

Review

QJM

The MTHFR 677C→T polymorphism and the risk of congenital heart defects: a literature review and meta-analysis

I.M. VAN BEYNUM¹, M. DEN HEIJER^{2,3}, H.J. BLOM⁴ and L. KAPUSTA¹

From the ¹Children's Heart Centre, ²Department of Endocrinology, ³Department of Epidemiology and Biostatistics and ⁴Laboratory of Pediatrics and Neurology, Radboud University Medical Centre, Nijmegen, The Netherlands

Summary

Background: Periconceptual folic acid supplementation may protect against congenital heart defects (CHDs). Identification of candidate genes in folate metabolism has suggested that the 677C→T polymorphism in the methylenetetrahydrofolate reductase (MTHFR) gene may be particularly associated with the risk of CHDs.

Aim: To assess the relationship between MTHFR 677C→T and CHDs by literature review and meta-analysis.

Methods: Studies were identified by searches of electronic literature for papers focussing on MTHFR 677C→T and the risk of any type of CHD. Both case-control comparisons and transmission-disequilibrium tests (TDTs) in family-based designs were included.

Results: We found 13 eligible studies. Of 10 case-control studies, four focused on the fetal polymorphism, two studied the maternal polymorphism, and a further four investigated both. Three further

publications used a family-based association study to assess the effect of the T allele on cardiac development. Overall analysis yielded odds ratios of 1.3 (95%CI 0.97–1.73) and 1.2 (95%CI 0.83–1.74) for fetal and maternal MTHFR TT genotypes, respectively. TDTs revealed no association between fetal 677T allele and CHDs.

Discussion: This relatively small meta-analysis found no substantial evidence of increased CHD risk in individuals with MTHFR 677CT and TT genotypes. Heterogeneity regarding population background, study design and type of heart defects complicates the pooling and comparison of the studies. The effect of modification by periconceptual folic acid intake should be taken into account. Further larger studies and well-defined phenotypic subcategory analyses are needed to decide whether the MTHFR 677C→T polymorphism of the affected child and/or their mother is truly a risk factor for the development of CHDs.

Introduction

Congenital heart defects (CHDs) are a common variety of birth defect, with a prevalence of confirmed defects of approximately 1:100 living births, although this varies throughout the world.^{1,2}

They account for approximately one third of all congenital anomalies, and are the single largest contributor to infant mortality attributable to birth defects.^{3,4}

Address correspondence to Dr I.M. van Beynum, Children's Heart Centre, Radboud University Nijmegen Medical Centre, PO Box 9101, 6500 HB Nijmegen, The Netherlands. email: i.vanbeynum@cukz.umcn.nl

CHDs mainly result from incomplete development of the heart during the first 6 weeks of pregnancy. Despite the advances in diagnosis and treatment, understanding of the developmental causes and aetiologies of CHDs has been limited. In <20% of the cases, a cause can be found, including 22q11 deletion, trisomy 21, and established environmental risk factors as maternal diabetes, exposure to certain drugs and infectious agents,^{3,5,6} but the cause is unknown for the vast majority. Most CHDs are thought to be of complex multifactorial origin, with one or more alleles at a number of loci interacting with environmental factors.

Maternal multivitamin supplementation containing folic acid reduces the risk of neural tube defects, and evidence suggests that it may be associated with other reproductive outcomes, including CHDs.^{7–12} Methylene tetrahydrofolate reductase (MTHFR) catalyses the reduction of 5, 10-methylene tetrahydrofolate into 5-methyl tetrahydrofolate, which is the circulating form of folate. 5-Methyl tetrahydrofolate donates its methyl group to homocysteine, forming methionine and tetrahydrofolate. In the MTHFR enzyme, a common C→T substitution at position 677 (referred to as 677TT) exists, resulting in a substitution of alanine to valine, causing impaired folate binding and reduced activity of the MTHFR enzyme.^{13,14} About 10–12% of Caucasians of Northern European descents carry this 677TT genotype, and they have ~25% higher homocysteine levels than those with the 677CC genotype. The effect of the MTHFR 677TT genotype on homocysteine levels is more pronounced at low folate status.^{15,16}

In 1999, Kapusta *et al.* were the first to relate maternal hyperhomocysteinaemia to an increased risk for CHDs.¹⁷ More recently, Hobbs *et al.* studied mothers with CHDs in their offspring and identified homocysteine, S-adenosylhomocysteine and methionine as the most important biomarkers predictive of case status.¹⁸ Elucidation of an association between the MTHFR 677C→T polymorphism and CHDs might be informative about the hypothesis that the homocysteine methylation cycle plays a causal role in the development of CHD.

Studies regarding MTHFR 677C→T polymorphism in relation to CHD have yielded conflicting conclusions.^{19–31} We reviewed the literature, and evaluated the evidence as to whether such association is present, using a meta-analysis of studies on the MTHFR 677 C→T polymorphism and CHDs.

Methods

Data sources, study selection and data extraction

Eligible studies were identified by searching the electronic literature (PubMed database) for relevant published reports (using the terms: MTHFR, methylene tetrahydrofolate reductase, congenital heart defects, congenital anomalies, birth defects) and by hand searching reference lists of articles on this topic. Only human studies in the English language were included in the analysis. Data were extracted from the original reports, or in the case of with the authors.

The search yielded 13 retrospective studies eligible for inclusion.^{19–31} Among these 13 individual studies, 10 used a case-control design,^{19–28} four of the ten provided data on the fetal MTHFR genotypes,^{19,20,22,23} two provided data on the maternal genotypes,^{27,28} and four provided data on both.^{21,24,25,26} The other three eligible studies used a family-based Transmission Disequilibrium Test (TDT) to calculate the transmission frequency of the putative T-allele from heterozygous parents (CT) to their CHD-affected child.^{29–31} Van Beynum *et al.* presented both a TDT analysis and a case-control study.²⁴

Statistical analysis

For the meta-analysis, information was extracted from each report on: the odds ratios (or risk ratio) and its confidence interval; the study design—retrospective case-control—and the number of cases and controls. All odds ratios were recalculated using tabular data provided in the articles, or data provided by the authors if the complete genotype distribution was not provided. Summary estimates were obtained by taking an inverse-variance weighted average of the log odds ratios from individual studies. In our meta-analysis, we compared genotypes, using the CC genotype as the reference group, and generated separate odds ratios (ORs) for the CT and TT genotypes. Heterogeneity was assessed using standard χ^2 tests.

For the TDT, the number of T-alleles transmitted from heterozygous parents (CT) for the MTHFR polymorphism was used, with the non-transmitted T-alleles of the heterozygous parents serving as internal controls. An OR of disease risk and its associated standard error (SE) can be obtained from TDT results using the proportion of transmitted high-risk T-alleles. This can be expressed as the number of transmitted T-alleles (M_1) divided

by the number of transmitted C-alleles (M_2); ($OR=M_1/M_2$). The SE is then calculated using the following derived function:

$$SE = \sqrt{1/M_1 + 1/M_2}$$

The overall risk estimate can be computed from these data, as described previously. We used the statistical method described by Kazeem *et al.* for integrating results from the pooled case-control and TDT studies to provide a combined weighted analysis of disease-marker association.³² We tested for publication bias using the tests for funnel-plot asymmetry proposed by Begg and Mazumdar and Egger *et al.*^{33,34}

Classification of heart defects

Primarily, all CHDs were analysed together, but because of the heterogeneity of the included CHDs, associations between the polymorphism and specific heart defects could be missed. For three case-control studies focusing on the maternal MTHFR 677C→T polymorphism, data were available that enables us to perform a combined analysis for the conotruncal heart defects. The categorization of conotruncal heart defects defined by the individual studies was used.

Description of published studies

The characteristics of all included studies on the association between MTHFR 677C>T polymorphism and CHD are extensively described in Table 1.

Case-control studies: fetal MTHFR genotypes

Studies focusing on fetal MTHFR genotypes are summarized in Table 2. Junker *et al.* were the first to suggest an association between the incidence of CHDs and MTHFR 677C→T polymorphism. The authors observed a higher frequency of the 677TT genotype versus the combined group of CT and CC genotypes among children ($n=114$) with a CHD, compared with 228 controls. In particular, the frequency of the 677TT genotype in patients with pulmonary valve stenosis, hypoplastic left heart syndrome, coarctation of the aorta and aortic valve stenosis was significantly elevated, but the number of patients in these subgroups was rather small.¹⁹

Wenstrom *et al.* analysed amniotic fluid from a small number of pregnancies complicated by any type of isolated fetal cardiac defect. They

did not provide the full genotype distribution, but compared the combined group of 677CT and TT genotypes with CC individuals in samples of amniotic fluid of 26 pregnancies complicated by a CHD, and with 93 controls. They found the 677CT and TT genotypes in 35% of the samples versus 13% in controls, but the genotype distribution was not consistent with Hardy-Weinberg equilibrium in the control group. Due to incomplete data, this study was excluded from the meta-analysis.²⁰

Storti *et al.* did not observe any association between the fetal MTHFR genotypes ($n=103$ versus $n=200$ controls) and the risk of conotruncal heart defects. The CHD-affected children were included consecutively, but methods of case ascertainment and definition of conotruncal heart defects were not described. Among the cases, 11 individuals had the 22q11 deletion, which is strongly associated with conotruncal heart defects.²¹

Lee *et al.* studied 213 patients and 195 controls. Overall, the 677TT and CT genotypes in the cases were not significantly associated with the risk of CHDs. They observed a significantly increased proportion of homozygous TT genotypes in specific subgroups of CHD patients (valvular pulmonary stenosis, $n=9$; or pulmonary atresia with an intact ventricular septum, $n=3$). Whether the CHD was isolated, or part of a syndrome or genetic defect was unknown.²²

Shaw *et al.* performed a population-based case-control study, including cases with an isolated conotruncal heart defects, defined as anomalies resulting from aorticopulmonary septation. A total of 238 cases were ascertained among 344 214 deliveries in the study period, yielding an incidence of 7 conotruncal heart defects per 10 000 births. This incidence is relatively low compared to the literature, suggesting that cases with conotruncal heart defects may have been missed.¹ They determined 32 allelic variants, including MTHFR 677C→T polymorphism, by genotyping 155 infants with conotruncal defects and 437 infants without malformations. They did not find an increased risk of conotruncal heart defects for the MTHFR 677CT or TT genotype. Analyses in that study, investigating a potential gene-nutrient interaction between maternal periconceptional vitamin use and MTHFR genotypes, did not indicate that the CT or TT genotype contributed to conotruncal heart defect risk in infants, even in the absence of maternal use of multivitamin supplements with folic acid.²³

We analysed the effect of MTHFR 677C→T variants on CHD risk in 165 children, with either conotruncal or other heart defects. The distribution of MTHFR polymorphism was not different in

Table 1 Characteristics of the included studies on MTHFR 677C→T polymorphism and congenital heart defects (CHDs)

Authors	Country	Year	Year of inclusion	Types of CHD	Case ascertainment	Exclusion criteria	Ethnicity of cases	Ethnicity of controls	CC fetal	CC maternal	TDT	HWE	Incidence of CHD	Use of folic acid ^a
Wenstrom	USA	2001	1988–1998	All types	E/D prenatal, verified postnatal	Genetic disorder syndromes, teratogens, DM	Black 27%/20%, White 69%/78%, Other 4%/2%		Yes	–	–	No	Unknown	Unknown ^b
Junker	Germany	2001	1995–2000	All types except PFO	E/D and/or HC	Chromosomal anomalies	Caucasian	Caucasian, same area	Yes	–	–	Yes	Unknown	Known/not performed
Storti	Italy	2003	2000–2001	Conotruncal 11 cases 22q11 del	Unknown	Not described	Caucasian	Caucasian	Yes	Yes	–	Yes	Unknown	Unknown
Shaw	USA	2005	1987–1988	Conotruncal according to Clark	E/D, HC, surgery, autopsy	Aneusomies, single-gene disorders	White Non-Hispanic 67%/58%, White Hispanic 23%/29%, Other 10%/13%		Yes	–	–	Yes	0.7/1000	Known/performed ^b
Lee	Taiwan	2005	2002–2003	All types	All types HC	Not described	Asian	Asian, same area	Yes	–	–	Yes	Unknown	Unknown
Van Beynum	Netherlands	2006	2002–2003	All types	E/D and HC and/or surgery	NTD, clefts, genetic disorder, syndromes, associations	Caucasian	Caucasian, same area	Yes	Yes	Yes	Yes	Unknown	Known/performed
Zhu	China	2006	unknown	ASD, PDA	E/D or surgery	DM, PKU, chemicals, X-ray	Asian, province in China	Asian, same area	Yes	Yes	–	Yes	Unknown	Unknown
Galdieri	Brazil	2007	unknown	Unknown	E/D or HC	Genetic syndromes, other malformations	White 22%/53%, non-White 78%/47%		Yes	Yes	–	Yes	Unknown	Unknown
Nurk	Norway	2004	1950–1952, 1967–1996	Unknown	Unknown	Not described	Unknown	Unknown	–	Yes	–	Yes	1.7/1000	Unknown
Hobbs	USA	2006	1998–2004	Septal, conotruncal, right-left sided CHD	E/D, surgery and/or autopsy	Gene disorder, syndromes, chromosomal anomalies	White	White	–	Yes	–	Yes	Unknown	Additional use known/performed ^c

(continued)

Table 1 Continued

Authors	Country	Year	Year of inclusion	Types of CHD	Case ascertainment	Exclusion criteria	Ethnicity of cases	Ethnicity of controls	CC fetal	CC maternal	TDT	HWE	Incidence of CHD	Use of folic acid ^a
McBride	USA	2004	1998–2003	Left-sided CHD	E/D, HC and/or surgery	Multiple other malformations, syndromes	65% Caucasian 29% Hispanic 5% African-American 1% Asian	NA	–	–	Yes	Yes	Unknown	Additional use unknown ^c
Hobbs	USA	2006	1998–2004	Septal, conotruncal, right-left sided CHD	E/D, surgery and/or autopsy	Gene disorder, syndromes, chromosomal anomalies	Known, but not described	NA	–	–	Yes	Yes	Unknown	Additional use known/performed ^c
Pereira	Brazil	2005	Unknown	All types	Unknown	Not described	Unknown	NA	–	–	Yes	Yes	Unknown	Unknown

^aInformation about and stratification for periconceptional use of folic acid supplements or multivitamin supplements containing folic acid. ^bPerformed before introduction of food fortification with folic acid. ^cPerformed after introduction of food fortification with folic acid. ASD, atrial septal defect; CC fetal, case-control study on fetal MTHFR polymorphism; CC maternal, case-control study on maternal MTHFR polymorphism; DM, diabetes mellitus; E/D, echocardiography; HWE, Hardy-Weinberg equilibrium; NA, not applicable; HC, heart catheterization; PDA, persistent ductus arteriosus; PFO, patent foramen ovale; PKU, phenylketonuria; TDT, transmission disequilibrium test.

these children, compared to 220 healthy controls. The interaction with maternal periconceptional folic acid use was also investigated, for the fetal MTHFR polymorphism, no interaction was found.²⁴

Zhu *et al.* genotyped 56 cases with an atrial septal defect or patent ductus arteriosus, and 103 controls. Individuals exposed to diabetes mellitus, phenylketonuria, chemicals and X-ray were excluded. Data on genetic abnormalities were not given. For these two specific lesions, the fetal MTHFR TT genotype was significantly associated with increased risk of CHD.²⁵

Galdieri *et al.* analysed 58 patients with isolated CHDs and 38 controls. Further specifications of the heart defects were not given. No difference was found in the distribution MTHFR genotypes between cases and controls. The result might be influenced by significant differences in the racial background.²⁶

Case-control studies: maternal MTHFR genotypes

Data on the MTHFR 677C→T polymorphism in mothers with a CHD-affected child were reported in six case-control studies, summarized in Table 3.

Storti *et al.* were the first to study the effect of the MTHFR 677C→T polymorphism, in 103 Italian mothers with conotruncal defects in offspring versus 220 controls, and found no association. Data on periconceptional folic acid supplementation or intake were lacking, which might explain the absence of the possible detrimental action of MTHFR 677C→T polymorphism.²¹

In the Hordaland homocysteine study, a population-based cohort of women were genotyped for the MTHFR 677C→T polymorphism, and this was linked with their 14 492 pregnancies as recorded in the Medical Birth Registry of Norway. They did not find an association between the polymorphism and CHDs. In that study, the incidence of CHDs was very low, with only 25 CHD cases among the 14 492 pregnancies. The low incidence of 1.7 per 1000 is probably due to under-registration because the birth registry covered only until the first 8 days of life, and consequently missed many heart defects that were diagnosed after this period. Furthermore, information concerning periconceptional folic acid use was not available.²⁷

Hobbs *et al.* did not find an independent effect of the MTHFR 677C→T polymorphism in 275 women vs. 118 controls on the estimated risk of having a CHD-affected pregnancy. This study was done after the introduction of food fortification with folic acid. Additional use of folic acid or folic-acid-containing multivitamins during the

Table 2 Fetal MTHFR 677C→T polymorphism and risk of congenital heart defect in case-control studies

Authors	Cases	TT	CT	CC	Controls	TT	CT	CC	CT vs. CC (OR, 95%CI)	TT vs. CC (OR, 95%CI)	Use of folic acid ^a	Reference
Wenstrom	26	9 ^c	–	17	93	12 ^c	–	81	–	–	Not performed ^b	20
Junker	114	21	42	51	228	21	78	129	1.4 (0.83–2.24)	2.5 (1.27–5.02)	Not performed	19
Storti	103	20	55	28	200	40	108	52	0.9 (0.54–1.66)	0.9 (0.46–1.88)	Not performed	21
Shaw	153	16	68	69	434	52	202	180	0.9 (0.59–1.30)	0.8 (0.43–1.50)	Performed, no effect ^b	23
Lee	213	14	89	110	195	13	68	114	1.4 (0.90–2.04)	1.1 (0.50–2.48)	Not performed	22
van Beynum	165	20	60	79	220	18	104	98	0.7 (0.46–1.11)	1.4 (0.68–2.78)	Performed, no effect	24
Zhu	56	27	22	7	103	24	57	22	1.2 (0.45–3.24)	3.5 (1.28–9.73)	Not performed	25
Galdieri	58	7	21	30	38	6	14	18	0.9 (0.37–2.20)	0.7 (0.20–2.41)	Not performed	26
<i>Total</i>	882				1511				1.0 (0.84–1.23)	1.3 (0.97–1.73)		

^aStratification for periconceptual use of folic acid supplements or multivitamin supplements containing folic acid.

^bPerformed before introduction of food fortification with folic acid. ^cFigure for TT and CT combined.

Table 3 MTHFR 677C→T polymorphism and risk of congenital heart defect in case-control studies

Authors	Cases	TT	CT	CC	Controls	TT	CT	CC	CT vs. CC (OR, 95%CI)	TT vs. CC (OR, 95% CI)	Use of folic acid ^a	Reference
Storti	103	23	53	27	200	40	108	52	0.9 (0.53–1.67)	1.1 (0.55–2.21)	Not performed	21
van Beynum	158	18	68	72	261	23	107	131	1.2 (0.76–1.76)	1.4 (0.72–2.81)	Performed, significant effect ^b	24
Zhu	56	23	27	6	102	25	57	20	1.6 (0.57–4.38)	3.1 (1.05–8.97)	Not performed	25
Galdieri	47	5	15	27	26	1	15	10	0.4 (0.13–1.03)	1.9 (0.19–17.86)	Not performed	26
Nurk	25	1	12	12	14484	1282	6037	7165	1.2 (0.53–2.64)	0.5 (0.06–3.59)	Not performed	27
Hobbs	275	30	118	127	118	14	56	48	0.8 (0.50–1.26)	0.8 (0.40–1.66)	Performed, no effect ^c	28
<i>Total</i>	664				15191				1.0 (0.79–1.31)	1.2 (0.83–1.74)		

^aStratification for periconceptual use of folic acid supplements or multivitamin supplements containing folic acid.

^bMaternal MTHFR 677CT and TT genotype contributed to conotruncal heart defect risk in infants in the absence of maternal use of periconceptual folic acid: OR 3.3 (95%CI 1.46–7.32) and 6.3 (95%CI 2.32–17.27), respectively. ^cPerformed after introduction of food fortification with folic acid. Additional use of folic acid or folic-acid-containing multivitamins during the periconceptual period was not significantly different between cases and controls.

periconceptual period was not significantly different between cases and controls.²⁸

Overall, we did not observe an association with the maternal MTHFR variants, but we found that the MTHFR 677CT and TT genotypes of the mother, when combined with no use of periconceptual folic acid supplements, appeared to be a risk factor for CHDs in offspring, especially for conotruncal heart defects.²⁴

Zhu *et al.* found only an association between maternal TT genotype, compared with CT and CC genotypes, with the occurrence of persistent ductus arteriosus in offsprings.²⁵

Galdieri *et al.* observed no difference in genotype distribution among 47 women and 26 controls. This was a relatively small study with different

racial background.²⁶ In both studies, the effect of periconceptual folic acid supplements was not investigated. Whether the cases with persistent ductus arteriosus were premature babies is unknown.

Family-based case-parental studies

Four family-based association studies were published, assessing the transmission distortion of the putative T-allele in 761 complete triads (Table 4). In two of these studies, a log-linear approach was used to analyse for asymmetric distribution of the variant T-allele for the maternal MTHFR genotype.

Pereira *et al.* found no association between this locus and CHDs in a relatively small group of

Table 4 Family-based case-parental studies: MTHFR 677T allele and risk of congenital heart defect

Authors	Transmitted T-alleles	Non-transmitted T-alleles	OR (95%CI)	Use of folic acid ^a	Reference
Pereira	Not available	Not available	–	Not performed	29
McBride	50	64	0.8 (0.54–1.13)	Not performed ^b	30
Hobbs	124	117	1.1 (0.82–1.36)	Performed, no effect ^{b,c}	31
van Beynum	59	67	0.9 (0.62–1.25)	Performed, no effect	24
Total			0.9 (0.79–1.12)		

^aStratification for periconceptional use of folic acid supplements or multivitamin supplements containing folic acid.

^bStudy was performed after introduction of food fortification with folic acid. ^cAdditional use of folic acid supplements had no effect.

patients ($n=91$) and parents ($n=147$). Very limited data on study design, case ascertainment and CHD specification were given. Data on the number of transmitted and non-transmitted T-alleles were not available, therefore this study was excluded from the meta-analysis.²⁹

Mc Bride *et al.* analysed 197 complete trios using the TDT. No significant relation was found between MTHFR 677C→T polymorphisms and the presence of a congenital left-sided heart malformation. A log-linear analysis did not find increased relative risk based on the maternal genotype. The study was performed after folate food fortification.³⁰

In 375 nuclear families, with non-syndromic septal, conotruncal, or right or left-sided obstructive CHD diagnosed, Hobbs *et al.* did not observe a distortion in the transmission of the 677T allele, nor was there evidence of a parent-of-origin effect.³¹

In our study of 133 triads, the TDT revealed no association of the fetal 677T allele with the development of a heart defect.²⁴

Data synthesis and meta-analysis

Case-controls studies: fetal MTHFR genotypes

Data were obtained from eight case-control studies, including a total of 882 cases with any type of congenital heart defect and 1511 controls. In seven of these case-control studies, we had access to the full genotype distribution, which enabled us to perform a meta-analysis.^{19,21–26} Using the CC genotype as the reference category, we computed summary odds ratios for the heterozygote variant CT genotype (OR 1.0, 95%CI 0.84–1.23) and for the homozygote variant TT genotype (OR 1.3, 95%CI 0.97–1.73). There was no evidence of

publication bias using the Egger method (p for bias 0.76) or Begg's test (p for bias 1.0).

Case-controls studies: maternal MTHFR genotypes

Data on the MTHFR 677C→T polymorphism in a total of 664 mothers with a CHD-affected child and 15191 controls was reported in six case-control studies.^{21,24–28} Combining these six studies, the estimated odds ratios comparing CT with CC, and TT with CC, were 1.0 (95%CI 0.79–1.31) and 1.2 (95%CI 0.83–1.74), respectively. Three studies gave the genotype distribution among individual heart defects or a group of defects.^{21,24,28} Analysing subgroups of conotruncal heart defects revealed ORs of 1.0 (95%CI 0.71–1.47) and 1.3 (95%CI 0.81–2.19) for the maternal CT genotype and the TT genotype, respectively. The Egger method (p for bias 0.87) and Begg's test (p for bias 0.85) revealed no evidence of publication bias.

Family-based case-parental studies

Of the four family-based association studies, three were included in the meta-analysis, revealing a summarized OR of 0.9 (95%CI 0.79–1.12). The odds ratio for the joint analysis of TDT and fetal case control studies was 1.1 (95%CI 0.94–1.29).^{24,30,31}

Discussion

We have reviewed, and performed a meta-analysis of all published studies on associations between single nucleotide polymorphism (SNP) of the 5,10-methylenetetrahydrofolatereductase (MTHFR) gene and congenital heart defects.

The data published thus far do not provide strong evidence for an association between CHD risk and the MTHFR 677C→T polymorphism,

either in mothers or in their offspring. Some individual studies observed an association for the fetal and/or maternal MTHFR 677C→T polymorphism with different CHD types, or with a subgroup of heart defects.^{19,20,22,24,25} The absence of an overall significant association might indicate a real negative observation, but there are several important factors underlying the inability to find and to replicate gene-disease associations. Methodological and statistical issues are differences in study design, inadequate sample sizes, confounding from population structure, inadequate control selection, publication bias, allelic heterogeneity, and true heterogeneity in gene-disease associations, misclassification, variety of outcome parameters, and lack of information on potential effect modifiers, e.g. the introduction of food fortification with folic acid and periconceptional use of folic acid supplements. Which factors may contribute to the inconsistency between studies and to the inability to find a risk effect of MTHFR 677C→T polymorphism on the complex trait of CHD will be discussed below.

Publication bias concerning the MTHFR 677C>T polymorphism in relation to CHD appeared to be low. Both positive and negative studies are published, and formal testing revealed no evidence of publication bias. Hopefully negative results will still be accepted for publication in the future, or else alternative ways of publishing negative studies should be provided.

One problem is the use of different study designs, which might aggravate the comparison of outcome and reduce the sample size of the pooled data. The case-control design is sensitive to population stratification due to different genetic populations. A useful robust method to confirm gene-disease associations detected in case-control studies is the TDT approach.³⁵ Regarding the fetal MTHFR genotypes, the TDT results might provide stronger evidence for a real negative association observed in case control studies. In our opinion, both strategies are useful to explore the MTHFR 677C→T polymorphism in relation to CHDs in offspring. For case-control designs, population stratification should be controlled by a proper selection of cases and controls from the same demographic background.

Serious study of genetic associations with complex diseases, as with CHDs, will need far larger case-control collections than are typically available to researchers at present.³⁶ A total number of 13 studies focusing on MTHFR 677C→T polymorphism and CHD risk, with relatively small individual studies, yields insufficient power to draw definite conclusions. To detect a small

underlying size effect of the different MTHFR genotypes, even the meta-analysis is underpowered, because for small effects the required sample size will be very large. Furthermore, the detrimental effect on the developing embryo might be related to the polymorphism in the fetal or in the maternal genotype, or in both. A single focus on either the fetal or the maternal genotype makes the power of the pooled data even weaker. Together with the small sample sizes of the individual studies, the combined data lose further power.

Another important point is the broad heterogeneity of heart defects, which also complicates comparison of results. A potential link between MTHFR 677C→T polymorphism and a CHD could be missed if only a specific group of heart defects are studied. Cardiac development is a complex process in early embryonic phase, and the true morphogenesis of the heart is still not completely unravelled. The pathophysiological mechanism of CHDs is even more uncertain. Due to the relatively low prevalence of single heart defects, and for power reasons, most studies grouped the heart defects together as left- or right-sided heart defects, or conotruncal and other heart defects. Misclassification of outcome might even further weaken the association with the genotype. There is a need for use of uniform definition and classification of various types of CHD. Furthermore, the sample sizes need to be large to estimate accurately the causal relationship for finer phenotype categories of CHD.

A further complicating issue is the geographical and demographical variability; the studies were carried out in seven different countries, including populations from North America, South America, Europe and Asia. There might be a true variability in gene-disease association, but more important are the differences between these populations concerning the potential effect modifiers. Differences in the level of susceptibility of individuals to potentially adverse effects of environmental influences due to MTHFR polymorphism may be an important contributor to the presence or absence of this gene-disease association. Maternal life style and nutritional factors and exposure to several drugs early in pregnancy seem to contribute to the complex aetiologies of CHDs.^{4,37,38} Foremost among these factors is the periconceptional use of vitamin supplements containing folic acid and food fortification with folic acid. There is substantial evidence that increased folic acid intake plays an important role in the prevention of CHD. Observational studies have demonstrated an association between periconceptional use of multivitamins containing folic acid, and a reduction

of CHD, both conotruncal and other heart defects.^{7–12} Additional support for the importance of folate in CHD risk reduction was provided by Hernandez-Diaz *et al.*³⁹ Their study showed that periconceptional intake of medications acting as folic acid antagonists, including anti-epileptic agents and the group of drugs consisting of dihydrofolate reductase inhibitors, doubled the risk of cardiovascular defects (i.e. conotruncal defects, ventricular septal, and other cardiovascular defects) (OR 2.2, 95%CI 1.4–3.5).

In experimental animal model systems, limiting availability of folate to the developing embryo by inactivating the folate transporter, or a defective folate receptor, increases the risk of several CHDs, such as improper septation, persistent truncus arteriosus, and double-outlet right ventricle.⁴⁰

The availability of folate also plays an important role in the methylation cycle in each cell of the human body, except for red blood cells. For the preventive effect of folic acid on neural tube defects, the so called methylation hypothesis has been developed, which suggests that folic acid stimulates cellular methylation reactions.⁴¹ A comparable mechanism might be present for folic acid in relation to the prevention of CHDs. MTHFR has a unique position in folate metabolism, as it makes one-carbon units available for methylation reactions at the expense of purine and thymidine synthesis.

The importance of the MTHFR 677C→T polymorphism and folate metabolism lies in the large number of reactions that may impact embryonic development in which the enzyme is involved. The presence of a gene-environment interaction, resulting in altered susceptibility, exist with respect to the MTHFR 677C→T polymorphism. Consequently, the MTHFR 677C→T homozygous genotypes (TT) and possibly the heterozygous mutant genotypes (CT) are more likely to be related to CHD risk in the absence of periconceptional folic acid supplementation. We found such an interaction for the maternal MTHFR 677C→T polymorphism in relation to CHD when the mother did not take folic acid periconceptionally. The maternal MTHFR 677CT and TT genotypes in combination with no use of periconceptional folic acid supplements were associated with, respectively, a three-fold (OR 3.3 95%CI 1.46–7.32) and six-fold (OR 6.3 95%CI 2.32–17.27) increased risk for conotruncal heart defects in offspring.²⁴ The studies, included in the meta-analysis and performed in the non-folate-fortified countries, did not investigate this gene-environment interaction. Information regarding dietary folic acid intake and the use of periconceptional folic

acid supplement was often unknown. Therefore the role of folic acid supplementation in relation to the MTHFR 677C→T polymorphism should be studied extensively.

In the US, fortification of flour and other enriched grain products with folic acid has been compulsory since 1998.⁴² The North American studies in the meta-analysis, except for one, were performed after the introduction of food fortification. A higher dietary intake of folic acid has been shown to be effective in increasing folate concentration in both serum and red blood cells among women of childbearing age since that period.⁴³ The absence of a detrimental effect of MTHFR 677C→T polymorphism on CHD risk, in the North American studies, might be due to this policy.

In this paper, we have reviewed all published studies focusing on the MTHFR 677C→T polymorphism and the risk of giving birth to an infant with a CHD. Our meta-analysis, including 13 studies, reveals no significant association between MTHFR 677C→T genotypes and CHD risk. In our opinion, these studies are heterogeneous with regard to population background, study design, and type of heart defect, and are also relatively small. Moreover, the effect of periconceptional folic acid supplementation, which potentially modified the relationship between MTHFR 677C→T polymorphism and CHD risk, was frequently not studied. Further studies are warranted to enable definite conclusions on the MTHFR 677C→T polymorphism and CHD. For such studies, sample sizes need to be large, to accurately estimate the causal relationship for finer phenotype categories of CHD. Whether the MTHFR 677C→T polymorphism is only a risk factor in the mother, or in the fetus as well, also needs to be further studied. The effect of potential effect modifiers such as periconceptional folic acid intake also has to be taken into account. Ideally, such studies will be performed in countries where food fortification with folic acid is not yet the norm.

References

1. Hoffman JI. Incidence of congenital heart disease: II. Prenatal incidence. *Pediatr Cardiol* 1995; **16**:155–65.
2. Bakker MK, van den Berg MD, Boonstra A, van Diem MT, Meijer WM, ter Beek L, Siemensma-Mühlenberg N, Pliester-Engel E, van der Werf MCR, Verheij JBG, de Walle HEK. *EUROCAT Northern Netherlands Registration of congenital anomalies (Tables 1981–2002)*. The Netherlands, Department of Medical Genetics University Medical Centre Groningen, 2005:1–56.
3. Tikkanen J, Heinonen OP. Risk factors for ventricular septal defects in Finland. *Public Health* 1991; **105**:99–112.

4. Centers for Disease Control and Prevention. Trends in infant mortality attributable to birth defects-United States, 1980-1995. *Morb Mortal Wkly Rep* 1998; **47**:773-8.
5. berg A, Westbom L, Källén B. Congenital malformations among infants whose mothers had gestational diabetes or preexisting diabetes. *Early Hum Dev* 2001; **61**:85-95.
6. Goldmuntz E. The epidemiology and genetics of congenital heart disease. *Clin Perinatol* 2001; **28**:1-10.
7. Czeizel AE, Dudas I. Prevention of the first occurrence of neural-tube defects by periconceptional vitamin supplementation. *N Engl J Med* 1992; **327**:1832-5.
8. Czeizel AE. Periconceptional folic acid containing multivitamin supplementation. *Eur J Obstet Gynecol Reprod Biol* 1998; **78**:151-61.
9. Botto LD, Khoury MJ, Mulinare J, Erickson JD. Periconceptional multivitamin use and the occurrence of conotruncal heart defects: results from a population-based, case-control. *Pediatrics* 1996; **98**:911-17.
10. Botto LD, Mulinare J, Erickson JD. Do multivitamin of folic acid supplements reduce the risk for congenital heart defects? *Am J Med Gen* 2003; **121A**:95-101.
11. Scalco KS, Ferencz C, Loffredo CA, Wilson PD, Correa-Villasenor A, Khoury MJ, Willett WC. Periconceptional folate intake and malformations of the cardiac outflow tract. *Epidemiology* 1998; **9**:95-8.
12. Shaw GM, O'Malley CD, Wasserman CR, Tolarova MM, Lammer EJ. Maternal periconceptional use of multivitamins and reduced risk for conotruncal heart defects and limb deficiencies among offspring. *Am J Med Genet* 1995; **59**:536-45.
13. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GHJ, den Heijer M, Kluijtmans LAJ, van den Heuvel LP, Rozen R. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet* 1995; **10**:111-13.
14. Guenther BD, Sheppard CA, Tran P, Rozen R, Matthews RG, Ludwig ML. The structure and properties of methylenetetrahydrofolate reductase from *Escherichia coli* suggest how folate ameliorates human hyperhomocysteinemia. *Nat Struct Biol* 1999; **6**:359-65.
15. Kluijtmans LAJ, Young IS, Boreham CA, Murray L, McMaster D, McNulty H, Strain JJ, McPartlin J, Scott JM, Whitehead AS. Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. *Blood* 2003; **101**:2483-8.
16. Jacques PF, Bostom AG, Williams RR, Ellison RC, Eckfeldt JH, Rosenberg IH, Selhub J, Rozen R. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation* 1996; **93**:7-9.
17. Kapusta L, Haagmans ML, Steegers EA, Cuyper MH, Blom HJ, Eskes TK. Congenital heart defects and maternal derangement of homocysteine metabolism. *J Pediatr* 1999; **135**:773-4.
18. Hobbs C, Cleves MA, Melnyk S, Zhao W, James JS. Congenital heart defects and abnormal maternal biomarkers of methionine and homocysteine metabolism. *Am J Clin Nutr* 2005; **81**:147-53.
19. Junker R, Kotthoff S, Vielhaber H, Halimeh S, Kosch A, Koch HG, Kassenbohmer R, Heineking B, Nowak-Gottle U. Infant methylenetetrahydrofolate reductase 677TT genotype is a risk factor for congenital heart disease. *Cardiovasc Res* 2001; **51**:251-4.
20. Wenstrom KD, Johanning GL, Johnston KE, DuBard M. Association of the C677T methylenetetrahydrofolate reductase mutation and elevated homocysteine levels with congenital cardiac malformations. *Am J Obstet Gynecol* 2001; **184**:806-17.
21. Storti S, Vittorini S, Iacone MR, Sacchelli M, Collavoli A, Ripoli A, Cocchi G, Biagini A, Clerico A. Association between 5,10-Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and conotruncal heart defects. *Clin Chem Lab Med* 2003; **41**:276-80.
22. Lee CN, Su YN, Cheng WF, Lin MT, Wang JK, Wu MH, Hsieh FJ. Association of the C677T methylenetetrahydrofolate reductase mutation with congenital heart diseases. *Acta Obstet Gynecol Scand* 2005; **84**:1134-40.
23. Shaw GM, Iovannisci DM, Yang W, Finnell RH, Carmichael SL, Cheng S, Lammer EJ. Risks of human conotruncal heart defects associated with 32 single nucleotide polymorphisms of selected cardiovascular disease-related genes. *Am J Med Genet A* 2005; **138**:21-6.
24. van Beynum IM, Kapusta L, den Heijer M, Vermeulen SHHM, Kouwenberg M, Daniëls O, Blom HJ. Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation. *Eur Heart Journal* 2006; **27**:981-7.
25. Zhu WL, Li Y, Yan L, Dao J, Li S. Maternal and offspring MTHFR gene C677T polymorphism as predictors of congenital atrial septal defect and patent ductus arteriosus. *Mol Hum Reprod* 2006; **12**:51-4.
26. Galdieri LC, Santiago RA, Silva CMC, Pedra CAC, Almeida VD. Homocysteine concentrations and molecular analysis in patients with congenital heart defects. *Arch Med Res* 2007; **38**:212-18.
27. Nurk E, Tell GS, Refsum H, Ueland PM, Vollset SE. Associations between maternal methylenetetrahydrofolate reductase polymorphisms and adverse outcomes of pregnancy: The Hordaland homocysteine study. *Am J Med* 2004; **117**:26-31.
28. Hobbs CA, James SJ, Jernigan S, Melnyk S, Lu Y, Malik S, Cleves MA. Congenital heart defects, maternal homocysteine, smoking, and the 677C>T polymorphism in the methylenetetrahydrofolate reductase gene: Evaluating gene-environment interactions. *Am J Obst Gyn* 2006; **194**:218-24.
29. Pereira AC, Xavier-Neto J, Mesquita SM, Mota GFA, Augusto Lopes A, Krieger JE. Lack of evidence of association between MTHFR C677T polymorphism and congenital heart disease in a TDT study design. *Int J Cardiol* 2005; **105**:15-18.
30. McBride KL, Fernbach S, Meneses A, Molinari L, Quay E, Pignatelli R, Towbin JA, Belmont JW. A family-based association study of congenital left-sided heart malformations and 5,10 methylenetetrahydrofolate reductase. *Birth Defects Res A Clin Mol Teratol* 2004; **70**:825-30.
31. Hobbs CA, James SJ, Parsian A, Krakowiak PA, Jernigan SL, Greenhaw JJ, Lu Y, Cleves MA. Congenital heart defects and genetic variants in the methylenetetrahydrofolate reductase gene. *J Med Genet* 2006; **43**:162-6.
32. Kazeem GR, Farrall M. Integrating case-control and TDT studies. *Ann Hum Genet* 2005; **69**:329-35.

33. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. *Biometrics* 1994; **50**:1088–101.
34. Egger M, Smith GD, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *Br Med J* 1997; **315**:629–34.
35. Spielman RS, McGinnis RE, Ewens WJ. Transmission test for linkage disequilibrium: the insulin gene region and insulin-dependent diabetes mellitus (IDDM). *Am J Hum Genet* 1993; **52**:506–16.
36. Colhoun HM, McKeigue PM, Smith GD. Problems of reporting genetic associations with complex outcomes. *Lancet* 2003; **361**:865–72.
37. Williams LJ, Correa A, Rasmussen S. Maternal lifestyle factors and risk of ventricular septal defects. *Birth Defects Res A Clin Mol Teratol* 2004; **70**:59–64.
38. Cooper WO, Hernandez-Diaz S, Arbogast PG, Dudley JA, Dyer S, Gideon PS, Hall K, Ray WA. Major congenital malformations after first-trimester exposure to ACE inhibitors. *N Engl J Med* 2006; **354**:2443–51.
39. Hernandez-Diaz S, Werler MM, Walker AM, Mitchell AA. Folic acid antagonists during pregnancy and the risk of birth defects. *N Engl J Med* 2000; **343**:1608–14.
40. Tang LS, Wlodarczyk BJ, Santillano DR, Miranda RC, Finnell RH. Development Consequences of abnormal folate transport during murine heart morphogenesis. *Birth Defects Res A Clin Mol Teratol* 2004; **70**:449–58.
41. Blom HJ, Shaw GM, den Heijer M, Finnell RH. Neural tube defects and folate: case far from closed. *Nat Rev Neurosci* 2006; **7**:724–31.
42. Centers for Disease Control and Prevention. Knowledge and use of folic acid by women of childbearing age—United States, 1995 and 1998. *Morb Mortal Wkly Rep* 1999; **48**:325–7.
43. FDA. Food Standards: amendment of standards of identity for enriched grain products to require addition of folic acid. *Fed Reg* 1996; **61**:8781–97.