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Original

Studies of palatine rugae and interferon regulatory factor 6 variations in a group of families with sporadic hypodontia

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Abstract: ***Irf6*** **(interferon regulatory factor 6) is expressed in tooth buds and palatine rugae during development in the mouse. Here we report the first study to investigate whether *IRF6* variation is associated with palatine rugae patterns in a population with sporadic tooth agenesis. Fifty-two individuals with sporadic tooth agenesis and their parents were studied. Palatine rugae were scored from casts available for a subset of 38 families. DNA samples were obtained from whole blood or saliva samples. Genotyping was performed using TaqMan assays. Linkage disequilibrium and transmission distortion analyses of the marker alleles were performed. Borderline results were obtained for *IRF6* genetic variation and having primary rugae larger on the right side than on the left (rs20131633, *P* = 0.07; rs642961, *P* = 0.06) and having fewer than eight primary palatine rugae (rs20131623, *P* = 0.07). However, no specific pattern of tooth agenesis was associated with the palatine rugae patterns studied. Our data suggest that *IRF6* may contribute to specific palatine rugae patterns in humans. (J Oral Sci 51, 521-526, 2009)**

Keywords: dental abnormalities; palate; gene expression.

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Introduction

The interferon regulatory factor (IRF) family of genes regulates transcription of interferon in mice (1-7), with the possible exception of *IRF6*. Mice that are *Irf6*-deficient show embryological abnormalities in skin, limb, and craniofacial development. As *Irf6*-null mice lack of a normal stratified epidermis, the major role of Irf6 is probably the regulation of keratinocye proliferation and differentiation (8). In zebrafish, *Irf6* is expressed in the pharyngeal arches, olfactory and otic placodes, and in the epithelial cells of endoderm-derived tissues (9).

In humans, mutations in *IRF6* lead to the Van der Woude or popliteal pterygium syndromes (10), and *IRF6* genetic variants have also been independently associated with isolated forms of cleft lip and palate (11) and isolated forms of hypodontia (12,13).

Whole *in situ* hybridization of mouse embryos on day 14.5 has clearly demonstrated *Irf6* expression in hair follicles, palatine rugae, and the medial edge of the secondary palate immediately before and during fusion, and in the mandibular molar tooth germs, thyroglossal duct, and penis (10). Based on these expression patterns, we studied a group of families with sporadic hypodontia and observed overtransmission of *IRF6* alleles to affected individuals (13). We expanded these studies and investigated whether *IRF6* variants were associated with specific palatine rugae patterns, since *IRF6* is expressed in the palatine rugae.

Materials and Methods

Our study group comprised 52 unrelated patients with

Table 1 Demographic characteristics of the studied population

Characteristics	*N (%)*
Gender distribution	
Males	25 (48)
Females	27 (52)
Average number of primary palatine rugae	7
Number of individuals with secondary and/or fragmentary palatine rugae	12 (38)
Number of individuals with larger primary palatine rugae on the right side of the palate	27 (71)
Number of teeth missing	
1	7 (14)
2	17 (33)
3 or more	27 (53)
Type of teeth more often missing	
(total teeth missing)	207
Second premolar	75 (36.2)
Lateral incisor	54 (26)
First premolar	8 (3.8)
Second molar	11 (5.3)
Central incisor	32 (15.4)
First molar	9 (4.3)
Canines	18 (8.6)
Number of cases missing incisors	29 (56.8)
Number of cases missing premolars	44 (86.2)
Number of cases missing molars	8 (15.6)
Number of cases missing canines	10 (19.6)

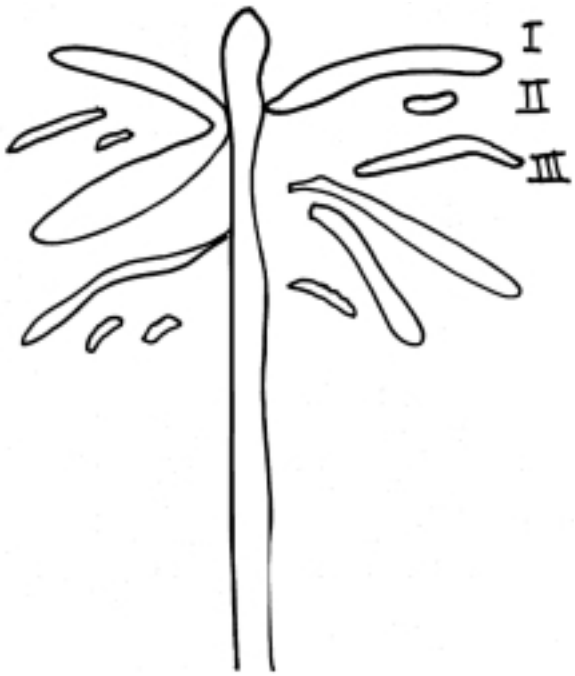

Fig. 1 Classification of palatine rugae based on size. Rugae were measured in a straight line between their origin and termination by a single examiner (A.M.M.) and grouped into three categories: (I) Primary, longer than 5 mm, (II) Fragmentary, between 2 and 3 mm, and (III) Secondary, between 3 and 5 mm.

sporadic tooth agenesis and their parents, who were recruited in the metropolitan area of Istanbul. This study was conduced with approval from both the University of Istanbul and the University of Pittsburgh Institutional Review Boards. None of the subjects reported any other relative affected by tooth agenesis, oral clefts, or anosmia. The probands had at least one developmentally missing tooth, excluding third molars. After informed consent had been obtained from all participants, peripheral blood samples were drawn or a saliva sample was collected from each individual. Clinical analyses, blood and saliva collection, and DNA extraction were performed using consolidated protocols. Dental casts were available for 38 nuclear families and we used the palatine rugae classification of Lysell (14) to classify our study group. Rugae were measured in a straight line between origin and termination by a single examiner (A.M.M.) and grouped into three categories: (a) primary rugae, those measuring 5 mm or more; (b) secondary rugae, those measuring between 3 and 5 mm; and (c) fragmentary rugae, measuring between 2 and 3 mm (Table 1; Fig. 1). Palatine rugae were analyzed for five different traits of interest: 1) Presence of secondary and fragmentary rugae; 2) Primary rugae on the right side of the palate being larger than those on the left side; 3) Primary rugae on the left side of the palate

Table 2 Information about assays for SNP analyzed in this study

SNP Marker	Approximate Location	Position on Chromosome 1*
rs4844880	90 kb 3' of *IRF6*	207937289
rs2235371 (V274I)	In *IRF6*	208030453
rs2013162	In *IRF6*	208035057
rs861019	In *IRF6*	208041759
rs2073487	In *IRF6*	208043019
rs642961	5' of *IRF6*	208055643
rs658860	5' of *IRF6*	208056922

* Based on the UCSC Genome Browser (http://genome.ucsc.edu), Human Mar. 2006 (hg18) assembly.

being larger than those on the right side; 4) Possessing fewer than 8 rugae; and 5) Possessing 8 or more rugae. Figure 2 presents examples of each of these subphenotyping groups. In addition, these characteristics were compared to the types of teeth that were missing in order to detect any preferential association between palatine rugae patterns and tooth agenesis.

Marker information is included in Table 2. Genotypes were obtained using an ABI PRISM 7900 Sequence Detection System and TaqMan chemistry. Reagents and SNP genotyping assays were supplied by Applied Biosystems. All SNPs showed Hardy-Weinberg equilibrium in both the affected probands and unaffected individuals. Pairwise calculations of linkage disequilibrium were computed with the Graphical Overview of Linkage Disequilibrium (GOLD) software package (15) for both the squared correlation coefficient (r^2, above the diagonal) and Lewontin's standardized disequilibrium coefficient (D', below diagonal). Markers showed weak to moderate linkage disequilibrium, suggesting that they would not provide redundant information. Alleles at each marker and haplotypes were tested for association with the proposed palatine rugae patterns with the use of the Family Based Association Test (FBAT) software package (16,17).

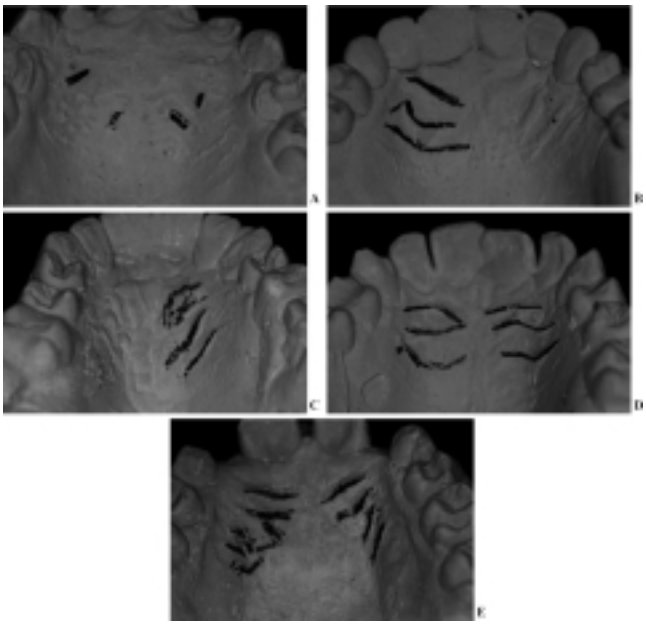

Fig. 2 Representative samples of the subphenotypes analyzed in this study. We used a pencil to highlight the traits of interest to facilitate visualization. Rugae were measured in a straight line between the origin and termination of the pencil marked line by a single examiner (A.M.M.). (A) Presence of secondary and fragmentary rugae; (B) Primary rugae on the right side of the palate are larger than rugae on the left side (a difference of approximately 1 to 2 mm); (C) Primary rugae on the left side of the palate are larger than rugae on the right side (difference of approximately 1 to 2 mm); (D) Number of rugae is less than 8 (6 rugae can be seen here); and (E) Number of rugae is 8 or more (9 can be seen here).

Results

Borderline associations were seen for *IRF6* genetic variation and having primary rugae larger on the right side than on the left (rs20131633, $P = 0.07$; rs642961, $P = 0.06$), and having less than eight primary palatine rugae (rs20131623, $P = 0.07$) (Table 3). However, no specific pattern of tooth agenesis was associated with the palatine rugae patterns studied.

Discussion

This is the first study to have investigated the possible association of palatine rugae with tooth agenesis, or with genetic variation. We used a population of subjects with sporadic tooth agenesis in which we had demonstrated that *IRF6* was a contributory factor (13). Since *Irf6* is expressed at the same time in tooth buds and palatine rugae in mice (8), we investigated whether palatine rugae patterns could be influenced by genetic variation in *IRF6*, as we described for tooth agenesis. Although the sample sizes were small, our results suggested a trend of association between certain

Table 3 Association results for palatine rugae

SNP	Allele	Test statistic S*	Expected value for S	*P*-value
Presence of secondary and fragmentary rugae				
rs4844880	A	9.000	10.333	0.42
	T	7.000	5.667	
rs2235371 (V274I)	C	5.000	4.500	0.56
	T	1.000	1.500	
rs20131623	A	9.000	10.000	0.62
	C	13.000	12.000	
rs861019	A	11.000	13.500	0.25
	G	15.000	12.500	
rs2073487	C	12.000	11.667	0.85
	T	6.000	6.333	
rs642961	A	4.000	3.000	0.41
	G	4.000	5.000	
rs658860	C	12.000	11.667	0.82
	T	4.000	4.333	
Primary right rugae larger than left rugae				
rs4844880	A	18.000	17.833	0.93
	T	8.000	8.167	
rs2235371 (V274I)	C	7.000	6.000	0.32
	T	1.000	2.000	
rs20131623	A	12.000	16.500	0.07
	C	26.000	21.500	
rs861019	A	19.000	21.500	0.33
	G	21.000	18.500	
rs2073487	C	26.000	22.667	0.16
	T	10.000	13.333	
rs642961	A	6.000	3.500	0.06
	G	4.000	6.500	
rs658860	C	20.000	18.667	0.5
	T	6.000	7.333	
Primary left rugae larger than right rugae				
rs4844880	A	8.000	8.167	0.92
	T	6.000	5.833	
rs2235371 (V274I)	C	4.000	4.500	0.56
	T	2.000	1.500	
rs20131623	A	11.000	12.000	0.64
	C	13.000	12.000	
rs861019	A	12.000	13.000	0.64
	G	12.000	11.000	
rs2073487	C	15.000	15.167	0.94
	T	11.000	10.833	
rs642961	A	1.000	1.000	1.0
	G	1.000	1.000	
rs658860	C	5.000	4.167	0.82
	T	3.000	3.833	
Less than 8 primary rugae				
rs4844880	A	9.000	9.833	0.6
	T	7.000	6.167	
rs2235371 (V274I)	C	4.000	4.500	0.56
	T	2.000	1.500	
rs20131623	A	11.000	15.500	0.07
	C	23.000	18.500	
rs861019	A	20.000	23.000	0.26
	G	18.000	15.000	
rs2073487	C	24.000	21.883	0.35
	T	10.000	12.167	
rs642961	A	5.000	3.500	0.26
	G	5.000	6.500	
rs658860	C	17.000	15.883	0.5
	T	7.000	8.167	
8 or more primary rugae				
rs4844880	A	17.000	16.167	0.65
	T	7.000	7.833	
rs2235371 (V274I)	C	7.000	6.000	0.32
	T	1.000	2.000	
rs20131623	A	12.000	13.000	0.64
	C	16.000	15.000	
rs861019	A	11.000	11.500	0.81
	G	15.000	14.500	
rs2073487	C	17.000	16.000	0.64
	T	11.000	12.000	
rs642961	A	2.000	1.000	0.16
	G	0.000	1.000	
rs658860	C	8.000	7.000	0.41
	T	2.000	3.000	

* Genotypic distribution in the offspring conditioned on affection status and parental genotypes.

palatine rugae patterns and *IRF6* variation, and therefore further investigations are warranted to address the hypothesis that *IRF6* contributes to the complexity of palatine rugae patterns.

The distribution of the number of primary rugae in our study population was similar to data reported previously (14). Almost half of the subjects (48%) had secondary rugae, and a much smaller proportion (approximately 6%) had fragmentary rugae (data not shown). We found an average total of 7 primary rugae in our study group. The suggestion that genetic variation contributes to the presence of up to seven primary rugae is intriguing. *Irf6* is clearly expressed in the palatine rugae of mice during development (8), and it can be hypothesized that the degree of *Irf6* expression may define the number of palatine rugae and their conformation.

Our study agrees with previously published data indicating that there are differences in the sizes of primary rugae (14), and we found larger rugae on the right side. Assuming that the genetic information is identical for each side, differences between sides can be interpreted as a consequence of environmental factors. On the other hand, one can propose that bilateral traits could be influenced by distinctive genes, depending on the particular side. Subtle random deviation from perfect bilateral symmetry is known as *fluctuating asymmetry* and is considered an appealing measure of developmental precision because of the apparent ease with which it may be measured and because its developmental origins seem so straightforward (18). In our study population, a larger number of individuals had larger primary palatine rugae on the right side (Table 1). A number of traits show differences in laterality. Cleft lip is more common on the left side (19), as is postaxial polydactyly (20,21), whereas microtia is found more commonly on the right side (22). Data also suggest that agenesis of mandibular second premolars may be more common on the right side (23). With regard to breast sizes, no significant differences between left and right have been described, although breast asymmetry is more common in healthy women who subsequently develop breast cancer than in those who remain disease-free, suggesting that breast asymmetry could be an indicator of future breast disease in women (24).

In summary, we have reported data related to palatine rugae patterns and their possible association with *IRF6* variation in humans.

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