Original Article

FGF10 and *FGF13* genetic variation and tooth-size discrepancies

Guido Artemio Marañón-Vásquez[a]; Alexandre Rezende Vieira[b]; Luiza Vertuan dos Santos[c]; Arthur Silva Cunha[d]; Suyany Gabriely Weiss[e]; Mônica Tirre de Souza Araujo[f]; Ana Maria Bolognese[g]; Rafaela Scariot[h]; Erika Calvano Küchler[h]; Maria Bernadete Sasso Stuani[i]

ABSTRACT

Objectives: To explore whether variations in odontogenesis-related genes are associated with tooth-size discrepancies.

Materials and Methods: Measurements of the width of permanent teeth were obtained from dental casts of 62 orthodontic patients (age 15.65 $\pm$ 6.82 years; 29 males and 33 females). Participants were classified according to the anterior and overall Bolton ratios as without tooth-size discrepancy or with maxillary or mandibular tooth-size excess. Genomic DNA extracted from buccal cells was used, and 13 single nucleotide polymorphisms (SNPs) across nine genes were genotyped by polymerase chain reaction using TaqMan chemistry. χ^2 or Fisher exact tests were applied to determine the overrepresentation of genotypes/alleles depending on the type of tooth-size discrepancy ($\alpha = .05$; corrected P value: $P < 5.556 \times 10^{-3}$). Odds ratios (ORs) and their correspondent 95% confidence intervals (CIs) were also calculated to investigate the risk of this phenotype for the SNPs having significant association.

Results: Individuals carrying the *FGF10* rs900379 T allele were more likely to have larger mandibular teeth (OR = 3.74; 95% CI: 1.65–8.47; $P = .002$). This effect appeared to be stronger when two copies of the risk allele (TT) were found (recessive model, OR = 6.16; 95% CI: 1.71–22.16; $P = .006$). On the other hand, *FGF13* rs5931572 rare homozygotes (AA, or male A hemizygotes) had increased risk of displaying tooth-size discrepancies when compared with the common homozygotes (GG, or male G hemizygotes; OR = 10.32; 95% CI: 2.20–48.26; $P = .003$).

Conclusions: The results suggest that *FGF10* and *FGF13* may contribute to the presence of tooth-size discrepancies. (*Angle Orthod.* 2021;91:356–362.)

KEY WORDS: Polymorphism; Single nucleotide; Fibroblast growth factors; Tooth abnormalities; Tooth; Dental arch

[a] PhD candidate, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[b] Professor, Department of Oral Biology, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA.

[c] Undergraduate student, School of Dentistry of Ribeirão Preto, University of São Paulo, São Paulo, Brazil.

[d] PhD candidate, Department of Orthodontics, State University of Rio de Janeiro, Rio de Janeiro, Brazil.

[e] PhD candidate, Department of Oral and Maxillofacial Surgery, Positivo University, Curitiba, Brazil.

[f] Associate Professor, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[g] Full Professor, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil.

[h] Professor, Department of Oral and Maxillofacial Surgery, Positivo University, Curitiba, Brazil.

[i] Associate Professor, Department of Pediatric Dentistry, School of Dentistry of Ribeirão Preto, University of São Paulo, Ribeirão Preto, Brazil.

Corresponding author: Guido Artemio Marañón-Vásquez, Department of Pediatric Dentistry and Orthodontics, School of Dentistry, Federal University of Rio de Janeiro, Rua. Prof. Rodolpho Paulo Rocco, 325 Cidade Universitária da Universidade Federal do Rio de Janeiro, Rio de Janeiro, RJ 21941-617, Brazil
(e-mail: guido_amv@hotmail.com)

Accepted: November 2020. Submitted: June 2020.
Published Online: January 25, 2021

INTRODUCTION

The lack of size correspondence between the upper and lower teeth, also known as tooth-size discrepancy, is a condition that contributes to the development of malocclusions.[1] It has been suggested that tooth-size discrepancy could be an inheritable trait.[2] In fact, tooth-size variability is largely caused by genetic factors, with or without a contribution from the environment.[3–6] Hundreds of genes participate in odontogenesis, being involved not only in growth, differentiation, and cell function but also in the patterning and morphogenesis of teeth.[7,8] Genetic variants in some of these genes have been associated with specific dental phenotypes.[9–12]

Tooth alterations are not isolated conditions. It was proposed that variation in tooth number and size were linked.[13] Various investigations reported the co-occurrence of hypodontia and supernumerary teeth, with size disparities in isolated teeth, as well as with consistent discrepancies in the size of the entire dentition, suggesting that these conditions could have a common etiologic origin.[14–20] Interestingly, previous studies showed that relatives of patients with hypodontia had decreased dental sizes, which could indicate a genetic link between both traits.[21,22] Based on this aggregate evidence, it was hypothesized that genetic variants suggested to contribute to the development of dental number anomalies could also be involved with the presence of altered tooth-size patterns. Thus, the objective of this study was to test for associations between odontogenesis-related genes and tooth-size discrepancies.

MATERIALS AND METHODS

The protocol of this study was reviewed and approved by the Research Ethics Committee at the School of Dentistry of Ribeirão Preto, University of São Paulo (01451418.3.0000.5419/3.150.551). The sample consisted of 62 healthy, unrelated individuals (age: 15.65 ± 6.82 years; 33 females, 29 males), self-reported as white, who were undergoing treatment at the orthodontic graduate clinic of the School of Dentistry of Ribeirão Preto. The main eligibility criterion was to have pretreatment dental casts with fully erupted permanent dentition (up to the first molar). Individuals with the presence of craniofacial congenital anomalies or syndromes (including tooth anomalies of number, size, or shape); preceding Class I, II, or III restorations; severe occlusal dental wear; interproximal caries; preceding orthodontic treatment including interproximal enamel reduction; extreme tooth misalignment hindering correct measurements; or poor-quality or fractured dental casts were not included. Signed written informed consent documents were obtained from all participants and their legal guardians.

Phenotyping

Tooth width was measured on all teeth for each of the upper and lower dental casts to the nearest 0.01 mm using an electronic handheld digital caliper (Digimatic CD-15DCX; Mitutoyo, Kawasaki, Japan) by the same individual. Tooth width was defined as the largest crown distance between the lateral contact points parallel to the occlusal plane. Measurements were performed three times consecutively and remeasured when they differed by more than 0.2 mm. Ten randomly chosen dental casts were measured twice at least 2 weeks apart to test intraexaminer reproducibility and agreement, using the intraclass correlation coefficient (ICC) for rater consistency in a two-way mixed model (95% confidence interval [CI]) and the estimation of the limits of agreement (LoA) using the Bland-Altman method. Intraexaminer reproducibility was high for all tooth measurements (ICC ranging from 0.888 to 0.996, all $P < .001$). Similarly, Bland-Altman tests showed small LoA.

Overall (B_{or}) and anterior (B_{ar}) Bolton ratios were calculated according to the following formulas[1]:

$$B_{or} = \frac{\text{Sum of mesiodistal width of 12 mandibular teeth (up to first molars)}}{\text{Sum of mesiodistal width of 12 maxillary teeth (up to first molars)}} \times 100$$

$$B_{ar} = \frac{\text{Sum of mesiodistal width of 6 mandibular anterior teeth (up to canines)}}{\text{Sum of mesiodistal width of 6 maxillary anterior teeth (up to canines)}} \times 100$$

Individuals were separately classified by their B_{or} and B_{ar}, according to the original values reported by Bolton,[1] as without tooth-size discrepancy ($89.39 \leq B_{or} \leq 93.21$; $75.55 \leq B_{ar} \leq 78.85$), with maxillary tooth-size excess ($B_{or} < 89.39$; $B_{ar} < 75.55$), or with mandibular tooth-size excess ($B_{or} > 93.21$, $B_{ar} > 78.85$).

Genotyping

Genomic DNA was extracted from buccal cells as previously described.[23] Genetic variants studied were single nucleotide polymorphisms (SNPs) selected based on the evidence of a previously reported association with tooth-related phenotypes and/or their known role in odontogenesis. A total of 13 SNPs across nine genes (Table 1) were blindly genotyped using TaqMan chemistry and end-point analysis in a real-time polymerase chain reaction (PCR) system (Prism QuantStudio 6 Flex PCR System, Applied

Table 1. Studied Single Nucleotide Polymorphisms[a]

Gene	Locus	Reference Sequence	Type of Alteration	Base Change (Context Sequence)	Global MAF
FGF3	11q13.3	rs1893047	Intron variant	CAC[**A**/G]TGA	0.4545/2276
FGF10	5p12	rs900379	Intron variant	CCT[C/**T**]ATA	0.4661/2334
FGF13	Xq26.3	rs12838463	Intron variant	ATC[A/**G**]TAG	0.4437/1675
FGF13	Xq27.1	rs5931572	Intron variant	ATT[A/**G**]TTT	0.4575/1727
GHR	5p12	rs2910875	Utr variant 3 prime	ATG[**A**/G]CTA	0.4557/2282
GHR	5p13.1	rs1509460	Intron variant	CAG[**G**/T]ACT	0.4407/2207
GLI2	2q14.2	rs2278741	Downstream variant 500B, utr variant	GAA[C/**G**]ACT	0.4922/2465
GLI2	2q14.2	rs3738880	Missense[b]	GAC[**G**/T]CCC	0.4910/2459
GLI3	7p14.1	rs846266	Missense[c]	CAG[C/**T**]GGG	0.3964/1985
GLI3	7p14.1	rs929387	Missense[d]	CCC[**A**/G]GCG	0.4215/2111
MSX1	4p16.2	rs1042484	Intron variant	TCC[A/**G**]ATG	0.1464/733
PAX9	14q13.3	rs8004560	Intron variant	TAA[**A**/G]TAT	0.3714/1860
TGFA	2p13.3	rs2902345	Intron variant	GGT[C/**T**]GCC	0.4022/2014

[a] MAF indicates minor allele frequency; Utr, untranslated region. Bold font indicates a lower-frequency allele. Sources of information: dbSNP from http://www.ncbi.nlh.nih.gov/snp/, http://genome.uscs.edu/, and https://www.thermofisher.com.
[b] Ala → Ser.
[c] Thr → Ala.
[d] Pro → Leu.

Biosystems, Thermo Fisher Scientific Inc, Foster City, Calif) following a standardized protocol.[24] Because of the exploratory nature of the study, only SNPs with a genotyping failure rate of up to 20% were included for further analyses. In addition, SNPs in Hardy-Weinberg disequilibrium at $P < 10^{-3}$ were not assessed. All retained SNPs had a minimum minor allele frequency >15%.

Statistical Analysis

The Pearson χ^2 (3 × 2 contingency tables; with continuity correction, when necessary) and Fisher exact (2 × 2 contingency tables) tests were performed to determine associations between genotype/allele frequencies on each SNP and the presence of tooth-size discrepancy. The absence of tooth-size discrepancy was considered as the reference phenotype for the analyses. For *FGF13* rs5931572 (located on the chromosome X), a multivariate logistic regression was performed to test associations, adjusting the analyses for the covariate sex. The threshold for statistical significance after Bonferroni correction for multiple testing was $P < 5.556 \times 10^{-3}$ (0.05/9 SNPs). The odds ratios (ORs) and their 95% CIs were also calculated to estimate the risk of tooth-size discrepancy when carrying variant alleles. Additional analyses were also performed for the dominant and recessive models for the significantly associated SNPs. All analyses were performed using two-tailed tests with a significance level of 5% using SPSS Statistics 23 (IBM, Armonk, NY).

RESULTS

Nine SNPs were included for genotype/phenotype analyses. *FGF10* rs900379, *GHR* rs1509460, *GLI3* rs929387, and *FGF3* rs1893047 showed association with the presence of tooth-size discrepancy at the nominal level ($P < .05$; Tables 2 and 3). *FGF10* rs900379 allele frequency was associated with the overall mandibular tooth-size excess, even after Bonferroni correction ($P < 5.556 \times 10^{-3}$; Table 2). Individuals carrying the rs900379 T allele were more likely to have larger mandibular teeth (OR = 3.74; 95% CI: 1.65–8.47; $P = .002$). This effect appeared to be stronger when two risk alleles (TT) were considered (recessive model, OR = 6.16; 95% CI: 1.71–22.16; $P = .006$).

Sex-adjusted analysis showed significant association of *FGF13* rs5931572 with the presence of tooth-size discrepancy ($P < 5.556 \times 10^{-3}$; Table 4). *FGF13* rs5931572 rare homozygotes (AA, or male A hemizygotes) had an increased risk of presenting this phenotype when compared with the common homozygotes (GG, or male G hemizygotes; OR = 10.32; 95% CI: 2.20–48.26; $P = .003$). Although only at the nominal level, subsequent analysis showed that rare homozygotes were more likely to have larger mandibular anterior teeth (OR = 4.25; 95% CI: 1.00–17.99; $P = .050$).

DISCUSSION

The importance of phenotype-genotype correlation data in determining the role of genes in dentofacial morphology variations associated with malocclusions has been suggested.[25] Although relevant phenotypes such as the Class II and Class III craniofacial patterns[26–30] have been widely investigated, studies regarding the underlying genetic component of variation in tooth-size patterns are still lacking. This is the

Table 2. Genotype and Allele Frequencies According to the Presence of TSD (B_{or})[a]

	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
TGFA rs2902345							
Without TSD	8 (26.7)	12 (40.0)	10 (33.3)	Reference	28 (46.7)	32 (53.3)	Reference
Maxillary tooth-size excess	1 (20.0)	3 (60.0)	1 (20.0)	.699	5 (50.0)	5 (50.0)	>.999
Mandibular tooth -size excess	7 (35.0)	9 (45.0)	4 (20.0)	.574	23 (57.5)	17 (42.5)	.314
	Genotype, n (%)				Allele, n (%)		
	CC	CG	GG	*P* Value	C	G	*P* Value
GLI2 rs2278741							
Without TSD	2 (6.1)	9 (27.3)	22 (66.7)	Reference	13 (19.7)	53 (80.3)	Reference
Maxillary tooth-size excess	0 (0.0)	2 (40.0)	3 (60.0)	.750[b]	2 (20.0)	8 (80.0)	>.999
Mandibular tooth-size excess	1 (5.0)	3 (15.0)	16 (80.0)	.559	5 (12.5)	35 (87.5)	.429
	Genotype, n (%)				Allele, n (%)		
	GG	GT	TT	*P* Value	G	T	*P* Value
GLI2 rs3738880							
Without TSD	11 (34.4)	8 (25.0)	13 (40.6)	Reference	30 (46.9)	34 (53.1)	Reference
Maxillary tooth-size excess	1 (20.0)	2 (40.0)	2 (40.0)	.728	4 (40.0)	6 (60.0)	.745
Mandibular tooth-size excess	6 (31.6)	2 (10.5)	11 (57.9)	.358	14 (36.8)	24 (63.2)	.409
	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
FGF10 rs900379							
Without TSD	15 (45.5)	13 (39.4)	5 (15.2)	Reference	43 (65.2)	23 (34.8)	Reference
Maxillary tooth-size excess	0 (0.0)	5 (100.0)	0 (0.0)	.041[b,c]	5 (50.0)	5 (50.0)	.484
Mandibular tooth-size excess	4 (19.0)	6 (28.6)	11 (52.4)	0.011[c]	14 (33.3)	28 (66.7)	.002[c,d]
	Genotype, n (%)				Allele, n (%)		
	GG	GT	TT	*P* Value	G	T	*P* Value
GHR 1509460							
Without TSD	5 (15.6)	19 (59.4)	8 (25.0)	Reference	29 (45.3)	35 (54.7)	Reference
Maxillary tooth-size excess	2 (40.0)	0 (0.0)	3 (60.0)	.047[a,b]	4 (40.0)	6 (60.0)	>.999
Mandibular tooth-size excess	4 (20.0)	12 (60.0)	4 (20.0)	.874	20 (50.0)	20 (50.0)	.689
	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
GLI3 rs846266							
Without TSD	10 (32.3)	9 (29.0)	12 (38.7)	Reference	29 (46.8)	33 (53.2)	Reference
Maxillary tooth-size excess	2 (40.0)	1 (20.0)	2 (40.0)	.902	5 (50.0)	5 (50.0)	>.999
Mandibular tooth-size excess	8 (50.0)	2 (12.5)	6 (37.5)	.349	18 (56.3)	14 (43.8)	.514
	Genotype, n (%)				Allele, n (%)		
	AA	AG	GG	*P* Value	A	G	*P* Value
GLI3 rs929387							
Without TSD	7 (25.0)	9 (32.1)	12 (42.9)	Reference	23 (41.1)	33 (58.9)	Reference
Maxillary tooth-size excess	1 (20.0)	2 (40.0)	2 (40.0)	.937	4 (40.0)	6 (60.0)	>.999
Mandibular tooth-size excess	0 (0.0)	5 (27.8)	13 (72.2)	.043[c]	5 (13.9)	31 (86.1)	.006[c]
	Genotype, n (%)				Allele, n (%)		
	AA	AG	GG	*P* Value	A	G	*P* Value
FGF3 rs1893047							
Without TSD	19 (70.4)	6 (22.2)	2 (7.4)	Reference	44 (81.5)	10 (18.5)	Reference
Maxillary tooth-size excess	3 (60.0)	2 (40.0)	0 (0.0)	.616[b]	8 (80.0)	2 (20.0)	>.999
Mandibular tooth-size excess	15 (71.4)	4 (19.0)	2 (9.5)	.941	34 (81.0)	8 (19.0)	>.999

[a] B_{or} indicates overall Bolton ratio; TSD, tooth-size discrepancy.
[b] χ^2 test conditions were not met.
[c] Significant association at the nominal level, $P < .05$.
[d] Significant association after Bonferroni correction, $P < 5.556 \times 10^{-3}$.

Table 3. Genotype and Allele Frequencies According to the Presence of TSD (B_{ar})[a]

	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
TGFA rs2902345							
Without TSD	12 (42.9)	9 (32.1)	7 (25.0)	Reference	33 (58.9)	23 (41.1)	Reference
Maxillary tooth-size excess	0 (0.0)	5 (62.5)	3 (37.5)	.072	5 (31.3)	11 (68.8)	.087
Mandibular tooth-size excess	4 (21.1)	10 (52.6)	5 (26.3)	.251	18 (47.4)	20 (52.6)	.298
	Genotype, n (%)				Allele, n (%)		
	CC	CG	GG	*P* Value	C	G	*P* Value
GLI2 rs2278741							
Without TSD	2 (6.5)	9 (29.0)	20 (64.5)	Reference	13 (21.0)	49 (79.0)	Reference
Maxillary tooth-size excess	1 (12.5)	1 (12.5)	6 (75.0)	.581[b]	3 (18.8)	13 (81.3)	>.999
Mandibular tooth-size excess	0 (0.0)	4 (21.1)	15 (78.9)	.394[b]	4 (10.5)	34 (89.5)	.273
	Genotype, n (%)				Allele, n (%)		
	GG	GT	TT	*P* Value	G	T	*P* Value
GLI2 rs3738880							
Without TSD	8 (26.7)	8 (26.7)	14 (46.7)	Reference	24 (40.0)	36 (60.0)	Reference
Maxillary tooth-size excess	3 (42.9)	1 (14.3)	3 (42.9)	.645	7 (50.0)	7 (50.0)	.556
Mandibular tooth-size excess	7 (36.8)	3 (15.8)	9 (47.4)	.604	17 (44.7)	21 (55.3)	.679
	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
FGF10 rs900379							
Without TSD	11 (35.5)	14 (45.2)	6 (19.4)	Reference	36 (58.1)	26 (41.9)	Reference
Maxillary tooth-size excess	3 (37.5)	3 (37.5)	2 (25.0)	.909	9 (56.3)	7 (43.8)	>.999
Mandibular tooth-size excess	5 (25.0)	7 (35.0)	8 (40.0)	.270	17 (42.5)	23 (57.5)	.157
	Genotype, n (%)				Allele, n (%)		
	GG	GT	TT	*P* Value	G	T	*P* Value
GHR 1509460							
Without TSD	7 (22.6)	16 (51.6)	8 (25.8)	Reference	30 (48.4)	32 (51.6)	Reference
Maxillary tooth-size excess	2 (28.6)	3 (42.9)	2 (28.6)	.909	7 (50.0)	7 (50.0)	>.999
Mandibular tooth-size excess	2 (10.5)	12 (63.2)	5 (26.3)	.540	16 (42.1)	22 (57.9)	.680
	Genotype, n (%)				Allele, n (%)		
	CC	CT	TT	*P* Value	C	T	*P* Value
GLI3 rs846266							
Without TSD	11 (40.7)	6 (22.2)	10 (37.0)	Reference	28 (51.9)	26 (48.1)	Reference
Maxillary tooth-size excess	3 (37.5)	2 (25.0)	3 (37.5)	.982	8 (50.0)	8 (50.0)	>.999
Mandibular tooth-size excess	6 (35.3)	4 (23.5)	7 (41.2)	.935	16 (47.1)	18 (52.9)	.827
	Genotype, n (%)				Allele, n (%)		
	AA	AG	GG	*P* Value	A	G	*P* Value
GLI3 rs929387							
Without TSD	4 (14.8)	10 (37.0)	13 (48.1)	Reference	18 (33.3)	36 (66.7)	Reference
Maxillary tooth-size excess	3 (42.9)	1 (14.3)	3 (42.9)	.230	7 (50.0)	7 (50.0)	.352
Mandibular tooth-size excess	1 (5.9)	5 (29.4)	11 (64.7)	.427	7 (20.6)	27 (79.4)	.232
	Genotype, n (%)				Allele, n (%)		
	AA	AG	GG	*P* Value	A	G	*P* Value
FGF3 rs1893047							
Without TSD	21 (77.8)	5 (18.5)	1 (3.7)	Reference	47 (87.0)	7 (13.0)	Reference
Maxillary tooth-size excess	3 (42.9)	2 (28.6)	2 (28.6)	.077[b]	8 (57.1)	6 (42.9)	.020[c]
Mandibular tooth-size excess	13 (68.4)	5 (26.3)	1 (5.3)	.776[b]	31 (81.6)	7 (18.4)	.560

[a] B_{ar} indicates anterior Bolton ratio; TSD, tooth-size discrepancy.
[b] χ^2 test conditions were not met.
[c] Significant association at the nominal level, $P < .05$.

Table 4. Genotype Frequencies for the ***FGF13* rs5931572** According to the Presence of TSD (B_{or} and B_{ar})[a]

Genotype[b] (Female/Male)	B_{or}				B_{ar}			
	TSD	No TSD	*P* Value[c]	OR (95% CI)	TSD	No TSD	*P* Value[c]	OR (95% CI)
AA / A-	9 (37.5)	8 (28.6)	.382	1.75 (0.49–6.16)	12 (50.0)	5 (17.9)	.003[d]	10.32 (2.20–48.26)
AG / –	6 (25.0)	6 (21.4)	.473	1.71 (0.39–7.50)	7 (29.2)	5 (17.9)	.078	4.07 (0.85–19.50)
GG / G-	9 (37.5)	14 (50.0)		Reference	5 (20.8)	18 (64.2)		Reference

[a] B_{ar} indictes anterior Bolton ratio; B_{or}, overall Bolton ratio; TSD, tooth-size discrepancy.
[b] Male participants could be A hemizygous or G hemizygous.
[c] *P* value adjusted by sex.
[d] Significant association after Bonferroni correction, $P < 5.556 \times 10^{-3}$.

first study to report genetic variants associated with the presence of tooth-size discrepancy.

The results indicated that *FGF10* rs900379 and *FGF13* rs5931572 were associated with the presence of tooth-size discrepancy, specifically with a larger size of mandibular teeth. *FGF10* rs900379 was previously reported as not associated with hypodontia, although another SNP in this gene was.[9] Mutations in *FGF10* cause the lacrimo-auriculo-dento-digital syndrome, an autosomal dominant multiple congenital anomaly disorder, which displays, among many other features, alterations in tooth size and structural defects.[31] The current findings, together with the information mentioned above, reinforce the idea that there would be a genetic link between tooth number anomalies and tooth-size variations and that *FGF10* and *FGF13* could be involved in this.

FGF10 and FGF13 are part of the family of fibroblast growth factors (FGFs) that participate in one of the most important signaling pathways during odontogenesis.[8,32–37] *Fgf10*-null mice have hypoplastic teeth[38] but no other significant dental defects.[39,40] For this reason, the functional redundancy of FGF10 in the dental formation process has been proposed.[33,41] However, on the other hand, it has been shown that the dental epithelium of these animals showed limited growth. Dental epithelium lacking a cervical loop exhibited a decreased growth rate.[35] Based on this, it may be assumed that genetic variation in *FGF10* could alter the rate of dental growth, which could explain variations in the size of the teeth.

FGF3 rs1893047 showed a trend for association with tooth-size discrepancy. Previous studies already demonstrated the possible involvement of markers of this gene (rs1893047, rs12574452, rs7932320) in the presence of hypodontia.[9,10] *Fgf3* is expressed in mesenchymal cells from E13.5 (bud stage) in regions adjacent to the inner enamel epithelium.[33,35] Unlike FGF10, FGF3 could stimulate cell proliferation in isolated dental mesenchyme.[33] Although it is not responsible for cervical loop formation, since *Fgf3*-/- mice present smaller molars with structural alterations compared with wild-type and *Fgf3* +/– molars,[42] *FGF3* may be involved in tooth-size–related alterations.

Further research is necessary to replicate the current findings in larger samples of different ethnic origin. The absence of positive associations for tested variations in the other genes could be due to a type II error due to an insufficient sample size (explorative study). In addition, future studies could use digital methods to possibly increase the accuracy of the acquisition processes and/or the measurement of the studied phenotype, as well as consider the study of individual tooth sizes expressed as continuous data or different approaches for tooth size analysis.

CONCLUSION

- *FGF10* and *FGF13* may contribute to the presence of tooth-size discrepancy.

REFERENCES

1. Bolton WA. Disharmony in tooth size and its relation to the analysis and treatment of malocclusion. *Angle Orthod*. 1958; 28:113–130.
2. Baydas B, Oktay H, Dagsuyu IM. The effect of heritability on Bolton tooth-size discrepancy. *Eur J Orthod*. 2005;27:98–102.
3. Osborne RH, Horowitz SL, De George FV. Genetic variation in tooth dimensions: a twin study of the permanent anterior teeth. *Am J Hum Genet*. 1958;10:350–356.
4. Townsend GC, Brown T. Heritability of permanent tooth size. *Am J Phys Anthropol*. 1978;49:497–504.
5. Dempsey PJ, Townend GC. Genetic and environmental contributions to variation in human tooth size. *Heredity (Edinb)*. 2001;86:685–693.
6. Kabban M, Fearne J, Jovanovski V, Zou L. Tooth size and morphology in twins. *Int J Paediatr Dent*. 2001;11:333–339.
7. Thesleff I. The genetic basis of tooth development and dental defects. *Am J Med Genet A*. 2006;140:2530–2535.
8. Bei M. Molecular genetics of tooth development. *Curr Opin Genet Dev*. 2009;19:504–510.
9. Küchler EC, Lips A, Tannure PN, et al. Tooth agenesis association with self-reported family history of cancer. *J Dent Res*. 2013;92:149–155.
10. Vieira AR, D'Souza RN, Mues G, et al. Candidate gene studies in hypodontia suggest role for FGF3. *Eur Arch Paediatr Dent*. 2013;14:405–410.
11. Vieira AR, Meira R, Modesto A, Murray JC. MSX1, PAX9, and TGFA contribute to tooth agenesis in humans. *J Dent Res*. 2004;83:723–727.

12. Liu H, Han D, Wong S, Nan X, Zhao H, Feng H. rs929387 of GLI3 is involved in tooth agenesis in Chinese Han population. *PLoS One*. 2013;8:e80860.
13. Brook AH. A unifying aetiological explanation for anomalies of human tooth number and size. *Arch Oral Biol*. 1984;19: 373–378.
14. Schalk-van der Weide Y, Steen WH, Beemer FA, Bosman F. Reductions in size and left-right asymmetry of teeth in human oligodontia. *Arch Oral Biol*. 1994;39:935–939.
15. Brook AH, Elcock C, al-Sharood MH, McKeown HF, Khalaf K, Smith RN. Further studies of a model for the etiology of anomalies of tooth number and size in humans. *Connect Tissue Res*. 2002;43:289–295.
16. Khalaf K, Robinson DL, Elcock C, Smith RN, Brook AH. Tooth size in patients with supernumerary teeth and a control group measured by image analysis system. *Arch Oral Biol*. 2005;50:243–248.
17. Khalaf K, Smith RN, Elcock C, Brook AH. Multiple crown size variables of the upper incisors in patients with supernumerary teeth compared with controls. *Arch Oral Biol*. 2009;54: S71–S78.
18. Brook AH, Griffin RC, Smith RN, et al. Tooth size patterns in patients with hypodontia and supernumerary teeth. *Arch Oral Biol*. 2009;54:S63–S70.
19. Gungor AY, Turkkahraman H. Tooth sizes in nonsyndromic hypodontia patients. *Angle Orthod*. 2013;83:16–21.
20. Higashihori N, Takada JI, Minami K, Takahashi Y, Moriyama K. Frequency of missing teeth and reduction of mesiodistal tooth width in Japanese patients with tooth agenesis. *Prog Orthod*. 2018;19:30.
21. Schalk-van der Weide Y, Bosman F. Tooth size in relatives of individuals with oligodontia. *Arch Oral Biol*. 1996;41:469–472.
22. McKeown HF, Robinson DL, Elcock C, al-Sharood M, Brook AH. Tooth dimensions in hypodontia patients, their unaffected relatives and a control group measured by a new image analysis system. *Eur J Orthod*. 2002;24:131–141.
23. Küchler EC, Tannure PN, Falagan-Lotsch P, Lopes TS, Granjeiro JM, Amorim LMF. Buccal cells DNA extraction to obtain high quality human genomic DNA suitable for polymorphism genotyping by PCR-RFLP and Real-Time PCR. *J Appl Oral Sci.* 2012;20:467–471.
24. Ranade K, Chang MS, Ting CT, et al. High-throughput genotyping with single nucleotide polymorphisms. *Genome Res*. 2001;11:1262–1268.
25. Moreno Uribe LM, Miller SF. Genetics of the dentofacial variation in human malocclusion. *Ortho Craniofac Res*. 2015;18:91–99.
26. Cruz CV, Mattos CT, Maia JC, et al. Genetic polymorphisms underlying the skeletal Class III phenotype. *Am J Orthod Dentofacial Orthop*. 2017;151:700–707.
27. Da Fontoura CSG, Miller SF, Wehby GL, et al. Candidate gene analyses of skeletal variation in malocclusion. *J Dent Res*. 2015;94:913–920.
28. Frazier-Bowers S, Rincon-Rodriguez R, Zhou J, Alexander K, Lange E. Evidence of linkage in a Hispanic cohort with a Class III dentofacial phenotype. *J Dent Res*. 2009;88:56–60.
29. Tobón-Arroyave SI, Jiménez-Arbeláez GA, Alvarado-Gómez VA, Isaza-Guzmán DM, Flórez-Moreno GA, Pérez-Cano MI. Association analysis between rs6184 and rs6180 polymorphisms of growth hormone receptor gene regarding skeletal-facial profile in a Colombian population. *Eur J Orthod*. 2018; 40:378–386.
30. Zebrick B, Teeramongkolgul T, Nicot R, et al. ACTN3 R577X genotypes associate with Class I and deepbite malocclusions. *Am J Orthod Dentofacial Orthop*. 2014;146:603–611.
31. Milunsky JM, Zhao G, Maher TA, Colby R, Everman DB. LADD syndrome is caused by FGF10 mutations. *Clin Genet*. 2006;69:349–354.
32. Porntaveetus T, Otsuka-Tanaka Y, Basson MA, Moon AM, Sharpe PT, Ohazama A. Expression of fibroblast growth factors (Fgfs) in murine tooth development. *J Anat*. 2011; 218:534–543.
33. Kettunen P, Laurikkala J, Itäranta P, Vainio S, Itoh N, Thesleff I. Association of FGF-3 and FGF-10 with signaling networks regulating tooth morphogenesis. *Dev Dyn*. 2000; 219:322–332.
34. Harada H, Kettunen P, Jung H, Mustonen T, Wang YA, Thesleff I. Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. *J Cell Biol*. 1999;147:105–120.
35. Harada H, Toyono T, Toyoshima K, et al. FGF10 maintains stem cell compartment in developing mouse incisors. *Development*. 2002;129:1533–1541.
36. Klein OD, Lyons DB, Balooch G, et al. An FGF signaling loop sustains the generation of differentiated progeny from stem cells in mouse incisors. *Development*. 2008;135:377–385.
37. Kettunen P, Furmanek T, Chaulagain R, Kvinnsland IH, Luukko K. Developmentally regulated expression of intracellular Fgf11-13, hormone-like Fgf15 and canonical Fgf16, -17 and -20 mRNAs in the developing mouse molar tooth. *Acta Odontol Scand*. 2011;69:360–366.
38. Ohuchi H, Hori Y, Yamasaki M, et al. FGF10 acts as a major ligand for FGF receptor 2IIIb in mouse multi-organ development. *Biochem Biophys Res Commun*. 2000;277:643–649.
39. Min H, Danilenko DM, Scully SA, et al. Fgf-10 is required for both limb and lung development and exhibits striking functional similarity to Drosophila branchless. *Genes Dev*. 1998;12:3156–3161.
40. Sekine K, Ohuchi H, Fujiwara M, et al. Fgf10 is essential for limb and lung formation. *Nat Genet*. 1999;21:138–141.
41. Kratochwil K, Galceran J, Tontsch S, Roth W, Grosschedl R. FGF4, a direct target of LEF1 and Wnt signaling, can rescue the arrest of tooth organogenesis in Lef1-/- mice. *Genes Dev*. 2002;16:3173–3185.
42. Charles C, Lazzari V, Tafforeau P, et al. Modulation of Fgf3 dosage in mouse and men mirrors evolution of mammalian dentition. *Proc Natl Acad Sci USA*. 2009;106:22364–22368.