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***IRF6* mutation screening in nonsyndromic orofacial clefting: analysis of 1521 families**

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Abstract

Van der Woude syndrome (VWS) is an autosomal dominant malformation syndrome characterized by orofacial clefting (OFC) and lower lip pits. The clinical presentation of VWS is variable and can present as an isolated OFC, making it difficult to distinguish VWS cases from individuals with nonsyndromic OFCs. About 70% of causal VWS mutations occur in *IRF6*, a gene that is also

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associated with nonsyndromic OFCs. Screening for *IRF6* mutations in apparently nonsyndromic cases has been performed in several modestly sized cohorts with mixed results. In the current study we screened 1521 trios with presumed nonsyndromic OFCs to determine the frequency of causal *IRF6* mutations. We identified seven likely causal *IRF6* mutations, although a posteriori review identified two misdiagnosed VWS families based on the presence of lip pits. We found no evidence for association between rare *IRF6* polymorphisms and nonsyndromic OFCs. We combined our results with other similar studies (totaling 2,472 families) and conclude that causal *IRF6* mutations are found in 0.24%-0.44% of apparently nonsyndromic OFC families. We suggest that clinical mutation screening for *IRF6* be considered for certain family patterns such as families with mixed types of OFCs and/or autosomal dominant transmission.

Keywords

nonsyndromic oral clefts; syndromic cleft; interferon regulatory factor 6; mutation screening

INTRODUCTION

Orofacial clefts (OFCs) are collectively the most common craniofacial birth defect in humans, and include cleft lip with or without cleft palate (CL/P) and cleft palate alone (CP) [1]. The majority, approximately 70%, of OFCs are nonsyndromic, while the other 30% are syndromic, i.e. they occur in combination with other significant structural or cognitive anomalies. Nonsyndromic OFCs are etiologically complex and are causally influenced by genetic risk factors, the combined action of genetic susceptibility and environmental exposures or from other unknown events. In contrast, syndromic OFCs are often caused by structural chromosomal anomalies or by coding mutations in a single gene [2]. The most common OFC syndrome is Van der Woude syndrome (VWS, MIM #119300), accounting for 2% of all OFCs with an overall prevalence of 1/34,000 live births [3]. The characteristic features of VWS are OFC congenital lower lip pits. While VWS is highly penetrant, there is remarkably variable expressivity [4]. Individuals with VWS mutations can present with CL, CLP (cleft lip with cleft palate), CP, or no OFC at all. Further CL/P and CP may both occur in VWS families; such “mixed” families are rare in nonsyndromic OFC. The range of lip pit phenotypes is also broad as they may be bilateral or unilateral, and can also appear as conical elevations or shallow openings [5, 6]. Significantly, approximately 15% of individuals diagnosed with VWS lack lip pits, making it difficult to distinguish some individuals with VWS from individuals with nonsyndromic OFC [4].

All causal VWS mutations identified to date are in either *IRF6* [7] or *GRHL3* [8]. Loss-of-function mutations in *IRF6* are responsible for about 70% of cases of VWS (VWS1, MIM # 119300), gain-of-function mutations in *GRHL3* account for another 5% (VWS2, MIM # 606713), with the remaining 25% of VWS cases having no known causal mutation(s) and no identified gene(s). As an autosomal dominant disorder, the expected recurrence risk for first degree relatives (FDRs) of individuals with mutations in these genes is 50%, although the observed risk is less (~35% [3]) due to non-penetrance and *de novo* mutations. In contrast, the empiric recurrence risk for FDRs of individuals with nonsyndromic OFC falls in the

3-5% range [9-11]. Thus the difficulty of distinguishing VWS from nonsyndromic OFC can lead to skewed estimates of FDR recurrence risk for some families of affected individuals.

A number of studies have screened the *IRF6* gene in nonsyndromic OFC cases in an effort to determine if clinical mutation screening should become routine or if unrecognized *IRF6* mutations underlie the well-known association between common variants in *IRF6* and nonsyndromic OFCs (summarized in Table 1) [11-22]. In this study, we report the results of our own *IRF6* mutation screen of 1,521 case-parent trios with apparently nonsyndromic OFCs and compare these findings with the existing literature.

MATERIALS AND METHODS

Subjects

Samples for the current study were drawn from a large cohort of families with OFCs assembled for the purpose of sequencing genomic regions previously identified through genome-wide association studies (GWAS) [23]. A total of 1521 case-parent trios were included where the affected subjects were diagnosed as having cleft lip alone (CL), cleft lip with cleft palate (CLP), or cleft palate alone (CP). This study included 1129 trios of Asian ancestry from China (N=497) and the Philippines (N=634); 383 trios of European ancestry from the United States (N=273), Hungary (N=65), Spain (N=27), Denmark (N=10), and Turkey (N=9); and 9 trios from Guatemala. Counts of trios by cleft type and population is available in Supplementary Table 1. All research work was approved by the Institutional Review Boards of participating institutions and informed consent was obtained for all individuals.

Sequencing

Thirteen regions with the best statistical and/or biological evidence for a role in nonsyndromic OFC [23] were selected for targeted sequencing. Full methodology including the exact coordinates was described in detail previously [23]. Sequencing was performed by the Genome Institute at Washington University-St. Louis. Briefly, Illumina multiplexed libraries were constructed with 1ug of genomic DNA as previously described [23]. NimbleGen (Roche NimbleGen, Madison, WI) custom target probes were designed to cover the target region, hybrid capture was performed on pools of 96 indexed samples, and each pool was sequenced on two lanes of Illumina HiSeq (Illumina Inc., San Diego, CA). Reads were mapped to the GRCh37-lite reference sequence using bwa v0.5.9, alignments were merged and duplicated marked with Picard v1.46, and germline and *de novo* variant calling was performed using Polymutt (v0.11). Candidate *de novo* mutations were further screened with VarScan trio and dbSNP 132 to remove likely germline variants that were under-called in parents.

Small indels were called in a three-step process. First, indels were called on a per-individual basis using VarScan v2.3.6 and filtered to remove likely artifacts [24]. Next, the BAM files for all samples were realigned with DINDEL at all indel positions identified in the cohort. Finally, consensus indel genotypes were generated for all samples simultaneously using the realigned BAM files and VarScan 2.

The sequence data described here is available in dbGaP (accession number phs000625.v1.p1). Note, at the time the targeted regions were selected (2010), numerous candidate gene and GWAS studies had found statistically significant association between common variants in *IRF6* and nonsyndromic OFC [2]. *GRHL3* had not yet been identified as the causative gene at the VWS2 locus [8] and had not shown evidence of association in GWAS studies of nonsyndromic OFC. Thus the complete *IRF6* region, including all exons and introns, was sequenced but not the *GRHL3* region.

RESULTS

After sequencing 1,521 OFC trios, we identified seven presumably pathogenic variants in the *IRF6* gene (Table 1).

Three of the mutations occurred *de novo*. In the proband of family A (from Spain), we identified NM_006147.3:c.250C>T; p.Arg84Cys. The proband had left unilateral CLP and no reported other major medical conditions. In family B, from China, we identified a *de novo* mutation, c.1060+1G>T [p.?] in the exon 7 splice donor site in a boy with left unilateral CLP. Family C was a nuclear family from the Philippines. The proband had bilateral CLP and was the only affected individual in the immediate family although the family also reported two distant relatives (7th degree) with unknown cleft types. The *de novo* mutation, c.379delG; p.Gly127Valfs*43, resulted in deletion of the last nucleotide of exon 4 and likely alters the location of the splice donor site leading to a shift in the reading frame and truncation of the protein. In each of these families, lip pits were not explicitly reported. However, no photographs or medical records are available for review and these families were not examined for other signs of VWS at the time of recruitment (e.g. syndactyly or hypodontia).

Family D was a nuclear family from the Philippines with mixed OFC; i.e. each of CL, CLP, and CP was present in one of the three siblings. The mutation, c.39G>A; p.Trp13*, identified in the proband, was also found in his father. *A posteriori* examination of the gathered clinical data revealed the proband was missing his upper lateral incisors. No lip pits were reported or identified from pictures. Although the father was initially reported to be unaffected because he did not have an OFC, *a posteriori* review of facial photographs revealed bilateral lower lip pits. In addition, the father reported having an extra right maxillary molar. Other family members did not participate in the study; i.e. DNA and phenotypic data were not available. No other family history of OFC, lip pits, or dental anomalies was reported. This *a posteriori* review of collected phenotypic data is consistent with a diagnosis of VWS for this family.

Family E is a multiplex family from China with CL in the child and CLP in the mother (Figure 1). We identified a novel *IRF6* mutation c.254G>T; p.Cys85Phe in both affected individuals. The proband's brother also had CL but no DNA was available. The family was not evaluated for lip pits nor other signs of VWS, however no other malformations were reported at the time of recruitment. A similar story pertains to Family F, also from China. We identified a 1bp deletion (c.165delC; p.Ile56Phefs*7) in the proband who had left CL

and her unaffected mother. A first-cousin on the paternal side was also reported to have an OFC. No photographs or medical records were available for review.

Family G was a multigenerational consanguineous family from Turkey (Figure 1). The proband had bilateral CLP and her father, grandfather, and great-grandmother all had bilateral CL. An aunt on her mother's side had a unilateral CL. Only the proband and her parents were sequenced, identifying an in-frame deletion (c.1289_1297del; p.Asp430_Ile432del) in the proband and her father. A posteriori review of the medical notes indicated the presence of a single pit on the lower lip in the proband. Although no other history of lip pits was reported, these data are consistent with a diagnosis of VWS.

In the 1521 trios, we identified 10 other rare variants (minor allele frequencies less than 1%) in *IRF6* that are unlikely to be causal for VWS (Table 2). Using the rare variant association extension of the Family Based Association Test (FBAT) [25] we found no evidence that these 10 rare *IRF6* variants were associated with nonsyndromic OFCs ($p=0.85$). Three of these rare variants in *IRF6* were present in a parent but not transmitted to the affected child, including a p.Arg45Trp variant that was previously reported as potentially pathogenic in a VWS family [26] despite the fact that the unaffected mother and unaffected twin sibling of the proband in the previous study also carried the Arg45Trp variant [26]. In addition, while Arg45 is absolutely conserved [27] and is predicted to contact the DNA, the DNA contact is limited to the phosphate backbone outside of the core binding site [28]. Finally, another missense mutation at this residue (Arg45Asn; rs121434229), was found previously in a control population. Overall, these data do not clearly support Arg45Trp as a causal variant for CL/P or VWS.

Previous estimates suggest VWS accounts for approximately 2% of all cases of OFC [29, 30]. Lip pits are absent in 15% of recognized VWS cases, resulting in a phenotype mimicking nonsyndromic OFC. Therefore, we estimate 0.3% of individuals with an OFC and no lip pits (i.e. those likely to be recruited for studies such as ours that target nonsyndromic OFC) will have a mutation in *IRF6*. In this current screen of 1,521 nonsyndromic CL/P trios, we identified seven likely causal *IRF6* mutations (0.46%), slightly higher than the expected frequency. To date, screening of *IRF6* in nonsyndromic cases has been undertaken in 12 studies, including this one (Table 3). Of the 2,472 families or singleton cases screened, only 17 carried causal mutations. In 6 of these, the proband was later determined to have lip pits, bulges, or other lip anomalies. Considering only those cases in which the proband did not have lip pits, the overall frequency of *IRF6* mutations was 0.44% (11 of 2,466). We note photos were unavailable for five of our probands found to carry a detrimental *IRF6* mutation so one or more of these individuals may have had lip pits. If we also exclude these families, the frequency of *IRF6* mutations is 0.24%. Therefore, in all of the families sequenced to date (Table 1, 3), *IRF6* mutations have been found in 0.24-0.44% of probands who otherwise appear nonsyndromic.

DISCUSSION

Variants in *IRF6* are associated with multiple phenotypes, e.g. common variants are associated with nonsyndromic OFC, while structural mutations cause VWS1 or popliteal

offspring pair, those segregating both CL/P and CP, and those where the presence or absence of a lip phenotype is unclear [12, 13, 15]. Others have suggested *IRF6* testing should be considered for families with apparently dominant transmission of OFC, or those with mixed types of OFC (ie both CL/P and CP in the family) to maximize the accuracy of genetic counseling [14]. In our seven families, only families D, E, and G would have been recommended for *IRF6* testing under the latter criteria because families A, B, C, and F had no reported history of OFC. We would recommend genetic counseling for each of these families, as the empiric risk for OFC in FDRs of VWS probands has been estimated as about 35% [3] in marked contrast to the empiric FDR risk of 4-5% for nonsyndromic CL/P and 3% for nonsyndromic CP.

In summary, we have shown that pathogenic *IRF6* mutations are likely to occur in a small fraction (~0.3%) of individuals with apparent nonsyndromic CL/P, across multiple ethnic groups. As others have shown, *a posteriori* review of phenotypic data or photographs will often reveal lip pits in first degree relatives. Therefore, we strongly recommend that research participants recruited for studies of nonsyndromic OFCs be photographed when possible and asked about personal and family histories of lip anomalies and other minor VWS signs, such as missing or extra teeth. Our data indicate that universal screening of *IRF6* in nonsyndromic OFCs would have low diagnostic yield. However, at this point it is difficult to propose a definite strategy for routine clinical screening of the *IRF6* gene, as none of the previously proposed strategies would have recommended that screening occur in all seven of our families with pathological *IRF6* variants. We agree with others who have suggested that *IRF6* testing should be considered for a subset of OFC families, such as those with mixed clefting or dominant inheritance patterns as such families are enriched with rare variants in *IRF6*.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Marazita ML. The Evolution of Human Genetic Studies of Cleft Lip and Cleft Palate. Annual Review of Genomics and Human Genetics. 2012; 13:263–83.
2. Leslie EJ, Marazita ML. Genetics of cleft lip and cleft palate. Am J Med Genet C Semin Med Genet. 2013; 163C(4):246–58. [PubMed: 24124047]

3. Burdick AB. Genetic epidemiology and control of genetic expression in van der Woude syndrome. *Journal of Craniofacial Genetics and Developmental Biology*. 1986; 2:99–105. supplement.
4. Burdick AB, Bixler D, Puckett CL. Genetic analysis in families with van der Woude syndrome. *Journal of Craniofacial Genetics and Developmental Biology*. 1985; 5(2):181–208. [PubMed: 4019732]
5. Onofre MA, Brosco HB, Taga R. Relationship between lower-lip fistulae and cleft lip and/or palate in Von der Woude syndrome. *Cleft Palate Craniofacial Journal*. 1997; 34(3):261–5. [PubMed: 9167079]
6. Rintala AE, Ranta R. Lower lip sinuses: I. Epidemiology, microforms and transverse sulci. *British Journal of Plastic Surgery*. 1981; 34(1):26–30. [PubMed: 7459520]
7. Kondo S, Schutte BC, Richardson RJ, et al. Mutations in IRF6 cause Van der Woude and popliteal pterygium syndromes. *Nature Genetics*. 2002; 32(2):285–9. [PubMed: 12219090]
8. Peyrard-Janvid M, Leslie EJ, Kousa YA, et al. Dominant mutations in GRHL3 cause Van der Woude Syndrome and disrupt oral periderm development. *Am J Hum Genet*. 2014; 94(1):23–32. [PubMed: 24360809]
9. Klotz CM, Wang X, Desensi RS, et al. Revisiting the recurrence risk of nonsyndromic cleft lip with or without cleft palate. *Am J Med Genet A*. 2010; 152A(11):2697–702. [PubMed: 20949506]
10. Sivertsen A, Wilcox AJ, Skjaerven R, et al. Familial risk of oral clefts by morphological type and severity: population based cohort study of first degree relatives. *BMJ*. 2008; 336(7641):432–4. [PubMed: 18250102]
11. Grosen D, Chevrier C, Skytthe A, et al. A cohort study of recurrence patterns among more than 54,000 relatives of oral cleft cases in Denmark: support for the multifactorial threshold model of inheritance. *Journal of Medical Genetics*. 2010; 47(3):162–168. [PubMed: 19752161]
12. Desmyter L, Ghassibe M, Revencu N, et al. IRF6 Screening of Syndromic and a priori Non-Syndromic Cleft Lip and Palate Patients: Identification of a New Type of Minor VWS Sign. *Molecular Syndromology*. 2010; 1(2):67–74. [PubMed: 21045959]
13. Jehee FS, Burin BA, Rocha KM, et al. Novel mutations in IRF6 in nonsyndromic cleft lip with or without cleft palate: when should IRF6 mutational screening be done? *American Journal of Medical Genetics Part A*. 2009; 149A(6):1319–22. [PubMed: 19449419]
14. Rutledge KD, Barger C, Grant JH, Robin NH. IRF6 mutations in mixed isolated familial clefting. *American Journal of Medical Genetics. Part A*. 2010; 152A(12):3107–9. [PubMed: 21082654]
15. Salahshourifar I, Wan Sulaiman WA, Halim AS, Zilfalil BA. Mutation screening of IRF6 among families with non-syndromic oral clefts and identification of two novel variants: review of the literature. *Eur J Med Genet*. 2012; 55(6-7):389–93. [PubMed: 22440537]
16. Zuccherro TM, Cooper ME, Maher BS, et al. Interferon regulatory factor 6 (IRF6) gene variants and the risk of isolated cleft lip or palate. *New England Journal of Medicine*. 2004; 351(8):769–80. [PubMed: 15317890]
17. Birnbaum S, Reutter H, Lauster C, et al. 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 A*. 2008; 146A(6):787–90. [PubMed: 18247422]
18. Butali A, Mossey PA, Adeyemo WL, et al. Novel IRF6 mutations in families with Van Der Woude syndrome and popliteal pterygium syndrome from sub-Saharan Africa. *Mol Genet Genomic Med*. 2014; 2(3):254–60. [PubMed: 24936515]
19. Larrabee YC, Birkeland AC, Kent DT, et al. Association of common variants, not rare mutations, in IRF6 with nonsyndromic clefts in a Honduran population. *Laryngoscope*. 2011; 121(8):1756–9. [PubMed: 21792966]
20. Wu-Chou YH, Lo LJ, Chen KT, Chang CS, Chen YR. A combined targeted mutation analysis of IRF6 gene would be useful in the first screening of oral facial clefts. *BMC Med Genet*. 2013; 14:37. [PubMed: 23510002]
21. Pegelow M, Peyrard-Janvid M, Zucchelli M, et al. Familial non-syndromic cleft lip and palate--analysis of the IRF6 gene and clinical phenotypes. *Eur J Orthod*. 2008; 30(2):169–75. [PubMed: 18209213]
22. Cuddapah SR, Kominek S, Grant JH 3rd, Robin NH. IRF6 Sequencing in Interrupted Clefting. *Cleft Palate Craniofac J*. 2015

23. Leslie EJ, Taub MA, Liu H, et al. Identification of Functional Variants for Cleft Lip with or without Cleft Palate in or near PAX7, FGFR2, and NOG by Targeted Sequencing of GWAS Loci. *Am J Hum Genet.* 2015
24. Koboldt DC, Zhang Q, Larson DE, et al. VarScan 2: somatic mutation and copy number alteration discovery in cancer by exome sequencing. *Genome Res.* 2012; 22(3):568–76. [PubMed: 22300766]
25. De G, Yip WK, Ionita-Laza I, Laird N. Rare variant analysis for family-based design. *PLoS One.* 2013; 8(1):e48495. [PubMed: 23341868]
26. Tan EC, Lim EC, Yap SH, et al. Identification of IRF6 gene variants in three families with Van der Woude syndrome. *International Journal of Molecular Medicine.* 2008; 21(6):747–51. [PubMed: 18506368]
27. Kent WJ, Sugnet CW, Furey TS, et al. The human genome browser at UCSC. *Genome Res.* 2002; 12(6):996–1006. [PubMed: 12045153]
28. Escalante CR, Yie J, Thanos D, Aggarwal AK. Structure of IRF-1 with bound DNA reveals determinants of interferon regulation. *Nature.* 1998; 391(6662):103–6. [PubMed: 9422515]
29. Dronamraju KR. Genetic studies of a cleft palate clinic population. *Birth Defects Orig Artic Ser.* 1971; 7(7):54–7. [PubMed: 5173242]
30. de Lima RL, Hoper SA, Ghassibe M, et al. Prevalence and nonrandom distribution of exonic mutations in interferon regulatory factor 6 in 307 families with Van der Woude syndrome and 37 families with popliteal pterygium syndrome. *Genetics in Medicine.* 2009; 11(4):241–7. [PubMed: 19282774]
31. Leslie E, Murray J. Evaluating rare coding variants as contributing causes to non-syndromic cleft lip and palate. *Clin Genet.* 2012 n/a-n/a.
32. Leslie EJ, Mansilla MA, Biggs LC, et al. Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. *Birth Defects Research Part A: Clinical and Molecular Teratology.* 2012; 94(11):934–42.
33. Robin NH, Biesecker LG. Considerations for a multiaxis nomenclature system for medical genetics. *Genet Med.* 2001; 3(4):290–3. [PubMed: 11478529]
34. Schutte, BC.; Saal, HM.; Goudy, S.; Leslie, E. IRF6-Related Disorders. In: Pagon, RA., et al., editors. *GeneReviews(R).* Seattle (WA): 1993.
35. Leslie EJ, Standley J, Compton J, et al. Comparative analysis of IRF6 variants in families with Van der Woude syndrome and popliteal pterygium syndrome using public whole-exome databases. *Genetics in Medicine.* 2013; 15(5):338–44. [PubMed: 23154523]
36. Matsuzawa N, Yoshiura K, Machida J, et al. Two missense mutations in the IRF6 gene in two Japanese families with Van der Woude syndrome. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontics.* 2004; 98(4):414–7.

Figure 1. Pedigrees of families with pathogenic *IRF6* mutations. The proband is indicated by an arrow. The proband and both parents from each pedigree were sequenced. Confirmed mutation carriers are indicated with an asterisk(*).

Table 1Likely pathogenic *IRF6* mutations identified in the present study

Population	Subjects	Mutation(s)	Cleft Phenotype of subject(s) with mutation	Lip Phenotype of subject with mutation
US, European, Philippines, China, Guatemala	1,521 trios with NSCL/P and NSCPO	p.Trp13X	Proband: CLP	Proband: no pits; Father: bilateral lower lip pits
		p.Arg84Cys (<i>de novo</i>)	Proband: CLP	Not examined
		p.Cys85Phe	Proband: CL, mother: CLP	Not examined
		c.1060+1G>T (<i>de novo</i>)	Proband: CLP	Not examined
		p.Ile56Phefs*7	Proband: CL	Not examined
		p.Gly127Valfs*43 (<i>de novo</i>)	Proband: CLP	Not examined
		p.Asp430_Ile423del	Proband: CLP	Proband: reported single lower lip pit; Father: none reported; Grandfather: none reported; Great-grandmother: none reported

Table 2Other *IRF6* coding variants identified in 1,521 trios

Nucleotide Change	Coding Change [NM_006147.3]	Protein Change [NP_006138.1]	Transmitted Count	Untransmitted Count	ExAC Counts (Frequency)*
chr1:209974626G>A	c.133C>T	p.Arg45Trp	0	1	Not found
chr1:209968641T>A	c.502A>T	p.Ile168Phe	0	1	Not found
chr1:209968637T>C	c.506A>G	p.Asn169Ser	2	1	7/121412 (0.006%)
chr1:209965751C>T	c.530G>A	p.Ser177Asn	1	1	Not found
chr1:209965664G>A	c.617C>T	p.Pro206Leu	0	1	Not found
chr1:209964095C>T rs112435119	c.805G>A	p.Val269Ile	1	0	6/121158 (0.005%)
chr1:209963903C>T	c.997G>A	p.Ala333Thr	1	0	10/121392 (0.008%)
chr1:209963840C>T	c.1060G>A	p.Asp354Asn	1	0	37/121246 (0.03%)
chr1:209961910C>T rs115777201	c.1259G>A	p.Arg420His	14	15	8/121202 (0.007%)

* counts from the Exome Aggregation Consortium (<http://exac.broadinstitute.org>)

Table 3Previous *IRF6* mutation screens in families/individuals with nonsyndromic orofacial clefts

Population	Subjects	Mutation(s)	Cleft Phenotype of subject(s) with mutation ¹	Lip Phenotype of subject with mutation ¹	Reference
US, Philippines	180 families with NSCL/P	None found			Zuccherro et al. 2004
Germany	63 families with NSCL/P and NSCPO	p.His30IlefsX9 p.Arg6ProfsX15	P: CLP; M: CP P,M,B,D: CLP	P: lip pit B, D: lip pit	Birnbaum et al. 2008
Sweden	17 families with NSCL/P and NSCPO	None found			Pegelow et al. 2008
Brazil	108 families	p.Arg9Trp p.Gln17Pro p.Val113Leu p.Asp354Asn*	P: CLP P, 2 sons: CLP P: micrognathia, CP, glossoptosis; F: unaffected P: CP; M: unaffected	P: atypical pit No pits P: irregular lower lip No pits	Jehee et al. 2009
African-American; Caucasian	2 families with NSCL/P and NSCPO	p.Met1Leu p.Phe369Ser	P: CP, bifid uvula P: submucous CP	No pits; family members not examined No pits; family members not examined	Rutledge et al. 2010
European	95 families with NSCL/P and NSCPO	p.Arg6Cys p.Arg250Glu p.Arg400Gln	P,B,F: CP P: CP; F,GF: CLP P: CL (<i>de novo</i>)	P: no pits; B: bilateral lip bulges F: lip pits at birth; P: lip bulges P: lip pit	Desmyter et al. 2010
Honduras	100 families with NSCL/P	None found			Larrabee et al. 2011
Malaysia	16 CL cases, 23 families with NSCL/P and NSCPO	None found			Salahshourifar et al. 2012
Taiwan	123 CL/P cases	None found			Wu-Chou et al. 2013
Nigeria; Ethiopia	220 cases with NSCL/P and NSCPO	None found			Butali et al. 2014
US	4 cases with CL/P	None found			Cuddapah et al. 2015
TOTAL	951 cases/families	10 cases/families			

¹ Abbreviations: P-proband, M-mother, F-father, B-brother, D-daughter, GF-grandfather