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Is the common 677C→T mutation in the methylenetetrahydrofolate reductase gene a risk factor for neural tube defects? A meta-analysis

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Summary

The common 677C→T mutation (+) in the 5,10-methylenetetrahydrofolate reductase gene, resulting in decreased activity of the enzyme, has been associated with spina bifida neural tube defects (NTD). We combined all known Dutch control groups, a total of 1273 individuals, and found a prevalence of the 677C→T mutation of 8.4%. When compared with the frequencies in 55 SB patients and to mothers with a child with SB their parents, this gave an OR of 1.9 [95% CI 1.1–3.3] for mothers and an OR of 1.5 [95% CI 0.74–3.1] for patients. The frequency of this mutation and its associated risk for NTD may be population-dependent. However, the frequencies of the 677C→T mutation in different national and international control groups are almost all in the same range. We therefore combined the observed frequencies of the 677C→T mutation in all reported studies. The mutation was present in 9.2% of controls, resulting in ORs for all reported NTD patients and their parents of: 1.7 [95% CI: 1.1–2.6]; 1.8 [95% CI: 1.1–3.1] and 1.9 [95% CI: 1.3–2.8] for mothers (combined prevalence 14.5%), fathers (combined prevalence 15.5%) and NTD patients (combined prevalence 16.4%), respectively, vs. all international controls. This meta-analysis confirms that the 677C→T mutation is a genetic risk factor for NTD.

Introduction

In our previous studies, a common 677C→T mutation in the 5,10-methylenetetrahydrofolate reductase (MTHFR) gene, resulting in reduced enzyme activity and an impaired homocysteine/folate metabolism, was investigated as a genetic risk factor for spina bifida (SB).[1,2] Homozygosity for the alanine to valine substitution (+/+) was observed in 4.8% of the controls, vs. 15.7% of the mothers, 10.0% of the fathers and 12.7% of the affected children of the families with SB offspring.[1] Significant odds ratios (ORs) of 3.7 for mothers and 2.9 for SB patients were observed in the first study, demonstrating an abnormality in folate metabolism as a genetic risk factor for SB. Our findings have been confirmed by two other studies on neural tube defects (NTD).[3,4] Recently, we showed that the OR increased to 4.1 [95% CI 1.5–11.1] if the mother has a +/+ genotype and her child a +/– genotype, and even to 6.1 [95% CI 1.0–35.5] if both the mother and her child have a +/+ genotype.[2]

MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a

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cosubstrate for the remethylation of homocysteine to methionine. The 677C→T mutation causes reduced MTHFR activity and a protein with thermolabile properties and is associated with elevated plasma homocysteine levels and a redistribution of folates, namely elevated red-cell folate (RCF) and lowered plasma folate.[1,2] This mutation explains a substantial part of the observed elevated plasma homocysteine levels in mothers of children with NTD. Although mean homocysteine levels were increased in those homozygous for this mutation, not all +/+ subjects had elevated homocysteine levels. Plasma folate is a very strong determinant of plasma homocysteine in individuals with the +/+ genotype. It has been suggested that individuals who are homozygous for the 677C→T mutation in the MTHFR gene have higher nutritional folate requirements.[2]

We reported previously that about 5% of the Dutch control group had the mutation in the homozygous form,[1] whereas an Irish study observed a frequency of 6.1% and an Atlanta group a frequency of 5% in their controls.[3,4] However, the prevalence of homozygosity for this mutation is also reported to be 12% in French Canadians,[5] 16.3% in Italians,[6] 10.7% in White Australians,[7] and (12% among British controls, 2% in Japanese and 9% in Utah Mormons).[8] In this study, we examined whether these reported prevalences of the 677C→T mutation in the MTHFR gene are population-dependent. If they were, the risk of NTD associated with this mutation might also be population-dependent. All known Dutch control groups were combined to estimate the prevalence of the 677C→T mutation in the Dutch population. Furthermore, all published data of control groups and NTD families were pooled to study the implications of this common mutation for the risk of NTD.

Methods

SB patients and their parents were recruited in collaboration with the Dutch society for patients with central nervous system defects and their parents (BOSK).[1] The study group consisted, as reported previously,[1,2] of 70 mothers, 60 fathers and 55 children with SB. Children younger than 3 years were excluded.

For a more reliable estimate of the prevalence of the 677C→T mutation among the Dutch population, the control group was composed of controls from several published and unpublished Dutch studies. Some 440 controls were from published studies: 318 controls (207 and 109 of whom had been used in our previous studies);[1,9] 99 controls[10] and 23 controls,[11] and 833 as yet unpublished controls. All the mutation analyses were performed by our laboratory. By combining these different studies, we obtained a large control group of 1273 unrelated Dutch individuals of both sexes with no history of NTD.

The investigated mutation in the MTHFR gene is a C to T substitution at base pair 677, altering an alanine to a valine residue. The prevalence of this mutation was investigated, as reported before.[1]

Statistics

Crude odds ratios (OR) and 95% confidence intervals (95% CIs) were calculated to estimate the relative risk of the 677C→T mutation for NTD offspring.

Results

Of 1273 Dutch controls, 107 (8.4%; 95% CI 6.9%–10.0%) had a +/+ genotype for the 677C→T mutation (Table 1). The +/– genotype was observed in 537 and the –/– genotype in 629 control. The observed frequency of the + allele was 0.3.

Figure 1 shows the frequencies and 95% CIs of the 677C→T mutation among the different Dutch controls and other international studies. Since there were no significant differences between the Dutch control groups, they were combined into one total Dutch control group.

We calculated ORs by comparing the prevalence of the 677C→T mutation in the combined Dutch control groups and in 55 SB patients and their parents. The ORs were: mothers ($n=70$), 1.9 [95% CI 1.1–3.3]; fathers ($n=60$), 1.1 [95% CI 0.5–2.4]; and patients ($n=55$), 1.5 [95% CI 0.7–3.1], all vs. combined Dutch controls ($n=1273$).

A large international control group was constructed by combining Dutch and other reported control groups.[1–12] The +/+ genotype was found in 235/254 international controls was found, giving a frequency of 9.2% (95% CI 8.1%–10.4%) (Figure 1). Only the Italian control group[6] had a significantly higher prevalence of this common mutation than the combined international control group.

The prevalences of the +/+ genotype among NTD patients[1,3,4,8] and their mothers[1,3,6,8] and fathers[1,3,8] were also calculated from combined groups. Overall, 36/219 NTD patients, 24/166 mothers and 17/110 fathers had a +/+ genotype. We calculated ORs for the combined NTD patients and their parents compared to the total international control group. These were: mothers, 1.6 [95% CI 1.1–2.3]; fathers, 1.7 [95% CI 1.1–3.6] and patients, 1.8 [95% CI 1.3–2.5], all vs. all international controls.

Discussion

The present data confirm our previous observations[1,2] that the 677C→T mutation in the MTHFR gene is a

Table 1 Prevalence of thermolabile MTHFR due to the 677C→T mutation among Dutch and international controls and NTD patients and their parents

Control groups	Group size	+/+ frequency (*n*)	Range 95% CI
Dutch controls			
Unpublished X[a]	833	8.9% (74)	7.0–10.8%
Put *et al.*[1]	318	7.9% (25)	4.7–11.0%
Verhoef *et al.*[10]	99	7.1% (7)	2.0–12.1%
Engbersen *et al.*[11]	23	4.3% (1)	0–13.0%
Total Dutch controls	1273	8.4% (107)	6.8–10.0%
Reported international controls			
Whitehead *et al.*[3]	99	6.1% (6)	2.0–10.1%
Ou *et al.*[4]	109	4.6% (5)	0.9–8.3%
Frosst *et al.*[5]	57	12.3% (7)	3.5–21.1%
Franchis *et al.*[6]	289	16.3% (47)	11.8–20.8%
Wilcken & Wang[7]	225	10.7% (24)	6.7–14.7%
Papapetrou et al.[8]	199	12.1% (24)	7.7–16.6%
Papapetrou *et al.*[8]	44	2.3% (1)	0–6.8%
Papapetrou *et al.*[8]	45	8.9% (4)	0–17.8%
Kang *et al.*[12]	202	5.0% (10)	2.0–7.9%
Total all controls	2542	9.2% (235)	8.1–10.4%
Reported NTD cases			
Put *et al.*[1]	55	12.7% (7)	3.6–21.8%
Whitehead *et al.*[3]	82	17.6% (15)	9.8–26.8%
Ou *et al.*[4]	41	22.0% (9)	9.8–34.1%
Papapetrou *et al.*[8]	41	12.2% (9)	2.4–22.0%
Total all NTD cases	219	16.4% (36)	11.4–21.5%
Reported mothers			
Put *et al.*[1]	70	15.7% (11)	7.1–24.3%
Whitehead *et al.*[3]	32	18.8% (6)	6.3–31.3%
Franchis *et al.*[6]	28	7.1% (2)	0–17.9%
Papapetrou *et al.*[8]	36	13.9% (5)	2.8–25.0%
Total all mothers	166	14.5% (24)	9.0–19.9%
Reported fathers			
Put *et al.*[1]	60	10.0% (6)	1.7–18.3%
Whitehead *et al.*[3]	24	29.2% (7)	12.5–45.8%
Papapetrou *et al.*[8]	26	15.4% (4)	3.8–26.9%
Total all fathers	110	15.5% (17)	8.2–22.7%

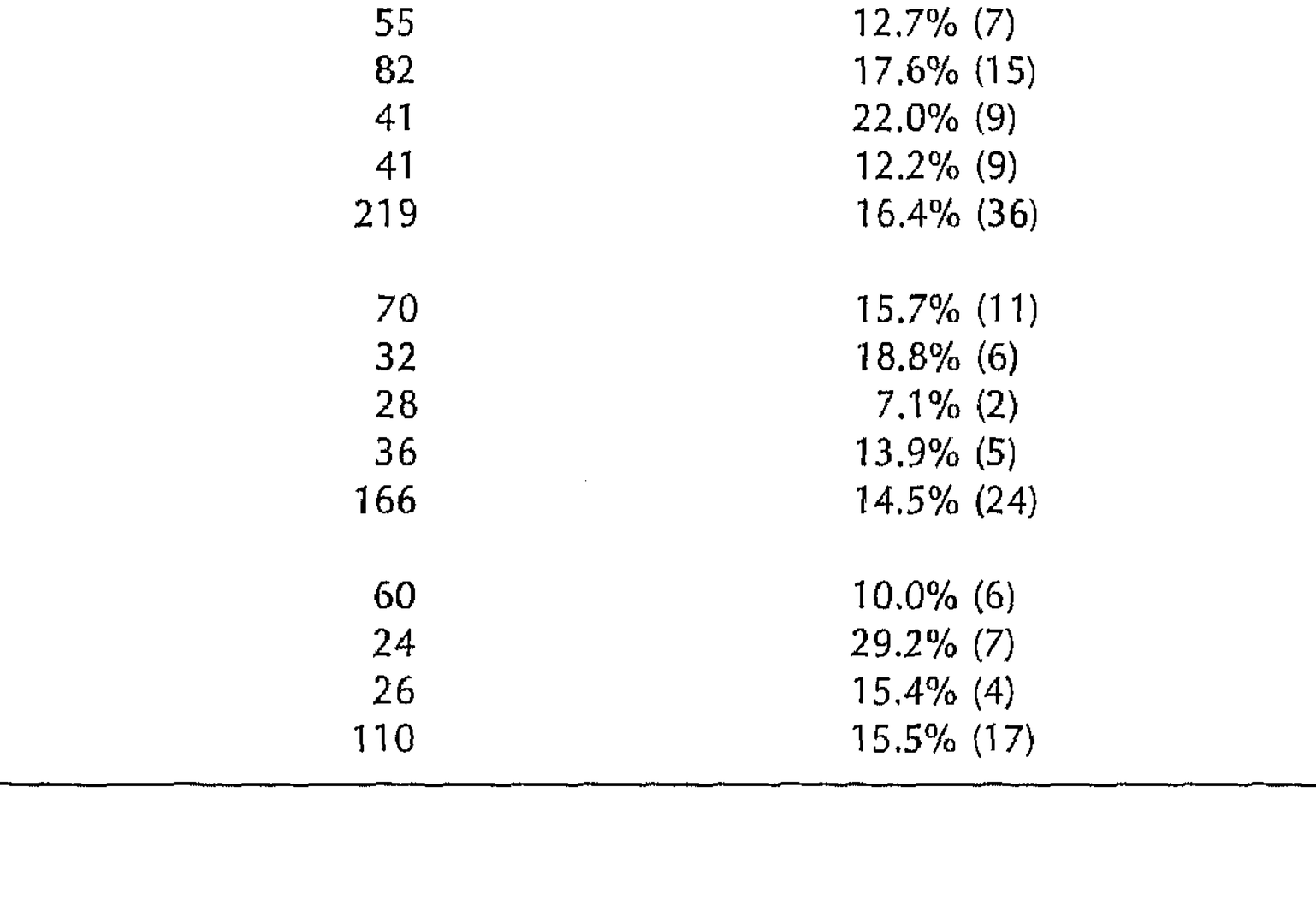

Figure 1. Prevalence of the 677C→T mutation among different control groups. The 95% CIs are shown by arrows. Reported prevalences among international control groups are shown as stars, those in Dutch controls are indicated by X, and the number indicates the relevant reference. X[a] represents the prevalence among unpublished Dutch controls. The combined prevalence of all Dutch controls is shown as ■ and the combined frequency among all Dutch and reported international control groups by ○.

genetic risk factor for NTD. The mutation analysis shows that homozygosity for this mutation is relevant not only in mothers, but also in the affected patients, and therefore their fathers. The risk of NTD increases if both the mother and her child have a +/+ genotype,[2] supporting this finding.

The observed ORs of 1.5–1.8 in patients and 1.6–1.9 in their mothers are modest, but from the point of view of general health care, the observed risk factors are of great importance because of the high prevalence of homozygous mutants of about 9% in the general population.

The 677C→T mutation causes an alanine to valine substitution in the MTHFR protein. This amino acid change results in decreased MTHFR activity in both the homozygous and the heterozygous state.[1] Individuals with a +/+ genotype demonstrate elevated plasma homocysteine at low folate levels, whereas those with a +/– or –/– genotype have normal plasma homocysteine levels, indicating that the +/+ genotype can cause a defective homocysteine methylation to methionine.[1,2] Embryonic tissues grow extremely rapidly, with high requirements for methyl groups from S-adenosylmethionine, which are provided by folate-dependent homocysteine remethylation. Individuals with a +/+ genotype may well need more folate to provide adequate amounts of methyl groups. While during early pregnancy fetal DNA is hypomethylated,[13] this relatively mild deficiency in folate metabolism may become important in pregnancy, when large amounts of methyl groups are required to methylate DNA. Disrupted gene expression, due to the undermethylation of parts of a gene, can explain how a relatively mild defect initiates the development of severe abnormalities in the embryo.

The 677C→T mutation analysis of all control groups known to us revealed few significant differences in population-specific frequencies; only one Italian study recorded a frequency that was significantly different from most, but not all, other control groups. The prevalences of the mutation among all different reported NTD patients and their parents were not significantly different between the studies.[1,3,4,6,8] Combining all international data on control groups and families with SB offspring, resulted in a twofold increased risk of the mutation for NTD. This increased risk was not only associated with the genotype of the mother but also with that of her child, and thus by implication the genotype of the father.

In conclusion, the observed frequencies of the 677C→T mutation in the MTHFR gene in the different control groups are within the same range, except for one Italian study.[6] Large numbers of controls and NTD families are required for a reliable estimate of the prevalence of this mutation. From this meta-analysis we conclude that homozygosity for this common mutation results in a twofold increased risk of NTD.

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