Candidate Gene Analyses of Skeletal Variation in Malocclusion

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Abstract

This study evaluated associations between craniofacial candidate genes and skeletal variation in patients with malocclusion. Lateral cephalometric radiographs of 269 untreated adults with skeletal classes I, II, and III malocclusion were digitized with 14 landmarks. Two-dimensional coordinates were analyzed using Procrustes fit and principal component (PC) analysis to generate continuous malocclusion phenotypes. Skeletal class classifications (I, II, or III) were used as a categorical phenotype. Individuals were genotyped for 198 single-nucleotide polymorphisms (SNPs) in 71 craniofacial genes and loci. Phenotype-genotype associations were tested via multivariate linear regression for continuous phenotypes and multinomial logistic regression for skeletal malocclusion class. PC analysis resulted in 4 principal components (PCs) explaining 69% of the total skeletal facial variation. PC1 explained 32.7% of the variation and depicted vertical discrepancies ranging from skeletal deep to open bites. PC1 was associated with a SNP near *PAX5* ($P = 0.01$). PC2 explained 21.7% and captured horizontal maxillomandibular discrepancies. PC2 was associated with SNPs upstream of *SNAI3* ($P = 0.0002$) and *MYO1H* ($P = 0.006$). PC3 explained 8.2% and captured variation in ramus height, body length, and anterior cranial base orientation. PC3 was associated with *Twist1* ($P = 0.000076$). Finally, PC4 explained 6.6% and detected variation in condylar inclination as well as symphysis projection. PC4 was associated with *PAX7* ($P = 0.007$). Furthermore, skeletal class II risk increased relative to class I with the minor alleles of SNPs in *FGFR2* (odds ratio [OR] = 2.1, $P = 0.004$) and declined with SNPs in *EDN1* (OR = 0.5, $P = 0.007$). Conversely, skeletal class III risk increased versus class I with SNPs in *FGFR2* (OR 2.2, $P = 0.005$) and *COL1A1* (OR = 2.1, $P = 0.008$) and declined with SNPs in *TBX5* (OR = 0.5, $P = 0.014$). *PAX5*, *SNAI3*, *MYO1H*, *Twist1*, and *PAX7* are associated with craniofacial skeletal variation among patients with malocclusion, while *FGFR2*, *EDN1*, *TBX5*, and *COL1A1* are associated with type of skeletal malocclusion.

Keywords: cephalometry, genetics, angle class III, angle class II, polymorphisms, association study

Introduction

Malocclusion is a heterogeneous condition that affects populations worldwide and results in impaired aesthetics and function and reduced quality of life (Claudino and Traebert 2013). Genetic studies have focused on class III malocclusion and a few genome linkage scans, and 1 genomewide association study (GWAS) has identified class III loci, including 1p36, 1p22.3, 1q32.2, 3q26.2, 4p16, 6q25, 11q22, 12q13.13, 14q24, and 19q13.2, in Asian and Hispanic families (Yamaguchi et al. 2005; Frazier-Bowers et al. 2009; Li et al. 2010; Ikuno et al. 2014). Fine mapping efforts within loci 1p22-p36 and 12q13-q24 have reported associations of mandibular prognathism with *EPB41*, *MATN1*, *COL2A1*, *MYO1H*, *TGFB3*, and *LTBP2* (Xue et al. 2010; Li et al. 2011; Tassopoulou-Fishell et al. 2012). Recently, full exome sequencing identified a likely causal heterozygous missense mutation c.545C>T (p.Ser182Phe) in *DUSP6* (12q21.3) for maxillary hypoplasia (Nikopentis et al. 2013). The few genetic studies of class II and I malocclusion have detected associations between *NOGGIN* and mandibular hypoplasia (Gutierrez et al. 2010) and between *HOXB*, *EDA*, *XEDAR*, and *BMP2* and dental irregularities or severe crowding (Pillas et al. 2010; Ting et al. 2011).

While informative, these studies are limited by modest sample sizes, unclear generalizability to populations of non-Asian ancestry, and exclusive use of categorical phenotypes (i.e., mandibular prognathism) that do not capture the phenotypic

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Figure 1. Skeletal classification was based on cephalometric and dental criteria. The total sample included 53 class I, 128 class II, and 88 class III individuals.

complexity of malocclusion. Thus, additional genetic studies coupled with comprehensive phenotyping to reduce heterogeneity and increase power for detecting genetic associations are needed (Robinson 2012).

Pretreatment orthodontic records constitute a rich source of phenotypic data. Among these, 2-dimensional lateral cephalometric radiographs can be utilized to generate quantitative and categorical phenotypes via cephalometric approaches (Moreno Uribe et al. 2014) or landmark-based shape methods such as geometric morphometrics (GM). GM approaches provide greater resolution in detecting shape variation of complex structures than do cephalometric methods (Zelditch et al. 2004).

The purpose of this study was to evaluate genetic associations between craniofacial candidate genes and skeletal variation in patients with malocclusion.

Materials and Methods

Sample

The study protocol was reviewed and approved by the University of Iowa Institutional Review Board and conforms to STROBE guidelines. All participants signed consent forms. The sample consisted of 269 self-reported Caucasian and untreated individuals aged 15 to 68 y (mean, 30.7) with malocclusion who were seeking orthodontic treatment at the University of Iowa and private practices in eastern Iowa. Exclusion criteria included individuals with 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, history of facial trauma or facial surgery, missing or impacted teeth other than third

molars, and radiographs with poor quality or missing landmarks. The total sample was classified into skeletal class I ($n = 53$; without maxillomandibular skeletal discrepancies), II ($n = 128$), and III ($n = 88$) malocclusions according to criteria in Figure 1.

Phenotyping

Digital, analog, and cone beam computed tomography (CBCT)-derived pretreatment lateral cephalometric radiographs were utilized for phenotyping. Analog films were scanned in a flat-bed scanner with a 100-mm ruler added for scaling. In analog films, centroid sizes were calculated as the square root of the sum of the squared distances of each landmark to the center of the object (i.e., centroid) and were adjusted for 12% (multiplied by 0.8929) and 13% (multiplied by 0.8850) magnifications according to cephalostat specifications to match digital- and CBCT-derived films, which were not corrected for magnification (Cohen 2005). Images were digitized with 14 skeletal landmarks (Appendix Table 1 and Fig. 1) by 3 raters utilizing Dolphin Imaging software, version 11.5.04.35. Reliability assessment of landmark location was described previously (Moreno Uribe et al. 2014). Briefly, 15 randomly chosen lateral cephs were traced twice by each rater at least 2 wk apart. Intraclass correlation methods (Shrout and Fleiss 1979) were used to test inter- and intraexaminer agreement. Calibration rounds continued until an intraclass correlation >0.85 was obtained for all landmarks.

The x and y coordinates were exported from Dolphin for GM analyses in the MorphoJ software package (Klingenberg 2011). GM procedures included a Generalized Procrustes superimposition step for rotation, scaling, and translation of the data to remove any variation not due to shape. Subsequently, Procrustes residuals were submitted to a principal component

(PC) analysis to identify the most important aspects of skeletal variation in the data. In addition, type of skeletal class was evaluated as a categorical phenotype to assess the risk of developing skeletal malocclusion class II or class III compared to class I.

Genotyping

Genomic DNA was extracted from saliva samples collected using Oragene kits (DNA Genotek, Ontario, CA, USA).

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 and/or biological functions in the craniofacial complex, 3) known role in etiology of craniofacial conditions with phenotypic spectra that include skeletal malocclusion, and 4) previous GWAS indicating association with craniofacial variation (details in Appendix Table 2).

For single-nucleotide polymorphism (SNP) selection, haplotype blocks (Gabriel et al. 2002) were reconstructed for all genes/loci, including 10-kb regions flanking intended intervals via Haploview, using genotypic data from a Caucasian population (HapMap CEU; HapMap release 27 based on February 2009 assembly, phase 2 and 3 versions). A total of 216 tagging SNPs across 71 candidate genes and loci were selected. Of these, 24 SNPs were genotyped using 5- μ L reaction volumes and TaqMan chemistry (Applied Biosystems, Foster City, CA, USA) and detected on a 7900HT Sequence Detection Instrument. A 96.96 Dynamic Array was used to generate genotypes for the 192 remaining SNPs using competitive allele-specific PCR KASPar chemistry (KBioscience Ltd., Hoddesdon, UK) on a Fluidigm (Fluidigm Corp., South San Francisco, CA, USA) nanofluidic platform (Wang et al. 2009). For genotype calling, default settings of the Fluidigm SNP genotyping software, version 4.1.2, were utilized, 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 individuals who failed on ≥ 10 SNPs ($\sim 5\%$ failure rate), and removing SNPs with $>5\%$ genotyping failure rate or Hardy-Weinberg disequilibrium test at $P < 10^{-4}$. All retained SNPs had a minimum minor allele frequency $>6\%$. A total of 198 of 216 SNPs remained for analyses (Appendix Table 3).

Genotype-phenotype Associations

PCs explaining $>5\%$ of the facial skeletal variation and centroid size were selected for genotype-phenotype correlation analyses. SNPs were coded as 0, 1, or 2 according to the number of minor allele copies. Multivariate linear regressions adjusting for age, sex, and ceph source (analog film, digital, and CBCT) were performed using Stata (StataCorp 2011,

Figure 2. The scree plot illustrates the amount of variance explained by each principal component (PC). The results of the PC analysis showed that 4 PCs accounted for 69% of the total variation in the data.

College Station, TX, USA) to test for association between each SNP (1 at a time) and the selected PCs and centroid size. The formal threshold for statistical significance after Bonferroni correction for multiple testing was $P < 0.00025$ ($0.05/198$ SNPs). Moreover, the type of skeletal malocclusion (i.e., skeletal class) was modeled using multinomial logistic regression to evaluate the association between SNPs and risk of skeletal malocclusion (class II or III) relative to class I as the reference category adjusting for the same covariates described above.

Results

Phenotypic Variability

PC analysis resulted in 4 PCs (PC1 to PC4), each explaining $>5\%$ of the total shape variation and together explaining about 69% of the variation (Fig. 2). PC1 explained 32.7% of the variation and depicted vertical discrepancies ranging from skeletal open bites for individuals with very negative PC scores to skeletal deep bites for individuals with high positive PC scores. PC2 explained 21.7% of the variation and captured horizontal maxillamandibular discrepancies, ranging from extremely convex profiles and class II skeletal malocclusions in individuals with more negative PC scores to extreme concave profiles and class III skeletal malocclusions in individuals with high positive PC scores (Fig. 3A). PC3 explained 8.2% of the variation and captured shapes ranging from a large ramus height, a small mandibular body, and a flat anterior cranial base orientation for individuals with more negative PC scores to a small ramus height, increased mandibular body, and steep anterior cranial base orientation for those with high positive PC scores. Finally, PC4 explained 6.6% of the variation ranging from posterior condylar inclination and reduced chin anteroposterior projection for individuals with more negative PC scores to anterior condylar inclination and increased chin projection for individuals with more positive PC scores (Fig. 3B).

Genotype-phenotype Associations

Associations significant at $P < 0.05$ for the 4 PCs are displayed in Figure 3; full results are shown in Figure 4. Complete results for all phenotypic variables including centroid size (i.e., proxy for facial size) are shown in Appendix Tables 4 and 5.

Figure 3. Phenotype-genotype correlations significant at $P < 0.05$ with (A) PC1 and PC2 and (B) PC3 and PC4 along with the skeletal phenotypic variation displayed by individuals with most negative ($-\beta$) or positive ($+\beta$) PC scores. Light blue represents the average configuration, whereas dark blue represents the deformation of interest. PC, principal component; SNP, single-nucleotide polymorphism. This figure is available in color online at <http://jdr.sagepub.com>.

The strongest associations for PC1 were with rs3780138 near the *PAX5* gene ($P = 0.01$), rs7438559 within the 4p16.1 linkage interval ($P = 0.015$), and rs1576593 in the *ABCA4-ARHGAP29* locus ($P = 0.019$). PC2 was associated with rs4287555 upstream of *SNAI3* ($P = 0.00026$) and rs11066446

upstream of *MYO1H* ($P = 0.006$). PC3 was associated with *TWIST1* rs2189000 ($P = 0.000076$) and rs985246 ($P = 0.005$). PC4 was associated with rs766325 near *PAX7* ($P = 0.007$). Finally, centroid size was associated with rs3742794 near *LTBP2* ($P = 0.0008$) and rs1233560 in *SHH* ($P = 0.004$).

Figure 4. Display of all association results obtained for the 4 selected principal components and respective P values ($\log_{10}$ transformed). The red line runs through the value that corresponds to $P = 0.05$. This figure is available in color online at <http://jdr.sagepub.com>.

After Bonferroni correction, only *TWIST1* rs2189000 associated with PC3 ($P = 0.000076$) remained significant at $P = 0.00025$, while *SNAI3* rs4287555 associated with PC2 ($P = 0.00026$) was highly suggestive. As indicated by the negative β coefficient (Fig. 3A), the minor allele of rs4287555 upstream of *SNAI3* was associated with a severe class II phenotype and accentuated convex profile. The minor allele of *TWIST1* rs2189000 was associated with a shorter ramus, a larger body length, and a steep anterior cranial base orientation. To further support our association results for *SNAI3* rs4287555 and *TWIST1* rs2189000, canonical variate analyses were performed to test for overall shape differences based on these SNPs. Significance was tested using the intergroup Procrustes distances and 10,000 permutations. We found significant differences ($P < 0.05$) in average shape by SNP genotype (detailed results available upon request). These results corroborate that genetic variants in both *TWIST1* and *SNAI3* are associated with overall craniofacial variation.

A few interesting associations (at $P < 0.05$) were observed between the SNPs and type of skeletal malocclusion from the multinomial logit regression modeling risk for class II or III skeletal malocclusion compared to skeletal class I, but none were significant after Bonferroni correction (Appendix Table 5). The minor allele of rs11200014 in *FGFR2* was associated with an increased risk of class II versus I (odds ratio [OR] = 2.1, $P = 0.004$). In contrast, the minor allele of rs2070699 in

EDN1 was associated with a reduced risk of class II versus I (OR = 0.5, $P = 0.007$). Each minor allele of rs2249492 in *COL1A1* was associated with an increased risk of class III versus I (OR = 2.1, $P = 0.008$), while the minor allele of rs1248046 in *TBX5* was associated with reduced risk (OR = 0.5, $P = 0.014$). Interestingly, *FGFR2* SNPs rs2162540 and rs11200014 were associated with increased risk for classes II and III skeletal malocclusion compared to class I.

Discussion

Our study evaluated the associations of facial skeletal variation and the type of skeletal malocclusion with 71 craniofacial candidate genes/loci in patients with malocclusion. Two genes, *SNAI3* and *TWIST1*, were particularly suggestive as relevant for craniofacial variation after Bonferroni correction for multiple testing. While statistically insignificant after Bonferroni correction, SNPs within *FGFR2*, *EDN1*, *TBX5*, and *COL1A1* showed suggestive associations with type of skeletal malocclusion.

The findings suggest that the evaluated genes and loci are more strongly tied to horizontal shape variation captured by PC2 and both vertical and horizontal shape variation captured by PC3 instead of mandibular rotation and condylar angulation or chin projection captured by PC1 and PC4. These results are consistent with previous findings of traditional cephalometric studies suggesting that different craniofacial features may be

Figure 5. Genotype-phenotype associations that remained suggestive and significant at $P = 0.00025$: *SNAI3* with PC2 ($P = 0.00026$) and *TWIST1* with PC3 ($P = 0.000076$). Also, mouse models and craniofacial expression of *Snai3* and *Twist1* genes. PC, principal component.

influenced by different genetic mechanisms (Vanco et al. 1995; Manfredi et al. 1997). It is possible that phenotypes represented in PC1 and PC4 are more complex in their genetic etiology than those captured in PC2 and PC3 and are associated with a larger number of genetic variants, each with a small effect. If so, GWAS using large samples may be a more effective approach to identify loci for such phenotypes, since there is little evidence that they are associated with the evaluated candidate genes and loci.

The main findings indicate that *SNAI3* is associated with craniofacial variation ranging from severely concave to convex profiles, while *TWIST1* is related to variation from short to long mandibular bodies. *SNAI3* is a member of the SNAIL family of zinc-finger transcription factors, which are important in epithelial to mesenchymal transitions that contribute to the formation of the mesoderm and the neural crest (Nieto 2002). *Snai3* is expressed in the facial prominences that give rise to upper and lower jaws (EMAGE <http://www.emouseatlas.org/emage/home.php>; Fig. 5). Interestingly, *Snai3*-null mice do not show significant anomalies, suggesting that Snail genes complement each other in their biological functions (Pioli et al. 2013). *Snai2* and *Snai3* double-knockout mice present anomalies beyond the *Snai2*^{-/-} null, including eye deformities and stunted growth (Pioli et al. 2013; Fig. 5). Neural crest-specific deletion of *Snai1* on a *Snai2*^{-/-} background leads to multiple craniofacial defects, including mandibular deficiency similar to Pierre Robin sequence (Murray et al. 2007) indicating that SNAIL genes may modulate jaw growth (Murray et al. 2007). Thus, future studies of this gene family and its role in malocclusion are warranted.

TWIST1 encodes for a basic helix-loop-helix transcription factor that plays an important role in activating downstream mesodermal genes during early development in *Drosophila* (Leptin 1991). Mutations and deletions in human *TWIST1* are found in patients with Saethre-Chotzen syndrome (Johnson et al. 1998), a condition associated with a broad spectrum of craniofacial anomalies, including craniosynostosis, maxillary hypoplasia, narrow palates, facial asymmetry with a deviated nasal septum, and cleft palate (Jabs 2004). *Twist1*^{+/-} mice exhibit craniosynostosis primarily affecting the coronal sutures (Carver et al. 2002). Given that maxillary hypoplasia is a common finding in patients with craniosynostosis, one could speculate that genetic variation in *TWIST1* may also result in premature ossification of the maxillary sutures leading to class III malocclusion due to maxillary hypoplasia. *Twist1* inactivation in mandibular arch neural crest cells results in mandibular shortening and abnormal ramus formation with missing or malformed condylar and coronoid processes (Fig. 5). In addition, *Twist1* is required for molar development, cusp formation, and mandibular ossification (Zhang et al. 2012).

The phenotypic associations that we observe with both *SNAI3* and *TWIST1* are especially interesting in light of research findings supporting genetic interactions between *Twist* and members of the *Snail* family during formation of the mesoderm (Leptin 1991) and in cranial suture ossification. The craniosynostosis phenotype of the double heterozygous mice for *Twist1*^{+/-} with *Snail1*^{+/-} or *Snai2*^{+/-} showed increased premature fusion and complete penetrance compared to *Twist1*^{+/-} heterozygous (Oram and Gridley 2005). Whether *TWIST* and *SNAIL* act independently or interactively to confer risk for

abnormal maxillomandibular relations in malocclusion warrants future research using a larger sample.

Other suggestive results worth discussing include the association of the *ABCA4-ARHGAP29* locus with *PC1* representing facial variation ranging from skeletal open bites to skeletal deep bites (Appendix Fig. 2). The *ARHGAP29* gene is associated with orofacial clefting in humans (Leslie et al. 2012; Fontoura et al. 2013) and with facial features that are part of the human cleft phenotypic spectrum (Miller et al. 2014). In addition, a craniofacial enhancer region (mm435) that presumably regulates the expression of *ARHGAP29* in the facial prominences was recently located within an intron of *ABCA4* (hg19:94580867-9458352; Attanasio et al. 2013). The SNP analyzed in the current study (rs1576593 hg19: 94631751 bp) is upstream of *ABCA4* and 50 kb from this enhancer region. Future work is needed to understand whether this locus has a regulatory role in human craniofacial variation and malocclusion. The association of *PC2* with *MYOHI* is consistent with previous results for class III malocclusion (Frazier-Bowers et al. 2009; Tassopoulou-Fishell et al. 2012), providing further support for a potential role of *MYOHI* in regulating maxillo-mandibular growth.

The multinomial logistic regression for type of skeletal malocclusion showed that SNPs within *FGFR2*, *EDN1*, *TBX5*, and *COL1A1* were associated with developing class II or III of skeletal malocclusion relative to class I, with the *FGFR2* SNPs conferring risk for classes II and III. *Fgfrs* belong to the *Fgf* signaling pathway, which is essential in craniofacial morphogenesis, particularly for outgrowth and shape of maxillary prominences, cranial suture function, as well as endochondral and intramembranous bone development (Jin et al. 2012). *FGFR2* mutations are found in patients with Apert (OMIM 101200) and Crouzon syndrome (OMIM 123500). In both conditions, maxillary hypoplasia and relative mandibular prognathism (pseudoprognathism) are observed. The associations that we observe between the *FGFR2* SNPs and increased risk of classes II and III of skeletal malocclusion could indicate that these SNPs modulate risk for abnormal maxillomandibular discrepancies in general. Alternatively, since maxillary hypoplasia can be seen in patients with class II or III malocclusions, it is possible that the observed associations are driven by patients who have maxillary hypoplasia within both skeletal malocclusion groups. In any case, findings for *FGFR2* deserve future follow-up.

Edn1 is important in dorsoventral patterning of the first branchial arch (Clouthier et al. 2010). Loss or inhibition of *Edn1* in multiple animal models results in the loss or partial transformation of the lower jaw. In contrast, *Edn1* misexpression alters upper jaw development (Medeiros and Crump 2012). Mutations in *EDN1* cause auriculocondylar syndrome (OMIM 615706), which presents with severe retrognathia.

Mutations in *TBX5* have been reported in >50% of patients presenting with Holt-Oram syndrome (OMIM 142900), which primarily affects the heart and limbs. However, specific facial features have been identified in patients with this condition, including squared faces with a broad lower jaw, parietal bossing, and micrognathia. Last, *COL1A1* encodes the proalpha

chain of type I collagen. In human midterm fetuses, *COL1A1* is found in the perichondrium of Meckel's cartilage; the periosteum of the mandibular bone; the mandibular condylar cartilage; and the perichondrium, periosteum, and bone collar of the cranial base cartilage (Shibata et al. 2013). Mutations in *COL1A1* have been found in patients with osteogenesis imperfecta (OMIM 166200) and Ehlers-Danlos syndrome (OMIM 130000), both of which commonly involve facial anomalies, including micrognathia, frontal bossing, and midface hypoplasia. Mice deficient for *Mmp2* and simultaneously carrying the mutant *Colla1r* gene show multiple craniofacial anomalies beyond the *Mmp2* nulls, including shorter snouts and bulging skulls (Egeblad et al. 2007).

Conclusion

This study characterized craniofacial skeletal phenotypes among patients with malocclusion and generated genetic data on craniofacial genes/loci to identify phenotype-genotype associations of clinical relevance. Results suggest genetic pathways that play a role in anteroposterior and vertical skeletal variation observed in patients with malocclusion and in type of skeletal malocclusion. The study supports several previously reported linkage and association findings for malocclusion phenotypes. The observed associations are encouraging and underscore the importance of pursuing this research in more powerful data sets to confirm these findings and identify functional variants. Also future studies should consider examining soft tissue differences to uncover the genetic etiology of skeletal and soft tissue variation in patients with malocclusion. The identification of the genetic influence on these phenotypes will enhance our understanding of the molecular regulation of postnatal facial growth and can inform clinical practices to improve treatment effectiveness for patients with malocclusion.

Author Contributions

C.S.G. da Fontoura, contributed to conception, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; S.F. Miller, contributed to design and data analysis, drafted and critically revised the manuscript; G.L. Wehby, contributed to conception, design, and data analysis, drafted and critically revised the manuscript; B.A. Amendt, contributed to conception, data acquisition, and interpretation, critically revised the manuscript; N. Holton, contributed to conception and data interpretation, critically revised the manuscript; T.E. Southard, V. Allareddy, contributed to conception and data acquisition, critically revised the manuscript; L.M. Moreno Uribe, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agree to be accountable for all aspects of the work.

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References

- Attanasio C, Nord AS, Zhu Y, Blow MJ, Li Z, Liberton DK, Morrison H, Plajzer-Frick I, Holt A, Hosseini R, et al. 2013. Fine tuning of craniofacial morphology by distant-acting enhancers. *Science*. 342(6157):1241006.
- Carver EA, Oram KF, Gridley T. 2002. Craniosynostosis in Twist heterozygous mice: a model for Saethre-Chotzen syndrome. *Anat Rec*. 268(2):90–92.
- Claudino D, Traebert J. 2013. Malocclusion, dental aesthetic self-perception and quality of life in a 18 to 21 year-old population: a cross section study. *BMC Oral Health*. 13:3.
- Clouthier DE, Garcia E, Schilling TF. 2010. Regulation of facial morphogenesis by endothelin signaling: insights from mice and fish. *Am J Med Genet A*. 152(12):2962–2973.
- Cohen JM. 2005. Comparing digital and conventional cephalometric radiographs. *Am J Orthod Dentofacial Orthop*. 128(2):157–160.
- Egeblad M, Shen HC, Behonick DJ, Wilmes L, Eichten A, Korets LV, Kheradmand F, Werb Z, Coussens LM. 2007. Type I collagen is a genetic modifier of matrix metalloproteinase 2 in murine skeletal development. *Dev Dyn*. 236(6):1683–1693.
- Fontoura C, Silva RM, Granjeiro JM, Letra A. 2013. Further evidence of association of the ABCA4 gene with cleft lip/palate. *Eur J Oral Sci*. 120(6):553–557.
- Frazier-Bowers S, Rincon-Rodriguez R, Zhou J, Alexander K, Lange E. 2009. Evidence of linkage in a Hispanic cohort with a class III dentofacial phenotype. *J Dent Res*. 88(1):56–60.
- Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, et al. 2002. The structure of haplotype blocks in the human genome. *Science*. 296(5576):2225–2229.
- Gutiérrez SJ, Gómez M, Rey JA, Ochoa M, Gutiérrez SM, Prieto JC. 2010. Polymorphisms of the noggin gene and mandibular micrognathia: a first approximation. *Acta Odontol Latinoam*. 23(1):13–19.
- Ikuno K, Kajii TS, Oka A, Inoko H, Ishikawa H, Iida J. 2014. Microsatellite genome-wide association study for mandibular prognathism. *Am J Orthod Dentofacial Orthop*. 145(6):757–762.
- Jabs EW. 2004. TWIST and the Saethre-Chotzen syndrome. In: Epstein CJ, Erickson RP, Wynshaw-Boris A, editors. *Molecular basis of inborn errors of development*. New York (NY): Oxford University Press. p. 401–409.
- Jin M, Du X, Chen L. 2012. Cross-talk between FGF and other cytokine signalling pathways during endochondral bone development. *Cell Biol Int*. 36(8):691–696.
- Johnson D, Horsley SW, Moloney DM, Oldridge M, Twigg SR, Walsh S, Barrow M, Njolstad PR, Kunz J, Ashworth GJ, et al. 1998. A comprehensive screen for TWIST mutations in patients with craniosynostosis identifies a new microdeletion syndrome of chromosome band 7p21.1. *Am J Hum Genet*. 63(5):1282–1293.
- Klingenberg CP. 2011. MorphoJ: an integrated software package for geometric morphometrics. *Molecular ecology resources*. 11(2):353–357.
- Leptin M. 1991. Twist and snail as positive and negative regulators during *Drosophila* mesoderm development. *Genes Dev*. 5(9):1568–1576.
- Leslie EJ, Mansilla MA, Biggs LC, Schuette K, Bullard S, Cooper M, Dunnwald M, Lidral AC, Marazita ML, Beaty TH, et al. 2012. Expression and mutation analyses implicate ARHGAP29 as the etiologic gene for the cleft lip with or without cleft palate locus identified by genome-wide association on chromosome 1p22. *Birth Defects Res A Clin Mol Teratol*. 94(11):934–942.
- Li Q, Li X, Zhang F, Chen F. 2011. The identification of a novel locus for mandibular prognathism in the Han Chinese population. *J Dent Res*. 90(1):53–57.
- Li Q, Zhang F, Li X, Chen F. 2010. Genome scan for locus involved in mandibular prognathism in pedigrees from China. *PLoS One*. 5(9):e12678.
- Manfredi C, Martina R, Grossi GB, Giuliani M. 1997. Heritability of 39 orthodontic cephalometric parameters on MZ, DZ twins and MN-paired singletons. *Am J Orthod Dentofacial Orthop*. 111(1):44–51.
- Medeiros DM, Crump JG. 2012. New perspectives on pharyngeal dorsoventral patterning in development and evolution of the vertebrate jaw. *Dev Biol*. 371(2):121–135.
- Miller SF, Weinberg SM, Nidey NL, Defay DK, Marazita ML, Wehby GL, Moreno Uribe LM. 2014. Exploratory genotype-phenotype correlations of facial form and asymmetry in unaffected relatives of children with non-syndromic cleft lip and/or palate. *J Anat*. 224(6):688–709.
- Moreno Uribe LM, Vela KC, Kummet C, Dawson DV, Southard TE. 2014. Phenotypic diversity in white adults with moderate to severe class III malocclusion. *Am J Orthod Dentofacial Orthop*. 144(1):32–42.
- Murray SA, Oram KF, Gridley T. 2007. Multiple functions of Snail family genes during palate development in mice. *Development*. 134(9):1789–1797.
- Nieto MA. 2002. The snail superfamily of zinc-finger transcription factors. *Nat Rev Mol Cell Biol*. 3(3):155–166.
- Nikopensius T, Saag M, Jagomagi T, Annilo T, Kals M, Kivistik PA, Milani L, Metspalu A. 2013. A missense mutation in DUSP6 is associated with class III malocclusion. *J Dent Res*. 92(10):893–898.
- Oram KF, Gridley T. 2005. Mutations in snail family genes enhance craniosynostosis of Twist1 haplo-insufficient mice: implications for Saethre-Chotzen syndrome. *Genetics*. 170(2):971–974.
- Pillas D, Hoggart CJ, Evans DM, O'Reilly PF, Sipila K, Lahdesmaki R, Millwood IY, Kaakinen M, Netuveli G, Blane D, et al. 2010. Genome-wide association study reveals multiple loci associated with primary tooth development during infancy. *PLoS Genet*. 6(2):e1000856.
- Pioli PD, Dahlem TJ, Weis JJ, Weis JH. 2013. Deletion of Snai2 and Snai3 results in impaired physical development compounded by lymphocyte deficiency. *PLoS One*. 8(7):e69216.
- Robinson PN. 2012. Deep phenotyping for precision medicine. *Hum Mutat*. 33(5):777–780.
- Shibata S, Sakamoto Y, Baba O, Qin C, Murakami G, Cho BH. 2013. An immunohistochemical study of matrix proteins in the craniofacial cartilage in midterm human fetuses. *Eur J Histochem*. 57(4):e39.
- Shrout PE, Fleiss JL. 1979. Intraclass correlations: uses in assessing rater reliability. *Psychol Bull*. 86(2):420–428.
- StataCorp. 2011. Stata statistical software: release 12. College Station (TX): StataCorp LP.
- Tassopoulou-Fishell M, Deeley K, Harvey EM, Sciote J, Vieira AR. 2012. Genetic variation in myosin 1H contributes to mandibular prognathism. *Am J Orthod Dentofacial Orthop*. 141(1):51–59.
- Ting TY, Wong RW, Rabie AB. Analysis of genetic polymorphisms in skeletal class I crowding. *Am J Orthod Dentofacial Orthop*. 140(1):e9–15.
- Vanco C, Kasai K, Sergi R, Richards LC, Townsend GC. 1995. Genetic and environmental influences on facial profile. *Aust Dent J*. 40(2):104–109.
- Wang J, Lin M, Crenshaw A, Hutchinson A, Hicks B, Yeager M, Berndt S, Huang WY, Hayes RB, Chanock SJ, et al. 2009. High-throughput single nucleotide polymorphism genotyping using nanofluidic Dynamic Arrays. *BMC Genomics*. 10:561.
- Xue F, Wong R, Rabie AB. 2010. Identification of SNP markers on 1p36 and association analysis of EPB41 with mandibular prognathism in a Chinese population. *Arch Oral Biol*. 55(11):867–872.
- Yamaguchi T, Park SB, Narita A, Maki K, Inoue I. 2005. Genome-wide linkage analysis of mandibular prognathism in Korean and Japanese patients. *J Dent Res*. 84(3):255–259.
- Zelditch M, Swiderski D, Sheets DH. 2004. *Geometric morphometrics for biologists*. Burlington (MA): Academic Press.
- Zhang Y, Blackwell EL, McKnight MT, Knutsen GR, Vu WT, Ruest LB. 2012. Specific inactivation of Twist1 in the mandibular arch neural crest cells affects the development of the ramus and reveals interactions with hand2. *Dev Dyn*. 241(5):924–940.