Published in final edited form as:
Birth Defects Res A Clin Mol Teratol. 2009 February ; 85(2): 125–129. doi:10.1002/bdra.20501.

Evaluation of Potential Modifiers of the Cardiac Phenotype in the 22q11.2 Deletion Syndrome

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Abstract

BACKGROUND—The phenotype associated with deletion of the 22q11.2 chromosomal region is highly variable, yet little is known about the source of this variability. Cardiovascular anomalies, including tetralogy of Fallot, truncus arteriosus, interrupted aortic arch type B, perimembranous ventricular septal defects, and aortic arch anomalies, occur in approximately 75% of individuals with a 22q11.2 deletion.

METHODS—Data from 343 subjects enrolled in a study of the 22q11.2 deletion syndrome were used to evaluate potential modifiers of the cardiac phenotype in this disorder. Subjects with and without cardiac malformations, and subjects with and without aortic arch anomalies were compared with respect to sex and race. In addition, in the subset of subjects from whom a DNA sample was available, genotypes for variants of four genes that are involved in the folate-homocysteine metabolic pathway and that have been implicated as risk factors for other birth defects were compared. Five variants in four genes were genotyped by heteroduplex or restriction digest assays. The chi-square or Fisher's exact test was used to evaluate the association between the cardiac phenotype and each potential modifier.

RESULTS—The cardiac phenotype observed in individuals with a 22q11.2 deletion was not significantly associated with either sex or race. The genetic variants that were evaluated also did not appear to be associated with the cardiovascular phenotype.

CONCLUSIONS—Variation in the cardiac phenotype observed between individuals with a 22q11.2 deletion does not appear to be related to sex, race, or five sequence variants in four folate-related genes that are located outside of the 22q11.2 region.

Keywords

birth defect; congenital heart disease; conotruncal defect; folate; genetic

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INTRODUCTION

The 22q11.2 deletion syndrome is the most common chromosomal deletion syndrome identified to date and is estimated to occur in 1 in 4,000–6,000 livebirths (Botto et al., 2003). The phenotype is highly variable, and may include congenital heart disease, palate anomalies, hypocalcemia, immunodeficiency, cognitive difficulties, and dysmorphic facies (Goldmuntz, 2005). Approximately 75% of patients with a 22q11.2 deletion are diagnosed with a cardiovascular anomaly. However, the severity of the cardiac phenotype varies substantially between individuals (McDonald-McGinn et al., 1997; Ryan et al., 1997). The most common types of cardiac malformations observed in affected individuals include tetralogy of Fallot, truncus arteriosus, interrupted aortic arch type B, perimembranous ventricular septal defects, and aortic arch anomalies (McDonald-McGinn et al., 1997; Ryan et al., 1997). Aortic arch anomalies can be seen either in conjunction with other intracardiac anomalies like tetralogy of Fallot, or in isolation with normal intracardiac anatomy. In the latter case, a patient may have a clinically significant aortic arch anomaly causing a vascular ring (e.g., double aortic arch or right aortic arch with an aberrant left subclavian artery) or a silent anomaly of the aortic arch (e.g., a left aortic arch with an aberrant right subclavian artery) that would not be detected unless appropriate imaging studies were performed.

The etiology of the phenotypic variability in the 22q11.2 deletion syndrome is poorly understood. Neither the size nor the parental origin of the 22q11.2 deletion appear to explain the variable phenotype (Carlson et al., 1997; Desmaze et al., 1993; Morrow et al., 1995; Saitta et al., 2004; Scambler et al., 1991). Potential mechanisms underlying this variability include modifier genes that reside outside the deleted region, allelic variation of genes within the deleted region of the nondeleted chromosome, somatic mutations, epigenetic phenomena, individual characteristics (e.g., sex, race), environmental factors, and chance.

There is some evidence that the cardiac phenotype observed in the 22q11.2 deletion syndrome may be influenced by genetic modifiers that lie outside of the region of the deletion. Specifically, variants of the vascular endothelial growth factor (**VEGF**) gene, which are known to alter expression of **VEGF**, have been associated with cardiovascular anomalies in the presence of a 22q11.2 deletion (Stalmans et al., 2003). However, the population attributable fraction for these variants is only 14% (Stalmans et al., 2003), which suggests that additional factors contribute to the variability in the cardiac phenotype observed in the 22q11.2 deletion syndrome. Interestingly, variants of **VEGF** also appear to be associated with the risk of CHD in individuals who do not have a deletion of 22q11.2 (Lambrechts et al., 2005), suggesting that syndromic and nonsyndromic forms of CHD may share common risk factors.

The present analyses were undertaken to determine whether factors known or suspected to be associated with the risk of CHD in nondeleted individuals might also be associated with the cardiac phenotype in the 22q11.2 deletion syndrome. These analyses considered sex and race, both of which are known to be associated with the prevalence of CHD (Jenkins et al., 2007; Perry et al., 1993), and five variants in four genes involved in folate-homocysteine metabolism (*MTHFR*, *CBS*, *MTR*, and *MTRR*). Genes involved in folate-homocysteine metabolism were considered of interest because of the evidence linking maternal periconceptional folic acid supplementation, which is known to be protective against neural tube defects (NTD) in offspring (MRC, 1991; Czeizel and Dudas, 1992), with reduced risk of CHD, including outflow tract/conotruncal malformations, in offspring (Botto et al., 1996, 2000; Czeizel, 1996). The methylenetetrahydrofolate reductase (**MTHFR**) C677T variant was of particular interest, given early reports that this functional variant was associated with the risk of CHD (Junker et al., 2001; Wenstrom et al., 2001) as well as NTD (van der Put et al., 1995; Whitehead et al., 1995). Although at the time these studies were initiated there were no other reported associations between folate-related genes and CHD, the **MTHFR** A1298C variant and

functional variants in cystathionine β-synthase (**CBS**), methionine synthase (**MTR**), and methionine synthase reductase (**MTRR**) were selected for inclusion in this study based on the reported associations between these variants and the risk of NTD (Botto and Mastroiacovo, 1998; Christensen et al., 1999; Wilson et al., 1999).

MATERIALS AND METHODS

Clinical Evaluation

Subjects were ascertained through the 22q11.2 Deletion Center at The Children's Hospital of Philadelphia according to a protocol approved by the Institutional Review Board for the Protection of Human Subjects. Each patient had a deletion of chromosome 22q11.2 confirmed by fluorescence *in situ* hybridization using standard techniques (Driscoll et al., 1993). Study subjects were examined by a geneticist for dysmorphic features and by other clinical subspecialists as clinically indicated after informed consent was obtained.

A pediatric cardiologist (E. G.) examined each subject and/or reviewed available cardiovascular records. Patients examined by the pediatric cardiologist underwent an imaging study (echocardiogram and/or cardiac MRI) to define both intracardiac and aortic arch anatomy (including aortic arch sidedness, cervical location, and branching pattern of the brachiocephalic vessels). Each patient was classified as either having or not having a cardiac defect and a primary cardiac diagnosis was assigned to each subject with a cardiac defect. In addition, for each subject, the aortic arch anatomy was classified as normal, abnormal, or unspecified. Upon review of outside records, if the aortic arch anatomy was not explicitly defined, then the aortic arch sidedness and/or branching pattern were considered unspecified. This approach may have underestimated the number of subjects with normal aortic arch anatomy, but avoided mis-classifying a patient with a subtle, unrecognized variant of aortic arch anatomy as "normal".

Genotyping Methods

The collection of blood samples for the purposes of DNA extraction and genotyping was not included in the initial study protocol. Consequently, DNA for genotyping was available for only a subset of the study subjects. Further, DNA samples were added to the genotyping runs as they became available, such that more samples were available for genotypes that were analyzed later in the study period than for genotypes that were analyzed earlier.

Genomic DNA was extracted from whole blood or lymphoblastoid cell lines obtained from the subset of study subjects for whom such a sample had been collected. The available DNA samples were genotyped for the following variants using previously described methods. The **MTHFR** C677T single nucleotide polymorphism was genotyped using either a restriction digest assay (Frosst et al., 1995) or heteroduplex analysis (Barbaux et al., 2000). The **MTHFR** A1298C, **MTR** A2756G (Leclerc et al., 1996), **MTRR** A66G (Wilson et al., 1999), and the **CBS** 844ins68 polymorphisms (Sebastio et al., 1995) were genotyped using heteroduplex analysis (Barbaux et al., 2000). Genotyping assays were validated by bidirectional sequencing of 10 control samples.

Statistical Methods

The chi-square or the Fisher's exact test was used to evaluate the association between cardiac phenotype and each potential modifier. For these analyses, two definitions of the cardiac phenotype were considered. Under the first definition, individuals identified as having any cardiac abnormality (intracardiac or aortic arch) were compared to individuals with no identified cardiac abnormality, including those for whom the aortic arch anatomy was unspecified. Under the second definition, individuals with abnormal aortic arch anatomy were compared to individuals with confirmed normal aortic arch anatomy (i.e., individuals for whom

aortic arch anatomy was unspecified were excluded under this definition). Although other definitions of the cardiac phenotype could have been considered (e.g., confirmed normal intracardiac and aortic arch anatomy vs. intracardiac abnormality only), the numbers available for such analyses were generally small and precluded meaningful analyses. For comparisons involving a potential genetic risk factor, the full genotype distribution was considered (i.e., genotype categories were not pooled). For simplicity, the alleles of each biallelic variant were assigned a numeric code (i.e., 1, 2). All analyses were conducted using SAS version 8.02 (SAS Institute, Cary, NC). An association was judged to be statistically significant when the unadjusted *p* value for the chi-square or Fisher's exact text was less than .05. However, due to the relatively large number of comparisons that were evaluated, these analyses should be considered exploratory in nature and the statistical significance of any single test must be considered in this context.

RESULTS

Information on the cardiac phenotype was available for 343 study subjects, of whom 264 (77%) had a cardiac malformation. The most common defect was tetralogy of Fallot (Table 1). Of the 343 study subjects, aortic arch anatomy was explicitly assessed or reported in 235, of whom 71% (166/235) had an aortic arch anomaly. As many of the subjects with unspecified aortic arch anatomy were likely to have a normal phenotype, this percentage is likely to overestimate the true proportion of individuals with the 22q11.2 deletion syndrome who have an aortic arch anomaly. A lower estimate of this proportion is obtained by assuming that all subjects with unspecified aortic arch anatomy had normal aortic arch anatomy. Under this scenario, the estimated proportion of subjects with a 22q11.2 deletion that have an aortic arch anomaly is only 48% (166/343).

The study subjects included 171 males and 172 females. Neither of the cardiac phenotypes was significantly associated with subject sex (Table 2). The study subjects were predominantly Caucasian ($n = 278$; 81%) with the next largest group being African-American ($n = 34$; 10%). Cardiac malformations were more common in African-American (88%) than in Caucasian (77%) subjects, but this difference was not statistically significant (Table 2). Abnormalities of the aortic arch were also slightly more common in African-Americans (78%) than in Caucasians (72%), but again this difference was not statistically significant.

Because genotype frequencies can differ by race and there were some differences in the frequency of the study outcomes by subject race, there was concern that analyses of the potential genetic modifiers might be biased as a result of population admixture. Due to this concern, and the small number of non-Caucasian subjects (African-American, 34; Other, 31), all statistical analyses of the genetic variants were conducted using data only from the subgroup of Caucasian subjects. DNA samples were available and genotyped for at least one variant in 185 Caucasian subjects. The number of subjects genotyped for each variant ranged from 140 to 174, reflecting the progressive accumulation of subject samples over the course of this study.

Five variants in four genes were evaluated as potential genetic modifiers of the cardiac phenotype in patients with the 22q11.2 deletion (Tables 3 and 4). A single significant association involving the **MTHFR** C677T variant and aortic arch anatomy was observed (Table 4). However, this association was of only borderline statistical significance ($p = .04$), and the difference in the genotype distributions of those with and without arch anomalies was not consistent with an underlying genetic model (i.e., subjects with an aortic arch malformation were more likely to be heterozygous for this variant, and less likely to be either type of homozygote than were subjects with normal arch anatomy). Hence, this association is likely to represent a false-positive finding.

DISCUSSION

The cardiovascular manifestations of the 22q11.2 deletion are highly variable. They range from normal intra-cardiac and aortic arch anatomy, to severe malformations such as tetralogy of Fallot with pulmonary atresia, multiple aortopulmonary collaterals, and right-sided aortic arch with an aberrant left subclavian artery (Goldmuntz, 2005; Goldmuntz et al., 1998). While the latter diagnosis is clinically apparent, isolated aortic arch anomalies such as an aberrant right subclavian artery with a normal left-sided aortic arch may be asymptomatic and clinically unapparent unless specific imaging studies are performed. Therefore, explicit definition of the aortic arch anatomy is critical to the classification of subjects into either “normal” or “abnormal” subgroups.

This study was undertaken to determine whether factors that are known or suspected to be associated with CHD in nondeleted individuals might also modify the cardiac phenotype in the 22q11.2 deletion syndrome. Our analyses did not identify any convincing associations between sex, race, or five genetic variants from the folate-homocysteine metabolic pathway and the risk of cardiovascular disease in this cohort with a 22q11.2 deletion. There is no apparent association whether the risk of cardiac disease is considered overall or the risk of aortic arch anomalies is considered separately.

As with all studies, this study had limitations including the incomplete description of the aortic arch anatomy in a substantial number of study subjects. Many patients were referred from outside centers with limited cardiac records that often incompletely defined the aortic arch anatomy or simply described a “normal aortic arch”. To assume that these subjects had completely normal aortic arch anatomy with normal branching of the brachiocephalic vessels would, from clinical experience, underestimate the number of subjects with clinically silent abnormalities. Therefore, a small number of subjects with no apparent cardiac phenotype might have aortic arch anomalies. While this issue limited the ability to compare those with completely normal anatomy to those with any type of abnormality, we were able to classify and compare those with normal as compared to abnormal aortic arch anatomy by restricting the latter analysis to the subset of study subjects for whom aortic arch anatomy was completely defined. An additional limitation of this study is that these analyses considered only one or two variants per gene. When this study was designed (in the late 1990s), it was common for genetic association studies to focus on the known functional variants of a gene. Nonetheless, these focused studies are limited in scope and do not exclude possible associations between other variants in these genes and the cardiac phenotype in individuals with the 22q11.2 deletion syndrome. In addition, there are other potential sources of phenotypic variation (e.g., deletion size, parent of origin) that were not evaluated.

This study also had several strengths, including a relatively large, study population that allowed for consideration of intracardiac and aortic arch anatomy independently in a novel patient population. The extent to which there is overlap in the genes that influence intracardiac and aortic arch development is unknown. Therefore, analyses that consider different phenotypic definitions may yield biologically interesting results.

In summary, our analyses of these data indicate that the variation in the cardiac phenotype observed between individuals with the 22q11.2 deletion syndrome is not associated with sex, race, or five variants in four folate-related genes. These findings are in contrast to those from previous analyses of the same study population, aimed at identifying modifiers of the palatal phenotype (Driscoll et al., 2006). Specifically, analyses of the palatal phenotype in individuals with the 22q11.2 deletion syndrome indicated that this aspect of the phenotype (i.e., the palatal phenotype) is significantly associated with both sex and race, and possibly with the **MTR** A2756G variant. Hence, despite the negative findings in the present study, additional studies

of potential genetic modifiers of the 22q11.2 phenotype, including folate-related genes, are warranted.

Acknowledgments

Grant sponsor: National Institutes of Health; Grant numbers: DC002027, HL074731 and HL062177.

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Table 1

Cardiac Phenotypes in 343 Subjects with a 22q11.2 Deletion

Phenotype	*n* (%)
Cardiac malformation	
No	79 (0.23)
Yes	264 (0.77)
Primary diagnosis	
Tetralogy of Fallot	80 (0.30)
Ventricular septal defect	44 (0.17)
Interrupted aortic arch	41 (0.15)
Aortic arch anomaly	36 (0.14)
Truncus arteriosus	27 (0.10)
Other or unspecified	36 (0.14)
Aortic arch anatomy	
Unspecified	108 (0.31)
Normal	69 (0.20)
Abnormal	166 (0.48)

Table 2

Association between Cardiac Phenotypes and Sex and Race in Subjects with a 22q11.2 Deletion

		Sex			Race		
		Female (*n* = 172)	**Male (*n* = 171)**	**χ_1^2 (*p*-value)**	**Caucasian**	**African-American**	**χ_1^2 (*p*-value)**
Cardiac malformation	No	40 (0.23)	39 (0.23)		65 (0.23)	4 (0.12)	
	Yes	132 (0.77)	132 (0.77)	0.01 (0.92)	213 (0.77)	30 (0.88)	2.37 (0.12)
Aortic arch anatomy	Normal	38 (0.32)	31 (0.27)		52 (0.28)	6 (0.21)	
	Abnormal	82 (0.68)	84 (0.73)	0.63 (0.73)	135 (0.72)	22 (0.78)	0.50 (0.48)

Table 3

Association between Cardiac Malformation and Genotype for Variants of Folate-Related Genes in Subjects with a 22q11.2 Deletion

			Genotype n (%)			
Gene	**Cardiac malformation**	***n***	**11**	**12**	**22**	χ^2_2 **or Fisher's exact (FE) (*p*-value)**
MTHFR C677T	No	32	13 (0.41)	15 (0.47)	4 (0.12)	0.11 (0.95)
(1 = C, 2 = T)	Yes	142	62 (0.44)	64 (0.45)	16 (0.11)	
MTHFR A1298G	No	26	11 (0.42)	13 (0.50)	2 (0.08)	0.32 (0.85)
(1 = A, 2 = G)	Yes	126	47 (0.37)	66 (0.53)	13 (0.10)	
MTR	No	24	19 (0.79)	4 (0.17)	1 (0.04)	FE (0.26)
(1 = A, 2 = G)	Yes	121	84 (0.69)	35 (0.29)	2 (0.02)	
MTRR	No	23	10 (0.44)	7 (0.30)	6 (0.26)	4.14 (0.13)
(1 = A, 2 = G)	Yes	117	27 (0.23)	46 (0.39)	44 (0.38)	
CBS	No	27	25 (0.93)	2 (0.07)	0 (0.00)	FE (0.48)
(1 = wild-type, 2 = insertion)	Yes	134	109 (0.81)	22 (0.17)	3 (0.02)	

Table 4

Association between Aortic Arch Anatomy and Genotype for Variants of Folate-Related Genes in Subjects with a 22q11.2 Deletion

			Genotype n (%)			
Gene	**Aortic arch anatomy**	***n***	**11**	**12**	**22**	**χ^2_2 or Fisher's exact (FE) (*p*-value)**
MTHFR C677T	Normal	31	18 (0.58)	7 (0.23)	6 (0.19)	6.23 (0.04)
(1 = C, 2 = T)	Abnormal	88	39 (0.44)	41 (0.47)	8 (0.09)	
MTHFR A1298G	Normal	21	5 (0.24)	12 (0.57)	4 (0.19)	2.88 (0.24)
(1 = A, 2 = G)	Abnormal	86	35 (0.41)	43 (0.50)	8 (0.09)	
MTR	Normal	20	13 (0.65)	6 (0.30)	1 (0.05)	FE (0.40)
(1 = A, 2 = G)	Abnormal	81	57 (0.70)	23 (0.29)	1 (0.01)	
MTRR	Normal	18	3 (0.17)	11 (0.61)	4 (0.22)	4.32 (0.12)
(1 = A, 2 = G)	Abnormal	78	20 (0.26)	27 (0.34)	31 (0.40)	
CBS	Normal	24	23 (0.96)	1 (0.04)	0 (0.00)	FE (0.12)
(1 = wild type, 2 = insertion)	Abnormal	86	66 (0.77)	17 (0.20)	3 (0.03)	