Published in final edited form as:
Int J Genet Mol Biol. 2010 March 1; 2(3): 43–47.

MTHFR polymorphisms in Puerto Rican children with isolated congenital heart disease and their mothers

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Abstract

Congenital heart defects (CHD) are among the most common birth defects. There is evidence suggesting that polymorphisms in folate metabolism could alter susceptibility to CHD. The MTHFR 677TT genotype has been associated with the development of structural congenital heart malformations. The objective of this study was to identify common polymorphisms in the MTHFR gene in children with isolated CHD and their mothers. The DNA analysis for the C677T and A1298C mutations was performed. The study group included 27 mothers, 27 children with CHD, and 220 controls. The prevalence of the TT polymorphism was higher in mothers (22%) than in controls (10%). Compound heterozygosity for both polymorphisms was 3.7 times more common in children with CHD than in the newborn controls. Mothers of children with CHD were more likely to be compound heterozygotes. The higher prevalence of C677T polymorphisms in mothers of children with CHD and of compound heterozygosity for both polymorphisms suggests the possible role of folic acid in the prevention of CHD. Due to the relation of this enzyme to folate metabolism, current folate recommendations for women in childbearing age in Puerto Rico to reduce neural tube defects may need to be extended to the prevention of CHD.

Keywords

Congenital heart disease; folic acid; MTHFR polymorphisms

Introduction

Congenital heart defects (CHD) are among the most common birth defects, with an incidence of 4 to 50 per 1,000 live births (Maschhoff 2004). In Puerto Rico, the prevalence of these defects was 9.5 /1,000 live births for the years 2003-2007 (PR Health Department 2009). CHD accounts for more than 25% of all infants' deaths, 10% of total admissions to neonatal intensive care units, and 26% of total neonatal intensive care mortality (Hall 1998). Although CHD has long been recognized as a component of complex genetic syndromes, a genetic cause has been less obvious.

Some studies have reported a reduced incidence of heart defects with maternal periconceptional use of multivitamins (Shaw et al., 1995; Botto et al., 1996; Botto et al.,

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2000$_a$). The role of folic acid in the prevention of congenital heart disease has been proposed by its association to the C677T mutation in the methylenetetrahydrofolate reductase (MTHFR) gene (Whitehead et al., 1995; Frosst et al., 1995; Bayley and Berry, 2005). Junker and colleagues showed for the first time in 2001 that the embryonal MTHFR 677TT genotype was significantly associated with the development of structural congenital heart malformations during early pregnancy (Junker et al., 2001). The objective of this study was to identify common polymorphisms in the MTHFR gene in children with CHD and their mothers and to test for an association between genotype and disease.

Materials and Methods

This project is a descriptive study where we screened blood samples from 27 children and their mothers for two common MTHFR gene polymorphisms (C677T and A1298C). The research proposal and consent forms were approved by the University of Puerto Rico, Medical Sciences Campus, Institutional Review Board. Parents signed an informed consent at enrollment. Demographic data was gathered upon enrollment, including maternal age, parents' education and their occupation. The Hollingshead social strata scale was used to assign social class (Hollingshead, 1975). There are V social strata, with class I being the highest.

All newborns admitted to the neonatal services at the University Pediatric Hospital, diagnosed with an isolated congenital heart defect, were candidates to be included in this study. Infants and children receiving services at the University Pediatric Hospital Cardiology clinics were also considered for the study. This hospital is a tertiary hospital receiving referrals from all over the island and serving mostly a low income population.

Exclusion criteria included infants and children with congenital heart disease associated to chromosomal anomalies and genetic syndromes. Newborns with patent ductus arteriosus associated to prematurity were excluded. Exclusion criteria for mothers included the use of antiepileptic drugs (valproic acid, phenytoin, carbamazepine) and conditions associated with food intolerance, malabsorption, or wasting syndromes, since all can alter folic acid metabolism and increase the risk of congenital anomalies. Mothers with diabetes were also excluded since they are at higher risk of having a child with congenital heart disease.

All the human DNA samples collected for this study were obtained from dried blood on Guthrie card filters. We have successfully analyzed these types of samples in other studies for establishing the prevalence of founder mutations among Puerto Rican newborns by polymerase chain reaction (PCR) followed by agarose gel analysis of the PCR products (Santiago-Borrero et al., 2006). The DNA analysis for the most common polymorphisms in the 5, 10 methylenetetrahydrofolate reductase gene (677 C→T and 1298 A→C) was performed using PCR amplification of the affected region and restriction enzyme digestion of the PCR product as described by Frosst et al. (1995) and Whitehead et al. (1995). Individuals were genotyped by comparison of restriction digests of the samples to those of the control DNA samples, and determined to be of TT genotype if homozygous for the C677T thermolabile allele, CT if heterozygous for the C677T thermolabile allele, and CC if no polymorphism was identified. The prevalence of these genotypes was compared to 220 healthy volunteers (controls).

The A1298C polymorphism was analyzed in a similar manner only for cases (mothers and children). We compared the frequency of combined heterozygotes in our CHD group with a group of 400 newborns previously screened at the same laboratory using the same methodology (Ayala, 1999). The polymorphism frequency study was carried out using dried

filters from the Newborn Screening Program. A randomized sampling method was used to ensure representation of newborns from all over the island.

Statistical Analysis

We examined the distributions of maternal age, maternal education, social strata, family history of CHD and NTD, use of folic acid, and type of congenital heart defect in all cases. Genotype frequencies were determined for the C677T and the A1298C variant in MTHFR and evaluation for Hardy-Weinberg equilibrium was performed. Allele frequencies were calculated by counting genes from the observed genotypes. We tested differences in the prevalence of the MTHFR variants between cases and controls using the Chi-square test. The level of significance was $p<0.05$.

Results

The study group included 27 Puerto Rican mothers and their children. The general characteristics of the mothers who participated in the study are shown in Table 1. Seventy percent (70%) of the children were male. The children primary cardiac diagnoses are presented in Table 2. The prevalence of conotruncal defects in this group of children with CHD was 48%. Eighty-nine percent (89%) of the mothers consumed folic acid during the pregnancy but none of them consumed it before pregnancy or before being aware of the pregnancy. Eighty-nine percent (89%) of the mothers knew that folic acid can prevent birth defects. The genotype frequencies of the study group were compared to those of 220 healthy Puerto Rican volunteers (controls).

Analysis of the C677T variant

Table 3 shows the prevalence of the different genotypes for the C677T polymorphism for all the individuals. The prevalence of the TT polymorphism was higher in mothers of affected children (22%) than in controls (10%) ($p=0.0463$). Homozygosity for the TT polymorphism was observed in 15% of the affected children. There was no difference in the prevalence of the TT variant in the children when compared to controls. Using simple allele association, we found no difference between the frequency of the variant allele (T) among cases and controls (Table 4). The distribution of the C677T genotype violated the Hardy-Weinberg equilibrium for controls ($p=0.0395$). On the other hand, it was in equilibrium in both mothers ($p=0.386$) and infants ($p=0.701$).

The TT variant was identified in six mothers. Their infants had hypoplastic left heart (3 cases), ventricular septal defect (1 case), pulmonary atresia (1 case) and transposition of great vessels (1 case). On the other hand, the TT variant was identified in four infants (2 ventricular septal defects, pulmonary atresia, and hypoplastic left heart). In three cases, the polymorphism was found in the mothers and in their infants as well.

Analysis of the A1298C variant

No homozygotes for the A1298C polymorphism were found in the study group. The prevalence of heterozygosity was 41% in the mothers and 44% in the children. The distribution of the A1298C genotype was in Hardy-Weinberg equilibrium in controls ($p=0.078$), mothers ($p=0.184$) and infants ($p=0.138$).

Analysis of combined heterozygosity

The frequency of combined heterozygotes for both polymorphisms was 26% in the mothers and 33% in the children (Table 5). Compound heterozygosity was 3.7 times more common in children with CHD than in the general population of newborns. Mothers of children with CHD were also more likely to be compound heterozygotes.

Discussion

There is strong evidence to support that the use of folic acid can prevent the occurrence of neural tube defects. In 1995, Shaw and colleagues (1995) reported that women who take multivitamins from one month before until two months after conception have 30% to 35% lower risk of delivering offspring with conotruncal defects. A study by Hernández and co-workers (2000) evaluated data on exposure to folic acid antagonists, finding a correlation with cardiovascular defects and oral clefts. Another study reported the greatest reduction in risk for transposition of the great arteries (Botto et al., 1996). Botto and his study group (2000a) suggested that approximately one in four major cardiac defects could be prevented by periconceptional multivitamins use. Recently, investigators from Canada reported a 6% decrease per year in the rates of severe congenital heart defects after folic acid fortification of grain products (lonescu-lttu et al., 2009). The American Academy of Pediatrics has endorsed a statement by the American Heart Association Council on Cardiovascular Disease in the Young. They concluded that periconceptional intake of multivitamin supplements that contain folic acid may reduce the risk of congenital cardiovascular defects in offspring, similar to the known risk reduction for neural tube defects that is seen with folic acid intake (AAP, 2007).

The evidence suggesting a role for folic acid in the development of CHD has led to the hypothesis that mutations in folate metabolism could alter susceptibility to CHD. MTHFR catalyzes the conversion of 5, 10 methylenetetrahydrofolate into 5-methyltetrahydrofolate. The C677T polymorphism causes the gene product to be thermolabile and, consequently, higher homocysteine levels (Botto and Yang, 2000b). This polymorphism has been studied extensively in relation to the occurrence of neural tube defects which have been shown to be prevented by periconceptional folic acid supplementation. In a meta-analysis, van der Put and colleagues (1997) studied the reported frequencies of the C677T MTHFR gene polymorphism to examine whether they were population-dependent. The prevalence of homozygosity for this polymorphism is reported to be 5% to 16% in different populations. This allele appears to be very common among Hispanics (Botto and Yang, 2000b). In this study we report a prevalence of 10% in the control group.

A report on the incidence of heart malformations in murine embryos showed that mild MTHFR deficiency, low dietary folate, or both increase the incidence of fetal loss and heart defects (Li et al., 2005). Although previous studies suggested a relation between MTHFR polymorphisms and CHD, a study by Mc Bride in 2004 could not replicate the association. The study only included children with left ventricular outflow tract malformations (McBride et al., 2004). A study performed in the Netherlands with 158 mothers with a CHD-affected child reported that the maternal MTHFR 677CT and TT genotypes in combination with no use of periconceptional folate supplements were associated with a three-fold and six-fold increased risk for conotruncal heart defects in offspring van Beynum et al., 2006). The data obtained from these studies supported the conclusion that folic acid is essential for normal fetal cardiac development during early embryogenesis, and that periconceptional folic acid use may reduce the risk for congenital cardiac anomalies (Bayley and Berry, 2005).

A second common polymorphism in the methylenetetrahydrofolate reductase gene (1298 A→C) was reported by van der Put and colleagues (van der Put et al., 1998) suggesting that a combined heterozygosity for the two MTHFR common polymorphisms accounts for a proportion of folate-related neural tube defects. Hobbs et al (2006) examined the relation between CHD and maternal MTHFR polymorphisms and reported the 1298C allele to be transmitted less often than expected suggesting an apparent protective effect against CHD. More recently, a study done in Brazil (Galdieri et al., 2007) did not find a difference in allele frequencies among patients with CHD, mothers, or controls and included the C677T,

A1298C, and the A2756G MTHFR polymorphisms. Using data from a California population-based registry, investigators studied 118 single nucleotide polymorphisms associated with the folate pathway (Shaw et al., 2009). They did not find a particular folate transport or metabolism gene to be strongly associated with risks for conotruncal defects. A meta-analysis by Verkleij-Hagoort and colleagues (2007) concluded that the MTHFR polymorphisms in mothers and children are not independently associated to CHD. The role of compound heterozygosity was not analyzed. Our study supports a relation between compound heterozygosity and the occurrence of CHD, both for mothers and for infants with CHD.

To our knowledge, this is the first report of MTHFR polymorphisms in a group of Hispanic children with CHD. This study is limited by the small sample of children with CHD which precluded further analysis of the association between conotruncal defects and the presence of the MTHFR polymorphisms. Nevertheless, the higher prevalence of the TT variant in mothers of these children supports the association between homozygosity for the C677T allele and a higher risk of having a child with CHD. Due to the relation of these enzymes to folate metabolism, current folate consumption recommendations for women in childbearing age in Puerto Rico to reduce neural tube defects may need to be extended for the prevention of CHD.

Acknowledgments

We would like to acknowledge the contributions made by the UPR School of Medicine, Clinical Research Center personnel. We would also like to express our gratitude to Dr. Pedro Santiago-Borrero and the Newborn Screening Program for their contribution to the success of this study. This study was supported in part by the UPR School of Medicine, Associate Deanship for Biomedical Sciences and Graduate Studies; and the Clinical Research Center, NIH Grant P20RR11126, National Center for Research Resources (NCRR). The RCMI Human Molecular Genetics Unit is supported by NCRR-NIH RCMI Grant G12RR03051.

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Table 1

General characteristics of the participants (N=27)

Characteristics	Participants
Maternal age in years, mean	25 (17-38)
Maternal Education	
Less than high school	22%
High school	19%
University	59%
Social Strata (Hollingshead)	
Class I	11.1%
Class II	14.8%
Class III	29.6%
Class IV	7.4%
Class V	37.0%
Use of folic acid during pregnancy	89%
Family history of NTD[a]	3.7%
Family history of CHD[b]	11.1%

[a] NTD - neural tube defects

[b] CHD - congenital heart disease

Table 2

Children principal cardiac diagnoses (N=27)

Characteristics	Participants
Hypoplastic left heart	6
Tetrallogy of Fallot	4
Double outlet right ventricle	3
Transposition of Great Vessels	4
Ebstein anomaly	2
Ventricular septal defect	3
Pulmonary stenosis/atresia	2
Coarctation of the aorta	2
Atrial septal defect	1

Table 3

Distribution of genotypes for the C677T MTHFR[c] polymorphism

Participants	N	CC	CT	TT	p value
Infants with CHD	27	9(33%)	14(52%)	4(15%)	0.3916
Mothers	27	10(37%)	11(41%)	6(22%)	0.0463
Controls	220	84(38%)	115(52%)	21(10%)	

[c]MTHFR = methylenetetrahydrofolate reductase

Table 4

Allele frequency for the C677T MTHFR[c] polymorphism

Participants	C	T	p value
Infants	32 (59%)	22 (41%)	0.4655
Mothers	31 (57%)	23 (43%)	0.3193
Controls	283 (64%)	157 (36%)	

[c] MTHFR = methylenetetrahydrofolate reductase

Table 5

Distribution of compound heterozygosity for the C677T and A1298C MTHFR[c] polymorphisms

Participants	N	Frequency	unadjusted OR[d] (95% CI[e])	p value
Infants with CHD	27	33%	3.7 (1.6-8.6)	0.0016
Mothers	27	26%	2.6 (1.0-6.4)	0.0366
Controls	400	12%		

[c] MTHFR = methylenetetrahydrofolate reductase

[d] OR = odds ratio

[e] CI = confidence interval