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Malocclusion May Be Attributed to Variation among 10 Genes

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Abstract

Introduction: The goal of this study was to utilize physical characteristics instead of placing subjects in arbitrary diagnostic categories to test for associations with genetic variants. **Methods:** Forty-four single nucleotide polymorphisms were tested for association with specific cephalometric measurements in thirty-nine University of Pittsburgh Dental Registry and DNA Repository orthodontic subjects. Cephalometric measurements included an evaluation of FMA, a Wits appraisal, and a Steiner's ANB analysis. Genetic markers were genotyped using polymerase chain reaction and Taqman chemistry. Chi-square and Fischer's exact tests (α = 0.05) were used in investigation of overrepresentation of marker alleles. Samples were divided into groups based upon having an FMA, Wits, or ANB measurement above or below the mean of the cohort studied. Secondary analysis was done for sex and ethnicity to determine their effect on FMA, Wits, or ANB. **Results:** An association between FMA measurements was discovered in the following genes: *ACTN3*, *CASP4*, *ESR1*, *FGF13*, *KRT7*, and *PITX2*. An association between Wits measurements was discovered in the following genes: *ACTN2*, *BTBD11*, *CASP4*, *FGF3*, and *FGF10*. No associations were found with ANB. **Conclusions:** Genetic markers in several genes at different loci may contribute to craniofacial deformities in humans. This approach of using physical measurements may be an advantage to placing patients in arbitrary diagnostic categories.

Keywords

Malocclusion, Multifactorial Inheritance, Genetic Association Studies, Linkage Disequilibrium, Genes

1. Introduction

Craniofacial abnormalities erupting during postnatal growth and development

are often a result of both genetic and environmental influences [1]. Some of the most common types include cleft lip and/or cleft palate, craniosynostosis, and hemifacial microsomia. It has been documented that there is a link between certain craniofacial abnormalities and genetics [2] [3].

Cephalometric analyses can be used to measure vertical and horizontal relationships of craniofacial development, with the degree of departure from normal corresponding to the severity of the abnormality [4]. Three common cephalometric analyses include an evaluation of FMA (Frankfort mandibular angle), a Wits appraisal, and a Steiner's ANB analysis.

An FMA evaluation determines the skeletal vertical pattern of the patient, and is measured at the intersection of the Frankfort horizontal and mandibular plane [5]. FMA profiles include a normal vertical relationship at an angle of 25˚ ± 5˚, an open vertical pattern at an angle greater than 30˚, and a deep vertical relationship at an angle less than 20˚ [6].

A Wits appraisal measures the linear maxillary and mandibular disharmony in the anteroposterior direction along the functional occlusal plane (FOP) [7]. A perpendicular line is dropped from the most anterior point on the maxilla (PtA) and the most anterior point on the mandible (PtB) to the (FOP) and the difference is measured. Class I Wits values range from −1 ± 2 for males and 0 ± 2 for females. A Wits value less than −3 for males (−2 for a females) would indicate Class III, whereas a Wits value greater than +1 for males (+2 for females) would indicate Class II. Wits values differ depending on the sex of the patient. A limitation of this appraisal is that this measurement of jaw disharmony has no reference to external cranial morphology and is affected by the cant of the occlusal plane [7] [8].

In contrast to the linear measurement of Wits, the Steiner's ANB analysis measures the angular discrepancy between the maxilla and the mandible [9]. This analysis measures the angle (ANB) between PtA and PtB registered at Nasion. Steiner's ANB profiles include an ideal of 2˚ ± 2˚ angle indicating a class I skeletal relationship, angles greater than 4˚ indicating a class II skeletal relationship, and angles less than 0˚ indicating a class III skeletal relationship. Limitations of this analysis include the position of the nasion, jaw rotations, and facial height [10].

The goal of this study was to use cephalometric measurements to identify genetic variants associated with craniofacial deformities among orthodontic patients. Preliminary work has shown associations when combining three cephalometric analyses to define the phenotype [11]. We aim to find genetic associations with these assessments analyzed individually.

2. Materials and Methods

The subjects in this study were patients treated in the Department of Orthodontics at the University of Pittsburgh School of Dental Medicine who had also volunteered to participate in the Dental Registry and DNA Repository project [12].

This project is approved by the University of Pittsburgh Institutional Review Board. All subjects provided written informed consent. Of the 95 patients that had been chosen for a previous study [11], 39 of those subjects were selected for this study. These subjects were chosen based upon their clinical and radiographic records and were included for having good quality images and measurements done and entered in the database. No individuals were excluded. The average age of participants was 17.8 years; 28 were female and 11 male; 20 were White, 13 were African American, two were Hispanic, and four were Asian. Table 1 displays all information used in this study. We followed the STROBE guidelines.

The mean of the cohort studied was calculated for each of the three measurements (FMA, Wits, and Steiner's), and subjects were placed into categories either above or below the mean for FMA, Wits, and Steiner's measurements. Significance from the mean was an arbitrary guide in comparing the difference in distribution of genotypes. Those found to be significant were further analyzed based upon sex and ethnic background, which were independently conducted and used to determine if the true significance was driven by either of these variables.

Forty-four single nucleotide polymorphisms were chosen for each subject based upon genes of interest previously found to be linked to prognathism [11]. These genetic markers were tested using end-point analysis of polymerase chain reactions using Taqman chemistry [13]. Chi-square and Fischer's exact tests (α = 0.05) were used in investigation of overrepresentation of marker alleles.

3. Results

An association between FMA measurements and markers in the genes of *ACTN3*, *CASP4*, *ESR1*, *FGF13*, *KRT7*, and *PITX2* was discovered (Table 2). Ethnicity was found to influence the associations with *ESR1* and *KRT7*. Sex was not found to influence the association in any of the above genes.

An association between Wits measurements and markers in the genes of *ACTN2*, *BTBD11*, *CASP4*, *FGF3*, and *FGF10* was discovered (Table 2). Ethnicity was found to influence the association with *ACTN2*. Sex was not found to influence the association in any of the above genes.

No associations with ANB measurements were detected.

4. Discussion

In this study, 44 single nucleotide polymorphisms were selected to uncover a common genetic variation in horizontal and vertical malocclusion. Our study provides evidence that a number of genes were associated with hyperdivergent facial patterns, or contribute to sagittal maxillomandibular discrepancies.

Estrogen (ESR1) is a key hormone for maintaining bone mass, maturation and skeletal growth. One function of the increase in serum estrogen during puberty would be to decrease the activity of osteoclasts, while increasing osteoblastic activity, which supports the importance of estrogen for jaw mass and growth [14].

Table 1. Summary of the subject's measurements (n = 39) used in this study.

	Sex	Age (Years)	Ethnicity	FMA (˚)	Wits	ANB (˚)
1	M	22	Asian	31.2	−6.6	0.3
2	M	17	White	22.2	−12	−8.1
3	F	23	White	24	−17.5	−10.1
4	F	19	African American	39.8	−6.4	3.4
5	F	21	African American	26.8	−4.5	−1.9
6	F	20	White	23.3	−6.8	−4.9
7	F	18	White	28.8	−6.5	−1.9
8	M	19	White	31.1	−7.3	−1.1
9	F	15	African American	19.3	−3.4	2.5
10	F	14	Hispanic	31.6	−6.2	−2.7
11	F	22	White	22.6	−15.7	−6.6
12	F	12	Asian	28.8	−5	−2.3
13	F	20	White	26.9	−7.6	0.7
14	M	15	White	18.3	−1.3	0.3
15	F	12	Hispanic	28.2	−8.1	0.6
16	F	15	White	23.1	−13.3	−7.5
17	F	18	White	33	−10.6	−4.7
18	M	18	White	23.4	−7	−1.9
19	F	11	African American	34.6	−8.8	2.3
20	M	11	White	19.3	−7.8	−4.4
21	F	16	African American	25.3	−5	−0.9
22	F	15	White	20.6	−4.6	−0.7
23	F	18	White	22.5	−4.4	−2.9
24	F	28	African American	20.8	−5.9	−1.0
25	F	13	White	20.9	−5.4	−0.9
26	F	23	African American	22.6	−8.4	−1.2
27	F	14	White	30.1	−4.7	−0.3
28	M	24	Asian	34.7	−4.1	−3.4
29	F	18	African American	23.7	−10	−6
30	M	19	White	33.2	−7.8	−1.4
31	F	15	African American	17.6	−6.4	0.2
32	M	12	African American	21.5	−8.2	−3.8
33	F	21	African American	23.3	−4.3	−4.8
34	F	15	White	32.7	−10.7	−2.7
35	M	28	White	22	−8.3	−1.9
36	F	13	African American	18.5	−12.8	−4.1
37	F	13	African American	25.3	−12.2	−5.3
38	F	24	White	22.3	−3.5	−1.4
39	M	24	Asian	14.9	−4.2	−3.6
Mean	-	17.82	-	25.35	−7.52	−2.41

Table 2. Summary of the association results.

Gene	Marker	Test	Associated Allele/Genotype	p-value	Trait
ACTN3	rs1671064	FMA	G vs. A	0.003	G associated with tendency to have a higher-angle FMA, A associated with tendency to have a lower-angle FMA
CASP4	rs571407	FMA	T vs. C	0.04	T associated with tendency to have a higher-angle FMA, C associated with tendency to have a lower-angle FMA
ESR1	rs2077647	FMA	CC vs. TT	0.03	CC associated with tendency to have a higher-angle FMA, TT associated with tendency to have a lower-angle FMA
FGF13	rs489895	FMA	CT vs. TT	0.03	Having at least one copy of C associated with tendency to have a higher-angle FMA, TT associated with tendency to have a lower-angle FMA
FGF13	rs489895	FMA	C vs. T	0.04	C associated with tendency to have a higher-angle FMA, T associated with tendency to have a lower-angle FMA
KRT7	rs7300317	FMA	A vs. G	0.05	A associated with tendency to have a higher-angle FMA, G associated with tendency to have a lower-angle FMA
PITX2	rs2595110	FMA	A vs. G	0.04	A associated with tendency to have a higher-angle FMA, G associated with tendency to have a lower-angle FMA
ACTN2	rs4659704	Wits	CC vs. AC	0.04	CC associated with tendency to have a less negative Wits value, having at least one copy of A associated with tendency to have a more negative Wits value
BTBD11	rs11113231	Wits	GG vs. AA	0.03	GG associated with tendency to have a less negative Wits value, AA associated with tendency to have a more negative Wits value
CASP4	rs571407	Wits	T vs. C	0.04	T associated with tendency to have a less negative Wits value, C associated with tendency to have a more negative Wits value
FGF3	rs1893047	Wits	AA vs. GG	0.04	AA associated with tendency to have a less negative Wits value, GG associated with tendency to have a more negative Wits value
FGF3	rs1893047	Wits	A vs. G	0.02	A associated with tendency to have a less negative Wits value, G associated with tendency to have a more negative Wits value
FGF10	rs1448037	Wits	T vs. C	0.004	T associated with tendency to have a less negative Wits value, C associated with tendency to have a more negative Wits value

Notes: Higher FMA angles (greater than 30°) correlate to open-bite skeletal patterns, while lower FMA angles (fewer than 20°) correlate to deep-bite skeletal patterns. Less negative Wits values correlate to mandibular retrognatism, while more negative Wits values correlate to mandibular prognatism. Larger ANB angles (greater than 2°) correlate to the mandible being located behind the maxilla, while smaller ANB angles (fewer than 2°) correlate to the mandible being located ahead of the maxilla.

This would be compatible with the functional matrix theory of Moss, where growth and the fundamental matrix are linked.

KRT7 encodes a protein which is a member of the keratin gene family. This protein is a type II cytokeratins consisting of basic or neutral proteins, arranged in pairs of heterotypic chains, coexpressed during differentiation of simple and stratified epithelial tissues. This type II cytokeratin is specifically expressed in the simple epithelia lining the cavities of the internal organs and in the gland ducts and blood vessels. *KRT7* is clustered in a region of chromosome 12q12-q13. Its role in vertical jaw discrepancy remains unclear [15]. Similarly, the role of *CASP4*, *FGF13*, *PITX2*, *BTBD11*, *FGF3*, and *FGF10* in post-natal craniofacial growth are not known.

ACTN2, located on the long arm of chromosome 1, is expressed in both slow type 1 and fast type 2 fibers. In skeletal muscle, alpha-Actinin-2 crosslinks actin filaments. *ACTN2* expression remains relatively constant irrespective of the changes in the *ACTN3* genotype. However, *ACTN3* R577X was associated with Class II and deepbite malocclusions [16]. As alpha-Actinin-2 and -3 are closely related isoforms, a compensatory effect may have occurred.

The small sample size of our study can be suggested as a limitation, however the exercise of characterizing the sample carefully aims to decrease heterogeneity and allow for more statistical power. Still, the associations reported here should be interpreted with caution and ideally independently replicated in other cohorts. Future studies will aim to predict postnatal craniofacial patterns and treatment outcomes from genomic profiles.

5. Conclusion

ACTN3, *CASP4*, *ESR1*, *FGF13*, *KRT7*, and *PITX2* were associated with hyperdivergent skeletal pattern. *ACTN2*, *BTBD11*, *CASP4*, *FGF3*, and *FGF10* were associated with sagittal maxillomandibular discrepancies. *FGF10* and *GHR* were associated with ANB.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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