POLYMORPHISMS IN GENES RELATED TO METABOLISM OF GLUCOCORTICOIDS AND THE OUTCOME OF CHILDHOOD LYMPHOBLASTIC LEUKEMIA TREATMENT

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INTRODUCTION

The prognosis of children with acute lymphoblastic leukemia (ALL) has improved in the last decades, achieving a 5-year survival rate about 80%. An important advance in treatment during this period was the introduction of glucocorticoids in the therapeutic protocols. Despite the improvement in prognosis, ALL remains an important cause of death and the treatment still results in high toxicity rates. Polymorphisms in glucocorticoids metabolism genes may alter the efficacy and toxicity of therapy, constituting an important cause of variability in treatment response. The **Figure 1** summarize the studied genes and their relation with the glucocorticoid metabolism. The understanding of the association between these polymorphisms and the outcome of ALL patients is fundamental to the institution of individualized therapies, with less toxic events and greater efficacy. This study aims to investigate the effect of polymorphisms in genes related to the metabolism of glucocorticoids in the toxicity and survival of pediatric ALL patients.

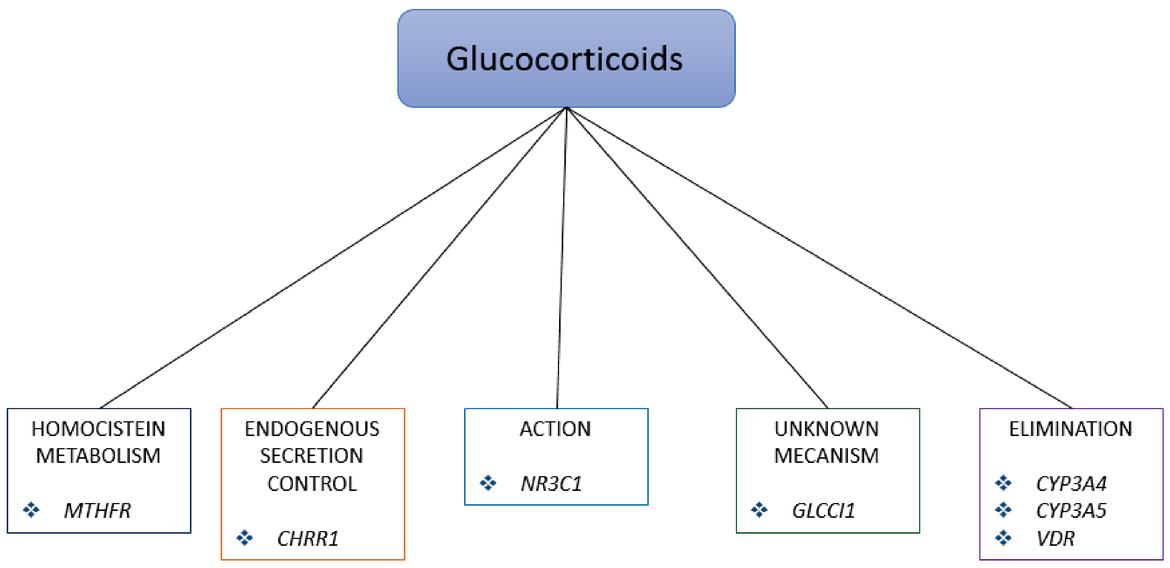


Figure 1: Genes related to Glucocorticoids metabolism. Glucocorticoids act by binding to their nuclear receptor NR3C1. To be eliminated, glucocorticoids are metabolized by CYP3A4 and CYP 3A5, whose expression is induced by activation of the vitamin D receptor VDR. The endogenous synthesis of glucocorticoids is mediated by the hypothalamic-pituitary-adrenal axis, including the action of corticotrophin-releasing hormone on its receptor CRHR1. Alterations in homocysteine metabolism such as polymorphisms in *MTHFR* may be associated with glucocorticoid toxicity. Polymorphisms in Glucocorticoid-induced transcript 1 (*GLCCI1*) are associated with glucocorticoid response in asthma and rheumatoid arthritis, but protein function is not known.

METHODS AND RESULTS

The study flowchart is summarized in **Figure 2**. The study will include samples from patients aged up to 21 years diagnosed with ALL between 2012 and 2015 and treated in seven Brazilian medical centers. It will be considered exclusion criteria the presence of Down syndrome. An electronic questionnaire will be sent to collaborating centers containing questions regarding the following clinical data: 1) treatment toxicity (hematological, hepatic, digestive and corticoid related); 2) patient's vital state; 3) occurrence of relapses; 4) risk group; 5) bone marrow transplantation; 6) response to prednisone (D8); 7) minimal residual disease. Genomic DNA will be purified by QI Aamp DNA Mini Kit® or Trizol®. The genotyping will be performed through Polymerase Chain Reaction-High Resolution Melting (PCR-HRM) for the following polymorphisms: CYP3A4 rs2740574; CYP3A5 rs776746; VDR rs2228570 and rs1544410; MTHFR rs1801133; GLCCl3 rs37972; CRHR1 rs242941; and NR3C1 rs41423247. The **Figure 3** explains the HRM technique. A group of healthy individuals will be also genotyped to estimate the frequency distributions of polymorphisms in the Brazilian population and test the Hardy-Weinberg equilibrium. Statistical analysis will involve multivariate logistic regression and Kaplan–Meier survival curves.

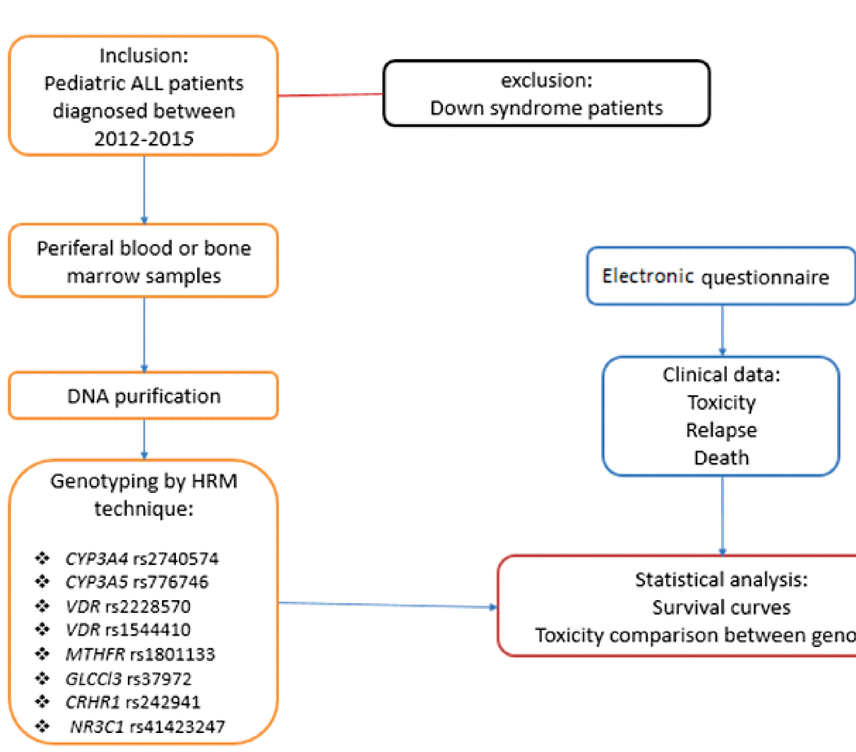
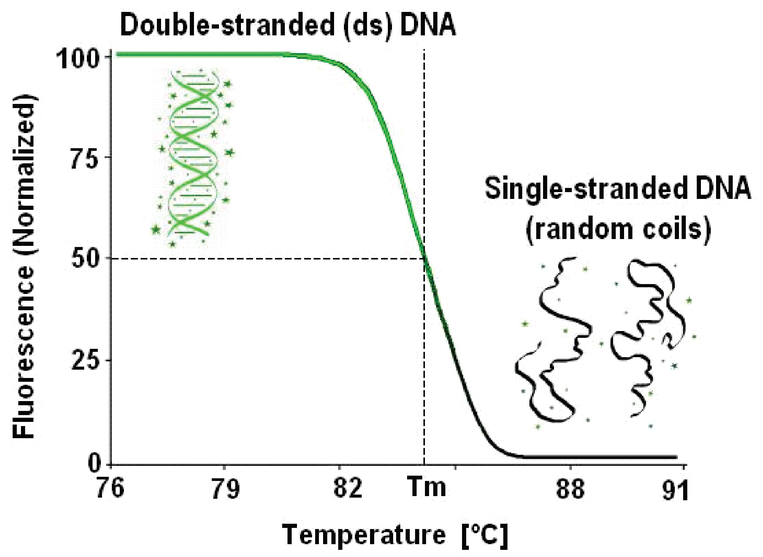


Figure 2: Study design flowchart.



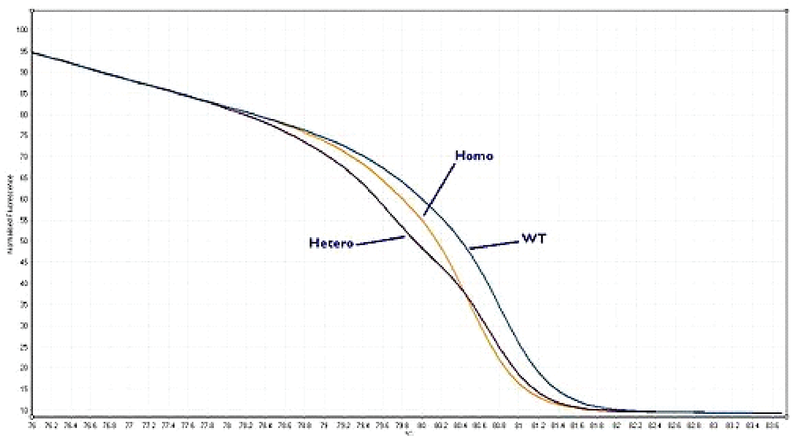


Figure 3: HRM curves. The HRM is a post-PCR technique that detects small differences between denaturation temperatures of DNA (Druml & Cichna-Markl 2014).

PARTIAL RESULTS

The study participants were selected according to eligibility criteria (490 cases). A group of 400 samples of cord blood was also selected. Genomic DNA was purified by QI Aamp DNA Mini Kit® or Trizol®. Forty-six samples were genotyped for *MTHFR* rs1801133. HRM technique is being standardized to other genetic polymorphisms. The collection of clinical data are still ongoing.

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