

Relationship between P561T and C422F polymorphisms in growth hormone receptor gene and mandibular prognathism

Sinem Bayram^a; Faruk Ayhan Basciftci^b; Ercan Kurar^c

ABSTRACT

Objective: To evaluate the allele and genotype frequencies of the P561T and C422F polymorphic sites of the growth hormone receptor (GHR) gene and the relationship between mandibular prognathism (MP) and these two single-nucleotide polymorphisms (SNPs).

Materials and Methods: A total of 99 subjects with severe skeletal Class III MP who planned to undergo orthognathic surgery and 99 subjects with Class I occlusion were examined in this study to evaluate the relationship between MP and two SNPs in exon 10 of the GHR gene. GHR was chosen as a candidate gene because growth hormone plays an important role in cartilage growth. A blood sample was used to extract genomic DNA, and the polymerase chain reaction–restriction fragment length polymorphism method was used to determine genotypes of P561T and C422F. The Minitab 14.0 packet program was used to perform statistical analysis.

Results: Allele frequencies of the C422F and P561T variants were determined. Because of the low allele frequency of the control group, statistical analysis could not be performed to test the difference between MP and control groups. Therefore, the data were combined to determine the association between the P561T polymorphism and craniofacial measurements. Effective mandibular length (condylion-gnathion) and lower face height (anterior nasal spina-menton) were associated with the P561T variant.

Conclusion: This finding supports that the GHR might be a candidate gene for mandibular morphogenesis in this population. (*Angle Orthod.* 2014;84:803–809.)

KEY WORDS: Mandibular prognathism; Single-nucleotide polymorphism; Growth hormone receptor

INTRODUCTION

Skeletal Class III malocclusion may be related to excessive mandibular growth (OMIM #176700), inefficient maxillary growth, or a combination of both, which is one of the most severe maxillofacial skeletal deformities in orthodontics.¹ The prevalence of mandibular prognathism (MP) varies among different populations. It is reported that the prevalence of MP

is the highest in East Asian populations (approximately 15%–23%), moderate in Sub-Saharan Africans (3%–8%), and lowest in white populations (0.48%–4%).^{2,3} Sari et al.⁴ and Sayin and Turkkahraman⁵ reported the incidence of Class III malocclusion in Turkish population to be 10.2% and 12%, respectively. However, these data include not only mandibular prognathic patients but also patients with maxillary retrusion.

Although it has been mentioned in a number of previous studies that various environmental factors have contributed to the development of Class III malocclusion, MP has a significant genetic component.^{6–10} Craniofacial growth in patients who show more pronounced characteristics of mandibular development is difficult to predict.¹¹

Growth hormone (GH) is a peptide hormone produced in the anterior pituitary gland that plays a major role in regulating the growth and development of the craniofacial complex.¹² GH must bind to its specific cell surface receptor (growth hormone receptor [GHR]) to commence these processes and activate various intracellular signaling pathways.¹³ GHRs are present in

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Accepted: January 2014. Submitted: September 2013.

Published Online: March 21, 2014

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Table 1. Demographic Characteristics of All Subjects

Variable	MP (n=99)		Control (n=99)		p*
Sex	Female	Male	Female	Male	
	47	52	52	47	
Age	(21.9± 2.2)		(22.4±2.2)		0.856
	22.1± 2.1	21.8± 1.9	22.2± 2	22.6± 2.1	
ANB angle	(-3.27±2.63)		(2.20±1.90)		0.000
	(-3.45±2.87)	(-3.05±2.41)	2.35±1.70	2.05±2.10	
Wits value	(-7.43±2.55)		(0.54±1.55)		0.000
	(-7.55±2.75)	(-7.15±2.25)	0.68±1.45	0.52±1.54	

* p value of 2 sample t test.

regions with cartilage growth, particularly the mandibular condyle.¹⁴

The human growth hormone receptor (*hGHR*) gene is encoded by a single gene on chromosome 5p13.1-p12, is about 87 kilobases (kb) long, and consists of 10 exons, nine of which are coding.¹⁵ Exon 2 encodes the signal peptide, exons 3–7 the extracellular domain, exon 8 the transmembrane domain, and exon 9 and part of exon 10 the intracellular domain.¹⁶ To date, 88 different GHR gene mutations have been recorded in the Human Gene Mutation Database.¹⁷ These gene mutations were found to be related to idiopathic short stature,¹⁸ Laron syndrome (GH insensitivity syndrome), severe growth retardation, and undetectable serum GH-binding protein.¹⁹

Previous research indicated possible effects of GHR gene mutations on MP, and these studies were usually carried out in Asian populations because of the high incidence (approximately 15%–23%).³ According to the results of the first report about GHR gene variants and MP, a relationship has been reported between mandibular ramus length and a heterozygous missense polymorphism of P561T.²⁰ It has been reported that adult Chinese Han individuals with a genomic polymorphism I526L of the GHR gene have a greater mandibular ramus length.²¹ In another study, 33 children with mandibular protrusion and 27 normal children were evaluated, and the authors remarked that the P561T heterozygous polymorphism may have an effect on mandibular growth during early childhood.²² Tomayasu et al.²³ examined the association between five single-nucleotide polymorphisms (SNPs) in exon 10 of GHR and MP in 167 normal Japanese adult subjects and in a multiethnic population of Han Chinese, African Americans, European Americans, and Hispanics to evaluate the effects of ethnic differences.

Although these findings support the idea that sequence variations in GHR may be associated with differences in mandibular morphology and that GH plays an important role in cartilage growth, there is no study in the literature examining the minor allele

frequencies of the P561T and C422F SNPs of GHR in a Turkish population. The aim of this study was to evaluate the allele and genotype frequencies of P561T and C422F polymorphic sites of GHR and the relationship between MP and these two SNPs in this population.

MATERIALS AND METHODS

Subjects

A total of 101 mandibular prognathic patients with severe skeletal Class III malocclusion who planned to undergo or had undergone orthognathic surgery and 99 patients with normal occlusion at the Orthodontics Department of Selcuk University Faculty of Dentistry participated in this study. Mandibular prognathic patients were chosen according to these inclusion criteria:

- Patients older than 16 years who have completed their growth and development
- Patients with severe Class III malocclusion with MP
- Patients with no congenital anomalies such as cleft lip and palate, hereditary diseases, or endocrine problems
- ANB angle and Wits value less than 0°

Two subjects were excluded from the study because of the poor quality of the lateral cephalograms. The control group consisting of 99 patients was chosen according to these inclusion criteria:

- Patients older than 16 years who have Class I occlusion with orthognathic profile
- Patients with no congenital anomalies, hereditary diseases, or endocrine problems
- ANB angle 2°–4° and Wits value of 0–2

All of the MP subjects and Class I subjects who participated in this study are unrelated and Turkish. All patients participated in a protocol approved by the Ethical Committee of Selcuk University, Faculty of Medicine, and all participating subjects gave written informed consent before blood samples were taken.

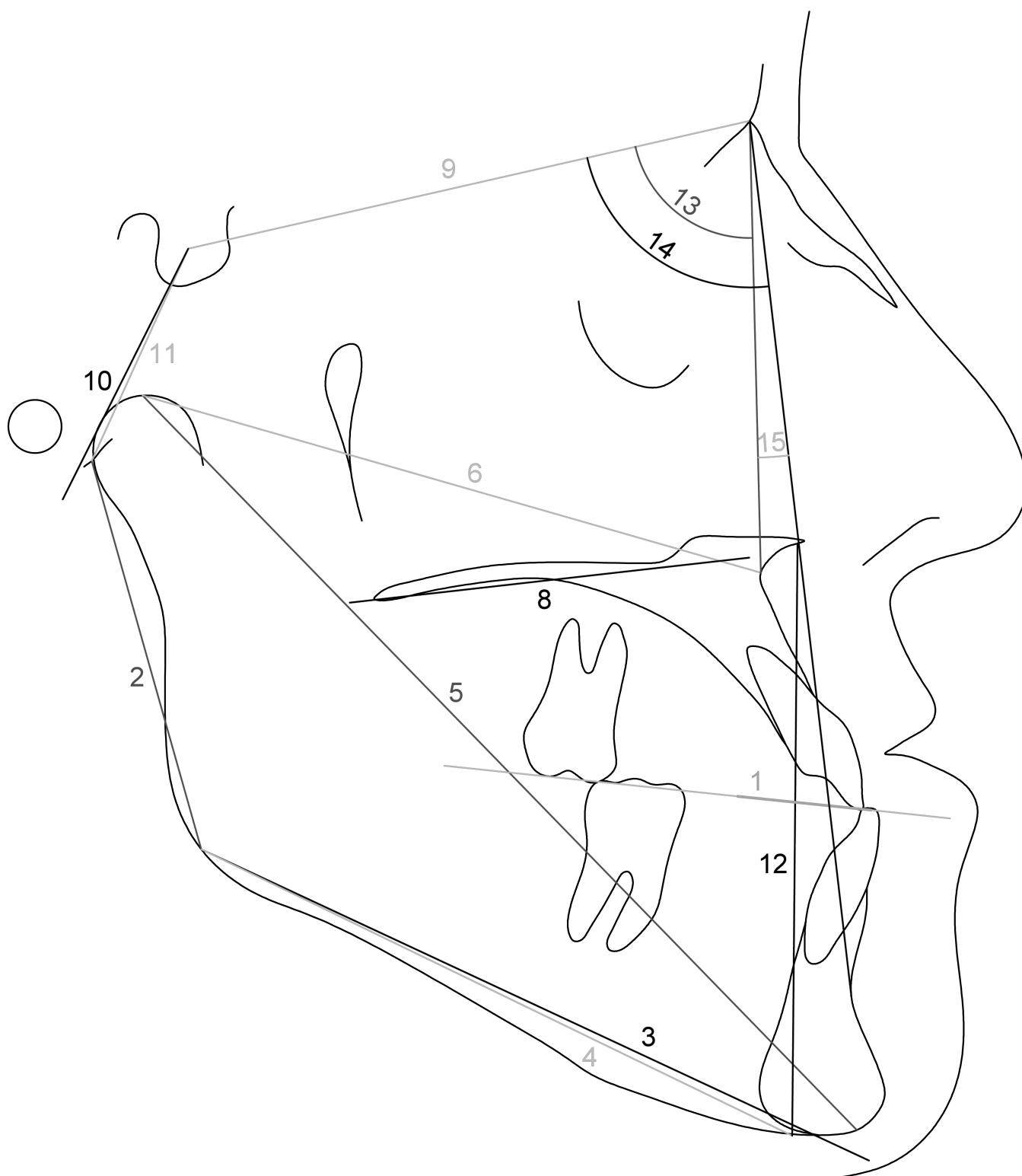


Figure 1. Linear and angular measurements. 1. Wits appraisal. 2. Ramus height (articulare-gonion; Ar-Go). 3. Corpus length (gonion-pogonion'; Go-Pog'). 4. Mandibular length (gonion-menton; Go-Me). 5. Effective length of mandible (condylion-pogonion; Co-Pog). 6. Effective midface length (condylion-A point; Co-A). 7. Maxillomandibular difference. 8. Maxillary length (A point'-pterygomaxillary point'; A'-Ptm'). 9. Sella-nasion (S-N). 10. Sella-basion (S-Ba). 11. Sella-articulare (S-Ar). 12. Lower face height (anterior nasal spina-menton; ANS-Me). 13. SNA angle. 14. SNB angle. 15. ANB angle.

Table 2. Comparison of Lateral Cephaometric Measurements between MP and Control Groups

Parameter		Mean ^a / Median ^b	Minimum ^c / Q ₁ ^d	Maximum ^e / Q ₃ ^f	p ^g /p ^h
SNB	MP	82.79±3.89 ^a	75.80 ^c	92.50 ^e	.000 ^{g****}
	Control	78.44±2.97 ^a	71.40 ^c	83.60 ^e	
Ar-Go	MP	48.96±4.82 ^a	41.50 ^c	60.00 ^e	.013 ^{g*}
	Control	46.00±5.00 ^a	34.90 ^c	55.50 ^e	
Go-Pog'	MP	77.35±5.44 ^a	68.00 ^c	90.60 ^e	.000 ^{g****}
	Control	73.95±4.84 ^a	62.40 ^c	82.50 ^e	
Go-Me	MP	72.74±4.89 ^a	64.50 ^c	84.20 ^e	.000 ^{g****}
	Control	67.29±4.95 ^a	57.40 ^c	78.00 ^e	
ANS-Me	MP	69.66±6.78 ^a	57.70 ^c	88.80 ^e	.003 ^{g**}
	Control	66.86±6.51 ^a	53.80 ^c	81.50 ^e	
SNA	MP	79.53±3.34 ^a	72.40 ^c	85.90 ^e	.277 ^g
	Control	80.91±2.96 ^a	73.90 ^c	85.70 ^e	
A'-Ptm'	MP	47.80 ^b	38.00 ^d	42.40 ^f	.288 ^h
	Control	48.50 ^b	46.30 ^d	51.70 ^f	
Co-A	MP	82.10 ^b	80.10 ^d	85.30 ^f	.559 ^h
	Control	84.00 ^b	80.10 ^d	86.80 ^f	
ANB	MP	-2.60 ^b	-4.60 ^d	-1.30 ^f	.000 ^{h***}
	Control	2.70 ^b	1.50 ^d	3.40 ^f	
Wits	MP	-8.40 ^b	-9.20 ^d	-6.00 ^f	.000 ^{h****}
	Control	0.54 ^b	0.20 ^d	1.90 ^f	
CoGn-CoA	MP	39.80 ^b	38.00 ^d	42.40 ^f	.000 ^{h****}
	Control	27.70 ^b	24.40 ^d	29.80 ^f	
S-N	MP	65.80 ^b	64.20 ^d	68.80 ^f	.762 ^h
	Control	66.40 ^b	63.80 ^d	68.90 ^f	
S-Ba	MP	39.60 ^b	37.30 ^d	42.00 ^f	.957 ^h
	Control	38.90 ^b	37.90 ^d	41.30 ^f	
S-Ar	MP	34.10 ^b	31.50 ^d	36.00 ^f	.039 ^{h*}
	Control	34.80 ^b	32.40 ^d	37.60 ^f	
Co-Gn	MP	122.10 ^b	119.60 ^d	124.40 ^f	.000 ^{h****}
	Control	111.20 ^b	107.10 ^d	115.90 ^f	

* p<0.05; ** p<0.01; *** p<0.001; **** p<0.0001.

Table 3. Hardy-Weinberg Equilibrium

Population	Locus	df	χ^2	Probability	Significance ^a
MP	C422F	1	0.010	.919	NS
MP	P561T	1	0.066	.797	NS
Control	C422F	1	0.003	.960	NS
Control	P561T	1	0.003	.960	NS

^a NS indicates nonsignificant.

Lateral cephalometric radiographs and blood samples were obtained from 198 subjects. Demographic characteristics of all participants are shown in Table 1.

Genotyping of P561T and C422F Codons

Whole blood was collected in a sterile tube with K3-EDTA and stored at -20°C for later examination. Genomic DNA was extracted from whole blood samples using a standard phenol/chloroform method.²⁴ The partial sequence of exon 10 of the GHR gene, which encodes the cytoplasmic domain of GHR, was amplified by polymerase chain reaction (PCR). The PCR amplification was performed in a 30-μL PCR volume containing 100 ng template DNA, 0.750 units *Taq* polimeraz (Pittsburgh, PA, USA) 1× Mg++ free PCR buffer (Fermentas), 200 μM dNTP (Fermentas), 1.5 mM MgCl++, 10 pMol of each primers (5'-GGGAAGCAGATCTCTTATGC-3' and 5'-TAGTCTGGGACAGGCATCT-3'). A touchdown-PCR profile was carried out on the BioRad MyCycler-Thermal Cycler (BioRad Laboratories Inc, Hercules, Calif). After agarose gel electrophoresis, the 10-μL PCR-amplified DNA was digested with *Eco147I* (*StuI*) and *Cac8I*, which digests the GHR gene at codon 561 and 422, respectively. Digested PCR products were electrophoresed on 1.5% gels and visualized after ethidium bromide staining and allele genotypes of 561 and 422 codons were determined.

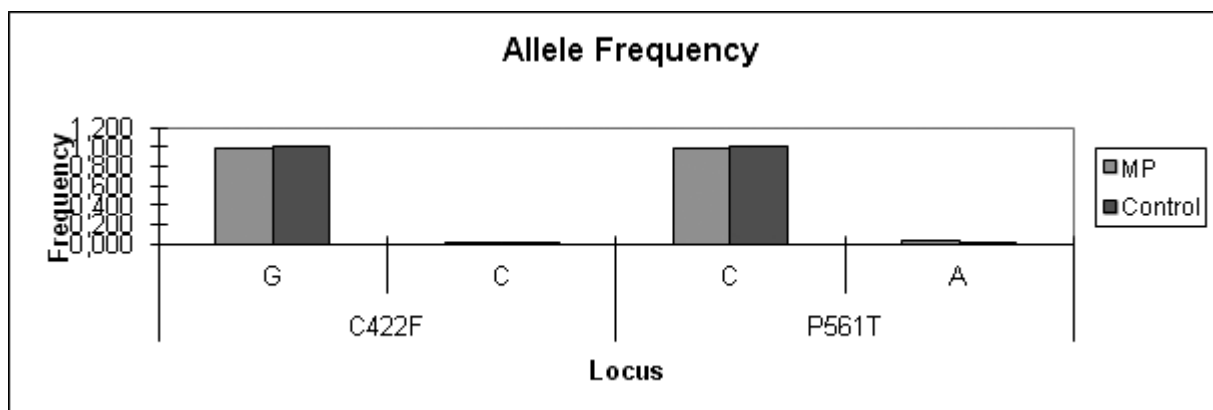
**Figure 2.** Allele and genotype frequencies of P561T and C422F regions.

Table 4. Association Between P561T Mutation and Mandibular and Maxillary Measurements

P561T	n	Ar-Go, mm		Go-Pog', mm		Go-Me, mm		Co-Gn, mm		A'-Ptm', mm		Co-A, mm	
		Median	P	Median	P	Median	P	Median	P	Median	P	Median	P
CC	94	48.5	.164	77.4	.637	72.0	.962	122.0	.019**	47.8	.587	82.1	.438
CA	5	53.1		76.6		72.4		126.0		48.3		82.2	

** $P < .01$; Mann-Whitney U -test.

Craniofacial Measurements

Lateral cephalometric radiographs were used to classify subjects into two groups and to determine the maxillary, mandibular, and cranial lengths. Cephalometric radiographs of 198 subjects were traced and analyzed with Quick Ceph Studio (Quick Ceph Systems, San Diego, Calif) by the same researcher. Twenty randomly selected radiographs were retraced and remeasured after 2 weeks, and method error was calculated according to Dahlberg's formula.²⁵ Angular and linear measurements used in our study are shown in Figure 1.

Statistical Analysis

The experiment, with the current sample size (99 MP, 99 control), was established to detect the differences with 95% chance at the usual level of statistical significance ($\alpha = .05$) for the ANB angle to differentiate groups. The Minitab 14.0 (Minitab Inc, State College, Penn) packet program was used to perform all statistical analyses. Allele and genotype frequencies of P561T and C422F polymorphic sites and Hardy-Weinberg equilibrium were calculated using the GenAlEx6 (Peakall and Smouse 2006) program. The Mann-Whitney U -test was performed to analyze the association between polymorphisms and craniofacial measurements.

RESULTS

A comparison of the lateral cephalometric measurements between the MP and the control groups is shown in Table 2. If the data showed normal distribution, a two-sample t -test was used to analyze the difference between two groups; otherwise, the Mann-Whitney U -test was performed. Although there was no difference between the two groups in maxillary measurements, all mandibular measurements were

statistically significant between the two groups, indicating Class III malocclusion with MP.

There was no subject with the C422F and P561T homozygous polymorphism. Two of the MP subjects and one of the control subjects had the C422F heterozygous polymorphism. In 561T locus, five MP subjects and one of the control subjects were heterozygous. Allele and genotype frequencies are shown in Figure 2. C422F and P561T genotypes were also confirmed by forward and reverse sequencing, indicating that PCR restriction fragment length polymorphism analyses were in concordance.

The haplotype distribution of both polymorphic sites was evaluated with the Hardy-Weinberg equilibrium and found to be stable ($P > .05$; Table 3). Because of the lack of heterozygous genotype at both the C422F and P561T codons in the control group, statistical analysis could not be performed to evaluate the difference between the two groups. Therefore, the data were integrated to define the effect of P561T SNP on craniofacial growth.

According to the Mann-Whitney U statistical analysis, two craniofacial measurements including effective mandibular length (condylion-gnathion [Co-Gn]) and lower face height (anterior nasal spina-menton [ANS-Me]) were found to be associated with the P561T variant ($P < .05$; Tables 4 and 5).

DISCUSSION

Class III malocclusion with MP has attracted the attention of many researchers because it is frequently encountered in the orthodontic clinic and relatively difficult to treat. MP is important to the understanding of the molecular pathogenesis of skeletal Class III malocclusion. In particular, etiological factors of MP described in the literature⁵⁻⁹ and genetic susceptibilities on mandible growth have been defined in recent studies.^{3,20-23,27-31} Although none of the genomewide

Table 5. Association Between P561T Mutation and Craniofacial Measurements

P561T	n	S-N, mm		S-Ba, mm		S-Ar, mm		ANS-Me, mm	
		Median	P	Median	P	Median	P	Median	P
CC	94	65.8	.743	39.7	.943	34.3	.121	69.4	.01**
CA	5	66.8		39.1		32.8		73.6	

** $P < .01$; Mann-Whitney U -test.

studies found a significant marker on chromosome 5 for MP, we analyzed two SNPs (C422F and P561T) located on exon 10 of the GHR in Turkish subjects. We chose GHR as a candidate gene because GH plays an important role in cartilage growth and GHRs were identified in the mandibular condyle.³¹ These polymorphisms can cause variance in the signal transduction by affecting the expression of IGF-1, which can affect endochondral bone growth.

The first polymorphism of *hGHR* was defined in a study consisting of 76 patients with Laron syndrome, and heterozygous polymorphisms were associated with this syndrome.³² Initially, allele and genotype frequencies of P561T and C422F polymorphic sites of the GHR gene in the Turkish population were determined in the present study. No difference in the frequency of the C422F and P561T variants was found between the two groups. The lack of heterozygous polymorphism at the C422F region in both the MP and control group showed that this polymorphism was not associated with MP in this population. Positive correlations were observed between body height and all mandibular measurements, but it was the highest in effective mandibular length. In this study, an association was also determined between the P561T polymorphism and effective mandibular length and lower face height. Even though a previous study²³ of 167 Japanese adults reported an association between the C422F locus and mandibular ramus height, no such association was observed in this study. Findings of a study of 95 Chinese adult subjects did, however, agree with our findings at the C422F locus.²¹ Zhou et al.²¹ reported an association between the I526L polymorphism of the GHR and mandibular ramus height. The findings from both Turkish and Chinese populations did not show any relationship between mandibular length and C422F polymorphism, which is different from findings in the Japanese population. This discrepancy may be related to the ethnic differences of the groups.

According to the first study examining the association between MP and the single polymorphism of GHR, the P561T heterozygous polymorphism and ramus height were found to be associated.²⁰ Sasaki et al.²² reported differences in all cranial measurements except maxillary length between heterozygous subjects and subjects without polymorphism. Another study of 167 Japanese subjects reported an association between the P561T variant and mandibular ramus height.²³ Unlike those results, we found that the P561T variant was associated with effective mandibular length and lower face height in the Turkish population. Previous studies showed that ethnicity is a risk factor for MP.^{2,3} According to these results, it can be thought that GHR can affect not only the longitudinal development but also the horizontal development of the

mandible. Therefore, statistically significant results can be obtained in ramus height if the sample size is increased. From these data, it can be concluded that there is a relationship between the GHR polymorphism and mandibular growth.

In this study, subjects with the genotype CA had a longer mandible and a higher lower face than those with the genotype CC, whereas cranial measurements of heterozygous subjects tend to be lower in other studies.^{20,22,23}

Although a number of etiological factors have been found to contribute to mandibular morphology, genetic factors play a significant role.³³ Bailey³⁴ found that 30 genes can affect the morphogenesis of the mandible in mice. Different sites of GHR and new candidate genes must be examined to explain the relationship between different phenotypes and genetic variants as well as to understand the effects of genetic factors in the multifactorial etiology of MP.

The advantage of determining candidate genes for MP is to predict mandibular length in growing patients by using DNA from a simple blood sample. This genotyping could also be done in a less invasive manner from buccal cells collected in saliva, but the quality and quantity of DNA were inefficient in this method. We planned an association study to investigate the relationship between GHR and MP, but a linkage study will be helpful to understand the effect of this gene and its promoter region on MP.

In the past decade, interest in growth factors and other agents that may control cartilage proliferation and differentiation has increased. In the near future, this knowledge could be used for growth regulation in patients with MP and might be useful for orthodontic diagnosis and orthopedic treatment of the mandible.

CONCLUSIONS

- C422F and P561T heterozygous polymorphisms of the GHR gene did not justify the difference between the MP group and control group in this population.
- Subjects with the CA genotype of P561T have a greater effective mandibular length (Co-Gn) and lower face height (ANS-Me) than those with genotype CC.
- This finding supports that the GHR might be a candidate gene for mandibular morphogenesis.

ACKNOWLEDGMENTS

This study was produced from the PhD thesis of Sinem Bayram and supported by Selcuk University Research Projects (11202032).

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