

## SHORT REPORT

## MTHFR 677TT genotype increases the risk for cervical artery dissections

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The methylene tetrahydrofolate reductase (MTHFR) C677T polymorphism was studied in 174 German patients with cervical artery dissection (CAD). The results were compared with published data on 927 healthy German controls. In the series of patients, the frequency of T alleles and of TT carriers was slightly higher (13.8%) than among the healthy controls (10.6%). In patients with multiple dissections ( $n=50$ ), the proportion of TT carriers (18%) was found to be even higher and correlated with the number of events. The MTHFR C677T polymorphism was suggested to modify the risk for CAD.

The methylene tetrahydrofolate reductase (MTHFR) 677T allele codes for the thermolabile form of MTHFR, a key enzyme in the conversion pathway of homocysteine to methionine. In people with the homozygous MTHFR 677TT genotype, mean plasma levels of homocysteine are mildly raised. As such, mild hyperhomocysteinaemia is a risk factor for cardiovascular diseases; the MTHFR 677TT genotype is considered to be a risk factor for arterial diseases too. The MTHFR 677TT genotype was found to be associated with increased carotid intima-media thickness,<sup>1</sup> ischaemic events<sup>2</sup> and stroke.<sup>3</sup>

The potential contribution of the MTHFR 677TT genotype to the risk for cervical artery dissection (CAD) is still unclear.<sup>4</sup> Pezzini and collaborators<sup>5</sup> found the homozygous MTHFR 677TT genotype associated with CAD. Another Italian group found an increased, albeit non-significant, percentage of TT carriers among 26 patients with CAD.<sup>6</sup> A subsequent German study on 95 patients with CAD and 95 healthy controls did not confirm these findings.<sup>7</sup> It was speculated that the effect of the MTHFR 677TT genotype on the risk for CAD does indeed exist, but is modest.<sup>8</sup> Mean plasma levels of homocysteine were consistently found to be raised in patients with CAD, even in those studies that did not find an association with the TT allele.<sup>6–7</sup> As folic acid and vitamin B<sub>12</sub> act as cofactors for the MTHFR enzyme, the plasma level of homocysteine is modified by the intake of these substances too. Hence, the genetic association is not likely to be strong.

## MATERIALS AND METHODS

We assessed the MTHFR C677T single-nucleotide polymorphism in patients with CAD by restriction fragment length polymorphism analysis of genomic DNA amplified by polymerase chain reaction. The clinical diagnosis of CAD was approved by fat suppression MRI in all patients. A total of 180 German patients who had been admitted to our hospital or referred to our institution from other German centres were enrolled in this study. Owing to medical histories being incomplete in five patients and our inability to amplify the MTHFR sequence in one patient, we analysed the MTHFR gene in 174 well-documented patients with CAD. The data from these patients were compared with data on

MTHFR C677T alleles in the healthy German adult population, as reported in five published studies.<sup>7–9–12</sup>

## RESULTS

Table 1 shows the published genotypes in 927 healthy controls and the assessed genotypes in 174 patients with CAD. The frequency of TT carriers among controls varies between 8.7% and 11.3% (mean 10.6%). The frequency of TT carriers in our series of patients with CAD is slightly increased (13.8%), but this difference is not significant ( $\chi^2$ ,  $p=0.21$ ). Further differentiation between patients shows an association between the number of affected vessels and the MTHFR genotypes. Among 50 patients with multiple dissections (recurrent dissections,  $n=17$ ; multiple simultaneous dissections,  $n=33$ ), we found nine carriers of the 677TT genotype (18%;  $\chi^2$ ,  $p=0.10$ ), and among 14 patients with three or more events, we found four TT carriers (28%;  $\chi^2$ ,  $p=0.032$ ).

## DISCUSSION

The number of patients with multiple dissections in our series is higher than in other published series. Two reasons for this are that some of our patients were followed over a time span longer than 10 years and that other German centres referred mainly patients with multiple dissections to the Heidelberg Neurology Department. Most patients with single-vessel dissections were from Heidelberg and were recruited from hospital-based consecutive series of all patients with CAD who were willing to participate in the study. Patients with multiple CAD were referred to the Heidelberg Neurology Department from other German centres for diagnostic investigation. We cannot exclude a bias towards multiple patients with CAD with more severe neurological deficits among patients from other centres. The data on healthy controls were selected from various published German association studies. All control series in these studies were composed of healthy German adults. We consider these samples to be representative of the general population. The frequency of the T alleles varies among the different control samples, but in the series of patients with CAD we found allelic frequencies outside this range of variation.

Our data suggest an association between the TT genotype and an increased risk for CAD. The analysis of all patients with CAD showed a somewhat larger, albeit non-significant, proportion of TT carriers among patients. To reach significant  $p$  values (5% level) with genotype (or allele) frequencies similar to those in our study (OR 1.35), a much larger series of about 700 patients had to be genotyped. As we studied only 174 patients with CAD, the slightly higher frequency of TT carriers in our series is not significant. Addition of the data from the three published genetic association studies on CAD and MTHFR results in a similar, somewhat increased

**Abbreviations:** CAD, cervical artery dissection; MTHFR, methylene tetrahydrofolate reductase

**Table 1** Methylene tetrahydrofolate reductase genotypes in healthy controls and in patients with cervical artery dissection

	n	Genotype			Frequency of T allele
		CC, n	CT, n	TT, n (%)	
<b>Controls</b>					
From Schnakenberg <i>et al</i> <sup>a</sup>	379*	184	152	43 (11.3)	0.31
From Koch <i>et al</i> <sup>†</sup>	153†	75	61	17 (11.1)	0.31
From Reinhardt <i>et al</i> <sup>‡</sup>	104‡	49	46	9 (8.7)	0.31
From Meyer <i>et al</i> <sup>§</sup>	196§	94	82	20 (10.2)	0.36
From Konrad <i>et al</i> <sup>¶</sup>	95¶	49	37	9 (9.5)	0.29
All controls	927	451	378	98 (10.6)	0.31
<b>Patients</b>					
With single dissection	124	61	48	15 (12.1)	0.31
With two dissections	36	16	15	5 (13.9)	0.35
With three dissections	10	4	4	2 (20)	0.40
With four dissections	4	1	1	2 (50)	0.63
All patients with CAD	174	82	68	24 (13.8)	0.33
<b>Patients with multiple dissections</b>					
Simultaneous dissections	33	14	13	6 (18.2)	0.39
Recurrent dissections	17	7	7	3 (17.6)	0.38

\*Healthy blood donors (aged between 18 and 68 years).

†Randomly selected from the population.

‡Apparently healthy volunteers (mean age 55.8 years (SD 13.9); 70 men and 34 women) without signs of coronary artery disease.

§196 healthy, non-related blood donors (101 men and 95 women) from Heidelberg, Germany.

¶Age-matched and sex-matched (to a series of patients with cervical artery dissection (CAD)) healthy controls drawn from the ongoing Prospective Cardiovascular Münster (PROCAM) study.

frequency of TT carriers among the patients (23/146 (15.8%) patients compared with 16/161 (9.9%) controls). These studies with comparable allele frequencies in the control groups suggest a picture similar to that observed from our data: a slight, but (owing to insufficient numbers of patients and controls) non-significant increase of TT carriers among patients.

Our analysis of patients with multiple dissections yielded an independent argument for a role of the TT genotype (or the T allele) in the aetiology of CAD. These data showed that the proportion of TT carriers increases with the number of dissections. Although the number of patients with multiple dissections is small and the statistical power of these results is modest, we consider the correlation of the T allele frequencies with the number of dissections in the patients to be suggestive of a causal relationship.

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Ethical approval: The local ethical committee of the medical faculty of the Heidelberg University approved the protocol and blood was sampled from all participants after written informed consent was obtained.

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