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Impact of polymorphisms in apoptosis-related genes on the outcome of childhood acute lymphoblastic leukaemia

SHORT TITLE: Polymorphisms and outcome of acute lymphoblastic leukaemia

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ABSTRACT

Identification of children with acute lymphoblastic leukaemia (ALL) that have poor response to chemotherapy is important to individualize therapy. In the present study, we sought to determine if polymorphisms in genes coding for proteins involved in drug metabolism or apoptosis have an impact on the therapeutic outcome of childhood ALL patients. We observed that genetic variants in two apoptosis-related genes, such as *GSMT1* and *TP53*, contribute to differences in response to therapy in childhood ALL.

KEYWORDS

Polymorphisms, apoptosis, outcome, chemotherapy, acute lymphoblastic leukaemia

Despite intensification of therapy, 20% of children with acute lymphoblastic leukaemia (ALL) relapse (Ceppi *et al*, 2015). The different response to chemotherapy may be partially explained by inherited genetic variants, e.g. single nucleotide polymorphisms (SNPs) and copy number variants (CNVs). However, clinically useful genetic predictors of ALL treatment response are yet to be identified.

We investigated the association between polymorphisms in genes encoding drug-metabolizing enzymes or apoptosis-related proteins and early-treatment response, minimal residual disease (MRD), relapse, overall survival (OS), and event-free survival (EFS) in 173 Caucasian children with ALL. Patients were genotyped for CNVs at *CYP2D6*, *GSTT1*, *GSTM1*, *UGT2B17*, and *SULT1A1* genes and for SNPs at *CYP2D6*, *SULT1A1*, and *TP53* genes (see methods in Supporting Information). This is the first report searching for an association between the exact number of copies (0, 1, 2, ≥ 3) in selected candidate genes and ALL prognosis, and the first study analysing the *TP53* Arg72Pro polymorphism as a possible predictor of treatment response in childhood ALL.

As expected for the Caucasian population, around 50% of children with ALL presented a *GSTM1* null genotype, whereas most patients had one copy of *GSTT1* and *UGT2B17*, and two copies of *CYP2D6* and *SULT1A1*. G allele was predominant in *CYP2D6* and *SULT1A1* SNPs, and C allele was predominant in *TP53* SNP (Tables S1 and S2).

The association analysis between genotypes and early-treatment response, MRD or relapse revealed no gene dose or SNP effect with any of the genetic models tested. However, interestingly, patients with 1, 2 or ≥ 3 copies of *GSTM1* had worse outcome than patients with *GSTM1* null genotype, both when genotypes were analysed separately (OS $p=0.013$, Figure S1A; EFS $p=0.047$, Figure S1B) and when all non-null genotypes were grouped together (OS $p=0.002$, Figure 1A; EFS $p=0.005$, Figure S1C). Differences between null and non-null individuals were confirmed in multivariate analysis for OS (hazard ratio [HR]=16.51; 95% confidence interval [CI] 2.13-128.18; $p=0.007$) and EFS (HR=3.80; 95% CI 1.40-10.30; $p=0.009$), and after false discovery rate (FDR) adjustment. Therefore, *GSTM1* non-null genotype may be a determinant factor to shift the balance

from good to poor prognosis, regardless of the number of copies. So far, *GSTM1* null genotype has been associated with improved childhood ALL outcome in some studies but not in others (Bolufer *et al*, 2006 and Lopez-Lopez *et al*, 2014). One explanation for these discrepancies is that association might be dependent on the multidrug strategy used, as childhood ALL protocols can include up to 13 different chemotherapeutic agents, with variations in drug dosage, glucocorticoid pre-phase and prophylaxis of central nervous system relapse. Although *GSTM1* and *GSTT1* can have overlapping substrate affinity sometimes, no effect on outcome was observed for *GSTT1* or the double-null genotype *GSTT1/GSTM1*, which could indicate that *GSTM1* may influence outcome by additional mechanisms, different from drug metabolism, possibly involving apoptosis. Moreover, as *GSTM1* null genotype is present in half the Caucasian population, other gene/s could modulate its impact on childhood ALL outcome. Interestingly, we found that carriers of at least one Pro variant of *TP53* Arg72Pro polymorphism had worse survival, although non-significant (OS $p=0.208$, Figure 1B; EFS $p=0.110$, Figure S1D).

We next analysed the combination of *GSTM1* and *TP53* genotype, being both genes implicated in apoptosis. Patients having both non-null genotype of *GSTM1* and *TP53* Pro/Pro or Pro/Arg genotype had a higher risk of relapse ($p=0.043$, non-significant after FDR adjustment) and poorer survival (OS $p=0.0005$, Figure 1C; EFS $p=0.003$, Figure S1E). Association with survival remained significant after FDR adjustment and after multivariate analysis, either in OS (HR=19.10; 95% CI, 2.44-149.21; $p=0.005$) or EFS (HR=4.13; 95% CI, 1.49-11.49; $p=0.006$). Differences were still significant if patients were censored at the time of transplantation, even after FDR adjustment (OS $p=0.001$, EFS $p=0.004$). Since drugs inducing apoptosis are used at every phase of treatment, it could be that drug-induced cell death plays an important role in all treatment phases, influencing final patient outcome. Our data indicated that the combination of *GSTM1* and *TP53* genotypes can predict survival with higher significance than *GSTM1* alone.

GSTM1 inhibits the activity of ASK1 and MEKK1, two kinases that activate the JNK and p38 pro-apoptotic pathways under cellular stress (Dorion *et al*, 2002; Ryou *et al*, 2004). Moreover, the *TP53* Arg variant is

stronger and faster in inducing apoptosis, whereas the Pro variant is a more efficient activator of cell cycle arrest and DNA damage repair and thus may protect tumour cells against chemotherapy-induced apoptosis (Hrstka *et al*, 2009). Therefore, both having some production of GSTM1 protein (at least one copy of *GSTM1* gene) and having the *TP53* Pro variant (enough in heterozygosis) would lead to less efficient apoptosis of ALL lymphoblasts after antileukemic treatment and this could translate into shorter survival.

We also investigated *in vitro* the effect of *GSTM1* expression and *TP53* variant on cellular sensitivity to dexamethasone, a glucocorticoid, using leukemic Jurkat cells (Jurkat p53Arg *GSTM1* null, Jurkat p53Arg *GSTM1* non-null, Jurkat p53Pro *GSTM1* null, and Jurkat p53Pro *GSTM1* non-null) (see Supporting Information). Jurkat p53Arg *GSTM1* null cells exhibited the highest sensitivity to dexamethasone and Jurkat p53Pro *GSTM1* non-null the lowest (Figure 2), with statistically significant differences ($p=0.006$ for 1 and 10 μM , and $p=0.004$ for 100, 200 and 400 μM).

Glucocorticoids are the keystone in the treatment of childhood ALL due to their ability to induce extensive apoptosis in ALL cells, through activation of p38-MAPK and Bim (Lu *et al*, 2006). Response to these agents is highly predictive of ALL outcome (Distelhorst, 2002; Kfir-Erenfeld *et al*, 2010). Notably, *GSTM1* inhibits dexamethasone-induced apoptosis in an ALL cell line by suppression of Bim through downregulation of p38-MAPK and upregulation of NF-KB p50 (Hosono *et al*, 2010). Consequently, *GSTM1* and *TP53* polymorphisms may contribute to differences in response to glucocorticoids in childhood ALL.

Our study, if confirmed in larger series, may provide tools to individualize drug therapy in childhood ALL. Patients with *GSTM1* non-null genotype and the *TP53* Pro/Pro or Pro/Arg genotype at codon 72 could be identified as having high-risk of resistance to apoptotic-based agents and could benefit from other therapies.

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AUTHOR CONTRIBUTIONS

G.A. and M.R.C. conceived and designed the experiments, M.Cabezas conducted all the experiments. All authors analysed the results and wrote and reviewed the manuscript.

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FIGURE LEGENDS

Figure 1. Kaplan–Meier survival curves of childhood ALL patients according to the *GSTM1* genotype (A), to the *TP53* genotype at codon 72 (B), or to the combination of *GSTM1* and *TP53* genotypes (C). (A) Patients with *GSTM1* null genotype had better survival than the other genotypes (OS 99% vs. 83%, $p=0.002$, significant after FDR). (B) Patients with the Arg/Arg genotype at codon 72 of *TP53* had better survival than the others (OS 100% vs. 89%, $p=0.208$). (C) Patients with ≥ 1 copies of *GSTM1* and *TP53* Pro/Pro or Pro/Arg genotype ($n=62/149$ patients with both genes successfully genotyped) had worse survival than the others (OS 79% vs. 99%, $p=0.0005$, significant after FDR).

Figure 2. Survival analysis of Jurkat cells with the *TP53* Arg or Pro variants and *GSTM1* null/non-null treated with varying concentrations of dexamethasone by MTT assay. Cells with p53Pro and *GSTM1* non-null showed significantly less sensitivity than cells with p53Arg and *GSTM1* null at all concentrations ($p<0.006$). The other cell lines presented an intermediate sensitivity. Bars correspond to standard deviations from six separate measurements. This experiment is representative of two independent experiments.

FIGURES

Figure 1

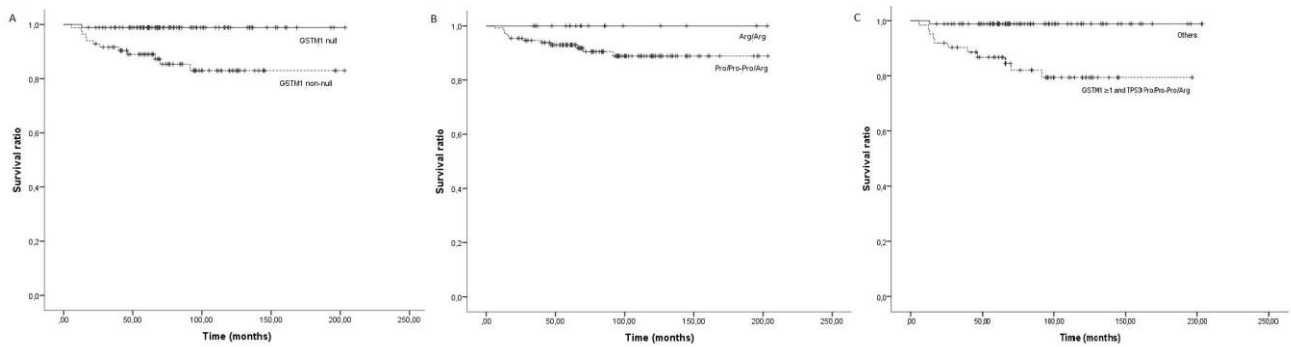


Figure 2

