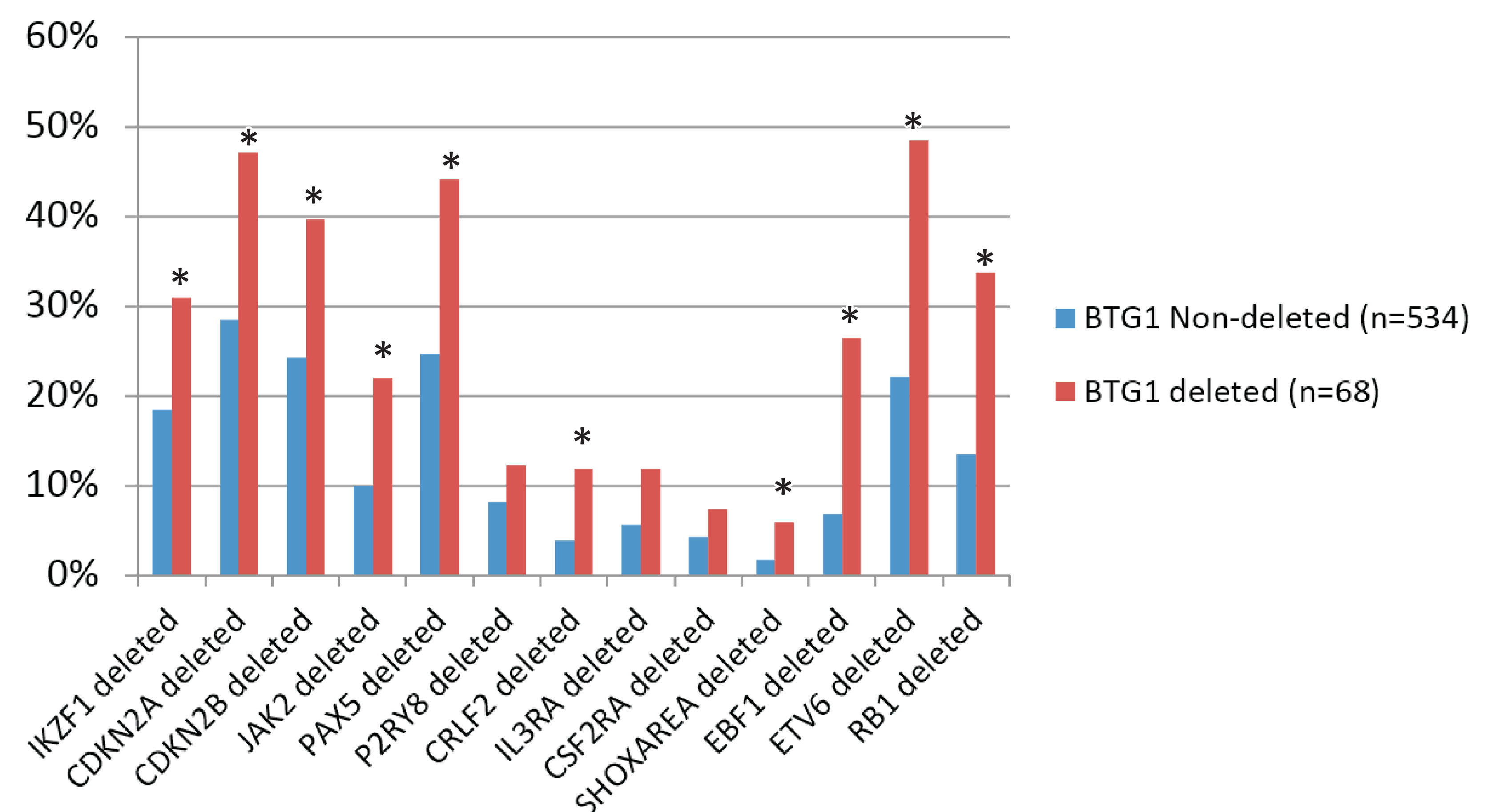

Fig 3: Correlation between *BTG1* status and other additional alterations in BCP-ALL.

[a] IKZF1 status was determined using MLPA (P335 and P202) and MP-PCR. All the other alterations were determined solely using MLPA (P335) technique.

[b] Although this work included 602 cases with BCP-ALL, the identification of CNAs in *JAK2* (n = 272) and *P2RY8* (n = 296) presented missing values because these MLPA probes were added in updated versions of MLPA assays.

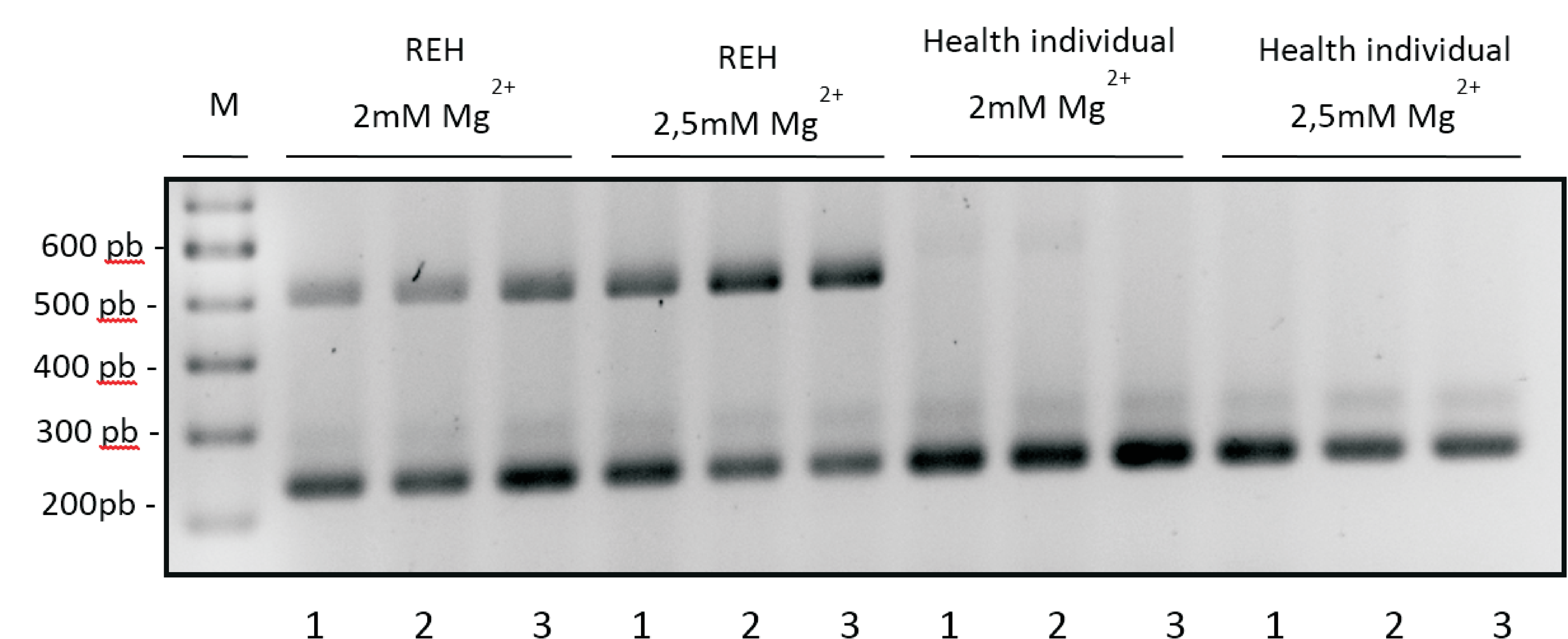

Fig 4: Agarose gel. MP-PCR standardization using concentrations of 2mM and 2.5mM Mg^{2+} and annealing temperatures of 57°C, 60°C, and 63°C (1, 2, 3, respectively). Samples of REH and a negative sample to *BTG1* deletions were used..

Discussion and Perspectives

BTG1 deletions occur simultaneously with lesions in several genes involved with differentiation of lymphoid cells and cell cycle regulation. For the continuity of the study, *BTG1* deletions will be confirmed by MP-PCR, and a ROC curve will compare MLPA and MP-PCR results to describe the most accurate assay for the detection of *BTG1* deletions. RT-qPCR analysis will be performed for comparison of gene expression according to *BTG1* status.

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[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."

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