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Ethnic heterogeneity of IRF6 AP-2 α binding site promoter SNP association with nonsyndromic cleft lip and palate

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Abstract

Objective—The goal of this study was to confirm the reported association between a noncoding SNP (rs642961) in IRF6 and nonsyndromic cleft lip and palate (NSCLP).

Design, Setting and Participants—Two SNPs in IRF6 (rs2235371 and rs64296) were genotyped in Hispanic and nonHispanic white multiplex (122) and simplex (308) NSCLP families. Linkage and family-based association analyses were performed on the individual SNPs as well as the 2-SNP haplotype.

Results—We find only modest evidence for an association with rs642961 and the 2-SNP haplotype. In contrast, we found strong evidence for association with rs2235371; this was most evident in the nonHispanic white simplex families.

Conclusions—While we confirm that variation in IRF6 is associated with NSCLP, our results do not support the reported association with SNP rs64296. Importantly, the association varies between ethnic groups. This finding underscores the need for evaluating additional variations in IRF6 across multiple populations to better determine its role in NSCLP.

Keywords

NSCLP; IRF6; orofacial clefting; association study; genetics

Introduction

Nonsyndromic cleft lip and palate (NSCLP) is a common birth defect affecting 4000 newborns in the US and more than 133,000 infants worldwide (Gorlin et al., 2001; Hashmi

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et al., 2005). Surgical, dental and speech problems require a series of interventions throughout the growing years with healthcare costs estimated to be over \$100,000 per individual for complete craniofacial team care (CDC, 1995; CDC, 2007). The healthcare burden of NSCLP is substantial for families.

NSCLP is a complex disorder with a multifactorial etiology that involves both genetic and environmental factors; none of which have been fully elucidated (Murray, 2002; Lidral and Moreno, 2005). The number of genes contributing to the NSCLP phenotype is estimated to range from 2 to 14; association and linkage studies have identified some of these putative genes (Schliekelman and Slatkin, 2002; Lidral and Moreno, 2005; Carinci et al., 2007). Mutations in interferon regulatory factor 6 (IRF6) cause van der Woude and Popliteal Pterygium syndromes (VWS OMIM 119300; PPS OMIM 119500) (Kondo et al., 2002). Both are autosomal dominant conditions and are associated with either cleft lip/palate or cleft palate and/or lip pits (Kondo et al., 2002). Several studies have suggested that variation in IRF6 plays an etiologic role in NSCLP, although the results are often conflicting (Zuccherro et al., 2004; Blanton et al., 2005; Ghassibe et al., 2005; Scapoli et al., 2005; Srichomthong et al., 2005; Park et al., 2007; Jugessur et al., 2008; Diercks et al., 2009). A recent study by Rahimov et al. (2008) reported that overtransmission of a specific haplotype involving SNPs rs2235371, a silent mutation in IRF6, and rs642961, a putative functional mutation in an IRF6 AP-2 α DNA binding site in the promoter region, confers an 18% attributable risk for NSCLP (Rahimov et al., 2008). To further evaluate the role of the rs2235371 and rs642961 SNPs in NSCLP, we interrogated these two SNPs in our nonHispanic White (NHW) and Hispanic NSCLP multiplex and simplex families, a population in which we previously found association between SNPs in IRF6 and NSCLP (Blanton et al., 2005). While the results further support an association between variation in IRF6 and NSCLP, we did not find strong evidence for association with the haplotype and only nominal evidence for association with the functional variant.

Materials and Methods

Dataset

This study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston (HSC-MS-03-090). The dataset consisted of 122 families with two or more individuals with NSCLP. Of these, there were 80 extended multiplex families (65 nonHispanic white (NHW), 15 Hispanic and 42 multiplex trios/duos (23 NHW and 19 Hispanic) with a positive history of NSCLP. In addition, there were 308 simplex trios/duos (226 NHW, 82 Hispanic). These families, methods of ascertainment and inclusion criteria have been previously reported (Chiquet et al., 2007). Briefly, probands were identified at one of several collaborating centers. After informed consent, probands were examined to confirm the diagnosis of nonsyndromic cleft lip and palate and a family history was obtained. Additional family members were ascertained based on family history information, after informed consent. Blood or saliva samples were collected DNA was extracted from blood using Roche DNA Isolation Kit for Mammalian Blood (Roche, Basel, Switzerland) and from saliva using Oragene Purifier (DNA Genotek Inc., Ontario, Canada) following manufacturer's protocol.

IRF6 SNPs

Two SNPs, rs2235371 and rs642961, were genotyped using TaqMan Assays (ABI; Foster City, California) and detected using ABI 7900HT Sequence Detection System. All genotyping results were imported into Progeny Lab (South Bend, Indiana, USA) and PedCheck was run to identify Mendelian inconsistencies (O'Connell and Weeks, 1995).

Analysis

Allele frequencies and Hardy-Weinberg equilibrium were calculated using SAS (v9.1). Pairwise linkage disequilibrium values (D' and r^2) were calculated using GOLD (Abecasis and Cookson, 2000). To extract the maximum amount of information from the dataset, multiple approaches for assessing linkage and/or association were used. Results of genotyping were subjected to parametric and non-parametric linkage analyses using MERLIN (Abecasis et al., 2002). Evidence of altered transmission was assessed using the Pedigree Disequilibrium Test (PDT), Geno-PDT (G-PDT) and Association in the Presence of Linkage (APL) test (Martin et al., 2000; Martin et al., 2003; Chung et al., 2006). Relative risks were calculated using SAS (v9.1). APL was used to examine the data for altered transmission of haplotypes. Each ethnic group was analyzed as a whole and then stratified by the presence/absence of family history.

Results

Two SNPs in IRF6, rs2235371 and rs642961, were genotyped in both ethnic groups and found to be in Hardy-Weinberg equilibrium. Allele frequencies for rs2235371 (minor allele=A) were significantly different between NHW (MAF=0.07) and Hispanics (MAF=0.22) ($p=8.52 \times 10^{-16}$); however, there was no difference in allele frequencies for rs642961 (minor allele=A; NHW=0.25; Hispanic=0.22). For this reason, the data was stratified by ethnicity. The SNPs were in linkage equilibrium with $r^2 < 0.05$.

There was no evidence for linkage in either ethnic group (data not shown). Association analysis detected altered transmission for rs2235371 in the NHW group and this was the same trend in the Hispanic group (Table 1). When the data was stratified by family history, the altered transmission was observed only in the NHW and Hispanic families with no history of NSCLP (trios) (Table 1). There was no evidence for altered transmission of rs642961 in the NHW group; however, there was some evidence for altered transmission in Hispanics in the total group and in the subset with a positive family history.

Relative risks for the genotypes with one or two copies of the risk alleles are presented in Table 2. The 95% confidence intervals include 1.0 for all genotypes and ethnicities, except for the NHW rs2235371 GA genotype, where the relative risk is 1.93 (95%CI=1.27-2.91).

When the 2 SNPs were considered as a haplotype, both the G-A and G-G haplotypes were overtransmitted ($p=0.001$) (Table 3) in the NHW group. In the Hispanic group, only the G-A haplotype was overtransmitted and similarly only the A-G was undertransmitted ($p=.03$). Sample size did not allow for stratification by family history.

Discussion

Zuccherro et al. previously found evidence for overtransmission of several SNPs in IRF6 in NSCLP, several of which were confirmed by ourselves and others (Zuccherro et al., 2004; Blanton et al., 2005; Scapoli et al., 2005; Diercks et al., 2009; Jia et al., 2009). Based on that association, other SNPs in IRF6 and the promoter region were evaluated and the A allele in rs642961 was found to be significantly overtransmitted in multiple populations (Rahimov et al., 2008). However, in our dataset, the strongest evidence was for an association with the rs2235371 SNP. This was restricted to our negative family history sub-group as there were very few heterozygotes for this SNP in the multiplex families in either ethnicity. Importantly, the relative risks for the AG and AA genotypes for rs642961 for either ethnicity (NHW: 1.02 (0.89-1.17) and 0.95 (0.71-1.27); Hisp 0.91 (0.76-1.11) and 1.02 (0.68-1.54) respectively) were less than those reported by Rahimov et al. (2008) (1.47 (1.28-1.68) and 1.87 (1.47-2.38) respectively).

When Rahimov et al. (2008) considered the two SNPs as a haplotype, they found that the G-A haplotype (rs2235371-rs642961) was overtransmitted, whereas the G-G haplotype was undertransmitted (Rahimov et al., 2008). In contrast, we found that both the G-G and the G-A haplotypes were overtransmitted in our NHW group but only the G-A haplotype was overtransmitted in the Hispanic group. This suggests that it is only rs2235371 driving the association in the NHW group.

Rahimov and coworkers found that the association was strongest when they stratified the data by phenotype and considered complete clefts (CLP) versus cleft lip (CL) only (Rahimov et al., 2008). Within that framework, they considered the cleft lip only phenotype to include not only clefts restricted to the lip but also those with a cleft of the lip and a notch of the alveolus (Marazita, personal communication). Families were designated as “cleft lip only” if all affected individuals met this criteria. The comparison group was individuals and families with a complete cleft of the lip and palate. In our sample, any cleft involving the lip and palate was phenotypically classified as CLP and thus includes all individuals that have a notched alveolus. The clefts of “cleft lip only” individuals, therefore, involve only the lip. We do not have any multiplex families meeting our definition of cleft lip only and only 33 simplex trios (25 NHW, 8 Hispanic) who are cleft lip only. In this small subset, we were unable to detect altered transmission (data not shown). While this difference in phenotyping may explain some of the differences in the results, Rahimov and colleagues were clearly able to demonstrate an association in the nonstratified dataset, with overall p-value for altered transmission of rs642961 of $p=1E-11$. Nonetheless, in our dataset, rs642961 alone does not convey a significant risk. More importantly, we found only modest evidence for confirmation of the haplotype results in our Hispanic group. Nevertheless, our results do confirm that variation in IRF6 plays a role in NSCLP. Therefore, care should be taken when using this information to determine the extent of the role that variation in this gene plays in NSCLP and when developing risk models. Further studies in additional ethnic populations are clearly warranted.

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Table 1

IRF6 Single SNP Association Analysis Results

SNP	Sample Set	Subset	PDT	Geno-PDT	APL
rs2235371	NHW	All families	0.001	0.0004	0.00007
		Positive FH	0.23	0.28	0.12
		Negative FH	0.0006	0.0006	0.0002
	Hispanic	All Families	0.04	0.04	0.04
		Positive FH	0.56	0.81	0.77
		Negative FH	0.05	0.05	0.03
rs642961	NHW	All families	0.78	0.91	0.48
		Positive FH	0.31	0.59	0.49
		Negative FH	0.58	0.72	0.19
	Hispanic	All families	0.05	0.11	0.01
		Positive FH	0.03	0.11	--*
		Negative FH	0.41	0.59	0.08

* variance too small to calculate p-value, PDT=Pedigree Disequilibrium Test, Geno-PDT=Genotypic Pedigree Disequilibrium Test, APL= Association in the Presence of Linkage, FH= family history, NHW = nonHispanic white

Table 2
Genotype Relative Risk (95% CI) for Risk Alleles

	rs2235371		rs642961	
Sample	GA	GG	GA	AA
NHW	1.93 (1.27-2.91)	2.65 (0.29-23.73)	1.02 (0.89-1.17)	0.95 (0.71-1.27)
Hisp	1.45 (0.89-2.34)	1.59 (0.75-3.35)	0.91 (0.76-1.11)	1.02 (0.68-1.54)

Table 3
Haplotype Association Results

Sample Set	Overtransmitted Haplotype(s)	APL p-value
NHW ⁺	G-G, G-A	0.001
Hispanic	G-A	0.03

⁺NHW = nonHispanic white