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SHORT REPORT

The methylenetetrahydrofolate reductase C677T gene polymorphism decreases the risk of childhood acute lymphocytic leukaemia

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Summary. We have determined the prevalence of methylenetetrahydrofolate reductase (MTHFR) mutations C677T and A1298C in 71 children (⩽ 15 years) with acute lymphoblastic leukaemia (ALL) and in 71 control subjects. Odds ratio (OR) for ALL linked to MTHFR C677T was 0·4 (95% CI 0·2–0·8); for heterozygotes it was 0·5 (95% CI 0·2–0·9) and for homozygotes it was 0·3 (95%CI 0·09–0·8). MTHFR A1298C yielded an overall OR for ALL of 1·3 (95% CI: 0·7–2·6); for heterozygotes it was 1·3 (95% CI: 0·7–7·6) and for homozygotes it was 2·8 (95% CI 0·5–15·6). In conclusion, MTHFR C677T was linked to a significant 2·4-fold decreased risk of developing childhood ALL, whereas MTHFR A1298C did not significantly affect the risk of ALL in our population.

Keywords: acute lymphocytic leukaemia, MTHFR, polymorphisms, risk factor, folate metabolism.

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme involved in homocysteine intracellular metabolism. Gene defects in MTHFR may result in enzyme deficiency and hyperhomocysteinaemia. To date, several mutations in MTHFR have been identified; most of these are rare and only have clinical consequences in homozygosity. This condition (homocystinuria) is characterized by multiple neurological deficits, psychomotor retardation, seizures, skeletal abnormalities, lens dislocation, premature arterial disease and venous thromboembolism (Rozen, 1997). In contrast to the rarity of these defects, two mutations in the MTHFR are prevalent in the general population: C677T and A1298C.

MTHFR C677T is (in its homozygous state) associated with reduced enzyme activity, thermolability and mild to moderate hyperhomocysteinaemia (Rozen, 1997). A second polymorphism in MTHFR (A1298C) has been reported, which also exhibits a high-allele frequency in the general population (van der Put *et al*, 1998; Franco *et al*, 1999). On its own, MTHFR A1298C does not result in decreased enzyme activity or increased plasma homocysteine levels, but in association with MTHFR C677T it may lead to hyperhomocysteinaemia (van der Put *et al*, 1998).

In recent years, the two MTHFR polymorphisms have been investigated in their relation to vascular thrombotic diseases in several studies (Rozen, 1997; Franco *et al*, 1999). More recently, a study demonstrated an association between carriership of the two common MTHFR polymorphisms (C677T and A1298C) and protection from acute lymphocytic leukaemia (ALL) in adults (Skibola *et al*, 1999). It was hypothesized that both polymorphisms (which in the homozygous form leads to diminished enzyme activity and an increase of the methylene-THF pool at the expense of a decrease in the methyl-THF pool) may reduce uracil misincorporation in DNA, lowering the risk of chromosome breaks and consequently decreasing risk of developing leukaemia (Skibola *et al*, 1999; Ames, 1999). Whether the two MTHFR polymorphisms also exert a protective effect in childhood ALL is unknown. Therefore, in the present study, we investigated the influence of MTHFR C677T and A1298C on the risk of childhood ALL by determining their prevalence in children with ALL and in matched healthy control subjects.

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PATIENTS AND METHODS

Subjects. Included were 71 patients aged ⩽ 15 years with

Table I. General characteristics of patients with childhood ALL and healthy controls.

Characteristic	Patients	Control subjects
Number	71	71
Mean age (range)	7·6 years (2 months – 15 years)	7·6 years (2 months – 15 years)
Male/female ratio	0·4	0·4
Race		
White	64	64
Black	6	6
Mulatto	1	1
Immunophenotype		
B-phenotype ALL	55	–
T-ALL	16	–

Table II. MTHFR polymorphisms and the risk of childhood ALL.

	Patients ($n = 71$)	Controls ($n = 71$)	OR (95%CI)
MTHFR C677T			
CC	36	22	1·0*
CT	28	36	0·5 (0·2–0·9)
TT	6	13	0·3 (0·09–0·8)
CT + TT	34	49	0·4 (0·2–0·8)
MTHFR A1298C			
AA	36	41	1·0*
AC	30	28	1·3 (0·7–7·6)
CC	5	2	2·8 (0·5–15·6)
AC + CC	35	30	1·3 (0·7–2·6)

*Reference category (OR = 1·0).

a diagnosis of acute lymphocytic leukaemia, admitted to the University Hospital of the School of Medicine of Ribeirão Preto, University of São Paulo, between January 1991 and January 2000. Seventy-one age-, gender- and race-matched healthy children without evidence of malignancy were investigated as the control group. General characteristics of patients and controls are presented in Table I. Mean age in both study groups was 7·6 years (range: 2 months – 15 years), with a male to female ratio of 0·4. Based on phenotype characteristics, 64 patients were White, six were Black and one was Mulatto. Based on immunophenotyping studies, 55 cases were classified as B-phenotype ALL and 16 cases were diagnosed as T-ALL (Rego *et al*, 1999).

Methods. DNA was extracted from peripheral blood leucocytes using standard methods (Miller *et al*, 1988). Polymerase chain reaction (PCR) amplification followed by *Hin*fI and *Mbo*II restriction digestion was used to determine the MTHFR 677 and 1298 genotypes respectively (Frosst *et al*, 1995; van der Put *et al*, 1998; Franco *et al*, 1999). Odds ratios (OR) as an estimate of relative risks and 95% confidence intervals (95% CI) were calculated using standard methods.

RESULTS

MTHFR C677T was detected in 34 patients (48%) and in 49 control subjects (69%), yielding an overall odds ratio (OR) for ALL of 0·4 (95% CI 0·2–0·8). OR for heterozygotes was 0·5 (95% CI 0·2–0·9) and for homozygotes the OR was 0·3 (95% CI 0·09–0·8). PCR failure occurred in one case during MTHFR C677T genotyping (Table II).

MTHFR A1298C was detected in 35 patients (49%) and 30 control subjects (42%) yielding and overall OR for ALL of 1·3 (95% CI 0·7–2·6). The OR for heterozygotes was 1·3 (95% CI 0·7–7·6) and for homozygotes the OR was 2·8 (95% CI 0·5–15·6) (Table II).

We also recalculated the OR for leukaemia taking into account the combined inheritance of MTHFR A1298C and C677T (data not shown in the tables). In this context, no subject was homozygous for both polymorphisms. MTHFR A1298C and C677T were co-inherited in 13 patients (18.3%) and 15 control subjects (21·1%), with an OR of 0·8 (95% CI 0·4–1·9).

DISCUSSION

In the present study, carriership of MTHFR C677T was linked to a significant 2·4-fold decreased risk of developing childhood ALL. This finding supports the idea that increased availability of 5,10-MTHF, which is a feature of MTHFR 677T, influences the leukaemogenesis process by conferring protection against the occurrence of ALL. Our findings are in agreement with recent data on the impact of MTHFR C677T on the risk of adult ALL (Skibola *et al*, 1999) in so far as both investigations demonstrated a protective effect against leukaemia conferred by the mutant 677T allele. Skibola *et al* (1999) analysed the MTHFR polymorphisms in 69 adult ALL cases, and found an OR linked to heterozygosity for MTFHR C677T of 0·58 (95% CI 0·27–1·28), and an OR of 0·23 (95% CI 0·06–0·81) linked to the homozygous (TT) state. In the same study, the OR for heterozygous and homozygous MTHFR A1298C were 0·33 (95% CI 0·15–0·73) and 0·07 (95% CI 0·00–1·77) respectively.

MTHFR A1298C did not significantly affect the risk of ALL in our population, either isolated or in combination with MTHFR C677T. Conversely, a (non-significant) trend towards increased risk was observed for A1298C homozygosity. This finding contrasts with the data currently available on the role of MTHFR A1298C in adults (Skibola *et al*, 1999), for whom a protective effect linked to this polymorphism was detected. Several possibilities may be raised to explain these contrasting results. One possibility is that MTHFR A1298C does not influence the risk of leukaemia in children. Alternatively, our study may not have had statistical power to detect a mild effect of the polymorphism on leukaemic risk. The influence of ethnicity on the results should also be considered as the polymorphisms investigated here are known to exhibit a significantly heterogeneous ethnic distribution (Franco *et al*, 1998; van

der Put *et al*, 1998; Akar *et al*, 2000); we tried to minimize the impact of this issue by using ethnically matched controls. Finally, one may argue that the different MTHFR polymorphisms may exhibit differential impact on the risk of leukaemia in different populations due to specific gene–gene and gene–environment interactions. For instance, vitamin intake probably differs between different populations, a fact that will probably modulate protective effects for leukaemia conferred by MTHFR gene variations.

We did not detect a significant synergism between the two MTHFR polymorphisms in determining leukaemic risk, i.e. no supra-additive protective effect was observed when co-inheritance of A1298C and C677T was present. However, these calculations were based on small numbers of compound carriers and therefore need confirmation in larger samples of paediatric patients with ALL and control subjects.

The OR for MTHFR C677T homozygotes tended to be lower than the OR for heterozygotes, though the overlap between the confidence intervals do not allow us to definitively conclude that the protective effect is more intense when MTHFR C677T is present in homozygosity. It should be stressed, however, that in the study published by Skibola *et al* (1999) the same trend towards higher protection in homozygotes was detected in adult ALL. Thus, both studies agree in suggesting a gene-dosage effect linked to MTHFR C677T. Furthermore, given the proposed mechanism to explain the decreased leukaemic risk, it is conceivable that homozygosity would have a more pronounced impact than heterozygosity in affecting uracil incorporation into DNA, chromosome breakage and oncogenesis (Chen *et al*, 1996; Ma *et al*, 1997; Ames, 1999).

In conclusion, the data from the present investigation support the hypothesis that the MTHFR C677T polymorphism is protective against the development of ALL in children. Our findings should encourage further exploration of the role of MTHFR gene variations in lymphoid neoplastic disorders, with emphasis in aspects involving gene–environmental interactions in modulating leukaemic risk. Finally, the contribution of the MTHFR genotype of the parents to the protective effect for ALL in children detected in the present study is a point for exploration in future investigations, as the parental genotype may influence germ line damage and therefore contribute to ALL in children.

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REFERENCES

Akar, N., Akar, E., Akcay, R., Avcu, F., Yalcin, A. & Cin, S. (2000) Effect of methylenetetraydrofolate reductase 677 CT, 1298 AC, and 1317 TC on factor V 1691 mutation in Turkish deep vein thrombosis patients. *Thrombosis Research*, **97**, 163–167.

Ames, B.N. (1999) Cancer prevention and diet: help from single nucleotide polymorphisms. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 12216–12218.

Chen, J., Giovannucci, E., Kelsey, K., Rimm, E.B., Stampfer, M.J., Colditz, G.A., Spiegelmen, D., Willet, W.C. & Hunter, D.J. (1996) A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. *Cancer Research*, **56**, 4862–4864.

Franco, R.F., Araújo, A.G., Guerreiro, J.F., Elion, J. & Zago, M.A. (1998) Analysis of the 677 CT mutation of the methylenetetrahydrofolate reductase gene in different ethnic groups. *Thrombosis and Haemostasis*, **79**, 119–121.

Franco, R.F., Morelli, V., Lourenço, D., Maffei, F.H., Tavella, M.H., Piccinato, C.E., Thomazini, I.A. & Zago, M.A. (1999) A second mutation in the methylenetetrahydrofolate reductase gene and the risk of venous thrombotic disease. *British Journal of Haematology*, **105**, 556–559.

Frosst, P., Blom, H.J., Milos, R., Goyette, P., Sheppard, C.A., Mattheus, R.G., Boers, G.J.H., den Heijer, M., Kluijtmans, L.A.J., van den Heuvel, L.P. & Rozen, R. (1995) A candidate risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nature Genetics*, **10**, 111–113.

Ma, J., Stampfer, M.J., Giovannucci, E., Artigas, C., Hunter, D.J., Fuchs, C., Willett, W.C., Selhub, J., Hennekens, C.H. & Rozen, R. (1997) Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. *Cancer Research*, **57**, 1098–1102.

Miller, S.A., Dykes, D.D. & Polesky, H.F. (1988) A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Research*, **16**, 1215–1218.

van der Put, N.M., Gabreels, F., Stevens, E.M., Smeitink, J.A., Trijbels, F.J., Eskes, T.K., van den Heuvel, L.P. & Blom, H.J. (1998) A second mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? *American Journal of Human Genetics*, **62**, 1044–1051.

Rego, E.M., Tone, L.G., Garcia, A.B. & Falcão, R.P. (1999) CD10 and CD19 fluoresecence intensity of B-cell precursors in normal and leukemic bone marrow. Clinical characterization of CD10 (+strong) and CD10 (+weak) common acute lymphoblastic leukemia. *Leukemia Research*, **23**, 441–450.

Rozen, R. (1997) Genetic predisposition to hyperhomocysteinaemia: deficiency of methylenetetrahydrofolate reductase (MTHFR). *Thrombosis and Haemostasis*, **78**, 297–301.

Skibola, C.F., Smith, M.T., Kane, E., Roman, E., Rollison, S., Cartwright, R.A. & Morgan, G. (1999) Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 12810–12815.