ELECTRONIC LETTER

Human *T* and risk for neural tube defects

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Neural tube defects (NTDs) are likely to result from an interaction of several genes and environmental factors. A role for genetic factors is supported by the finding that first degree relatives of NTD patients have a significantly increased recurrence risk.^{1,2} Since periconceptional folic acid supplementation of the maternal diet has been shown to have a preventive effect on occurrence and recurrence of the malformation, one contributing factor is believed to involve an altered folate metabolism.³⁻⁵

Other potential candidate genes for human NTDs are offered by animal models. NTDs have been observed in a variety of mutant mice, spontaneously arisen or genetically engineered.^{6,7} One of the candidate genes for human NTDs, the murine *Brachyury* gene (*T* gene), was recognised by virtue of its mutant phenotype. Mice that are homozygous for null mutations in *Brachyury* die in midgestation because of abnormalities in notochord and defects in other mesoderm derived structures. Heterozygous mice have short tails, notochord anomalies, and fusion of neural tube and gut in the caudal region.⁸ Studies of *T* gene expression pattern show that it is maintained in those cells that are absent in mutant mouse embryos, early stage mesoderm, tailbud, and notochord.^{8,9}

Brachyury encodes a DNA binding protein that functions as a transcription activator.¹⁰ After the cloning of the human homologue of *Brachyury*, human *T*,¹¹ Morrison *et al*¹² were the first to determine its role in susceptibility to NTDs. A statistically significant transmission distortion ($p=0.02$) between the intronic TIVS7-2 allele and familial spina bifida aperta (SBA) patients was shown in their mixed UK/Dutch patient group. In contrast, a case-control study for sporadic SBA cases found no allelic association with the TIVS7-2 allele.¹² In a follow up study, the allelic association between the *T* gene polymorphism and familial SBA was confirmed.¹³ Equal transmission rates for this intronic polymorphism were described in a sporadic NTD population from Iowa, USA.¹⁴ An Irish study found no relationship between familial ($p=0.86$) or sporadic SBA cases ($p=0.063$) and TIVS7 genotypes.¹⁵ Since there has been a decline in the incidence of NTDs in Ireland in the last 20 years, the Irish study investigated whether the TIVS7-2 allele was clustered in families with a SBA case born before the year 1980. Indeed, the data provided evidence for a trend towards an excess of the TIVS7-2 allele among cases (familial and non-familial) born before the year 1980 ($p=0.03$). This finding is difficult to explain, especially without any knowledge of the biological consequence (that is, interaction with environmental factors) of the polymorphism in intron 7. It is conceivable that the site is, for instance, in linkage disequilibrium with an as yet undetected mutation and/or polymorphism which has effects on mRNA processing or directly on the function of the *T* protein.

In order to evaluate further the association of this polymorphism in a good sized NTD population with a different genetic background from other previous studies, a panel of 183 German NTD patients (95 males, 88 females, 168 non-familial, 15 familial) with non-syndromic neural tube defects (nine anencephalics, three encephaloceles, 171 spina bifida aperta) was investigated. The control population consisted of 266 unrelated healthy German volunteers (96 males, 170 females).

Table 1 Genotype distributions and allele frequencies for the human *T* polymorphism TIVS7

	Genotype			Allele frequency	
	1/1	1/2	2/2	Allele 1	Allele 2
Controls (n=266)	137	107	22	0.72	0.28
Patients (n=183)	88	81	14	0.70	0.30

The study was approved by the ethics committee of the University of Marburg and informed consent was obtained from all people tested.

METHODS AND RESULTS

Genotyping for the TIVS7 three allele polymorphism was performed using primers and single strand conformation analysis (SSCA) as previously described.¹² We detected the two common alleles corresponding to a T (allele 1) to C (allele 2) single base exchange at position 79 bp downstream from the 5' end of intron 7. The rare third allele was not detected in our population. Genotypes resulting from the nucleotide exchanges were confirmed by sequencing several independent subjects. Unlike Ireland the incidence of NTDs in Germany has not changed in the last three decades. We therefore did not divide our case population by year of birth. We found TIVS7 heterozygosity (homozygosity of allele 2) in 44.3% (7.6%) of cases and in 40.2% (8.3%) of controls (table 1). Genotype frequencies in cases and controls were compared using Cochran-Armitage trend tests.¹⁶ There was no significant difference in TIVS7 genotype distribution in our sample ($p=0.70$). Analysis of 101 trios (father, mother, SBA child) showed no transmission disequilibrium for the TIVS7-2 allele (transmitted:non-transmitted 45:49). A p value of 0.68 was calculated using the exact version of McNemar's test.¹⁷

Since failure of neural tube closure is likely to result from an interaction of several genes, we evaluated whether there is a gene-gene interaction between human *T* and folate/homocysteine pathway genotypes, namely 5,10-methylenetetrahydrofolate reductase (*MTHFR*) and cystathionine β -synthase (*CBS*) polymorphisms. Subjects homozygous for the *MTHFR* 677T allele in combination with homozygosity for the 1298A allele (677TT/1298AA) and compound heterozygotes for *MTHFR* 677CT/1298AC have been shown to be at a higher risk for NTD in some populations.¹⁸⁻²⁰ In an Italian sample, NTD patients homozygous for *MTHFR* 677TT were significantly more often heterozygous for the CBS 844ins68 allele than controls.²¹ We therefore tested if patients with the *MTHFR* 677TT/1298AA or 677CT/1298AC genotypes in combination with *CBS* 844ins heterozygosity carry TIVS7-2 alleles

Abbreviations: NTD, neural tube defects; SBA, spina bifida aperta

Table 2 Human T polymorphism TIVS7 in NTD cases (controls) with selected *MTHFR* haploid genotypes

<i>MTHFR</i> haploid genotypes			Human T genotypes		
			1/1	1/2	2/2
677	T	T	15/28	11/28	2/28
1298	A	A	(14/30)	(13/30)	(3/30)
677	C	T	21/44	16/44	7/44
1298	C	A	(24/48)	(18/48)	(6/48)

more often than controls (table 2). *MTHFR* and *CBS* genotyping of all subjects in this study has been reported previously.^{20–22}

Altogether 28 patients and 30 controls had the *MTHFR* risk genotype 677TT/1298AA. There was no TIVS7 genotype occurring more frequently with this *MTHFR* genotype ($p=0.71$, Cochran-Armitage trend tests¹⁶). The same was true for the *MTHFR* 677CT/1298AC genotype (44 cases, 48 controls) and the TIVS7 genotypes ($p=0.81$). No subject in the case or the control group had a combination of TIVS7-2 homozygosity, heterozygosity for the CBS 844ins68 allele, and a *MTHFR* 677TT/1298AA or 677CT/1298AC genotype. Thus, there is no evidence for an interaction between the human *T*, *MTHFR*, or *CBS* polymorphisms in our NTD population, although the study may have missed such an interaction owing to small sample sizes.

DISCUSSION

In conclusion, our results do not indicate an increased risk for sporadic SBA among homozygotes or heterozygotes for the human *T* TIVS7 genotypes. These data are supported by previous studies^{12–14–15} and therefore it is questionable if this polymorphism plays an important role in the susceptibility for sporadic NTD. In addition, the Irish data do not support the hypothesis for an association of the TIVS7-2 allele and familial SBA put forward by the combined British/Dutch NTD study.^{12–13–15} A larger study on familial SBA cases in an ethnically well defined case and control population or a carefully designed meta-analysis of different studies are necessary to provide convincing evidence for an association in familial cases only.

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