# Genetic polymorphisms underlying the skeletal Class III phenotype

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**Introduction:** Our goal was to verify the association between candidate polymorphisms and skeletal Class III malocclusion in a well-characterized homogeneous sample set. **Methods:** Thirty-five single-nucleotide polymorphisms were studied from 10 candidate loci in 54 Class III subjects and 120 controls. Skeletal Class III characteristics included ANB angle less than 0°, SNB angle greater than 83° (mandibular prognathism), SNA angle less than 79° (maxillary deficiency), Class III molar relationship, and negative overjet. Inclusion criteria for the controls were ANB angle between 0° and 4°, Class I molar relationship, and normal overjet. Chi-square and Fisher exact tests and principal component (PC) analysis were used to determine overrepresentation of marker alleles with alpha of 0.05. Odds ratios and 95% confidence intervals were calculated. **Results:** *MYO1H* (rs10850110 A<G) ($P$ <0.01; odds ratio, 7.44 [4.02-13.77]) was associated with an increased risk for the mandibular prognathism phenotype. These results were confirmed by PC analysis, which showed 4 PCs representing the sample variations (PC1, 37.24%; PC2, 20.02%; PC3, 12.18%; and PC4, 11.40%), and PC1 was associated with *MYO1H* ($P$ <0.001). We also found by PC analysis associations between *MYO1H* ($P$ <0.001) and *GHR* (rs2973015 A>G) ($P$ = 0.001) with PC2 and between *FGF10* (rs593307 A<G) ($P$ = 0.001) with PC4. **Conclusions:** Polymorphism in *MYO1H* could be used as a marker for genetic susceptibility to Class III malocclusion with mandibular prognathism, and polymorphisms in *GHR* and *FGF* were associated with maxillomandibular discrepancies. This study may contribute to improved diagnosis and further research assessing possible differences in treatment responses based on genetic polymorphisms. (Am J Orthod Dentofacial Orthop 2017;151:700-7)

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The skeletal Class III malocclusion phenotype is heterogeneous and is usually characterized by some combination of excessive mandibular growth (mandibular prognathism, Mendelian Inheritance in Man [MIM] #176700) and deficient maxillary growth and can occur as part of a syndrome or as an isolated trait.[1] The clinical aspects of Class III malocclusion can be perceived in childhood and become progressively more evident with growth, contributing to disturbances in both function and esthetics.[2] Prevalence varies according to different populations; it is higher in Asians (19%)[3] and lower in white people (1.0%).[4] Its etiology is still unknown[1] and has been attributed to many patterns of genetic inheritance and to environmental factors as well as to gene-environment interactions.[5]

Several candidate loci have been related to skeletal Class III malocclusion. According to the first genome-wide linkage analysis, there were mandibular prognathism susceptibility loci in chromosomes 1p36, 6q25, and 19p13.2 in Korean and Japanese families.[6] In Hispanic families, genome-wide linkage showed 5 suggestive loci to maxillary deficiency (1p22.1, 3p26.2,

11q22, 12q13.3, and 12q23).[7] Suggestive evidence of linkage in a Han Chinese pedigree was shown on the 14q24.3-31.2 locus for mandibular prognathism.[8] Based on the findings of genome-wide linkage studies, Tassopoulou-Fishell et al[9] and Fontoura et al[10] showed evidence that a polymorphism in *Myosin 1H* (*MYO1H*) was associated with mandibular prognathism. Furthermore, a genome-wide association study showed 2 loci (1p32.2 and 1p22.3) susceptible to mandibular prognathism in Japanese people.[11] According to Nikopensius et al,[12] the region 12q22-q23—harboring dual specificity phosphatase 6 (*DUSP6*)—may be genetically linked to both mandibular prognathism and maxillary deficiency. Growth hormone receptor (*GHR*) appears to play an important role in the mandibular growth in Japanese,[13,14] Chinese,[15] Korean,[16] and Turkish[17] peoples.

Furthermore, additional candidate genes can be proposed based on gene function and its potential impact on normal and abnormal postnatal growth. Fibroblast growth factors (*FGFs*) control the balance among skeletal cell growth, differentiation, and apoptosis. *FGFs* and fibroblast growth factor receptors (*FGFRs*) are essential for the control of endochondral and intramembranous bone formation during development.[18] A mutation in *FGFR1* at 8p11.2 is responsible for Pfeiffer syndrome (MIM #101600), which includes craniosynostosis as a phenotype.[19] Moreover, mutations in *FGFR2* at 10q26 cause Crouzon (MIM #123500) and Apert (MIM #101200) syndromes,[20] and both present a Class III malocclusion phenotype. Our previous work showed that genes that are linked to rare syndromes can give insight for the comprehension of isolated traits,[21] and *FGFs* and *FGFRs* may be involved in isolated forms of skeletal Class III malocclusion.

There is a consensus of a need to replicate previous genetic association findings because of the winner's curse effect,[22] and our initial report of an association between *MYO1H* and mandibular prognathism should be replicated. Hence, we aimed to verify the association between polymorphisms in loci identified in previous studies (1p22.1, 1p22.2, 1p36, 3q26.2, 5p13-p12, 6q26, 11q22.2-q22.3, 12q23, 12q13.13, and 19p13.2), and polymorphisms in several *FGF* family members for skeletal Class III malocclusion.

## MATERIAL AND METHODS

This study was approved by the research and ethics committee of Antônio Pedro University Hospital at the Universidade Federal Fluminense (number 314/2011) in Rio de Janeiro, Brazil, and by the University of Pittsburgh Institutional Review Board in Pittsburgh, Pa. Written consent was obtained from all adults or legal guardians, in the case of minors, before they entered this study.

The cohort of 652 subjects in active treatment from 3 reference centers for malocclusion treatment in Rio de Janeiro, Brazil (Departments of Pediatric Dentistry and Orthodontics at the Universidade Federal do Rio de Janeiro, Universidade Federal Fluminense, and Brazilian Dental Association) formed the initial sample. Patients were recruited for this study from January 2011 to January 2013.

Each subject's clinical aspects, pretreatment lateral cephalometric records, and cast records were assessed for eligibility. Two groups were selected from these patients based on preestablished characteristics: a Class III malocclusion group and a control group (Class I patients). The inclusion criteria for the Class III malocclusion group were cephalometric ANB angle (Point A-nasion-Point B) of centric jaw relationship less than 0.0°,[6,23] Class III molar malocclusion according to Angle's classification, negative overjet, and age over 6 years. To increase homogeneity, the maxillary deficiency and the mandibular prognathism were distinguished within skeletal Class III malocclusion. The cephalometric parameters used to classify the maxillary deficiency were SNA angle (sella-nasion-Point A) less than 79°, and the mandibular prognathism was SNB angle (sella-nasion-Point B) greater than 83°.[24] Inclusion criteria for the control group were skeletal Class I (ANB angle between 0° and 4°),[24] Class I molar occlusion according to Angle's classification, normal overjet, and age over 6 years. Exclusion criteria for both groups comprised growth disturbances, syndromes, cleft lip and palate, missing teeth, poor quality of radiographic records, consent form not signed, and trauma. The lateral cephalograms of all patients who had been previously classified in their records as having a Class III or Class I phenotype were retraced by the same examiner (C.V.C.) to check eligibility for the study. Reliability was determined by measurement of 20 cephalograms randomly selected from the sample. The intraexaminer agreement was assessed by a second cephalometric measurement after 2 weeks. An additional cephalometric parameter recorded was the sella-nasion–gonion-gnathion angle (SN-GoGn) of each subject to register the main facial growth direction.[24] Thus, a total of 14 cephalometric measurements were assessed to find the most significant components of variation representing a distinct Class III phenotype to reduce the genetic heterogeneity (SNA angle, maxillary unit length, anterior cranial base, SND angle, SNB angle, ANB angle, facial convexity, 3 measurements of the length of mandibular base, facial depth, maxillary depth, and facial axis).

**Table I.** Markers studied

| Locus | Gene | Marker public identification | Base pair change |
|---|---|---|---|
| 1p36.11 | Intergenic | rs4649030 | A/G |
| 3q26.32 | Intergenic | rs2087312 | G/T |
| | Intergenic | rs987526 | A/G |
| 5p12 | Growth hormone receptor (*GHR*) | rs2973015 | A/G |
| | Growth hormone receptor (*GHR*) | rs1509460 | A/C |
| | Growth hormone receptor (*GHR*) | rs2910875 | C/T |
| 5p13-12 | Fibroblast growth factor 10 (*FGF10*) | rs11750845 | C/T |
| | Fibroblast growth factor 10 (*FGF10*) | rs1448037 | A/G |
| | Fibroblast growth factor 10 (*FGF10*) | rs900379 | C/T |
| | Fibroblast growth factor 10 (*FGF10*) | rs1011814 | A/G |
| | Fibroblast growth factor 10 (*FGF10*) | rs593307 | C/T |
| | Fibroblast growth factor 10 (*FGF10*) | rs7708529 | C/T |
| 8p12.11.2 | Fibroblast growth factor receptor 1 (*FGFR1*) | rs13317 | C/T |
| 6q26 | Parkinson juvenile disease protein 2 (*PARK2*) | rs7750085 | A/T |
| | Parkinson juvenile disease protein 2 (*PARK2*) | rs12207168 | A/G |
| | Parkinson juvenile disease protein 2 (*PARK2*) | rs1884153 | C/T |
| 10q26 | Fibroblast growth factor receptor 2 (*FGFR2*) | rs2981582 | C/T |
| 11q13 | Fibroblast growth factor 3 (*FGF3*) | rs7932320 | A/G |
| | Fibroblast growth factor 3 (*FGF3*) | rs1893047 | A/G |
| | Fibroblast growth factor 3 (*FGF3*) | rs12574452 | A/G |
| | Fibroblast growth factor 3 (*FGF3*) | rs10796856 | C/T |
| | Fibroblast growth factor 3 (*FGF3*) | rs4980700 | A/G |
| | Fibroblast growth factor 3 (*FGF3*) | rs35420992 | C/T |
| 11q13.3 | Intergenic | rs4631909 | C/T |
| 11q22.3 | Caspase 4 isoform gamma precursor (*CASP4*) | rs571407 | A/G |
| 12q13.13 | Keratin 7 (*KRT7*) | rs1902768 | C/T |
| | Keratin 7 (*KRT7*) | rs7300317 | A/G |
| 12q23.3 | Intergenic | rs11113231 | A/G |
| 12q24.11 | Myosin 1H (*MYO1H*) | rs10850110 | A/G |
| 15q21.2 | Fibroblast growth factor 7 (*FGF7*) | rs2413958 | C/T |
| 19p13.2 | Fibrilin 3 precursor (*FBN3*) | rs7351083 | A/G |
| | Fibrilin 3 precursor (*FBN3*) | rs4804264 | C/T |
| | Fibrilin 3 precursor (*FBN3*) | rs8103218 | C/T |
| | Fibrilin 3 precursor (*FBN3*) | rs12327845 | C/T |
| 19p13.3 | Intergenic | rs10411185 | A/G |

After the inclusion and exclusion criteria were applied, 185 unrelated patients remained in the sample. There were missing data for 11 patients: 7 subjects moved to another city, 3 subjects dropped out of the study, and 1 subject died. The final sample comprised 174 subjects. Fifty-four subjects (34 white, 20 black; 27 male, 27 female; mean age, 19.65 ± 8.7 years) were included in the Class III malocclusion group. In this group, 31 subjects had mandibular prognathism, and 25 had maxillary deficiency. One hundred twenty subjects (82 white, 38 black; 53 male, 67 female; mean age, 20.46 ± 11.15 years) were included in the control group. Assuming D' = 1.0, a frequency of 20% of the high-risk marker allele, and genotypic relative risk of 1 copy of the high-risk allele of 2.0 and 2 copies of 3.0, our power calculations suggested that we would have 85% power to detect an association with an alpha of 0.05.[25]

Saliva was collected from all participants (they were asked to spit), and the genomic DNA was extracted according to published protocols.[26] All saliva samples were numbered with the patient's name hidden, and all analyses were performed blindly to the case-control status.

Thirty-five single-nucleotide polymorphism (SNP) markers were selected in candidate genes to mandibular prognathism from previous studies[6,7,9,15,16] and in genes related to the skeletal Class III phenotype (Table I). These markers were chosen based on information on the gene structure and linkage disequilibrium relationships available at the international HapMap Project Web site (http://www.hapmap.org/). Real-time polymerase chain reaction was performed using the TaqMan method; for all TaqMan assays, an end-point analysis was performed in an automatic sequence-detection instrument (ABI Prism 7900HT; Applied Biosystems, Foster City, Calif)

**Table II.** Descriptive statistics of the sample

| Characteristic | Skeletal Class III (n = 54) | Controls (n = 120) | P value |
|---|---|---|---|
| Mean age (SD) | 19.65 (±8.5) | 20.50 (±11.16) | 0.475 |
| Sex (%) | | | |
| Male | 27 (50) | 53 (44.2) | 0.764 |
| Female | 27 (50) | 67 (55.8) | |
| Ethnicity (%) | | | |
| White | 34 (63.0) | 82 (68.3) | 0.750 |
| Black | 20 (37.0) | 38 (31.7) | |
| Measurements (°) (SD) | | | |
| ANB | −3.60 (2.77) | 2.45 (1.39) | * |
| SNA | 79.77 (4.34) | 82.03 (4.04) | 0.002 |
| SNB | 83.40 (4.94) | 79.71 (4.16) | * |
| SN-GoGN | 33.56 (5.77) | 33.92 (6.07) | 0.597 |
| SN-GoGn white × black | $P = 0.067$ | $P = 0.872$ | - |

*P* values according to independent *t* test and chi-square test.
*$P < 0.001$.

to test for the presence of an allelic variant in the genes, and the results were recorded according to fluorescent signals from reporters VIC and FAM. Each reaction mixture contained 10 μL 1x TaqMan universal polymerase chain reaction master mix, 0.5 μL 1x Taq-Man SNP kit (probe/primer mix), 2 μL DNA obtained, and 7.5 μL DNase-free water in a final volume of 20 μL. Standard amplification conditions were 95°C for 10 minutes, and 40 cycles at 92°C for 15 seconds and at 60°C for 40 seconds; 2 negative controls with sterile water as the template were used in each reaction plate. For quality control for genotyping, 10% of the sample was genotyped, with greater than 99% concordance.

## STATISTICAL ANALYSIS

Reliability was calculated using the intraclass correlation coefficient and confirmed by a median value of 0.982.

The chi-square, Fisher exact, and independent *t* tests were carried out to compare sex and ethnicity frequencies and to assess deviations in the allele and genotype distributions between both skeletal Class III and Class I subjects. The variations of SNA, SNB, and ANB angles of the Class III subjects and controls were tested separately according to sex, ethnicity (independent *t* test), and age (correlation coefficient). Odds ratios were used to measure the strength of the association between the frequencies of genotype in the Class III malocclusion and Class I participants. All *P* values were 2-tailed, and 95% confidence intervals (95% CI) were calculated. After the Bonferroni correction (0.05/35), the established alpha was 0.0014286, to accommodate for the concern of multiple tests. Hardy-Weinberg equilibrium was tested by a goodness-of-fit test, with 1 degree of freedom (http://www.oege.org/software/hardy-weinberg.html), comparing observed genotype frequencies with expected genotype frequencies among subjects. A *P* value less than 0.05 was considered to be significant, and only the results that were in Hardy-Weinberg equilibrium were further analyzed. Furthermore, principal components explaining more than 5% of the facial skeletal variation were selected for genotype-phenotype correlation analyses. Data were normalized and standardized using a linear model to assess the possible effects of age and sex and to consider the possibility of age-by-sex interactions. SNPs were coded 0, 1, and 2 according to the number of minor allele copies. Multivariate linear regressions adjusting for age, sex, and ethnicity were performed to test for associations between each SNP (one at a time) and the selected principal components. The same Bonferroni threshold described above was used here. All analyses were performed with SPSS software for Windows (version 20.0; IBM, Armonk, NY).

## RESULTS

Differences in sex, ethnicity, and SN-GoGn angle between the skeletal Class III malocclusion subjects and the control subjects were not statistically significant (Table II). All markers studied were in Hardy-Weinberg equilibrium (data not shown). The genotype distribution of the *MYO1H* (rs10850110 A<G) polymorphism among the controls was in Hardy-Weinberg equilibrium ($P = 0.1088$).

The genotype and polymorphic allele frequencies of the studied markers between the skeletal Class III malocclusion and control subjects are shown in Supplemental Table I. The distribution of the *MYO1H* genotype in the Class III malocclusion patients was significantly different from that in the control group (*MYO1H* rs10850110 A<G; $P < 0.001$). The rs10850110 A<G genotype was associated with a significantly increased risk of skeletal Class III with mandibular prognathism (odds ratio, 7.44; 95% CI, 4.02-13.77; $P < 0.001$) (Table III).

The distribution of the subjects based on ethnic background suggested that the allelic frequencies between the skeletal Class III and control groups were not statistically significant in *MYO1H* ($P = 0.1$) and did not influence the results (Table IV). These same comparisons in the Class III subjects and Class I controls separately also showed no differences (Table V).

Principal component (PC) analysis comprised 4 PCs (PC1 to PC4), each explaining more than 5% of the total shape variation, and the cumulative variation of each PC explained 80.84% of the sample variability (Table VI). The genotype-phenotype correlations are shown in

**Table III.** Genotype frequencies of studied markers in Class III malocclusion with mandibular prognathism (n = 31) and control subjects (n = 120)

| | | | *Control* | *Mandibular prognathism* | | |
|---|---|---|---|---|---|---|
| *Locus* | *Gene* | *Genotype* | *n (%)* | *n (%)* | *Odds ratio (95% CI)* | P *value* |
| 12q24.11 | *MYO1H* | rs10850110 A<G | | | | |
| | | AA | 7 (5.8) | 11 (35.5) | | |
| | | AG | 30 (25.0) | 18 (58.1) | | |
| | | GG | 75 (62.5) | 2 (6.5) | | |
| | | A allele | 44 (19.64) | 40 (64.5) | 7.44 (4.02-13.77) | * |

Variations in the numbers of genotypes/alleles or subjects are due to missing values caused by polymerase chain reaction failures, which we do not believe influenced the results.
*$P$ <0.0001.

**Table IV.** Comparison of demographic variables related to the genotypes of *MYO1H* between skeletal Class III (n = 54) and control subjects (n = 120)

| | | MYO1H *rs10850110 A<G* | | | | | |
|---|---|---|---|---|---|---|---|
| *Ethnicity* | *Sex* | *Skeletal Class III* | | | *Control* | | |
| | | AA | AG | GG | AA | AG | GG |
| White | Male | 6 | 12 | - | 2 | 9 | 23 |
| | Female | 4 | 12 | - | 5 | 13 | 25 |
| Black | Male | 3 | 5 | 1 | - | 2 | 14 |
| | Female | 5 | 5 | 1 | - | 6 | 13 |
| Chi-square $P$ value black vs white in controls | | | | | | 0.11 | |

Variations in the numbers of genotypes/alleles or subjects are due to missing values caused by polymerase chain reaction failures, which we do not believe influenced the results.

**Table V.** Sample distribution related to SNA, SNB, and ANB angles with regard to sex, age, and ethnicity

| | *SNA angle* | | *SNB angle* | | *ANB angle* | |
|---|---|---|---|---|---|---|
| | *Skeletal Class III* | *Controls* | *Skeletal Class III* | *Controls* | *Skeletal Class III* | *Controls* |
| Sex | 0.130 | 0.707 | 0.452 | 0.751 | 0.227 | 0.708 |
| Ethnicity | 0.303 | 0.866 | 0.178 | 0.843 | 0.504 | 0.974 |
| Age | 0.951 | 0.733 | 0.983 | 0.528 | 0.878 | 0.572 |

$P$ value according to independent $t$ test or correlation coefficient.

**Table VI.** Summary results of the PC analysis

| | *Component* | | | |
|---|---|---|---|---|
| | *1* | *2* | *3* | *4* |
| Variance explained (%) | 37.24 | 20.02 | 12.18 | 11.40 |
| Cumulative variance (%) | 37.24 | 57.27 | 69.45 | 80.84 |
| Correlated variables | | | | |
| SNA (°) | 0.444 | 0.791 | 0.221 | 0.196 |
| Maxillary unit length (Co-A) (mm) | −0.039 | 0.130 | 0.865 | −0.276 |
| Anterior cranial base (SN) (mm) | −0.212 | −0.199 | 0.777 | −0.402 |
| SND (°) | 0.876 | 0.304 | 0.020 | −0.030 |
| SNB (°) | 0.884 | 0.317 | 0.042 | −0.050 |
| ANB (°) | −0.645 | 0.562 | 0.221 | 0.326 |
| Facial convexity (A-NAPg) (mm) | −0.536 | 0.594 | 0.208 | 0.400 |
| Length of mandibular base (Xi-Pm) (mm) | 0.760 | −0.413 | 0.230 | 0.216 |
| Length of mandibular base (Go-Pg) (mm) | 0.705 | −0.400 | 0.203 | 0.277 |
| Length of mandibular base (Co-Gn) (mm) | 0.663 | −0.428 | 0.246 | 0.282 |
| Facial depth (FH-NPg) (°) | 0.848 | 0.031 | −0.098 | 0.024 |
| Maxillary depth (FH-NA) (°) | 0.447 | 0.587 | 0.030 | 0.409 |
| Facial axis (BaN-PTGn) (°) | 0.371 | 0.399 | −0.122 | −0.609 |
| SN-GoGn (°) | −0.385 | −0.492 | 0.172 | 0.553 |

Supplemental Table II. PC1 explained 37.24% of the variance and showed anteroposterior discrepancies, with higher scores related to mandibular dimensions (position and length) and lower scores related to intermaxillary relationship (indicating skeletal Class III malocclusion). Regarding the genotype-phenotype correlation between the PC1 phenotype and genetic variations, *MYO1H* rs10850110 was statistically significantly associated with PC1 ($P$ <0.0001). PC2 explained 20.02% of the variance, disclosing horizontal and vertical maxillomandibular discrepancies (position and morphology), with higher scores for maxillary position and lower scores for mandibular length and vertical dimension (hypodivergent facial growth). The less frequent alleles of *MYO1H* rs10850110 and *GHR* rs2973015 were associated with PC2 ($P$ <0.001 and $P$ = 0.001, respectively). PC3 explained 12.18% of the variance and showed higher scores for maxillary dimensions (length) and anterior cranial base; however, it was not associated with the genetic variants studied. PC4 (11.40%) showed vertical dimensions, disclosing hyperdivergent facial growth (higher scores for SN-GoGn and lower scores for facial axis angle), and the minor allele of

*FGF*10 rs593307 was statistically significant ($P = 0.001$) with PC4.

## DISCUSSION

The results of genome-wide linkage[6-8,23,27,28] and genome-wide association[11] studies provide evidence that many genomic areas may harbor a large number of genes suggested to contribute to Class III malocclusion. There is a gap in the knowledge regarding which candidate genes are related to mandibular prognathism and maxillary deficiency in patients with skeletal Class III malocclusion because of different genetic backgrounds between populations. We typed 35 polymorphisms from 10 candidate loci in an attempt to unveil a common genetic variation related to skeletal Class III. We provide further evidence that the genetic variation in *MYO1H* (rs10850110 A<G) contributes to mandibular prognathism and horizontal maxillomandibular discrepancies based on both comparing qualitative descriptors of malocclusion (Class I vs Class III), and PC analysis (*MYO1H* rs10850110 A<G was associated with PC1). This analysis also showed an association between markers in *GHR* (rs2973015 A>G) and *MYO1H* (rs10850110 A<G) and maxillomandibular discrepancies (PC2). In addition, the genetic variation in *FGF10* (rs593307 A<G) was associated with hyperdivergent facial growth (PC4).

Myosins are molecular motors that, upon interaction with actin filaments, use adenosine triphosphate hydrolysis to generate mechanical force. Myosin I generates movement at the leading edge in cell motility, phagocytosis, and vesicle transport.[29] Since myosins are involved in these biologic pathways, we could speculate that muscular functions play an important role in mandibular growth. Possibly, there is more to craniofacial postnatal growth than strictly a skeletal role. This is consistent with the functional matrix hypothesis, in which skeletal growth is linked to its underlying muscular matrix.[30]

The genetic associations identified in 1 population may not be transferable to other populations because of different genetic backgrounds.[31] However, we found an association between *MYO1H* (rs10850110 A<G), mapped on locus 12q24.11, and mandibular prognathism (Supplementary Tables) in groups from 2 distinct geographic areas, western Pennsylvania in North America[9] and Brazil in South America. In addition, Frazier-Bowers et al[7] found evidence of linkage on chromosome 12 with Class III malocclusion in Hispanics; Nikopensius et al[12] reinforced that chromosome 12 was genetically linked to the Class III malocclusion in Estonians, and Fontoura et al[10] showed an association between horizontal maxillomandibular discrepancies and a marker upstream from *MYO1H* in North Americans from Iowa. According to Dohmoto et al,[32] the mouse chromosome 10 (chromosome 12 in humans) is linked to craniofacial growth in mice, determining mandibular length.

Growth hormone regulates metabolism through its binding to the growth hormone transmembrane receptor (GHR) and plays a major role in regulating growth during childhood and adolescence. Our results appear to replicate initial reports that have shown specifically in Asians an association between *GHR* and mandibular ramus length (Japanese,[3] Chinese,[15] and Turkish from Anatolia[17]). In this study, *GHR* was associated with PC2, showing that mandibular prognathism is related to smaller mandibular length and hypodivergent facial growth. This seems to have compensated for the smaller mandibular dimensions in the Class III phenotype. This is the first report to show an association between *GHR* and South Americans and illustrates the potential of implementing more sophisticated analysis of craniomandibular phenotypes.

As recommended in genetic association studies, we applied strict multiple testing corrections.[33] However, the Bonferroni adjustment may result in the loss of true associations because the type I error cannot decrease without enhancing type II error; this does not guarantee a prudent interpretation of results. Thus, the main Bonferroni adjustment weakness is that the interpretation of the findings depends on the number of the tests applied,[34] and the main objective of this study was to replicate alleged associations based on previous suggestive candidate loci to skeletal Class III malocclusion.

A limitation of this study was the small sample size; however, our strict inclusion criteria led to a homogeneous sample set, which possibly increased statistical power. In addition, we did not perform the geometric morphometric analysis, which could illustrate the skeletal Class III phenotype. This analysis was focused on pure shape, measuring morphologic similarities and differences, and required imaging such as cone-beam computed tomography. Nevertheless, size is completely absent from geometric morphometric analysis, and we considered size biologically relevant in studies about craniofacial discrepancies. Furthermore, routine use of cone-beam computed tomography in orthodontic practices is unlikely to replace traditional cephalometric analyses. Moreover, only single rigid structures can be easily analyzed, and our results suggested that muscular tissues may play an important role in craniofacial growth. Interestingly, the geometric morphometric analysis performed to date[10] did replicate our original finding in *MYO1H*,[9] which showed that the likely impact

of this gene pathway can be detected both by directly measuring the skeletal structures and when facial shape is considered.

The Class III malocclusion phenotype shows different skeletal types, and the skeletal origin of Class III malocclusion may be a complicating factor for genetic studies because of its heterogeneity.[35] The skeletal manifestation can be due to mandibular anterior positioning (mandibular prognathism), maxillary posterior positioning (maxillary retrognathism), or a combination of mandibular and maxillary discrepancies.[36] Thus, in this study, to reduce genetic heterogeneity and increase the probability of identifying candidate genes amplifying the true association features, we stratified skeletal Class III malocclusion into mandibular prognathism and maxillary deficiency. Mandibular prognathism was the principal and most frequent component of skeletal Class III malocclusion in this sample. This finding corroborates the study of Staudt and Kiliardis[36] in a population of Swiss white men with the skeletal morphology underlying Class III. In our study, there was no statistically significant difference with regard to age, sex, and ethnicity between the skeletal Class III and the control subjects (Tables II and V). Moreover, the measurement SN-GoGn was not statistically significant between the Class III malocclusion and Class I participants, so the mandibular growth direction seems not to influence our results.

On the other hand, the Class III phenotype can be influenced by growth direction, because of the variability in craniofacial morphology underlying Class III malocclusion.[37] In hyperdivergent patients, even an excessive mandibular size may be compensated leading to a normal mandibular position; that is why vertical components may play an important role in anteroposterior malocclusion. *FGF10* (rs593307 A<G) was associated with hyperdivergent patients in this study (PC4). The human *FGFs* family, which consists of 22 members (*FGF-1* to *FGF-14* and *FGF-16* to *FGF-23*), is linked to osteoblast formation and plays a subtler role in bone formation, because *FGFs* prevent osteoblast apoptosis and modulate osteoblast response to other growth factors.[38] Several *FGFs* are expressed in the endochondral bone development, and this plays an important role in the determination of mandibular growth and morphology.[18] However, the factors that regulate these *FGFs* and the identity and function of each member of *FGFs* family that function in skeletogenesis remain to be discovered.

In the genetic association studies with Class III malocclusion performed so far, most existing cohorts have been collected for case-control analysis and therefore can only provide a snapshot assessment of the association of a genetic variation and this trait. However, the natural progression of Class III cannot be accurately probed in such studies; neither can possible gene-environment interactions, as suggested by some authors.[1,5,6] In the long term, the ability to predict mandibular growth, as well as to classify mandibular prognathism based on genotypic information, will result in improved diagnosis and treatment.[35] Genetic therapy can have the potential to make clinical trials more cost-effective and time-efficient, whereas the developing craniofacial complex is more likely to be susceptible to prophylactic therapies. Identification of the genetic contribution to Class III malocclusion susceptibility could be useful for establishing a better understanding of the mechanisms underlying and protecting outcomes for Class III malocclusion as well as for the design of specific intervention strategies to prevent Class III malocclusion.[6] This study was designed to precede further research assessing possible differences in treatment responses based on genetic polymorphisms.

## CONCLUSIONS

This study provides further evidence that the polymorphism rs10850110 in *MYO1H*, mapped on locus 12q24.11, is associated with skeletal Class III malocclusion with mandibular prognathism, increasing its risk. In addition, polymorphisms in *MYO1H* (rs rs10850110), *GHR* (rs2973015, locus 5p12), and *FGF10* (rs593307, locus 5p13-12) were associated with horizontal and vertical maxillomandibular discrepancies. These results suggest that *MYO1H*, *GHR*, or *FGF10* could be used as a marker for genetic susceptibility to skeletal Class III malocclusion.

## SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at http://dx.doi.org/10.1016/j.ajodo.2016.09.013.

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