

Region 8q24 Is a Susceptibility Locus for Nonsyndromic Oral Clefting in Brazil

Luciano Abreu Brito,¹ Lívia Máris Ribeiro Paranaíba,^{2,3} Camila Fernandes Silva Bassi,¹ Cibele Masotti,¹ Carolina Malcher,¹ David Schlesinger,^{1,4} Katia Maria Rocha,¹ Lucas Alvizi Cruz,¹ Lígia Kobayashi Bárbara,¹ Nivaldo Alonso,⁵ Diogo Franco,⁶ Elizabete Bagordakis,² Hercílio Martelli Jr.,^{3,7} Diogo Meyer,⁸ Ricardo D. Coletta,² and Maria Rita Passos-Bueno^{1*}

¹Human Genome Research Center, Institute of Biosciences, University of São Paulo, São Paulo, Brazil

²Department of Oral Diagnosis, School of Dentistry, State University of Campinas, Piracicaba, São Paulo, Brazil

³Stomatology Clinic, Dental School, State University of Montes Claros, Montes Claros, Minas Gerais, Brazil

⁴Instituto do Cérebro, Instituto Israelita de Ensino e Pesquisa Albert Einstein, São Paulo, Brazil

⁵Department of Surgery, School of Medicine, University of São Paulo, São Paulo, Brazil

⁶Department of Plastic Surgery, University of Rio de Janeiro, Rio de Janeiro, Brazil

⁷Center for Rehabilitation of Craniofacial Anomalies, Dental School, University of Alfenas, Minas Gerais, Brazil

⁸Department of Genetics, Institute of Biosciences, University of São Paulo, São Paulo, Brazil

Received 28 January 2012; Revised 2 March 2012; Accepted 6 March 2012

BACKGROUND: Nonsyndromic cleft lip with or without cleft palate is a relatively common craniofacial defect with multifactorial inheritance. The association of the rs987525 single nucleotide variant, located in a gene desert at 8q24.21 region, has been consistently replicated in European populations. We performed a structured association approach combined with transcriptional analysis of the *MYC* gene to dissect the role of rs987525 in oral clefting susceptibility in the ethnically admixed Brazilian population. **METHODS:** We performed the association study conditioned on the individual ancestry proportions in a sample of 563 patients and 336 controls, and in an independent sample of 221 patients and 261 controls. The correlation between rs987525 genotypes and *MYC* transcriptional levels in orbicularis oris muscle mesenchymal stem cells was also investigated in 42 patients and 4 controls. **RESULTS:** We found a significant association in the larger sample ($p = 0.0016$; OR = 1.80 [95% confidence interval {CI}, 1.21–2.69], for heterozygous genotype, and 2.71 [95% CI, 1.47–4.96] for homozygous genotype). We did not find a significant correlation between rs987525 genotypes and *MYC* transcriptional levels ($p = 0.14$; $r = -0.22$, Spearman Correlation). **CONCLUSIONS:** We present a positive association of rs987525 in the Brazilian population for the first time, and it is likely that the European contribution to our population is driving this association. We also cannot discard a role of rs987515 in *MYC* regulation, because this locus behaves as an expression quantitative locus of *MYC* in another tissue. *Birth Defects Research (Part A) 94:464–468, 2012.* © 2012 Wiley Periodicals, Inc.

Key words: rs987525; rs1476165; rs2099897; heritability; cleft lip; cleft palate; 8q24 gene desert; *MYC* transcriptional levels; common disease-common variant; mesenchymal stem cells

INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (NS CL/P; OMIM 119530), which represents 70% of orofacial clefts and is the most common craniofacial anomaly, is always accompanied by physical and psychological impairments to affected children, with great family burden (Wehby and Cassell, 2010).

NS CL/P has a worldwide birth prevalence of approximately 1:700 live births (Dixon et al., 2011), varying according to ethnicity (African, 0.3:1000; European, 1.0:1000; Native American, 3.6:1000), geographic origin,

Additional Supporting Information may be found in the online version of this article.

Financial support from FAPESP/CEPID (98/14254-2), CNPq (401952/2010-S) and the Brazilian Ministry of Health.

*Correspondence to: Maria Rita Passos-Bueno, Rua do Matão, 277, sala 200, Cidade Universitária, São Paulo, SP 05508-090, Brazil. E-mail: passos@i-b.usp.br

Published online 18 April 2012 in Wiley Online Library (wileyonlinelibrary.com).

DOI: 10.1002/bdra.23011

and socioeconomic status (Carinci et al., 2007; Jugessur et al., 2009). In Brazil, NS CL/P prevalence has ranged from 0.28:1000 to 1.54:1000 (Menegotto and Salzano, 1991; Loffredo et al., 2001; Martelli-Junior et al., 2006). NS CL/P etiology is multifactorial and is determined by numerous interacting loci with additional environmental covariates (Mossey et al., 2009). The identification of genetic risk factors for NS CL/P has been the subject of intensive research, and genome-wide scans have shown a range of plausible candidate genes or chromosomal regions (Dixon et al., 2011). Of those, the most remarkable loci are the *IRF6* gene (Zuccherro et al., 2004; Rahimov et al., 2008) and the rs987525 single nucleotide variant (SNV) at 8q24.21 (Birnbaum et al., 2009), as their association with NS CL/P has been largely replicated (Park et al., 2007; Jugessur et al., 2008; Grant et al., 2009; Huang et al., 2009; Nikopensius et al., 2009; Beaty et al., 2010; Blanton et al., 2010a, 2010b). Despite the several reported associations between rs987525 and NS CL/P, it is unknown whether this SNV is the cause of the association or if it is in linkage disequilibrium with still some unknown gene or region; nevertheless, a regulatory function has been credited for this region (Dixon et al., 2011). Furthermore, patients of European origin seem to be more at risk from this SNV (Beaty et al., 2010), and it is certainly important to evaluate its worldwide effect. Although the Brazilian population has a trihybrid constitution of European, African, and Native American parental populations, European contribution is as high as 80% (Santos et al., 2010), and it is possible that rs987525 contributes to oral clefting in our population. To test this hypothesis, and to avoid population stratification bias, we have conducted a structured association analysis in a large sample of patients ascertained in different localities of the country (Sample 1) and in a minor replication sample ascertained in the state of Minas Gerais (southeastern region; Sample 2). To provide new insights into the functional role of rs987525, we have also investigated the correlation between its genotypes and the expression levels of the *MYC* gene, which is an important regulator of a variety of cellular processes necessary for normal cell growth and differentiation (Secombe et al., 2004), located at approximately 1.1 Mb of rs987525.

MATERIALS AND METHODS

Ethics

All samples were collected in accordance with the rules of the Research Ethics Committee of the Institute of Biosciences, University of São Paulo, Brazil, and the Human Research Ethics Committee of the University of Alfenas, Brazil. Informed consent was obtained from the patients or legal tutors.

Patients and Controls

We ascertained two independent samples of unrelated cases and controls. Sample 1 comprises 563 NS CL/P patients from medical programs of Operation Smile (<http://www.operationmile.org>) between 2007 and 2010 in five different Brazilian sites (Santarém, Fortaleza, Barbalha, Maceió, and Rio de Janeiro) and 336 controls from São Paulo (previously described by Errera et al., 2006), provided by our Biobank, because we were not able to ascertain local controls at the corresponding period.

Patients affected by cleft palate only were removed from the sample. Patient ascertainment methods were previously reported by Brito et al., (2011a). DNA extraction from an oral swab followed the manufacturer's protocols (Macherey-Nagel, Düren, Germany).

To replicate our findings, we recruited an independent sample of 221 patients and 261 controls (Sample 2) from the Center for Rehabilitation of Craniofacial Anomalies–Dental School, University of Alfenas, State of Minas Gerais (southeastern region of Brazil). Control individuals were subjects admitted as in-patients, but with conditions unrelated to clefting disorders and their endophenotypes. Genomic DNA of Sample 2 was extracted from oral mucosa cells, as described previously (Aidar and Line, 2007).

To verify the effect of rs987525 in the expression levels of the *MYC* gene, we established mesenchymal stem cell cultures (MSCs) from orbicularis oris muscle fragments (OOMF), discarded during surgical procedures of cheiloplasty, of an additional 42 NS CL/P patients and 4 controls submitted to surgery for other reasons than CL/P correction. These surgeries were performed in three different centers (Division of Plastic Surgery of University of São Paulo, SOBRAPAR Hospital–Campinas, and Operation Smile, Rio de Janeiro, Brazil).

Genotyping

All individuals were genotyped for the SNV rs987525 with the TaqMan method (Applied Biosystems, Foster City, CA, USA). To characterize the European, African, and Native American ancestry contributions, Samples 1 and 2 were genotyped for independent panels of 40 ancestry informative indels. FAM-labeled M13 tail was incorporated to forward primers, allowing multiplex PCR, with four to seven markers per reaction (sequences available upon request). These markers are spread across the genome and were accessed from the Marshfield database (<http://research.marshfieldclinic.org>), which holds a set of indel markers described by Weber et al. (2002). Sample 2 was genotyped for a panel extensively used for characterizing Brazilian populations (Bastos-Rodrigues et al., 2006), and Sample 1 was genotyped for an independent panel with matched marker selection criteria, as published previously (Brito et al., 2011a). Fragment analysis was performed with Gene Mapper software (Applied Biosystems) after capillary electrophoresis targeting the PCR products for the FAM-labeled M13 tail.

Cell Culture

The MSCs were established according to previously published protocol (Bueno et al., 2009) and expanded to 80% confluence. RNA and DNA extraction followed the manufacturer's protocols (Macherey-Nagel). We confirmed the homogeneous mesenchymal status of 10 cell cultures using five mesenchymal cell markers (CD29, CD90, CD105, SH3, and SH4), one hematopoietic (CD45), and one endothelial (CD31) through flow cytometric analysis (EasyCyte Flow Cytometer; Guava Technologies, Hayward, CA) according to standard protocol in our laboratory (Bueno et al., 2010).

Statistical Analyses

We used Structure 2.3.3 to infer ancestry components of each individual (Pritchard et al., 2000a; Falush et al.,

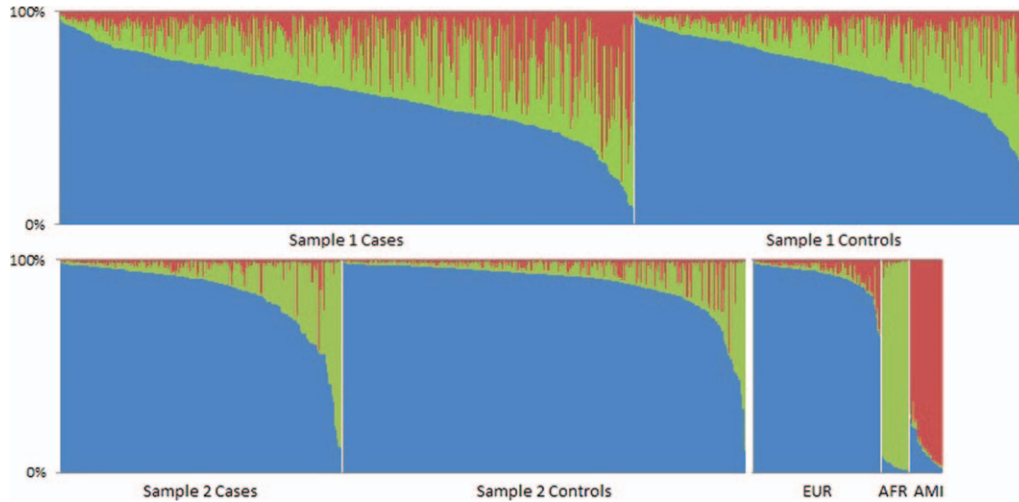


Figure 1. European (blue), African (green), and Native American (red) ancestry contributions for Samples 1 and 2. Each individual is represented by a single column. In the lower panel, EUR, AFR, and AMI represent the parental populations used as “learning samples” to assist Structure in estimating ancestry of the admixed individuals.

2003, 2007), in a run assuming $K = 3$ parental populations, based on the trihybrid origin of Brazilians; we also incorporated “learning samples” in the run, with prespecified population of origin (European, African, and Native American reference populations from Marshfield Clinic collection) to assist the software to estimate ancestry of the admixed individuals. Following the ancestry inference, STRAT was used to test the SNV for association, conditioning on the individual ancestry proportions (Pritchard et al., 2000b).

We obtained *MYC* expression values from microarray gene expression data (Human Gene 1.0 ST microarray chips; Affymetrix, Santa Clara-CA, USA), with the three-step robust multiarray average preprocessing method implemented in the software Expression Console (Affymetrix). We assessed the correlation between the rs987525 genotype and *MYC* expression levels with a Spearman correlation test (95% confidence interval [CI]).

RESULTS

Association Analyses

After excluding individuals with low genotype assignment (<80%), Sample 1 comprised 471 patients (18% presenting cleft lip only [CLO] and 82% presenting cleft lip and cleft palate [CLP]) and 326 controls. Average ancestry contributions were estimated at 22.2% African, 60.3% European, and 17.5% Native American for patients, and 18.8% African, 70.9% European, and 10.3% Native American for controls (individual ancestry profile is shown in Fig. 1). Ancestry analysis stratifying the patients for region of origin is shown in Table 1. Minor allele frequency (MAF) of rs987525 was similar in cases (34%) and controls (35%), and genotype distributions followed the Hardy-Weinberg expected proportions in both groups ($p > 0.48$). Despite the similar MAF between cases and controls, a significant association with the A allele was observed ($p = 0.006$) in the structured association test (Table 1). We further investigated whether the European ancestry would be driving this association by stratifying

the analysis into two groups: a European group with the 361 patients presenting more than 50% of European ancestry, and a non-European group with 110 patients with less than 50% of European ancestry, and then comparing both groups with the same 326 controls. We found a stronger association in the European group ($p = 0.008$) than in the non-European group ($p = 0.21$).

Sample 2 consisted of 196 patients (28% CLO and 72% CLP) and 263 controls, after excluding low-genotype assignment individuals. Average ancestry contributions of this sample were estimated at 13.4% African, 84.1% European, and 2.5% Native American for patients and 8.1% African, 89.0% European, and 2.9% Native American for controls (Fig. 1). Minor allele frequency was higher in patients (39.5%) than in controls (27.4%), and genotypes matched Hardy-Weinberg proportions in both groups ($p > 0.31$). A significant association between rs987525 and NS CL/P group was also observed in this sample ($p = 0.0016$). Because rs987525 allele frequencies vary greatly among continents, and differences in ancestry contribution between cases and controls are more pronounced in Sample 1, we considered that we would obtain a more reliable odds ratio (OR) estimates only for Sample 2, from where cases and controls come from a single location. For this group, OR was estimated at 1.80 (95% CI, 1.21–2.69) for the heterozygous genotype (AC versus CC) and 2.70 (95% CI, 1.47–4.96) for the homozygous genotype (AA versus CC), under an additive model.

MYC Expression Analyses

We found a nonsignificant correlation between rs987525 genotypes (Supporting Information Table S1) and *MYC* transcriptional levels in MSCs (Supporting Information Fig. S1; $p = 0.14$; $r = -0.22$, Spearman correlation).

DISCUSSION

It has been postulated that part of the susceptibility alleles for complex disorders might have a common origin

Table 1
 Estimates of Average Ancestry Contributions of Parental Populations (European, African, and Native American), rs987525 Genotype Frequencies, Risk Allele (A) Frequency, and *p* Value of the Structured Association Tests

Sample	Subsamples	N	Ancestry contribution (%)			Genotype frequencies (%)				<i>p</i> value (structured association)	<i>p</i> value (H-W test)	OR (95% CI)
			European	African	Native American	AA	AC	CC	f(A)			
Sample 1	All Patients	471	60	22	18	12	45	44	34	0.92	0.006	
	Santarém	82	54	14	32	7	38	55	26	0.99	0.07	
	Barbalha	52	64	24	12	58	30	41	41	0.09	0.005	
	Fortaleza	163	64	20	16	12	42	46	33	0.42	0.33	
	Maceió	62	59	23	19	12	44	44	34	0.92	0.21	
	Rio de Janeiro Controls	112 326	58 71	31 19	11 10	14 13	48 44	40 42	38 35	0.79 0.48	—	
Sample 2	Patients	196	84	13	3	16	47	37	40	0.69	0.0016	AC: 1.80 (1.21–2.69)
	Controls	263	89	8	3	9	37	57	21	0.31		AA: 2.70 (1.47–1.96)

The results are presented for Sample 1 as a whole and stratified by location of origin of the patients (Santarém, Barbalha, Fortaleza, Maceió, and Rio de Janeiro). The odds ratio was only estimated for Sample 2, since cases and controls from this sample are less stratified. The odds ratios for heterozygous (AC; *p* = 0.004) and homozygous (AA; *p* = 0.001) genotypes were estimated by comparing with CC genotype, under an additive model. CA, frequency of the risk allele (A); OR, odds ratio; CI, confidence interval; H-W, Hardy-Weinberg.

(Lander, 2011). Therefore, replicating the NS CL/P association studies results in other populations is relevant, not only to define the common or population-specific risk alleles, but to construct the best model to explain the disorder.

In this study, we show a positive association between rs987525 and NS CL/P in the Brazilian population through a structured association approach. It is interesting to note that the level of significance was much higher in Sample 2, which has a greater contribution of European ancestry compared with Sample 1. The higher OR for homozygous genotype (AA) suggested an additive model, similar to those observed in previous studies with European populations (Birnbäum et al., 2009; Nikopensius et al., 2009).

Although we did not observe a significant correlation between rs987525 genotypes and the expression levels of *MYC* (~ 1.1 Mb apart from rs987525) in MSCs, this locus behaves as an expression quantitative trait locus of *MYC* on adipose tissue according to the GENEVAR database (*p* = 0.01; MuTHER study, Yang et al., 2010; Nica et al., 2011). It is of note that other SNVs at 8q24, rs1476165 and rs2099897 (802 and 831 kb apart from the 5' end of *MYC*, respectively), were found to modulate *MYC* expression tissue-specifically, according to GENEVAR (Yang et al., 2010; Nica et al., 2011), suggesting that this region might be of great relevance to *MYC* regulation. Given the importance of *MYC* on cell proliferation control and the absence of other known genes within this region playing a role in craniofacial development, it would be of interest to conduct additional studies on the effect of rs987525 in *MYC* transcriptional regulation. It is possible that we did not detect the effect of rs987525 genotypes in the expression levels of *MYC* because of our limited sample size or, alternatively, MSCs of OOMF might not be the best cell type to conduct such studies.

Our results confirm the importance of this locus to NS CL/P predisposition and provide additional evidence that the true causal variant beyond this association might be of European origin, because a positive association between rs987525 and clefting patients has been shown in a variety of European patients of different regions of the world, such as Germany, Estonia, Lithuania, and Poland, and European descents in United States (Birnbäum et al., 2009; Grant et al., 2009; Nikopensius et al., 2009; Beaty et al., 2010; Mostowska et al., 2010), in contrast to the lack of association in patients of East Asian origin (Beaty et al., 2010). Considering the trihybrid origin of Brazilians and that the association observed in Sample 1 was driven by the subset with more than 50% of European contribution, it is also likely that this allele is not so relevant in populations of African descent. Indeed, a recent report by (Weatherley-White et al., 2011) showed no evidence of association of rs987525 in patients from Kenya; therefore, this SNV might track a functionally relevant variant only in the European haplotype block.

We have previously shown that heritability of NS CL/P in Brazil is high (up to 85%) and varies according to the geographic region. We have also predicted that heritability might be a confounding factor in association studies of heterogeneous diseases such as NS CL/P (Brito et al., 2011a). In this regard, we have recently observed a positive association between the traditional GWAS hit rs642961, in the *IRF6* gene, and NS CL/P only in the

sample with the highest heritability, which comes from Barbalha (Brito et al., 2012). We have therefore stratified the present association analysis by location of origin, and we have verified that the Barbalha group reached the highest significance ($p = 0.005$), compared with samples from Santarém ($p = 0.07$), Fortaleza ($p = 0.33$), Maceió ($p = 0.21$), and Rio de Janeiro ($p = 0.19$; Table 1). These results provide confirm heritability as a confounding factor in association analyses.

In summary, our results provided further evidence that rs987525 is involved with NS CL/P susceptibility in patients of European descent, and heritability seemed to be an important confounding factor. In addition, we did not find evidence of rs987525 modulating *MYC* expression.

ACKNOWLEDGMENTS

We thank the Operation Smile team and its executive director Clovis Brito, Constância Urbani and Márcia Teixeira for secretarial assistance, and the Brazilian Ministry of Health, FAPESP, and CNPq for financial support.

REFERENCES

- Aidar M, Line SR. 2007. A simple and cost-effective protocol for DNA isolation from buccal epithelial cells. *Braz Dent J* 18:148–152.
- Bastos-Rodrigues L, Pimenta JR, Pena SD, et al. 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, et al. 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, et al. 2009. 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, et al. 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, et al. 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, Cruz LA, Rocha KM, et al. 2011a. Genetic contribution for nonsyndromic cleft lip with or without cleft palate (NS CL/P) in different regions of Brazil and implications for association studies. *Am J Med Genet A* 155A:1581–1587.
- Brito LA, Bassi CFS, Masotti C, et al. 2012. IRF6 is a risk factor for nonsyndromic cleft lip in the Brazilian Population. *Am J Med Genet A*, in press.
- Bueno DF, Kerkis I, Costa AM, et al. 2009. New source of muscle-derived stem cells with potential for alveolar bone reconstruction in cleft lip and/or palate patients. *Tissue Eng Part A* 15:427–435.
- Bueno DF, Sunaga DY, Kobayashi GS, et al. 2010. Human stem cell cultures from cleft lip/palate patients show enrichment of transcripts involved in extracellular matrix modeling by comparison to controls. *Stem Cell Rev* 7:446–457.
- Carinci F, Scapoli L, Palmieri A, et al. 2007. Human genetic factors in nonsyndromic cleft lip and palate: an update. *Int J Pediatr Otorhinolaryngol* 71:1509–1519.
- Dixon MJ, Marazita ML, Beaty TH, et al. 2011. Cleft lip and palate: understanding genetic and environmental influences. *Nat Rev* 12:167–178.
- Errera FI, Silva ME, Yeh E, et al. 2006. Effect of polymorphisms of the MTHFR and APOE genes on susceptibility to diabetes and severity of diabetic retinopathy in Brazilian patients. *Braz J Med Biol Res* 39:883–888.
- Falush D, Stephens M, Pritchard JK, et al. 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. *Genetics* 164:1567–1587.
- Falush D, Stephens M, Pritchard JK, et al. 2007. Inference of population structure using multilocus genotype data: dominant markers and null alleles. *Mol Ecol Notes* 7:574–578.
- Grant SF, Wang K, Zhang H, et al. 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, et al. 2009. Association between IRF6 SNPs and oral clefts in West China. *J Dent Res* 88:715–718.
- Jugessur A, Farlie PG, Kilpatrick N, et al. 2009. The genetics of isolated orofacial clefts: from genotypes to subphenotypes. *Oral Dis* 15:437–453.
- Jugessur A, Rahimov F, Lie RT, et al. 2008. Genetic variants in IRF6 and the risk of facial clefts: single-marker and haplotype-based analyses in a population-based case-control study of facial clefts in Norway. *Genet Epidemiol* 32:413–424.
- Lander ES. 2011. Initial impact of the sequencing of the human genome. *Nature* 470:187–197.
- Loffredo LCM, Freitas JAS, Grigolli AAG, et al. 2001. Prevalence of oral clefts from 1975 to 1994, Brazil. *Rev Saúde Pública* 35:571–575.
- Menegotto BG, Salzano FM. 1991. Epidemiology of oral clefts in a large South American sample. *Cleft Palate Craniofac J* 28:373–376, discussion 376–377.
- Martelli-Junior H, Orsi-Junior J, Chaves MR, et al. 2006. Estudo epidemiológico das fissuras labiais e palatais em Alfenas - Minas Gerais - de 1986 a 1998. *RPG Rev Pos-grad* 13:31–35.
- Mossey PA, Little J, Munger RG, et al. 2009. Cleft lip and palate. *Lancet* 374:1773–1785.
- Mostowska A, Hozyasz KK, Wojcicki P, et al. 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.
- Nica AC, Parts L, Glass D, et al. 2011. The architecture of gene regulatory variation across multiple human tissues: the MuTHER study. *PLoS genetics* 7:e1002003.
- Nikopensius T, Ambrozaityte L, Ludwig KU, et al. 2009. Replication of novel susceptibility locus for nonsyndromic cleft lip with or without cleft palate on chromosome 8q24 in Estonian and Lithuanian patients. *Am J Med Genet A* 149A:2551–2553.
- Park JW, McIntosh J, Hetmanski JB, et al. 2007. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations. *Genet Med* 9:219–227.
- Pritchard JK, Stephens M, Donnelly P, et al. 2000a. Inference of population structure using multilocus genotype data. *Genetics* 155:945–959.
- Pritchard JK, Stephens M, Rosenberg NA, et al. 2000b. Association mapping in structured populations. *Am J Hum Genet* 67:170–181.
- Rahimov F, Marazita ML, Visel A, et al. 2008. Disruption of an AP-2alpha binding site in an IRF6 enhancer is associated with cleft lip. *Nat Genet* 40:1341–1347.
- Santos NP, Ribeiro-Rodrigues EM, Ribeiro-Dos-Santos AK, et al. 2010. Assessing individual interethnic admixture and population substructure using a 48-insertion-deletion (INSEL) ancestry-informative marker (AIM) panel. *Hum Mutat* 31:184–190.
- Secombe J, Pierce SB, Eisenman RN, et al. 2004. Myc: a weapon of mass destruction. *Cell* 117:153–156.
- Weatherley-White RC, Ben S, Jin Y, et al. 2011. Analysis of genome-wide association signals for nonsyndromic cleft lip/palate in a Kenya African Cohort. *Am J Med Genet A* 155A:2422–2425.
- Weber JL, David D, Heil J, et al. 2002. Human diallelic insertion/deletion polymorphisms. *Am J Hum Genet* 71:854–862.
- Wehby GL, Cassell CH. 2010. The impact of orofacial clefts on quality of life and healthcare use and costs. *Oral Dis* 16:3–10.
- Yang TP, Beazley C, Montgomery SB, et al. 2010. Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. *Bioinformatics (Oxford, England)* 26:2474–2476.
- Zuchero TM, Cooper ME, Maher BS, et al. 2004. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *New Engl J Med* 351:769–780.