

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

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 assess 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 \pm P group as compared to the control group ($P = 0.02$). Patients carrying the rs560426 G allele exhibited a moderately increased risk to CL \pm 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 \pm 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 \pm P in this same Brazilian sample [Brito et al., 2012a]. Since both rs987525 and rs1530300 showed a positive correlation with CL \pm 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 \pm P patients as compared to controls and showed a significant risk for having CL \pm 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 \pm 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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REFERENCES

- Bastos-Rodrigues L, Pimenta JR, Pena SD. 2006. The genetic structure of human populations studied through short insertion-deletion polymorphisms. *Ann Hum Genet* 70:658–665.
- Beaty TH, Murray JC, Marazita ML, Munger RG, Ruczinski I, Hetmanski JB, Liang KY, Wu T, Murray T, Fallin MD, Redett RA, Raymond G, Schwender H, Jin SC, Cooper ME, Dunnwald M, Mansilla MA, Leslie E, Bullard S, Lidral AC, Moreno LM, Menezes R, Vieira AR, Petrin A, Wilcox AJ, Lie RT, Jabs EW, Wu-Chou YH, Chen PK, Wang H, Ye X, Huang S, Yeow V, Chong SS, Jee SH, Shi B, Christensen K, Melbye M, Doheny KF, Pugh EW, Ling H, Castilla EE, Czeizel AE, Ma L, Field LL, Brody L, Pangilinan F, Mills JL, Molloy AM, Kirke PN, Scott JM, Arcos-Burgos M, Scott AF. 2010. A genome-wide association study of

- cleft lip with and without cleft palate identifies risk variants near MAFB and ABCA4. *Nat Genet* 42:525–529.
- Birnbaum S, Ludwig KU, Reutter H, Herms S, Steffens M, Rubini M, Baluardo C, Ferrian M, Almeida de Assis N, Alblas MA, Barth S, Freudenberg J, Lauster C, Schmidt G, Scheer M, Braumann B, Bergé SJ, Reich RH, Schiefke F, Hemprich A, Pötzsch S, Steegers-Theunissen RP, Pötzsch B, Moebus S, Horsthemke B, Kramer FJ, Wienker TF, Mossey PA, Propping P, Cichon S, Hoffmann P, Knapp M, Nöthen MM, Mangold E. 2009b. Key susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24. *Nat Genet* 41:473–477.
- Blanton SH, Burt A, Garcia E, Mulliken JB, Stal S, Hecht JT. 2010a. Ethnic heterogeneity of IRF6 AP-2a binding site promoter SNP association with nonsyndromic cleft lip and palate. *Cleft Palate Craniofac J* 47:574–577.
- Blanton SH, Burt A, Stal S, Mulliken JB, Garcia E, Hecht JT. 2010b. Family-based study shows heterogeneity of a susceptibility locus on chromosome 8q24 for nonsyndromic cleft lip and palate. *Birth Defects Res A Clin Mol Teratol* 88:256–259.
- Brito LA, Paranaíba LMR, Bassi CFS, Masotti C, Malcher C, Schlesinger D, Rocha KM, Cruz LA, Bárbara LK, Alonso N, Franco D, Bagordakis E, Martelli-Junior H, Meyer D, Coletta RD, Passos-Bueno MR. 2012a. Region 8q24 is a susceptibility locus for nonsyndromic oral clefting in Brazil. *Birth Defects Res A Clin Mol Teratol* 94:464–468.
- Brito LA, Bassi CF, Masotti C, Malcher C, Rocha KM, Schlesinger D, Bueno DF, Cruz LA, Barbara LK, Bertola DR, Meyer D, Franco D, Alonso N, Passos-Bueno MR. 2012b. IRF6 is a risk factor for nonsyndromic cleft lip in the Brazilian population. *Am J Med Genet Part A* 158A:2170–2175.
- Grant SF, Wang K, Zhang H, Glaberson W, Annaiah K, Kim CE, Bradfield JP, Glessner JT, Thomas KA, Garris M, Frackelton EC, Otieno FG, Chiavacci RM, Nah HD, Kirschner RE, Hakonarson H. 2009. A genome-wide association study identifies a locus for nonsyndromic cleft lip with or without cleft palate on 8q24. *J Pediatr* 155:909–913.
- Huang Y, Wu J, Ma J, Beaty TH, Sull JW, Zhu L, Lu D, Wang Y, Meng T, Shi B. 2009. Association between IRF6 SNPs and oral clefts in West China. *J Dent Res* 88:715–718.
- Ludwig KU, Mangold E, Herms S, Nowak S, Reutter H, Paul A, Becker J, Herberz R, AlChawa T, Nasser E, Böhmer AC, Mattheisen M, Alblas MA, Barth S, Kluck N, Lauster C, Braumann B, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Daratsianos N, Kreusch T, Murray JC, Marazita ML, Ruczinski I, Scott AF, Beaty TH, Kramer FJ, Wienker TF, Steegers-Theunissen RP, Rubini M, Mossey PA, Hoffmann P, Lange C, Cichon S, Propping P, Knapp M, Nöthen MM. 2012. Genome-wide meta-analyses of nonsyndromic cleft lip with or without cleft palate identify six new risk loci. *Nat Genet* 44:968–971.
- Mangold E, Ludwig KU, Birnbaum S, Baluardo C, Ferrian M, Herms S, Reutter H, de Assis NA, Chawa TA, Mattheisen M, Steffens M, Barth S, Kluck N, Paul A, Becker J, Lauster C, Schmidt G, Braumann B, Scheer M, Reich RH, Hemprich A, Pötzsch S, Blaumeiser B, Moebus S, Krawczak M, Schreiber S, Meitinger T, Wichmann HE, Steegers-Theunissen RP, Kramer FJ, Cichon S, Propping P, Wienker TF, Knapp M, Rubini M, Mossey PA, Hoffmann P, Nöthen MM. 2010. Genome-wide association study identifies two susceptibility loci for nonsyndromic cleft lip with or without cleft palate. *Nat Genet* 42:24–26.
- Marazita ML, Lidral AC, Murray JC, Field LL, Maher BS, Goldstein McHenry T, Cooper ME, Govil M, Daack-Hirsch S, Riley B, Jugessur A, Felix T, Morene L, Mansilla MA, Vieira AR, Doheny K, Pugh E, Valencia-Ramirez C, Arcos-Burgos M. 2009. Genome scan, fine-mapping, and candidate gene analysis of non-syndromic cleft lip with or without cleft palate reveals phenotype-specific differences in linkage and association results. *Hum Hered* 68:151–170.
- Mostowska A, Hozyasz KK, Wojcicki P, Biedziak B, Paradowska P, Jagodzinski PP. 2010. Association between genetic variants of reported candidate genes or regions and risk of cleft lip with or without cleft palate in the polish population. *Birth Defects Res A Clin Mol Teratol* 88:538–545.
- Mostowska A, Hozyasz KK, Wojcicka K, Biedziak B, Jagodzinski PP. 2012. Polymorphic variants at 10q25.3 and 17q22 loci and the risk of nonsyndromic cleft lip and palate in the polish population. *Birth Defects Res A Clin Mol Teratol* 94:42–46.
- Pan YC, Zhang WB, Du YF, Tong N, Han Y, Zhang HC, Wang ML, Ma JQ, Wan LZ, Wang L. 2011. Different roles of two novel susceptibility loci for nonsyndromic orofacial clefts in a Chinese Han population. *Am J Med Genet Part A* 155A:2180–2185.
- Pan YC, Zhang WB, Du YF, Tong N, Han Y, Zhang HC, Wang ML, Ma JQ, Wan LZ, Wang L. 2011. Different roles of two novel susceptibility loci for nonsyndromic orofacial clefts in a Chinese Han population. *Am J Med Genet Part A* 155A:2180–2185.
- Paranaíba LM, Bufalino A, Martelli-Junior H, de Barros LM, Graner E, Coletta RD. 2010. Lack of association between IRF6 polymorphisms [rs2235371 and rs642961] and non-syndromic cleft lip and/or palate in a Brazilian population. *Oral Dis* 16:193–197.
- Parra FC, Amado RC, Lambertucci JR, Rocha J, Antunes CM, Pena SD. 2003. Color and genomic ancestry in Brazilians. *Proc Natl Acad Sci USA* 100:177–182.
- Weatherley-White RC, Ben ST, Jin Y, Riccardi S, Arnold TD, Spritz RA. 2011. Analysis of genomewide association signals for nonsyndromic cleft lip/palate in a Kenya African cohort. *Am J Med Genet Part A* 155A:2422–2425.