Journal of Taibah University Medical Sciences (2025) 20(1), 112–119

Taibah University

Journal of Taibah University Medical Sciences

www.sciencedirect.com

Original Article

Polymorphisms in *SOX2*/*FGFR1* are associated with skeletal class III maxillary and mandibular dimensions: A preliminary study

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Received 6 August 2024; revised 28 November 2024; accepted 25 January 2025; Available online 24 February 2025

الملخص

أهداف البحث: كان الهدف من الدراسة الحالية تقييم الارتباط بين سوء الإطباق من الدرجة الثالثة والتعددات الشكلية الجينية في جينين هما سوكس2 (آر اس 4434184) و فجفر1 (آر اس 881301).

طريقة البحث: تم تضمين ما مجموعه 60 مريضا، بما في ذلك 30 مريضا يعانون من سوء الإطباق الهيكلي من الدرجة الأولى و30 مريضا يعانون من سوء الإطباق الهيكلي من الدرجة الثالثة، في الدراسة. تم جمع عينات الحمض النووي اللعابي وتحليلها باستخدام تسلسل سانجر. تم إجراء التتبع الرقمي على صور الأشعة السينية الجانبية المحملة في برنامج أوتوكاد (الإصدار 2017) لتقييم العلاقة الأمامية الخلفية والرأسية للأقواس العلوية والسفلية. تمت مقارنة توزيع النمط الجيني بين المجموعتين باستخدام اختبار مربع كاي لتقييم توازن هاردي وينبرج. تم إجراء تحليل الانحدار اللوجستي المتعدد.

النتائج: ارتبط تعدد أشكال سوكس2 (آر اس 4434184) بزيادة طول الفك السفلي. وعلى العكس من ذلك، ارتبط انخفاض طول الفك العلوي وزيادة طول الفك السفلي ووجه غير متباعد بتعدد أشكال فجفر1 (آر اس 881301). تم تحديد تعدد أشكال جديدة، بما في ذلك فجفر1 (آر اس 881300) و (آر اس 881299) و (آر اس 7829058)، بالاقتران مع أنماط ظاهرية مختلفة. والجدير بالذكر أن (آر اس 881300) و (آر اس 7829058) أظهرا ارتباطا كبيرا بالفئة الهيكلية الثالثة، بينما أظهر (آر اس 881299) ارتباطا كبيرا بوجه غير متباعد وزيادة طول الفك السفلي الأمامي الخلفي.

الاستنتاجات: أفادت الدراسة الحالية بوجود ارتباط محتمل بين السمات المرتبطة بسوء الإطباق الهيكلي من الفئة الثالثة وتعدد أشكال جينات سوكس2 (آر اس 4434184)، و فجفر1 (آر اس 881301) و (آر اس 881300) و (آر اس 881299) و (آر اس 7829058). ويحمل هذا الاكتشاف وعدا بتعزيز التنبؤ بالهيكل العظمي وإبلاغ تخطيط العلاج التقويمي.

الكلمات المفتاحية: سوء الإطباق الهيكلي من الفئة الثالثة؛ فجفر1؛ طول الفك السفلي؛ تسلسل سانجر؛ سوكس.

Abstract

Objectives: The aim of the present study was to assess the association between class III malocclusion and genetic polymorphisms in two genes: *SOX2* (rs4434184) and *FGFR1* (rs881301).

Methods: A total of 60 patients, 30 with skeletal class I and 30 with skeletal class III malocclusion, were included in this study. Salivary DNA samples were collected and analyzed with Sanger sequencing. Digital tracing was performed on lateral cephalometric radiographs loaded into AutoCAD software (Version 2017) to assess the anteroposterior and vertical relationships of the maxillary and mandibular arches. Genotype distribution was compared between groups with the chi-square test to assess Hardy–Weinberg equilibrium. Multiple logistic regression analysis was conducted.

Results: The *SOX2* rs4434184 polymorphism was associated with longer mandibular length. In contrast, shorter maxillary length, longer mandibular length, and hypodivergent face were correlated with the rs881301 polymorphism in *FGFR1*. New polymorphisms, including

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Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2025.01.004

FGFR1 rs881300, rs881299, and rs7829058, have been identified in association with various phenotypes. Notably, rs881300 and rs7829058 displayed a substantial association with skeletal class III, whereas rs881299 revealed a significant association with a hypodivergent face and longer mandibular anteroposterior length.

Conclusions: A potential association was observed between class III skeletal malocclusion-related traits and polymorphisms of *SOX2* (rs4434184) and *FGFR1* (rs881301, rs881300, rs881299, and rs7829058). This finding holds promise for enhancing skeletal prediction and informing orthodontic treatment planning.

Keywords: Class III malocclusion; *FGFR1*; Mandibular length; Sanger sequencing; *SOX*

Introduction

The sizes and shapes of dental arches vary according to racial and ethnic group, exposure to diverse influences from the environment, genetic makeup, and developmental characteristics.[1] Class III malocclusion is considered among the most challenging malocclusions to diagnose and treat.[2] This condition involves a complex three-dimensional facial skeletal imbalance between the differential growth of the mandible and maxilla.[3] Although class III malocclusion affects populations globally, it has elevated prevalence in Asian populations, which are often characterized by mandibular prognathism. This condition is observed in fewer than 1% of white individuals and in 10% of Japanese individuals.[4] Various anomalies in craniofacial and dentofacial structures can arise from both of environmental and genetic factors occurring during different developmental periods.[5] Twin and family genetic studies have provided strong evidence that hereditary factors contribute to the development of skeletal malocclusions.[6,7] In embryonic cranial morphogenesis, genetics has a significant influence: genetic variants have the potential to contribute to skeletal malformations during both prenatal and early postnatal ontogenesis.[8]

Research elucidating the genetic basis of class III malocclusion is essential for establishing preventive measures and improving treatment options for affected individuals.[9] Analysis of the Habsburgs, one of Europe's most prominent royal families, has revealed an autosomal dominant hereditary pattern in 23 generations, which has confirmed the long-standing hypothesis that heredity plays a major role in the etiology of mandibular prognathism.[10] Therefore, identifying the biological factors underlying the phenotypic deviation observed in patients with skeletal malocclusion is crucial for developing more powerful therapeutic strategies.[11] Potential candidates for skeletal malocclusions include genes involved in skeletogenesis, bone metabolism, and cartilage development. Examples include members of the family of candidate genes known as fibroblast growth factor (*FGF*) ligands and FGF receptors (*FGFR*), which play critical roles in angiogenesis, wound healing, and cell proliferation and differentiation.[12,13] *FGF* signaling is essential in regulating development and growth of endochondral and intramembranous bones, and has an inductive effect on the development of facial primordia.[14]

The *SOX2* gene is a member of the *SOX* family that is essential for embryonic development. *SOX2* is a high mobility group (HMG) domain-containing transcription factor that is highly expressed in the nervous system during development and is involved in various developmental processes.[15] Variants in *SOX2* have been associated with several craniofacial abnormalities, such as cleft palate and ocular malformations, because of this gene's critical role in sustaining stem cell pluripotency and facilitating neurogenesis.[16,17] People with *SOX2* mutations or haploinsufficiency frequently exhibit severe ocular and central nervous system problems, hormone deficits, and craniofacial abnormalities, including retrognathia and facial asymmetry.[18] According to Numakura et al., widely spaced teeth and supernumerary teeth are two examples of dental malformations suggesting that *SOX2* is involved in orodental development.[19] More recently, *SOX2* variants have been associated with elevated manifestation of a class III phenotype.[20]

A study by Weaver has found the single nucleotide polymorphisms (SNPs) rs4434184 in SOX2 and rs881301 in *FGFR1* in Americans with class III skeletal malocclusion and maxilla-mandibular deviations.[20] This study was aimed at investigating the relationships of SNPs in *SOX2* and *FGFR1* genes with class III malocclusion, skeletal variance in the vertical plane, and maxillary and mandibular dimensions.

Materials and Methods

Participants and study groups

Before taking part in this study, each participant provided informed consent. The study followed the checklist statement from the STREGA project aimed at strengthening the reporting of genetic associations.[21] Salivary samples from patients at the Orthodontic Department at the College of Dentistry, University of Baghdad, were used to extract genomic DNA, and lateral cephalometric radiographs taken before the orthodontic treatment were analyzed to determine eligibility. After a cephalometric analysis and a clinical evaluation, 60 participants (all of Arab ethnicity) of 278 clinically assessed individuals were included in the study. They were divided into two groups: 30 individuals

with class I occlusion (17 males and 13 females) and 30 individuals with class III malocclusion (19 males and 11 females). Patients with underlying inheritable disorders such as congenital anomalies, growth problems, or cleft lip and palate were excluded.

Phenotypic assessments

To evaluate each participant's phenotype, we collected lateral cephalometric radiographs before the dental treatment. For digitization, the lateral cephalograms were loaded into AutoCAD software (Version 2017). One examiner (AMB) who had received training from a qualified orthodontist performed the measurements. For evaluation of intra-examiner repeatability, a second examination was performed on ten randomly selected radiographs 1 month later. Reliability was verified with the intraclass correlation coefficient (ICC), which indicated high data reproducibility. Point A, point B, and the nasion (N), sella (S), gnathion (Gn), menton (Me), gonion (Go), and condylion (Co) were the eight anatomical hard tissue points, and condylion-point A (Co-A), condylion-gnathion (Co-Gn), nasion-menton (N−Me), and sella-fonion (S-Go) were the four linear measurements. The three angular measurements SNA, SNB, and ANB in Steiner analysis were used as the tracing landmarks and reference planes. For identification of the type of malocclusion (sagittal skeleton jaw relationship), the angles SNA, SNB, and ANB in Steiner analysis were used. Consequently, samples were categorized as having class I (2°−4°) or class III (<0°) malocclusions according to the ANB angle. Additionally, the vertical skeletal discrepancy was evaluated with the Jarabak ratio between the anterior facial height (N−Me) and the posterior facial height (S-Go). Faces with ratios of 59% or less were determined to be hyperdivergent, those with ratios of 65% or more were determined to be hypodivergent, and those with ratios between 60% and 64% were determined to be normal. Finally, the Co-A and Co-Gn planes were used to determine the maxillary and mandibular lengths. The normal values of angles, lines, and reference points were measured, according to McNamara, Jarabak, and Steiner.[22]

Genotype assessments

Genotyping analysis was conducted on salivary DNA. In accordance with the manufacturer's instructions, a ReliaPrepTM gDNA Miniprep System (Promega, WI, USA) was used to extract genomic DNA. Agarose gel electrophoresis was conducted to assess DNA integrity before samples were sent to Macrogen (Seoul, Korea) for Sanger sequencing with an automated DNA sequencer (ABI3730XL, Thermo Scientific, MA, USA). Sanger sequencing was performed on two SNPs rs4434184 (A > G) in *SOX2* and rs881301 (T > C) in *FGFR1*, that were previously associated with diseases or developmental abnormalities in the craniofacial region's bone and/or cartilage.[20] The Korean company Macrogen supplied the verified primers for the selected SNPs. The sequencing differences between samples of a particular gene were established with Geneious software, which analyzes data with both forward and reverse reading.

Statistical analysis

I. GraphPad Prism version 8 (GraphPad Software Inc., La Jolla, CA) was used to conduct statistical analysis. Genotypes and SNP alleles of genes are reported as frequency numbers and percentages. To compare the distribution of genotypes between groups, we used the chi-square test to estimate the Hardy−Weinberg equilibrium of group genotype frequencies. To evaluate the potential effects of the variable on a measurement with confidence intervals and odds ratios, we also performed multiple logistic regression analysis. $P \leq 0.05$ was considered to indicate a significant difference.
II. The ICC was used to verify the reliability.
III. Logistic regression analysis was used to determine which genotype polymorphisms had significant relationships with the studied traits.

Results

Study group characteristics

The mean age was 22.7 years (SD: 4.433) in the class III malocclusion group and 25.6 years (SD: 5.056) in the class I malocclusion group. The characteristics of the study participants are listed in Table 1.

Angular and linear measurements

The angular measurements (Table 2) comprised the mean, minimum, and maximum SNA, SNB, and ANB angles for the class I and class III groups. Table 2 also shows linear

Table 1: Population characteristics for each phenotype.

Phenotype	N%
Class I malocclusion age: mean ± SD	30 (50): 25.6 ± 5.056
Male/female	17 (56.7)/13 (43.3)
Class III malocclusion age: mean	30 (50): 22.7 ± 4.433
Male/female	19 (63.3)/11 (36.7)
Vertical dimension	
Normal face	29 (43.3) [20 Cl.I/9 Cl.III]
Hyperdivergent	10 (16.7) [2 Cl.I/8 Cl.III]
Hypodivergent	21 (40) [8 Cl.I/13 Cl.III]
Maxillary-mandibular antero-posterior measurement	
Normal	20 (33.3) [16 Cl.I/4 Cl.III]
Increased mandibular length	16 (26.7) [6 Cl.I/10 Cl.III]
Decreased maxillary length	24 (40) [8 Cl.I/16 Cl.III]

Note: % = percentage, Cl. = class, N = number, SD = standard deviation.

Table 2: Descriptive statistics of the angular and linear variables measured in the case and control groups.

Variables		Class I group				Class III group			
		Mean	S.D.	Min.	Max.	Mean	S.D.	Min.	Max.
Angular measurements	**SNA (°)**	81.34°	1.040°	79°	83°	80.1°	3.782	75°	87°
	SNB (°)	79.38°	0.873	78°	81°	83.48°	3.584	77°	89°
	ANB (°)	1.96°	0.880	1°	3°	−3.38°	2.083	0°	-9°
Linear measurements	**AFH (mm)**	129	9.920	117	141	121.2	11.391	97	139.6
	PFH (mm)	81	5.000	72	88	77.57	8.098	65	94.5
	P-AFH%	62.9%	2.795	59%	67.5%	64.2%	6.180	56%	76.3%
	Max. L (mm	95	4.623	86	100	87.47	6.388	70	97.6
	Mand. L (mm)	121.3	5.390	114	130	124.86	9.853	104	138.6

Note: S.D = Standard deviation, Min = minimum, Max = Maximum, mm = millimeter, AFH = Anterior facial height, PFH = Posterior facial height, P-AFH% = Posterio-anterior facial height percentage, Max. L = Maxillary length, Mand. L = Mandibular length.

measurements comprising the mean, minimum, and maximum anterior facial height (AFH) (N−Me), posterior facial height (PFH) (S-Go), posterio-anterior facial height percentage (P-AH%), maxillary anterio-posterior length, and mandibular anterio-posterior length for both class I and class III groups.

Genotype-phenotype associations

Table 3 illustrates the distribution of genotypes for each SNP associated with each trait. In individuals with the AG genotype, the *SOX2* SNP rs4434184 was significantly associated with longer mandibular anteroposterior length (12/16, 75%, $P = 0.0026$) (Figure 1A). Individuals with a lower anterior facial height (hypodivergent face) had a significantly greater SNP rs881301 in *FGFR1* with CC genotype (13/19, 62%, $P = -0.0038$) (Figure 1B). Additionally, the same SNP was shown to be substantially higher in individuals with a longer mandibular anteroposterior distance in TC genotype carriers (11/16, 69%, $P = 0.0223$) and a shorter maxillary anteroposterior distance in CC genotype carriers (10/24, 42%, $P = 0.0405$). Three additional *FGFR1* SNPs, rs881300, rs881299, and rs7829058, were identified during Sanger sequencing, as shown in Table 4.

Regression analysis results

All SNPs demonstrated significant correlations with various traits (Table 5). Both rs881300 and rs7829058 showed a significant correlation with skeletal class III with the corresponding genotypes AC (14/30, 46.3%,

Table 3: Genotype distribution of *SOX2* rs4434184 and FGFR1 rs881301 SNPs across phenotypes.

Gene and SNP		Phenotypes	Genotypes N (%)			*P* value
			AA	AG	GG	
SOX2 rs4434184	Sagittal relation	Class I	19 (63.33%)	10 (33.33%)	1 (0.33%)	Ref.
		Class III	22 (73.3%)	8 (26.7%)	0 (0%)	0.5154
	Vertical relation	Normal face	21 (63%)	7 (33%)	1 (4%)	Ref.
		Hypodivergent face	14 (66.7%)	7 (33.3%)	0 (0%)	0.5239
		Hyperdivergent face	6 (60%)	4 (40%)	0 (0%)	0.3737
	Maxillary-Mandibular length	Normal	15 (75%)	5 (25%)	0 (0%)	Ref.
		Decreased maxillary	23 (96%)	1 (4%)	0 (0%)	0.0752
		Increased mandibular	3 (19%)	12 (75%)	1 (6%)	0.0026*
			TT	TC	CC	
FGFR1 rs881301	Sagittal relation	Class I	8 (26.7%)	16 (53.3%)	6 (20%)	Ref
		Class III	6 (20%)	12 (40%)	12 (40%)	0.1826
	Vertical relation	Normal face	10 (34.5%)	15 (51.7%)	4 (13.8%)	Ref
		Hypodivergent face	2 (9.5%)	6 (28.5%)	13 (62%)	0.0038*
		Hyperdivergent face	2 (20%)	6 (60%)	2 (20%)	0.4303
	Maxillary-Mandibular length	Normal	9 (45%)	7 (35%)	4 (20%)	Ref
		Decreased maxillary	4 (16%)	10 (42%)	10 (42%)	0.0405*
		Increased mandibular	1 (6%)	11 (69%)	4 (25%)	0.0223*

Note: * significant at *P* value ≤ 0.05, A = adenine, C = cytosine, G = guanine, N = number, % = percentage, rs = reference of SNP, Ref = reference, T = thymine.

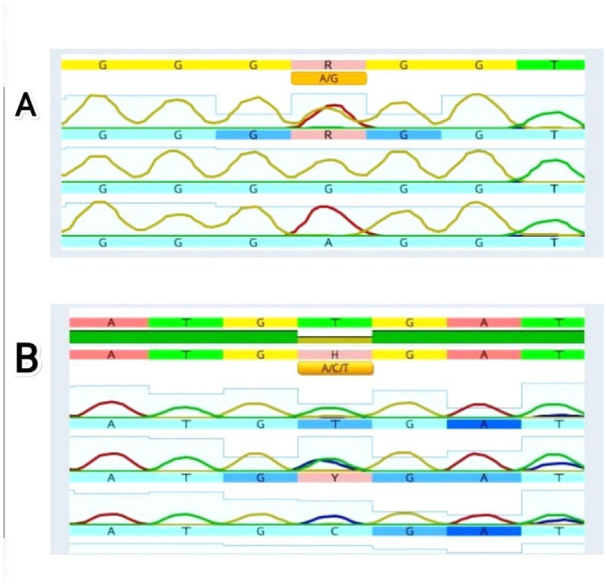

Figure 1: Chromatograms of SNPs. (A) Analysis of the rs4434184 SNP of *SOX2* with Sanger sequencing. Single "A" peak indicative of an A homozygous allele. Single "G" peak indicative of a G homozygous allele. Presence of "A" and "G" peaks indicative of an A/G heterozygous allele. **(B)** Analysis of the rs881301 SNP of *FGFR1* with Sanger sequencing. Single "T" peak indicative of a T homozygous allele. Single "C" peak indicative of a C homozygous allele. Presence of "T" and "C" peaks indicative of a T/C heterozygous allele.

P = 0.0150) and GC (13/30, 43.33%, p = 0.0229), respectively. Moreover, rs881299 was significantly associated with longer anteroposterior length of the mandible in the TC genotype (11/16, 69%, P = 0.0384) and was also significantly higher in individuals with shorter anterior facial height (hypodivergent face) with the CC genotype (13/21, 62%, P = 0.0022). Logistic regression analysis (Table 4) indicated a significant correlation of *SOX2* rs4434184 with the AG genotype and longer mandibular antero-posterior length (P = 0.0026, OR = 12.0000). *FGFR1* rs881301 was strongly associated with a hypodivergent face and the CC genotype (P = 0.0038, OR = 16.2500). Longer mandibular anteroposterior length was associated with the same rs881301 SNP with the TC genotype (P = 0.0223, OR = 14.1429). In individuals with shorter maxillary anteroposterior length, the CC genotype was considerably more prevalent (P = 0.0405, OR = 5.6250). Interestingly, skeletal class III was associated with *FGFR1* rs881300 and rs7829058 in individuals with the AC genotype (P = 0.0150, OR = 4.4800) and GC genotype (P = 0.0229, OR = 4.0625), respectively. Additionally, a significant increase in the mandibular anteroposterior length was observed with the TC genotype of *FGFR1* rs881299 (P = 0.0384, OR = 11.0000), and rs881299 was also significantly higher in individuals with shorter anterior

Table 4: Genotype distribution of the new rs881300, rs881299, and rs7829058 SNPs of the *FGFR1* gene across phenotypes.

Gene and SNP		Phenotypes	Genotypes N (%)			*P* value
			AA	AC	CC	
FGFR1 rs881300	Sagittal relation	Class I	24 (8%)	5 (16.7%)	1 (3.3%)	Ref
	Vertical relation	Class III	15 (50%)	14 (46.3%)	1 (3.33%)	0.0150*
	Maxillary-mandibular length	Normal face	20 (69%)	7 (24%)	2 (7%)	Ref
		Hypodivergent face	13 (61.9%)	8 (38.1%)	0 (0%)	0.3691
		Hyperdivergent face	6 (60%)	4 (40%)	0 (0%)	0.4092
		Normal	11 (55%)	8 (40%)	1 (5%)	Ref.
		Shorter maxillary length	16 (66.6%)	7 (29.2%)	1 (4.2%)	0.4336
		Longer mandibular length	12 (75%)	4 (25%)	0 (0%)	0.2925
			TT	TC	CC	
		Class I	9 (30%)	15 (50%)	6 (20%)	Ref.
FGFR1 rs881299	Sagittal relation	Class III	5 (16.7%)	13 (43.3%)	12 (40%)	0.5096
	Vertical relation	Normal face	10 (34.5%)	16 (55.1%)	3 (10.3%)	Ref.
	Maxillary-mandibular length	Hypodivergent face	2 (9.5%)	6 (28.5%)	13 (62%)	0.0022 *
		Hyperdivergent face	2 (20%)	6 (60%)	2 (20%)	0.3146
		Normal	8 (45%)	8 (35%)	4 (20%)	Ref
		Shorter maxillary length	4 (16%)	10 (42%)	10 (42%)	0.2368
		Longer mandibular length	1 (6%)	11 (69%)	4 (25%)	0.0384 *
			GG	GC	CC	
FGFR1 rs7829058	Sagittal relation	Class I	25 (83.3%)	5 (16.7%)	0 (0%)	Ref.
		Class III	16 (53.33%)	13 (43.33%)	1 (3.33%)	0.0229*
	Vertical relation	Normal face	22 (75.9%)	6 (20.7%)	1 (3.4%)	Ref.
		Hypodivergent face	13 (61.9%)	8 (38.1%)	0 (0%)	0.2060
		Hyperdivergent face	6 (60%)	4 (40%	0 (0%)	0.4289
	Maxillary-mandibular length	Normal	13 (65%)	7 (35%)	0 (0%)	Ref.
		Shorter maxillary length	16 (66.6%)	7 (29.2%)	1 (4.2%)	0.7501
		Longer mandibular length	12 (75%)	4 (25%)	0 (0%)	0.5190

Note: * significant at P value $\leq$ 0.05, A = adenine, C = cytosine, G = guanine, N = number, % = percentage, rs = reference of SNP, Ref = reference, T = thymine.

Table 5: Multiple logistic regression analysis of genotype distribution of SNPs and associated phenotypes.

Phenotype	Genes	SNPs	Reference	Genotype	Odds ratio (CI 95%)	*P* value
Longer mand. Ant-post length	*SOX2*	rs4434184	AA	AG	12.0000 (2.3743−60.6501)	0.0026*
skeletal class III	*FGFR1*	rs881300	AA	AC	4.4800 (1.3388−14.9913)	0.0150*
		rs7829058	GG	GC	4.0625 (1.2147−13.5869)	0.0229*
Shorter max. Length		rs881301	TT	CC	5.6250 (1.0772−29.3719)	0.0405*
Longer mand. Ant-post length		rs881301	TT	TC	14.1429 (1.4568−137.3042)	0.0223*
		rs881299	TT	TC	11.0000 (1.1369−106.4344)	0.0384 *
Shorter anterior facial height		rs881301	TT	CC	16.2500 (2.4622−107.2453)	0.0038*
		rs881299	TT	CC	21.6667 (3.0215−155.3686)	0.0022 *

Note: * significant at P value $\leq$ 0.05, A = adenine, C = cytosine, CI = confidence interval, G = Guanine, mand. = mandibular, max. = maxillary, rs = reference of SNP, T = thymine.

facial height (hypodivergent face) with the CC genotype ($P = 0.0022$, OR = 21.6667).

Discussion

Genetics is a highly reliable tool for predicting an individual's growth. According to Mossey, "the ability to ascertain the relative contribution of both genetics and environment will ultimately determine the success of treatment".[23] Class III malocclusion is widely believed to have a significant hereditary component. Previous studies have suggested correlations of certain gene variants with facial morphology, and maxillary or mandibular discrepancy.[24,25] Multiple risk loci have been associated with the phenotypic genetic predisposition of the maxilla and mandible. Mandibular prognathism has been associated with gene loci including *SOX2*,[20] and *FGFR1*.[26]

Using a case−control sample of Iraqi origin, we identified five polymorphisms—one in *SOX2* and four in *FGFR1*—that were significantly associated with class III malocclusion, as well as each jaw's sagittal and vertical craniofacial features. The highly conserved *SOX2* gene is located at 3q26. This single exon gene encodes a 317-residue protein with a DNA-binding HMG domain at the N-terminus and a transcriptional activation domain at the C-terminus.[27] The protein encoded by *SOX2* is crucial in embryogenesis.[17,28] The anophthalmia/microphthalmia condition, as well as other associated disorders such as anophthalmia-esophageal-genital syndrome, are associated with loss of function mutations or deletions in *SOX2*.[29,30] Studies are increasingly identifying that *SOX2* mutations lead to a variety of extra-ocular symptoms, including delayed growth, hearing loss, intellectual disability, and cleft palates, thereby suggesting direct effects of variations in *SOX2* on the development of the craniofacial complex.[17,31]

According to Weaver's study on *SOX2* (rs4434184), the A > G genotype increases the likelihood of a class III phenotype by 1.7−2.15 times.[20] Individuals with anophthalmia syndrome and a *SOX2* variant have been reported to have numerous affected supernumerary teeth.[19]

Our findings indicated that *SOX2* rs4434184 was associated with longer mandibular anteroposterior length. The AG genotype substantially correlated with longer mandibular anteroposterior length, according to multiple logistic regression analysis. *SOX2* rs4434184 was associated with class III malocclusion, because longer mandibular length is a typical trait in patients with this type of malocclusion.

The balance among the development, differentiation, and apoptosis of skeletal cells, as well as endochondral and intramembranous bone formation, is controlled by the *FGF* and *FGFR* genes. Numerous studies have demonstrated that variants in a single gene can provide insights into understanding disease phenotypes and normal development. Specifically, variants in *FGF* and *FGFR* genes may contribute to specific types of skeletal class III malocclusion.[32,33] *FGFR1* plays a major role in the development of the skull and face, by influencing components such as the craniomaxillofacial bone, facial and masticatory muscles, palate, teeth, and submandibular salivary gland.[34] This gene also serves as a pro-skeleton-formation regulator modifying osteoblast differentiation.[35] Gain-of-function mutations in *FGFR1* and *FGFR2* have been associated with craniosynostosis syndromes such as Apert, Crouzon, and Pfeiffer syndrome, all of which frequently exhibit mandibular prognathism.[26,36,37] Additionally, mutations in this gene can cause Kallmann syndrome or hypogonadotropic hypogonadism 2 with or without anosmia, a condition characterized by cleft palate, teeth deformities, and olfactory problems.[38]

Herein, rs881301 in *FGFG1* was tested and found to be associated with shorter anterior facial height (hypodivergent face), shorter maxillary and longer mandibular anteroposterior length, and increased mandibular prognathism. The longer mandibular length was associated with a considerably higher prevalence of the TC genotype, according to multiple logistic regression analysis; the CC genotype in participants with hypodivergent faces; and the CC genotype in participants with shorter maxillary anteroposterior length. Moreover, *FGFG1* rs881301 has also been identified by Weaver in patients from the United States with class III skeletal malocclusion and maxilla-mandibular deficits.[20] Alexander et al. have further found an association of *FGFR1* rs881301 with tooth agenesis phenotype in a Brazilian dataset.[32] Moreover, another investigation has identified three SNPs in *FGFR1, FGF12*, and *FGF20* that are nominally significantly associated with mandibular prognathism.[26] Collectively, the overall phenotype of the reported patients with class III malocclusion included an enlarged mandible and a shortened maxilla. These observations suggest that the rs881301 in *FGFR1* might be a candidate SNP that contributes to the longer mandibular anteroposterior dimension and shorter maxillary anteroposterior length in individuals with class III skeletal malocclusion.

Three additional SNPs in *FGFR1* (rs881300, rs881299, and rs7829058) identified in this study showed substantial correlation with various traits. Significant correlations between skeletal class III and rs881300 and rs7829058 were observed, whereas shorter anterior facial height (hypodivergent face) and longer mandibular antero-posterior length were significantly correlated with rs881299. The GG genotype of *FGFR1* rs881300 was more common in patients with skeletal class III malocclusion, according to multiple logistic regression analysis, and the TC genotype of rs881299 was significantly prevalent in patients with the CC genotype who exhibited longer mandibular anteroposterior length and shorter anterior facial height (hypodivergent face), thus suggesting a potential association of both rs881300 and rs881299 with class III malocclusion. No prior studies have tested the association of these SNPs with craniofacial disorders, to our knowledge, thus underscoring the novelty of our findings. Moreover, the TC genotype of rs7829058 in *FGFR1* was significantly associated with class III malocclusion. Many studies have supported a correlation between the rs7829058 and craniofacial phenotype. Studies by Lace et al., and Nikopensius et al. have shown significant associations between the rs7829058 in *FGFR1* and nonsyndromic cleft lip and/or palate[39] and nonsyndromic cleft palate in European populations.[40] On the basis of the above evidence, we suggest a potential correlation between class III malocclusion and rs7829058 in *FGFR1*.

The restricted sample size is one limitation of this study. A larger sample size would aid in comprehensive investigation of potential associations between newly identified SNPs and specific features. Notably, significant correlations were observed between *SOX2* (rs4434184) and *FGFR1* (rs881301) loci and the specific traits of interest in this study, which provides novel evidence establishing such associations. To validate our findings and assess the recently identified SNPs, additional studies in larger cohorts and diverse populations will be necessary.

Conclusions

In this study, genetic variations in *SOX2* and *FGFR1* were identified as potential contributors to class III malocclusion, particularly in individuals exhibiting a longer mandibular anteroposterior length and shorter maxillary anterioposterior length. Nevertheless, additional research in a larger, more diverse participant pool will be necessary to corroborate and establish the validity of these findings.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflicts of interest relevant to this article.

Ethical approval

The study was approved by the ethical committee of the College of Dentistry, University of XXX, and reference number 590422 at 10-4-2022.

Author contributions

AMB and MA participated in the study, helped interpret the data, and wrote and critically revised the text. KA and TP participated in data analysis, commented, and critically revised the manuscript. Each author is responsible for every part of the work and provided final approval. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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How to cite this article: Bahya AM, Abid MF, Aljohani KA, Porntaveetus T. Polymorphisms in *SOX2*/*FGFR1* are associated with skeletal class III maxillary and mandibular dimensions: A preliminary study. J Taibah Univ Med Sc 2025;20(1):112–119.