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

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Abstract

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Objective—To evaluate potential modifiers of the palatal phenotype in individuals with the 22q11.2 deletion syndrome.

Design—Data from 356 subjects enrolled in a study of the 22q11.2 deletion syndrome were used to evaluate potential modifiers of the palatal phenotype. Specifically, subjects with and without velopharyngeal inadequacy and/or structural malformations of the palate were compared with respect to gender, race, and genotype for variants of seven genes that may influence palatal development.

Methods—The chi-square test or Fisher exact test was used to evaluate the association between palatal phenotype and each potential modifier. Odds ratios and their associated 95% confidence intervals were used to measure the magnitude of the association between palatal phenotype, subject gender and race, and each of the bi-allelic variants.

Results—The palatal phenotype observed in individuals with the 22q11.2 deletion syndrome was significantly associated with both gender and race. In addition, there was tentative evidence that the palatal phenotype may be influenced by variation within the gene that encodes methionine synthase.

Conclusions—Variation in the palatal phenotype observed between individuals with the 22q11.2 deletion syndrome may be related to personal characteristics such as gender and race as well as variation within genes that reside outside of the 22q11.2 region.

Keywords

chromosome 22q11.2 deletion; genes; genotype; palate; penetrance; phenotype

Hemizygous deletion of the chromosome 22q11.2 region results in a syndromic phenotype that encompasses the DiGeorge, velocardiofacial, and conotruncal anomaly face syndromes. Population-based estimates of the prevalence of this deletion range from 1 in 4500 to 1 in 7100 births (Botto et al., 2003; Tezenas du Montcel et al., 1996; Oskarsdottir et al., 2004), making it the most frequent known interstitial deletion in humans. The majority of individuals with the 22q11.2 deletion syndrome have a *de novo* three-megabase deletion. However, approximately 10% of patients with the 22q11.2 deletion syndrome have an inherited deletion, and approximately 10% have a smaller deletion (1.5 Mb) (Saitta et al., 2004; Yamagishi and Srivastava, 2003).

The phenotypic consequences of hemizygous deletion of chromosome 22q11.2 are remarkably variable and can include cardiac, thymic, parathyroid, craniofacial, developmental, neurologic, and behavioral manifestations. None of these features appears to be fully penetrant, and each exhibits variable expressivity. For example, approximately 75% of patients with the 22q11.2 deletion syndrome have a cardiac defect, and a range of malformations (e.g., tetralogy of Fallot, interrupted aortic arch, septal defects) is observed among those who have such a defect (McDonald-McGinn et al., 1997; Ryan et al., 1997).

The variability in the 22q11.2 deletion syndrome phenotype is largely unexplained. Variability is observed across unrelated patients and between affected family members who have presumably inherited identical deletions (Desmaze et al., 1993; McLean et al., 1993; Scambler et al., 1991; Singh et al., 2002; Van Hemel et al., 1995). Moreover, the observed variability does not appear to be attributable to either deletion size or the parental origin of the deleted chromosome (Carlson et al., 1997; Desmaze et al., 1993; Morrow et al., 1995; Scambler et al., 1991). Potential mechanisms underlying this variability include: modifier genes that reside outside the deleted region; allelic variation of genes within the 22q11.2 region of the non-deleted chromosome; somatic mutations; epigenetic phenomena; individual characteristics (e.g., race, gender); environmental factors; and chance.

Evidence that the 22q11.2 deletion syndrome phenotype may be influenced by genetic modifiers that reside outside of the region of the deletion is provided by recent studies in animals and humans. Specifically, it has been shown that genetic background influences the penetrance of cardiovascular, thymic, and parathyroid anomalies in mice that are heterozygous for a deletion (i.e., Df1/+) that encompasses homologues of 18 of the genes that reside within the 22q11.2 region in humans (Taddei et al., 2001). There is also evidence that variation in the gene encoding vascular endothelial growth factor may modify the cardiovascular phenotype in humans who are hemizgyous for the 22q11.2 deletion (Stalmans et al., 2003). Moreover, it has been shown that attention deficit hyperactivity disorder (ADHD) in patients with 22q11.2 deletion syndrome is associated with both patient gender and family history of ADHD (Gothelf et al., 2004).

Individuals with the 22q11.2 deletion syndrome present with a range of structural malformations of the palate, including cleft lip, overt cleft palate, submucous cleft palate, and bifid uvula (McDonald-McGinn et al., 1997; Ryan et al., 1997). In addition, patients may present with velopharyngeal inadequacy, with or without an associated structural malformation of the palate (McDonald-McGinn et al., 1997; Ryan et al., 1997). The present analyses were undertaken in an attempt to identify factors that influence the palatal phenotype observed in the 22q11.2 deletion syndrome. These analyses considered individual characteristics (gender and race) as well as potential genetic modifiers of the palatal phenotype. Potential genetic modifiers were selected based on published reports providing evidence of linkage or association between a genetic variant and facial clefts in humans and included: transforming growth factor α (TGFA) (Ardinger et al., 1989; Machida et al., 1999; Mitchell, 1997), transforming growth factor β three (TGFB3) (Lidral et al., 1998), and muscle segment homeobox (MSX1) (Fallin et al., 2003; Jezewski et al., 2003; Lidral et al., 1998; reviewed in Marazita and Neiswanger, 2002). In addition, because there is evidence that the risk of facial clefts may be influenced by maternal folate intake (van Rooij et al., 2004), variants of genes involved in the folate-homocysteine metabolic pathway, including methylenetetrahydrofolate reductase (MTHFR), methionine synthase (MTR), methionine synthase reductase (MTRR), and cystathionine beta synthase (CBS) (reviewed in Finnell et al., 1998), were evaluated.

Materials and Methods

Study Subjects

The analyses described in this paper are based on data obtained from subjects enrolled in a study of velocardiofacial syndrome at The Children's Hospital of Philadelphia. Subjects with a 22q11.2 deletion, confirmed by fluorescence in situ hybridization, and who had undergone clinical examinations to identify structural abnormalities of the primary and secondary palate and to evaluate velopharyngeal function were eligible for this study. Information on subject sex and race was collected as part of the clinical evaluation and was available for all subjects. However, DNA for genotyping was not available for all subjects, because the collection of blood samples for the purposes of DNA extraction and genotyping was not included in the initial study protocol. This study was approved by the Institutional Review Board of the Children's Hospital of Philadelphia. Informed consent was obtained for each study subject.

Clinical Evaluation

Subjects were evaluated by a pediatric plastic surgeon and a speech pathologist. The palate of each subject was inspected for the presence of an overt or submucous cleft and bifid uvula. Velopharyngeal function was assessed by perceptual speech examination. When velopharyngeal inadequacy was suspected on the basis of the speech evaluation, it was confirmed by nasendoscopy or multiview videofluoroscopy. Subjects with clinical evidence of velopharyngeal inadequacy who had not yet undergone nasendoscopy or videofluoroscopy

were classified as "suspected velopharyngeal inadequacy." All other subjects were classified as unknown (e.g., prelingual subjects with no clinical symptoms of velopharyngeal inadequacy).

Genotyping Methods

Genomic DNA was obtained from whole blood or lymphoblastoid cell lines and genotyped for the following variants using previously described methods.

MTHFR—The MTHFR C677T single nucleotide polymorphism (SNP) was genotyped using either a restriction digest assay (Frosst et al., 1995) or heteroduplex analysis (Barbaux et al., 2000). The MTHFR A1298C SNP was genotyped using heteroduplex analysis (Barbaux et al., 2000).

CBS—The 68-bp insertion/deletion CBS variant (Sebastio et al., 1995) was genotyped using heteroduplex analysis (Barbaux et al., 2000).

MTR—The MTR A2756G SNP (Leclerc et al., 1996) was genotyped using heteroduplex analysis (Barbaux et al., 2000).

MTRR—The MTRR A66G SNP (Wilson et al., 1999) was genotyped using heteroduplex analysis (Barbaux et al., 2000).

TGFA—The TGFA *Taq*I restriction fragment length polymorphism was genotyped using a polymerase chain reaction–based method (Basart et al., 1994).

MSX1—A MSX1 CA dinucleotide repeat was genotyped using a polymerase chain reaction–based method (Padanilam et al., 1992).

TGFB3—A TGFB3 CA dinucleotide repeat was genotyped as described in Lidral et al. (1997).

Statistical Methods—Subjects were classified as having an abnormality of palatal structure if they had cleft lip with or without cleft palate, overt cleft palate, submucous cleft palate, and/or a bifid uvula. All other subjects were classified as having normal palatal structure. Subjects demonstrating incomplete closure of the velum by nasendoscopy or multiview videofluoroscopy were classified as having velopharyngeal inadequacy, and subjects demonstrating complete closure of the velum by either of these methods were classified as having normal velopharyngeal function. Subjects who had symptoms of velopharyngeal inadequacy but who had not undergone confirmatory testing were classified as "suspected velopharyngeal inadequacy." All other subjects were classified as having unknown velopharyngeal function.

For the purpose of the present analyses, subjects with complete data for palatal structure and velopharyngeal function were divided into three mutually exclusive categories: normal palatal structure and normal velopharyngeal function (normal), normal palatal structure and velopharyngeal inadequacy (non-cleft-VPI), and abnormal palatal structure with or without velopharyngeal inadequacy (palate). Subjects with a suspected diagnosis of velopharyngeal inadequacy and an abnormality of palatal structure were included in the third group, whereas subjects with a suspected diagnosis of velopharyngeal inadequacy and normal palatal structure were excluded from all analyses.

Data for the noncleft-VPI and palate groups were analyzed separately, using the normal group as the reference category for both sets of analyses. In addition, the analyses were repeated after combining the data from the noncleft-VPI and palate groups (total), again using the normal group as the reference category. Although cleft palate is the most common cause of velopharyngeal inadequacy (Riski, 2002), the relationship between velopharyngeal inadequacy in the absence of cleft palate and structural malformations of the palate has not been defined. Data from the noncleft-VPI and palate groups were pooled based on the possibility that these two conditions may share common developmental pathways.

The chi-square or Fisher exact test was used to evaluate the association between velopharyngeal function and/or structural palatal malformations and each potential modifier. For comparisons involving a potential genetic risk factor, the full genotype distribution was considered (i.e., genotype categories were not pooled). Odds ratios (OR) and their associated 95% confidence interval (CI) were used to measure the magnitude of the association between velopharyngeal function and/or structural palatal malformations, subject sex and race, and each of the bi-allelic variants. For simplicity, the alleles of each bi-allelic variant were assigned a numeric code (i.e., 1, 2) and the '11' genotype was arbitrarily selected as the reference category for the calculation of ORs. ORs were not calculated for the multi-allelic variants because of the small number of observations for several of the genotype categories.

All analyses were conducted using SAS (version 8.02; SAS Institute Inc, Cary, NC). An association was judged to be statistically significant when the unadjusted *p* value for the chi-square or Fisher exact text was less than .05. However, because of 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

Complete data on palatal structure and velopharyngeal function were available for 356 study subjects. Of these subjects, 58 had suspected velopharyngeal inadequacy with normal palatal structure and were omitted from all analyses. The remaining 298 unrelated subjects included 91 (30.5%) with normal palatal structure and normal velopharyngeal function, 67 (22.5%) with velopharyngeal inadequacy and normal palatal structure, and 140 (47.0%) with abnormal palatal structure with or without velopharyngeal inadequacy (Table 1). The abnormalities identified in the group of subjects with abnormal palatal structure (n = 140) were: overt cleft palate (n = 24, 17.1%), submucous cleft palate (n = 88, 62.8%), cleft lip with cleft palate (n = 1, 0.7%), bifid uvula (n = 24, 17.1%), and unspecified conditions (n = 3, 2.1%).

The study sample included 148 (49.7%) boys and 150 (50.3%) girls. Velopharyngeal inadequacy and structural palatal malformations were significantly more common in girls (113/150 = 0.75) than in boys (94/148 = 0.63) with the 22q11.2 deletion (total versus normal: $\chi^2_1 = 4.91$, $p = .03$) (Table 1). When the two case groups were analyzed separately, velopharyngeal inadequacy was 2.6 times more common in girls (43/80 = 0.54) than in boys (24/78 = 0.31), and this association was statistically significant (noncleft-VPI versus normal: $\chi^2_1 = 8.54$, $p = .004$). Structural malformations of the palate also appeared to be more common in girls (70/107 = 0.65) than in boys (70/124 = 0.56), but this association was not statistically significant (palate versus normal: $\chi^2_1 = 1.94$, $p = .16$).

The study subjects were predominantly Caucasian (n = 246, 82.6%), with the next largest group being African-American (n = 24, 8.05%). Velopharyngeal inadequacy and structural malformations of the palate were more common in Caucasians (180/246 = 0.73) with the 22q11.2 deletion than in African-Americans (8/24 = 0.33) with this deletion (total versus normal: Fisher exact test, $p = .0002$). Despite the relatively small number of African-American

subjects in the noncleft-VPI and palate groups, the association with race was statistically significant in each group (Table 1).

Because the study outcomes (i.e., velopharyngeal inadequacy and structural malformations of the palate) were related to subject race, and genotype frequencies can also differ by race, there was concern that analyses of the potential genetic modifiers might be biased as a result of population admixture. Because of this concern, and the small number of non-Caucasian subjects (African-American, 24; Other, 28), all statistical analyses of the genetic variants were conducted using data only from the subgroup of Caucasian subjects (n = 246).

Eight variants in seven genes were evaluated as potential genetic modifiers of the palatal phenotype (Tables 2 and 3). A single significant association involving the A2756G variant of MTR and velopharyngeal inadequacy was observed (Table 2). This association suggested that the risk of velopharyngeal inadequacy in subjects with a 22q11.2 deletion is significantly lower in individuals with the '12' (MTR 2756-AG) genotype than in individuals with the '11' (AA) genotype (noncleft-VPI versus normal: OR = 0.16, 95% CI 0.03, 0.79). However, this association is based on only two individuals with VPI and the '12' genotype.

Discussion

Our analyses of these data indicate that the palatal phenotype (i.e., velopharyngeal inadequacy and/or structural malformations of the palate) associated with the 22q11.2 deletion syndrome may be influenced by both gender and race. Compared to boys, girls with the 22q11.2 deletion were more likely to have velopharyngeal inadequacy and/or a palatal malformation, and compared to Caucasians, African-Americans with the 22q11.2 deletion were less likely to have velopharyngeal inadequacy and/or a palatal malformation. Although relatively little information has been published with regard to the epidemiologic characteristics of velopharyngeal inadequacy, these associations are consistent with the patterns observed for cleft palate in the general population (Mossey and Little, 2002). Hence, as-yet unidentified aspects of gender and race may influence susceptibility to palatal anomalies arising from a range of development insults, including chromosomal imbalances such as the 22q11.2 deletion.

To our knowledge, this is the first study aimed at the identification of genetic variants that may modify the palatal phenotype in individuals with the 22q11.2 deletion syndrome. Our analyses of eight genetic variants that are potentially associated with facial clefts revealed one statistically significant association in Caucasians with the 22q11.2 deletion. Specifically, the A2756G variant of MTR was significantly (i.e., $p < .05$) related to velopharyngeal inadequacy. This gene (MTR) encodes methionine synthase, which catalyzes the remethylation of homocysteine to methionine. Several studies have assessed the relationship between neural tube defects and variants of MTR, with conflicting results (e.g., Brody et al., 1999; De Marco et al., 2002; Johanning et al., 2000; Shaw et al., 1999; Doolin et al., 2002; Wilson et al., 1999; Zhu et al., 2003).

The results of the analyses summarized in this paper should be interpreted with caution, given the number of comparisons that were performed and the relatively small numbers on which many of the comparisons are based. It is possible that the associations that were detected in our subjects may represent false-positive findings. Moreover, given the relatively small sample sizes available for these analyses, it is possible that true associations have been missed. Further, since these analyses considered only one or two variants per gene, associations between other variants in these genes and the palatal phenotype in individuals with 22q11.2 deletion syndrome cannot be excluded. In addition, there are other potential sources of phenotypic variation (e.g., deletion size, parent of origin) that were not evaluated.

In summary, our analyses of these data indicate that the variation in the palatal phenotype observed between individuals with the 22q11.2 deletion syndrome may be related to both gender and race. Our analyses also provide tentative evidence that phenotypic differences between individuals with this deletion may be related to variation in genes that lie outside of the deleted region.

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TABLE 1

Summary of Associations Between Noncleft-VPI and Palate and Subject Sex and Race

		Sex				Race			
Group	**All Subjects**	**Males (n = 148)**	**Females (n = 150)**	**χ^2_1 (p Value)**	**Odds Ratio (95% CI)**	**African-American (n = 24)**	**Caucasian (n = 246)**	**Fisher Exact Test (p Value)**	**Odds Ratio (95% CI)**
Normal	91	54	37	—	—	16	66	—	—
Noncleft-VPI	67	24	43	8.54 (.004)	2.62 (1.36, 5.02)	1	64	(.0005)	15.52 (2.00, 120.44)
Palate	140	70	70	1.92 (.16)	1.46 (0.86, 2.49)	7	116	(.003)	4.01 (1.57, 10.26)
Total*	207	94	113	4.91 (.03)	1.75 (1.06, 2.89)	8	180	(.0002)	5.45 (2.23, 13.34)

*Total = Noncleft-VPI plus Palate.

TABLE 2

Summary of Associations Between Noncleft-VPI and Palate and Subject Genotype for Bi-allelic Genetic Variants

		Genotypes			Fisher Exact p Value	Odds Ratio (95% confidence intervals)	
Gene	**Group**	**11**	**12**	**22**		**OR_{12}**	**OR_{22}**
MTHFR C677T (1=C, 2=T)	Normal	16	23	4			
	Noncleft-VPI	18	16	2	.51	0.70 (0.30, 1.60)	1.63 (0.44, 5.98)
	Palate	27	27	11	.38	0.62 (0.24, 1.56)	0.44 (0.07, 2.76)
	Total*	45	43	13	.51	0.66 (0.31, 1.42)	1.16 (0.33, 4.06)
MTHFR A1298C (1=A, 2=C)	Normal	18	16	4			
	Noncleft-VPI	8	18	2	.21	2.53 (0.87, 7.34)	1.12 (0.17, 7.45)
	Palate	26	33	3	.41	1.43 (0.61, 3.33)	0.52 (0.10, 2.60)
	Total	34	51	5	.26	1.69 (0.76, 3.76)	0.66 (0.16, 2.77)
CBS 69bp ins (1=wt, 2=ins)	Normal	39	4	1			
	Noncleft-VPI	25	7	0	.18	2.73 (0.72, 10.29)	—
	Palate	48	7	1	.88	1.42 (0.39, 5.21)	0.81 (0.05, 13.41)
	Total	73	14	1	.48	1.87 (0.58, 6.06)	0.53 (0.03, 8.78)
MTR A2756G (1=A, 2=G)	Normal	25	12	0			
	Noncleft-VPI	26	2	0	.02	0.16 (0.03, 0.79)	—
	Palate	40	17	2	.69	0.88 (0.36, 2.16)	—
	Total	66	19	2	.32	0.60 (0.25, 1.41)	—
MTRR A66G (1=A, 2=G)	Normal	11	14	11			
	Noncleft-VPI	5	10	9	.75	1.57 (0.41, 5.96)	1.80 (0.45, 7.12)
	Palate	17	23	17	1.00	1.06 (0.39, 2.91)	1.00 (0.34, 2.92)
	Total	22	33	26	.94	1.18 (0.45, 3.07)	1.18 (0.43, 2.25)
TGFA TaqI (1=117bp, 2=113bp)	Normal	13	2	0			
	Noncleft-VPI	17	6	0	.44	2.29 (0.40, 13.28)	—
	Palate	30	5	1	1.00	1.08 (0.18, 6.32)	—
	Total	47	11	1	1.00	1.52 (0.30, 7.74)	—

*Total = Noncleft-VPI plus Palate.

TABLE 3

Summary of Associations Between Noncleft-VPI and Palate and Subject Genotype for Multi-allelic Genetic Variants

MSX1				
Genotype	**Normal**	**Noncleft-VPI**	**Palate**	**Total***
168/168	11	9	26	35
168/170	5	2	1	3
168/172	10	10	13	23
168/174	2	2	3	5
170/170	4	4	3	7
170/172	5	2	5	7
170/174	3	1	5	6
170/176	1	1	0	1
172/172	1	2	3	5
172/174	1	1	3	4
174/174	1	1	1	2
Fisher exact test		.98	.43	.71
p value				

TGFβ3				
Genotype	**Normal**	**Noncleft-VPI**	**Palate**	**Total***
166/166	3	6	7	13
166/168	6	4	10	14
168/170	3	2	3	5
168/172	0	0	0	0
168/168	11	12	15	27
168/170	14	4	17	21
168/172	1	4	1	5
170/170	1	1	6	7
170/172	0	2	2	4
172/172	0	0	0	0
Fisher exact test		.12	.79	.61
p value				

* Total = Noncleft-VPI plus Palate.