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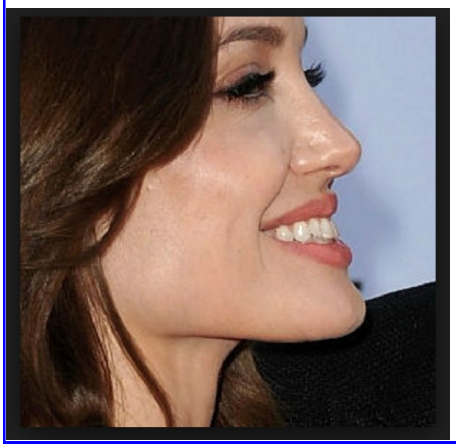
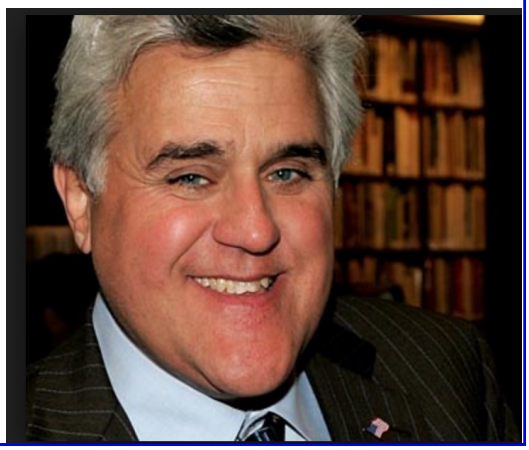
Vieira Lab Newsletter

Lab News

The original discovery that an unconventional myosin gene (myosin 1H) associated with mandibular prognathism *(Genetic variationin Myosin 1H contributes to mandibular prognathism)* has been independently confirmed in a cohort of orthodontic patients that were analyzed based on their specific maxillomandibular discrepancies *(Genetic polymorphisms underlying the skeletal Class III phenotype).* Results point to the possibility that genetic markers in myosin 1H could be used to identify cases that have class III malocclusion due to mandibular prognathism, in contrast to other cases that may be class III due to maxillomandibular discrepancies.





Upcoming Events

CODA Accreditation Site Visit
School of Dental Medicine May 9-11

17th Annual University of Pittsburgh SDM Research Symposium
Thursday, May 18
Alumni Hall, 7th floor

Data & Dine
Tuesday, May 16
O'Hara Student Center
5:00 p.m.

DRDR Update

Tables show running totals of patient recruitment

Recruitment Summary

Subjects Recruited	5962	
Subjects Declined	1209	
Compliance Rate	80%	
Gender	47% male	53% female

Subject Recruitment Location

Module 1	1526	**Emergency Care**	74	**UDHS**	8
Module 2	1815	**Oral Surgery**	32	**Orthodontics**	206
Module 3	702	**Pediatric Dentistry**	315	**Other**	140
Module 4	467	**Implant Center**	15	**AEGD**	26
Dental Hygiene	384	**Prosthodontics**	1112	**Perio**	69
Endo	71				

Oral Microbiome Study

Affected by Digestive Disease	57	**Not Affected by Digestive Disease**	105

For newsletter questions, comments or suggestions please e-mail Alexandre Vieira at arv11@pitt.edu

Genetic variation in *Myosin 1H* contributes to mandibular prognathism

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Introduction: Several candidate loci have been suggested as influencing mandibular prognathism (1p22.1, 1p22.2, 1p36, 3q26.2, 5p13-p12, 6q25, 11q22.2-q22.3, 12q23, 12q13.13, and 19p13.2). The goal of this study was to replicate these results in a well-characterized homogeneous sample set. **Methods:** Thirty-three single nucleotide polymorphisms spanning all candidate regions were studied in 44 prognathic and 35 Class I subjects from the University of Pittsburgh School of Dental Medicine Dental Registry and DNA Repository. The 44 subjects with mandibular prognathism had an average age of 18.4 years; 31 were female and 13 male; and 24 were white, 15 African American, 2 Hispanic, and 3 Asian. The 36 Class I subjects had an average age of 17.6 years; 27 were female and 9 male; and 27 were white, 6 African American, 1 Hispanic, and 2 Asian. Skeletal mandibular prognathism diagnosis included cephalometric values indicative of Class III such as an ANB smaller than 2°, a negative Wits appraisal, and a positive A-B plane. Additional mandibular prognathism criteria included negative overjet and visually prognathic (concave) profile as determined by the subject's clinical evaluation. Orthognathic subjects without jaw deformations were used as the comparison group. The mandibular prognathic and orthognathic subjects were matched by race, sex, and age. Genetic markers were tested by polymerase chain reaction with TaqMan chemistry. Chi-square and Fisher exact tests were used to determine overrepresentation of marker allele with an alpha of 0.05. **Results:** An association was unveiled between a marker in *MYO1H* (rs10850110) and the mandibular prognathism phenotype ($P = 0.03$). *MYO1H* is a Class I myosin that is in a different protein group than the myosin isoforms of muscle sarcomeres, which are the basis of skeletal muscle fiber typing. Class I myosins are necessary for cell motility, phagocytosis, and vesicle transport. **Conclusions:** More strict clinical definitions might increase homogeneity and aid the studies of genetic susceptibility to malocclusions. We provide evidence that *MYO1H* can contribute to mandibular prognathism. (Am J Orthod Dentofacial Orthop 2012;141:51-9)

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In orthodontics, one of the most challenging aspects in treating patients is predicting mandibular growth, especially in patients who show more pronounced characteristics of mandibular development. Through studies predominantly conducted with family members and twin siblings, it is well documented that there is a strong link between mandibular prognathism and genetics.[1,2] More specifically, there is evidence of autosomal-dominant inheritance, with incomplete penetrance associated with this phenotype.[1,3] The expression of the phenotype is a product of genetics and environmental factors.[4] The multifactorial nature of mandibular prognathism makes it difficult to study and understand.

Mandibular prognathism has a prevalence as low as 1% in white people but as high as 15% in Asian populations.[5,6]

Dohmoto et al[7] found that in mice the size of the mandible was controlled by genes located at chromosomes 10 and 11 that correspond to human chromosomal regions 12q21 and 2p13, respectively.

In humans, genome-wide linkage analysis provided evidence for linkage to mandibular prognathism at chromosomes 1p36, 6q25, and 19p13.2.[8] The presence of P56IT variant in the growth hormone receptor gene has been shown to affect mandibular morphology in Chinese and Japanese populations, especially in regard to mandibular height.[9,10] In a study with a Hispanic cohort, Class III (due primarily to maxillary deficiency) was confirmed to be inherited in an autosomal-dominant pattern and linked to 5 loci: 1p22.1, 3q26.2, 11q22, 12q13.13, and 12q23.[11] Li et al[12] detected a specific locus, 14q24.3-31.2, associated with the mandibular prognathism phenotype in a Han Chinese population. In addition to the strong role of heredity, there has been evidence suggesting the contribution of certain environmental factors to mandibular prognathism such as enlarged tonsils,[13] endocrine imbalances,[14] posture, trauma, and disease.[15]

It is well documented that the first studies of complex traits suggest a stronger genetic effect that was found by subsequent studies. Both bias and genuine population diversity might explain why early studies tended to overestimate the disease predisposition conferred by candidate gene polymorphisms.[16] If there is a true effect of any of the previously described loci in mandibular prognathism, the expectation is that those results can be replicated. Therefore, we typed markers in 8 loci and measured the associations between genetic variations and mandibular prognathism using a population from Pittsburgh, Pennsylvania.

MATERIAL AND METHODS

The subjects in this study were active orthodontic patients from the Department of Orthodontics of the School of Dental Medicine at the University of Pittsburgh. They were identified through the Dental Registry and DNA Repository project. In this project, since September 2006, people who seek treatment at the University of Pittsburgh School of Dental Medicine have been invited to be part of the registry. They provide written informed consent authorizing the extraction of information from their dental records. Also, they provide a saliva sample from which DNA can be extracted. Unstimulated saliva samples were obtained from all participants (they were asked to spit) and stored in Oragene DNA Self-Collection kits (DNA Genotek, Ottawa, Ontario, Canada) at room temperature until processing. No centrifugation was performed on the saliva samples. DNA was extracted according to the manufacturer's instructions. This project was approved by the University of Pittsburgh Institutional Review Board.

In September 2009, data from 1630 subjects were extracted from the registry for this pilot project. The 95 patients treated at the orthodontics department were considered eligible for this study. The assessment of the eligible subjects consisted of a careful review of each subject's clinical and radiographic records. Clinical records consisted of digital orthodontic models (orthoCAD, Cadent, Carlstadt, NJ), digital tracings of lateral cephalograms (Dolphin, Chatsworth, Calif), and digital photographs. The first steps in the assessment process were to obtain all subjects' soft-tissue profile photos (concave or straight profile) and certain cephalometric values to classify them as either orthognathic or prognathic. Specifically, we looked at Steiners' ANB, Wits appraisal. and Downs' A-B plane. As described in the Steiner analysis, ANB angles of less than 2° indicate that the mandible is located ahead of the maxilla. For this study, subjects with ANB values less than 2° were reviewed further to clarify whether the discrepancy was attributed to a smaller than average maxilla according to the SNA values. In that case, the subjects did not represent true prognathism but, rather, a regular-size mandible appearing to be protrusive because of a small maxilla. Therefore, they were excluded from the true prognathic group. The Wits appraisal was another measurement we reviewed because this value indicates the anteroposterior jaw relationship in the facial complex regardless of intracranial references. A negative Wits indicates a Class III skeletal relationship, and the more negative the value, the more severe the Class III. A Downs' A-B plane angle larger than −4.6° indicates a skeletal Class III diagnosis, although this measurement might appear more severe in subjects with a pronounced bony pogonion. Additional Class III criteria included dental classifications, such as Class III molar and canine relationships, and negative overject based on the digital models and clinical examinations. We excluded subjects with facial clefting, abnormal anterior cranial base growth defects such as or similar to achondrodysplasia, or midfacial growth deficiencies caused by other pathologies such as tumors, cysts, or trauma that might scar midfacial periosteal surfaces and limit normal growth potentials. After analyzing the clinical information, we selected 44 mandibular prognathism and 36 orthognathic subjects for this study. The 44 mandibular prognathism subjects had an average age of 18.4 years; 31 were female and 13 male; and 24 were white, 15 African American, 2 Hispanic, and 3 Asian. The 36 Class I subjects had an average age of 17.6 years; 27 were female and 9 male; and 27 were white, 6 African American, 1 Hispanic, and 2 Asian. Table I describes all measurements used in the study. The Figure shows examples of the subjects in each comparison group.

We selected 33 single nucleotide polymorphisms covering the 8 candidate regions studied (Table II) from

Table I. Summary of the subjects' measurements

Profile	*Sex*	*Age (y)*	*Ethnicity*	*Steiner's ANB (°)*	*Wits appraisal (mm)*	*Downs' A-B plane (°)*
Prognathic	Female	18	White	−1.9	−6.5	1.6
	Female	20	White	−4.9	−6.8	6.6
	Male	17	White	−8.1	−12	12.7
	Female	23	African American	−1.2	−8.4	1.3
	Female	20	White	0.7	−7.6	−0.1
	Female	23	White	−10.4	−33.4	14.1
	Female	14	Hispanic	−2.7	−6.2	2.7
	Female	22	White	−6.6	−15.7	4.5
	Female	19	African American	−3.4	−6.4	−2.8
	Male	19	White	−1.1	−7.3	1
	Female	30	African American	−1.2	−12.2	3.7
	Female	15	African American	2.4	−3.4	−2.4
	Male	15	White	0.3	−1.3	−1
	Female	16	African American	−0.9	−5	2.4
	Female	12	Asian	−2.3	−5	2.9
	Female	12	Hispanic	0.6	−8.1	0.7
	Female	21	African American	−1.9	−4.5	−1.7
	Female	13	African American	−4	−12	4.9
	Female	21	African American	−4.8	−4.3	4
	Female	15	White	−7.5	−13.3	9.1
	Female	28	African American	−1	−5.9	2.3
	Female	14	White	−0.3	−4.7	2
	Male	11	White	−4.4	−7.8	7.3
	Female	18	White	−4.7	−10.6	4.1
	Male	18	White	−1.9	−7	0.6
	Female	25	White	−1.6	−5.5	2.1
	Male	26	White	−8.8	−12.1	4.3
	Female	18	African American	−6	−10	7
	Female	11	African American	−2.3	−8.8	2.7
	Female	18	White	−2.9	−4.4	5.5
	Male	24	Asian	−3.4	−4.1	4.7
	Female	13	White	−0.9	−5.4	−0.3
	Female	15	White	−0.7	−4.6	0.4
	Female	15	African American	−1.2	−7	2.6
	Male	22	Asian	0.3	−6.6	−1.7
	Female	15	White	−3	−10.6	3
	Male	28	White	−1.9	−8.3	2
	Male	12	African American	−3.8	−8.2	4.6
	Male	19	White	−2.6	−7.7	1.2
	Male	17	African American	−2.2	−3.6	1.8
	Female	13	African American	−4.1	−12.5	5.8
	Male	25	White	−0.3	−4.1	−0.9
	Female	15	White	−4.7	−10	5.6
	Female	24	White	−1.1	−3.5	2.1
Orthognathic	Female	14	White	1.8	−0.2	−3.4
	Female	14	White	1.7	1.3	−4.5
	Female	13	African American	2.4	−0.9	−2.7
	Male	14	African American	2.6	−1.7	−2.7
	Female	17	White	2	−0.6	−4.1
	Male	36	White	1.4	−0.7	−3.7
	Male	52	White	0.8	−1.3	−5.4
	Female	14	White	2.8	−0.5	−5.3
	Female	13	White	2.5	1.4	−5
	Female	18	White	2.6	−1.5	−3.2
	Male	19	White	2.9	−0.3	−6.8
	Female	14	White	3.8	1.4	−8.7
	Female	15	White	1.8	1	−5
	Male	11	African American	2.3	−2.9	−3.4

Table I. Continued

Profile	Sex	Age (y)	Ethnicity	Steiner's ANB (°)	Wits appraisal (mm)	Downs' A-B plane (°)
	Female	24	White	2.4	−2.7	−3.7
	Female	15	White	0.1	−2.7	−0.3
	Female	15	White	2.5	−3.2	−2.5
	Female	15	African American	2.8	−1.3	−3.9
	Female	12	White	0.8	−0.1	−1.9
	Female	17	White	0.7	−0.9	−1.7
	Male	16	White	1.9	−0.6	−3.6
	Female	13	White	1.6	−0.2	−4.8
	Female	14	White	3.3	0.1	−7.4
	Female	12	White	1.7	0.5	−3.6
	Female	24	White	3.3	0.5	−7.7
	Male	16	White	2.1	−1.1	−5.4
	Female	17	Asian	2.2	−0.4	−4.6
	Female	15	White	1.2	−0.6	−3.1
	Female	15	White	1	−1.2	−3.5
	Female	11	Hispanic	3.7	0.1	−6
	Female	13	White	1.7	1.8	−4.5
	Male	28	Asian	3.7	0.9	−5.1
	Female	11	African American	1.9	−1	−1
	Female	11	White	3	−0.1	−4.6
	Female	16	African American	3.8	0.1	−4.8
	Male	22	White	2.7	−4	−2.6

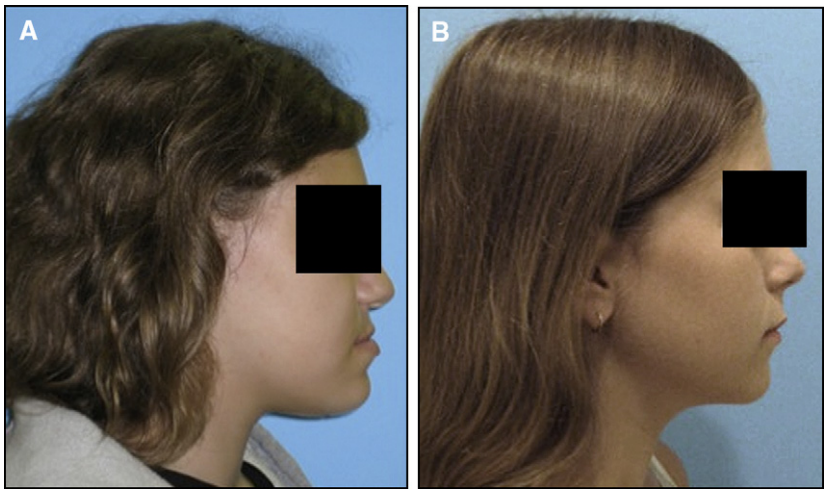

Fig. Examples of subjects in study groups: **A**, prognathic profile; **B**, orthognathic profile.

the International HapMap Project database (http://www.hapmap.org). We used the function "Download tag SNP data" and selected 26 polymorphisms as representative of the polymorphisms in the region. We selected polymorphisms that maximally represented the linkage disequilibrium structure of a region to avoid redundant information.[17] Preference was given to polymorphisms with high heterozygosity levels and different minor allele frequencies to avoid intermarker linkage disequilibrium.

The selected single nucleotide polymorphisms were genotyped by using TaqMan chemistry on an automatic sequence-detection instrument (ABI Prism 7900HT, Applied Biosystems, Foster City, Calif).[18] Assays and reagents were supplied by Applied Biosystems.

Statistical analysis

Chi-square or Fisher exact calculations were used to assess Hardy-Weinberg equilibrium and significance in

Table II. Markers studied

Marker	Locus	Gene	Original study suggesting as a candidate region
rs2503243	1p22.2	Intergenic	Frazier-Bowers et al, 2009[11]
rs972054	1p22.2	Intergenic	Frazier-Bowers et al, 2009[11]
rs1413533	1p22.2	Intergenic	Frazier-Bowers et al, 2009[11]
rs4649030	1p36.11	Intergenic	Yamaguchi et al, 2005[8]
rs2101560	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs1490055	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs2087312	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs987526	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs1601948	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs1387168	3q26.32	Intergenic	Frazier-Bowers et al, 2009[11]
rs2940913	5p12	Growth hormone receptor (*GHR*)	Yamaguchi et al, 2001[9]; Zhou et al, 2005[10]; Kang et al, 2009[20]; Tomayasu et al, 2009[21]
rs2973015	5p12	Growth hormone receptor (*GHR*)	Yamaguchi et al, 2001[9]; Zhou et al, 2005[10]; Kang et al, 2009[20]; Tomayasu et al, 2009[21]
rs1509460	5p12	Growth hormone receptor (*GHR*)	Yamaguchi et al, 2001[9]; Zhou et al, 2005[10]; Kang et al, 2009[20]; Tomayasu et al, 2009[21]
rs2910875	5p12	Growth hormone receptor (*GHR*)	Yamaguchi et al, 2001[9]; Zhou et al, 2005[10]; Kang et al, 2009[20]; Tomayasu et al, 2009[21]
rs7718944	5p12	Growth hormone receptor (*GHR*)	Yamaguchi et al, 2001[9]; Zhou et al, 2005[10]; Kang et al, 2009[20]; Tomayasu et al, 2009[21]
rs7750085	6q26	Parkinson juvenile disease protein 2 (*PARK2*)	Yamaguchi et al, 2005[8]
rs3016534	6q26	Parkinson juvenile disease protein 2 (*PARK2*)	Yamaguchi et al, 2005[8]
rs12207168	6q26	Parkinson juvenile disease protein 2 (*PARK2*)	Yamaguchi et al, 2005[8]
rs9458378	6q26	Parkinson juvenile disease protein 2 (*PARK2*)	Yamaguchi et al, 2005[8]
rs1884153	6q26	Parkinson Juvenile disease protein 2 (*PARK2*)	Yamaguchi et al, 2005[8]
rs571407	11q22.3	Caspase 4 isoform gamma precursor (*CASP4*)	Frazier-Bowers et al, 2009[11]
rs1902768	12q13.13	Keratin 7 (*KRT7*)	Frazier-Bowers et al, 2009[11]
rs7300317	12q13.13	Keratin 7 (*KRT7*)	Frazier-Bowers et al, 2009[11]
rs11113231	12q23.3	Intergenic	Frazier-Bowers et al, 2009[11]
rs4964541	12q23.3	Intergenic	Frazier-Bowers et al, 2009[11]
rs10850110	12q24.11*	Myosin 1H (*MYO1H*)	Frazier-Bowers et al, 2009[11]
rs10850364	12q24.21*	T-box 3 (*TBX3*)	Frazier-Bowers et al, 2009[11]
rs7351083	19p13.2	Fibrilin 3 precursor (*FBN3*)	Yamaguchi et al, 2005[8]
rs4804264	19p13.2	Fibrilin 3 precursor (*FBN3*)	Yamaguchi et al, 2005[8]
rs8103218	19p13.2	Fibrilin 3 precursor (*FBN3*)	Yamaguchi et al, 2005[8]
rs12327845	19p13.2	Fibrilin 3 precursor (*FBN3*)	Yamaguchi et al, 2005[8]
rs10411185	19p13.3	Intergenic	Yamaguchi et al, 2005

*These loci flank the region suggested by Frazier-Bowers et al[11] and were included to more comprehensively study this candidate region.

all comparisons. The numbers of copies of each allele and each genotype per marker were compared between the subjects with mandibular prognathism and the Class I subjects (Table II). An α of 0.05 was considered statistically significant.

RESULTS

Differences in sex and ethnic background between the 2 groups were not statistically significant. All makers were in Hardy-Weinberg equilibrium in both the Class I and the mandibular prognathism groups. Markers rs2503243, rs972054, rs1413533, rs1490055, rs2101560, rs1601948, rs1387168, rs2940913, rs7718944, rs3016534, rs9458378, and rs4964541 were not informative and could not be analyzed. The G allele of marker rs10850110 (5′ of myosin 1H-*MYO1H*) was overrepresented in the mandibular prognathism subjects ($P = 0.03$; Table III). In Table IV, details of the samples contributing to the rs10850110 are shown. The distribution of samples based on ethnic background suggests that a disproportionate number of samples from a specific subgroup does not influence the results (of the 27 mandibular prognathism subjects, 16 were white, 8 were African American, and 3 belonged to other groups; of the Class I subjects, 19 were white, 4 were African American, and 3 belonged to other groups).

DISCUSSION

This is the first report that independently attempted to replicate the recent results of 2 genome-wide scans

Table III. Summary of the association results

Locus	*Marker*	*Genotype*	*Mandibular prognathism*	*Class I*	*Allele*	*Mandibular prognathism*	*Class I*
1p22.2	rs4649030	AA	3	3	A	13	18
		AG	7	12	G	43	46
		GG	18	17		$P = 0.54$	
			$P = 0.58$				
3q26.32	rs2087312	AA	30	26	A	73	61
		AC	13	9	C	13	19
		CC	0	1		$P = 0.16$	
			$P = 0.41$				
	rs987526	CC	1	0	C	7	3
		CT	5	3	T	61	59
		CC	28	28		$P = 0.24$	
			$P = 0.5$				
5p12	rs2973015	AA	9	10	A	27	31
		AG	9	11	G	27	21
		GG	9	5		$P = 0.32$	
			$P = 0.5$				
	rs1509460	GG	5	3	G	35	29
		GT	25	23	T	39	43
		TT	7	10		$P = 0.4$	
			$P = 0.58$				
	rs2910875	AA	4	5	A	18	27
		AG	10	11	G	20	31
		GG	5	10		$P = 0.94$	
			$P = 0.69$				
6q26	rs7750085	AA	8	13	A	35	39
		AT	19	13	T	31	31
		TT	6	9		$P = 0.75$	
			$P = 0.24$				
	rs12207168	AA	8	12	A	35	39
		AG	19	15	G	41	33
		GG	11	9		$P = 0.32$	
			$P = 0.49$				
	rs1884153	AA	0	0	A	2	3
		AG	2	3	G	80	67
		GG	39	32		$P = 0.53$	
			$P = 0.52$				
11q22.3	rs571407	CC	1	0	C	7	3
		CT	5	3	T	61	59
		TT	28	28		$P = 0.24$	
			$P = 0.5$				
12q13.13	rs1902768	AA	1	2	A	11	14
		AG	9	10	G	59	50
		GG	25	20		$P = 0.36$	
			$P = 0.67$				
	rs7300317	AA	8	7	A	35	33
		AG	19	19	G	35	37
		GG	8	9		$P = 0.92$	
			$P = 0.94$				
12q23.3	rs11113231	AA	6	5	A	38	30
		AG	26	20	G	42	38
		GG	8	9		$P = 0.68$	
			$P = 0.8$				
12q24.11	rs10850110	AA	1	5	A	8	17
		AG	6	7	G	46	35
		GG	20	14		$P = 0.03$	
			$P = 0.15$				
12q24.21	rs10850364	AA	3	7	A	19	30
		AG	13	16	G	29	34
		GG	8	9		$P = 0.44$	
			$P = 0.66$				

Table III. Continued

Locus	Marker	Genotype	Mandibular prognathism	Class I	Allele	Mandibular prognathism	Class I
19p13.2	rs7351083	AA	6	6	A	34	29
		AG	22	17	G	42	41
		GG	10	12		$P = 0.69$	
			$P = 0.7$				
	rs4804264	CC	9	6	C	33	27
		CT	15	15	T	49	45
		TT	17	15		$P = 0.73$	
			$P = 0.82$				
	rs8103218	CC	6	7	C	25	30
		CT	13	16	T	25	28
		TT	6	6		$P = 0.86$	
			$P = 0.96$				
	rs12327845	CC	25	15	C	63	45
		CT	13	15	T	23	23
		TT	5	4		$P = 0.34$	
			$P = 0.42$				
19p13.3	rs10411185	AA	11	5	A	43	29
		AG	21	19	G	37	39
		GG	8	10		$P = 0.18$	
			$P = 0.35$				

Values under the "Mandibular prognathism" and "Class I" columns indicate the numbers of subjects with each genotype in each group and the number of alleles found in each group of subjects. Variations in the number of subjects or the number of alleles are due to polymerase chain reaction failure. These failures are related to the purity of the genomic DNA used and the uniqueness of polymerase chain reaction primer probes. These aspects are hard to be predicted but should not dramatically influence the data.

Table IV. Demographic variables of the samples contributing to the rs10850110 marker analysis

		Number of subjects with genotype mandibular prognathism			Number of subjects with genotype Class I		
Ethnicity	Sex	AA	AG	GG	AA	AG	GG
White	Male	1	2	4	-	1	2
	Female	-	4	5	5	5	6
African American	Male	-	-	1	-	-	1
	Female	-	-	7	-	-	3
Other	Male	-	-	-	-	-	1
	Female	-	-	3	1	-	1

for mandibular prognathism and Class III (due primarily to maxillary deficiency).[8,11] Our findings corroborate the previous suggestive linkage results with 12q23 (logarithm of the odds score, 2.93[11]). The most common allele of a marker flanking *MYO1H* (rs10850110) was overrepresented in the mandibular prognathism subjects. The frequency of the less common rs10850110 allele varies from 0.008 in sub-Saharan Africans to 0.089 in Japanese, 0.148 in Han Chinese, and 0.275 in Europeans (National Center for Biotechnology Information, http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10850110). *MYO1H* is a class I myosin that is in a different protein grouping than the myosin heavy chain isoforms found in the skeletal muscle sarcomeres, which are the basis of fiber typing. Class I myosins are necessary for cell motility, phagocytosis, and vesicle transport.[19]

Since the sample size of our study was modest and some genetic markers were uninformative, we cannot conclude that the chromosomal regions studied here do not play a role in the development of mandibular prognathism. However, the definitions used to select subjects for this study most likely increased the homogeneity, and the evidence of a role of 12q24 in mandibular prognathism is likely to be true. In an interval of 397,305 base pairs in 12q24.11, another 4 genes flank *MYO1H*: *ACACB* (acetyl-CoA carboxylase beta), *FOXN4* (forkhead box N4), *KCTD10* (potassium channel tetramerisation domain containing 10), and *UBE3B* (ubiquitin protein ligase E3B). These genes are involved in different aspects of metabolism, and, although *MYO1H* is the best candidate for a role in mandibular prognathism, one cannot discard the possibility that these other genes also have roles.

The cohort selected for this study reflects the ethnic breakdown of Pittsburgh. According to its census of 2000 (http://www.hellopittsburgh.com/Census.Cfm), the inhabitants included 67.6% of European descent and

27.1% of African descent. The remaining 5.3% were Hispanics, Asians, and other groups. There was no statistically significant difference between the mandibular prognathism and orthognathic subjects in regard to the distribution of age, sex, and ethnic background. However, an undetected population substructure could have shown a spurious association, even though when this effect is present, we tend to see several positive associations. The small percentage of Asians in our sample most likely did not allow us to detect the reported association between growth hormone receptor and mandibular height that has been described primarily in Japanese, Chinese, and Koreans.[9,10,20,21] Future investigations also should expand further the variants studied in the growth hormone receptor locus. One ideal variant to be studied because of its allelic frequency is the d3/fl-growth hormone receptor variant, which has minor allele frequencies of 47.9% in African Americans and 31.3% in European Americans.[20]

Although concerned about multiple testing, we did not apply the strict Bonferroni correction because it would have increased type II errors, and a major focus of this study was to replicate putative associations with the previously described contributing loci to mandibular prognathism. For example, under the Bonferroni correction, we would have lowered the α to 0.0025 (0.05/20) or 0.00125 (0.05/40) (number of tests in Table III). Our previous work with other craniofacial defects has shown that true associations will be missed if nominal *P* values are not considered as well. One example is the known association between *IRF6* (interferon regulatory factor 6) and isolated cleft lip and palate that would have been missed in a project we performed in which we tested 1489 genetic markers.[22] Therefore, we reported here results with *P* values below 0.05. However, our data must be carefully interpreted, since some *P* values below 0.05 could be due to chance.

One biologic pathway recently identified in contributing to skeletal height in humans is the nicotinic acetylcholine receptor signaling pathway with 3 associated genes included in the unconventional myosin grouping: *MYO1F*, *MYO6*, and *MYO9B*.[23] *MYO1F* is a similar gene to the *MYO1H* identified as associated with mandibular prognathism in our study. Future studies should consider all genes in the family as candidates for a role in the development of mandibular prognathism.

CONCLUSIONS

We provide further evidence of a role of the 12q24 locus in the development of mandibular prognathism. The possible role on a type I myosin is intriguing, since it suggests that muscle function might have a more important role than previously thought in the development and deviations of the bone structures of the craniofacial complex. Since the sample size of this study was modest, replication of these findings in independent populations is necessary. We will continue our efforts to expand the population studied to allow the results of our study to be replicated and to perform these analyses by sex, age groups, and ethnicity.

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Genetic polymorphisms underlying the skeletal Class III phenotype

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

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

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

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

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

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

MATERIAL AND METHODS

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

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

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

Table I. Markers studied

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

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

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

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

Table II. Descriptive statistics of the sample

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

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

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

STATISTICAL ANALYSIS

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

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

RESULTS

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

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

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

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

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

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

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

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

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

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

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

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

P value according to independent t test or correlation coefficient.

Table VI. Summary results of the PC analysis

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

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

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

DISCUSSION

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

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

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

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

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

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

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

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

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

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

CONCLUSIONS

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

SUPPLEMENTARY DATA

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

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