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Short communication

Analysis of the genetic ancestry of patients with oral clefts from South American admixed populations

Camilla D. Vieira-Machado[1,2], Flavia M. de Carvalho[2,3], Luiz C. Santana da Silva[2,4], Sidney E. dos Santos[4], Claudia Martins[5], Fernando A. Poletta[2,6], Juan C. Mereb[7], Alexandre R. Vieira[8,9], Eduardo E. Castilla[2,3,6], Iêda M. Orioli[1,2]

[1]ECLAMC at Department of Genetics, Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro; [2]INAGEMP (National Institute of Population Medical Genetics), Rio de Janeiro; [3]ECLAMC at LEMC (Laboratory of Congenital Malformations Epidemiology), Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro; [4]Institute of Biological Sciences, Federal University of Pará, Belém, Pará; [5]Speech Therapy Division, Ophir Loyola Hospital, Belém, Pará, Brazil; [6]ECLAMC at CEMIC (Center for Medical Education and Clinical Research) and CONICET (National Council for Scientific and Technical Investigation), Buenos Aires; [7](In memoriam) ECLAMC at Hospital Zonal El Bolsón, El Bolsón, Argentina; [8]Department of Oral Biology and Pediatric Dentistry, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA; [9]Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA

Iêda Maria Orioli, Genetics Department, Federal University of Rio de Janeiro, Caixa Postal 68.011, 21949-900, Rio de Janeiro, RJ, Brazil

E-mail: orioli@centroin.com.br

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Increased susceptibility to cleft lip, with or without cleft palate (CL±P) has been observed in South America, as related to Amerindian ancestry, using epidemiological data, uniparental markers, and blood groups. In this study, it was evaluated whether this increased risk remains when Amerindian ancestry is estimated using autosomal markers and considered in the predictive model. Ancestry was estimated through genotyping 62 insertion and deletion (INDEL) markers in sample sets of patients with CL±P, patients with cleft palate (CP), and controls, from Patagonia in southern Argentina and Belém in northern Brazil. The Amerindian ancestry in patients from Patagonia with CL±P was greater than in controls although it did not reach statistical significance. The European ancestry in patients with CL±P from Belém and in patients with CP from Belém and Patagonia was higher than in controls and statistically significant for patients with CP who were from Belém. This high contribution of European genetic ancestry among patients with CP who were from Belém has not been previously observed in American populations. Our results do not corroborate the currently accepted risks for CL±P and CP estimated by epidemiological studies in the North American populations and probably reflect the higher admixture found in South American ethnic groups when compared with the same ethnic groups from the North American populations.

Key words: cleft lip and palate; cleft palate; genetic ancestry; INDEL; South American populations

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Oral clefts are typically subdivided into cleft lip with or without cleft palate (CL±P) and cleft palate (CP) (1), and cases can also be classified as isolated or associated according to the absence or presence, respectively, of other malformations. Approximately 70% of all cases of CL±P and 50% of all cases of CP are isolated (2, 3).

There are differences in the birth prevalence of oral clefts across populations. Of 23 studies, conducted in American populations (Table S1), reporting the birth prevalence of oral clefts in different ethnic groups only six reported the prevalence of CL±P and CP in all three of the following ethnic groups: Native American; European American; and African American (4–9). In North American populations, the prevalence of CL±P has been reported as being significantly higher in Native American subjects and significantly lower in African American subjects when compared with European American subjects (5–9). The prevalence of CP in the same populations was reported as being significantly lower in African American than in European American subjects but was not significantly different between Native American and European American subjects (4–9).

The main ancestry contribution to the Argentinean population is European; the percentages of Amerindian ancestry vary according to the geographic region, and that of African ancestry is low nationwide. The provinces located in the Patagonian region show a high contribution of Amerindian ancestry (10). The same pattern of high European ancestry is seen in the Brazilian populations. The northern region of Brazil shows a significant percentage of Amerindian and African ancestries (11).

Poletta *et al*. (12) identified four clusters with a high prevalence of CL±P in material from the Latin American Collaborative Study of Congenital Malformations (ECLAMC), one of them in Patagonia. Amerindian ancestry has been proposed as a risk factor for CL±P in areas identified as clusters of a high prevalence of this defect (12, 13) as well as in the general population in South America (14, 15). The Amerindian ancestry in the studies cited was estimated by ethnic self-declaration (12), uniparental ancestry informative markers located in the mitochondrial DNA, and the non-recombinant region of the Y-chromosome (13, 15) or blood groups (14). Ancestry studies using only ethnic self-declaration, blood groups, or uniparental markers might be less comprehensive than studies using markers located in autosomal chromosomes (16, 17), probably because autosomal chromosomes represent a larger proportion of the genome.

This study tested the hypothesis that the increased risk for CL±P based on Amerindian ancestry estimated using epidemiological data, blood groups, and uniparental markers is comparable with that estimated using autosomal markers in South American populations.

Material and methods

Subjects with isolated CL±P and CP, and controls, were studied in samples from two populations with a high contribution of Amerindian ancestry. One population was from a region with a high prevalence of CL±P and where the risk for this malformation has been observed as 2.5 times higher in Amerindians than in non-Amerindians (Patagonia; southern Argentina) (13) when using uniparental markers. The other population was from a region with a more typical prevalence of CL±P but with no information about risk for this malformation among Amerindians (Belém; northern Brazil).

A total of 143 patients with CL±P, 21 with CP, and 92 controls, from Patagonia, were studied. All subjects studied were unrelated and were drawn from a special study in the Patagonia region, in southern Argentina, conducted by ECLAMC. All patients and controls reside in cities in the region, located between 36°53′S and 43°29′S and 63°0′W and 71°32′W and belonging to the provinces of Neuquén, Rio Negro, and Chubut.

Another group of 210 subjects – 103 patients with CL±P, 33 with CP, and 74 controls – from Belém, was studied. All study subjects were unrelated and were from the metropolitan region of Belém and nearby towns in the state of Pará in northern Brazil, located in the area between 0°44′S and 4°58′S and 47°46′W and 52°44′W, and including the municipalities of Ananindeua, Belém, Marituba, Benevides, Santa Isabel do Pará, Santa Bárbara do Pará, and Castanhal.

Syndromic cases of CL±P and CP were excluded from both samples (Patagonia and Belém). The two control groups consisted of unrelated individuals with no history of oral clefts in the family and were residents in the same region as the patients. Patients and controls were informed of the study objectives and they signed an informed consent form when agreeing to participate. The study protocol and terms of free and informed consent signed by patients and controls from Patagonia were approved by the Ethics Committee in Investigation of the Medical and Clinical Research Education Center (Dr Norberto Quirno) in Buenos Aires, Argentina (IRB 1745, IORG-0001315; approval number: # 238). The study protocol and terms of free and informed consent signed by patients and controls from Belém were approved by the Ethics Committee on Human Research of the Institute of Health Sciences of the Federal University of Pará (CAAE 4879.0.000.073-10 and Opinion 140/10 – CEP-ICS/UFPA).

Patients with CL±P and CP, and controls, were genotyped using a panel of 62 insertion and deletion (INDEL) markers (Table S2), which were selected based on their greatest absolute frequency difference (δ) between African and Amerindian, European and Amerindian, or European and African populations. The ancestral populations used to select the INDEL markers, as well as part of this panel, have been previously described (18).

A sample of 701 individuals had been previously genotyped using the 62 INDEL markers: 222 Native Americans from nine tribes of the Brazilian Amazon (Tiriyó, Waiãpi, Zoé, Urubu-Kaapor, Awa-Guajá, Parakanã, Wai Wai, Gavião, and Zoró) (19); 211 Africans (from Angola, Mozambique, Republic of the Congo, Cameroon, and Ivory Coast) (20); and 268 Europeans (from Portugal and Spain). This sample has been used in previous investigations on Brazilian ancestry (18, 21). The δ and fixation index (F_{ST}; a measure of population differentiation caused by genetic structure) values for these markers are presented in Table S2.

Genetic diversity parameters, such as allele frequencies, and Hardy–Weinberg equilibrium of the INDEL markers were estimated separately in each group of patients and controls using the ARLEQUIN v3.5.1.3 software (22).

The percentage of ancestry contributions of each parental population in the individuals studied was calculated using the STRUCTURE v2.3.3 software (23) considering three parental populations ($K = 3$) and using the 701 individuals, described above, as proxies for the parental populations (Amerindian, African, and European). Runs consisted of 100,000 burn-in steps followed by 100,000 Markov chain Monte Carlo (MCMC) iterations, using the ancestry model 'Use population information to test for migrants' with correlated allele frequencies.

The Mann–Whitney *U*-test was used to determine whether there were significant differences in the estimates of Amerindian, European, and African ancestry between patients with each type of oral cleft and controls in each studied population.

Results

The frequency of the 62 INDEL markers in the parental populations and in each sample of patients and controls from Patagônia and Belém are presented in Table S2. All 62 INDELs were in Hardy–Weinberg equilibrium in each sample of patients and controls.

The average values of Amerindian, European, and African ancestry contributions, estimated by STRUCTURE, in each population studied, are presented in Table 1.

In Patagonia, there was a trend towards Amerindian ancestry (44.1% vs. 40.0%) but not European ancestry (45.2% vs. 49.8%) in patients with CL±P compared

Table 1

Ancestry estimates for patients with cleft lip, with or without cleft palate (CL±P) or cleft palate (CP), and their respective controls, in the two study regions

			Ancestry								
			AMR			EUR			AFR		
Study region	Study group	*n*	%	95% CI	*P**	%	95% CI	*P**	%	95% CI	*P**
Patagônia	CL±P	143	44.1	40.7–46.8	0.12	45.2	42.1–48.7	0.05	10.7	9.9–11.5	0.16
	CP	21	36.5	29.8–42.8	0.19	53.8	46.5–59.7	0.15	9.8	8.2–11.3	0.47
	Controls	92	40.0	37.0–42.5		49.8	46.6–52.5		10.1	9.1–11.1	
Belém	CL±P	103	32.6	30.1–34.6	0.18	44.6	42.1–46.8	0.18	22.8	20.9–24.4	0.21
	CP	33	28.0	23.9–31.6	**0.02**	49.8	44.8–53.9	**0.01**	22.1	19.2–24.9	0.32
	Controls	74	34.0	30.6–36.7		42.1	39.1–45.9		23.2	20.0–25.7	

AFR, African; AMR, Amerindian; EUR, European.
*Comparison between patients with CL±P or CP and controls (Mann–Whitney *U*-test); bold values are statistically significant ($P < 0.05$).

with controls (Table 1; Fig. 1A); however, this difference was not significant. The difference in African ancestry (10.7% vs. 10.1%) between patients with CL±P and controls was also non-significant. The comparison between patients with CP and controls from Patagonia showed a trend of increased European ancestry in those with CP (53.8% vs. 49.8%); however, this difference was not significant.

In Belém, the patients with CL±P showed a trend towards European ancestry (44.6% vs. 42.1%), although all three parental contributions in this group were not significantly different from that observed in the control group. In Belém, the patients with CP showed significantly more European ancestry (49.8% vs. 42.1%) and less Amerindian ancestry (28.0% vs. 34.0%) than the controls (Table 1; Fig. 1B); however, no difference in African ancestry (22.1% vs. 23.2%) between patients with CP and controls was observed.

Figure 1 shows the results of individual ancestry estimates based on allele frequencies of INDEL markers analysed using the STRUCTURE software. These results showed low variation in African ancestry among individuals in the Patagonian population (Fig. 1A); this ancestry was as low in subjects with CL±P and with CP as in controls. However, the Amerindian and European ancestries varied greatly, even among individuals with the same defect (CL±P and CP) or among control subjects. In Belém, a high variation in the contributions of European, African, and Amerindian ancestries was observed among individuals (Fig. 1B).

In the triangle plots, each vertex indicates 100% contribution from a specified parental population. Therefore, the closer an individual is to a vertex, the greater the contribution of that population to his ancestry. The triangle plot in Fig. 1A shows that patients with CL±P (yellow) were closer to the Amerindian vertex compared with controls (pink) in the Patagonian population, demonstrating the greatest Amerindian ancestry in subjects with CL±P despite the lack of statistical significance. In Fig. 1B, the triangle plots show that patients with CL±P and controls from Belém showed no particular association with European, Amerindian or African vertexes; however, patients with CP were placed in closer proximity to the European vertex.

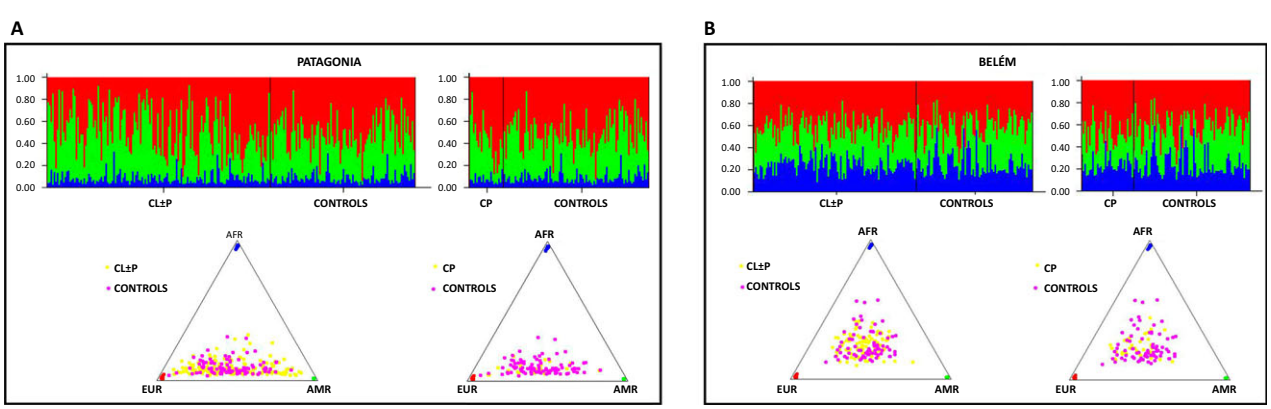

Fig. 1. Plots generated for patients with cleft lip, with or without cleft palate (CL±P), cleft palate (CP), and controls are shown for the two populations studied: Patagonia (A) and Belém (B). The bar plots (upper images) in each panel show Amerindian (green), European (red), and African (blue) ancestry contributions in patients and controls. Each column represents one individual; the contribution of each parental population can be seen for each individual studied. The triangle plots (lower images) in each panel show the proximity of each individual studied to each parental population. Patients are represented in yellow and controls in are pink. AFR, African; AMR, Amerindian; EUR, European.

Discussion

The ancestry informative INDEL panel, used in the present study, consists of highly informative markers with high δ and pairwise F_{ST} values for Amerindian/European, Amerindian/African, and African/European comparisons (Table S2), and is indicated for ancestry estimation in admixed populations with contributions of Amerindian, European, and African ancestry, such as those in this study (24, 25). However, this is the first time that it has been used to investigate differences in ancestral contributions between patients affected by a disease and unaffected controls.

The Patagonian region has been identified as a cluster with a high prevalence of CL±P (1.76/1000) in an epidemiological study using ECLAMC material, which suggests that the high incidence of Amerindian ancestry in the population could be contributing to this high prevalence (12).

A study conducted with samples from four observed clusters of high CL±P prevalence (12) (Patagonia, Northwest Argentina, the Bolivian Altiplano, and Ecuador including two contiguous cities in southern Colombia), which used the same patients with CL±P from Patagonia who were evaluated in the present study, estimated, using uniparental markers, that individuals with Amerindian ancestry have 2.5-times higher risk for CL±P than do individuals without Amerindian ancestry (13). Nevertheless, despite the trend of finding, in Patagonia, greater Amerindian ancestry in patients with CL±P than in controls, this difference was not significant in the present study.

If confirmed in future studies, the apparent lack of difference in Amerindian ancestry evaluated by autosomal markers between patients with CL±P and controls from Patagonia could indicate an aetiological contribution to CL±P linked to genes that are present in mitochondrial DNA or the X-chromosome, which result in inheritance patterns completely different from that of autosomal chromosomes. Genome-wide studies have identified variants in regions and genes of the X-chromosome that are associated with CL±P (26). The association between variants of the dystrophin gene (*DMD*; Xp21.2-21.1) and CL±P was recently confirmed in a sample of 26 families, with a high contribution of Amerindian ancestry and from the same Patagonian population evaluated in the present study. This association indicates that these variants may be in linkage disequilibrium with genes on the X-chromosome that contribute to the aetiology of CL±P in this population (27). Epidemiological data showing a higher prevalence of CL±P in men than in women (28) also favour the hypothesis of causal genes for CL±P that are specific for the X-chromosome.

One previous study conducted in samples from two Brazilian hospitals located in the city of Alfenas in Minas Gerais State and the city of Salvador in Bahia State found higher African ancestry and lower European ancestry among patients with CL±P than in controls (29). This result is uncommon and might be related to the high European/African admixture of the subjects with CL±P. An admixed origin of mother or proband has already been suggested (30) and dismissed (31) as a risk factor for oral clefts. This unexpected result observed by Aquino *et al.* (29), in addition to our findings, suggests that association between the risk for CL±P and genomic ancestry might be more complex than previously estimated.

The pooled relative risks (RR) of CL±P in each ethnic group, considering data published by Greene *et al.* (4), Emanuel *et al.* (5), Croen *et al.* (6), DeRoo *et al.* (7), Hashmi *et al.* (8), and Canfield *et al.* (9), indicated risk for CL±P as significantly higher for individuals declared as Native American (RR = 1.92) and significantly lower for individuals declared as African American (RR = 0.59) when compared with individuals declared as European American. The pooled relative risks of CP in African American subjects compared with European American subjects, using data from the same studies (4–9), confirmed a low birth prevalence of CP in African American subjects (RR = 0.68), similar to that observed in CL±P. However, there was no high birth prevalence of CP in Native American subjects compared with European American subjects, as shown for CL±P (RR = 1.08). According to these previous epidemiologic studies in North American populations, we should expect significantly higher Amerindian ancestry and lower African ancestry in patients with CL±P than in controls, and significantly lower African ancestry in patients with CP than in controls, which was not found in the populations from Patagonia and Belém. In addition, we found a significantly higher contribution of European ancestry to patients from Belém with CP than to controls, which has not been reported in previous studies.

We could assume that individuals declared as African, Native, or European American in the previously mentioned North American populations show a different genomic ancestry composition from those found in the same ethnic groups in South American populations. In the US population, self-declared European American subjects have higher European genomic ancestry (98.6%) (32) than those from Latin America (Mexico, Colombia, Peru, Brazil, and Chile) who are self-declared as White (in whom European genomic ancestry ranges from 47% in Peru to 85% in Brazil) (17). The same is observed for African ancestry, which shows a higher frequency in self-declared African American subjects from the USA (73.2%) than in Black people from Latin America (in whom African genomic ancestry ranges from 13% in Chile to 69% in Colombia). This difference demonstrates that the population in Latin America is more admixed. Therefore, the relative risks for CP and CL±P in the same ethnic groups in Latin American populations might be different from those reported in North American populations.

The currently accepted risks for CL±P are high in Native Americans subjects, intermediate in European American subjects, and low in African American subjects (2), but the low risk in African American subjects seen in the epidemiologic studies with subjects with CP

(4–9), could not be demonstrated using autosomal markers in the populations analysed from Patagonia and Belém. The high European genetic ancestry noted in subjects from Belém with CP has not been reported in American populations. Further studies in other populations with a similar, high contribution of Native, African, and European American ancestries are still needed for a better understanding of the relationship between European ancestry and the risk for CP.

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Supporting Information

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

Table S1. Articles found in the literature search reporting birth prevalence of oral clefts (per 1,000 births).

Table S2. Frequencies of INDEL markers in cleft cases and controls and parental populations; paired F_{ST} and δ (absolute difference) frequencies among the three parental populations.