Frequency of copy number abnormalities in common genes associated with T-cell acute lymphoblastic leukemia in Brazilian Children

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BACKGROUND

Pediatric T-cell acute lymphoblastic leukemia (T-ALL) is a heterogeneous disease resulting from a multistep oncogenic process, which promotes the arrest of cellular differentiation, reflected in different immunophenotypic and molecular subtypes. The genomic landscape includes mutations and copy number alterations (CNA) disrupting relevant cellular pathways such as RAS signalling (*KRAS*, *NRAS*, *FLT3*, *NF1* mutations), haematopoietic development (*TAL1*, *MYB*, *LMO2*, *TLX3*, *TLX1*, *LEF1*), PI3K-AKT (*PTEN* mutations and CNA), NOTCH1 signalling (*NOTCH1*, *FBXW7*, *IL7R* mutations), cell cycle (*CDKN2A/B*, *CASP8AP2*) and epigenetic regulation (*EZH2*, *SUZ12*, *EED*, *PHF6*). In contrast with B-cell precursor ALL, the immunophenotype-genotype and predictive outcome associations are not fully understood. In order to test possible associations of T-ALL distinct profiles we have investigated gene mutations and CNA in a cohort of pediatric T-ALL.

METHODS

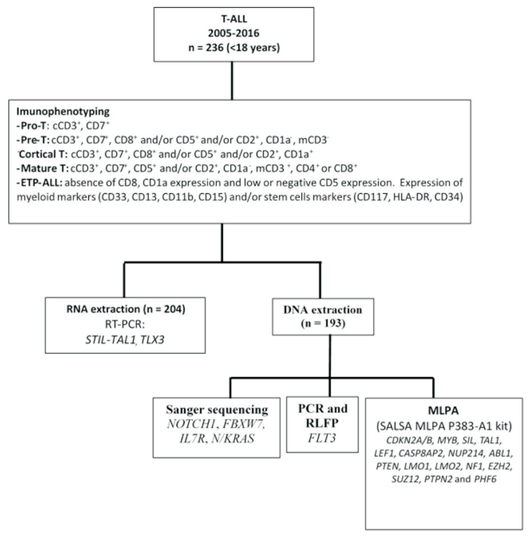


Figure 1: Summary of the study design. Abbreviations: c: cytoplasmatic, m: membrane, RFLP: restriction fragment length mediated PCR assay, MLPA: *Multiplex Ligation dependent Probe Amplification.*

STATISTICAL METHODS

To compare the distribution of categorical variables, the Chi-square test was used, and Fisher`s exact test was used when the expected count in at least one cell of the table was less than five. Overall survival (OS) was measured from the date of diagnosis to the date of last follow-up or death from any cause. Event-free survival (EFS) was measured from the date of diagnosis to the date of relapse. Patients who did not experience an event were censored at the time of last follow-up and those with lost to follow-up were censored at their date of last known contact. The Kaplan-Meier survival analysis method was used to calculate the 5-year of OS and EFS, and estimated survival values were compared using the log-rank test in order to verify the differences in outcome. P-values of < 0.05 were considered statistically significant. All analysis were performed using SPSS 21.0 (SPSS, Chicago, IL, USA, 2004).

RESULTS

The frequencies of immunophenotype subtypes in total cohort consisted of 9.3% (n = 22) early T cell precursor ALL (ETP-ALL), 19.9% (n= 47) pre-T, 38.5% (n=91) T cortical and 32.2% (n =76) T mature subtype.The frequencies of mutated genes were: *NOTCH1* 47% (89/189), *FBXW7* 16.5% (31/188), *N/KRAS* 8.8% (17/193), *FLT3-internal tandem duplication* (ITD) 4.3% (8/187), *IL7R* 7% (13/186), *STIL-TAL1* 20.4% (39/191) and *TLX3* 10.3% (21/183). CNA were tested in 133 samples and identified in at least one gene in 95% of all cases. The most common deletions found in *CDKN2A/B* locus (66%), *PHF6* (36.8%), *STIL* (21.8%), *LEF1* (14.3%), *PTPN2* (13.5%) while, gains were frequently found in *MYB* (15.8%), *TAL1* (12.8%), *EZH2* (12.8%), *NUP214/ABL1* (12.8%) and *LMO2* (9.8%) (Figure 2). The concomitance of gene alterations (CNA and mutations) is demonstrated in Figure 3 and associations of gender, age and WBC in relation to CNA in Figures 4, 5 and 6. Regarding OS and EFS analysis of all cases evaluated were 41.7% and 40.3%, respectively. *NOTCH*^{mut} and/or *FBXW7*^{mut} were associated with better EFS (45.3%) when compared to cases without mutations (35.1%) (p = 0.025) and cases positive to STIL-TAL1 rearrangement were associated to worse EFS (26.6%) compared to *STIL-TAL1* negative (44.2%) (p<0.001) (Figure 7). Immunophenotype (Figure 8) or CNA profile was not associated with OS or EFS. The major differences regarding CNA were shown in Figure 9.

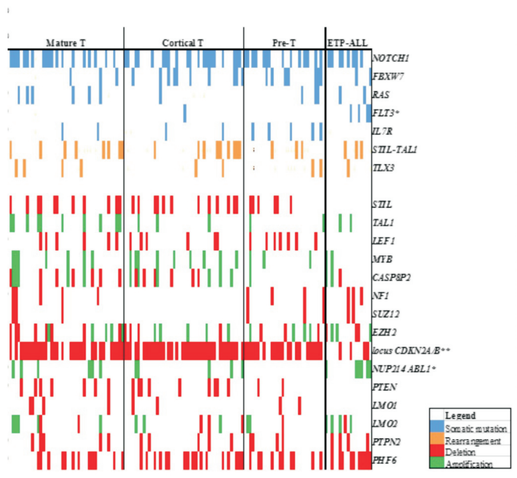


Figure 2: Overview of molecular alterations identified in 133 samples according to T-ALL subtypes. Vertical lines represent each patient. **NUP214/ABL1* amplification and FLT3 mutations were found in high frequency in ETP-ALL cases (p<0.001) and deletions in *CDKN2A/B* locus was rarely found (p<0.001) in this subtype. Frequencies of others alterations did not differ according to T-ALL subtypes.

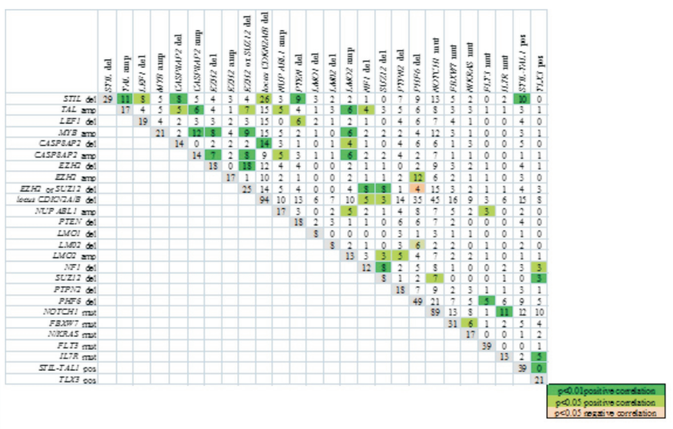


Figure 3: Concomitance of gene alterations (CNA and mutations) in T-ALL cases. Grey boxes indicate the total number of patients with a respective genetic alteration. Other boxes indicate the number of patients with two specific abnormalities. Green or orange boxes indicate a significant overlap association (p < 0.01 or <0.05), respectively.

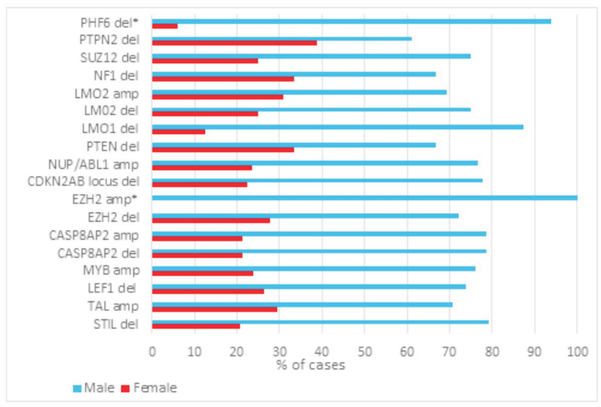


Figure 4: Distributions of CNA according do gender. **EZH2* amplification were not found in female (p<0.001) and *PHF6* deletions were rarely found (p< 0.001).

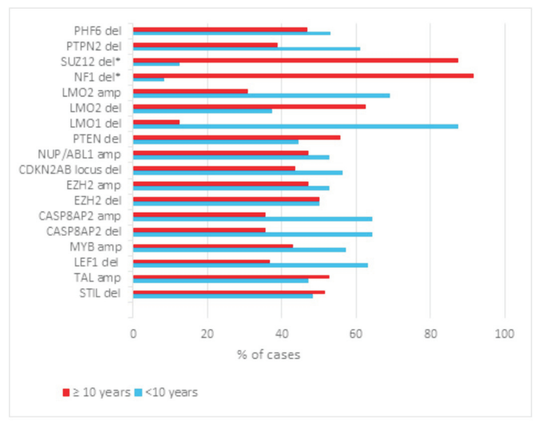


Figure 5: Distributions of CNA according to age. **SUZ12* and *NF1* deletions were associated to age ⩾10 years (p<0.001).

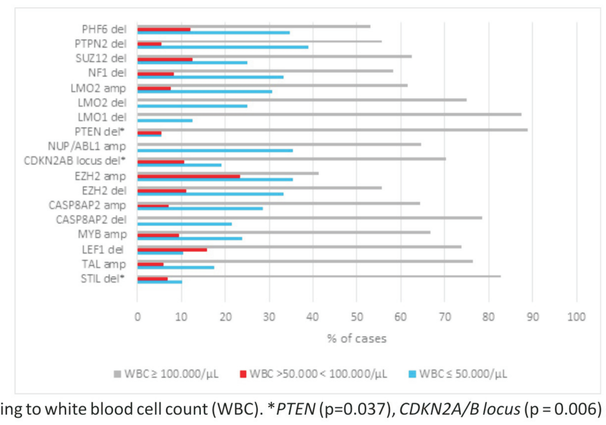
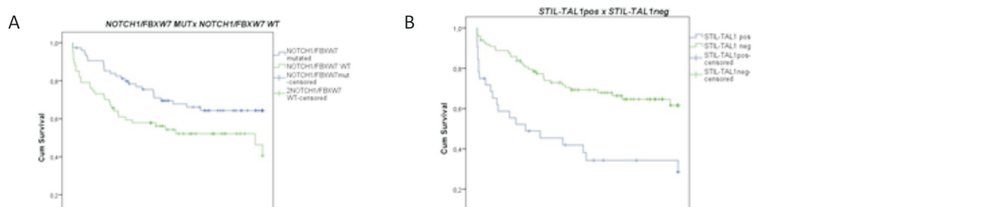
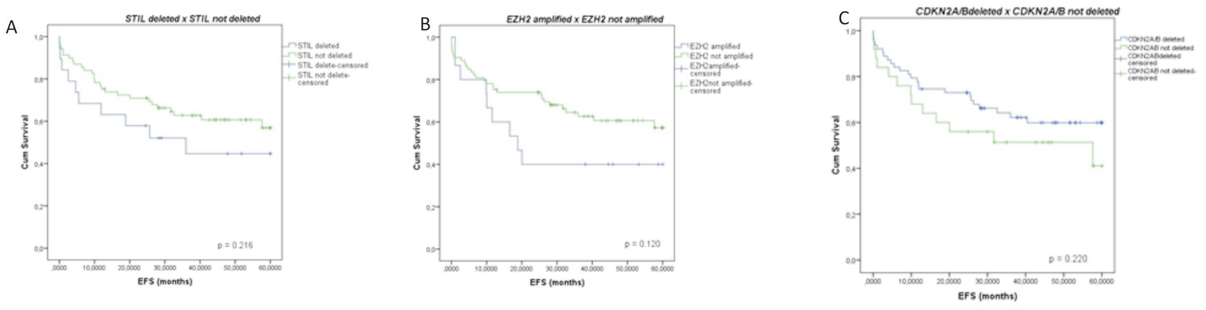


Figure 6: Distributions of CNA according to white blood cell count (WBC). **PTEN* (p=0.037), *CDKN2A/B locus* (p = 0.006) and *STIL* deletions (p = 0.030) were associated to high WBC (⩾100.000/µL).



Gene	NE/TN	Mutated EFS%(95%CI)	NE/TN	WT EFS% (95%CI)	p-*value*
NOTCH1/FBXW7	25/75	44.5(39.4-49.5)	33/67	35.4(29-41.9)	0.021
STIL-TAL1	21/32	26.6(17.2-35.9)		44.1(39.6-48.7)	<0.001

Figure 7: Event free survival curves in 60 months according to *NOTCH1/FBXW7* (A) and *STIL-TAL1* (B) status.
Abbreviations: Mut: mutated, WT: wild type, pos: positive, neg: negative, NE: N of events, TN: Total N, EFS, event free survival; CI, confidential interval



Alteration	NE/TN	With alteration EFS%(95%CI)	NE/TN	Without alteration EFS% (95%CI)	p-*value*
STIL deleted	10/19	33.3(21.5-45.1)	27/69	41.5(35.8-47.3)	0.216
EZH2 amplified	9/15	30.1(17.5-42.8)	28/73	41.7(36.1-47.3)	0.120
CDKN2A/B deleted	24/63	41.6(35.7-47.6)	13/25	35.3(25.2-45.5)	0.220

Figure 9: Event free survival curves in 60 months according to status of *STIL* (A), *EZH2* and *CDKN2A/B* (C) status.
NE: N of events, TN: Total N, EFS, event free survival; CI, confidential interval

CONCLUSION

Through the MLPA technique we identified a variety of CNA in several genes involved in the pathogenesis of T-ALL and shown associations with clinical and demographic variables. Regarding the association of immunophenotype and genotype, we identify a molecular profile in ETP-ALL group that were characterized by *FLT3*^{mut} and *NUP214-ABL1*^{gain} while *CDKN2A/B*^{del} was rarely found. Furthermore, frequencies of others alterations did not differ according to T-ALL subtypes. *NOTCH1* and/or *FBXW7*^{mut} were associated with better EFS and *STIL-TAL1* to worse EFS. So far, no particularly CNA or immunophenotype profile was associated with OS or EFS. The prognostic value of CNAs needs further analysis in larger cohorts of ETP-ALL.

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