Published in final edited form as:

Am J Med Genet A. 2009 December ; 149A(12): 2673–2679. doi:10.1002/ajmg.a.33089.

Whorl Patterns on the Lower Lip are Associated with Nonsyndromic Cleft Lip with or without Cleft Palate

Katherine Neiswanger^{1,2}, Kevin W. Chirigos¹, Cherise M. Klotz¹, Margaret E. Cooper^{1,2}, Kathleen M. Bardi¹, Carla A. Brandon¹, Seth M. Weinberg^{1,2}, Alexandre R. Vieira^{1,2,3}, Rick A. Martin⁴, Andrew E. Czeizel⁵, Eduardo E. Castilla^{6,7}, Fernando A. Poletta⁷, and Mary L. Marazita^{1,2,8,9}

¹Center for Craniofacial and Dental Genetics, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA

²Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA

³Department of Pediatric Dentistry, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA

⁴Division of Medical Genetics, School of Medicine, St. Louis University, St. Louis, MO

⁵Genetikai Ártalmak Társadalmi Megelőzése, Budapest, Hungary

⁶ECLAMC (Latin-American Collaborative Study of Congenital Malformations) at Laboratório de Epidemiologia de Malformações Congênicas, Instituto Oswaldo Cruz, FIOCRUZ, Rio de Janeiro, Brazil

⁷CEMIC: Centro de Educación Médica e Investigación Clínica, Buenos Aires, Argentina

⁸Clinical and Translational Sciences Institute, School of Medicine, University of Pittsburgh, Pittsburgh, PA

⁹Department of Psychiatry, School of Medicine, University of Pittsburgh, Pittsburgh, PA

Abstract

Nonsyndromic cleft lip with or without cleft palate (CL/P) is a common birth defect due to both genetic and environmental factors. Whorl lip print patterns are circular grooves on the central upper lip and/or the left and right lower lip. To determine if whorls are more common in families with CL/P than in controls, the Pittsburgh Orofacial Cleft Study collected lip prints from over 450 subjects, i.e., individuals with CL/P, their relatives, and unrelated controls—from the U.S., Argentina, and Hungary. Using a narrow definition of lower-lip whorl, the frequency of whorls in the U.S sample was significantly elevated in cleft individuals and their family members, compared to unrelated controls (14.8% and 13.2% versus 2.3%; $P = 0.003$ and 0.001 , respectively). Whorls were more frequent in CL/P families from Argentina than in CL/P families from the U.S. or Hungary. If these results are confirmed, whorl lip print patterns could be part of an expanded phenotypic spectrum of nonsyndromic CL/P. As such, they may eventually be useful in a clinical setting, allowing recurrence risk calculations to incorporate individual phenotypic information in addition to family history data.

Keywords

lip prints; lip whorls; cheiloscropy; cleft lip; cleft palate

INTRODUCTION

Nonsyndromic cleft lip with or without cleft palate (CL/P) is a common birth defect that stems from both genetic and environmental factors [Marazita, 2002; Hayes, 2002; Jugessur and Murray, 2005; Olasoji et al., 2005; Lidral and Moreno, 2006; Carinci et al., 2007; Vieira, 2008]. Prevalences range as high as 3.6:1,000 in some Native American groups [Gorlin et al., 2001] to 1.2:1,000 in Asians [Cooper et al., 2006], approximately 1:1,000 in Caucasians [Gorlin et al., 2001; Mossey and Little, 2002], and 0.3:1,000 in African-derived populations [Gorlin et al., 2001; Mossey and Little, 2002]. Reasons for the population variation in CL/P remain obscure, although genetic variation in different racial groups is one possible factor.

Cheiloscropy—the study of the patterns of lines and grooves on the vermilion borders of the lips—has continued sporadically for a century [Hirth et al., 1975]. Whorl lip print patterns can occur on the upper and/or lower lips. An upper lip whorl is a single circular pattern of grooves centered on the midline of the upper lip. Lower lip whorls are circular patterns located on the left and/or right of the lower lip midline. Whorls were first noted by Hirth and his colleagues in Germany over thirty years ago as part of their extensive study of the variability and genetics of lip print patterns [Hirth et al., 1974; Hirth et al., 1975; Hirth et al., 1976; Hirth and Goedde, 1977; Hirth et al., 1977; Hirth et al., 1978].

Hirth et al. [1976; 1977; 1978] observed that the frequency of whorls on the lower lip was increased in nonsyndromic CL/P patients and their families, and speculated that the whorls they observed in families with clefts might represent a mild form of lower lip pits or fistulae [Hirth et al., 1976; Hirth et al., 1978]. If whorl lip prints are indeed more frequent in families with CL/P, they may be part of an expanded phenotypic spectrum of nonsyndromic CL/P [Weinberg et al., 2006]. To test this hypothesis, we estimated the frequency of lip print whorl patterns in nonsyndromic CL/P families and controls from three different populations in the U.S., Argentina, and Hungary. If whorls are associated with clefting, this easily identifiable trait could eventually prove useful in the clinic by helping to tailor recurrence risk estimates to each CL/P family member based on both their subclinical phenotypic features and their family history.

MATERIALS AND METHODS

Subjects

The Pittsburgh Orofacial Cleft Study is a multinational research effort that collects extensive phenotypic data and DNA on families with nonsyndromic CL/P and controls, in order to identify cleft susceptibility genes [Weinberg et al., 2006]. For this study, individuals with a clinical diagnosis of nonsyndromic cleft lip $\pm$ cleft palate who attended cleft or surgical clinics in the U.S.A. (Pittsburgh, PA and St. Louis, MO), Hungary (Budapest), and in a geographic cluster of oral clefting in Patagonia, Argentina [Poletta et al., 2007], were screened for a positive family history of nonsyndromic CL/P (after informed consent approved by the U.S. and local IRBs). Multiplex CL/P families, i.e., those with more than one affected individual, were preferentially recruited. Individuals with known syndromic forms of clefting, as well as those with multiple anomalies that did not fit any recognizable syndrome, were excluded. The nuclear families of eligible subjects, as well as all available extended relatives, were invited to participate. Unrelated controls were recruited in the U.S.

and Hungary through marketing agencies and advertising flyers, and by word of mouth. Controls with any known family history of clefting in relatives as distant as cousins were excluded. Subjects from the U.S. and Hungary were of Caucasian ancestry, while in Argentina the subjects were Caucasian/Amerindian (Mapuche) mestizos.

Lip prints

Lip prints were collected from over 450 subjects. After obtaining informed consent (University of Pittsburgh IRB 0607057), we used the Faurot inkless fingerprinting system (Faurot™ Forensic Products, Inc., Raleigh, NC) to obtain lip prints. Subjects rubbed their lips with non-toxic, invisible ink and then pressed their mouths against chemically treated paper to create the lip prints. We obtained four or more sets of prints from each subject, using varied amounts of ink and different mouth expressions. To better visualize the prints, we scanned the raw data at high resolution and contrast-enhanced the prints by substituting blue for the original grey color. See Figure 1 for examples of contrast-enhanced lip print patterns.

Three raters who were blind to the subjects' cleft status scored the upper midline, and lower left and right paramedial regions of the lip independently, using the following rating system: 1) Definite whorl; 2) Unusual whorl (asymmetric or laterally-positioned circular patterns or "U"-shaped whorls on upper lip); 3) Possible whorl (incomplete, faint, or unclear circular patterns); 4) No whorl; and 5) Unknown (relevant region of the print missing or too unclear to rate). The raters then reached a consensus for each of the three lip regions, so that every subject's lip print was assigned three ratings.

The reliability of this system was assessed by having two different groups of raters score 50 lip prints (150 ratings) twice in two years. The proportion of overall agreement between old and new ratings ranged from 78% for the upper lip to 90% for the lower-left lip. κ statistics ranged from 0.51 to 0.72, indicating generally fair to good inter-rater agreement [Altman, 1991]. For those ratings that differed between the first and second groups of raters, the second rating was used. One subject in the reliability study had completely discrepant ratings and was excluded from further analysis.

Phenotype Definition

Two definitions of whorl were developed from the lip ratings. The first was a narrow definition that only included whorls rated "Definite." The second was a broad definition that included whorls rated "Definite," "Unusual," or "Possible." For both whorl definitions, we correlated the ratings of the upper, lower left, and lower right lip. As expected, whorls on the lower left and right lip were highly correlated (tetrachoric correlations 0.90 – 0.97). On the other hand, upper lip whorls did not correlate strongly with either lower left or lower right whorls (tetrachoric correlations 0.18 – 0.29). This was consistent with Hirth et al., [1975], who noted that whorls could be present on the upper, lower, or both upper and lower lips. Because of the low correlations, and because the upper lip patterns of individuals with cleft lips were often obscured as a result of surgery, upper lip whorls were not included in the phenotype definition. Instead, whorls were analyzed as a dichotomous trait determined by the presence of a whorl (narrow or broad definition) on the left and/or right sides of the lower lip.

Data Analysis

The frequencies of whorls on the lower lip were compared among individuals from the U.S., Argentina, and Hungary, including subjects with CL/P, their unaffected relatives, and unrelated controls. For these case-control comparisons, subjects were selected from families

to create unrelated groups. Statistical analyses, including standard χ^2 and Fisher Exact tests, were performed using SAS [SAS Institute, 2002–2003] or web-based GUIs.

RESULTS

We selected 113 unrelated individuals with CL/P, 198 unrelated non-cleft family members, and 142 unrelated controls for this case-control study. Note that since CL/P individuals and non-cleft relatives came from the same families, subjects in the first group had relatives in the second. Table I presents the sample by population and sex, while Table II provides the frequencies of lower-lip whorls by population and phenotype definition. The whorl status of a few individuals was unknown for some portion of their lip ratings, resulting in slightly smaller sample sizes in Table II than Table I.

Whorl frequencies varied extensively, depending on both the phenotype definition and the population (Table II). Because of these differences, as well as the lack of Argentinean and Hungarian controls, all analyses were performed separately by population. Under the narrow phenotype, 14.8% of the U.S. subjects with CL/P and 13.2% of their family members had whorls, compared to only 2.3% of the controls ($P = 0.003$ and 0.001 , respectively; Fisher Exact test); whorl frequencies did not differ significantly between individuals with CL/P and their non-cleft relatives. In contrast, Argentinean subjects with CL/P had significantly more whorls than their unaffected family members (32.3% versus 10.9%, $P = 0.04$; Fisher Exact test). Unrelated controls were not available from Argentina, so the general population frequency of whorls could not be estimated. None of the participants from Hungary had a definite whorl on their lower lip.

When the broad definition of whorl status was employed, the frequency of lower-lip whorls increased considerably in each population—to 22.4% in the U.S. sample, 60.0% in Argentina, and 9.8% in Hungary (Table II). In the U.S. sample, whorl frequencies were again elevated in CL/P families, although the increase was significant only for non-cleft family members compared to controls (29.4% versus 15.0%, $P = 0.008$; Fisher Exact test). The frequency of whorls was very high in the Argentinean families; even so, CL/P cases had significantly more whorls than their non-cleft relatives (73.5% versus 50.0%, $P = 0.04$; Fisher Exact test). In Hungary, the frequency of whorls in CL/P individuals jumped to 21.1% (4/19), and whorls were more frequent in CL/P cases than in non-cleft family members (21.1% versus 2.9%, $P = 0.05$; Fisher Exact test), although the small sample sizes from Hungary make these observations tentative.

DISCUSSION

We have observed that whorls on the lower lip are associated with nonsyndromic CL/P, no matter which definition of whorls is employed. Not surprisingly, the frequency of whorls differs widely depending on phenotype definition (Table II). The narrow definition includes only definite whorls, thus representing the core whorl phenotype. Definite whorls were five-fold more common in cleft families (~14%) than unrelated controls (2.3%) from the U.S. The broad definition, on the other hand, encompasses all possible whorls, which adds a number of subjects whose phenotypic status is less certain. Individuals from U.S. cleft families have a whorl frequency of ~27% under the broad definition, almost double the 15% seen in controls. Controls were not available from either Argentina or Hungary for comparison.

These findings are similar to those of Hirth et al. [1976], who reported that lower-lip whorls were present in 59% of the relatives of CL/P individuals ($N = 22$) from Germany, compared to 15% in German controls ($N = 500$). Finally, a Polish study found one lower-lip whorl in

twenty individuals with CL/P (5%), but did not examine any controls [Pruszewicz et al., 1988]. There is no description of exactly how the whorls in the above reports were scored; therefore a direct comparison between their observations and this study is not possible. Taken together, however, the literature and the current study demonstrate that whorls on the lower lip are increased in cleft individuals and their families, regardless of methodological or racial differences, and thus may be part of the expanded phenotypic spectrum of nonsyndromic CL/P.

The field of cheiloscopy has not been extensively developed. Basic research has demonstrated that, similar to fingerprints, lip prints are unique to an individual, constant over time, and very similar in monozygotic twin pairs [Suzuki and Tsuchihashi, 1970; Tsuchihashi, 1974; Hirth et al., 1975]. Hirth et al. [1974; 1975; 1977] and Tsuchihashi [1974] have suggested that lip print patterns are inherited. Furthermore, whorls might be inherited as an autosomal dominant trait [Hirth et al., 1975, 1977; Hirth and Goedde, 1977]. However, a formal genetic segregation analysis of lip prints has yet to be performed. To our knowledge, this summarizes the extent of the general research on lip print patterns, excluding forensic and other personal identification applications [e.g., Tsuchihashi, 1974; Williams, 1991; Alvarez et al., 2000; Ball, 2002; Caldas et al., 2007].

Because of the relative lack of information concerning lip prints, it was necessary to develop protocols to collect, rate, and analyze lip print data in the absence of well-established procedures. Reported protocols for collecting lip prints include taking photographs and two-dimensional impressions, which are then traced under magnification onto cellophane [Suzuki and Tsuchihashi, 1970; Tsuchihashi, 1974], or applying lipstick and transferring the pattern to cellophane tape for analysis and storage [Sivapathasundharam et al., 2001]. Lip print recognition architecture is also being developed to include lip prints in conjunction with other physical features for biometric identification [Kim et al., 2004].

Following Hirth et al. [1974; 1975], we employed the Faurot method, developed originally to obtain dermatoglyphics using invisible, non-toxic ink and chemically sensitive paper. The Faurot method was chosen because it is simple, safe, relatively reliable, and the prints are easy to store and analyze by computer. It was not completely satisfactory, however, because the prints were often incomplete or over-/under-inked. To compensate for these drawbacks, four or more prints were taken from most subjects. However, some subjects find the ink irritating. Thus, we are investigating alternative methods, such as photographs with an intra-oral camera or digital scanning, which will allow us to reliably capture the three-dimensional surface of the lips with greater clarity and contrast than the Faurot system permits.

Several rudimentary schemes have been proposed to classify lip print variation, e.g., simple versus compound branching patterns [Santos, 1967; Hirth et al., 1974], a set of distinct branching patterns [Suzuki and Tsuchihashi, 1970; Hirth et al., 1975; Sivapathasundharam et al., 2001], and formulas derived from each specific groove [Renaud, 1973]. None of these systems has gained widespread acceptance. The first rating system we used was a modification of Suzuki and Tsuchihashi [1970], who distinguished five pattern types: 1) Straight vertical lines; 2) Branched lines; 3) Intersected lines; 4) Reticular lines; 5) Others. The lip prints in Figures 1A and 1B illustrate straight vertical and branched types, respectively. The whorl pattern type was added to this classification system by Hirth et al. [1975], and is shown in Figure 1D. To rate a lip print, the upper and lower lips are divided into four horizontal sections and one of the above types is assigned to each section, so that a single lip print is characterized by eight numbers.

This system proved difficult to implement. Many prints had different patterns on their lateral and central regions or on their upper and lower lips. Furthermore, the lip pattern often resembled a combination of two distinct types (see Figure 1C). Finally, the Type 5 “Other” pattern was over-used, and included both different/unusual and unclear pattern types. The two original raters (KN and KWC) did not agree on the complete set of eight scores for at least half of the first hundred prints rated.

Since our primary interest was in whorls, we devised a simplified system (described above in Methods—Lip Prints), in which the central upper lip and paramedial lower left and right lip were scored only for the presence/absence of whorls. The inter-rater reliability of this simplified protocol was adequate (proportion of overall agreement: 78 – 90%; κ s: 0.51–0.72), which, while not completely satisfactory, was sufficient to proceed with the analysis. Because raters were blind to the cleft status of the subjects, we felt that any issues with reliability would lower the power of the sample, but not bias the results. The positive results that we have observed are thus conservative, and may well become stronger as the reliability of the data improves.

With these methodological caveats in mind, our data show that lower-lip whorls are more common in cleft families from the Patagonian region in Argentina than in cleft families from two sites in the U.S., and least common in cleft families from Hungary. For the broad definition of whorl, both Argentinean cleft individuals and their unaffected relatives had significantly more whorls than their counterparts from the U.S. (CL/P: 73.5% versus 25.4%, respectively, $P = 0.00001$; non-cleft relatives: 50% versus 29.3%, respectively, $P = 0.017$; Fisher Exact tests). Although sample sizes from Hungary were small, U.S. non-cleft relatives had significantly more whorls than Hungarian relatives (29.3% versus 2.9%, $P = 0.00009$; Fisher Exact test). Differences between the populations were similar using the narrow definition, but were not significant. Larger samples will be needed to verify these observations.

The magnitude of the differences in lip whorl frequencies across the U.S., Argentinean, and Hungarian samples precluded pooling them for analysis. Prevalence rates of nonsyndromic CL/P also vary in these three groups, with Patagonians from Argentina and Chile showing an unusually high rate of 1.75:1,000 [Poletta et al., 2007], compared to 1.03 and 1.05 per 1,000 for Hungarians [Czeizel, 1997] and U.S. Caucasians from California [Croen et al., 1998], respectively. It is tempting to speculate that the extremely high rates of lip whorls in the Argentinean sample are a direct reflection of the high frequency of nonsyndromic CL/P seen in the Patagonian region of Argentina. However, larger samples of cleft families, as well as a sizeable set of unrelated controls from Argentina will be needed to explore this notion further. It will also be important to collect a large set of unrelated controls from Hungary, to establish the baseline rate of whorls in this country. Finally, it will be of great interest to obtain lip print data from nonsyndromic cleft families and ethnically matched controls in additional countries around the world, to better understand the extent of the variation in this phenotype and how it correlates with clefting.

Among the genes which have been associated with nonsyndromic clefting (e.g., *MSX1*, *TGFA*, *PVRL1*, *FGFR1* [Vieira, 2008]), the interferon regulatory factor 6 gene (*IRF6*) is an obvious candidate for lip whorls. Mutations in *IRF6* cause Van der Woude syndrome (VWS; OMIM # 119300) [Kondo et al., 2002; Du et al., 2006], while different variants in the gene have been associated with nonsyndromic CL/P in numerous populations around the world [Zuccherro et al., 2004; Blanton et al., 2005; Ghassibé et al., 2005; Scapoli et al., 2005; Srichomthong et al., 2005; Morküniené et al., 2006; Park et al., 2007; Vieira et al., 2007; Jugessur et al., 2008; Suazo et al., 2008; Diercks et al., 2009; Tang et al., 2009]. VWS is a rare, autosomal dominant form of clefting that is phenotypically quite similar to

nonsyndromic CL/P. Affected individuals may have clefts of the lip and/or palate, along with hypodontia and/or lip pits (present in about 80% of VWS cases [Schinzel and Klauser, 1986]). Lip pits can be elevated or indented, and vary widely in their severity [Rizos and Spyropoulos, 2004], to the extent that mild lip pits have been missed during clinical evaluation of cleft families, and only found with the knowledge that *IRF6* variants were segregating in the families [Birnbbaum et al., 2008]. It will be of great interest to examine VWS families for lip whorls, in order to determine if they represent the most attenuated form of lip pits.

Recently, SNPs from the *IRF6* gene region have been associated with isolated tooth agenesis, another phenotype closely related to VWS [Vieira et al. 2007; Vieira et al., 2008]. With larger samples, we likewise plan to analyze the lip whorl phenotype for associations with *IRF6*. If whorls are associated with *IRF6*, they may represent an extremely attenuated form of the lip pits seen in VWS, and thus be a phenotypic marker of the *IRF6* contribution to the risk of nonsyndromic CL/P. In any event, the association of lower-lip whorls and nonsyndromic CL/P, if confirmed, suggests a connection between the development of the superficial lower lip and the upper lip/palate that will be of great interest to explore.

Acknowledgments

We thank the families and individuals in Pittsburgh, PA, and St. Louis, MO, U.S.A., Hungary, and throughout Patagonia, Argentina, who have participated in the Pittsburgh Oral Facial Cleft Study (POFC), without whom this work would not be possible. Lip print data was collected from Patagonia with the assistance and hard work of Drs. Ieda Orioli, Jorge Lopez-Camelo, and Juan Carlos Mereb, from ECLAMC. Lip prints were collected through the efforts of Eszter Nyulászi and Julia Métneki in Hungary, and of Sybil Naidoo in St. Louis. In Pittsburgh, Rebecca DeSensi and Toby McHenry provided data management, Megan Branning processed images for scoring, and Megan, Judith Resick, Pooja Gandhi, Anna Kamelin, and Jennifer Moeller scored lip prints from all sites. Dr. Karen T. Cuenco's discussions of this project were most helpful. This work was supported by NIH grants R01-DE016148, R21-DE016930, P50-DE016215, and partially supported by Agencia Nacional de Promoción Científica y Tecnológica (ANPCyT), Argentina, Grant number: PICTO-CRUP 2005 # 31101; and Edital N ° 15/2008-MCT/CNPq/Institutos Nacionais de Ciência e Tecnologia, INAGEMP (Instituto Nacional de Genética Médica Populacional); Processo 573993/2008-4. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Dental and Craniofacial Research, or the National Institutes of Health.

REFERENCES

- Altman, DG. Practical Statistics for Medical Research. London: Chapman and Hall; 1991. p. 611
- Ball J. The current status of lip prints and their use for identification. *J Forens Odonto-Stomat*. 2002; 20:43–46.
- Alvarez M, Miquel M, Castello A, Verdu FA. Persistent lipsticks and their lip prints; new hidden evidence at the crime scene. *Forens Sci Int*. 2000; 112:41–47.
- Birnbbaum S, Reutter H, Lauster C, Scheer M, Schmidt G, Saffar M, Martini M, Hemprich A, Henschke H, Kramer FJ, Mangold E. Mutation screening in the *IRF6*-gene in patients with apparently nonsyndromic orofacial clefts and a positive family history suggestive of autosomal-dominant inheritance. *Am J Med Genet Part A*. 2008; 146A:787–790. [PubMed: 18247422]
- Blanton SH, Cortez A, Stal S, Mulliken JB, Finnell RH, Hecht JT. Variation in *IRF6* contributes to nonsyndromic cleft lip and palate. *Am J Med Genet Part A*. 2005; 137A:259–262. [PubMed: 16096995]
- Caldas IM, Magalhães T, Afonso A. Establishing identity using cheiloscopy and palatoscopy. *Forens Sci Int*. 2007; 165:1–9.
- Carinci F, Scapoli L, Palmieri A, Zollino I, Pezzetti F. Human genetic factors in nonsyndromic cleft lip and palate: An update. *Int J Pediatr Otorhinolaryng*. 2007; 71:1509–1519. [PubMed: 17606301]
- Cooper ME, Ratay JS, Marazita ML. Asian Oral-Facial Cleft Birth Prevalence. *Cl Pal Craniof J*. 2006; 43:580–589.

- Croen LA, Shaw GM, Wasserman CR, Tolarová MM. Racial and ethnic variations in the prevalence of orofacial clefts in California, 1983–1992. *Am J Med Genet.* 1998; 79:42–47. [PubMed: 9738868]
- Czeizel AE. First 25 years of the Hungarian Congenital Abnormality Registry. *Teratol.* 1997; 55:299–305.
- Diercks GR, Karnezis TT, Kent DT, Flores C, Su GH, Lee JH, Haddad J Jr. The association between interferon regulatory factor 6 (IRF6) and nonsyndromic cleft lip with or without cleft palate in a Honduran population. *Laryngoscope.* 2009; 119 published online.
- Du X, Tang W, Tian W, Li S, Li X, Liu X, Zheng X, Chen X, Lin Y, Tang Y. Novel IRF6 mutations in Chinese patients with Van der Woude Syndrome. *J Dent Res.* 2006; 85:937–940. [PubMed: 16998136]
- Ghassibè M, Benedicte B, Revencu N, Verellen-Dumoulin C, Gillerot Y, Vanwijck R, Vikkula M. Interferon regulatory factor-6: a gene predisposing to isolated cleft lip with or without cleft palate in the Belgian population. *Eur J Hum Genet.* 2005; 13:1239–1242. [PubMed: 16132054]
- Gorlin, RJ.; Cohen, MM.; Hennekam, RCM. *Syndromes of the Head and Neck.* Oxford: Oxford University Press; 2001. p. 850-876.
- Hayes, C. Environmental risk factors and oral clefts. In: Wyszynski, DF., editor. *Cleft Lip & Palate.* Oxford: Oxford University Press; 2002. p. 159-169.
- Hirth L, Goedde HW. Zur variabilität und formalin genetik der lippenfurchen. *Anthrop Anz.* 1977; 36:51–57.
- Hirth L, Goedde HW, Pfeifer G, Kastein I. Lippenfurchen und hautleisten bei zwilligen mit lippenkiefer-(gaumen)-spalten. *Z Morph Anthropol.* 1978; 69:197–204.
- Hirth L, Goedde HW, Pfeifer G, Kreybig T. Besonderheiten der Lippenfurchen bei den eltern von patienten mit lippenkiefer-(gaumen)-spalten. *Z Morph Anthropol.* 1976; 67:345–352.
- Hirth L, Göttische H, Goedde HW. Untersuchungen zur variabilität und genetik der Lippenfurchen. *Z Morph Anthropol.* 1974; 65:362–366.
- Hirth L, Göttische H, Goedde HW. Lippenfurchen — variabilität und genetik. *Humangenetik.* 1975; 30:47–62. [PubMed: 1237462]
- Hirth L, Göttische H, Goedde HW. Lip prints — variability and genetics. *J Hum Evol.* 1977; 6:709–710.
- Jugessur A, Murray JC. Orofacial clefting: recent insights into a complex trait. *Curr Opin Genet Devel.* 2005; 15:270–278. [PubMed: 15917202]
- Jugessur A, Rahimov F, Lie1 RT, Wilcox AJ, Gjessing HK, Nilsen RM, Truc Trung Nguyen TT, Murray JC. 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.* 2008; 32:413–424. [PubMed: 18278815]
- Kim JO, Lee W, Hwang J, Baik KS, Chung CH. Lip print recognition for security systems by multi-resolution architecture. *Fut Gener Comp Sys.* 2004; 20:295–301.
- Kondo S, Schutte BC, Richardson RJ, Bjork BC, Knight AS, Watanabe Y, Howard E, Ferreira de Lima RLL, Daack-Hirsch S, Sander A, McDonald-McGinn DM, Zackai EH, Lammer EJ, Aylsworth AS, Ardinger HH, Lidral AC, Pober BR, Moreno L, Arcos-Burgos M, Valencia C, Houdayer C, Bahuau M, Moretti-Ferreira D, Richieri-Costa A, Dixon MJ, Murray JC. Mutations in *IRF6* cause van der woude and popliteal pterygium syndromes. *Nature Genet.* 2002; 32:285–289. [PubMed: 12219090]
- Lidral AC, Moreno LM. Progress toward discerning the genetics of cleft lip. *Curr Opin Pediatr.* 2006; 17:731–739. [PubMed: 16282779]
- Marazita, ML. Segregation analyses. In: Wyszynski, DF., editor. *Cleft Lip & Palate.* Oxford: Oxford University Press; 2002. p. 222-233.
- Morkūnienė A, Steponavičiūtė D, Kasnauskienė J, Kucinskas V. Nucleotide sequence changes in the *MSX1* and *IRF6* genes in Lithuanian patients with nonsyndromic orofacial clefting. *Acta Médica Lituanica.* 2006; 13:219–225.
- Mossey, PA.; Little, J. Epidemiology of oral clefts: an international perspective. In: Wyszynski, DF., editor. *Cleft Lip & Palate.* Oxford: Oxford University Press; 2002. p. 127-159.
- Olasoji HO, Ukiri OE, Yahaya A. Incidence and aetiology of oral clefts: a review. *Afr J Med Med Sci.* 2005; 34:1–7. [PubMed: 15971547]

- Park JW, McIntosh I, Hetmanski JB, Jabs EW, Vander Kolk CA, Wu-Chou YH, Chen PK, Chong SS, Yeow V, Jee SH, Park BY, Fallin MD, Ingersoll R, Scott AF, Beaty TH. Association between IRF6 and nonsyndromic cleft lip with or without cleft palate in four populations. *Genet Med*. 2007; 9:219–227. [PubMed: 17438386]
- Poletta FA, Castilla EE, Orioli IM, López-Camelo JS. Regional Analysis on the Occurrence of Oral Clefts in South America. *Am J Med Genet Part A*. 2007; 143A:3216–3227. [PubMed: 18000905]
- Pruszewicz A, Gąsiorek J, Obrębowski A. Use of cheiloscropy in the clinical evaluation of cleft palate patients. *Otolaryng Pol*. 1988; 42:265–270.
- Renaud M. Cheiloscopic identification in forensic medicine. *Nouv Presse Med*. 1973; 2:2617–2620. [PubMed: 4793319]
- Rizos M, Spyropoulos MN. Van der Woude syndrome: a review. Cardinal signs, epidemiology, associated features, differential diagnosis, expressivity, genetic counselling and treatment. *Eur J Orthodont*. 2004; 26:17–24.
- Santos CM. Queiloscropy, a supplementary stomatological means of identification. *Int Microform J Leg Med*. 1967; 2:1–15.
- SAS Institute, Inc.. SAS® Procedures Guide, version 9.1.3. Cary, NC: SS Institute Inc; 2002–2003.
- Scapoli L, Palmieri A, Martinelli M, Pezzetti F, Carinci P, Tognon M, Carinci F. Strong evidence of linkage disequilibrium between polymorphisms and the IRF6 locus and nonsyndromic cleft lip with or without cleft palate, in an Italian population. *Am J Hum Genet*. 2005; 76:180–183. [PubMed: 15558496]
- Schinzel A, Kläuser M. The Van der Woude syndrome (dominantly inherited lip pits and clefts). *J Med Genet*. 1986; 23:291–294. [PubMed: 3746828]
- Sivapathasundharam B, Ajay Prakash P, Sivakumar G. Lip prints (cheiloscropy). *Int J Dent Res*. 2001; 12:234–237.
- Srichomthong C, Siriwan P, Shotelersuk V. Significant association between IRF6 820G→A and nonsyndromic cleft lip with or without cleft palate in the Thai population. *J Med Genet*. 2005; 42(7):e46. [PubMed: 15994871]
- Suazo J, Santos JL, Jara L, Blanco R. Linkage disequilibrium between *IRF6* variants and nonsyndromic cleft lip/palate in the Chilean population. *Am J Med Genet Part A*. 2008; 146A:2706–2708. [PubMed: 18798331]
- Suzuki K, Tsuchihashi Y. New attempt of personal identification by means of lip print. *J Ind Dent Assoc*. 1970; 42:8–9.
- Tang W, Du X, Feng F, Long J, Lin Y, Li P, Liu L, Tian W. Association analysis between the *IRF6* G820A polymorphism and nonsyndromic cleft lip and/or cleft palate in a Chinese population. *CI Pal Craniof J*. 2009; 46:89–92.
- Tsuchihashi Y. Studies on personal identification by means of lip prints. *Forens Sci*. 1974; 3:233–248.
- Vieira A. Unraveling human cleft lip and palate research. *J Dent Res*. 2008; 87:119–125. [PubMed: 18218836]
- Vieira AR, Cooper ME, Marazita ML, Orioli IM, Castilla EE. Interferon regulatory factor 6 (*IRF6*) is associated with oral-facial cleft in individuals that originate in South America. *Am J Med Genet Part A*. 2007; 143A:2075–2078. [PubMed: 17702008]
- Vieira AR, Modesto A, Meira R, Barbosa ARS, Lidal AC, Murray JC. Interferon regulatory factor 6 (*IRF6*) and fibroblast growth factor receptor 1 (*FGFR1*) contribute to human tooth agenesis. *Am J Med Genet Part A*. 2007; 143A:538–545. [PubMed: 17318851]
- Vieira AR, Seymen F, Patir A, Menenzes R. Evidence of linkage disequilibrium between polymorphisms at the IRF6 locus and isolate tooth agenesis, in a Turkish population. *Arch Oral Biol*. 2008; 53:780–784. [PubMed: 18452891]
- Weinberg SM, Neiswanger K, Martin RA, Mooney MP, Kane AA, Wenger SL, Losee J, Deleyiannis F, Ma L, De Salamanca JE, Czeizel AE, Marazita ML. The Pittsburgh Oral-Facial Cleft study: Expanding the cleft phenotype. Background and justification. *CI Pal Craniof J*. 2006; 43:7–20.
- Williams TR. Lip prints – another means of identification. *J Forens Ident*. 1991; 41:190–194.
- Zuccherro TM, Cooper ME, Maher BS, Daack-Hirsch S, Nepomuceno B, Ribeiro L, Caprau D, Christensen K, Suzuki Y, Machida J, Natsume N, Yoshiura KI, Vieira AR, Orioli IM, Castilla EE, Moreno L, Arcos-Burgos M, Lidal AC, Field LL, Liu YE, Ray A, Goldstein TH, Schultz RE, Shi

M, Johnson MK, Kondo S, Schutte BC, Marazita ML, Murray JC. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *NEJM*. 2004; 351:19–30.

A. Straight Vertical **B. Branching** **C. Diamond + Reticular** **D. Lower Lip Whorls** **Figure 1.**

Table 1

Sample for Case-Control Analysis, by Sex and Population

Cleft Status by Sex		U.S.	Argentina	Hungary	All Populations
CL/P Cases	Male	32	21	9	62
	Female	25	16	10	51
Non-Cleft Relatives	Male	54	13	15	82
	Female	63	34	19	116
Controls	Male	48	0	4	52
	Female	86	0	4	90
Total		308	84	61	453

Table II
Frequency (%) of Lower Lip Whorls in CL/P Families and Controls, by Population and Phenotype Definition

Sample	Phenotype Definition	Population			Total
		U.S.	Argentina	Hungary	
CL/P Cases	Narrow	8/54 ^a * (14.8%)	10/31 ^e (32.3%)	0/18 (0%)	18/103 (17.5%)
	Broad	14/55 ^b (25.4%)	25/34 ^f (73.5%)	4/19 (21.1%)	43/108 (39.8%)
CL/P Family Members	Narrow	15/114 ^c (13.2%)	5/46 ^e (10.9%)	0/34 (0%)	20/194 (10.3%)
	Broad	34/116 ^d (29.3%)	23/46 ^f (50.0%)	1/34 (2.9%)	58/196 (29.6%)
Controls	Narrow	3/132 ^{a,c} (2.3%)	—	0/8 (0%)	3/140 (2.1%)
	Broad	20/133 ^{b,d} (15.0%)	—	1/8 (12.5%)	21/141 (14.9%)
Total	Narrow	26/300 (8.7%)	15/77 (19.5%)	0/60 (0%)	41/437 (9.4%)
	Broad	68/304 (22.4%)	48/80 (60.0%)	6/61 (9.8%)	122/445 (27.4%)

* Fisher Exact tests: Narrow definition Broad definition

- ^aP = 0.003
- ^bP = 0.10
- ^cP = 0.001
- ^dP = 0.008
- ^eP = 0.037
- ^fP = 0.04