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

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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 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 phenotpye-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. (Am J Orthod Dentofacial Orthop 2017;151:539-58)

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

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All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest, and none were reported.
Funding was provided by the American Association of Orthodontists Foundation (grants OFDFA_2008-2011 and BRA 2012). Also supported by the National Center for Advancing Translational Sciences and the National Institutes of Health through Grants 2 UL1 TR000442-06 and T32-DE014678-09, and the National Institutes of Health/National Institute of Dental and Craniofacial Research (grant R90 DE024296). The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH.
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Submitted, December 2015; revised and accepted, August 2016.
0889-5406/$36.00

http://dx.doi.org/10.1016/j.ajodo.2016.08.027

prognathism has identified 4 etiologic mutations.[7] The first mutation identified was a heterozygous missense change rs139318648 (c.545C>T, p.Ser182Phe) in the duel 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 (c7421>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 (c7421>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 variations[18] 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]