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Candidate gene analyses of 3-dimensional dentoalveolar phenotypes in subjects with malocclusion

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Abstract

Introduction—Genetic studies of malocclusion etiology have identified 4 deleterious mutations in genes, *DUSP6*, *ARHGAP21*, *FGF23*, and *ADAMTS1* in familial Class III cases. Although these variants may have large impacts on Class III phenotypic expression, their low frequency (<1%) makes them unlikely to explain most malocclusions. Thus, much of the genetic variation underlying the dentofacial phenotypic variation associated with malocclusion remains unknown. In this study, we evaluated associations between common genetic variations in craniofacial candidate genes and 3-dimensional dentoalveolar phenotypes in patients with malocclusion.

Methods—Pretreatment dental casts or cone-beam computed tomographic images from 300 healthy subjects were digitized with 48 landmarks. The 3-dimensional coordinate data were submitted to a geometric morphometric approach along with principal component analysis to

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SUPPLEMENTARY DATA

Supplementary data related to this article can be found online at <http://dx.doi.org/10.1016/j.ajodo.2016.08.027>.

generate continuous phenotypes including symmetric and asymmetric components of dentoalveolar shape variation, fluctuating asymmetry, and size. The subjects were genotyped for 222 single-nucleotide polymorphisms in 82 genes/loci, and phenotype-genotype associations were tested via multivariate linear regression.

Results—Principal component analysis of symmetric variation identified 4 components that explained 68% of the total variance and depicted anteroposterior, vertical, and transverse dentoalveolar discrepancies. Suggestive associations ($P < 0.05$) were identified with *PITX2*, *SNAI3*, 11q22.2-q22.3, 4p16.1, *ISL1*, and *FGF8*. Principal component analysis for asymmetric variations identified 4 components that explained 51% of the total variations and captured left-to-right discrepancies resulting in midline deviations, unilateral crossbites, and ectopic eruptions. Suggestive associations were found with *TBX1*, *AJUBA*, *SNAI3*, *SATB2*, *TP63*, and 1p22.1. Fluctuating asymmetry was associated with *BMP3* and *LATS1*. Associations for *SATB2* and *BMP3* with asymmetric variations remained significant after the Bonferroni correction ($P < 0.00022$). Suggestive associations were found for centroid size, a proxy for dentoalveolar size variation with 4p16.1 and *SNAI1*.

Conclusions—Specific genetic pathways associated with 3-dimensional dentoalveolar phenotypic variation in malocclusions were identified.

Malocclusion is a common disarrangement of teeth or jaws that affects populations worldwide,^{1–5} resulting in impaired oral function, increased susceptibility to dental trauma, periodontal disease, and reduced dentofacial esthetics.⁶ Genetic studies of malocclusion etiology have focused on Class III malocclusion; so far, full exome sequencing of large families segregating maxillary hypoplasia or mandibular prognathism has identified 4 etiologic mutations.⁷ The first mutation identified was a heterozygous missense change rs139318648 (c.545C>T, p.Ser182Phe) in the dual specific phosphatase 6 (*DUSP6*) gene on chromosome 12q22-q23 carried by 5 siblings of an Estonian family with maxillary hypoplasia.⁸ The second mutation, rs111419738 (c.3361G>A, p.Gly1121Ser), in the rho GTPase-activating protein 21 (*ARHGAP21*) gene on 10p12.1 was found in a large Italian family segregating mandibular prognathism.⁹ The third mutation, c.35C>A (no rs assigned yet, p.Ala12Asp), in the fibroblast growth factor 23 (*FGF23*) gene (12p12.3) gene was discovered in a Chinese family and also found in 3 of 65 isolated cases of mandibular prognathism.¹⁰ The fourth mutation, rs200052788 (c.7421>T c.2225T>C, p.Ile742Thr), was found in a disintegrin-like and metalloproteinase with thrombospondin type 1 motif, 1 (*ADAMTS1*) gene (21q21.3) also in a large Chinese family. The latter (c.7421>T rs200052788) was also detected in 3 of 230 unrelated persons with mandibular prognathism. Moreover, significant associations were found with single-nucleotide polymorphisms (SNPs) rs2768 and rs229038 in *ADAMTS1*, indicating that both rare and common variants of this gene are associated with mandibular prognathism.¹¹

As indicated above, progress toward the identification of deleterious mutations for Class III malocclusion is evident; yet future research is needed to continue to unravel the genetic etiology of malocclusion conditions. For instance, the mutations described above are likely to have a large impact in the maxillary hypoplasia and mandibular prognathism phenotypes of persons carrying these mutations. Thus, functional studies to understand their specific roles in jaw growth are essential. However, their low frequency in global populations (<1%)

implies that these mutations are not likely to explain most malocclusions. Also to our knowledge, these mutations have not been replicated in other populations; thus, it is uncertain whether the results can be generalized to other ancestries. Moreover, the above studies have only used discrete phenotypes (ie, maxillary hypoplasia or mandibular prognathism), which lack resolution to identify phenotype-genotype correlations underlying the multitude of dentofacial phenotypic variations that in persons with these conditions.^{12,13} In an effort to address some of these knowledge gaps, a recent genetic association study of 71 craniofacial genes/loci in white subjects with severe malocclusion used both categorical (skeletal Class II or Class III vs Class I) and quantitative skeletal malocclusion phenotypes derived from cephalometric tracings and geometric morphometrics methods, respectively.¹⁴ This study showed that the risk for skeletal Class II relative to Class I was modulated by rare alleles in variants near *FGFR2* and *EDN1*, whereas Class III risk was modulated by variants in *FGFR2*, *COL1A1*, and *TBX5*. In addition, SNPs near *SNAI3* were highly associated with skeletal variations ranging from severely concave to convex skeletal profiles and SNPs near *TWIST1* were associated with variations ranging from a large to a short mandibular body.¹⁴ Collectively, these findings suggest that malocclusion is a complex trait in both its phenotypic expression and its genetic etiology; therefore, continuing efforts to characterize phenotype-genotype correlations underlying the large variations of malocclusion phenotypes are warranted.

Orthodontic pretreatment records are rich sources of phenotypic information for genetic studies of malocclusion. For example, dental casts trimmed based on the patient's bite registrations reproduce the patient's pretreatment occlusion and are routinely taken for orthodontic diagnosis and treatment planning. Dental casts have been used in cross-sectional and longitudinal studies to document 3-dimensional (3D) variations via linear measurements of arch width,¹⁵ perimeter and length,^{16,17} and arch-shape variations¹⁸ as well as other malocclusion indicators such as overjet, overbite,¹⁹ curve of Spee,²⁰ tooth size-arch length discrepancies,²¹ and various indexes to measure crowding and other dental irregularities.²²

With the recent technological advancements, it is possible to digitize dental casts and expedite dentoalveolar measurements beyond hand-held techniques. In addition, cone-beam computed tomography (CBCT) images have increasingly become more common in orthodontic and orthognathic practices facilitating the generation of 3D skeletal and dentoalveolar images that can provide abundant 3D phenotypic information.²³

Both digitized dental casts and CBCT images are amenable to analysis with landmark-based shape methods such as geometric-morphometric approaches. These techniques allow the study of an object's shape independent of size and orientation, facilitating the evaluation of causal factors behind a given shape.²⁴ Steps for geometric-morphometric analyses include a generalized least squares Procrustes superimposition applied to coordinate data to remove variations in landmarks due to size, position, and rotation. Once completed, any residual information in the positional relationships between landmarks is due purely to differences in shape. These standardized residuals can then be submitted to principal components analyses to reduce the multidimensional data into a few independent axes or principal components, simplifying subsequent analyses and yet retaining most of the shape variations in the data.²⁵ The derived components of shape variation resemble quantitative phenotypes that can then

be correlated with genetic data. The purpose of this study was to explore genetic associations between known craniofacial candidate genes/loci and 3D dentoalveolar shape phenotypes derived from digital casts or CBCT images of patients with moderate or severe malocclusion.

MATERIAL AND METHODS

The study protocol was reviewed and approved by the ethics committee of the University of Iowa, and signed consents were obtained from all participants. A sample of 300 untreated healthy subjects (average age, 29.9 years; age range, 12–68 years) seeking orthodontic treatment at the Department of Orthodontics of the University of Iowa was recruited and classified into skeletal Class I ($n = 63$), Class II ($n = 154$), or Class III ($n = 83$) according to criteria specified previously¹⁴ and shown in Supplemental Figure 1. Of these, about 80% were white ($n = 239$), and the remaining subjects had other ancestries (Table I). Exclusion criteria were craniofacial syndromes or chronic conditions that would limit physical activities according to the American Society of Anesthesiologists physical status classification system for dental care (http://www.dhed.net/ASA_Physical_Status_Classification_SYSTEM.html), previous orthodontic treatment, a history of facial trauma or facial surgery, missing or impacted teeth other than third molars, and CBCT images or dental casts of poor quality or with missing landmarks.

The total data set was procured from 2 subsets. CBCT images ($n = 131$) or digitized dental casts ($n = 169$) in occlusion were taken as pretreatment orthodontic records from the study sample. To select a group of landmarks that could capture similar aspects of shape on both data subsets and allow the analyses of the combined data, a test was used with subjects who had both pretreatment digitized dental casts and CBCT images. A sample of 7 subjects was selected, and a set of 136 dentoalveolar landmarks was digitized in both data subsets for each subject via the 3D module of Dolphin software (version 11.5; Dolphin Imaging, Chatsworth, Calif) and the Landmark Editor program (version 3.0; Institute for Data Analysis and Visualization, University of California, Davis, Calif)²⁶ for CBCT images and digital casts, respectively (Supplemental Fig 2). To test for compatibility between landmarks in CBCT images and digital casts, Euclidean distance differences were calculated between each landmark placed on both images. An error of 1.5 mm or less was considered acceptable; according to this criterion, 24 landmarks on each arch (48 of 136 total) were deemed compatible between CBCT images and digital casts, and therefore useful for shape analysis of both data subsets combined (Fig 1; Table II).

For the 48 compatible landmarks, an additional step of intraobserver reliability was conducted using 15 subjects with CBCT images and 15 with digital casts. These 30 subjects were landmarked using the 48 landmarks in 2 separate attempts 2 weeks apart. Intraclass correlation methods, Euclidean distances, and t tests were used to evaluate discrepancies in landmark location between the 2 attempts.²⁷ Intraclass correlation results indicated good to excellent reliability, Euclidean distances were less than 0.5 mm, and no error trends were detected for most landmarks except for landmarks 11, 12, 14, and 15 in the maxillary CBCT images and landmarks 5, 11, 19, and 20 for the mandibular CBCT images. For these problematic landmarks, intraclass correlation values of less than 0.8 and Euclidean distances

greater than 0.6 mm were obtained; therefore, the average data of the 2 digitizing sessions were used to resolve inaccuracies.

After the landmark selection and reliability above, the CBCT images or digital casts of the 300 subjects were landmarked with 48 landmarks. Detailed steps for CBCT digitizing were described in Supplemental Table I. To prevent interference in landmark placement caused by the overlap between teeth, the maxillary and mandibular digital casts were landmarked separately at first. Subsequently, both digital casts were brought into occlusion via 14 matching points (7 on the maxilla, 7 on the mandible) via DVL.R.msi (NYCEP Morphometrics Group; <http://pages.nycep.org/nmg/programs.html>) (Supplemental Fig 3). Occlusions were verified with the dental casts trimmed using each patient's bite registration and confirmed against the patient's photographic pretreatment records. The CBCT images, taken in maximum intercuspation, did not pose this problem, since the observer can scroll through multiple slices to locate each landmark even with dental superimposition.

The 3D coordinate data derived from the 48 landmarks digitized in the total sample ($n = 300$) and the white subsample ($n = 239$) were analyzed in 2 shape analyses, with Morpho J.²⁸ The 3D data have object symmetry, which means that both the left and the right sides of the dental arches are connected by the midline plane, and the data are made of paired and unpaired landmarks. A 3D shape analysis of data with object symmetry includes independent analyses of both symmetric and asymmetric aspects of shape variation.^{29,30} The symmetric component of shape portrays all variations of paired landmarks in all directions of space, whereas the unpaired landmarks can only vary along the midline plane. Thus, in the symmetric component, anteroposterior, transverse, and vertical dentoalveolar shape variations are illustrated. The asymmetric component only depicts shape differences between the left and right side of the arches. According to the Procrustes fit of the coordinate data, covariance matrices are generated for both the symmetric and asymmetric components of shape variation and then submitted independently to principal component analyses. The symmetric and asymmetric principal components explaining at least 5% of the dentoalveolar shape variation were used as quantitative phenotypes for phenotype-genotype correlations with the genetic data.

In addition, we estimated fluctuating asymmetry Mahalanobis scores, which represent the overall magnitude to which a person's dental arch shows differences between the left and right sides based on a zero mean value, without considering laterality or directionality.³¹ We also measured dental arch size via centroid size, calculated as the square root of the sum of the squared distances between the centroid and all other points in the landmark configurations. The resulting phenotypic data consisted of the principal components of symmetric and asymmetric shape variations, fluctuating asymmetry, and centroid size.

Genomic DNA was extracted from saliva samples collected with Oragene kits (DNA Genotek, Ontario, Calif). As described in our previous study, selection criteria for genes/loci were based on at least 1 of the following: (1) evidence of genetic association or linkage to malocclusion phenotypes in previous studies, (2) known expression patterns or biologic functions in the craniofacial complex, (3) known role in the etiology of craniofacial conditions with phenotypic spectra that include skeletal malocclusion, and (4) previous

Genome Wide Association Analysis indicating association with craniofacial variation.¹⁴ For SNP selection, haplotype blocks were reconstructed for all genes/loci, including 10-kb regions flanking intended intervals via Haploview, using genotypic data from a white population (HapMap CEU; HapMap release 27 based on February 2009 assembly, phase 2 and 3 versions).³² A total of 224 tagging SNPs across 82 candidate genes and loci were selected. Of these, 24 SNPs were genotyped using 5-mL reaction volumes and TaqMan chemistry (Applied Biosystems, Foster City, Calif) and detected on a 7900HT sequence detection instrument (Applied Biosystems). A 96.96 or 192.24 dynamic array was used to generate genotypes for the 200 remaining SNPs using competitive allele specific PCR KASPar chemistry (KBioscience, Hoddesdon, United Kingdom) on a nanofluidic platform (Fluidigm, South San Francisco, Calif). For genotype calling, default settings of the Fluidigm SNP genotyping software (version 4.1.2) were used, including a calling algorithm confidence threshold of 65%, a nontemplate control normalization method, and a K-means clustering method. Genotyping quality was assessed by manually inspecting all genotyping plots, excluding subjects who failed on 10 or more SNPs (>5% failure rate) and removing SNPs with greater than a 5% genotyping failure rate or Hardy-Weinberg disequilibrium test at $P < 10^{-4}$. All retained SNPs had a minimum minor allele frequency greater than 3%. Of the 224 SNPs attempted, 5 SNPs (rs10801834, rs2235371, rs12106771, rs1374961, rs2852458) in the total sample and 2 SNPs (rs2235371, 2852458) in the white subsample were dropped. Thus, totals of 219 and 222 SNPs remained for analyses, respectively (Supplemental Tables II and III).

Principal components explaining 5% or more of the symmetric and asymmetric dentoalveolar variations were selected for genotype-phenotype correlation analyses along with centroid size and fluctuating asymmetry for the total sample and the white subsample. SNPs were coded as 0, 1, or 2 according to the number of minor allele copies on a subject's genotype. Multivariate linear regressions were performed in both samples, adjusting for age, sex, and data source (CBCT or dental cast). An adjustment for self-reported ancestry was also included for the total sample. Regressions were performed using software (version 2011; StataCorp, College Station, Tex) to test for associations between each SNP (1 at a time) and the phenotypic data. The formal threshold for statistical significance after the Bonferroni correction for multiple testing was $P < 0.00022$ ($0.05/222$ SNPs).

RESULTS

Phenotypic analyses of the total sample and the white subsample resulted in almost identical dentoalveolar phenotypic variations. Thus, for ease of description and visualization, we only show the components of shape variation for the white subsample (Figs 2–9). Shape components of the total sample are available upon request.

Principal component analysis of the symmetric dentoalveolar shape variations resulted in 4 components that together explained 68% of the total symmetric variations. The scree plot and histograms depicting the sample distribution in each symmetric component of variation are shown in Supplemental Figures 4 and 5, respectively. The first component explained 39.1% of the variation and depicted discrepancies ranging from severe overjet and increased overbite mostly due to mandibular incisors stepped up for subjects with negative principal

component scores to end-on or anterior crossbite relationships and decreased overbites in those with highly positive principal component scores (Fig 2).

Symmetric component 2 explained 15.5% of the variations ranging from a severe open-bite tendency with increased vertical dimension, proclined maxillary and mandibular incisors, and narrow arches for subjects with more negative principal component scores to a deepbite tendency with decreased vertical dimensions, retroclined maxillary and mandibular incisors, and broad arches for those with highly positive principal component scores (Fig 3).

Symmetric component 3 explained 7.5% of the variations and depicted retroclined maxillary central incisors and proclined and mesially tipped lateral incisors, and also retroclined mandibular incisors for subjects with negative principal component scores. Conversely, proclined maxillary central incisors and retroclined lateral incisors with uprighed mandibular incisors were found in subjects with highly positive principal component scores (Fig 4). Interestingly, in symmetric component 3, anterior crossbites between maxillary lateral incisors and mandibular incisors are common in those with highly positive principal component scores.

Symmetric component 4 explained 6.2% of the variations and ranged from features of a Class II Division 2 malocclusion on the negative end to a Class II Division 1 malocclusion on the positive end of the spectrum. Subjects with negative principal component scores had all 4 maxillary incisors stepped down and retroclined with the central incisors slightly more retroclined than the lateral incisors and also wider maxillary arches. In contrast, those with positive principal component scores showed proclined and stepped up maxillary incisors and narrow arches (Fig 5).

Principal component analysis of the asymmetric dentoalveolar shape variations resulted in 4 components that together explained 51% of the total asymmetric variations. The scree plot and histograms depicting the sample distribution of each asymmetric principal component are shown in Supplemental Figures 5 and 6, respectively. Asymmetric principal component 1 explained 32.4% of the variation and depicts left or right rotations of the maxillary and mandibular arches. Subjects with negative principal component scores had the maxillary arch rotated to the left and the mandibular arch rotated to the right. Conversely, those with positive principal component scores had the exact opposite morphology. This leads to dental midline deviations, asymmetries in the anteroposterior occlusion relationships, and a tendency for increased lateral overjet on 1 side and a transverse tight relationship or unilateral crossbite on the other side. As depicted in Figure 6, subjects with negative principal component scores can show a Class II subdivision right, whereas those on the other end can display a Class II subdivision left.

Asymmetric principal component 2 explained 8.2% of the variations with a unilateral maxillary arch transverse collapse and misalignment in the mandibular arch resulting in both maxillary and mandibular lateral incisors shifted palatally or lingually, respectively, and blockage of the maxillary canine with ectopic eruption gingivally and buccally. Also, a crossbite or end-on relationship involving the maxillary lateral incisor and the mandibular canine on the same side was often seen. Thus, subjects with negative principal component

scores had the maxillary right side collapsed, the maxillary right canine blocked buccally and gingivally, and the maxillary right lateral incisor in a crossbite relationship, whereas subjects with highly positive principal component scores had the exact opposite morphology (Fig 7).

Asymmetric principal component 3 explained 5.6% of the variations and portrayed unilateral transverse collapse of the maxillary arch along with right or left mandibular arch rotations leading to unilateral posterior crossbites. Subjects with negative principal component scores had unilateral right posterior crossbites, and those with positive principal component scores had the opposite morphology (Fig 8).

Asymmetric principal component 4 explained 5% of the variations and illustrates severe maxillary and mandibular incisor crowding with blocked right or left central incisors that appeared to erupt buccally and gingivally compared with the rest of the arch. Subjects with negative principal component scores had maxillary and mandibular right central incisors blocked, whereas those with negative principal component scores had the opposite morphology (Fig 9).

Fluctuating asymmetry scores were regressed on the skeletal classification variable Class II or Class III with Class I as the reference category and adjusting for the variables of sex, age, and data source as indicated above. No significant differences were found in the total sample or the white subsample, indicating no difference in overall dentoalveolar asymmetry when comparing Class II or Class III subjects with Class I.

Similar to fluctuating asymmetry, centroid size was regressed on the skeletal classification variable Class II or Class III with Class I as the reference category and also adjusting for the variables of sex, age, and data source as indicated above. No significant differences were found in the arch sizes in the total sample or the white subsample when comparing Class II or Class III subjects with Class I.

Multivariate linear regressions were performed separately for the total sample and the white subsample with 219 and 222 SNPs, respectively. Most results in both samples indicated suggestive associations ($P < 0.05$) with only a few signals significant at $P < 0.00022$ after the Bonferroni correction as shown below. Comparison of results between both samples showed that most association signals point to the same SNPs and or gene/loci, and most alleles were associated in the same direction (according to b regression coefficient) with few exceptions. Therefore, to facilitate visualization, we will focus on the results of the white subsample, pointing out result differences and similarities between samples when appropriate. Figures 2 through 9 show associations at $P < 0.05$ for the white subsample along with the direction of the associated minor allele. Figure 10 shows all regression results for all phenotypes studied, and Table III summarizes the key findings in the white subsample. Complete phenotype-genotype correlation results are shown in Supplemental Tables IV (total sample) and V (white subsample).

Symmetric principal component 1 showed the best associations ($P < 0.05$) with *SNAI3* (rs4287555), *PITX2* (rs2595104), and *BMP3* (rs1390319) in the white subsample and with *PITX2* and *BMP3* for the total sample ($P < 0.01$). More copies of the rare allele for *SNAI3*

(rs4287555) on a person's genotype were associated with an end-on or anterior crossbite relationship, whereas more copies of the rare allele for PITX2 (rs2595104) and BMP3 (rs985328, rs1390319) were associated with severe overjet and increased overbite mostly due to stepped up mandibular incisors.

Symmetric principal component 2 was associated ($P < 0.01$) with loci 11q22.2–q22.3 (rs6591100) and *SNAI3* (rs4287555) in the white subsample and with *SNAI3* (rs4287555), 8q24 (rs11994831), and *MAFB* (rs1124794) in the total sample. More copies of the rare allele for 11q22.2–q22.3 (rs6591100) were associated with an open-bite tendency with increased vertical dimension, proclined maxillary and mandibular incisors, and narrow arches, whereas more copies of the rare alleles for *SNAI3* (rs4287555) in the white sample and of 8q24 (rs11994831) and *MAFB* (rs1124794) in the total sample were associated with deepbite tendencies with decreased vertical dimensions, retroclined maxillary and mandibular incisors, and broader arches. Of note, *SNAI3* (rs4287555) represented an exception in which the regression coefficient of the association was in the opposite direction in the total sample, with more copies of the rare allele associated with an open-bite tendency with increased vertical dimension, proclined maxillary and mandibular incisors, and narrow arches.

Symmetric principal component 3 showed the best associations ($P < 0.01$) with loci 4p16.1 (rs13152451) and *ISL1* (rs3811911) for the white subsample and with *ISL1* (rs3811911) in the total sample albeit with lower significance ($P < 0.05$). More copies of the rare allele for 4p16.1 (rs13152451) were associated with retroclined maxillary central incisors and mandibular incisors and proclined and mesially tipped maxillary lateral incisors, whereas more copies of the rare allele for *ISL1* (rs3811911) were associated with the opposite morphology.

Symmetric principal component 4 was associated with *FGF8* (rs10786648, rs4919593) in both samples ($P < 0.01$) and with *FOXL2* (rs9809852) in the white subsample, where more copies of the rare alleles on both *FGF8* and *FOXL2* SNPs were associated with Class II Division 2 features.

Asymmetric principal component 1 was associated ($P < 0.01$) with *TBX1* (rs6518580) and *SATB1* (rs9713269) in the white subsample and with *TBX1* (rs6518580) and *RUNX2* (rs1997992) in the total sample. More copies of the rare allele for *TBX1* (rs6518580) were associated with the maxillary arch rotated to the right and the mandibular arch rotated to the left, leading to an asymmetric occlusal relationship (ie, the mandibular right ahead of the mandibular left) and a maxillary and mandibular midline discrepancy (ie, mandible deviated left from maxilla), increased transverse overjet on the right side, and a transverse tight relationship or unilateral crossbite on the left side. In contrast, more copies of the rare allele for *SATB1* (rs9713269) and *RUNX2* (rs1997992) were associated with the opposite morphology.

Asymmetric principal component 2 showed the best associations ($P < 0.01$) with *SNAI3* (rs4287555) and *AJUBA* (rs997154) for the white subsample and with *AJUBA* (rs997154), *LEFTY1* (rs360101), *PAX5* (rs1360165), and *SNAI3* (rs4287555) for the total sample. More

copies of the rare alleles for *SNAI3* (rs4287555), *AJUBA* (rs997154), and *PAX5* (rs1360165) were associated with collapse in the maxillary right side of the arch with the maxillary and mandibular lateral incisors shifted palatally or lingually, respectively, with blockage and ectopic eruption of the maxillary right canine. Also, a crossbite or end-on relationship involving the maxillary right lateral incisor and mandibular right canine is often seen. In contrast, rare alleles in *LEFTY1* (rs360101) in the total sample were associated with the opposite morphology.

Asymmetric principal component 3 was highly associated with *SATB2* (rs7593422) in the white subsample ($P = 0.0009$) and remained significant after the Bonferroni correction in the total sample ($P = 0.0001$). The second best signal ($P < 0.01$) for asymmetric principal component 3 was found with locus 12q13.13 (rs2085502). More copies of the rare alleles for *SATB2* (rs7593422) and locus 12q13.13 (rs2085502) were associated with a unilateral posterior crossbite on the right side.

Asymmetric principal component 4 was associated ($P < 0.01$) with locus 1p22.1 (rs10801834) and TP63 (rs9817981) in the white sample and TP63 (rs9817981) in the total sample. More copies of the rare allele for locus 1p22.1 (rs10801834) are associated with maxillary and mandibular right central incisors blocked buccally, whereas more copies of the rare allele of TP63 (rs9817981) are associated with the opposite morphology.

Regression results for Mahalanobis fluctuating asymmetry showed significant associations with *BMP3* SNPs (rs1495643, rs985328) in the white sample. Of these, *BMP3* (rs1495643; $P = 0.00008$) remained significant after the Bonferroni correction ($P < 0.00022$). For the total sample, Mahalanobis fluctuating asymmetry showed associations ($P < 0.01$) with *LATS1* (rs3798761) and *BMP3* (rs1495643, rs985328). More copies of the rare alleles for *BMP3* SNPs (rs1495643, rs985328) reduced asymmetry, whereas more copies of rare alleles in *LATS1* (rs3798761) increased the overall asymmetry.

Regression results for centroid size showed associations ($P < 0.01$) between centroid size and *SNAIL* (rs6012791) and *FOXL2* (rs12106771) for the white sample and for locus 4p16.1 (rs4524456), *FOXO6* (rs10889961), and *EDNI* (rs2859338) for the total sample. More copies of the rare alleles for all these SNPs in a subject's genotype are associated with decreases in the size of the dental arches.

DISCUSSION

In our previous candidate gene study in patients with malocclusion, we used lateral cephalograms to derive strictly skeletal 2-dimensional phenotypes in a white adult cohort with moderate or severe malocclusion.¹⁴ We identified significant associations ($P < 0.00025$) with *SNAI3* and *TWIST1* along with other modest signals ($P < 0.05$) for 21 of the 71 craniofacial genes/loci attempted. In this study, we used a similar cohort (about 80% of the samples overlap) to evaluate the association of 3D dentoalveolar shape phenotypic variation against 82 craniofacial candidate genes and loci (71 genes/loci were included in our previous study).

This study brings a novel approach to the study of phenotype-genotype correlations of dentoalveolar variations in malocclusion via the use of geometric morphometric methods with 3D coordinate information from landmarks placed along the dental arches. Because the landmark data set was analyzed from dental casts trimmed in occlusion or CBCT images with the patient in maximum intercuspation, both data sets are expected to capture intra-arch dentoalveolar and interarch occlusal relationships. Since the landmark data set has object symmetry as explained in the methods section, aspects of 3D variation are analyzed separately into symmetric and asymmetric components of variation.

Our results for the symmetric shape variations indicate that about 54% of these variations were explained by severe horizontal and vertical discrepancies often seen in patients with severe malocclusion. In our sample, increased horizontal discrepancies resulting in large overjets and also severe open-bite tendencies as displayed in symmetric principal components 1 and 2, respectively, are usually accompanied by severe transverse deficiencies. In contrast, severe underbites and deepbites were seen along with broader arches. The last 13.5% of the symmetric variations seemed focused on horizontal and vertical discrepancies of the maxillary and mandibular incisor segments. For instance, symmetric principal component 3 depicts proclined and mesially tipped maxillary lateral incisors and retroclined maxillary central incisors and mandibular incisors on the negative end and the opposite morphology on the positive end. Symmetric principal component 4 depicts the maxillary incisor segment largely stepped down on the negative end and stepped up on the positive end. Interestingly, once again the anterior deepbite phenotype had broad maxillary and mandibular arches, whereas subjects with open bite had narrow maxillary and mandibular arches with clear evidence of crowding. These features illustrated by symmetric principal component 4 are similar to those in a Class II Division 2 malocclusion compared with a Class II Division 1 malocclusion.

Genotype-phenotype correlations with the symmetric components of dentoalveolar variation identified modest signals ($P < 0.05$) for 36 of the 82 genes/loci attempted. Of these, 13 genes/loci including 4p16.1, *ABCA4-ARHGAP29*, *BMP3*, *COL1A1*, *FGFR2*, *IRF6*, *LEFTY2*, *PAX7*, *PRRX1*, *PRRX2*, *SNAI3*, *TBX5*, and *TLX1* were found in common between this study and our previous one. Thus, although this study cannot be considered an independent replication of our previous findings, the consistency of results for loci such as 4p16.1, *BMP3*, *PAX7*, and *SNAI3* despite the use of different records and landmarks (skeletal vs dentoalveolar landmarks) indicates that these loci underlie both skeletal and dentoalveolar variations in malocclusion. For instance, loci 4p16.1 was associated with principal component 1 in the previous study, which depicts skeletal variation ranging from a skeletal open bite to a skeletal deepbite, whereas *BMP3*, *PAX7*, and *SNAI3* were associated in the previous study with principal component 2, which features skeletal variations ranging from severe profile convexity to profile concavity. Similarly, in this study, symmetric principal component 1, which depicts variations ranging from severe overjet to anterior crossbite, was associated with *BMP3*, *PAX7*, and *SNAI3*, and symmetric principal component 2, with variations ranging for severe open bite to deepbite, was associated with both 4p16.1 and *SNAI3*.

Although no association signals were significant after the Bonferroni correction ($P < 0.00022$) for any symmetric shape components, the best associations observed in the total sample and the white subsample for the 4 symmetric components included *PITX2*, *SNAI3*, 11q22.2-q22.3, 4p16.1, *ISL1*, and *FGF8*. Loci 11q22.2-q22.3 and 4p16.1 were identified initially in genetic linkage scans of families segregating maxillary hypoplasia and mandibular prognathism, respectively.^{33,34} Significant genetic associations were identified by our group in our previous study for *SNAI3* with a phenotypic continuum of horizontal maxillomandibular discrepancies, indicating that *SNAI3* is largely associated with skeletal and dentoalveolar variations in malocclusion. Mutations in *PITX2* caused Rieger syndrome, which is associated with maxillary hypoplasia.³⁵ Mutations in *FGF8* are seen in patients with Kallmann's syndrome, which is associated with orofacial clefting along with maxillary and mandibular retrognathism.³⁶ Also, mouse models of ciliopathies, which can result in craniofacial dysmorphology, indicate that FGF8 dysregulation is associated with the maxillary phenotypic anomalies in these conditions.³⁷ *ISL1*, a LIM-homeodomain transcription factor, is expressed in cell lineages that contribute to facial muscle formation³⁸ and the oral epithelium that gives rise to incisor formation.³⁹ Recently, *Isl1* was shown to contribute to the facial epithelium and mesenchymal survival during mandibular jaw formation via regulation of β catenin and *Fgf8*.⁴⁰

Our results for the asymmetric shape variations indicated that most variations (about 32%) were explained by maxillary and mandibular arch rotations resulting in midline deviations and asymmetric occlusion patterns that clinically are often referred to as right or left subdivisions (ie, Class II subdivision right). Beyond this, subsequent components explained a maximum of 8% each and were mostly focused on unilateral ectopic eruptions or malposition at the canines and lateral incisors resulting in same-side blocked canines and lateral incisors, unilateral collapse of the maxillary arch with mandibular arch rotations resulting in unilateral posterior crossbites, and unilateral ectopic eruption or malposition of the central incisors. Interestingly, Sprowls et al⁴¹ found significant positive correlations between the extent of fluctuating asymmetry in tooth sizes and the extent of transverse discrepancies and crowding. Although we did not capture fluctuating asymmetry in tooth sizes as did that study, the phenotypes displayed in our asymmetric components seem to be dominated by severe crowding, supporting those findings.

Genotype-phenotype correlations with the asymmetric components of dentoalveolar variation identified modest association signals ($P < 0.05$) for 24 of the 82 genes/loci attempted. Of these, the best association signals were observed for *TBX1*, *AJUBA*, *SNAI3*, *SATB2*, *TP63*, and 1p22.1. The association with *SATB2* remained significant after the Bonferroni correction. *TBX1* is a major candidate gene causal for 22q11 deletion syndrome associated with congenital heart defects resulting from incorrect left-to-right patterning important for asymmetric cardiac morphogenesis⁴² as well as craniofacial and dental anomalies including cleft palate.⁴³ The LIM protein *AJUBA* has previously shown an association with soft tissue facial width phenotypes,^{44,45} and it is also required for expression of early developmental genes that determine left-right body axis patterning.⁴⁶ As discussed above, *SNAI3* was largely associated with skeletal phenotypes in malocclusion patients in our previous study. Although to our knowledge there is no known direct role for *SNAI3* in left-to-right patterning, other members of the snail family of genes are required for

left-right patterning during development.⁴⁷ *SATB2* is expressed in the maxillary and mandibular mesenchymes, where it regulates the size of distal jaw elements. *Satb2* null mice die of cleft palate, whereas heterozygotes exhibit jaw-size reduction.⁴⁸ Moreover, early during jaw development, *Satb2* expression is random along the left-right axis, and *Satb2*^{+/-} exhibits random side asymmetries of the craniofacial complex.⁴⁹ Human mutations in TP63 are found in patients with syndromic forms of clefting such as ectrodactyly-ectodermal dysplasia-cleft lip/palate, ankyloblepharon-ectodermal dysplasia-clefting, and nonsyndromic cleft lip.⁵⁰ SNPs in TP63 were associated with the distance between the eyes in a Genome-Wide Association Analysis of facial shape in Europeans⁵¹; moreover, P63 regulates asymmetric cell division in the epidermis; yet its role in asymmetric craniofacial shape has not been reported.⁵² Finally, loci 1p22.1 was also identified previously by Frazier-Bowers et al³³ in a linkage scan of families segregating maxillary hypoplasia. The associated SNP rs10801834 in the 1p22.1 region lies within the ZNF644 gene implicated in myopia; yet no role in left-right asymmetries has been attributed to ZNF644 so far.

Regression results for Mahalanobis fluctuating asymmetry identified 8 modest signals ($P < 0.05$), with the best associations observed for *LATS1* and *BMP3* for the total sample and the white subsample, respectively. The association for *BMP3* SNPs (rs1495643, rs985328) in the white subsample remained significant after the Bonferroni correction. *LATS1* is a member of the hippo pathway known to control organ size; more recently, evidence suggests that the hippo pathway may also regulate tooth development.⁵³ According to DECIPHER, patients with chromosome abnormalities including the *LATS1* gene have facial and dental anomalies; yet no asymmetric phenotypes have been described. BMP signaling regulates left to right patterning in mammalian development.⁵⁴ *BMP3* variation is associated with brachycephalic skull shapes in dogs and is essential for jaw development in zebrafish.⁵⁵ Recently, a *BMP3* missense variant Tyr67Ala was found in 6 family members with mandibular prognathism in a large Italian family.⁹ Findings of genetic associations between loci such as *BMP3*, *SNAI3*, and *SATB2* with both symmetric and asymmetric phenotypes are not surprising, since these genes participate in the development of bilateral structures and have key roles in left-to-right patterning during development.

The results for centroid size identified 14 modest association signals ($P < 0.05$) for both samples. However associated genes/loci were less consistent between samples. The best signals were observed at 4p16.1 and *SNAIL1* for the total sample and the white subsample, respectively, and only 5 associated genes/loci overlapped between the 2 samples, indicating population genetic heterogeneity for dentoalveolar size phenotypes.

Overall, this study demonstrates that continuous dentoalveolar phenotypes are informative for genotype-phenotype correlation studies of malocclusion and thus could continue to be pursued in more powerful data sets than this study. Moreover, our 2 phenotype-genotype correlations that remained significant after the Bonferroni correction, *SATB2* and *BMP3*, together with other suggestive findings in genes and loci previously determined by our group and others, lend support to future research of these loci with larger samples.

CONCLUSIONS

This study presents the first comprehensive phenotype-genotype correlation study of 3D dentoalveolar phenotypic variations in patients with malocclusion. The results indicated that several craniofacial genes/loci involved in skeletal phenotypes derived from patients with malocclusion are also associated with dentoalveolar phenotypic variations. Moreover, this study indicates that genetic determinants of left-to-right patterning during early embryogenesis—in particular *BMP3* and *STAB2*—are highly associated with asymmetric phenotypic variations in the dental arches, pointing toward new genetic mechanisms associated with human malocclusion. Future research is needed to confirm these findings in larger data sets and uncover the identities of the etiological variants that can then best be tested in animal models of malocclusion.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Emrich RE, Brodie AG, Blayney JR. Prevalence of Class 1, Class 2, and Class 3 malocclusions (Angle) in an urban population. An epidemiological study. *J Dent Res.* 1965; 44:947–53. [PubMed: 5213215]
2. Lew KK, Foong WC, Loh E. Malocclusion prevalence in an ethnic Chinese population. *Aust Dent J.* 1993; 38:442–9. [PubMed: 8110079]
3. Thilander B, Pena L, Infante C, Parada SS, de Mayorga C. Prevalence of malocclusion and orthodontic treatment need in children and adolescents in Bogota, Colombia. An epidemiological study related to different stages of dental development. *Eur J Orthod.* 2001; 23:153–67. [PubMed: 11398553]
4. Soh J, Sandham A, Chan YH. Occlusal status in Asian male adults: prevalence and ethnic variation. *Angle Orthod.* 2005; 75:814–20. [PubMed: 16279828]
5. Borzabadi-Farahani A, Eslamipour F. Malocclusion and occlusal traits in an urban Iranian population. An epidemiological study of 11- to 14-year-old children. *Eur J Orthod.* 2009; 31:477–84. [PubMed: 19477970]
6. Claudino D, Traebert J. Malocclusion, dental aesthetic self-perception and quality of life in a 18 to 21 year-old population: a cross section study. *BMC Oral Health.* 2013; 13:3. [PubMed: 23295063]
7. Moreno Uribe LM, Miller SF. Genetics of the dentofacial variation in human malocclusion. *Orthod Craniofac Res.* 2015; 18(Suppl 1):91–9. [PubMed: 25865537]
8. Nikopensius T, Saag M, Jagomagi T, Annilo T, Kals M, Kivistik PA, et al. A missense mutation in *DUSP6* is associated with Class III malocclusion. *J Dent Res.* 2013; 92:893–8. [PubMed: 23965468]

9. Perillo L, Monsurro A, Bonci E, Torella A, Mutarelli M, Nigro V. Genetic association of ARHGAP21 gene variant with mandibular prognathism. *J Dent Res*. 2015; 94:569–76. [PubMed: 25691070]
10. Chen F, Li Q, Gu M, Li X, Yu J, Zhang YB. Identification of a mutation in FGF23 involved in mandibular prognathism. *Sci Rep*. 2015; 5:11250. [PubMed: 26059428]
11. Guan X, Song Y, Ott J, Zhang Y, Li C, Xin T, et al. The ADAMTS1 gene is associated with familial mandibular prognathism. *J Dent Res*. 2015; 94:1196–201. [PubMed: 26124221]
12. Moreno Uribe LM, Vela KC, Kummet C, Dawson DV, Southard TE. Phenotypic diversity in white adults with moderate to severe Class III malocclusion. *Am J Orthod Dentofacial Orthop*. 2013; 144:32–42. [PubMed: 23810043]
13. Moreno Uribe LM, Howe SC, Kummet C, Vela KC, Dawson DV, Southard TE. Phenotypic diversity in white adults with moderate to severe Class II malocclusion. *Am J Orthod Dentofacial Orthop*. 2014; 145:305–16. [PubMed: 24582022]
14. da Fontoura CS, Miller SF, Wehby GL, Amendt BA, Holton NE, Southard TE, et al. Candidate gene analyses of skeletal variation in malocclusion. *J Dent Res*. 2015; 94:913–20. [PubMed: 25910506]
15. DeKock WH. Dental arch depth and width studied longitudinally from 12 years of age to adulthood. *Am J Orthod*. 1972; 62:56–66. [PubMed: 4503706]
16. Bishara SE, Khadivi P, Jakobsen JR. Changes in tooth size-arch length relationships from the deciduous to the permanent dentition: a longitudinal study. *Am J Orthod Dentofacial Orthop*. 1995; 108:607–13. [PubMed: 7503038]
17. Thilander B. Dentoalveolar development in subjects with normal occlusion. A longitudinal study between the ages of 5 and 31 years. *Eur J Orthod*. 2009; 31:109–20. [PubMed: 19304760]
18. Papagiannis A, Halazonetis DJ. Shape variation and covariation of upper and lower dental arches of an orthodontic population. *Eur J Orthod*. 2016; 38:202–11. [PubMed: 25840587]
19. Francisconi MF, Janson G, Freitas KM, Oliveira RC, Oliveira RC, Freitas MR, et al. Overjet, overbite, and anterior crowding relapses in extraction and nonextraction patients, and their correlations. *Am J Orthod Dentofacial Orthop*. 2014; 146:67–72. [PubMed: 24975000]
20. Marshall SD, Caspersen M, Hardinger RR, Franciscus RG, Aquilino SA, Southard TE. Development of the curve of Spee. *Am J Orthod Dentofacial Orthop*. 2008; 134:344–52. [PubMed: 18774080]
21. Bishara SE, Jakobsen JR. Individual variation in tooth-size/archlength changes from the primary to permanent dentitions. *World J Orthod*. 2006; 7:145–53. [PubMed: 16779973]
22. Silvola AS, Rusanen J, Tolvanen M, Pirttiniemi P, Lahti S. Occlusal characteristics and quality of life before and after treatment of severe malocclusion. *Eur J Orthod*. 2012; 34:704–9. [PubMed: 21750239]
23. Li J, He Y, Wang Y, Chen T, Xu Y, Xu X, et al. Dental, skeletal asymmetries and functional characteristics in Class II subdivision malocclusions. *J Oral Rehabil*. 2015; 42:588–99. [PubMed: 25944587]
24. Zelditch, M., Swiderski, D., Sheets, DH. *Geometric morphometrics for biologists*. Burlington, Mass: Academic Press; 2004.
25. Lindeman, RH., Merenda, PF., Gold, RZ. *Introduction to bivariate and multivariate analysis*. Glenview, Ill: Scott, Foresman; 1980.
26. Wiley DF, Amenta N, Alcantara DA, Ghosh D, Kil YJ, Delson E, et al. Evolutionary morphing. *Proceedings of the IEEE Conference on Visualization*. 2005:55.
27. Landis JR, Koch GG. The measurement of observer agreement for categorical data. *Biometrics*. 1977; 33:159–74. [PubMed: 843571]
28. Klingenberg CP. MorphoJ: an integrated software package for geometric morphometrics. *Mol Ecol Resour*. 2011; 11:353–7. [PubMed: 21429143]
29. Klingenberg CP, Barluenga M, Meyer A. Shape analysis of symmetric structures: quantifying variation among individuals and asymmetry. *Evolution*. 2002; 56:1909–20. [PubMed: 12449478]
30. Mardia KV, Bookstein FL, Moreton IJ. Statistical assessment of bilateral symmetry of shapes. *Biometrika*. 2000; 87:285–300.

31. Klingenberg C. Analyzing fluctuating asymmetry with geometric morphometrics: concepts, methods, and applications. *Symmetry*. 2015; 7:843.
32. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, et al. The structure of haplotype blocks in the human genome. *Science*. 2002; 296(5576):2225–9. [PubMed: 12029063]
33. 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. [PubMed: 19131318]
34. Li Q, Zhang F, Li X, Chen F. Genome scan for locus involved in mandibular prognathism in pedigrees from China. *PloS One*. 2010; 5:e12678. [PubMed: 20844756]
35. Semina EV, Reiter R, Leysens NJ, Alward WL, Small KW, Datson NA, et al. Cloning and characterization of a novel bicoid-related homeobox transcription factor gene, RIEG, involved in Rieger syndrome. *Nat Genet*. 1996; 14:392–9. [PubMed: 8944018]
36. Molsted K, Kjaer I, Giwercman A, Vesterhauge S, Skakkebaek NE. Craniofacial morphology in patients with Kallmann's syndrome with and without cleft lip and palate. *Cleft Palate Craniofac J*. 1997; 34:417–24. [PubMed: 9345610]
37. Tabler JM, Barrell WB, Szabo-Rogers HL, Healy C, Yeung Y, Perdiguero EG, et al. Fuz mutant mice reveal shared mechanisms between ciliopathies and FGF-related syndromes. *Dev Cell*. 2013; 25:623–35. [PubMed: 23806618]
38. Tzahor E. Heart and craniofacial muscle development: a new developmental theme of distinct myogenic fields. *Dev Biol*. 2009; 327:273–9. [PubMed: 19162003]
39. Mitsiadis TA, Angeli I, James C, Lendahl U, Sharpe PT. Role of *Islet1* in the patterning of murine dentition. *Development*. 2003; 130:4451–60. [PubMed: 12900460]
40. Akiyama R, Kawakami H, Taketo MM, Evans SM, Wada N, Petryk A, et al. Distinct populations within *Isl1* lineages contribute to appendicular and facial skeletogenesis through the beta-catenin pathway. *Dev Biol*. 2014; 387:37–48. [PubMed: 24424161]
41. Sprowls MW, Ward RE, Jamison PL, Hartsfield JK. Dental arch asymmetry, fluctuating dental asymmetry, and dental crowding: a comparison of tooth position and tooth size between antimeres. *Semin Orthod*. 2008; 14:157–65.
42. Nowotschin S, Liao J, Gage PJ, Epstein JA, Campione M, Morrow BE. *Tbx1* affects asymmetric cardiac morphogenesis by regulating *Pitx2* in the secondary heart field. *Development*. 2006; 133:1565–73. [PubMed: 16556915]
43. Gao S, Moreno M, Eliason S, Cao H, Li X, Yu W, et al. *TBX1* protein interactions and microRNA-96-5p regulation controls cell proliferation during craniofacial and dental development: implications for 22q11.2 deletion syndrome. *Hum Mol Genet*. 2015; 24:2330–48. [PubMed: 25556186]
44. Fatemifar G, Hoggart CJ, Paternoster L, Kemp JP, Prokopenko I, Horikoshi M, et al. Genome-wide association study of primary tooth eruption identifies pleiotropic loci associated with height and craniofacial distances. *Hum Mol Genet*. 2013; 22:3807–17. [PubMed: 23704328]
45. Moreno Uribe LM, Ray A, Blanchette DR, Dawson DV, Southard TE. Phenotype-genotype correlations of facial width and height proportions in patients with Class II malocclusion. *Orthod Craniofac Res*. 2015; 18(Suppl 1):100–8. [PubMed: 25865538]
46. Nagai Y, Asaoka Y, Namae M, Saito K, Momose H, Mitani H, et al. The LIM protein *Ajuba* is required for ciliogenesis and left-right axis determination in medaka. *Biochem Biophys Res Commun*. 2010; 396:887–93. [PubMed: 20457130]
47. Murray SA, Gridley T. Snail family genes are required for left-right asymmetry determination, but not neural crest formation, in mice. *Proc Natl Acad Sci U S A*. 2006; 103:10300–4. [PubMed: 16801545]
48. FitzPatrick DR, Carr IM, McLaren L, Leek JP, Wightman P, Williamson K, et al. Identification of *SATB2* as the cleft palate gene on 2q32-q33. *Hum Mol Genet*. 2003; 12:2491–501. [PubMed: 12915443]
49. Fish JL, Villmoare B, Kobernick K, Compagnucci C, Britanova O, Tarabykin V, et al. *Satb2*, modularity, and the evolvability of the vertebrate jaw. *Evol Dev*. 2011; 13:549–64. [PubMed: 23016939]

50. Rinne T, Brunner HG, van Bokhoven H. p63-associated disorders. *Cell Cycle*. 2007; 6:262–8. [PubMed: 17224651]
51. Liu F, van der Lijn F, Schurmann C, Zhu G, Chakravarty MM, Hysi PG, et al. A genome-wide association study identifies five loci influencing facial morphology in Europeans. *PLoS Genet*. 2012; 8:e1002932. [PubMed: 23028347]
52. Gunschmann C, Stachelscheid H, Akyuz MD, Schmitz A, Missero C, Bruning JC, et al. Insulin/IGF-1 controls epidermal morphogenesis via regulation of FoxO-mediated p63 inhibition. *Dev Cell*. 2013; 26:176–87. [PubMed: 23906066]
53. Li CH, Li CZ. The role of hippo signaling in tooth development. *J Formos Med Assoc*. 2016; 115:295–7. [PubMed: 25890496]
54. Mine N, Anderson RM, Klingensmith J. BMP antagonism is required in both the node and lateral plate mesoderm for mammalian left-right axis establishment. *Development*. 2008; 135:2425–34. [PubMed: 18550712]
55. Schoenebeck JJ, Hutchinson SA, Byers A, Beale HC, Carrington B, Faden DL, et al. Variation of BMP3 contributes to dog breed skull diversity. *PLoS Genet*. 2012; 8:e1002849. [PubMed: 22876193]

Fig 1.

A, The 48 landmarks and the wire frame used in this study; **B**, landmarks digitized on dental casts and CBCT images.

Fig 2.

Symmetric principal component 1 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 3.

Symmetric principal component 2 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 4.

Symmetric principal component 3 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 5.

Symmetric principal component 4 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 6.

Asymmetric principal component 1 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 7.

Asymmetric principal component 2 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 8.

Asymmetric principal component 3 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

Fig 9.

Asymmetric principal component 4 phenotype-genotype correlations are significant at $P < 0.05$ along with the dentoalveolar phenotypic variations of subjects with the most negative ($-\beta$) or positive ($+\beta$) principal component scores. *Light blue* represents the average configuration; *dark blue* represents the deformation of interest.

A)

B)

Fig 10.

All association results obtained in the white subsample for **A**, the 4 symmetric principal components; **B**, the 4 asymmetric principal components; **C**, the Mahalanobis fluctuating asymmetric (*FA*) scores; and **D**, centroid size with respective *P* values (log10 transformed). The *yellow* and *purple* lines run through the value that corresponds to $P=0.05$ and $P=0.01$, respectively.

Table 1

Sample description

Total sample, n = 300					
White subsample, n = 239					
Sex	Data source	Skeletal Class	Ethnicity	Sex	Data source
Male, 93	CBCT images, 131	I, 63	White, 239	Male, 71	CBCT images, 100
Female, 207	Cast, 169	II, 154	African American, 15	Female, 168	Cast, 139
		III, 83	Asian, 18		
			Hispanic, 15		
			Other, 13		
Age range, 12–68 y					
Average age, 29.9 y					
Age range, 12–68 y					
Average age, 30.7 y					

Table II

Description of the 48 landmarks digitized on dental models or CBCT images

<i>Wire frame number</i>	<i>Landmark name</i>	<i>Description</i>
Maxilla		
1	s0	Most cervical point of tooth on edge of gingiva-right first molar
2	s1	Most cervical point of tooth on edge of gingiva-right second premolar
3	s2	Most cervical point of tooth on edge of gingiva-right first premolar
4	s3	Lateral edge of right canine
5	s4	Medial edge of right canine
6	s5	Most cervical point of tooth on edge of gingiva-right canine
7	s6	Lateral incisal edge of right lateral incisor
8	s7	Medial incisal edge of right lateral incisor
9	s8	Most cervical point of tooth on edge of gingiva-right lateral incisor
10	s9	Lateral incisal edge of right central incisor
11	s10	Medial incisal edge of right central incisor
12	s11	Most cervical point of tooth on edge of gingiva-right central incisor
13	s12	Lateral incisal edge of left central incisor
14	s13	Medial incisal edge of left central incisor
15	s14	Most cervical point of tooth on edge of gingiva-left central incisor
16	s15	Lateral incisal edge of left lateral incisor
17	s16	Medial incisal edge of left lateral incisor
18	s17	Most cervical point of tooth on edge of gingiva-left lateral incisor
19	s18	Lateral edge of left canine
20	s19	Medial edge of left canine
21	s20	Most cervical point of tooth on edge of gingiva-left canine
22	s21	Most cervical point of tooth on edge of gingiva-left first premolar
23	s22	Most cervical point of tooth on edge of gingiva-left second premolar
24	s23	Most cervical point of tooth on edge of gingiva-left first molar
Mandible		
25	s0	Most cervical point of tooth on edge of gingiva-left first molar
26	s1	Most cervical point of tooth on edge of gingiva-left second premolar
27	s2	Most cervical point of tooth on edge of gingiva-left first premolar
28	s3	Lateral edge of left canine
29	s4	Medial edge of left canine
30	s5	Most cervical point of tooth on edge of gingiva-left canine
31	s6	Lateral incisal edge of left lateral incisor
32	s7	Medial incisal edge of left lateral incisor
33	s8	Most cervical point of tooth on edge of gingiva-left lateral incisor
34	s9	Lateral incisal edge of left central incisor
35	s10	Medial incisal edge of left central incisor

<i>Wire frame number</i>	<i>Landmark name</i>	<i>Description</i>
36	s11	Most cervical point of tooth on edge of gingiva-left central incisor
37	s12	Lateral incisal edge of right central incisor
38	s13	Medial incisal edge of right central incisor
39	s14	Most cervical point of tooth on edge of gingiva-right central incisor
40	s15	Lateral incisal edge of right lateral incisor
41	s16	Medial incisal edge of right lateral incisor
42	s17	Most cervical point of tooth on edge of gingiva-right lateral incisor
43	s18	Lateral edge of right canine
44	s19	Medial edge of right canine
45	s20	Most cervical point of tooth on edge of gingiva-right canine
46	s21	Most cervical point of tooth on edge of gingiva-right first premolar
47	s22	Most cervical point of tooth on edge of gingiva-right second premolar
48	s23	Most cervical point of tooth on edge of gingiva-right first molar

Table III

Summary of the main association findings for the white subsample (n = 239)

<i>Phenotype</i>	<i>Mean</i>	<i>SD</i>	<i>Minimum</i>	<i>Maximum</i>	<i>Gene/loci</i>	<i>Best SNP</i>	<i>Phenotypic description</i>
Symm PC 1	0.00	0.07	-0.22	0.21	<i>SNAI3</i>	rs4287555	Neg PC scores: severe overjets and deepbites Pos PC scores: negative overjets and open bites
Symm PC 2	0.00	0.05	-0.18	0.19	11q22.2-q22.3	rs6591100 [*]	Neg PC scores: severe open bites, proclined maxillary and mandibular incisors and narrow arches Pos PC scores: severe deepbite, retroclined maxillary and mandibular incisors and broad arches
Symm PC 3	0.00	0.03	-0.10	0.08	4p16.1	rs13152451 [*]	Neg PC scores: retroclined maxillary central incisors and proclined and mesially tipped maxillary lateral incisors Pos PC scores: proclined maxillary central incisors and retroclined lateral incisors
Symm PC 4	0.00	0.03	-0.07	0.10	<i>FGF8</i>	rs10786648 [*]	Neg PC scores: maxillary incisors stepped down and retroclined (Class II Division 2 relationship) Pos PC scores: maxillary incisors proclined and stepped up (Class II Division 1)
Asymm PC 1	0.00	0.03	-0.10	0.09	<i>TBX1</i>	rs6518580 [*]	Dental midline deviations, asymmetries anteroposterior occlusion relationship and increased lateral overjet on 1 side and transverse tight relationship or unilateral crossbite on the other side
Asymm PC 2	0.00	0.02	-0.09	0.06	<i>SNAI3</i>	rs4287555 [*]	Maxillary left or right canine blocked buccally and gingivally; maxillary left or right lateral incisor in a crossbite relationship
Asymm PC 3	0.00	0.01	-0.04	0.04	<i>SATB2</i>	rs7593422 [†]	Left or right unilateral posterior crossbite
Asymm PC 4	0.00	0.01	-0.05	0.04	1p22.1	rs10801834 [*]	Severe maxillary and mandibular incisor crowding with blocked right or left central incisors that erupt buccally and gingivally
Mahalanobis FA	8.16	1.45	4.72	12.85	<i>BMP3</i>	rs1495643 [†]	Increased FA associated with more copies of the rare allele for BMP3 SNP rs1495643
Centroid size	122.8	5.7	106.3	138.9	<i>SNAI1</i>	rs6012791 [*]	Increased arch size associated with more copies of the rare allele for SNAI1 SNP rs6012791

Symm, Symmetric; *PC*, principal component; *Asymm*, asymmetric; *FA*, fluctuating asymmetry; *Neg*, negative; *Pos*, positive.^{*} Significant at $P < 0.01$; [†] significant after the Bonferroni correction ($P < 0.00022$).