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Family-based study shows heterogeneity of a susceptibility locus on chromosome 8q24 for nonsyndromic cleft lip and palate

Susan H. Blanton[1], **Amber Burt**[1], **Samuel Stal**[2], **John B. Mulliken**[3], **Elizabeth Garcia**[4], and **Jacqueline T. Hecht**[4]

[1] University of Miami Miller School of Medicine, Miami, FL, USA

[2] Texas Children's Hospital, Houston, TX, USA

[3] Children's Hospital, Boston, MA, USA

[4] University of Texas Medical School at Houston, Houston, TX, USA

Abstract

BACKGROUND—Nonsyndromic cleft lip with or without cleft palate is a common birth defect. While a number of susceptibility loci have been reported, replication has often been lacking. This is likely due, in part, to heterogeneity of datasets and methodologies employed. Two independent genome-wide association studies of individuals of largely western European extraction have identified a possible susceptibility locus on 8q24.21.

METHODS—In order to determine the overall impact of this locus, we genotyped six of the previously associated SNPs in our Hispanic and nonHispanic white family-based datasets and evaluated them for linkage and association. In addition, we genotyped a large African-American NSCLP family that we had previously mapped to the 8q21.3-24.12 region to test for linkage.

RESULTS—There was no evidence for linkage to this region in any of the three ethnic groups. Nevertheless, strong evidence for association was noted in the nonHispanic white group, whereas none was detected in the Hispanic dataset.

CONCLUSION—These results confirm the previously reported association and provide evidence suggesting that there is ethnically-based heterogeneity for this locus.

Keywords

NSCLP; nonsyndromic cleft lip and palate; linkage; association; 8q24

Introduction

Nonsyndromic cleft lip with or without cleft palate (NSCLP) is a common birth defect affecting 4000 newborns in the US and more than 133,000 infants world wide (www.marchofdimes.com) (Gorlin, 2001; Hashmi et al., 2005). NSCLP is a complex disorder with both genetic and environmental underpinnings. While some progress has been made in elucidating the underlying genetic susceptibility, results have been variable, often as a function of study design and ethnicity. Two recent genome wide association studies

*To whom correspondence should be sent: Jacqueline T. Hecht, PhD, University of Texas –Houston Medical School, Department of Pediatrics, PO Box 20708, Houston, TX 77225, USA, jacqueline.t.hecht@uth.tmc.edu.

(GWAS) identified associations between SNPs on chromosome 8q24.21 and NSCLP (Birnbaum et al., 2009; Grant et al., 2009). Birnbaum and colleagues conducted a case-control GWAS of NSCLP cases ascertained in Germany. Associations with $p<0.001$ were identified to 34 SNPs in the region of 8q24.21. Odds ratios for the top 10 SNPs ranged from 1.91 to 2.57 and 3.55 to 16.85 for heterozygotes and homozygotes, respectively. In a second GWAS of cases of European descent ascertained in the US, associations were found with two SNPs (both have been shown in the Birnbaum study). An odds ratio of 2.1 (95%CI 0.59 – 2.36) was reported for SNP rs987525 (Grant et al., 2009). The first study also evaluated the significant SNPs in a family-based dataset, and confirmed the case-control results (Birnbaum et al., 2009). Nevertheless, there was significant overlap between the case-control data and the family data, so that it did not represent an independent confirmation. Although both studies employed methods to correct for possible population stratification, confirmation in a family-based dataset is warranted. Moreover, investigation of this region in other ethnic groups is important to determine the overall impact of this region on NSCLP susceptibility.

Therefore, we studied six of the SNPs from this region reported by Birnbaum in our well-characterized family-based datasets of nonHispanic whites and Hispanics. In addition, as we had previously reported suggestive evidence (LOD = 2.98) for linkage to the region 8q21.3-24.12 in a large NSCLP African-American family, we genotyped this family for the same six SNPs (Chiquet et al., 2008). Lastly, we examined our data for an interaction between two previously genotyped SNPs in IRF6 (rs2235371 and rs642961) and the six 8q SNPs. Both of the IRF6 SNPs have shown significant association with NSCLP (Rahimov et al., 2008; Zucchero et al., 2003).

Methods

The study sample was composed of 120 multiplex NSCLP families (83 non-Hispanic white and 37 Hispanic) and 325 simplex parent-child trios (234 non-Hispanic white and 91 Hispanic), which have been described previously (Chiquet et al., 2007). Families were ascertained through a proband with NSCLP from three craniofacial centers: Children's Hospital Boston, Texas Children's Hospital in Houston, and the University of Texas Craniofacial Clinic at Houston. This study was approved by the Institutional Review Boards of all participating centers. Cases were examined and all families with syndromic clefting were excluded from analysis. Ethnicity was self-reported and all Hispanic cases were of Mexican ancestry. One large African-American family composed of seven affected individuals in three generations was also included (Chiquet et al., 2008). Blood or saliva samples were collected after obtaining informed consent and DNA was extracted using either the Roche DNA Isolation Kit for Mammalian Blood (Roche, Switzerland) or the Oragene Purifier kit for saliva (DNA Genotek Inc., Ontario, Canada) following the manufacturer's protocol.

Six SNPs were selected from the top 10 reported by Birnbaum et al. (2009) for testing based on association results, allele frequencies and location (coverage of the region). The SNPs were genotyped using the SNPlex Genotyping System [Applied Biosystems (ABI), Foster City, CA] and detected with the 3730 DNA Analyzer (ABI). Allele calls were determined using the GeneMapper analysis software (ABI). The data was imported into Progeny Lab (South Bend, IN, USA) and checked for Mendelian inconsistencies using PedCheck (O'Connell and Weeks, 1998).

Results

The data was stratified initially by ethnicity and then by family history. Hardy-Weinberg equilibrium (HWE) and allele frequency differences were evaluated using SAS (v9.1). Pair-wise linkage disequilibrium values (D′ and r^2) were calculated using GOLD (Abecasis and Cookson, 2000). Parametric and non-parametric linkage analyses were conducted using MERLIN (Abecasis et al., 2002). Parametric linkage parameters have been previously described (Blanton et al., 2004a; Blanton et al., 2004b). Evidence for association for individual SNPs was tested using Pedigree Disequilibrium Test (PDT), Geno-PDT and Association in the Presence of Linkage (APL) test (Chung et al., 2006; Martin et al., 2000; Martin et al., 2006). Pairwise haplotypes were evaluated for overtransmission with APL. Gene-gene interactions between SNPs in 8q24.21 and IRF6 were tested using Generalized Estimating Equations (GEE), as implemented in SAS (v9.1) (Hancock et al., 2007).

All SNPs were in Hardy Weinberg Equilibrium (HWE). Allele frequencies differed significantly ($p < 0.00009$) between nonHispanic whites and Hispanics for all SNPs except rs17821251 ($p<0.02$). In contrast, LD patterns were similar between the two ethnicities. In general, there was significant LD as measured by r^2 and/or D′ ($0.26 < r^2 < 0.69$; $0.75 < D' < 0.94$; nonHispanic white) among all SNPs except for rs17821251. There was no evidence for linkage in either the Hispanic or nonHispanic white extended families or in the African-American family. Moreover, there was no evidence for association between any of the SNPs and NSCLP in the Hispanic dataset, regardless of family history. In contrast, altered transmission was found for five of the six SNPs in the nonHispanic white dataset (Table 1). The strongest evidence for association was for SNPs rs17241253 and rs987525 in the complete dataset, as well as after stratification by the presence or absence of family history. For all but two of the SNPs (rs3857888 and rs17821251), the minor allele was over-transmitted. The relative risk for each of the over-transmitted SNPs ranged from 1.01 (95% CI 0.83–1.23, rs1530300) to 1.18 (95% CI 0.73 –1.9, rs17241253) for heterozygotes and 1.18 (95% CI 0.98–1.4 rs1530300) to 1.55 (95% CI 1.01–2.36, rs1721253) for homozygotes, suggesting a modest effect.

Analysis of two-SNP haplotypes revealed overtransmission of a haplotype for every two-SNP pairing; the same alleles were over-transmitted as in the single SNP analyses.

There was significant interaction between the IRF6 rs642961 SNP and three of the 8q24.21 SNPs (rs1530300, p=0.004; rs17241253, p=0.0072; and rs987525; p=0.0038) in the nonHispanic white dataset. Even after Bonferoni correction, two of these associations remained significant ($p<0.004$).

Discussion

We and others have suggested that the 8q24 region contains a locus for NSCLP susceptibility (Chiquet et al., 2008; Marazita et al., 2004; Prescott et al., 2000). In this study, we demonstrate evidence supporting the association between SNPs in 8q24.21 and NSCLP in nonHispanic whites. Rs987252 and rs1741253, the two most significant SNPs reported by Birnbaum et al. (2009) were also the most significantly associated SNPs in our dataset individually. Additionally, a haplotype involving the minor alleles of those two SNPs was the most significantly overtransmitted haplotype in our dataset. We calculated lower relative risks for the over-transmitted alleles than did Birnbaum et al (2009); our risks were more similar to those reported by Grant et al. (2009). That five of the six SNPs, as well as all haplotypes were significant is not unexpected given the high degree of LD among most of the markers. Our evidence for an interaction between SNPs in 8q24.21 and IRF6 is in

contrast to that observed by Birnbaum et al. (2009) who were unable to detect an interaction with IRF6 when examining the single SNP, rs642961.

Interestingly, there was no association detected in the Hispanic dataset to any of the six SNPs. Moreover, the allele frequencies of all six SNPs were significantly different between the two ethnicities. The Hispanics in the study are of Mexican origin, with combined European, African-American, and Native American ancestry. One possible interpretation of this difference in the allele frequencies between the two datasets is that this region of the genome in our Hispanics arises largely from one of the non-European ancestries. This then might explain why there is no observed association.

The maximum multipoint LOD score in the African-American family was to a SNP located approximately 1Mb from this group of SNPs (Chiquet et al., 2008). The lack of linkage to these SNPs suggests that another susceptibility locus may lay centromeric to this region, and may not play a significant role in NSCLP in individuals of western European descent.

Many of the studies involving NSCLP have yielded conflicting results. Confirmation of findings from a GWAS in a family-based dataset is an important step in identifying susceptibility loci for this complex disorder. Here, we are able to confirm the results of two different case-control studies in an independent family-based dataset. Moreover, our results strongly suggest that there is genetic heterogeneity with no association identified in our Hispanic dataset. In addition, linkage results in our large African-American family suggest that this region does not play a role in the NSCLP susceptibility in this family. The 8q24.21 region does not contain any known genes. Nevertheless, the strength of the associations and the replication in multiple datasets indicates that this region is extremely important in NSCLP susceptibility. Future studies will need to focus on identifying the mechanism of this susceptibility.

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Table 1

Results of association testing in nonHispanic white dataset

Dataset	SNP	Basepair	PDT	GENOPDT	APL	var_APL
All Families	rs3857888	129894043	**0.0042**	0.02	**0.0004**	82.6
	rs17241253	129959370	**0.000003**	**0.000002**	**0.000004**	54.4
	rs1530300	129988640	**0.004**	**0.008**	**0.0003**	70.5
	rs987525	130015336	**0.000003**	**0.000003**	**0.00003**	63.4
	rs1372449	130019572	**0.007**	0.024	**0.0004**	85.9
	rs17821251	130078415	0.56	0.62	0.81	35.3
Positive Family History	rs3857888	129894043	0.04	0.12	0.035	33.1
	rs17241253	129959370	**0.001**	**0.002**	**0.004**	16.3
	rs1530300	129988640	0.15	0.32	0.19	23.1
	rs987525	130015336	**0.001**	**0.0012**	0.024	20.9
	rs1372449	130019572	0.19	0.28	0.079	36.5
	rs17821251	130078415	0.73	0.69	0.81	8.4
Negative Family History	rs3857888	129894043	0.05	0.15	**0.0004**	51.0
	rs17241253	129959370	**0.0009**	**0.001**	**0.000004**	41.7
	rs1530300	129988640	**0.007**	0.012	**0.0003**	45.2
	rs987525	130015336	**0.0009**	**0.002**	**0.00003**	45.0
	rs1372449	130019572	**0.006**	**0.001**	**0.0004**	47.3
	rs17821251	130078415	0.72	0.19	0.93	25.82

Bold indicates significant