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Maternal MTHFR 677C>T is a risk factor for congenital heart defects: effect modification by periconceptional folate supplementation

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Aims Periconceptional folate supplementation prevents neural tube defects and possibly congenital heart defects (CHD) as well. The search for candidate genes involved in the folate metabolism includes the methylenetetrahydrofolate reductase (MTHFR) 677C>T polymorphism. We studied the association between MTHFR 677C>T variants and CHD risk. The interaction with periconceptional folate supplementation was also investigated.

Methods and results A case–control study and a family-based transmission disequilibrium test (TDT) were conducted to explore this association. In 133 triads, the TDT revealed no association of the fetal 677T allele with the development of a heart defect. In 158 mothers with a CHD-affected child, the maternal MTHFR 677CT and TT genotypes in combination with no use of periconceptional folate 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. In a case-only study, the interaction between periconceptional folate supplementation and maternal MTHFR genotype was significant ($P = 0.012$).

Conclusion The maternal MTHFR 677C>T variants are a risk factor for CHD in offspring, confined to conotruncal heart defects. A gene-environment interaction between maternal MTFHR 677CT and TT genotypes with periconceptional folate supplementation was observed. These findings provide a mechanism of the protective role of folate and support the thesis that periconceptional folate supplementation might prevent CHD.

Introduction

Congenital heart defects (CHD) are among the most common congenital anomalies worldwide, occurring in approximately one in hundred living newborns.[1] Heart defects at birth occur as an isolated malformation, but are also associated with other anomalies or occur as part of a syndrome. The aetiology of CHD is only partly illuminated, multifactorial causes are presumed in which both genetic and environmental risk factors play a role.[2]

Several studies have proposed that maternal periconceptional use of folic acid protects against the occurrence of congenital anomalies, including CHD.[3–5] In a Hungarian randomized intervention trial, primarily designed to study the reduction of the first occurrence of neural tube defects (NTD), CHD, which were mainly conotruncal heart defects and ventricular septal defects, were reduced by 58% (2–81%) in the women receiving multivitamin containing folate compared with the group receiving trace elements.[3] Also observational studies have shown an association between periconceptional use of multivitamins and a reduction of CHD, both conotruncal and other heart defects, in offspring.[4,5] The precise role of folate during cardiac morphogenesis remains unclear. Biochemically, folate is a one-carbon donor, as such involved in many important cellular reactions, including the synthesis of nucleotides and methyl transfer reactions important for methylation of DNA, proteins, and lipids.[6]

The protective effect of periconceptional folate on CHD has led to the search for candidate genes involved in its metabolic pathway. Methylenetetrahydrofolate reductase (MTHFR) is a promising candidate because it is a regulating key enzyme for the availability of active folate by catalyzing

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the reduction of 5,10-methylenetetrahydrofolate into 5-methyltetrahydrofolate. Reduced MTHFR activity results in a decreased availability of 5-methyltetrahydrofolate for the remethylation of homocysteine to methionine. The MTHFR 677C>T polymorphism results in a thermolabile enzyme with reduced activity.[7–9] We found that this polymorphism is associated with an increased risk of NTD.[10]

Three studies regarding the MTHFR 677C>T polymorphism in relation to CHD yielded conflicting results.[11–13] Junker *et al.*[11] observed a higher frequency of the 677TT genotype among children with a CHD. Wenstrom *et al.*[12] reported higher prevalence of 677CT and TT genotypes in samples of amniotic fluid of pregnancies complicated by a CHD. Storti *et al.*[13] did not observe any association between either the parental or fetal MTHFR 677C>T genotypes and the risk of CHD. These studies were relatively small and did not study the influence of periconceptional folate supplementation.

In 1999, we were the first to relate maternal hyperhomocysteinemia to an increased risk for CHD.[14] Recently, Hobbs *et al.*[15] studied mothers with CHD in offspring and identified homocysteine, *S*-adenosylhomocysteine and methionine as the most important biomarkers predictive of case status.

In several experimental animal studies, a disturbed folate and homocysteine metabolism have been related to congenital anomalies, including CHD. Folate deficiency has been shown to cause CHD and adding folate in case of folate deficiency or hyperhomocysteinemic state counteracts the development of congenital anomalies.[16–18] Chicken embryos exposured to homocysteine induced several kinds of heart defects.[18] *In vitro*, folic acid and homocysteine affect outgrowth and differentiation of neural crest cells.[19,20] For the development of the heart, in particular, critical components of the outflow tract, migration of neural crest cells through pharyngeal arches are essential. Ablation of the cardiac neural crest in chicken embryos leads chiefly to conotruncal heart defects.[21–23] Herein, we investigated the MTHFR 677C>T polymorphism as a risk factor for CHD by conducting a case–control comparison and a transmission disequilibrium test (TDT) in a family-based design. Furthermore, we studied the risk of the MTHFR genotype in relation to the use of periconceptional folate supplementation.

Methods

Patients and data collection

This study was carried out between August 2002 and December 2003. The CHD families were recruited through the Children's Heart Centre of the Radboud University Nijmegen Medical Centre. Cases with a CHD from the north, south, and east part of the country are referred to this tertiary centre. Children diagnosed with the CHD after birth and admitted for a heart catheterization or cardiac operation were asked to take part in this study. Type of CHD, associated abnormalities, age, sex, and medical history of the child were extracted from the medical records and through a written questionnaire filled out by one or both parents. The mothers were asked about index pregnancy and use of medication, multivitamins, and/or folate in the period before pregnancy until delivery. Use of periconceptional folate supplements was considered adequate if started before conception until 8 weeks thereafter. Women who started to use folate supplements after it was known that they were pregnant were categorized as non-users.

In total, 197 unrelated families with a child with a structural heart defect were eligible for this study. In 191 children, 171 mothers, and 147 fathers, a blood sample was available and DNA isolation and genotyping were successful. Two families were deleted because of Mendelian inconsistency for the MTHFR 677C>T polymorphism. The exclusion criteria were closure defects like NTD and cleft palate/lip, detected genetic abnormalities, known syndromes, and Vacterl-association. In total, 133 complete triads were present for TDT analysis. While the TDT, in contrast to case–control design, is not sensitive to population admixture, both Caucasian and non-Caucasian families were included. After exclusion of the non-Caucasian individuals, the case–control study was performed in 165 CHD-affected children [91 (55%) males, 74 (45%) females, median age 3.4 (range 0.04–18.0)] and in 158 mothers [median age 36 (range 22–52)]. The control group consisted of 220 apparently normal Caucasian unrelated children [108 (49%) males, 112 (51%) females, median age 9.4 (range 0.0–19.3)], without congenital abnormalities. The group consisted of apparently volunteers from secondary schools in the surrounding area of Nijmegen (aged 11–19). Younger children under 11 year were recruited in the Pediatric Clinic of the Radboud University Medical Centre for ethical reasons. When blood was drawn for diagnostic or follow-up investigations, the children and/or their parents were asked to donate some blood for this study in the same vena puncture session. The exclusion criteria were overt organ dysfunction, neoplastic disease, genetic defects, closure defects like cleft lips, NTD, and CHD.[24]

The 261 Caucasian women [median age 50 (range 21–84)] serving as controls, who gave birth to an apparently healthy child, were recruited through a general practice in The Hague. They were approached to take part in a health survey of risk factors for cardiovascular disease.[25]

The study complies with the Declaration of Helsinki, the study protocol was reviewed and approved by the local medical Ethics Committee and written consent was obtained from patients and/or parents, prior to commencement.

Classification of the CHD

All heart defects were diagnosed by at least one echocardiography. Anatomical findings during the cardiac operation were considered to be most reliable regarding the exact type of the defect. The CHD were categorized into conotruncal and other CHD based on the characteristic features of the heart defects as result of ablation of cardiac neural crest in animal studies.[21–23] The heart defects in the group of other CHD were pooled together, because subgroup analysis did not reveal a difference in genotype distribution in a specific heart defect.

Blood sampling and genetic analysis

One blood sample in 3 or 1 mL EDTA tube was collected from CHD-affected children and from their parents. The EDTA sample was centrifuged at 2000 *g* for 10 min. The plasma was separated and the remaining cells were stored at -20°C and used for DNA isolation. Genomic DNA was isolated from the leucocytes using an established procedure described by Miller *et al.*[26] The mutation in the *MTHFR* gene is a C to T substitution at base pair 677 and was investigated by polymerase-chain reaction, followed by restriction enzyme digestion with *Hin*fI and detection by agarose gel electrophoresis.[7]

Statistical analysis: case–control study

To evaluate the association of MTHFR 677C>T polymorphism with CHD, a case–control study was performed. The sample size was initially determined for an overall risk estimation, whereby the estimated frequency of TT genotype among controls was 10%, specifying $\alpha = 0.05$ and $\beta = 0.10$. The sample sizes required to detect a relative risk of 2.5 was 200 cases and 200 controls.[27] The genetic risk on CHD was assessed by the calculation of odds ratios (OR), with corresponding 95% confidence intervals (95% CI), as estimates

of the relative risk, for the MTHFR 677CT and TT vs. CC genotypes using logistic regression analysis. In addition, OR (95% CI) for maternal MTHFR 677C>T genotypes stratified by periconceptional folate supplementation and conotruncal and other CHD were calculated.

Information regarding periconceptional folate supplementation in control woman was absent; therefore, the interaction between MTHFR genotypes and periconceptional folate supplementation was tested in a case-only approach. The calculated ORs (95% CI) were adjusted for potential confounders of periconceptional folate use such as maternal age at delivery of index pregnancy, body mass index, smoking, and education. The case-only approach assumes independence between genotype and environmental factors of interest in the population, which is valid for the MTHFR 677C>T polymorphism and periconceptional folate supplementation.[28] The distribution of the MTHFR 677C>T polymorphism is not influenced by folate use in the general population, which was tested in the control group. Genotype frequencies were compared by χ^2 test and OR (95% CI) were calculated.

Statistical analysis: TDT

The putative risk allele 677T was tested for linkage and linkage disequilibrium with CHD by using the family-based TDT. The TDT design is considered more valid for genetic associations than the case–control design, as it is unbiased by population stratification or admixture.[29,30] The TDT assessed the relationship between fetal genotype and the risk of CHD by evaluating the frequency with which an allele is transmitted from heterozygous parents to an affected offspring. The non-transmitted alleles of the heterozygous parents serve as internal controls. The transmission frequency of an allele responsible for an increased risk of CHD should exceed 50%. The McNemar χ^2 test statistic (number of transmitted alleles − number of non-transmitted alleles)^n/(number of transmitted alleles + number of non-transmitted alleles) was used to evaluate the equality of the two transmission frequencies. The *P*-values are two-sided, and statistical significance was accepted at $P < 0.05$. Statistical analysis was performed using SPSS-software package (version 11.5).

Results

Frequencies of different types of CHD presented in this study, categorized by conotruncal and other CHD are listed in *Table 1*. Demographic and clinical characteristics of the study population are described in *Table 2*.

The MTHFR 677C>T polymorphism

The transmission frequencies of the alleles of MTHFR 677C>T polymorphism from heterozygous parents to offspring with CHD are shown in *Table 3*. In the 133 complete triads eligible for the TDT, 126 parents were heterozygous for the MTHFR 677C>T polymorphism (CT). The T-allele was transmitted from a heterozygous parent to the CHD offspring 59 times (47%) and was not transmitted 67 times (53%). No linkage disequilibrium for the T-allele with CHD could be demonstrated.

The distributions of the MTHFR 677C>T polymorphism in the controls were compatible with the Hardy–Weinberg equilibrium. The genotype distribution among the control children and woman were representative for the Dutch population.[31,32] From 165 CHD-affected children, 20 (12.1%) were homozygous (TT), 66 (40.0%) were heterozygous (CT), and 79 (47.9%) were wild-type (CC) for the MTHFR 677 C>T polymorphism. Among the 220 control children, 18 (8.2%) had TT, 104 (47.3%) CT and 98 (44.5%) CC genotype. Crude genetic analysis of MTHFR 677CT and 677TT genotypes compared with 677CC genotype in CHD-affected children vs. controls revealed an OR of 0.8 (95% CI 0.51–1.21) and 1.4 (95% CI 0.68–2.78), respectively.

The distribution of MTHFR 677CC, CT, and TT genotypes among the mothers of CHD-affected children was 72 (45.6%), 68 (43.0%), and 18 (11.4%) vs. 131 (50.2%), 107 (41.0%), and 23 (8.8%) in controls, respectively (*Table 2*). These frequencies did not reveal significantly increased ORs for 677CT and TT genotypes compared with the 677CC genotype (*Table 4*).

Periconceptional folate supplementation

Among the group of 158 women, 55 (34.8%) used the recommended periconceptional folate supplementation adequately and 103 (65.2%) used no periconceptional supplements. Periconceptional folate supplementation is recommended by the Dutch authority to prevent the risk

Table 1 Frequencies of different types of congenital heart defects represented in this study, categorized by conotruncal and other-heart defects ($n = 158$)

Conotruncal heart defects[a]	*n* (%)	Other heart defects[a]	*n* (%)
Neural crest related defects		*Possibly neural crest related defects/inlet segment*	
Tetralogy of Fallot	23 (14.5)	Tricuspid valve atresia	7 (4.4)
Complex heart defect[b]	20 (12.7)	Atrioventricular septal defect/inlet VSD	9 (5.7)
Outlet ventricular septal defect	11 (7.0)		
Pulmonary atresia and VSD	7 (4.4)	*Possibly neural crest related defects/outlet segment*	
Truncus arteriosus	2 (1.3)	Valvular pulmonary and aorta stenosis/atresia	31 (19.6)
Isolated transposition great arteries	2 (1.3)	Hypoplastic left heart syndrome	10 (6.3)
Aortic pulmonary window	1 (0.6)	Coarctation aorta and valvular aorta stenosis	12 (7.6)
		Non-neural crest related defects	
		Atrial septal defect type II	11 (7.0)
		Persistent ductus arteriosus	9 (5.7)
		Miscellaneous	3 (1.9)

Based on Caucasian mothers with CHD-affected children where the maternal MTHFR genotype was available.
[a]Classification based on the characteristic features of the heart defects as result of ablation of cardiac neural crest in animal studies.[21–23]
[b]Complex heart defects; double-outlet right ventricles and/or transposition of the great arteries with VSD and a variety of other anomalies.

Table 2 Characteristics of the study population

	Woman with CHD in offspring ($n = 158$)	Control–woman ($n = 261$)
Age (median (range))	36 year (22–52)	50 year (21–84)
Body mass index (median (range))	24.1 (18.3–50.4)	25.1 (17.1–46.2)
Current smoking		
No	73.9%	66.6%
Yes	23.6%	32.6%
Unknown	2.5%	0.4%
Current B-vitamin use		
No	88.5%	84.3%
Yes	9.6%	15.3%
Unknown	1.9%	0.4%
MTHFR		
677CC	45.6%	50.2%
677CT	43.0%	41.0%
677TT	11.4%	8.8%

Table 3 Transmission frequencies of the C and T alleles of MTHFR 677C>T genotypes from parents to children with isolated congenital heart defect classified by periconceptional folate supplementation

Transmitted alleles	Non-transmitted alleles		χ^2-square	*P*-value
Users and non-users	C	T		
C	116	67	0.51	0.48
T	59	24		
Non-users				
C	66	38	0.44	0.51
T	44	18		
Users				
C	50	29	4.45	0.04
T	15	6		

P-value for interaction between periconceptional folate supplementation and MTHFR genotype, tested in a case-only study, was 0.003 (χ^2 test).

of NTD, since 1993.[33] In total, 37 women delivered their child before this advice, none of them used periconceptional folate supplements. Since 1994, 121 women gave birth to a child with a CHD of which 54 (44.6%) took folate for the entire advised period and 67 (55.4%) did not use it. From questionnaires, no cases were found where the mothers with CHD offspring had taken any anti-folate medications.

The TDT stratified for maternal periconceptional folate supplementation did not reveal a preferential transmission of the T-allele to CHD-affected children in non-users (*Table 3*) and even showed a high transmission of C-alleles in users. In *Table 4*, the ORs are presented for MTHFR 677C>T genotypes of mothers of CHD-affected children vs. controls stratified for maternal periconceptional folate supplementation. Mothers carrying MTHFR 677TT genotype in combination with no periconceptional folate supplementation had a two-fold (OR = 2.1 95% CI 1.02–4.48) increased risk of any CHD in offspring. No risk was present, if the mothers used periconceptional folate supplements adequately. The interaction between maternal MTHFR 677C>T genotypes and periconceptional folate supplementation, studied in a case-only design, is shown in *Table 5*. The interaction between MTHFR genotypes of the CHD-affected children and maternal periconceptional folate use was also significant ($P = 0.003$). The distribution of the MTHFR 677C>T polymorphism was not significantly influenced by periconceptional folate use in the control population ($P = 0.75$).

Conotruncal and other CHD

Table 4 showed the calculated OR for the MTHFR 677CT and TT genotypes of mothers with CHD in offspring classified by conotruncal/other CHD and periconceptional folate supplementation. The maternal MTHFR TT genotype revealed an increased OR of 2.5 (95% CI 1.09–5.78) for conotruncal heart defects, independent of periconceptional folate use. The association between MTHFR 677C>T genotypes and conotruncal/other CHD was significant for mothers with CHD-affected children ($P = 0.041$) but not for the children themselves ($P = 0.377$). We did not observe a significant association between the MTHFR 677C>T genotypes and the subgroups of CHD which are possibly related to the cardiac neural crest.

Discussion

This is the first study revealing a gene–environment interaction between the maternal MTHFR 677C>T polymorphism and periconceptional folate supplementation on the risk of CHD in offspring. Mothers with MTHFR 677TT genotype who did not use periconceptional folate supplements had a two-fold increased risk of a CHD-affected child. The maternal MTHFR 677CT and TT genotypes were no genetic risk factors for CHD in women using folate supplements during the entire advised period.

The association between fetal MTHFR 677C>T genotypes and the risk of CHD has only been studied in case–control studies.[11–13] Junker *et al.*[11] reported in 114 Caucasian patients with either a conotruncal or other CHD a two-fold increased frequency (18.4%) of the MTHFR 677TT genotype compared with 228 controls (9.2%). Wenstrom *et al.*[12] found the 677CT or TT genotypes in 35% of in samples of amniotic fluid of 26 pregnancies complicated by a CHD vs. 13% in 116 controls. We applied the TDT to distinguish between the fetal and maternal genotypic effect on the developing embryo. To differentiate between fetal and maternal genotypic effects based on case–control design is complicated, as allele frequencies in children are dependent of those in the parents.[29,30] The results of the TDT analysis revealed no association of the fetal 677T allele with CHD risk for the whole group of non-syndromic heart defects. Considering these results, it is likely that CHD risk is related to the maternal MTHFR genotype. In our study, the maternal MTHFR 677TT genotype was associated with an increased risk of CHD in offspring, especially for a conotruncal heart defect if mothers did not use folate supplements (OR 6.3 95% CI 2.32–17.27). In addition, we observed a three-fold increased risk (OR 3.3 95% CI 1.46–7.32) of a conotruncal heart defect in offspring in mothers carrying the MTHFR 677CT genotype in combination with no use of periconceptional folate supplements. These

Table 4 Calculated odds ratios (95% confidence interval) for MTHFR 677C>T genotypes in mothers with congenital heart defects affected children vs. controls, classified by conotruncal/other heart defects and by periconceptional folate supplementation

	All heart defects		Conotruncal heart defects		Other heart defects	
	n	OR (95% CI)	*n*	OR (95% CI)	*n*	OR (95% CI)
Users and non-users						
MTHFR 677TT	18	1.4 (0.72–2.81)	11	2.5 (1.09–5.78)	7	0.8 (0.34–2.11)
MTHFR 677CT	68	1.2 (0.76–1.76)	30	1.5 (0.82–2.65)	38	1.0 (0.60–1.63)
MTHFR 677CC	72	Referent	25	Referent	47	Referent
Non-users						
MTHFR 677TT	15	2.1 (1.02–4.48)	10	6.3 (2.32–17.27)	5	0.9 (0.32–2.61)
MTHFR 677CT	48	1.5 (0.90–2.40)	24	3.3 (1.46–7.32)	24	0.9 (0.53–1.71)
MTHFR 677CC	40	Referent	9	Referent	31	Referent
Users						
MTHFR 677TT	3	0.5 (0.15–1.89)	1	0.4 (0.05–2.82)	2	0.7 (0.15–3.31)
MTHFR 677CT	20	0.8 (0.41–1.41)	6	0.5 (0.17–1.21)	14	1.1 (0.50–2.29)
MTHFR 677CC	32	Referent	16	Referent	16	Referent

P-value for interaction between folate supplementation and MTHFR genotype, tested in a cases only, was 0.012 and the *P*-value for the association between conotruncal/other-CHD and MTHFR genotype was 0.041, respectively (χ^2 test).

Among the control women, 23 (8.8%) were homozygous (TT), 107 (41.0%) were heterozygous CT, and 131 (50.2%) were wild-type (CC) for the MTHFR 677C>T polymorphism. Use of periconceptional folate supplements was considered adequate if started before pregnancy until 8 weeks after conception of the index pregnancy. Women who started to use folate supplements after conception were categorized as non-users.

Table 5 Odds ratios (95% confidence interval) for interaction between maternal MTHFR 677C>T genotype and periconceptional folate supplementation in cases only

MTHFR genotypes and maternal periconceptional folate supplementation[a]				
MTHFR genotypes	Non-users	Users	OR for interaction (95% CI)	Adjusted OR (95% CI)[b]
677TT	15	3	4.0 (1.06–15.03)	5.5 (1.13–26.69)
677CT	48	20	1.9 (0.96–3.86)	1.9 (0.87–4.17)
677CC	40	32	Referent	Referent

[a]*P*-values for the overall interaction (χ^2 test), was 0.012.

[b]Adjusted for maternal age at delivery of index pregnancy, body mass index, smoking, and education.

results are in contrast to the results described in the only published study on maternal MTHFR 677C>T polymorphism in relation to conotruncal heart defects by Storti *et al.*[13] who observed no differences. They reported a prevalence of 20% MTHFR 677TT genotype among CHD-affected children and 22% among their mothers and 20% in controls. The prevalence of 677TT homozygosity was evidently higher compared with our study, but in line with the reported higher prevalence of MTHFR 677TT genotype among the Italian population.[34] The differences with our results are unlikely to be explained by other types of CHD studied, because they studied the same type of conotruncal heart defects.

In our study, a detrimental action of the MTHFR 677CT and TT genotypes on the development of CHD in offspring was only present in mothers not using periconceptional folate supplements. In the Italian study, data on periconceptional folate supplementation or intake were lacking, which might explain the absence of the detrimental action.[13] In the MTHFR 677C>T polymorphism, an alanine is altered to a valine residue. Homozygosity (TT) and heterozygosity (CT) result in a thermolabile enzyme with reduced activity and is associated with mildly elevated plasma homocysteine levels, especially in individuals with low-folate levels. Folate supplementation ameliorates, the effect of reduced MTHFR activity due to the 677C>T polymorphism.[7–10] We can expect that in particular women carrying the MTHFR 677TT genotype, as well as the 677CT genotype, will benefit from periconceptional folate supplementation to protect against CHD in offspring.

Studies on the relation between MTHFR 677C>T polymorphism and congenital anomalies mainly focused on NTD, a neural crest derivate.[10,35] In our study, the maternal MTHFR 677CT and TT genotypes appeared to be genetic risk factors, in particular, for the conotruncal heart defects. This observation supports the hypothesis that an impaired folate and/or homocysteine metabolism interferes with the developing heart, possibly by affecting neural crest cells. *In vitro* studies found that an impaired folate and homocysteine metabolism affect the neural crest cells formation and migration.[19,20] Tang *et al.*[17] observed that reduced availability of folate by inactivating the folate transporter, *Folbp1*, in mice leads to an extensive reduction of migrating cardiac neural crest cells. The authors observed neural crest cell-associated CHD, including the improper septation, persistent truncus arteriosus, and double-outlet right ventricle. The cardiac neural crest cells are a group of cells that migrate from the dorsal side of the neural tube during a specific time window and contribute to the septation of the developing heart, specifically the outflow tract. Ablation of the neural crest cells results mainly in the absence of the outflow septum and malalignment of the outflow tract classified as conotruncal heart defects.[21–23] These cardiac anomalies resemble those observed in patients with the 22q11 deletion phenotype, which is considered as a neural crest related disease as well.[36] In recent years, in experimental studies, the neural crest cells have been traced to several other parts of the developing heart including the inlet of the heart, semilunary valves,

and distal aortic arch.[37,38] The exact role of the neural crest cells in the development of CHD related to these structures remains to be elucidated.

The Dutch government advices a daily additional intake of 0.4–0.5 mg folate in women whishing to become pregnant at least 4 weeks before conception until 2–3 months of pregnancy to prevent against NTD, since 1993.[33] Since that time, in our study group, only 44.6% of the mothers with CHD-affected children used periconeptional folate supplements adequately. We observed from 1993 to 2002 no increase in use of periconceptional folate supplements. Considering the high prevalence of about 50% of maternal MTHFR 677CT and TT genotypes in the general population, a substantial proportion of CHD might be prevented by increased folate intake by either periconceptional folate supplementation or food folate fortification. As long as folate fortification of food products is not applied in most countries, the benefits of periconceptional folate supplementation must be proclaimed with more strength.

Some limitations of our study must be acknowledged. Periconceptional folate supplements use was obtained from retrospective questionnaire information which is prone to recall bias. Therefore, the interaction between MTHFR genotypes and periconceptional folate supplementation was also analysed in a case-only study not sensitive to differences in reporting between affected and non-affected cases. Providing falsified information about the use of periconceptional folate supplementation intentionally was unlikely, because the mothers were unaware of the specific hypotheses of this study.

In a case-only approach, interaction is being evaluated, but not the independent effects of the MTHFR CT and TT genotypes alone and the risk reduction by the use of periconceptional supplementation itself. From a pathophysiological point of view, multiplicative effects of MTHFR 677C>T polymorphism and periconceptional folate supplementation can be expected, which is observed in our study. Another issue of this study is the potential environmental and lifestyle differences between folate and non-folate users in the study population. However, after adjustment for potential confounders of periconceptional folate use, the interaction remained unchanged or even increased.

In conclusion, the MTHFR 677C>T variant of the mother is a risk factor for CHD in offspring, confined to conotruncal heart defects. An evident gene–environment interaction between the maternal MTHFR 677C>T polymorphism and periconeptional folate supplementation was found. Our observations provide a mechanism of the protective role of folate and support the thesis that periconceptional folate supplementation will prevent CHD.

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