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Interferon Regulatory Factor 6 (*IRF6*) and Fibroblast Growth Factor Receptor 1 (*FGFR1*) Contribute to Human Tooth Agenesis

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Abstract

Phenotypic characteristics expressed in syndromes give clues to the factors involved in the cause of isolated forms of the same defects. We investigated two genes responsible for craniofacial syndromes, *FGFR1* and *IRF6*, in a collection of families with isolated tooth agenesis. Cheek swab samples were obtained for DNA analysis from 116 case/parent trios. Probands had at least one developmentally missing tooth, excluding third molars. In addition, we studied 89 cases and 50 controls from Ohio to replicate any positive findings. Genotyping was performed by kinetic polymerase chain-reaction or TaqMan assays. Linkage disequilibrium analysis and transmission distortion of the marker alleles were performed. The same variants in the *IRF6* gene that are associated with isolated orofacial clefts are also associated with human tooth agenesis (rs861019, $P = 0.058$; rs17015215—V274I, $P = 0.0006$; rs7802, $P = 0.004$). Mutations in *IRF6* cause Van der Woude and popliteal pterygium syndromes. The craniofacial phenotypic characteristics of these syndromes include oral clefts and preferential tooth agenesis of incisors and premolars, besides pits on the lower lips. Also it appears that preferential premolar agenesis is associated with *FGFR1* ($P = 0.014$) and *IRF6* ($P = 0.002$) markers. There were statistically significant data suggesting that *IRF6* interacts not only with *MSX1* ($P = 0.001$), but also with *TGFA* ($P = 0.03$).

Keywords

hypodontia; oligodontia; cleft lip and palate; orofacial clefts; Kallmann syndrome; Van der Woude syndrome; PAX9; MSX1; TGFA; paired-box; muscle segment; transforming growth factor alpha

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Introduction

The discovery of genes that are responsible for rare syndromes provides insights to the understanding of more common isolated traits. For facial anomalies, the genes for three rare autosomal syndromes were identified in recent years.

Loss-of-function mutations of fibroblast growth factor receptor 1 (*FGFR1*) cause Kallmann syndrome (OMIM 147950), which is characterized by an impaired sense of smell and incomplete or delayed puberty. Approximately 10% of individuals with Kallmann syndrome have mutations in the *FGFR1* gene. Cleft lip and palate is associated in 30% of these cases and tooth agenesis in 5–10% of these cases [Dodé et al., 2003]. In addition, *FGFR1* missense mutations cause two skeletal diseases, type I Pfeiffer syndrome (OMIM 101600) and osteoglophonic dysplasia (OMIM 166250). The altered FGFR1 protein appears to cause prolonged signaling, which promotes premature fusion of bones in the skull, hands, and feet in the case of Pfeiffer syndrome, and alters its function as negative regulator of long-bone growth in the case of osteoglophonic dysplasia [Muenke et al., 1994; White et al., 2005]. Finally, the t(8:9)(p11;q33) translocation fuses the centrosomal protein 1 (CEP1) in chromosome 11 and FGFR1 in chromosome 8 in the 8p11 myeloproliferative disorder. The demonstration that FGFR1 is disrupted by a translocation in a stem cell myeloproliferative disorder indicates that in addition to its involvement in Kallmann and Pfeiffer syndromes, it can also have oncogenic potential [Guasch et al., 2000].

Interferon regulatory factor 6 (*IRF6*) gene deletions and point mutations are responsible for Van der Woude (OMIM 119300) and popliteal pterygium syndromes (OMIM 119500) [Kondo et al., 2002]. In general, these syndromes affect about 1 in 100,000–200,000 people. About 1–2% of patients with cleft lip or palate have Van der Woude syndrome. Van der Woude syndrome is an autosomal dominant disorder in which lower-lip pits and less frequently tooth agenesis are the only features distinguishing the syndrome from isolated cleft lip with or without cleft palate (CL/P). CL/P occurs with wide geographic distribution with an average birth prevalence of 1 in 700 [Mossey and Little, 2002].

More recently, a significant association between CL/P and single nucleotide polymorphisms (SNPs) at the *IRF6* locus was reported in a large collection of cases from many geographic origins [Zucchero et al., 2004] and replicated by four independent studies [Blanton et al., 2005; Ghassibe et al., 2005; Scapoli et al., 2005; Srichomthong et al., 2005]. Variation at *IRF6* was responsible for 12% of the genetic contribution to CL/P and tripled the risk of recurrence in families that had already had one affected child. *IRF6* is another gene in the list of candidate genes for CL/P that includes *MSX1* and *TGFA*.

Similarly to CL/P, there is evidence that human tooth agenesis is a complex disorder with likely many genes involved. The frequency of tooth agenesis varies with the tooth class. Failure of one or more of the third molars to form occurs in 20% of the population. The reported incidence of teeth other than third molars being missing varies from 1.5 to 10% [Eidelman et al., 1973; Graber, 1978]. Autosomal dominant forms of oligodontia (agenesis of six or more teeth) have been linked to mutations or deletions in *PAX9*, *MSX1* [reviewed by Vieira, 2003], and *AXIN2* [Lammi et al., 2004]. One of the *MSX1* mutations that affected a Dutch family [van den Boogaard et al., 2000] segregates with a combination of tooth agenesis, cleft lip and palate, and cleft palate only. This family presents the most compelling evidence that some of the same mechanisms are shared for the development of teeth, lip, and palate. Recently, we showed that *MSX1* and *TGFA*, candidate genes for CL/P, are associated with tooth agenesis in humans and that *MSX1* may interact with *PAX9* [Vieira et al., 2004].

Additional evidence for common pathways in tooth, lip, and palate development comes from other studies that showed an association between having a cleft and tooth agenesis outside the cleft area, as well as studies that have shown that as clefting increases in severity, a greater number of teeth are missing [Ranta, 1984, 1988; Quezada et al., 1998; Ranta and Tulensalo, 1988; Lopes et al., 1991; Roth and Hirschfelder, 1991; Vichi and Franchi, 1995; Larson et al., 1998; Dewinter et al., 2003; Slayton et al., 2003]. In the present investigation, we examined samples of individuals with tooth agenesis to investigate the involvement of *FGFR1* and *IRF6* —genes involved in forms of CL/P—in cases of tooth agenesis, an even more common craniofacial phenotype.

Subjects and Methods

Our study group consisted of 116 patients with tooth agenesis and their parents. All of them were from Rio de Janeiro, Brazil, which is an admixed population of Europeans (from Portugal) and Africans, with a very small percentage of Native South Americans. This population contains 71 sporadic cases and 45 familial cases. Tooth agenesis was the sole disorder affecting these patients. None of the families reported history for clefts. Details of the study population are presented in Table I. The study was approved by the appropriate Institutional Review Board (IRB) and appropriate informed consent was obtained from human subjects. After informed consent was obtained, cheek swabs were collected from each individual. Clinical analysis, check swab collection, and DNA extraction were performed using a consolidated protocol described elsewhere [Vieira et al., 2004]. Cases were analyzed not only as a total group, but also in three subgroups: cases with positive family history, cases with at least one missing incisor, and cases with at least one missing premolar. To facilitate comparisons between studies, we selected ten polymorphisms that had been used before to investigate *IRF6* and CL/P populations [Zucchero et al., 2004]. Additional four polymorphisms were selected to study the *FGFR1* gene. Marker information is included in Table II. Genotypes were obtained using an ABI PRISM 7900 Sequence Detection System (Valencia, CA) and TaqMan or SIBR Green chemistries. Reagents and SNP genotyping assays were supplied by Applied Biosystems (Valencia, CA). All SNPs showed Hardy–Weinberg equilibrium in both the affected and unaffected individuals. Pairwise calculations of linkage disequilibrium (Table III) were computed with the Graphical Overview of Linkage Disequilibrium (GOLD) software for both the squared correlation coefficient (r^2, above the diagonal) and Lewontin's standardized disequilibrium coefficient (D′, below diagonal) [Abecasis and Cookson, 2000]. Alleles at each marker and haplotypes were tested for association with tooth agenesis with the use of the Family Based Association Test (FBAT) software [Horvath et al., 2001,2004]. Bonferroni correction was applied and *P*-values below 0.003 (0.05/14) were considered significant.

To infer the overall contribution of *IRF6* to human tooth agenesis, we calculated the attributable fraction (AF) for the associated *IRF6* allele [V of the V274I (rs17015215) marker]—that is, the proportion of tooth agenesis cases in a population that can be attributed to the V allele. We calculated the AF according to the formula

$$\mathrm{AF}=\frac{\mathrm{f}\,(\mathrm{R}-1)}{1+\mathrm{f}\,(\mathrm{R}-1)}$$

where f is the frequency of the risk factor in the population [frequency of the associated haplotype composed by all 10 markers; f = 0.286 (data not shown)] and R is the measure of relative risk. We used the odds ratio of the proportion of transmitted and non-transmitted V274I (rs17015215) alleles (data not shown) as an estimate of the relative risk.

We also studied a population from Ohio, USA, to replicate any association between the *FGFR1* and *IRF6* locus and the tooth agenesis phenotype. Details about these samples are reported elsewhere [Lidral and Reising, 2002]. A total of 89 samples from individuals affected with congenital tooth agenesis recruited from the Ohio State University College of Dentistry and Children's Hospital in Columbus were used. Written consent was obtained from all individuals, and the study was approved by the Ohio State University IRB. The inclusion criterion was congenital agenesis of at least one permanent tooth, not including third molars, as verified by radiographs and dental history. In addition, 50 Caucasian controls from Ohio, who were not affected with tooth agenesis, were also recruited. Genotypes for the *IRF6* marker rs764093 of the 89 tooth agenesis cases were compared to the 50 controls. This marker was chosen because it showed higher heterozygosity among Caucasians, compared to the markers V274I (rs17015215) and rs861019 [Zucchero et al., 2004]. We also studied the *FGFR1* rs881301 marker, which showed evidence of association in the Brazilian dataset.

Finally, we tested for possible gene-gene interactions between the two genes studied (*FGFR1* and *IRF6*) and other candidate genes for tooth agenesis using genotypes performed in the same Brazilian study population [Vieira et al., 2004]. We tested for *FGFR1-IRF6*, *FGFR1-MSX1*, *FGFR1-PAX9*, *FGFR1-TGFA*, *IRF6-MSX1*, *IRF6-PAX9*, and *IRF6-TGFA* interactions by observing the transmission of the marker alleles from parents heterozygous for both of the markers.

Results

Significant linkage disequilibrium with the *IRF6* locus was apparent for the marker rs17015215 (V274I) ($P = 0.0006$) and borderline for the markers rs7802 and rs861019 ($P = 0.004$, and 0.058 respectively). The estimated AF for the associated *IRF6* V274I (rs17015215) V allele was 16.4%.

Borderline results were found for the *FGFR1* marker rs881301 ($P = 0.03$); in all these markers, the common allele was overtransmitted to the affected individuals (Table IV). While markers rs861019 and V274I (rs17015215) are in linkage disequilibrium, V274I (rs17015215) and rs7802 are only weakly linked (Table II). Haplotype analysis was performed with the HBAT function of FBAT, using windows of three adjacent SNPs across the *IRF6* locus (Table V). Haplotypes carrying the frequent allele of the V274I (rs17015215) marker were always found to be overtransmitted to patients. No association was found when *FGFR1* haplotypes were analyzed. When *IRF6* V274I (rs17015215) was analyzed in cases with positive family history for tooth agenesis, cases with at least one missing incisor, and cases with at least one missing premolar, the association is apparent with the group that lacks premolars. The same was found for the *FGFR1* rs881301 marker (Table VI).

No differences in allele and genotype frequencies were seen between tooth agenesis cases and unaffected controls from Ohio regarding the *FGFR1* rs881301 and *IRF6* rs764093 markers (Table VII).

The *IRF6* V274I (rs17015215) V allele and the *MSX1*-CA 169-base-pair allele were transmitted together more often than expected ($P = 0.001$), as well as the *IRF6* V274I (rs17015215) V allele and the *TGFA* common haplotype Taq1- rs2166975-rs1058213 ($P = 0.03$) (Table VIII).

Discussion

We found a significant association between a marker in the *IRF6* gene and tooth agenesis in an admixed population from Brazil who is predominantly Caucasian with Portuguese ancestry. The estimated AF for the associated *IRF6* marker allele was 16.4%. This AF is based on the assumption that the risk factor is causal and is not correlated with other risk factors, so it should be interpreted cautiously because the *IRF6* contribution could happen in the background of other genes.

We also found a suggestive association between a marker in the *FGFR1* gene and this same population.

We were unable to replicate these findings in a population from Ohio, the US, who is predominantly of Caucasian origin. However, they are Caucasians more likely to be descendents of Northern Europeans, which may suggest genetic heterogeneity. In our previous studies with *IRF6* in cleft populations [Zucchero et al., 2004], we have also found only an effect in transmission-disequilibrium analyses and not in case-control comparisons. It may be an indication that much larger case-control studies are needed to find evidence of a genetic effect indicated by candidate-gene analysis and linkage disequilibrium.

It is remarkable that the same gene locus appears to contribute to phenotypes varying from very rare syndromic forms of clefting (frequency 1 to 100,000 to 200,000 live births) to the more common isolated forms of clefting (frequency 1 to 500 to 2,000) to the very common tooth agenesis phenotype (frequency 1 to 10 to 100) as these defects were part of the same clinical spectrum. Furthermore, 20–40% of the cases of Van der Woude syndrome present with tooth agenesis, preferentially involving incisors and premolars [Jones, 1997]. The relationship between premolar agenesis and clefting has been noted by previous studies [Larson et al., 1998], but its nature remains unclear. The association with cases lacking at least one premolar suggests that the *IRF6* spectrum may include isolated forms of clefting and tooth agenesis with preferential missing premolars. We also found evidence that *FGFR1* contributes to lacking premolars. These findings provide further evidence that human tooth agenesis is probably caused by several independent defective genes, acting alone or in combination with other genes, and leading to a specific phenotypic pattern.

We have reasons to believe that the V allele of the *IRF6* V274I marker, which is significantly overtransmitted in the Brazilian dataset, does not itself cause tooth agenesis. There is strong disequilibrium seen between particular *IRF6* haplotypes and cleft lip and palate in European populations [Zucchero et al., 2004], in which the I variant allele is rare. This suggests either that the V allele is not causal, or that it may share causality with variants at other sites within or near *IRF6*. It is possible that more than one variant might contribute to tooth agenesis or a specific combination of variants on a single chromosome may be required for a person to exhibit biologic effect (tooth agenesis or cleft lip and palate).

The occurrence of a mixed clefting phenotype in the same family (cleft lip and palate cases and cleft palate only cases) is common in families affected with Van der Woude syndrome, but very rare in families with isolated orofacial clefts, and is not seen in most other syndromic forms of orofacial clefts. It is, however, also seen in the cleft lip and palate-oligodontia disorder caused by a mutation in *MSX1* [van den Boogaard et al., 2000] suggesting that *IRF6* and *MSX1* may be involved in a common genetic pathway. In support of a common pathway, Kondo et al. [2002] found two *IRF* binding sites in the promoter of *MSX1* and one in the intron, all of which are conserved between human and mouse. In mice, high levels of *Irf6* expression are observed in the hair follicles, palatal rugae, medial edge epithelia of the secondary palate immediately before and during fusion, tooth germs, thyroglossal duct, penis, and skin [Kondo et al., 2002]. According to the COGENE project (a consortium involved in describing human gene expression changes that occur during early stages of craniofacial development; http://hg.wustl.edu/COGENE/), *IRF6*, *MSX1*, and *TGFA* are expressed in the human mandible at 6 weeks of gestation and *IRF6* and *MSX1* are expressed in the human dental lamina at 8.5 weeks of gestation. Our results tied with the expression data are suggestive that interactions between *IRF6* and *MSX1* and *IRF6* and *TGFA* or altered expression of these combinations of genes can lead to tooth agenesis in humans.

There are reports of a same gene family that contributes to both rare and more common traits. Mutations in *COL11A1* and *COL2A1* genes cause Marshall and Stickler syndromes, rare autosomal dominant disorders that affect cartilaginous tissues [Ahmad et al., 1991; Annunen et al., 1999a]. Also, an association between an allele of the *COL9A2* gene bearing a putative mutation and intervertebral disc disease, a phenotype that can affect up to 5% of the Finn population, was reported [Annunen et al., 1999b]. It is likely that genes or gene families that cause other rare disorders also contribute to more common, but genetically complex phenotypes.

In summary, we reported that genetic variation in the *IRF6* locus, which has been implicated in the rare Van der Woude and popliteal pterygium syndromes as well as in the more common isolated cleft lip with or without CL/P, is associated with human tooth agenesis, a complex trait that affects 1 in every 10–100 individuals. This association appears to be related to premolar agenesis. Also, a marker in *FGFR1*, in which mutations cause Kallmann syndrome, was associated with premolar agenesis. We believe that rare diseases can serve as models for genetic susceptibility of more common traits in the population.

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Table I

Aspects of the Population Studied

Family characteristic	**N (%)**
Gender distribution	
Males	42 (36)
Females	74 (64)
Average age of the probands (variation)	20 years (6–50 years)
Number of teeth missing	
1	44 (38)
2	46 (40)
3 or more	26 (22)
Type of teeth more often missing (total teeth missing = 274)	
Second premolar	104 (37.9)
Lateral incisor	82 (29.9)
First premolar	26 (9.7)
Second molar	24 (8.7)
Central incisor	16 (5.8)
First molar	11 (4)
Canines	11 (4)
Associated dental anomalies	16 (13.8)
Number of cases missing incisors	55 (47.4)
Number of cases missing premolars	63 (54.3)
Number of cases missing molars	14 (12)
Number of cases missing canines	7 (6)
Positive family history	41 (35.3)

Table II

Information about Assays for SNP Analyzed in this Study

SNP marker	Approximate location	Type of assay	Assay ID
FGFR1			
rs13317	In *FGFR1*	TaqMan OD[a]	C_1358324_10
rs6996321	In *FGFR1*	TaqMan OD	C_2080144_10
rs10958704	2 kb 5′ of *FGFR1*	TaqMan OD	C_2080139_10
rs881301	7 kb 5′ of *FGFR1*	TaqMan OD	C_8844845_10
IRF6			
rs2073487	In *IRF6*	TaqMan OD	C_2500179_1
rs861019	In *IRF6*	TaqMan BD[b]	NA
rs764093	In *IRF6*	TaqMan OD	C_2500165_10
rs2235375	In *IRF6*	TaqMan BD	NA
rs17015215 (V274I)	In *IRF6*	Kinetic PCR	See below
rs7802	In *IRF6*	TaqMan BD	NA
hCV16191297	60 kb 3′ of *IRF6*	TaqMan BD	NA
hCV2502441	90 kb 3′ of *IRF6*	TaqMan BD	NA
rs2235543	100 kb 3′ of *IRF6*	TaqMan BD	NA
hCV2501174	200 kb 3′ of *IRF6*	TaqMan BD	NA

For V274I: Common primer sequence: CAGACCATGACCGTGAGCAA.

V-specific primer: TGCTCAGGACCTGGGAATTTGTC.

I-specific primer: TATGCTCAGGACCTGGGAATTTTTAT.

NA = not available.

[a] Assay-on-demand.

[b] Assay-by-design.

Table III

Linkage Disequilibrium between Markers Genotyped in the Study

Marker	rs2073487	rs861019	rs764093	rs2235375	rs17015215 (V274I)	rs7802	hCV16191297	hCV2502441	rs2235543	hCV2501174
IRF6										
rs2073487	—	0.041	0.412	0.093	0.034	0.0	0.072	0.0	0.013	0.004
rs861019	0.406	—	0.107	0.679	0.878	0.001	0.017	0.002	0.005	0.0
rs764093	0.714	0.684	—	0.168	0.111	0.003	0.047	0.022	0.009	0.013
rs2235375	0.669	0.963	1.0	—	0.595	0.003	0.033	0.0	0.028	0.002
rs17015215 (V274I)	0.375	0.985	0.732	0.853	—	0.047	0.039	0.001	0.005	0.0
rs7802	0.033	0.029	0.091	0.084	0.284	—	0.0	0.001	0.003	0.0
hCV16191297	0.591	0.581	0.439	1.0	1.0	0.037	—	0.007	0.005	0.004
hCV2502441	0.016	0.373	0.224	0.038	0.192	0.254	0.106	—	0.059	0.063
rs2235543	0.733	0.233	0.674	0.468	0.219	0.064	0.999	0.614	—	0.295
hCV2501174	0.085	0.027	0.178	0.075	0.006	0.042	0.993	0.586	0.548	—
Marker	rs13317	rs6996321	rs10958704	rs881301						
FGFR1										
rs13317	—	0.105	0.036	0.059						
rs6996321	0.484	—	0.450	0.373						
rs10958704	0.430	0.979	—	0.787						
rs881301	0.515	0.917	0.894	—						

r^2 is above the diagonal; D′ is below the diagonal.

Table IV

Association (FBAT) Results for Isolated Tooth Agenesis

SNP	Allele	S	E (S)	*P*-value
FGFR1				
rs13317	T	92.0	89.5	0.547
	C	36.0	38.5	
rs6996321	G	60.0	59.5	0.905
	A	46.0	46.5	
rs10958704	A	69.0	72.5	0.458
	G	67.0	63.5	
rs881301	T	39.0	32.0	0.030
	C	25.0	32.0	
IRF6				
rs2073487	A	70.0	64.0	0.163
	G	40.0	46.0	
rs861019	A	10.0	7.5	0.058
	G	2.0	4.5	
rs764093	G	72.0	68.0	0.358
	T	44.0	48.0	
rs2235375	C	77.0	72.5	0.323
	G	45.0	49.5	
rs17015215 (V274I)	V	91.0	75.0	0.0006
	I	29.0	45.0	
rs7802	C	16.0	12.0	0.004
	T	0.0	4.0	
hCV16191297	C	3.0	3.0	1.0
	T	1.0	1.0	
hCV2502441	A	58.0	58.0	1.0
	G	28.0	28.0	
rs2235543	T	72.0	72.0	1.0
	C	32.0	32.0	
hCV2501174	A	53.0	52.0	0.772
	T	27.0	28.0	

Table V

Haplotype Results for Sliding Three-Marker Window Across the *IRF6* Region Studied

	SNP											
Window	**rs2073487**	**rs861019**	**rs764093**	**rs2235375**	**rs17015215 (V274I)**	**rs7802**	**hCV16191297**	**hCV2502441**	**rs2235543**	**hCV2501174**	**Haplotype frequency**	***P*-value**
1	A	A	G								0.63	0.053
2		A	G	C							0.63	0.094
3			G	C	V						0.49	0.005
4				C	V	C					0.51	0.002
5					V	C	C				0.74	0.001
6						C	C	A			0.77	0.490
7							C	A	T		0.72	0.476
8								A	T	A	0.63	0.330

Table VI

IRF6 V274I and *FGFR1* rs881301 Results (FBAT) for Subpopulations of Tooth Agenesis Cases

Subpopulation	Allele	S	E (S)	*P*-value
IRF6				
Positive family history for tooth agenesis (n = 45)	V	33.0	29.5	0.262
	I	17.0	20.5	
Missing at least one incisor (n = 55)	V	39.0	34.5	0.149
	I	15.0	19.5	
Missing at least one premolar (n = 63)	V	54.0	43.0	0.002
	I	16.0	27.0	
FGFR1				
Positive family history for tooth agenesis (n = 45)	T	18.0	14.0	0.073
	C	14.0	18.0	
Missing at least one incisor (n = 55)	T	19.0	16.667	0.282
	C	15.0	17.333	
Missing at least one premolar (n = 63)	T	23.0	17.0	0.014
	C	13.0	19.0	

Table VII

Case-Control Analysis of the Ohio Tooth Agenesis Population

Alleles	Controls (n = 50)	Tooth agenesis cases (n = 89)
FGFR1 rs881301		
C	32	63
T	44	97
P-value	0.68	
Odds ratio (95% confidence intervals)	1.12 (0.64–1.95)	
Genotypes		
CC	4	15
CT	24	33
TT	10	32
P-value	0.08	
CC	4	15
CT + TT	34	65
P-value	0.25	
Odds ratio (95% confidence intervals)	0.51 (0.16–1.66)	
IRF6 rs764093		
G	66	112
T	34	56
P-value	0.91	
Odds ratio (95% confidence intervals)	0.97 (0.57–1.64)	
Genotypes		
GG	22	33
GT	22	46
TT	6	10
P-value	0.67	
GG + GT	44	79
TT	6	10
P-value	0.89	
Odds ratio (95% confidence intervals)	0.93 (0.32–2.72)	

Table VIII

IRF6- and *FGFR1*-Other Candidate Genes for Tooth Agenesis Interactions

Interaction	**# Informative families**	**T/NT**	***P*-value**
IRF6-FGFR1	2	1/0[a]	0.31
IRF6-MSX1	32	12/1[b]	0.001
IRF6-PAX9	18	6/3[c]	0.30
IRF6-TGFA	21	10/2[d]	0.03
FGFR1-MSX1	18	4/2[e]	0.41
FGFR1-PAX9	5	3/0[f]	0.08
FGFR1-TGFA	7	3/1[g]	0.31

T = number of transmitted alleles; NT = number of non-transmitted alleles.

[a] *IRF6* V274I (rs17015215) V allele with *FGFR1* rs881301 C allele.

[b] *IRF6* V274I (rs17015215) V allele with *MSX1*-CA 169-base-pair allele.

[c] *IRF6* V274I (rs17015215) V allele with *PAX9* rs11847165 C allele.

[d] *IRF6* V274I (rs17015215) V allele with *TGFA* common haplotype Taq1-rs2166975-rs1058213.

[e] *FGFR1* rs881301 C allele with *MSX1*-CA 171-base-pair allele.

[f] *FGFR1* rs881301 T allele with *PAX9* rs11847165 C allele.

[g] *FGFR1* rs881301 T allele with *TGFA* common haplotype Taq1-rs2166975-rs1058213.