

REPORT

Mutations in *BMP4* Are Associated with Subepithelial, Microform, and Overt Cleft Lip

Satoshi Suzuki,^{1,2,4} Mary L. Marazita,^{5,6} Margaret E. Cooper,⁵ Nobutomo Miwa,^{7,8} Anne Hing,⁹ Astanand Jugessur,¹⁰ Nagato Natsume,¹ Kazuo Shimozato,³ Naofumi Ohbayashi,³ Yasushi Suzuki,¹ Teruyuki Niimi,¹ Katsuhiko Minami,¹ Masahiko Yamamoto,⁴ Tserendorj J. Altannamar,¹¹ Tudevдорj Erkhembaatar,¹¹ Hiroo Furukawa,¹ Sandra Daack-Hirsch,¹² Jamie L'Heureux,² Carla A. Brandon,⁵ Seth M. Weinberg,^{5,13} Katherine Neiswanger,⁵ Frederic W.B. Deleyiannis,¹⁴ Javier E. de Salamanca,¹⁵ Alexandre R. Vieira,^{5,16} Andrew C. Lidral,² James F. Martin,¹⁷ and Jeffrey C. Murray^{2,12,18,*}

Cleft lip with or without cleft palate (CL/P) is a complex trait with evidence that the clinical spectrum includes both microform and subepithelial lip defects. We identified missense and nonsense mutations in the *BMP4* gene in 1 of 30 cases of microform clefts, 2 of 87 cases with subepithelial defects in the *orbicularis oris* muscle (OOM), 5 of 968 cases of overt CL/P, and 0 of 529 controls. These results provide confirmation that microforms and subepithelial OOM defects are part of the spectrum of CL/P and should be considered during clinical evaluation of families with clefts. Furthermore, we suggest a role for *BMP4* in wound healing.

Isolated clefts of the lip with or without cleft palate (CL/P [MIM 119530]) comprise about 70% of all children born with an orofacial cleft. Cases of overt CL/P display a range of severity, from notches in the vermilion to complete bilateral clefts of the lip and palate.^{1,2} Also observed are more subtle expressions of the CL/P phenotype that are sometimes termed “microforms” and typically involve small defects of the lip, alveolar arch, or scar-like ridges above the lip.^{3,4} Such microforms extend to the muscle fibers of the superior *orbicularis oris* muscle (OOM).^{5,6} Microform cleft lip (congenital healed cleft lip [MIM 600625]) is a rarely reported birth defect that is suggested to occur in 0.06 /10,000 live births.⁷ Even more subtle than the visible microforms are subepithelial defects of the OOM. Martin et al.⁸ observed such defects in 18-week-old fetuses and developed a method to visualize OOM defects by using ultrasonography.⁹ A significant increase in the frequency of OOM defects has been reported in people who are related to individuals with CL/P but who do not have overt clefts.⁹ The fact that subepithelial defects of the OOM are part of the spectrum of orofacial cleft expression was also confirmed by Neiswanger et al.¹⁰ Furthermore, histologic studies of OOMs visualized as abnormal by ultrasound showed both disor-

ganized OOM fibers and excess connective tissue in comparison to normal OOMs.¹¹

Informative mouse models for cleft lip and cleft palate have aided the search for genes involved in human CL/P^{12–14}, as have rare Mendelian CL/P forms that are close phenocopies of isolated CL/P.^{15,16} The current study was motivated by a mouse model in which a conditional knockout for *Bmp4* (MIM 112262) had an unusual “healed” cleft-lip phenotype¹⁷; Liu et al.^{17,18} found that all embryos had bilateral cleft lip at 12 days after conception but that by 14.5 days only 22% still exhibited cleft lip. They hypothesized that many of the initial cleft lips had healed in utero. In this report we evaluate the *BMP4* gene in individuals born with overt CL/P, microform CL/P, and subepithelial OOM defects.

Clinical aspects of the sample collection have been described elsewhere.^{10,19,20} The University of Iowa Institutional Review Board (IRB) gave approval for sample collection (approval numbers 199804081, 200003065, and 200109094) in conjunction with local approval in the Philippines, Colombia, and Children's Hospital and Regional Medical Center, Seattle, Washington. The Ohio State University approved sample collection in Ohio (approval number 98H0041). The University of Pittsburgh

¹Division of Research and Treatment for Oral and Maxillofacial Congenital Anomalies, School of Dentistry, Aichi-Gakuin University, 2-11 Suemori-Dori, Chikusa-Ku, Nagoya 464-8651, Japan; ²University of Iowa, Department of Pediatrics, S Grand Avenue, 2182 ML, Iowa City, IA 52242, USA; ³Department of Maxillofacial Surgery, School of Dentistry, Aichi-Gakuin University, 2-11 Suemori-Dori, Chikusa-Ku, Nagoya 464-8651, Japan; ⁴Faculty of Psychological and Physical Science, Aichi-Gakuin University, 12 Araike, Iwasaki-cho, Nisshin 470-0195 Japan; ⁵Center for Craniofacial and Dental Genetics, and Department of Oral Biology, and Department of Human Genetics, University of Pittsburgh, Pittsburgh, PA 15219, USA; ⁶Department of Psychiatry, University of Pittsburgh, Pittsburgh, PA 15219, USA; ⁷Department of Human Genetics, Nagasaki University Graduate School of Biomedical Sciences, Sakamoto 1-12-4, Nagasaki 852-8523, Japan; ⁸Solution Oriented Research for Science and Technology, Japan Science and Technology Agency, Tokyo, Japan; ⁹University of Washington, Center on Human Development and Disability, Seattle, WA 35632, USA; ¹⁰Murdoch Childrens Research Institute, Royal Children's Hospital, Victoria 3052, Australia; ¹¹Maternal and Children's Health Research Center Hospital, Ulaanbaatar 210624, Mongolia; ¹²College of Nursing, The University of Iowa, Iowa City, IA 52242, USA; ¹³Department of Psychiatry, Carver College of Medicine, University of Iowa, Iowa City, IA 52242, USA; ¹⁴Division of Plastic Surgery, Department of Surgery, School of Medicine, University of Pittsburgh, Pittsburgh, PA 15219, USA; ¹⁵Department of Plastic Surgery, Niño Jesús University Hospital, Universidad Autónoma, Madrid 28009, Spain; ¹⁶Department of Pediatric Dentistry, University of Pittsburgh, Pittsburgh, PA 15219, USA; ¹⁷Institute of Biosciences and Technology, Texas A&M Health Science Center, 2121 Holcombe Boulevard, Houston, TX 77030, USA; ¹⁸University of Iowa, Departments of Pediatric Dentistry, Epidemiology and Biology, Iowa City, IA 52242

*Correspondence: jeff-murray@uiowa.edu

DOI 10.1016/j.ajhg.2009.02.002. ©2009 by The American Society of Human Genetics. All rights reserved.

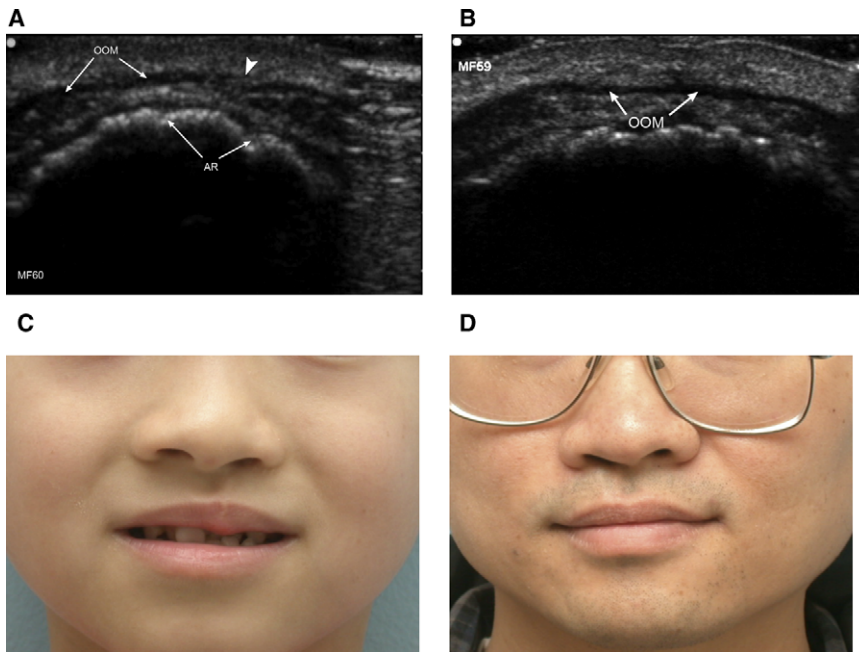


Figure 1. Subepithelial and Microform Defects

Ultrasound Images of the Proband S91C, Who Has an *Orbicularis Oris* Muscle Defect, and Her Husband

Ultrasound images are taken in the transverse plane; the top of the image corresponds to anterior structures; OOM = *orbicularis oris* muscle; AR = alveolar ridge. The arrowhead in (A) points to the discontinuity in the OOM of the proband (A). In contrast, note the continuous appearance of the husband's OOM (B). (C) A child (A346V) has left microform CLP. (D) His father (A346V) has subtle right microform cleft lip and a "heart-shaped" bifid uvula. See Table S1 for a summary of the cases and controls sequenced for *BMP4*.

approved sample collection in Pittsburgh, Spain, Guatemala, and the Philippines (approval numbers 0405013, 0511198, and 0607057) after approval by the IRBs of the local sites. The ethics committee of the School of Dentistry, Aichi-Gakuin University, approved the sample collection in Mongolia (approval number 11) in conjunction with local approval in Mongolia. DNA samples from 30 individuals with microform cleft lip (27 Filipinos, two Americans, and one Colombian), from 87 cases with OOM defects (nine Filipinos, 31 Americans, 34 Guatemalans, and 13 Europeans), from 968 cases with overt CL/P (537 Filipinos and 431 Mongolians) and from 529 controls (345 Filipinos, 90 Mongolians, and 94 Europeans) were sequenced according to published protocols.¹⁶ Five primer pairs (Table S2) were used for examining all four *BMP4* exons and 50 bp of flanking sequence (NP_001193). M.L.M., A.C.L., A.H., A.R.V., J.C.M., or S.D.H. reviewed subjects with CL/P to exclude any with syndromic features. OOM defects were assessed by high-resolution ultrasound according to the methods of Neiswanger et al.¹⁰ (Figures 1A and 1B). Note that all cases and controls are unrelated; only one cleft or OOM defect case was taken from any particular family.

Table S1 in the Supplemental Data summarizes the origins of the cases and controls studied. The mutations identified in the cases and their relatives are provided in Table S2 and depicted in Figure 2. No mutations were found in the controls. Five of the total 968 cases (0.51%) with overt nonsyndromic CL/P were found to have previously unreported missense or nonsense mutations. Two of the total 87 cases with OOM defects and one of the 30 cases with microform CL/P had novel missense mutations. One common missense-encoding SNP (p.A152V, c.455 C > T, SNP rs17563) was found in both cases and controls, with a borderline-significant difference in

frequency (the frequency of the C allele for the case and control is 0.20 and 0.26, respectively, and that for the T allele for the case and control is 0.80 and 0.74, respectively) ($p = 0.04$).

Differences between groups were assessed with Fisher's exact tests (see Web Resources). The frequency of *BMP4* mutations was greater for all cases (overt CL/P plus microforms plus OOM defects) than for all controls (8/1085 = 0.74% versus 0/529 = 0%) but was not statistically significant ($p = 0.06$). Notably, the *BMP4* mutation frequency for overt CL/P cases alone was not significantly greater than for controls (5/968 = 0.52% versus 0/529, $p = 0.17$), whereas the frequency for microform plus OOM cases was significantly greater than for controls (3/117 = 2.56% versus 0/529, $p = 0.006$). Furthermore, the *BMP4* mutation frequency in overt CL/P cases was significantly less than the frequency in microform plus OOM cases (5/968 versus 3/117, $p = 0.04$).

Comparative amino acid sequence analysis (Figure 2) showed complete conservation of the amino acid sequence in eight species (human, chimpanzee, cow, rat, dog, chicken, frog, and zebrafish) at positions p.S91C (c.271 A > T), p.R162Q (c.485 G > A), p.R287H (c.860 G > A), and p.A346V (c.1037 C > T). The missense mutation S91C was predicted to be possibly damaging by POLYPHEN (see Web Resources)²¹, and the R162Q and A346V variants are predicted to be intolerant amino acid changes by SIFT (see Web Resources).²² In addition, one nonsense mutation, p.R198X (c.592 C > T), was identified. The microform case with the missense mutation A346V is shown along with his son in Figures 1C and 1D. The father (1D) has a bifid uvula (MIM 192100) and microform cleft lip and the son with the same mutation has a microform cleft lip and a cleft palate. Two other cases with missense mutations (S91C and R287H) were parents who had

($p = 0.006$), but the frequency in overt CL/P cases did not reach significance ($p = 0.17$).

A recent report demonstrates that disruptions in BMP4 and Hedgehog signaling result in craniofacial developmental anomalies of the brain, eye, and digits (MCOPS6 [MIM 607932]).²⁴ They demonstrate ocular clefting-like phenotypes (coloboma [MIM 120200]) in some cases and found both frameshift and missense *BMP4* mutations that generate a more severe and general phenotype than the mutations reported here. In addition both *SHH* [MIM 600725]²⁵ and *PTCH1* [MIM 601309]²⁶ have been associated with clefting in mice and humans.

The results reported here are consistent with findings in the *Bmp4* mouse model^{17,18}, suggesting either that amino acid alterations in BMP4 result in delayed lip closure (resulting in the appearance of a healed scar) or that actual healing of the cleft has occurred through an unknown mechanism. The mouse model is a conditional knockout of craniofacial tissues and so is not directly analogous to the heterozygous amino acid variants observed here, but the striking phenotypic overlap of the unusual clinical appearances adds support to the connections between the genotypes and phenotypes observed. Bmp signaling has been implicated in dorsal closure in *Drosophila*, a model system for wound healing. Our finding that *BMP4* has a role in microform and subepithelial clefting is consistent with the speculation that a conserved genetic pathway might be involved in both wound healing and CL/P.²⁷ Identifying factors that modulate wound healing in the embryo would afford opportunities both for identification of groups at high risk for poor wound healing (where surgical approaches or timing might be influenced) and for the use of these factors to enhance the wound repair itself.

It can be difficult to prove that any particular rare variant observed is etiologic in a complex trait unless those variants are strong candidates on the basis of expression and arise de novo, as was seen in a recent report suggesting that spontaneous mutations in *VANGLI* (MIM 610132) could be a risk factor for neural-tube defects (NTD [MIM 182940]).²⁸ Kryukov²⁹ has provided empiric evidence that about 70% of missense mutations present at population frequencies of 1% or less probably contribute to the phenotype in which they are first identified. Identifying eight such rare missense variants in this cleft-related population (and identifying none in controls) would mean that there is a probability of more than 99.9% that at least one of these variants contributes to clefting. A range of other genes has been reported in which rare missense or nonsense mutations also appear to contribute to isolated CL/P.^{12–14,16,30–32} In this report we found a significant overrepresentation of *BMP4* mutations in cases with a range of lip and OOM defects and an absence of such variants in more than 500 control samples. These findings support a role for *BMP4* in the pathogenesis of isolated CL/P and/or in wound healing.

Furthermore, our observation that amino acid variations in *BMP4* are associated with microform and subepithelial

OOM defects provides confirmation that such subtle defects are part of the clinical spectrum of CL/P. Given that children with overt clefts had parents with microform cleft or OOM defects, genetic evaluation and counseling might now benefit from a full family phenotypic evaluation assessing such minor forms of clefts and including a high-resolution ultrasound of the upper-lip OOM. Mutations in *BMP4* should therefore be considered in any family whose non-overt-cleft members exhibit microform clefts or OOM defects. Prospective evaluation of the impact of these and other subclinical phenotypes on the occurrence of cleft lip and palate will increasingly be important in genetic counseling of nonsyndromic orofacial cleft families.³³

Supplemental Data

Supplemental Data include two tables and are available with this article online at <http://www.ajhg.org/>.

Acknowledgments

We are particularly grateful to the many patients and their families who willingly participated in this study. We were greatly assisted by many people, including those in the Department of Maxillofacial Surgery, School of Dentistry, Aichi-Gakuin University; those in the Japanese Cleft Palate Foundation (Aichi-Gakuin University, Nagoya, Japan), which is a medical organization that provides clinical care for underserved populations in Japan, Vietnam, Mongolia and Cambodia; and the many physicians and volunteers in Mongolia. We thank the outstanding technicians in our team, members of the Operation Smile Foundation for their invaluable assistance, Children of the Americas (in Guatemala), Kathy Bardi, Judith Resick, Edith Villanueva, Min Shi, Susie McConnell, Nancy Davin, and Daniel Benton. This work was supported by National Institutes of Health grants R37-DE08559 (J.C.M., M.L.M.), R01-DE016148 (M.L.M.), R21-DE016930 (M.L.M., F.W.B.D.), R01 DE/HD012324, P50-DE016215 (J.C.M., M.L.M.), CRR/CTSA Grant UL1-RR024153 (M.L.M.), and P30 ES05605, as well as AGU High-Tech-Research Center Project, KAKENHI, Grant-in-Aid for Young Scientists (S.S.) No. 20791560 (Aichi-Gakuin). Also, this work was made possible by a donation from the Iowa Order of the Eastern Star.

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.

Received: September 26, 2008

Revised: January 10, 2009

Accepted: February 2, 2009

Published online: February 26, 2009

Web Resources

The URLs for the data and analytic approaches presented herein are as follows:

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/>

PolyPhen, <http://genetics.bwh.harvard.edu/pph/>

SIFT, http://blocks.fhcr.org/sift/SIFT_BLink_submit.html

UCSC Genome Browser, <http://genome.ucsc.edu/>

Kanehisa Laboratories, <http://www.kegg.org/>

Preacher KJ. Calculation for the chi-square tests: An interactive calculation tool for chi-square tests of goodness of fit and independence [computer software], <http://www.quantpsy.org>

References

- Gorlin, R.J., Cohen, M.M., and Hennekam, R.C.M. (2001). Orofacial Clefting Syndromes. In *Syndromes of the Head and Neck*, M. Bobrow, P.S. Harper, and C. Scriver, eds. (New York: Oxford University Press), pp. 850–860.
- Eppley, B.L., van Aalst, J.A., Robrey, A., Havlik, R.J., and Sadove, A.M. (2005). The spectrum of orofacial clefting. *Plast. Reconstr. Surg.* *115*, 101e–114e.
- Cosman, B., and Crikelair, G.F. (1966). The minimal cleft lip. *Plast. Reconstr. Surg.* *37*, 334–340.
- Carstens, M.H. (2000). The spectrum of minimal clefting: process oriented cleft management in the presence of an intact alveolus. *J. Craniofac. Surg.* *11*, 270–294.
- Lehman, J.A., and Artz, J.S. (1976). The minimal cleft lip. *Plast. Reconstr. Surg.* *58*, 306–309.
- Heckler, F.R., Oesterle, L.G., and Jabaley, M.E. (1979). The minimal cleft lip revisited: clinical and anatomic correlations. *Cleft Palate J.* *6*, 240–247.
- Castilla, E.E., and Martinez-Frias, M.L. (1995). Congenital healed cleft lip. *Am. J. Med. Genet.* *58*, 106–112.
- Martin, R.A., Jones, K.L., and Benirschke, K. (1993). Extension of the cleft lip phenotype: The subepithelial cleft. *Am. J. Med. Genet.* *47*, 744–747.
- Martin, R.A., Hunter, V., Neufeld-Kaiser, W., Flodman, P., Spence, M.A., Furnas, D., and Martin, K.A. (2000). Ultrasonographic detection of orbicularis oris defects in first-degree relatives of isolated cleft lip patients. *Am. J. Med. Genet.* *90*, 155–161.
- Neiswanger, K., Weinberg, S.M., Rogers, C.R., Brandon, C.A., Cooper, M.E., Bardi, K.M., Deleyiannis, F.W., Resick, J.M., Bowen, A., Mooney, M.P., et al. (2007). Orbicularis oris muscle defects as an expanded phenotypic feature in nonsyndromic cleft lip with or without cleft palate. *Am. J. Med. Genet. A.* *143A*, 1143–1149.
- Rogers, C.R., Weinberg, S.M., Smith, T.D., Deleyiannis, F.W.B., Mooney, M.P., and Marazita, M.L. (2008). Anatomical basis for apparent subepithelial cleft lip: A histological and ultrasonographic survey of the *orbicularis oris* muscle. *Cleft Palate Craniofac. J.* *45*, 518–524.
- van den Boogaard, M.J., Dorland, M., Beemer, F.A., and van Amstel, H.K. (2000). *MSX1* mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat. Genet.* *24*, 342–343.
- Jezewski, P.A., Vieira, A.R., Nishimura, C., Ludwig, B., Johnson, M., O'Brien, S.E., Daack-Hirsch, S., Schultz, R.E., Weber, A., Nepomucena, B., et al. (2003). Complete sequencing shows a role for *MSX1* in non-syndromic cleft lip and palate. *J. Med. Genet.* *40*, 399–407.
- Watanabe, A., Akita, S., Tin, N.T., Natsume, N., Nakano, Y., Niikawa, N., Uchiyama, T., and Yoshiura, K. (2006). A mutation in *RYK* is a genetic factor for nonsyndromic cleft lip and palate. *Cleft Palate Craniofac. J.* *43*, 310–316.
- Zuccherro, T.M., Cooper, M.E., Maher, B.S., Daack-Hirsch, S., Nepomuceno, B., Ribeiro, L., Caprau, D., Christensen, K., Suzuki, Y., Machida, J., et al. (2004). Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *N. Engl. J. Med.* *351*, 769–780.
- Riley, B.M., Mansilla, M.A., Ma, J., Daack-Hirsch, S., Maher, B.S., Raffensperger, L.M., Russo, E.T., Vieira, A.R., Dode, C., Mohammedi, M., et al. (2007). Impaired FGF signaling contributes to cleft lip and palate. *Proc. Natl. Acad. Sci. USA* *104*, 4512–4517.
- Liu, W., Sun, X., Braut, A., Mishina, Y., Behringer, R.R., Mina, M., and Martin, J.F. (2005). Distinct functions for *Bmp* signaling in lip and palate fusion in mice. *Development* *132*, 1453–1461.
- Liu, W., Selever, J., Murali, D., Sun, X., Brugger, S.M., Ma, L., Schwartz, R.J., Maxson, R., Furuta, Y., and Martin, J.F. (2005). Threshold-specific requirements for *BMP4* in mandibular development. *Dev. Biol.* *283*, 282–293.
- Marazita, M.L., Murray, J.C., Lidral, A.C., Arcos-Burgos, M., Cooper, M.E., Goldstein, T., Maher, B.S., Daack-Hirsch, S., Schultz, R., Mansilla, M.A., et al. (2004). Meta-analysis of 13 genome scans reveals multiple cleft lip/palate genes with novel loci on 9q21 and 2q32–35. *Am. J. Hum. Genet.* *75*, 161–173.
- Murray, J.C., Daack-Hirsch, S., Buetow, K.H., Munger, R., Espina, L., Paglinawan, N., Villanueva, E., Rary, J., Magee, K., and Magee, W. (1997). Clinical and epidemiologic studies of cleft lip and palate in the Philippines. *Cleft Palate Craniofac. J.* *34*, 7–10.
- Ramensky, V., Bork, P., and Sunyaev, S. (2002). Human non-synonymous SNPs: Server and survey. *Nucleic Acids Res.* *30*, 3894–3900.
- Ng, P.C., and Henikoff, S.T. (2003). SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* *31*, 3812–3814.
- Lin, J.Y., Chen, Y.J., Huang, Y.L., Tang, G.P., Zhang, L., Deng, B., Li, M., Ma, H., and Luan, R.S. (2008). Association of bone morphogenetic protein 4 gene polymorphisms with nonsyndromic cleft lip with or without cleft palate in Chinese children. *DNA Cell Biol.* *27*, 601–605.
- Bakrania, P., Efthymiou, M., Klein, J.C., Salt, A., Bunyan, D.J., Wyatt, A., Ponting, C.P., Martin, A., Williams, S., Lindley, V., et al. (2008). Mutations in *BMP4* cause eye, brain, and digit developmental anomalies: Overlap between *BMP4* and hedgehog signaling pathways. *Am. J. Hum. Genet.* *82*, 304–319.
- Thomason, H.A., Dixon, M.J., and Dixon, J. (2008). Facial clefting in *Tp63* deficient mice results from altered *Bmp4*, *Fgf8* and *Shh* signaling. *Dev. Biol.* *321*, 273–282.
- Mansilla, M.A., Cooper, M.E., Goldstein, T., Castilla, E.E., Lopez Camelo, J.S., Marazita, M.L., and Murray, J.C. (2006). Contributions of *PTCH* gene variants to isolated cleft lip and palate. *Cleft Palate Craniofac. J.* *43*, 21–29.
- Martin, P., and Parkhurst, S.M. (2004). Parallels between tissue repair and embryo morphogenesis. *Development* *131*, 3021–3034.
- Kibar, Z., Torban, E., McDearmid, J.R., Reynolds, A., Berghout, J., Mathieu, M., Kirillova, I., De Marco, P., Merzell, E., Hayes, J.M., et al. (2007). Mutations in *VANGL1* associated with neural-tube defects. *N. Engl. J. Med.* *356*, 1432–1437.
- Kryukov, G.V., Pennacchio, L.A., and Sunyaev, S.R. (2007). Most rare missense alleles are deleterious in humans: Implications for complex disease and association studies. *Am. J. Hum. Genet.* *80*, 727–739.
- Vieira, A.R., Avila, J.R., Daack-Hirsch, S., Dragan, E., Felix, T.M., Rahimov, F., Harrington, J., Schultz, R.R., Watanabe, Y.,

- Johnson, M., et al. (2005). Medical sequencing of candidate genes for nonsyndromic cleft lip and palate. *PLoS Genet.* *1*, e64.
31. Chiquet, B.T., Lidral, A.C., Stal, S., Mulliken, J.B., Moreno, L.M., Arcos-Burgos, M., Valencia-Ramirez, C., Blanton, S.H., and Hecht, J.T. (2007). CRISPLD2: A novel NSCLP candidate gene. *Hum. Mol. Genet.* *16*, 2241–2248.
32. Scapoli, L., Palmieri, A., Martinelli, M., Vaccari, C., Marchesini, J., Pezzetti, F., Baciliero, U., Padula, E., Carinci, P., and Carinci, F. (2006). Study of the PVRL1 gene in Italian nonsyndromic cleft lip patients with or without cleft palate. *Ann. Hum. Genet.* *70*, 410–413.
33. Weinberg, S.M., Neiswanger, K., Martin, R.A., Mooney, M.P., Kane, A.A., Wenger, S.L., Losee, J., Deleyiannis, F., Ma, L., De Salamanca, J.E., et al. (2006). The Pittsburgh oral-facial cleft study: expanding the cleft phenotype. Background and justification. *Cleft Palate Craniofac. J.* *43*, 7–20.

Note Added in Proof

In the version of this paper published online on February 26, the legend of [Figure 2](#) contained descriptions for panels A and B, although no such panels were included in the figure. The legend has since been corrected online and in print through the removal of these panel labels. No additional text changes were made.