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 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-related 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 sagittal skeletal malocclusion (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).
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.

### 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 |
| TGFα  | 2p13.3      | rs2902345          | Intron variant     | GGT[C/T]GCC                   | 0.4022/2014 |
| FGF3  | 11q13.3     | rs1893047          | Intron variant     | CAC[A/G]TTA                   | 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.nlm.nih.gov/snp/, http://genome.uscs.edu/, and https://www.thermofisher.com

Bold: lower frequency allele

### 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 12 (66.7) AG 3 (16.7) GG 3 (16.7) | 0.414 | A 27 (75.0) G 9 (25.0) | 0.475 |
| Non-TA 239 (70.5) AG 73 (21.5) GG 27 (8.0) | | | | |
| PAX9 rs8004560       | AA 11 (57.9) AG 6 (31.6) GG 2 (10.5) | 0.783 | A 28 (73.7) G 10 (26.3) | 0.740 |
| Non-TA 214 (65) AG 81 (24.6) GG 34 (10.3) | | | | |
| TGFα rs2902345       | CC 6 (23.1) CT 12 (46.2) TT 8 (30.8) | 0.495 | C 24 (46.2) T 28 (53.8) | 0.317 |
| Non-TA 111 (30) CT 180 (48.6) TT 79 (21.4) | | | | |
| FGF3 rs1893047       | AA 17 (68) AG 7 (28) GG 1 (4) | 0.507 | A 41 (82.0) G 9 (18.0) | 0.881 |
| Non-TA 359 (74.4) AG 92 (19) GG 32 (6.6) | | | | |
| FGF10 rs900379       | CC 10 (35.7) CT 11 (39.3) TT 7 (25) | 0.775 | C 24 (46.2) T 28 (53.8) | 0.555 |
| Non-TA 164 (29.4) CT 244 (43.8) TT 149 (26.8) | | | | |
| FGF13 rs12838463     | AA 19 (51.4) AG 9 (24.3) GG 9 (24.3) | 0.828 | A 47 (63.5) G 27 (36.5) | 0.306 |
| Non-TA 360 (46.2) AG 212 (27.2) GG 207 (26.6) | | | | |
| FGF13 rs5931572      | AA 9 (32.1) AG 10 (35.8) GG 9 (32.1) | 0.783 | A 28 (50.0) G 28 (50.0) | 0.340 |
| Non-TA 170 (32.1) AG 160 (30.2) GG 200 (37.7) | | | | |
| FGF13 rs5974804      | AA 8 (40) AG 6 (30) GG 6 (30) | 0.884 | A 22 (55.0) G 18 (45.0) | 0.520 |
| Non-TA 194 (43.5) AG 112 (25.10) GG 140 (31.4) | | | | |

*Statistical significance (p ≤ 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 FG3 rs1893047 were significantly different between class III and class I individuals (Table 3). Analysis

| Genetic polymorphism | Genotypes, n (%) | \( p \) value | Alleles, n (%) | \( p \) value |
|----------------------|------------------|--------------|---------------|--------------|
| **MSX1 rs1042484**   |                  |              |               |              |
| Class I              | 118 (69.8)       | 38 (22.5)    | 13 (7.7)      | Reference    |
| Class II             | 106 (71.1)       | 29 (19.5)    | 14 (9.4)      | 0.730        |
| Class III            | 26 (70.3)        | 8 (21.6)     | 3 (8.1)       | 0.990        |
| **PAX9 rs8004560**   |                  |              |               |              |
| Class I              | 91 (62.3)        | 35 (24.0)    | 20 (13.7)     | Reference    |
| Class II             | 100 (67.1)       | 39 (26.2)    | 10 (6.7)      | 0.140        |
| Class III            | 25 (62.5)        | 10 (25.0)    | 5 (12.5)      | 0.975        |
| **TGFα1 rs2902345**  |                  |              |               |              |
| Class I              | 51 (28.5)        | 89 (49.7)    | 39 (21.8)     | Reference    |
| Class II             | 46 (28.8)        | 77 (48.1)    | 37 (23.1)     | 0.947        |
| Class III            | 16 (37.2)        | 22 (51.2)    | 5 (11.6)      | 0.261        |
| **FGF3 rs1893047**   |                  |              |               |              |
| Class I              | 157 (76.6)       | 36 (25.4)    | 12 (5.9)      | Reference    |
| Class II             | 165 (75.3)       | 39 (19.5)    | 15 (4.6)      | 0.932        |
| Class III            | 28 (59.6)        | 16 (34.0)    | 5 (10.3)      | 0.038*       |
| **FGF10 rs900379**   |                  |              |               |              |
| Class I              | 70 (29.8)        | 107 (45.5)   | 58 (24.7)     | Reference    |
| Class II             | 72 (30.5)        | 115 (48.7)   | 49 (20.8)     | 0.985        |
| Class III            | 19 (33.3)        | 20 (35.1)    | 18 (31.6)     | 0.336        |
| **FGF13 rs12838463** |                  |              |               |              |
| Class I              | 110 (47.8)       | 65 (28.3)    | 55 (23.9)     | Reference    |
| Class II             | 112 (46.9)       | 60 (25.1)    | 67 (28.0)     | 0.541        |
| Class III            | 29 (50.9)        | 14 (24.6)    | 14 (24.6)     | 0.850        |
| **FGF13 rs5931572**  |                  |              |               |              |
| Class I              | 72 (32.6)        | 63 (28.5)    | 86 (38.9)     | Reference    |
| Class II             | 67 (29.5)        | 65 (28.6)    | 95 (41.9)     | 0.749        |
| Class III            | 16 (30.8)        | 15 (28.8)    | 21 (40.4)     | 0.966        |
| **FGF13 rs5974804**  |                  |              |               |              |
| Class I              | 83 (42.1)        | 50 (25.4)    | 64 (32.5)     | Reference    |
| Class II             | 80 (44.7)        | 48 (26.8)    | 51 (28.5)     | 0.897        |
| Class III            | 22 (46.8)        | 9 (19.1)     | 16 (34.0)     | 0.678        |

*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 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 | AA          | AG          | GG         | p value | Alleles, n (%) | p value |
|----------------------|-------------|-------------|------------|---------|----------------|---------|
| **MSX1 rs1042484**   |             |             |            |         |                |         |
| Mesofacial           | 119 (69.6)  | 35 (20.5)   | 17 (9.9)   | Reference | 273 (79.8)     |         |
|                     | 35 (20.5)  | 17 (9.9)    | Reference  |         | 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**   |             |             |            |         |                |         |
| Mesofacial           | 103 (66.5) | 37 (23.9)   | 15 (9.7)   | Reference | 243 (78.4)     |         |
|                     | 37 (23.9)  | 15 (9.7)    | Reference  |         | 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 |
| **TGFα rs2902345**   |             |             |            |         |                |         |
| Mesofacial           | 51 (27.4)  | 95 (51.1)   | 40 (21.5)  | Reference | 197 (53.0)     | 175 (47) | Reference |
|                     | 95 (51.1)  | 40 (21.5)   | Reference  |         | 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 rs11893047**  |             |             |            |         |                |         |
| Mesofacial           | 166 (75.1) | 39 (17.6)   | 16 (7.2)   | Reference | 371 (83.9)     | 71 (16.1) | Reference |
|                     | 39 (17.6)  | 16 (7.2)    | Reference  |         | 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**   |             |             |            |         |                |         |
| Mesofacial           | 78 (31.0)  | 118 (46.8)  | 56 (22.2)  | Reference | 274 (54.4)     | 230 (45.6) | Reference |
|                     | 118 (46.8) | 56 (22.2)   | Reference  |         | 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 |
|                     | 63 (25.5)  | 68 (27.5)   | Reference  |         | 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**  |             |             |            |         |                |         |
| Mesofacial           | 70 (29.2)  | 69 (28.8)   | 101 (42.1) | Reference | 209 (43.5)     | 271 (56.5) | Reference |
|                     | 69 (28.8)  | 101 (42.1)  | Reference  |         | 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**  |             |             |            |         |                |         |
| Mesofacial           | 84 (42.6)  | 49 (24.9)   | 64 (32.5)  | Reference | 217 (55.1)     | 177 (44.9) | Reference |
|                     | 49 (24.9)  | 64 (32.5)   | Reference  |         | 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 ≤ 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.

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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. ECK: 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. ARV: analysis and interpretation 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. MAO: acquisition of data, analysis and interpretation 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. AGCR: 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. LSA: conception and design of the study, acquisition of data, analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. ASR: conception and design of the study, acquisition of data, analysis and interpretation of data, revising the article for important intellectual content, and final approval of the version to be submitted. LAA: 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. 33791314.0000.5243), School of Dentistry of Ribeirão Preto at the University of São Paulo (no. 50765715.30000.5419), and the Institutional review board committee at the University of Pittsburgh (no. 12980056). 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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