Published in final edited form as:

Am J Med Genet A. 2011 July ; 155(7): 1646–1653. doi:10.1002/ajmg.a.34063.

Microdeletion 20p12.3 involving *BMP2* contributes to syndromic forms of cleft palate

Trilochan Sahoo^{1,*}, Aaron Theisen¹, Pedro A. Sanchez-Lara^{2,3}, Michael Marble⁴, Daniela N. Schweitzer², Beth S. Torchia¹, Allen N. Lamb¹, Bassem A. Bejjani¹, Lisa G. Shaffer¹, and Yves Lacassie⁴

¹ Signature Genomics, Spokane, WA, USA

² Keck School of Medicine, University of Southern California, Los Angeles, CA, USA

³ Childrens Hospital Los Angeles, Los Angeles, CA, USA

⁴ Louisiana State University Health Sciences Center and Children's Hospital New Orleans, New Orleans, LA, USA

Abstract

Orofacial clefts of the lip and/or palate comprise one of the most common craniofacial birth defects in humans. Though a majority of cleft lip and/or cleft palate (CL/P) occurs as isolated congenital anomalies, there exist a large number of Mendelian disorders in which orofacial clefting is part of the clinical phenotype. Here we report on two individuals and one multi-generational family with microdeletions at 20p12.3 that include the bone morphogenetic protein 2 (*BMP2*) gene. In two probands the deletion was almost identical at ~600 kb in size, and *BMP2* was the only gene deleted; the third case had a ~5.5-Mb deletion (20p13p12.2) that encompassed at least 20 genes including *BMP2*. Clinical features were significant for cleft palate and facial dysmorphism in all three patients, including Pierre-Robin sequence in two. Microdeletion 20p13p12 involving *BMP2* is rare and has been implicated in Wolff-Parkinson-White (WPW) syndrome with neurocognitive deficits and with Alagille syndrome when the deletion includes the neighboring *JAG1* gene in addition to *BMP2*. Despite a significant role for the BMPs in orofacial development, heterozygous loss of *BMP2* has not been previously reported in patients with syndromic clefting defects. Because *BMP2* was the sole deleted gene in Patients 1 and 2 and one of the genes deleted in Patient 3, all of whom had clinical features in common, we suggest that haploinsufficiency for *BMP2* is a crucial event that predisposes to cleft palate and additional anomalies. Lack of significant phenotypic components in family members of Patient 1, suggests variable expressivity for the phenotype.

Keywords

Cleft palate; *BMP2*; microdeletion; 20p12.3; Wolff-Parkinson-White syndrome

INTRODUCTION

Cleft lip and/or palate (CL/P) comprise the most common congenital craniofacial anomalies in humans; over two-hundred syndromes have CL/P as a feature(s) [Gorlin et al., 2001]. The etiology is complex with genetic and environmental factors playing significant roles. With a

For correspondence and reprints: Trilochan Sahoo, MD, Quest Diagnostics Nichols Institute, 33608 Ortega Highway, San Juan Capistrano, CA 92690, Ph: 949.728.4760, Fax: 949.728.4979, tsahoo001@gmail.com.

*current affiliation: Quest Diagnostics Nichols Institute, San Juan Capistrano, CA

prevalence of >1/1000 live births, CL/P contributes significantly to physical and psychosocial morbidity in those affected. High familial recurrence and observed concordance rates in monozygotic twins suggest a strong genetic component in the etiology of orofacial clefting.

Normal development of the palate is complex. It requires synergy between growth and patterning of different facial primordia. Developmental processes of the vertebrate face are a well-orchestrated interplay of complex genetic cascades that involves a number of crucial developmental genes such as transforming growth factor- β family and their receptors (*TGF- β* , *TGFBR*), including members of the bone morphogenetic protein (*BMP*) family; fibroblast growth factors and their receptors (*FGFs* and *FGFRs*); and T-box transcription factors (*TBX*) [Schutte and Murray, 1999; Spritz, 2001; Stanier and Moore, 2004; Jugessur et al., 2009].

The *TGF- β* superfamily of growth factors regulates many components of skeletal development, including cartilage and bone formation, mesoderm patterning, and craniofacial and limb development [Zhang et al., 2002; Wan and Cao, 2005; Meng et al., 2009]. BMPs are members of the *TGF- β* superfamily and important participants in craniofacial development. Binding to and functioning via BMP receptors, BMPRI and BMPRII, they result in phosphorylation-dependent activation of the SMAD proteins and downstream signaling cascades. In addition to SMAD proteins, BMPs activate multiple kinase pathways (MAPK, PI3 kinase, etc.) [Kishigami and Mishina, 2005]. Starting from early stages of neural crest formation and until development of the complete orofacial musculoskeletal system, BMPs, in concert with other genes, are prominently expressed in the facial primordia and palate. Another significant interaction is that between BMPs and homeobox gene *MSX1* on 4p16.3. *MSX1* is required for *BMP4* and *BMP2* expression. *Msx1* deficiency results in cleft secondary palate and tooth agenesis in mice that can be rescued by *Bmp4*. Similarly, *MSX* deficiency causes nonsyndromic cleft palate with tooth agenesis in humans [van den Boogaard et al., 2000; Zhang et al., 2002]. Interestingly, *MSX1* appears to be an important genetic element in 4p16.3 microdeletions that result in Wolf-Hirschhorn syndrome [Nieminen et al., 2003]. Additional evidence linking orofacial clefting to members of the *Fgf* family and their receptors has been obtained from studies in transgenic mice [Abu-Issa et al., 2002; Nie et al., 2006].

Evidence from knockouts of conserved homologs in mouse and *drosophila* [Dudas et al., 2004; Liu et al., 2005; Le Gloan et al., 2008; Lalani et al., 2009] suggest a critical role in skeletal, cardiac and neural development. Expression of *Bmp2–5* in palatal epithelia and mesenchyme is crucial during multiple stages of palatogenesis [Lu et al., 2000; Nie, 2005]. Because BMP signaling is also conserved in developing craniofacial skeleton, changes in expression result in alterations of these structures [Dudas et al., 2004; Nie et al., 2006]. In addition, a significant role for Bmps in cardiovascular development is derived from mouse models deficient in *Bmp2* [Zhang and Bradley, 1996; Ma et al., 2005] signaling.

Although no deletions of *BMP2* have been reported in individuals with isolated or syndromic CL/P, variably sized microdeletions within 20p12 that include *BMP2* have been proposed to result in diverse phenotypes including Alagille (AGS), hypoplastic left heart and Wolff-Parkinson-White (WPW) syndromes, a bypass reentrant tachycardia that results from an abnormal connection between the atria and ventricles [Robert et al., 2007; Le Gloan et al., 2008; Lalani et al., 2009]. Inclusion of neighboring gene *JAG1* in one case resulted in a complex phenotype including Alagille and WPW syndrome [Le Gloan et al., 2008]. Deletions involving *JAG1* within the AGS critical region have been reported to result in features of AGS and developmental delay, autism, scoliosis, and bifid uvula [Kamath et al., 2009].

Here we report the clinical and molecular characterization of two individuals and one multi-generational family with microdeletions within chromosomal region 20p13p12.

METHODS

Patient ascertainment

Study Patients 1–3 in this study were referred for microarray testing to Signature Genomic Laboratories for physical and intellectual disabilities and/or dysmorphic features. An Institutional Review Board-approved informed consent was obtained from the appropriate parents or legal guardians of the patients to participate in this study.

Molecular analysis

A 105K-feature whole-genome oligonucleotide microarray (SignatureChipOS v1.0, custom-designed by Signature Genomics, Spokane, WA, manufactured by Agilent Technologies, Santa Clara, CA) was used for microarray analysis of DNA from study Patient 1 according to previously published methods [Ballif et al., 2008b]. A 135K-feature whole-genome oligonucleotide microarray (SignatureChipOS v2.0, custom-designed by Signature Genomics, manufactured by Roche NimbleGen, Madison, WI; coordinates based on UCSC Genome Browser build NCBI Build 36.1/hg18) was used for microarray analysis of DNA from Patient 2 according to previously published methods [Duker et al., 2010]. DNA from study Patient 3 was initially tested using a >4,600-clone whole-genome bacterial artificial chromosome (BAC)-based microarray (SignatureChipWG, Signature Genomics) using previously described methods [Ballif et al., 2008a]. Additional microarray analysis was performed for study Patient 3 using the 105K-feature oligonucleotide microarray to characterize the deletion breakpoints in more detail.

Deletions in the three probands and extended family members of Patient 1 were confirmed and visualized by fluorescence *in situ* hybridization (FISH) with BAC clones from the deletion regions using previously published methods [Shaffer et al., 1994].

RESULTS

Clinical summaries

Patient 1, the probanda in the multi-generational family, is a Caucasian female first referred at 13 days of age for evaluation of cleft palate (Fig 1A, B; Study Patient IV-1, Supplementary Fig 1). She was born at 39 ½ weeks to a 22-year-old G1P0 mother. On physical examination at age 13 days her height was 45 cm (<5th centile), weight was 2.60 kg (<5th centile), and head circumference was 33.3 cm (<5th centile). Besides the cleft palate, micrognathia and glossoptosis suggestive of Pierre Robin sequence, she also had large communicating fontanelles, a long well-formed philtrum, pectus excavatum, diastasis recti, gap between her halluces and second toes with vertical creases, deep palmar flexion creases and short 5th fingers. On examination at age 3 months and 3 weeks, the posterior fontanelle was still open and the anterior fontanelle measured 5 cm × 5 cm. She has a patent foramen ovale. An electrocardiogram was normal.

The family history was significant for a mother (III-1, Supplementary Fig 1) with trochlear nerve palsy (CNP); a high palate; small congenital lumbosacral hemangioma; and hands with short 5th fingers and some unusual dermatoglyphic patterns (Table I). Her height was 172.3 cm and her OFC 54.8 cm. The maternal grandmother (II-1, Supplementary Fig 1) measured 154.5 cm (5th–10th centile), and had trochlear nerve palsy, high palate, white spots in the irises similar to Brushfield spots, and hands with hard-to-see ridges and short 5th finger. The maternal great-grandmother (I-1, Supplementary Fig 1) had short stature

(137.1 cm, <2nd centile), high palate, trochlear nerve palsy, and hands with single palmar flexion crease (Supplementary Fig 1). There were no other significant physical findings or dysmorphism in these extended family members.

Patient 2 is a 7-year-old female who was first referred for re-evaluation of cleft palate, deafness and cholesteatoma. She was born at 37 weeks to a 24-year-old mother. Birth weight was 2.84 kg (25th–50th centile). Birth length was 45.1 cm (< 10th centile). Early childhood medical history was significant for bilateral cholesteatoma and chronic middle ear fluid. She underwent surgical removal of the cholesteatomas followed by prosthetic reconstruction of inner ear bones. The surgery improved her hearing, but she requires hearing aids bilaterally. The patient has had no major language deficits, although she is repeating the second grade owing to problems with reading comprehension. On exam at age 7 years her height was 114.6 cm (10th centile), weight was 19.4 kg (10th–25th centile), and head circumference was 48 cm (< 5th centile). She was noted to have submucous cleft palate, very high-arched hard palate, bifid uvula, large eyes, synophrys, long philtrum, upturned nose and microstomia. There were no significant limb anomalies. She was found to have a zygodactylous triradius between the 2nd and 3rd right toes (Fig 1C, D). She had no cardiovascular problems at primary examination or on continuing follow-up examinations.

Patient 3 is a 2-year-old female born at 41 weeks to a 30-year-old G1 P0–1 mother. Her birth weight was 3.15 kg (25th centile) and length was 47 cm (10th–25th centile). She had a U-shaped cleft palate and micrognathia noted at birth. She failed her newborn hearing exam, although a repeat ABR was normal (Fig 1E, F). On physical exam at 3 months of age, her length was at the 10th centile, weight was <3rd centile, and head circumference was 40.3 cm (50th–75th centile). Dysmorphic facial features included flat facial profile, downslanting palpebral fissures, depressed nasal bridge, small, upturned nose with anteverted nares, pinpoint hemangioma on the tip of the nose, long philtrum, transverse crease across the chin, U-shaped midline cleft palate and micrognathia (Fig 1E,F). She had a prominent forehead with subtle transient vertical skin gyrata. During the first year of life she was noted to have significant motor deficits with a head lag and wobble, delayed/poor reflexes, central hypotonia and feeding difficulties. She had decreased muscle mass and subtle hyperextensibility of her hands and feet with recurrent hip dislocations and developmental dysplasia of the hips. Cardiovascular exam was normal. High-resolution chromosome analysis revealed a *de novo* and apparently balanced translocation t(3;5)(q10;q10)dn.

Molecular analysis

Microarray analysis identified a microdeletion within 20p13p12 in each of the three study patients. Patient 1 had a 592.7-kb deletion (chr20: 6,222,266–6,814,990, based on UCSC hg18 coordinates); Patient 2 had a 566.4-kb deletion (chr20: 6,265,253–6,831,640); and Patient 3 had a 5.37-Mb deletion at 20p13p12.2 (chr20:3,672,605–9,042,183) (Fig 2A–C). For Patient 3, high-resolution chromosome analysis had previously revealed a *de novo* balanced translocation [t(3;5)(q10;q10)dn]. We did not detect any copy number loss or gain at the putative breakpoints in chromosomes 3 and 5 within the limits of resolution of the array used, which has substantial coverage of the pericentromeric regions of chromosomes 3 and 5. The deletions in Patients 1 and 2 encompassed one known gene, *BMP2*. The deletion in Patient 3 encompassed at least 20 known genes (Fig 2D–F).

The deletions in Patients 1 and 2 were confirmed by FISH using BAC probe RP11-184L8, and the deletion in Patient 3 was confirmed by FISH using BAC probes RP11-481K14 and RP11-177P14 (Supplementary Fig 2). FISH analysis was performed on PHA-stimulated peripheral blood lymphoblasts from the mother, maternal grandmother and maternal great-grandmother for Patient 1, and all three individuals were identified to have an apparently identical deletion. FISH on parental samples for Patient 3 showed the deletion to be

apparently *de novo* in origin. Parental samples were unavailable for Patient 2. In addition, no CNVs in the deleted region were identified in the Database of Genomic Variants (<http://projects.tcag.ca/variation/>), suggesting that there are no significant copy-number polymorphisms in this genomic region.

DISCUSSION

CL/P is one of the most common orofacial anomalies occurring as isolated defect(s) or part of complex syndromic disorders. However, identifying monogenic causes for CL/P has been of limited success. The probands reported on here share some significant clinical features, most notably cleft palate and variable facial dysmorphism including long philtrum and micrognathia. Short stature and developmental delay were variable and not seen in all the patients; microcephaly was obvious in only Patients 1 and 2. In addition, Patients 1 and 3 have features of Pierre Robin sequence. The overlapping phenotypes in Patients 1 and 2, both of whom have a deletion that encompasses only one gene, *BMP2*, support a causative relationship between *BMP2* and syndromic cleft palate. The third patient had a ~5.5-Mb deletion that included 20 OMIM annotated genes including *BMP2*. The multiple genes involved in the deletion in this case may contribute to the additional dysmorphism. The identification in Patient 3 of an apparently balanced t(3;5)(q10;q10), although unlikely, cannot definitively rule out the possibility of the disruption of one or more genes at the translocation breakpoints. Nonetheless, the three patients reported here, Patient 1 and 2 in particular, provide novel evidence in support of the hypothesis that hemizygosity for *BMP2* might be one important event in the pathogenesis of syndromic cleft palate. In addition, a clefting deformity, cleft lip, has been reported for one patient with a 10.7-Mb deletion at 20p12p13 that included the *BMP2* gene [Lalani et al., 2009]. The coincidental finding of congenital trochlear nerve palsy in Patients III-1, II-1 and I-1 was intriguing; there was no history of head trauma noted for any of the Patients (Supplementary Fig 1). Any clear correlation of the trochlear nerve palsy to the molecular defect and cleft palate remains remote.

A causative association between haploinsufficiency for *BMP2* and WPW syndrome has recently been suggested from studies in five patients harboring deletions at 20p12 and dysmorphic features and variable neurocognitive deficits [Le Gloan et al., 2008; Lalani et al., 2009]. The four probands and one parent reported by Lalani et al. had deletions ranging in size from 1.1 Mb to 10.7 Mb. Patient 1 in that study had a 1.1-Mb deletion that encompassed *BMP2* but no other genes; this patient had, in addition to WPW, motor and neurocognitive delays and dysmorphic features including downslanting palpebral fissures, bilateral epicanthal folds, broad nasal root and bridge, malar hypoplasia, full cheeks with microstomia, pectus deformity with a carinatum appearance superiorly and excavatum appearance inferiorly, and persistent fetal pads. Some of these features were shared with the patients described in the present study. Interestingly, none of the patients were reported to have had cleft palate [Le Gloan et al., 2008; Lalani et al., 2009], although maxillary hypoplasia was reported in three patients and cleft lip was noted in one patient. Cardiovascular problems were not evident in any of the patients reported here, including normal ECG in Patients 1 and 3. Of note, detailed cardiac electrophysiological studies in a *Bmp2*^{+/-} mouse model did not recapitulate any conduction defects or dysrhythmias [Lalani et al., 2009]. [Lalani et al. 2009] suggest the neurocognitive and musculoskeletal phenotypes are fully penetrant. However, our results suggest variable expressivity of the musculoskeletal phenotype because short stature was present only in Patient 1 and her maternal great-grandmother. In addition, our results suggest *BMP2* may not be associated with neurocognitive delays, because only one of our patients had developmental delay. Mutations of *BMP2* may also show incomplete penetrance for clefting anomalies, as three members of the multi-generational family had a milder form, high palate, and none of the

patients reported by Lalani et al. [2009] were reported to have clefting anomalies. The clefting abnormality in the three probands may result from a combination of *BMP2* haploinsufficiency and additional genetic or non-genetic factors.

Studies in mice suggest endogenous Bmp2 and Bmp6 cooperatively play significant roles in bone formation under both physiological and pathological conditions. Additionally, Bmp2 and Bmp6 are the main subtypes expressed in hypertrophic chondrocytes that induce endochondral bone formation. Fetal and adult compound-deficient mice (Bmp2 +/–; Bmp6 –/–) showed a reduction in the trabecular bone volume with suppressed bone formation with normal bone resorption. However, the single deficient mice (Bmp2 +/– or Bmp6 –/–) did not show the same effects [Kugimiya et al., 2005]. Therefore, *BMP2* haploinsufficiency in these patients might be contributory to growth deficiency, and short stature might be an indirect outcome.

Although haploinsufficiency of *BMP2* may be solely responsible for the phenotypes evident for the patients described above, mutational events might be contributory. Nonetheless, based on the phenotypes of our patients and previous expression studies of the mouse homolog, hemizygous loss of *BMP2* likely contributes to clefting abnormalities. There are few studies using microarray analysis to identify genomic copy number losses and gains in syndromic and nonsyndromic CL/P. Osoegawa et al. [2008] reported whole-genome microarray analysis in 83 syndromic (including 20 with Van der Woude syndrome) and 103 nonsyndromic CL/P cases with an abnormality detection rate of ~1%. One syndromic and two nonsyndromic cases had pathogenic microdeletions suggestive of a contiguous gene deletion syndrome. Our report demonstrates the utility of comprehensive genomic analysis in the genetic diagnosis of CL/P.

The highly conserved BMP signaling pathway and its functional and regulatory interactions with multiple growth factors and pathways deems it reasonable to speculate that perturbation of the above pathways would result in orofacial and skeletal anomalies. The three cases with cleft palate and additional anomalies presented here support a causative relationship between *BMP2* and syndromic forms of cleft palate. Additional cases with similar microdeletion and mutational analysis of BMPs in clefting anomalies with or without short stature will further delineate this genotype-phenotype correlation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors are grateful to all of the participating families which made this research possible. We would like to thank Dr. Ruthanne R Gallagher, MD (Bayou Pediatrics, Houma, LA) for referring Patient 1. PAS is supported by the Harold Amos Faculty Development Program through the Robert Wood Johnson Foundation and the CHLA-USC Child Health Research Career Development Program (NIH K12-HD05954).

References

- Abu-Issa R, Smyth G, Smoak I, Yamamura K, Meyers EN. Fgf8 is required for pharyngeal arch and cardiovascular development in the mouse. *Development*. 2002; 129:4613–4625. [PubMed: 12223417]
- Ballif BC, Theisen A, Coppinger J, Gowans GC, Hersh JH, Madan-Khetarpal S, Schmidt KR, Tervo R, Escobar LF, Friedrich CA, McDonald M, Campbell L, Ming JE, Zackai EH, Bejjani BA, Shaffer LG. Expanding the clinical phenotype of the 3q29 microdeletion syndrome and characterization of the reciprocal microduplication. *Mol Cytogenet*. 2008a; 1:8. [PubMed: 18471269]

- Ballif BC, Theisen A, McDonald-McGinn DM, Zackai EH, Hersh JH, Bejjani BA, Shaffer LG. Identification of a previously unrecognized microdeletion syndrome of 16q11.2q12.2. *Clin Genet*. 2008b; 74:469–475. [PubMed: 18811697]
- Dudas M, Sridurongrit S, Nagy A, Okazaki K, Kaartinen V. Craniofacial defects in mice lacking BMP type I receptor *Alk2* in neural crest cells. *Mech Dev*. 2004; 121:173–182. [PubMed: 15037318]
- Duker AL, Ballif BC, Bawle EV, Person RE, Mahadevan S, Alliman S, Thompson R, Traylor R, Bejjani BA, Shaffer LG, Rosenfeld JA, Lamb AN, Sahoo T. Paternally inherited microdeletion at 15q11.2 confirms a significant role for the SNORD116 C/D box snoRNA cluster in Prader-Willi syndrome. *Eur J Hum Genet*. 2010
- Gorlin, RJ.; Cohen, MM.; Levin, LS. Syndromes of the head and neck. New York: Oxford University Press; 2001.
- Jugessur A, Farlie PG, Kilpatrick N. The genetics of isolated orofacial clefts: from genotypes to subphenotypes. *Oral Dis*. 2009; 15:437–453. [PubMed: 19583827]
- Kamath BM, Thiel BD, Gai X, Conlin LK, Munoz PS, Glessner J, Clark D, Warthen DM, Shaikh TH, Mihci E, Piccoli DA, Grant SF, Hakonarson H, Krantz ID, Spinner NB. SNP array mapping of chromosome 20p deletions: genotypes, phenotypes, and copy number variation. *Hum Mutat*. 2009; 30:371–378. [PubMed: 19058200]
- Kishigami S, Mishina Y. BMP signaling and early embryonic patterning. *Cytokine Growth Factor Rev*. 2005; 16:265–278. [PubMed: 15871922]
- Kugimiya F, Kawaguchi H, Kamekura S, Chikuda H, Ohba S, Yano F, Ogata N, Katagiri T, Harada Y, Azuma Y, Nakamura K, Chung UI. Involvement of endogenous bone morphogenetic protein (BMP) 2 and BMP6 in bone formation. *J Biol Chem*. 2005; 280:35704–35712. [PubMed: 16109715]
- Lalani SR, Thakuria JV, Cox GF, Wang X, Bi W, Bray MS, Shaw C, Cheung SW, Chinault AC, Boggs BA, Ou Z, Brundage EK, Lupski JR, Gentile J, Waisbren S, Pursley A, Ma L, Khajavi M, Zapata G, Friedman R, Kim JJ, Towbin JA, Stankiewicz P, Schnittger S, Hansmann I, Ai T, Sood S, Wehrens XH, Martin JF, Belmont JW, Potocki L. 20p12.3 microdeletion predisposes to Wolff-Parkinson-White syndrome with variable neurocognitive deficits. *J Med Genet*. 2009; 46:168–175. [PubMed: 18812404]
- Le Gloan L, Pichon O, Isidor B, Boceno M, Rival JM, David A, Le Caignec C. A 8.26Mb deletion in 6q16 and a 4.95Mb deletion in 20p12 including *JAG1* and *BMP2* in a patient with Alagille syndrome and Wolff-Parkinson-White syndrome. *Eur J Med Genet*. 2008; 51:651–657. [PubMed: 18775522]
- Liu W, Sun X, Braut A, Mishina Y, Behringer RR, Mina M, Martin JF. Distinct functions for *Bmp* signaling in lip and palate fusion in mice. *Development*. 2005; 132:1453–1461. [PubMed: 15716346]
- Lu H, Jin Y, Tipoe GL. Alteration in the expression of bone morphogenetic protein-2,3,4,5 mRNA during pathogenesis of cleft palate in BALB/c mice. *Arch Oral Biol*. 2000; 45:133–140. [PubMed: 10716617]
- Ma L, Lu MF, Schwartz RJ, Martin JF. *Bmp2* is essential for cardiac cushion epithelial-mesenchymal transition and myocardial patterning. *Development*. 2005; 132:5601–5611. [PubMed: 16314491]
- Meng L, Bian Z, Torensma R, Von den Hoff JW. Biological mechanisms in palatogenesis and cleft palate. *J Dent Res*. 2009; 88:22–33. [PubMed: 19131313]
- Nie X, Luukko K, Kettunen P. FGF signalling in craniofacial development and developmental disorders. *Oral Dis*. 2006; 12:102–111. [PubMed: 16476029]
- Nie XG. Differential expression of *Bmp2*, *Bmp4* and *Bmp3* in embryonic development of mouse anterior and posterior palate. *Chin Med J (Engl)*. 2005; 118:1710–1716. [PubMed: 16313756]
- Nieminen P, Kotilainen J, Aalto Y, Knuutila S, Pirinen S, Thesleff I. *MSX1* gene is deleted in Wolf-Hirschhorn syndrome patients with oligodontia. *J Dent Res*. 2003; 82:1013–1017. [PubMed: 14630905]
- Osoegawa K, Vessere GM, Utami KH, Mansilla MA, Johnson MK, Riley BM, L'Heureux J, Pfundt R, Staaf J, van der Vliet WA, Lidral AC, Schoenmakers EF, Borg A, Schutte BC, Lammer EJ, Murray JC, de Jong PJ. Identification of novel candidate genes associated with cleft lip and palate

- using array comparative genomic hybridisation. *J Med Genet.* 2008; 45:81–86. [PubMed: 17873121]
- Robert ML, Lopez T, Crolla J, Huang S, Owen C, Burvill-Holmes L, Stumper O, Turnpenny PD. Alagille syndrome with deletion 20p12.2-p12.3 and hypoplastic left heart. *Clin Dysmorphol.* 2007; 16:241–246. [PubMed: 17786115]
- Schutte BC, Murray JC. The many faces and factors of orofacial clefts. *Hum Mol Genet.* 1999; 8:1853–1859. [PubMed: 10469837]
- Shaffer LG, McCaskill C, Han JY, Choo KH, Cuttillo DM, Donnenfeld AE, Weiss L, Van Dyke DL. Molecular characterization of de novo secondary trisomy 13. *Am J Hum Genet.* 1994; 55:968–974. [PubMed: 7977360]
- Spritz RA. The genetics and epigenetics of orofacial clefts. *Curr Opin Pediatr.* 2001; 13:556–560. [PubMed: 11753106]
- Stanier P, Moore GE. Genetics of cleft lip and palate: syndromic genes contribute to the incidence of non-syndromic clefts. *Hum Mol Genet.* 2004; 13(Spec No 1):R73–81. [PubMed: 14722155]
- van den Boogaard MJ, Dorland M, Beemer FA, van Amstel HK. MSX1 mutation is associated with orofacial clefting and tooth agenesis in humans. *Nat Genet.* 2000; 24:342–343. [PubMed: 10742093]
- Wan M, Cao X. BMP signaling in skeletal development. *Biochem Biophys Res Commun.* 2005; 328:651–657. [PubMed: 15694398]
- Zhang H, Bradley A. Mice deficient for BMP2 are nonviable and have defects in amnion/chorion and cardiac development. *Development.* 1996; 122:2977–2986. [PubMed: 8898212]
- Zhang Z, Song Y, Zhao X, Zhang X, Fermin C, Chen Y. Rescue of cleft palate in *Msx1*-deficient mice by transgenic *Bmp4* reveals a network of BMP and *Shh* signaling in the regulation of mammalian palatogenesis. *Development.* 2002; 129:4135–4146. [PubMed: 12163415]

Figure 1.

Facial profiles for the three patients. (A, B) Patient 1 at 13 days and 9.5 months. Note cleft palate in frame (A); micrognathia, long philtrum in frame (B). (C, D) Patient 2 at 7 years. Note deep cleft palate (frame C); micrognathia, long philtrum (frame D). (E, F) Patient 3 at 3 months and 19 months. Note flat facial profile, downslanting palpebral fissures, depressed nasal bridge, small, upturned nose with anteverted nares, long philtrum and micrognathia.

Figure 2.

Molecular cytogenetic characterization of microdeletions at 20p12.3. **(A)** In Patient 1, oligonucleotide microarray analysis identified a single-copy loss of 34 oligonucleotide probes from the short arm of chromosome 20 at 20p12.3, approximately 592.7 kb in size (chr20: 6,222,266–6,814,990; UCSC Genome Browser coordinates, build hg18, 2006). **(B)** In Patient 2, oligonucleotide microarray analysis identified a single-copy loss of 38 oligonucleotide probes from the short arm of chromosome 20 at 20p12.3, approximately 566.4 kb in size (chr20: 6,265,253–6,831,640). **(C)** In Patient 3 oligonucleotide microarray analysis identified a single-copy loss of 266 probes at 20p13p12.2, approximately 5.37 Mb in size (chr20: chr20:3,672,605–9,042,183). **(D)** Chromosome 20 ideogram for segment p13->p12.2. **(E)** Schematic of microdeletions at 20p13p12.3 identified in study Patients 1–3, and compared to Patients 1–5 reported by Lalani et al. [2009]. Green bars represent the deleted interval in each of the current and previously reported cases. **(F)** The blue vertical bars represent genes within the interval. *BMP2* highlighted by red circle.

Clinical characterization of individuals with microdeletions at 20p12.3 and comparison with Patients 1–3 reported by Lalani et al.,

Table 1

	Patient IV-1	Patient III-1	Patient II-1	Patient I-1	Patient 2	Patient 3	Lalani Patient 1	Lalani Patient 2	Lalani Patient 3
Age	3 mo				7 y	2y	20 mo	5 y	37 y
Sex	F	F	F	F	F	F	M	M	F
Deletion size	592.7 kb (chr20: 6,222,266–6,814,990)	592.7 kb (chr20: 6,222,266–6,814,990)	592.7 kb (chr20: 6,222,266–6,814,990)	592.7 kb (chr20: 6,222,266–6,814,990)	566.4 kb (chr20: 6,265,253–6,831,640)	5.37 Mb (chr20: 3,672,605–9,042,183)	1.1 Mb	2.3 Mb	2.3 Mb
Inheritance	Maternal	Maternal	Maternal	Unknown	Unknown	<i>de novo</i>	<i>de novo</i>	Maternal	<i>de novo</i>
Height	53.3 cm (<5 th centile)	172.7 cm	154.9 cm (5 th –10 th centile)	137.2 cm	114.6 cm (10 th centile)	10 th centile	–2 SD	25 th centile	N/A
Weight	4.17 kg (<5 th centile)	N/A	N/A	N/A	19.4 kg (10 th –25 th centile)	<3 rd centile	–2 SD	75 th centile	
Head circumference	38.0 cm (5 th –10 th centile)	54.8 cm	N/A	N/A	48 cm (<5 th centile)	40.3 cm (50 th –75 th centile)	25 th centile	+2 SD	+2.25 SD
Facial dysmorphism	Micrognathia; long philtrum	N/A	Brushfield’s spots	N/A	Large eyes; synophrys; long philtrum; microstomia	Flat facial profile; downslanting palpebral fissures; low nasal bridge; small, upturned nose with anteverted nares; pinpoint hemangioma on tip in middle of nose; long philtrum; transversecrease across chin; micrognathia	Downslanting palpebral fissures; bilateral epicanthal folds; broad nasal root and bridge; malar hypoplasia; full cheeks with microstomia	Frontal upsweep; downslanting palpebral fissures; epicanthal folds; hypertelorism; long philtrum; microstomia; small ears with thickened helices	Hypertelorism; malar hypoplasia
Craniofacial	Cleft palate; open posterior fontanelle; large anterior fontanelle	High palate	High palate	High palate	Submucous cleft palate; bifid uvula, high arched hard palate	Long U-shaped midline cleft of soft palate	N/A	Macrocephaly	Macrocephaly
Heart	Patent foramen ovale	–	–	–			ASD; WPW	WPW	–
Psychomotor and behavioral development	–	–	–	–	Problems with reading comprehension repeated second grade	–	Neurocognitive delay	Neurocognitive delay; motor delay	Neurocognitive delay; motor delay
Neurology	NA	4 th central nerve palsy	4 th central nerve palsy	4 th central nerve palsy	NA	Feeding difficulties; significant head lag and wobble; delayed/poor reflexes; significant central hypotonia	–	–	–
Hearing loss	–	–	–	–	+	Failed newborn hearing exam	N/A	N/A	N/A

	Patient IV-1	Patient III-1	Patient II-1	Patient I-1	Patient 2	Patient 3	Lalani Patient 1	Lalani Patient 2	Lalani Patient 3
Hands/feet	Deep palmar flexion creases, short 5 th fingers with mild mesobrachydactyly bilaterally, clinodactyly	Hands with short 5 th fingers; central pocket loop in the left 3 rd finger; tendency to central pocket radial loop in the right 3 rd finger; and to central pocket loop in the right 5 th finger	Hands with hard-to-define ridges and short 5 th fingers with evident mesobrachydactyly	Hands with tendency to single palmar flexion crease transitional 2 and single flexion crease on both 5 th fingers	Zygodactylous triiradius between the second and third right toes		Persistent fetal pads	Broad thumbs/toes with persistent fetal pads	Persistent fetal pads
Other	GERD	Small congenital lumbosacral hemangioma	–	–	Cholesteatoma	Lacrimal duct stenosis	–	–	–