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Association between craniofacial morphological patterns and tooth agenesis-related genes

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Abstract

Background: The aim of the present study was to assess if genetic polymorphisms in tooth agenesis (TA)-related genes are associated with craniofacial morphological patterns.

Methods: This cross-sectional, multi-center, genetic study evaluated 594 orthodontic Brazilians patients. The presence or absence of TA was determined by analysis of panoramic radiography. The patients were classified according to their skeletal malocclusion and facial growth pattern by means of digital cephalometric analysis. Genomic DNA was extracted from squamous epithelial cells of buccal mucosa and genetic polymorphisms in *MSX1* (rs1042484), *PAX9* (rs8004560), *TGF-α* (rs2902345), *FGF3* (rs1893047), *FGF10* (rs900379), and *FGF13* (rs12838463, rs5931572, and rs5974804) were genotyped by polymerase chain reaction using TaqMan chemistry and end-point analysis.

Results: Genotypes ($p = 0.038$) and allele ($p = 0.037$) distributions for the *FGF3* rs1893047 were significantly different according to the skeletal malocclusion. Carrying at least one G allele increased in more than two times the chance of presenting skeletal class III malocclusion (OR = 2.21, CI 95% = 1.14–4.32; $p = 0.017$). There was no association between another skeletal craniofacial pattern and some polymorphism assessed in the present study.

Conclusion: Our results suggest that the genetic polymorphism rs1893047 in *FGF3* might contribute to variations in the craniofacial sagittal pattern.

Keywords: Anodontia, Maxillofacial development, Polymorphism, Genetic

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Background

Tooth agenesis (TA) is the congenital absence of one or more teeth. This condition results from disturbances at early stages of odontogenesis. Several studies have demonstrated that mutations and genetic polymorphisms within specific genes may contribute to the presence of TA; among them are *MSX1* [1], *PAX9* [1], *TGF-α* [2] and genes from the *FGF* family [3, 4].

MSX1 (muscle segment homeobox 1) is expressed during epithelial-mesenchymal interactions that occur at the beginning of tooth formation [5]. Mutations on this gene are related to failures in the development of multiple teeth, preferentially premolars [6]. *Msx1* deficient mice showed cleft palate, alveolar bone defects, anomalies in various facial bones, and failure of tooth development [7], suggesting that TA could be genetically related to the development of cranium, maxillary, and mandibular complex [8]. On the other hand, *PAX9* (paired box gene 9) is expressed in the neural-crest-derived mesenchyme of the maxillary/mandibular arches and also contributes to tooth and palate formation [9]. Mutation in *PAX9* gene was associated with autosomal dominant oligodontia, usually affecting the majority of permanent molars [10]. *Pax9* deficient mice presented agenesis of all teeth, cleft palate, and other craniofacial anomalies [5].

TGF-α (transforming growth factor-alpha) is a gene expressed during craniofacial development [11]. Although, mice with *Tgf-α* deficiency did not show tooth anomalies [12], human studies evidenced that genetic polymorphisms in *TGF-α* contributes to the presence of TA [2]. Regarding the *FGF* (fibroblast growth factor) signaling, its role in craniofacial development has extensively investigated [13, 14]. This has an inductive function on craniofacial primordia formation and controls the balance among cell growth, differentiation, and apoptosis [13]. *FGF* signaling is expressed during tooth development [15] and it was associated with isolated TA [3, 4].

Some previous studies have reported an association between TA and craniofacial morphological patterns [16–23], including retrognathic maxilla [18], class III skeletal malocclusion [18, 22], and concave profile [21]. Additionally, our recent study demonstrated that TA-associated *GLI2* and *GLI3* genes might play a role in the development of skeletal malocclusions [24]. We therefore reaffirm the hypothesis that TA could share a similar genetic background with specific craniofacial morphologies or skeletal malocclusions, and that genetic polymorphisms on additional TA-associated genes may contribute to the establishment of both conditions. In the present study, we aimed to evaluate whether polymorphisms in TA-related *MSX1*, *PAX9*, *TGF-α*, *FGF3*, *FGF10*, and *FGF13* genes contribute to the development of different craniofacial morphological patterns.

Materials and methods

The protocol of this study was approved by the Research Ethics Committees of the Antônio Pedro University Hospital at the Fluminense Federal University (n° 33791314.3.0000.5243), School of dentistry of Ribeirão Preto at the University of São Paulo (n° 50765715.3.0000.5419), and the Institutional review board committee at the University of Pittsburgh (no. 12080056). An informed consent form was obtained from all participants or legal guardians.

Orthodontic dental records from 766 ethnically heterogeneous Brazilians were assessed for recruitment (from July 2015 to August 2017). Five hundred and ninety-four orthodontic patients (mean age = 23.1; 238 males, 356 females) from private and graduate orthodontic clinics in Rio de Janeiro (n = 325), São Paulo (n = 140), and Amazonas cities (n = 129) were selected following convenience sampling. Location setting and ethnic composition of each city were already described in previous studies [24, 25]. Patients with one of the following conditions were excluded: previous orthodontic treatment, medical systemic conditions, craniofacial congenital or syndromic anomalies, permanent teeth lost or extracted, and previous facial trauma. Individuals were classified according to the presence/absence of TA, 5.22% presented agenesis of at least one permanent tooth, excluding third molars [24].

Cephalometric assessment

Pretreatment lateral cephalometric radiographs of patients were scanned using the HP Scanjet G4050 scanner (L1957A; Hewlett Packard, Washington, USA). The images were imported to the Dolphin® Imaging 11.0 software (Dolphin Imaging and Management Solutions, Chatsworth, CA, USA) and then traced and analyzed by calibrated orthodontists. Ten percent of the radiographs were randomly selected and examined twice to test intra- and inter-examiner reproducibility (4-week interval) using the Intraclass Correlation Coefficient (ICC). ICC for repeated measurements ranged from 0.79–0.87.

The ANB angle was used to classify the individuals by their sagittal skeletal malocclusion (class I 0° to 4.0°; class II > 4.0°; class III ANB < 0°). On the other hand, the NaBa-PtGn angle was used to categorize the subjects by their facial growth pattern (mesofacial 87.0°–93.0°, dolichofacial < 87.0°, and brachyfacial > 93.0°).

DNA extraction and Genotyping

Genomic DNA was extracted from saliva samples for molecular analysis according to a previously reported method [26]. Quantification of the concentration and purity of the DNA was determined using a spectrophotometer (Nanodrop 1000; Thermo Scientific, Wilmington, DE, USA).

Table 1 Studied genetic polymorphisms

Gene	Locus	Reference sequence	Type of alteration	Base change (context sequence)	Global MAF
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
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
FGF13	Xq27.1	rs5974804	Intron variant	CAT[C/**T**]GGT	0.4967/1875

Source of information: dbSNP from: https://www.ncbi.nlh.nih.gov/snp/, http://genome.uscs.edu/, and https://www.thermofisher.com
Bold: lower frequency allele

Eight genetic polymorphisms, located in intronic regions, were assessed: *MSX1* (rs1042484), *PAX9* (rs8004560), *TGF-α* (rs2902345), *FGF3* (rs1893047), *FGF10* (rs900379), and *FGF13* (rs12838463, rs5931572, and rs5974804) (Table 1). The polymorphisms were blindly genotyped by polymerase chain reactions (PCR) using the TaqMan method (ABI Prism 7900HT, Applied Biosystems, Foster City, CA, USA) [27] and end-point analysis. The interpretation of the data was performed using software provided by Applied Biosystems (Foster City, CA, USA) for allelic discrimination.

Statistical analysis

Statistical analyses were performed on Epi Info 3.5.2 (www.cdc.gov/epiinfo) and Plink (http://zzz.bwh.harvard.edu/plink/), using an established α of 0.05. Chi-square

Table 2 Genotype and allele distribution between TA and non-TA participants

Genetic polymorphism	Genotypes, *n* (%)			*p* value	Alleles, *n* (%)		*p* value
***MSX1* rs1042484**	**AA**	**AG**	**GG**		**A**	**G**	
TA	12 (66.7)	3 (16.7)	3 (16.7)	0.414	27 (75.0)	9 (25.0)	0.475
Non-TA	239 (70.5)	73 (21.5)	27 (8.0)		551 (81.3)	127 (18.7)	
***PAX9* rs8004560**	**AA**	**AG**	**GG**		**A**	**G**	
TA	11 (57.9)	6 (31.6)	2 (10.5)	0.783	28 (73.7)	10 (26.3)	0.740
Non-TA	214 (65)	81 (24.6)	34 (10.3)		509 (77.4)	149 (22.6)	
***TGFα1* rs2902345**	**CC**	**CT**	**TT**		**C**	**T**	
TA	6 (23.1)	12 (46.2)	8 (30.8)	0.495	24 (46.2)	28 (53.8)	0.317
Non-TA	111 (30.0)	180 (48.6)	79 (21.4)		402 (54.3)	338 (45.7)	
***FGF3* rs1893047**	**AA**	**AG**	**GG**		**A**	**G**	
TA	17 (68)	7 (28)	1 (4)	0.507	41 (82.0)	9 (18.0)	0.881
Non-TA	359 (74.4)	92 (19)	32 (6.6)		810 (83.8)	156 (16.2)	
***FGF10* rs900379**	**CC**	**CT**	**TT**		**C**	**T**	
TA	10 (35.7)	11 (39.3)	7 (25)	0.775	24 (46.2)	28 (53.8)	0.555
Non-TA	164 (29.4)	244 (43.8)	149 (26.8)		572 (51.3)	542 (48.7)	
***FGF13* rs12838463**	**AA**	**AG**	**GG**		**A**	**G**	
TA	19 (51.4)	9 (24.3)	9 (24.3)	0.828	47 (63.5)	27 (36.5)	0.306
Non-TA	360 (46.2)	212 (27.2)	207 (26.6)		932 (59.8)	626 (40.2)	
***FGF13* rs5931572**	**AA**	**AG**	**GG**		**A**	**G**	
TA	9 (32.1)	10 (35.8)	9 (32.1)	0.783	28 (50.0)	28 (50.0)	0.340
Non-TA	170 (32.1)	160 (30.2)	200 (37.7)		500 (47.2)	560 (52.8)	
***FGF13* rs5974804**	**AA**	**AG**	**GG**		**A**	**G**	
TA	8 (40)	6 (30)	6 (30)	0.884	22 (55.0)	18 (45.0)	0.520
Non-TA	194 (43.5)	112 (25.10)	140 (31.4)		500 (56.1)	392 (43.9)	

*Statistical significance ($p \leq 0.05$). For genetic polymorphisms in *FGF13*, analyses were adjusted by the gender

test (with Yates' correction for continuity, when necessary) or Fisher's exact test were performed to determine association between allele/genotype frequencies and the craniofacial phenotypes assessed. For the polymorphisms located in the chromosome X (polymorphisms in *FGF13*), an analysis adjusted by the gender was performed. Due to the multiple comparisons made, a Bonferroni correction was applied for each evaluated outcome (corrected p value = 0.00625; 0.05/8 genetic polymorphisms assessed). Genotype/phenotype associations were also tested in the dominant and recessive models. Chi-square test was also used to evaluate the Hardy-Weinberg equilibrium.

Results

The distribution of genotypes followed Hardy-Weinberg equilibrium (data not shown). Information regarding the association between skeletal malocclusion and TA was reported in a previous published paper [24]; individuals presenting class II skeletal malocclusion showed lower frequency of TA. There was no significant association between genotype/allele distributions and the presence of TA for any polymorphism assessed in the present study ($p > 0.05$) (Table 2).

Genotype ($p = 0.038$) and allele ($p = 0.037$) distributions for the *FGF3* rs1893047 were significantly different between class III and class I individuals (Table 3). Analysis

Table 3 Genotype and allele distribution among class I, class II and class III skeletal malocclusions

Genetic polymorphism	Genotypes, *n* (%)			*p* value	Alleles, *n* (%)		*p* value
***MSX1* rs1042484**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	118 (69.8)	38 (22.5)	13 (7.7)	Reference	274 (81.1)	64 (18.9)	Reference
Class II	106 (71.1)	29 (19.5)	14 (9.4)	0.730	241 (80.9)	57 (19.1)	0.999
Class III	26 (70.3)	8 (21.6)	3 (8.1)	0.990	60 (81.1)	14 (18.9)	0.863
***PAX9* rs8004560**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	91 (62.3)	35 (24.0)	20 (13.7)	Reference	217 (74.3)	75 (25.7)	Reference
Class II	100 (67.1)	39 (26.2)	10 (6.7)	0.140	239 (80.2)	59 (19.8)	0.108
Class III	25 (62.5)	10 (25.0)	5 (12.5)	0.975	60 (75.0)	20 (25.0)	0.999
***TGFα1* rs2902345**	**CC**	**CT**	**TT**		**C**	**T**	
Class I	51 (28.5)	89 (49.7)	39 (21.8)	Reference	191 (53.4)	167 (46.6)	Reference
Class II	46 (28.8)	77 (48.1)	37 (23.1)	0.947	169 (52.8)	151 (47.2)	0.999
Class III	16 (37.2)	22 (51.2)	5 (11.6)	0.261	54 (62.8)	32 (37.2)	0.144
***FGF3* rs1893047**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	157 (76.6)	36 (17.6)	12 (5.9)	Reference	350 (85.4)	60 (14.6)	Reference
Class II	165 (75.3)	39 (17.8)	15 (6.8)	0.932	369 (84.2)	69 (15.8)	0.964
Class III	28 (59.6)	16 (34.0)	3 (6.4)	0.038*	72 (76.6)	22 (23.4)	0.037*
***FGF10* rs900379**	**CC**	**CT**	**TT**		**C**	**T**	
Class I	70 (29.8)	107 (45.5)	58 (24.7)	Reference	247 (52.6)	223 (47.4)	Reference
Class II	72 (30.5)	115 (48.7)	49 (20.8)	0.585	259 (54.9)	213 (45.1)	0.509
Class III	19 (33.3)	20 (35.1)	18 (31.6)	0.336	58 (50.9)	56 (49.1)	0.103
***FGF13* rs12838463**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	110 (47.8)	65 (28.3)	55 (23.9)	Reference	285 (62.0)	175 (38.0)	Reference
Class II	112 (46.9)	60 (25.1)	67 (28.0)	0.541	284 (59.4)	194 (40.6)	0.765
Class III	29 (50.9)	14 (24.6)	14 (24.6)	0.850	72 (63.2)	42 (36.8)	0.876
***FGF13* rs5931572**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	72 (32.6)	63 (28.5)	86 (38.9)	Reference	207 (46.8)	235 (53.2)	Reference
Class II	67 (29.5)	65 (28.6)	95 (41.9)	0.749	199 (43.8)	255 (56.2)	0.823
Class III	16 (30.8)	15 (28.8)	21 (40.4)	0.966	47 (45.2)	57 (54.8)	0.091
***FGF13* rs5974804**	**AA**	**AG**	**GG**		**A**	**G**	
Class I	83 (42.1)	50 (25.4)	64 (32.5)	Reference	216 (54.8)	178 (45.2)	Reference
Class II	80 (44.7)	48 (26.8)	51 (28.5)	0.897	208 (58.1)	150 (41.9)	0.820
Class III	22 (46.8)	9 (19.1)	16 (34.0)	0.678	53 (56.4)	41 (43.6)	0.784

*Statistical significance ($p \leq 0.05$). For genetic polymorphisms in *FGF13*, analyses were adjusted by the gender.

in the dominant model (AG + GG vs. AA) demonstrated that carrying at least one G allele increased in more than two times the chance of presenting skeletal class III malocclusion (OR = 2.21, 95% CI = 1.14–4.32; p = 0.017). There was no association between the facial growth pattern and any polymorphism assessed in the present study ($p > 0.05$) (Table 4). No reported associations remained significant after the Bonferroni correction.

Discussion

Many human and animal studies support that dental anomalies, mainly TA, and craniofacial alterations could share a common genetic background [7, 9, 12–24, 28, 29]. The identification of genes contributing to the establishment of craniofacial morphological patterns can impact the clinical practice, allowing genetic counseling of individuals carrying specific variants and their families, and to work on preventive strategies. To the best of our knowledge, this is the first report investigating the association between genetic polymorphisms in TA-related genes—*MSX1*, *PAX9*, *TGF-α*, and *FGF* signaling–and craniofacial morphological patterns (associated or not with TA).

MSX1, *PAX9*, *TGF-α*, and *FGF* genes are responsible for the patterning of tooth development [7, 9, 15, 30]. Previous studies performed in Brazilian families (trio designs) and case-controls studies demonstrated that TA

Table 4 Genotype and allele distribution among mesofacial, dolichofacial, and brachyfacial growth patterns

Genetic polymorphism	Genotypes, *n* (%)			*p* value	Alleles, *n* (%)		*p* value
***MSX1* rs1042484**	**AA**	**AG**	**GG**		**A**	**A**	
Mesofacial	119 (69.6)	35 (20.5)	17 (9.9)	Reference	273 (79.8)	69 (20.2)	Reference
Dolichofacial	77 (70.6)	22 (20.2)	10 (9.2)	0.975	176 (80.7)	42 (19.3)	0.888
Brachyfacial	54 (72.0)	18 (24.0)	3 (4.0)	0.271	126 (84)	24 (16)	0.335
***PAX9* rs8004560**	**AA**	**AG**	**GG**		**A**	**G**	
Mesofacial	103 (66.5)	37 (23.9)	15 (9.7)	Reference	243 (78.4)	67 (21.6)	Reference
Dolichofacial	70 (63.1)	28 (25.2)	13 (11.7)	0.811	168 (75.7)	54 (24.3)	0.527
Brachyfacial	43 (62.3)	19 (27.5)	7 (10.1)	0.823	105 (76.1)	33 (23.9)	0.680
***TGFα1* rs2902345**	**CC**	**CT**	**TT**		**C**	**T**	
Mesofacial	51 (27.4)	95 (51.1)	40 (21.5)	Reference	197 (53.0)	175 (47)	Reference
Dolichofacial	35 (30.2)	55 (47.4)	26 (22.4)	0.815	125 (53.9)	107 (46.1)	0.888
Brachyfacial	27 (33.8)	38 (47.5)	15 (18.8)	0.574	92 (57.5)	68 (42.5)	0.383
***FGF3* rs1893047**	**AA**	**AG**	**GG**		**A**	**G**	
Mesofacial	166 (75.1)	39 (17.6)	16 (7.2)	Reference	371 (83.9)	71 (16.1)	Reference
Dolichofacial	111 (75.5)	28 (19.0)	8 (5.4)	0.765	250 (85.0)	44 (15.0)	0.865
Brachyfacial	73 (70.9)	24 (23.3)	6 (5.8)	0.464	170 (82.5)	36 (17.5)	0.886
***FGF10* rs900379**	**CC**	**CT**	**TT**		**C**	**T**	
Mesofacial	78 (31.0)	118 (46.8)	56 (22.2)	Reference	274 (54.4)	230 (45.6)	Reference
Dolichofacial	49 (30.2)	73 (45.1)	40 (24.7)	0.873	171 (52.8)	153 (47.2)	0.871
Brachyfacial	34 (29.8)	51 (44.7)	29 (25.4)	0.658	119 (52.2)	109 (47.8)	0.798
***FGF13* rs12838463**							
Mesofacial	116 (47.0)	63 (25.5)	68 (27.5)	Reference	295 (59.7)	199 (40.3)	Reference
Dolichofacial	79 (47.6)	43 (25.9)	44 (26.5)	0.911	201 (60.5)	131 (39.5)	0.981
Brachyfacial	56 (49.6)	33 (29.2)	24 (21.2)	0.624	145 (64.2)	81 (35.8)	0.678
***FGF13* rs5931572**	**AA**	**AG**	**GG**		**A**	**G**	
Mesofacial	70 (29.2)	69 (28.8)	101 (42.1)	Reference	209 (43.5)	271 (56.5)	Reference
Dolichofacial	54 (35.3)	40 (26.1)	59 (38.6)	0.443	148 (48.4)	158 (51.6)	0.746
Brachyfacial	31 (29.0)	34 (31.8)	42 (39.3)	0.830	96 (44.9)	118 (55.1)	0.104
***FGF13* rs5974804**	**AA**	**AG**	**GG**		**A**	**G**	
Mesofacial	84 (42.6)	49 (24.9)	64 (32.5)	Reference	217 (55.1)	177 (44.9)	Reference
Dolichofacial	60 (46.9)	33 (25.8)	35 (27.3)	0.602	153 (59.8)	103 (40.2)	0.139
Brachyfacial	41 (41.8)	25 (25.5)	32 (32.7)	0.989	107 (54.6)	89 (45.4)	0.912

*Statistical significance ($p \leq 0.05$). For genetic polymorphisms in *FGF13*, analyses were adjusted by the gender.

was associated with *MSX1*, *PAX9*, *TGF-α* [2], *FGF3* [2], and *FGF10* [3]. In the present study, none of the studied genetic polymorphisms were associated with TA. These results should be carefully evaluated, because they may be due to methodological limitations of the present study (small sample size of individuals with TA, sampling limitations, failure rate of genotyping procedures), which could have led to type II error.

On the other hand, it has been demonstrated that *Msx1* deficient mice show shortened mandibles, anteroposterior deficiency in the middle third of the face, and subtle abnormalities in overall head size and cranial shape [7]. Also, *Pax9* deficient mice present agenesis of all teeth, cleft palate, and other craniofacial anomalies [5]. *TGF-α* is a gene expressed during craniofacial development [11]; mice with *Tgf-α* deficiency presented eye and hair anomalies [12]. Despite the above-described roles of these genes, the genetic polymorphisms studied within *MSX1*, *PAX9*, and *TGF-α* were not associated with none of the craniofacial morphological patterns assessed (skeletal malocclusions and facial type). Considering that previous studies support that genetic polymorphisms and mutations in these genes did show association with craniofacial patterns [2, 6, 10, 31–33], it is possible that other genetic variants within these genes are involved in the etiology of the craniofacial phenotypes in this population.

Fgf signaling is involved in various regulatory processes during embryogenesis as well as in the adult organism [34, 35]. This signal pathway has key roles in suture and synchondrosis regulation; mutations in *FGF* receptors cause craniosynostosis, which is the premature suture fusion [36, 37]. On the other hand, Fgf signal participates in multiple stages of palatogenesis, from palatal shelf elevation to the completion of fusion [38]. Therefore, disturbances in Fgf-related pathways are possible mechanisms of palatal cleft. Based on the above, it is clear that *FGF* is considered a candidate gene for study in relation to variations in the morphology of the craniofacial skeleton. In our study, carrying at least one G allele in the polymorphism rs1893047 within *FGF3* increased the chance of presenting skeletal class III malocclusion. Considering that multiple members of this gene family are expressed mainly during midfacial region development [39], among them *FGF3*, we hypothesize that variations on this gene could contribute to the development of class III due to maxillary growth deficiency. A stratified analysis according to the maxilla contribution on class III was not possible due to the low prevalence of class III individuals in our sample, and the consequent significant reduction that would have existed in the power of the analyses.

It has been shown that *Fgf3* is expressed during development and outgrowth of the facial primordia and branchial arches [39–41], and during odontogenesis [42, 43]. Additionally, this gene has been associated with oral cleft [31] and TA [2], suggesting that *FGF3* plays a role in both craniofacial phenotypes. Our data must be carefully interpreted since significant results could be due to chance. Statistical significance did not persist for the reported associations after the Bonferroni correction.

Briefly, the knowledge regarding the role of genetic polymorphisms on craniofacial development offers the possibility of establishing new strategies to prevent these disorders. Further investigations with other genetic polymorphisms in these genes are necessary to confirm our results.

Conclusion

Our result suggests that genetic polymorphism rs1893047 in *FGF3* might contribute to variations in the craniofacial sagittal pattern, specifically to the establishment of the Class III skeletal malocclusion.

Abbreviations

TA: Tooth agenesis; ICC: Intraclass correlation coefficient; PCR: Polymerase chain reaction

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Not applicable.

Authors' contributions

ASR: conception and design of the study, acquisition of data, analysis and interpretation of data, drafting the article, and final approval of the version to be submitted. ECT: acquisition of data, analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. LSA: conception and design of the study, revising the article for important intellectual content, and final approval of the version to be submitted. PNF: analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. AC: acquisition of data, revising the article for important intellectual content, and final approval of the version to be submitted. SCL: acquisition of data, revising the article for important intellectual content, and final approval of the version to be submitted. MTSA: analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. AGCR: acquisition of data, revising the article for important intellectual content, and final approval of the version to be submitted. GVC: analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. MAO: acquisition of data, revising the article for important intellectual content, and final approval of the version to be submitted. MANM: analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. ARV: analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. ECK: conception and design of the study, analysis and interpretation of data, drafting the article, and final approval of the version to be submitted. GAMV: acquisition of data, analysis and interpretation of data, drafting the article, and final approval of the version to be submitted. LAAA: conception and design of the study, analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted.

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Availability of data and materials

The data will be made available upon request to the authors.

Ethics approval and consent to participate

The protocol of this study was approved by the Research Ethics Committees of the Antônio Pedro University Hospital at the Fluminense Federal University (no. 33791314.3.0000.5243), School of dentistry of Ribeirão Preto at the University of São Paulo (no. 50765715.3.0000.5419), and the Institutional review board committee at the University of Pittsburgh (no. 12080056). An informed consent form was obtained from all participants or legal guardians.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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