RESEARCH LETTER

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Polymorphisms at Regions 1p22.1 (rs560426) and 8q24 (rs1530300) Are Risk Markers for Nonsyndromic Cleft Lip and/or Palate in the Brazilian Population

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TO THE EDITOR:

Recent genetic risk factors for nonsyndromic cleft lip and/or palate (NSCL/P) have been identified in genome-wide association studies (GWAS), however, few of them have been replicated in different populations. Exceptions include the genetic variants in *IRF6*, particularly the rs642961 polymorphism, and the rs987525 polymorphism at locus 8q24, which demonstrated a strong association with NSCL/P across samples of multiple populations [Birnbaum et al., 2009; Grant et al., 2009; Huang et al., 2009; Beaty et al., 2010; Blanton et al., 2010a, b; Mostowska et al., 2010]. Our previous studies confirmed the association of the 8q24 rs987525 polymorphism with NSCL/P susceptibility in the Brazilian population [Brito et al., 2012a], but the involvement of *IRF6* polymorphisms in the pathogenesis of NSCL/P is still unclear in Brazilians [Paranaiba et al., 2010; Brito et al., 2012b]. As the Brazilian population is ethnically heterogeneous, mainly comprised of European colonizers, African slaves, and the autochthonous Amerindians [Parra et al., 2003], it is possible that risk markers identified in genetically homogenous populations, such as those utilized in large-scale genetic approaches, are not substantiated in the Brazilian population.

The aim of this study was to validate the association of eight polymorphisms, which were identified as risk markers to NSCL/P in previous GWAS, with NSCL/P in a Brazilian population. To avoid population stratification bias, the polymorphic markers at regions 1p22.1, 1q32, 8q24, 9q21, 10q25.3, 18q22, and 20q12 were analyzed in a case–control study taking into account the genetic ancestry variation of each individual (structured analysis). In addition, we examined the haplotype interactions of polymorphisms at 8q24, including rs1530300 (being tested here for the first time) and rs987525 (previously genotyped) [Brito et al., 2012a].

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A total of 299 patients with nonsyndromic cleft lip with or without palate (CL ± P) and 384 healthy controls were included in this study (more details in the online supplementary file). Eight polymorphic sites at regions 1p22.1 (rs560426), 1q32 (rs2013162), 8q24 (rs1530300), 9q21 (rs1443434 and rs3758249), 10q25.3 (rs7078160), 18q22 (rs17085106), and 20q12 (rs13041247) were

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TABLE I. Association of the Polymorphic Sites of This Study With the Risk of NSCL/P

	HWE (*P* value)	Control group	CL ± P group	OR_{allele} (95% CI) *P* value	CL group	OR_{allele} (95% CI) *P* value	CLP group	OR_{allele} (95% CI) *P* value
rs560426 (1p22.1)								
Allele (A/G)	0.07	426/342	288/310	1.34 [1.08–1.66] 0.02	103/107	1.29 [0.95–1.76] 0.39	185/203	1.37 [1.07–1.75] 0.029
Genotype (AA/AG/GG)		127/172/85	74/140/85		29/45/31		45/95/54	
rs2013162 (1q32)								
Allele (C/A)	0.59	421/195	332/134	0.87 [0.67–1.13] 0.16	112/34	0.65 [0.43–0.99] 0.19	220/100	0.98 [0.73–1.31] 0.20
Genotype (CC/CA/AA)		142/137/29	122/88/23		45/22/6		77/66/17	
rs1530300 (8q24)								
Allele (T/C)	0.92	558/210	370/228	1.64 [1.31–2.06] <0.0001	123/87	1.88 [1.37–2.58] <0.0001	247/141	1.52 [1.17–1.97] 0.012
Genotype (TT/TC/CC)		203/152/29	131/108/60		40/43/22		91/65/38	
rs1443434 (9q21)								
Allele (T/G)	0.31	397/219	306/162	0.96 [0.74–1.23] 0.67	95/51	0.97 [0.66–1.42] 0.94	211/111	0.95 [0.72–1.26] 0.62
Genotype (TT/TG/GG)		132/133/43	106/94/34		32/31/10		74/63/24	
rs3758249 (9q21)								
Allele (G/A)	0.12	392/224	315/153	0.85 [0.66–1.09] 0.21	98/48	0.86 [0.58–1.25] 0.57	217/105	0.85 [0.54–1.12] 0.46
Genotype (GG/GA/AA)		131/130/47	106/103/25		32/34/7		74/69/18	
rs7078160 (10q25.3)								
Allele (G/A)	0.17	498/118	369/97	1.11 [0.82–1.50] 0.89	102/26	0.91 [0.57–1.46] 0.49	249/71	1.20 [0.86–1.67] 0.74
Genotype (GG/GA/AA)		205/88/15	142/85/6		47/26/0		95/59/6	
rs17085106 (18q22)								
Allele (G/T)	0.07	588/28	447/17	0.80 [0.43–1.48] 0.54	137/9	1.38 [0.63–2.99] 0.39	310/8	1.11 [0.49–2.50] 0.069
Genotype (GG/GT/TT)		282/24/2	216/15/1		65/7/1		151/8/0	
rs13041247 (20q12)								
Allele (T/C)	0.47	398/212	327/141	0.81 [0.62–1.05] 0.21	108/38	0.66 [0.44–0.99] 0.16	219/103	0.88 [0.66–1.17] 0.56
Genotype (TT/TC/CC)		127/144/34	114/99/21		39/30/4		75/69/17	

HWE, Hardy-Weinberg equilibrium; CL ± P, cleft lip with or without palate; CL, cleft lip; CLP, cleft lip and palate.

selected based on recent genome-wide scans [Grant et al., 2009; Marazita et al., 2009; Beaty et al., 2010; Mangold et al., 2010] and examined using the TaqMan 5′-exonuclease allelic discrimination assay (Applied Biosystems, Foster City, CA), with the researchers blinded to group status. Genotyping analyses were randomly repeated in 10% of the samples for all polymorphisms. To assessment to genomic ancestry, each subject was independently genotyped for 40 biallelic short insertion-deletion polymorphisms (INDELs) after the methods of Bastos-Rodrigues et al. [2006]. Deviation from Hardy-Weinberg equilibrium in the control group was assessed through the Chi-squared test. To determine the genomic ancestry of each individual, Structure software was utilized in a model assuming $K = 3$ parental populations based on the tri-hybrid origin of the Brazilian population. Following ancestry assessment, STRAT was used to test the association, conditioning on the individual ancestry proportions. The odds ratio (OR) and associated 95% confidence intervals (95% CI) were also calculated. Haplotype frequencies and linkage disequilibrium between rs1530300 and rs987525 [Brito et al., 2012a] polymorphisms located at 8q24 were estimated using the HaploView software.

The genotype frequencies observed for all studied polymorphisms in controls did not identify statistically significant differences compared to those expected under Hardy-Weinberg equilibrium (Table I). The percentage of the G allele of rs560426 (1p22.1) polymorphism was found to be significantly higher in the CL ± P group as compared to the control group ($P = 0.02$). Patients carrying the rs560426 G allele exhibited a moderately increased risk to CL ± P as revealed by an OR of 1.34 (95% CI 1.08–1.66). Compared to the control group, the rs1530300C allele at 8q24 was significantly more frequent in the CL ± P group with an OR of 1.64 (95% CI 1.31–2.06; $P < 0.0001$). Whereas rs560426 was significantly correlated only with the CLP group, the rs1530300 polymorphism was associated with both the CL and CLP groups. Recently, we demonstrated a strong association of the rs987525 polymorphism, which is also located at 8q24 and at a distance of ~26 kb from rs1530300, with nonsyndromic CL ± P in this same Brazilian sample [Brito et al., 2012a]. Since both rs987525 and rs1530300 showed a positive correlation with CL ± P, we performed haplotype analysis (Supplementary eTable I—see Supporting Information online). There was significant linkage disequilibrium among the risk alleles as measured by $r^2 = 0.65$ and $D' = 0.84$. Carriers of the risk alleles (the C-A haplotype) were found to be more prevalent among CL ± P patients as compared to controls and showed a significant risk for having CL ± P (OR 1.38; 95% CI 1.01–1.87; $P = 0.04$).

Carriers of the rs560426 G allele exhibited a 1.34-fold increased risk for CL ± P compared with carriers of the A allele. This finding is similar to the original results (OR 1.432, 95% CI 1.292–1.587) of the GWAS of Beaty et al. [2010] and the recent meta-analyses (OR 1.344 for Europeans) of Ludwig et al. [2012]. Although the strongest association of the study by Beaty and collaborators was with Asian families, a recent study with a Chinese Han population did not confirm this association [Pan et al., 2011]. Moreover, rs560426 was not found to be significantly associated with oral cleft in populations from Poland [Mostowska et al., 2012] and Kenya–Africa [Weatherley-White et al., 2011], but power to detect any effect was very limited in the latter study. The association of the 8q24 locus with NSCL/P susceptibility, mostly through the rs987525 marker, has been confirmed in different populations [Birnbaum et al., 2009b; Grant et al., 2009; Huang et al., 2009; Beaty et al., 2010; Blanton et al., 2010b; Mostowska et al., 2010; Brito et al., 2012a]. Interestingly, the original GWAS of Grant et al. [2009] supported the association rs1530300 with NSCL/P, which was in linkage disequilibrium with rs987525 and showed an even stronger association with NSCL/P susceptibility in Irish trios. The findings of the present study confirmed the significant association of rs1530300 as a single-marker and as a haplotype with rs987525. As a single marker, the association of rs1530300 with NSCL/P was stronger compared with rs987525. Blanton et al. [2010b] have also demonstrated that the 8q24 region is a locus for NSCL/P susceptibility, as shown by the positive association of the risk alleles contained in rs1530300 and rs987525. No significant associations among six other polymorphic susceptibility markers were obtained in the examined Brazilian population, demonstrating that they may not play an important role in the etiology of NSCL/P in this highly admixed population. However, the number of available samples is a limitation of the study, particularly with regard to the CL number; therefore modest associations of polymorphisms and oral cleft risk may have been missed.

In conclusion, the results of the present study confirm the importance of the 8q24 locus in the NSCL/P susceptibility in the Brazilian population, and show for the first time the importance of 1p22.1 in this population. In accordance with our previous studies [Brito et al., 2012a, b], the current results reinforce the suitability of ancestral markers in association studies of ethnically mixed populations. The findings have relevance beyond Brazil, because admixed populations are found in many other countries. Thus, our findings should encourage the application of similar approaches in samples from other countries that contain ancestrally admixed populations, to increase our understanding and knowledge of the genetic events that contribute to this complex disease.

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