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ORIGINAL INVESTIGATION

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Screening of the C677T mutation on the methylenetetrahydrofolate reductase gene in French patients with neural tube defects

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Abstract We report the analysis of the distribution of the C677T mutation on the methylenetetrahydrofolate reductase (MTHFR) gene in prenatally diagnosed neural tube defects (NTD) cases and controls. In contrast to previous reports, we found the same distribution in fetuses with NTD and controls, which suggests that the MTHFR C677T mutation cannot be regarded as a genetic risk factor for NTD.

The relation between mutations in the gene coding for 5,10-methylenetetrahydrofolate reductase (MTHFR) and neural tube defects (NTD) has been recently questioned (Ou et al. 1995; Van der Put et al. 1995; Whitehead et al. 1995). Posey et al. (1996) calculated that 13% of NTD cases can be attributed to MTHFR gene C677T mutation homozygosity. The authors highlighted the correlation between this frequency and the 50%–70% reduction in NTD rates that occurs with adequate periconceptional consumption of folic acid. We retrospectively analyzed the distribution of the MTHFR C677T mutation in 43 prenatally diagnosed severe NTD cases.

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Forty-three cases of myelomeningocele spina bifida or anencephaly without associated malformations or maternal diabetes were examined. All karyotypes were found to be normal by standard techniques. Prenatal diagnosis in all cases was based on ultrasound examination and confirmed by amniotic fluid acetylcholinesterase electrophoresis and at autopsy. Myelomeningocele was observed in 31 cases and anencephaly in 12. All pregnancies were terminated at the request of the parents and in accordance with French law. These cases were compared with 133 unrelated controls from CEPH (Centre d'Etudes du Polymorphisme Humain) or a panel of French volunteers.

DNA was extracted from lymphocytes and lymphoblastoid cell lines (controls) or frozen fetal blood, hepatic or lung tissues, amniotic cells, and embedded paraffin blocks (cases). The C677T mutation was analyzed by polymerase chain reaction (PCR) followed by *Hin*fI restriction was previously described (Frosst et al. 1995). Digested PCR products migrated on a 2% low melting-1% regular agarose gel with co-amplified and co-digested homozygous and heterozygous DNA controls.

The distribution of the mutation was the same in fetuses with myelomeningocele or anencephaly and controls (Table 1). Homozygosity for the mutation was found in 10% of controls (13 cases) and 7% of NTDs (3 cases). These results differ markedly from previous reports (Ou et al. 1995; Van der Put et al. 1995; Whitehead et al. 1995) and suggest that the MTHFR C677T mutation cannot be regarded as a genetic risk factor for NTD. The severity of the disease cannot explain the difference between our study and others since Ou et al. (1995) also studied prenatally detected cases (live cases may correspond to meningocele spina bifida, a clinical form not lethal in the first years of life, while prenatally detected cases may represent severer clinical forms with anencephaly or myelomeningocele spina bifida). But the frequency of C677T seems to exhibit great variability among Caucasian populations, regarding both genotypic and allelic frequencies (Stevenson et al. 1997). Homozygosity for the mutation was found ranging from 5%–6% in the Netherlands and in Ireland (Van der Put et al. 1995; Whitehead et

Table 1 Distribution of genotypes and alleles in neural tube defects (NTDs) and controls (+ corresponds to the presence of the *Hin*fI restriction site generated by the mutation C677T, – to its absence)

	Genotypes			Alleles	
	–/–	–/+	+/+	–	+
Controls (n = 133)	50 (38%)	70 (53%)	13 (10%)	170 (64%)	96 (36%)
Spina myelomeningocele (n = 31)	14 (45%)	14 (45%)	3 (10%)	42 (68%)	20 (32%)
Anencephalies (n = 12)	5 (42%)	7 (58%)	0	17 (71%)	7 (29%)
Total NTDs (n = 43)	19 (44%)	21 (49%)	3 (7%)	59 (69%)	27 (31%)
Chi-square values (P values)	$\chi^2 = 0.73$ ($P > 0.5$)			$\chi^2 = 0.63$ ($P > 0.5$)	

al. 1995) to 10% in France (this study) and 16% in Italy (De Franchis et al. 1995). As emphasized by De Franchis et al. (1995), these variations do not correlate with the incidence of NTDs in these countries since, for instance, the birth prevalence of spina bifida in Italy and France is lower than in the Netherlands and Ireland, yet C677T is more frequent. This absence of a correlation does not support the hypothesis of an increased risk associated with C677T. In our control group, no significant difference was found between controls from CEPH (103 cases) and the 30 controls from our panel of French volunteers (not shown). This control population is probably heterogeneous with regard to geographic origin, but the distribution of C677T alleles and genotypes was similar to that in other Caucasian populations (Frosst et al. 1995; Wilcken and Wang 1996; Jacques et al. 1996), suggesting that the absence of a significant difference between NTD cases and controls is not due to control selection.

On the other hand, C677T clearly results in MTHFR thermolability, as shown by the expression of mutagenized cDNA in *E. coli* (Frosst et al. 1995), and MTHFR thermolability was found in patients with hyperhomocysteinemia, a recessive autosomal trait which increases the risk for coronary artery disease (Kang et al. 1991). Thus, the mutation cannot be considered neutral. Although the role of the C667T genotype in MTHFR thermolability is now clearly demonstrated, the effect of the MTHFR thermolability on NTDs is not established. It must be considered that NTDs are multifactorial diseases with probably both environmental and genetic causes. Among the genetic factors are the genetic background of both the fetus and the mother (diabetes, chromosomal abnormality), as well as the multiple genes involved in the folate pathway which may also harbor mutations increasing the risk for spina bifida. This is corroborated by a recent study (Trembath et al. 1996) reporting mutations in the folate receptor alpha gene in patients with NTDs. Taking account of the strong heterogeneity of both the human populations and the causal factors of NTDs, looking for NTD risk factors will probably result more in regional specific risk factors than in global causes of NTDs. Another possible explanation of the C677T-NTD association found in only certain populations would be that another mutation in the MTHFR gene is the genuine associated risk factor for NTD. If this is so, C677T would be a linked polymorphism associated with the risk-carrying mutation in only certain populations and would play the role of genetic marker of the causal mutation. This is corroborated by a recent study reporting chromosomes with both C677T and another rare mutation in the MTHFR gene in patients with hyperhomocysteinemia (Goyette et al. 1996). Extensive analysis of the gene in patients would be a way of confirming or rejecting this hypothesis. It also would be interesting to determine the frequency of C677T in NTDs from other populations and the maternal and fetal MTHFR activity in prenatally detected cases, a prospective study now in progress in our laboratory.

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