Folate Metabolic Pathway and Susceptibility to Childhood Acute Lymphoblastic Leukaemia in North Portugal. The role of C677T and A1298C MTHFR Polymorphisms.

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SUMMARY

This study examines the effect of C677T and A1298C MTHFR polymorphisms on childhood acute lymphoblastic leukaemia in North Portugal. Blood samples of 103 children and 111 healthy controls were analysed. The presence of the MTHFR C677T and A1298C polymorphisms were screened using PCR/RFLP based approaches. Multivariate analysis did not reveal any significant association between different MTHFR genotypes and susceptibility to childhood ALL. The adjusted odds ratio and 95% confidence intervals for MTHFR C677T were 0.82 (0.47-1.44) for 677CT versus 677CC wild type, 0.52 (0.16-1.67) for 677TT versus 677CC, and 0.78 (0.46-1.34) for 677CT/TT versus 677CC. The corresponding values for A1298C were 1.81 (1.03-3.20) for 1298AC versus 1298AA wild type, 1.50 (0.54-4.14) for 1298CC versus 1298AA, and 1.76 (1.02-3.06) for 1298AC/CC versus 1298AA.

Despite the absence of significant associations, the frequency of MTHFR677*T among cases was lower comparatively to controls supporting previous indications that it might confer protection against ALL. Evidences are more difficult to interpret concerning the role of variation at MTHFR1298 in susceptibility to ALL.

INTRODUCTION

In acute lymphoblastic leukaemia (ALL), the most common pediatric cancer, research is being increasingly placed on the elucidation of the etiological factors and mechanisms involved in the disease development. Since leukaemias are malignancies arising from rapidly proliferating clones of haematopoietic cells, thus having greatest requirements for DNA synthesis, attention has recently been focused on genes or environmental factors that can play a role in an individual’s susceptibility to DNA damage (Robien et al., 2003).

Folate, a key element in the one-carbon group metabolism, is essential for normal mammalian cell growth. The folate metabolic pathway (Figure 1) is crucial in purine and pyrimidine synthesis, as well as in the provision of methyl groups for DNA, RNA and protein methylation. Not surprisingly, disruption of homeostasis in the one-carbon pool, has been shown to affect the risk of several kind of cancers (Wiemels et al., 2001), including ALL.

Alterations in this metabolic pathway can occur due to genetic variation at any of the enzymes directly involved in maintaining homeostasis of the one-carbon pool. Methylenetetrahydrofolate reductase (MTHFR) is a central enzyme in the metabolism of folate and methionine, both important factors in methylation and DNA synthesis in humans. The enzyme catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and it has been shown that reduction in its activity resulting from polymorphisms in the MTHFR gene can modify the susceptibility to some cancers.

Recently, the role of two common MTHFR variants, C677T and A1298C, in ALL susceptibility has been investigated. A significant association was reported between carriers...
of the two common MTHFR polymorphisms and protection against ALL (Skibola et al., 1999; Krajinovic et al., 2004); however other studies failed to detect such an association (Franco et al., 2001; Chiusolo et al., 2004).

Accordingly, the major aim of this study was to assess the role of MTHFR variation in the risk for childhood ALL in North Portugal.

**Figure 1** - Human folate metabolic pathway and the role of the MTHFR (Methylenetetrahydrofolate reductase), MS (Methionine Synthetase) and TS (Thymidylate Synthetase).

**MATERIALS AND METHODS**

Patients enrolled in the study were 103 children diagnosed with acute lymphoblastic leukemia in the Hospital Geral S.João or in the Portuguese Institute of Oncology, both in Oporto. All patients were submitted to the same chemotherapy protocol. As controls, 111 non related healthy subjects were analysed. They were not following any therapeutic treatment and were born in the same geographic area as patients, North Portugal.

All samples were collected under medical supervision and informed consent, and the ethic boards of the follow-up institutions approved the research protocol.

The presence of the MTHFR C677T and A1298C polymorphisms were screened using PCR/RFLP based approaches.

Genetic differences between ALL children and control population samples were evaluated applying exact tests to allelic contingency tables with the program STRUC of the software package Genepop (ver. 3.1c). Odds ratios (OR) and 95% Confidence Intervals (CI) were calculated applying Mantel-Haenzel tests, with the software package PEPI (routine MANTELX). ORs were computed taking always the homozygous wild type genotype as reference.
## RESULTS

Table 1– Distribution of MTHFR C677T and A1298C genotypes in ALL cases and controls.

<table>
<thead>
<tr>
<th></th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Cases</th>
<th>Controls</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=103</td>
<td>n=111</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>677 CC</td>
<td>48 (46.60)</td>
<td>45 (40.54)</td>
<td>1</td>
<td>n.a.</td>
<td>C = 0.709</td>
<td>0.662</td>
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<td>CT</td>
<td>50 (48.54)</td>
<td>57 (51.35)</td>
<td>0.82 (0.47–1.44)</td>
<td>0.59</td>
<td>T = 0.291</td>
<td>0.338</td>
<td>0.353</td>
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<tr>
<td>TT</td>
<td>5 (4.85)</td>
<td>9 (8.11)</td>
<td>0.52 (0.16–1.67)</td>
<td>0.41</td>
<td></td>
<td></td>
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<tr>
<td>CT+TT</td>
<td>55 (53.40)</td>
<td>66 (59.46)</td>
<td>0.78 (0.46–1.34)</td>
<td>0.45</td>
<td>P_hw = 0.098</td>
<td>P_hw = 0.138</td>
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<thead>
<tr>
<th></th>
<th>Cases (%)</th>
<th>Controls (%)</th>
<th>OR (95% CI)</th>
<th>P</th>
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<td>n=103</td>
<td>n=111</td>
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<tr>
<td>1298 AA</td>
<td>36 (34.95)</td>
<td>54 (48.65)</td>
<td>1</td>
<td>n.a.</td>
<td>A = 0.631</td>
<td>0.703</td>
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<tr>
<td>AC</td>
<td>58 (56.31)</td>
<td>48 (43.24)</td>
<td>1.81 (1.03–3.20)</td>
<td>0.06</td>
<td>C = 0.369</td>
<td>0.297</td>
<td>0.114</td>
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<tr>
<td>CC</td>
<td>9 (8.74)</td>
<td>9 (8.11)</td>
<td>1.50 (0.54–4.14)</td>
<td>0.60</td>
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<tr>
<td>AC+CC</td>
<td>67 (65.05)</td>
<td>57 (51.35)</td>
<td>1.76 (1.02–3.06)</td>
<td>0.06</td>
<td>P_hw = 0.056</td>
<td>P_hw = 0.824</td>
<td></td>
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</table>

(%) number of individuals with a given genotype/ total number of individuals;  
OR – crude odds ratios;  
CI – confidence interval with 95% of probability.  
n.a. – not applicable  
P\*– P value for genetic frequency difference’s test between cases and controls

## DISCUSSION AND CONCLUSION

In this case-control study we have evaluated whether the two common polymorphisms in the MTHFR gene, C677T and A1298C, may have a role in the risk of developing pediatric ALL. The affected children and the healthy controls were born and lived in the same geographic area, North Portugal, which means that all subjects enrolled in the analysis shared identical environmental conditions and a common genetic background since the northern Portuguese population does not present any signs of genetic heterogeneity.

While we did not observe any statistically significant association between 677 or 1298 MTHFR polymorphic variations and altered risk of leukaemia, our results do not completely contradict previous reports of a significant connection between genetic variation at MTHFR and the leukemogenic process, claiming that both the variants MTHFR677*T and 1298*C are protective for the development of infantile (Franco et al., 2001; Krainovic et al., 2004) or adult (Skibola et al., 1999) ALL. In the North Portuguese population, we have registered a non-significant trend to decreased frequency of 677CT or 677TT genotypes among ALL children. Therefore, similarly to all other studies addressing the role of genetic variation at 677 and risk of leukaemia, in the case sample we also observed a lower frequency of the 677*T allele than in the control one, supporting other findings that the presence of this allele confers a lower risk of developing ALL. According to the presumed protective mechanism of 677*T, its impact in cancer susceptibility can be compensated by environmental interactions related with the “population” folate status. This might explain the absence for MTHFR677 of statistical significant results in our study since, in Portugal folate supplementation during pregnancy is usually recommended.

Concerning the effect of variation at MTHFR1298, contrarily to previous evidences that the MTHFR1298*C also predisposes to a lower risk of leukaemia (Skibola et al., 1999), in the Portuguese ALL sample the allele exhibited a non-significant increased frequency comparatively to healthy individuals. Identical results were obtained by Franco et al.; taken
together the available data for the association of MTFHR1298*C and ALL are quite conflicting suggesting that its effect, if any, in the leukemogenesis must be more modest than that of MTFHR677*T, being difficult to be captured in traditional case-control approaches. As elsewhere recommended (Franco et al., 2001; Krajnović et al., 2004, Skibola et al., 1999; Chiusolo et al., 2004; Robien et al., 2003), larger epidemiological studies are needed to fully clarify the role of MTHFR genetic variations in ALL development and they should be accompanied by the simultaneous folate assessment, because its levels can mask or alter the influence of genetic variations.

ACKNOWLEDGMENTS

This work was partially supported by a research grant from Fundação para a Ciência e Tecnologia to Elisabete Oliveira (BI in the project POCTI/MGI/45076/2002), and by IPATIMUP by Programa Operacional Ciência, Tecnologia e Inovação (POCTI), Quadro Comunitário de Apoio III.

REFERENCES