

## Association of the P561T and C422F polymorphisms of the growth hormone receptor gene with facial dimensions.

Asociación de los polimorfismos P561T y C422F del gen receptor de la hormona del crecimiento con dimensiones faciales.

Kazem Dalaie.<sup>1</sup>  
Mohammad Behnaz.<sup>1</sup>  
Samin Banihashem.<sup>2</sup>  
Saeed Reza Motamedian.<sup>1</sup>  
Vahid Reza Yassaee.<sup>3</sup>  
Feyzollah Hashemi-Gorji.<sup>3</sup>  
Arash Khojasteh.<sup>4</sup>

**Affiliations:** <sup>1</sup>Department of Orthodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran. <sup>2</sup>Private practice, Zahedan, Iran. <sup>3</sup>Genomic Research Center, Department of Medical Genetics, Faculty of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. <sup>4</sup>Department of Oral and Maxillofacial Surgery, School of Dentistry, Shahid Beheshti University of Medical Sciences, Tehran, Iran.

**Corresponding author:** Saeed Reza Motamedian. Department of Orthodontics, School of Dentistry, Shahid Beheshti University of Medical Sciences, Daneshjou Boulevard, Evin, Tehran, Iran, P.O. 19839. Phone: (98) 9125176083. Email: motamedian@sbmu.ac.ir

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**Abstract:** Background: Growth hormone plays a significant role in determining craniofacial morphology. Mutations of its receptor gene might be associated with mandibular prognathism (MP). Purpose: The aim of the current study was to evaluate growth hormone receptor (GHR) gene polymorphisms in relation to facial dimensions. Material and Method: The study enrolled 65 participants with class III profile in MP group and 60 orthognathic control participants. Genomic DNA was extracted from a blood sample from the patients and the P561T and C422F polymorphisms of GHR gene were screened by PCR-RFLP method followed by Sanger sequencing of randomly selected samples to validate the genotyping results. Chi square was used to compare distribution of polymorphism in MP and control groups ( $p < 0.05$ ). Results: Heterozygous P561T mutation was found in 10.77% and 8.33% of MP and control groups, respectively ( $p = 0.644$ ) while none of the subjects had the C422F mutation. Sanger sequencing confirmed the genotyping results from the PCR-RFLP method. P561T polymorphism was significantly associated with ramus and lower facial height in MP patients and with ramus height in orthognathic patients ( $p < 0.05$ ). Conclusion: The results indicate that the P561T polymorphism of the GHR gene is associated with the vertical dimension of the mandible in an Iranian population.

**Keywords:** Growth hormone; polymorphism, genetic; mandible; malocclusion; Malocclusion, Angle Class III; Iran.

**Resumen:** Antecedentes: La hormona del crecimiento desempeña un papel importante en la determinación de la morfología craneofacial. Las mutaciones de su gen receptor podrían estar asociadas con el prognatismo mandibular (PM). Propósito: El objetivo del presente estudio fue evaluar dos polimorfismos del gen del receptor de la hormona del crecimiento (RHC) en relación con las dimensiones faciales. Materiales y Métodos: El estudio incluyó a 65 participantes con perfil de clase III en el grupo MP y 60 participantes de control ortognático. El ADN genómico se extrajo de una muestra de sangre de los pacientes y los polimorfismos P561T y C422F del gen RHC se seleccionaron mediante el método PCR-RFLP seguido de la secuenciación por Sanger de muestras seleccionadas al azar para validar los resultados del genotipo por RFLP. El test chi cuadrado se utilizó para comparar la distribución del polimorfismo en el grupo MP y grupo control ( $p < 0.05$ ). Resultados: Se encontró mutación heterocigota P561T en 10.77% y 8.33% de los grupos PM y control, respectivamente ( $p = 0.644$ ) mientras que ninguno de los sujetos tenía la mutación C422F. La secuenciación de Sanger confirmó los resultados de genotipado por el método PCR-RFLP. El polimorfismo P561T se asoció significativamente con la rama y la altura facial más baja en pacientes con PM y con la altura de la rama en pacientes ortognáticos ( $p < 0.05$ ). Conclusión: Los resultados indican que el polimorfismo P561T del gen RHC está asociado con la dimensión vertical de la mandíbula en una población iraní.

**Palabras Clave:** Hormona del Crecimiento; polimorfismo genético; mandíbula; maloclusión; maloclusión de Angle clase III; Irán.

## INTRODUCTION.

The clinical presentation of Class III malocclusion is highly heterogeneous and as such, the term "Class III" is used to describe pure mandibular prognathism (MP), maxillary retrognathism, or a combination of both.<sup>1-3</sup> When this type of craniofacial skeletal deformity is severe enough, it can lead to both social and functional disability.<sup>4-6</sup> The prevalence of class III malocclusion is reported to be high in Asians and relatively lower in Caucasians.<sup>7,8</sup> Based on a recent meta-analysis, the prevalence of class III malocclusion in Iranian children is 6.01%.<sup>9</sup>

It seems that this malocclusion is a poly-genetic anomaly caused by interaction of both environmental and genetic factors; however MP seems to be more related to genetic factors.<sup>10</sup> Although familial studies revealed an autosomal dominant inheritance of MP,<sup>11</sup> still it is difficult for clinicians to predict mandibular overgrowth.<sup>2</sup>

Growth hormone (GH) is a peptide secreted by the anterior pituitary gland and plays a considerable role during childhood and adolescence and also in craniofacial complex growth and development.<sup>12</sup> GH binds to its specific receptor in order to initiate internal cell signaling.<sup>13</sup>

The growth hormone receptor (GHR) is found in areas with endochondral growth such as mandibular condyle.<sup>14</sup> The GHR gene (NM\_000163.4) is located on chromosome 5 and has 10 exons of which nine exons encode the receptor (NP\_000154.1). Until now, 88 different mutations of GHR gene have been recorded.<sup>15</sup> The effect of some of these mutations on mandibular growth has been investigated. Yamaguchi *et al.*,<sup>16</sup> reported the association between mandibular height growth and the P561T variant in a Japanese population. In addition, the C422F and P561T polymorphisms of the GHR gene were reported to be related to effective mandibular length and lower face height in a Turkish population.<sup>17</sup> Tomoyasu *et al.*,<sup>18</sup> evaluated the C422F, S473S, P477T, I526L and P561T polymorphisms in a Japanese population and reported that only the C422F and P561T mutations were associated with ramus height. The same results were reported in a Korean population.<sup>19</sup>

However, in a Japanese population study using cone beam computed tomography (CBCT), the P561T mutation was not associated with cephalometric measurements of the mandible.<sup>20</sup> Mandibular growth was negatively influenced by the P561T polymorphism during early childhood in a

study by Sasaki *et al.*<sup>21</sup> These differences among studies are partly due to the fact that the P561T variation occurs at different frequencies in different ethnic backgrounds (*i.e.* less than 1% prevalence in Caucasian populations and 5.2-15% in Japanese populations).<sup>16-21</sup>

Due to this inconsistency of the results of previous studies and the necessity of assessment of genetic factors in different populations, the current study was performed to investigate the prevalence of the P561T and C422F polymorphisms and their association with mandibular dimensions in an Iranian population.

## MATERIALS AND METHODS.

### Study designs and population

This observational case control study was performed at the Genomic Research Center of Shahid Beheshti University of Medical Sciences, Tehran, Iran during 2015-16. Samples were collected from orthodontic patients at four private clinics in Tehran and patients who had orthognathic surgery in Taleghani and Shariati hospitals, both in Tehran. The study protocol was approved by the ethical committee of the university and an informed consent in accordance with Helsinki Declaration was taken from all participants.

This study included two groups of subjects: Patients with a class III appearance who were candidate for orthognathic surgery were included in the mandibular prognathism (MP) group, and patients with class I appearance served as control group. Only patients older than 16 years were included. In the MP group, patients with skeletal class III appearance (ANB and Wits less than zero) and mandibular prognathism (SNB>82°) were included while patients with class III appearance due to maxillary retrognathism (SNA<80°) were excluded. In the control group, patients with orthognathic appearance (2° ≤ ANB ≤ 4° and 0 mm ≤ Wits ≤ 2mm) were included. In both groups, patients with syndromes, endocrine or systemic diseases that affect craniofacial morphology were excluded.

A sample size of 57 subjects in each group was calculated considering proportion of polymorphism 0.2 and 0.02 in case and control groups, respectively, with confidence level of 0.95 and 80% power.

Sample collection, DNA extraction and genotyping

Five milliliters of fresh venous blood were obtained

from the participants and stored in EDTA/K tubes, kept at  $-20^{\circ}\text{C}$  until DNA extraction. Genomic DNA was prepared using salting out method.<sup>22</sup> The DNA concentration was estimated at 260 nm using a spectrophotometer.

Genotyping of the partial sequence of exon 10 of the GHR gene was performed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method as previously described, with optimized PCR condition.<sup>17</sup> PCR amplifications were performed in a total volume of 25  $\mu\text{l}$ , containing 100ng template DNA, 12.5  $\mu\text{l}$  Taq DNA Pol. 2\_Master mix Red (Ampliqon, Denmark), 0.5  $\mu\text{l}$  of each primer pair (10pmol/

$\mu\text{l}$ ) and distilled deionized water up to 25  $\mu\text{l}$ . PCR thermal parameters consisted of initial denaturation at  $95^{\circ}\text{C}$  for 3 min, and 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 30s, annealing of primers  $57^{\circ}\text{C}$  for 30s and extension at  $72^{\circ}\text{C}$  for 40s followed by a final extension step at  $72^{\circ}\text{C}$  for 5 min. The following primers were used to assess polymorphism on rs6182 and rs6184: 5'-GGGAAGCAGATCTCTTATGC-3' and 5'-TATAGTCTGGGACAGGCATCT-3'.

Following digestion of the amplicon DNA with Eco147I and Cac8I, which digests the GHR gene at codon 561 and 422, respectively, the products were separated by electrophoresis on a 2.5% agarose gel and finally allele genotypes at 561 and 422 codons were recorded. Then, the PCR products of randomly selected samples were sequenced to corroborate the genotyping data by RFLP method

#### Cephalometric analysis

Lateral cephalograms of patients were traced using Dolphin software version 10 (Patterson Inc., Chatsworth, CA) by two trained examiners. The average values of the measurements by the two examiners were calculated. Intraclass correlation coefficient (ICC) was used to assess inter-examiner reliability. All the cephalograms had proper quality and a ruler for linear measurement calibration. Linear measurements included Wits appraisal, ramus height (articulare-gonion; Ar-Go), mandibular length (gonion-menton; Go-Me), effective length of mandible (condylion-gnathion; Co-Gn), anterior cranial base length (sella-nasion; S-N), lower face height (anterior nasal spina-menton; ANS-Me) as well as Wits appraisal and angular measurements comprising SNA, SNB and ANB. In order

to assess intra-examiner reliability,<sup>15</sup> measurements were performed by one of the examiners after two weeks and ICC was calculated.

#### Statistical analysis

Cephalometric measurements were calculated as mean and standard deviation (SD) while polymorphism occurrence was reported as percentage. In order to compare polymorphism between groups a chi square analysis was used and to compare cephalometric measurements between groups' independent samples the Student's t-test and Mann-Whitney U test were applied. Statistical analysis was performed using SPSS version 21 (SPSS, Chicago, IL) with a significant level of 0.05.

### RESULTS.

A total of 65 patients (23 males and 42 females) with a mean age of  $24.64 \pm 5.86$  years were included in the MP group, and 60 patients (22 males and 38 females) with a mean age of  $22.41 \pm 7.49$  years were included in the control group. No significant difference was found between the two groups regarding age and gender ( $p > 0.05$ ). Mean cephalometric measurements of both groups are demonstrated and compared between MP and control groups in Table 1. All measurement except Go-Me and S-N were significantly different between the two groups. ICC was 0.879 and 0.812 for intra-examiner and inter-examiner reliability, respectively, which are considered an excellent correlation.

No subject with a C422F polymorphism was found. Regarding P561T, no homozygous polymorphisms were detected while 7 (10.77%) and 5 (8.33%) cases of heterozygous polymorphism was found in MP and control groups, respectively. The difference was not statistically significant ( $p = 0.644$ ). In order to combine results of polymorphism with cephalometric analysis, average measurements of cephalometric indices were compared based on presence of polymorphisms.

The results showed that the P561T polymorphism was significantly associated with larger Ar-Go and ANS-Me in MP group and larger Ar-Go in control group ( $p < 0.05$ ). Further, the patients were divided based on P561T variation and the results showed the polymorphism is related with larger Ar-Go and ANS-Me in the whole study population, regardless of group. (Table 2)

**Table 1.** Comparison of cephalometric measurements between MP and control groups.

Index	Group	Mean	Std. Deviation	p-value
Witts	Mandibular prognathism	-6.7538	4.32468	<0.001
	Control	.8400	.62415	
ANB	Mandibular prognathism	-3.2292	2.28129	<0.001
	Control	1.0200	.62675	
SNA	Mandibular prognathism	83.4677	4.37822	<0.001
	Control	80.9583	.81184	
SNB	Mandibular prognathism	86.6031	3.82949	<0.001
	Control	80.0133	.60013	
Ar-Go	Mandibular prognathism	46.6077	2.83578	<0.001
	Control	44.7383	2.05782	
Go-Me	Mandibular prognathism	67.3985	7.37990	.339
	Control	66.4467	2.90980	
ANS-Me	Mandibular prognathism	66.2615	3.94128	.026
	Control	64.8800	2.77390	
Co-Gn	Mandibular prognathism	134.5485	4.33949	<0.001
	Control	111.4300	5.09151	
S-N	Mandibular prognathism	64.9600	3.49409	.198

**Table 2.** Cephalometric measurements based on P561T polymorphism.

Index	561 region	Mandibular prognathism			Control			Total		
		Mean	SD	p-value	Mean	SD	p-value	Mean	SD	p-value
Ar-Go	C/A	50.2286	2.74695	.001	47.1000	1.15542	.000	48.93	2.68	<0.001
	C/C	46.1707	2.53585		44.5236	1.99109		45.37	2.42	
Go-Me	C/A	63.9000	4.67761	.172	65.0000	2.57585	.282	64.36	3.83	.067
	C/C	67.8207	7.56067		66.5782	2.92370		67.22	5.80	
ANS-Me	C/A	70.9429	2.35291	<0.001	66.1600	3.42170	.145	68.95	3.65	<0.001
	C/C	65.6966	3.72054		64.7636	2.71555		65.24	3.29	
Co-Gn	C/A	132.8714	4.15039	.204	113.1000	4.81041	.449	124.63	11.02	.890
	C/C	134.7509	4.35241		111.2782	5.13109		123.33	12.70	
S-N	C/A	64.4286	3.73529	.688	66.9800	3.69959	.836	65.49	3.78	.927
	C/C	65.0241	3.49292		65.6145	3.06168		65.31	3.29	

## DISCUSSION.

The present study investigated a correlation of mandibular morphology with GHR gene polymorphisms in an Iranian population. The reason for choosing C422F and P561T polymorphisms of the GHR gene was that a recent study in Turkey, which has a relatively similar population to Iran, showed an association between these polymorphisms and facial dimensions.<sup>17</sup> The GHR gene was selected due to its influence on craniofacial growth. Laron syndrome (GH insensitivity syndrome), which is

associated with underdeveloped facial bones, is caused by mutation in the GHR gene.<sup>23</sup> In addition, GHR deficiency would result in relatively poor vertical growth of the face.<sup>24</sup>

The results showed that none of the subjects had genotype GT of variants rs6182 (C422F) while genotype CA of rs6184 (P561T) was found in 10.77% and 8.33% patients in the MP and control groups, respectively. Although the P561T variation was more common in the MP group, the difference was not significant. This finding is in harmony with previous studies.<sup>21</sup> Previously, P561T heterozygosity



was reported in approximately 5.2%-15% of a Japanese population,<sup>18,20,21,25</sup> 1% of a Turkish population<sup>17</sup> and more than 10% of a Chinese population<sup>26</sup> with normal occlusion while it was found in 15% of Japanese<sup>21</sup> and 5% of a Turkish population<sup>17</sup> with class III malocclusion. In contrast to the results of the current study, the C422F polymorphism was found in 5.9% of Japanese<sup>18</sup> and 1-2% of Turkish<sup>17</sup> people.

A noteworthy finding was relatively larger ramus and lower facial height in association with P561T polymorphism. This polymorphism is defined by transversion of cytosine to adenine (C to A) which changes the codon 561 from proline to threonine (P561T) and alters the intracellular domain of the GHR. Similar to these results, a study in a Turkish population of 200 normal and class III Angle patients showed a relationship between the P561T variant of the GHR gene and effective mandibular length and lower face height.<sup>17</sup>

In contrast, Sasaki *et al.*,<sup>21</sup> assessed the P561T polymorphism in 60 Japanese children (age range 3 to 13 years old) and revealed that this GHR gene mutation is related with decreased mandibular dimensions. However, in their study patients' growth was not evenly distributed. In another study, the P561T polymorphism was associated with smaller mandibular ramus height in 167 Japanese individuals with normal occlusion.<sup>18</sup>

A study of Kang *et al.*,<sup>19</sup> in a group of 159 Koreans with normal occlusion also revealed an association between P561T polymorphism and smaller ramus height. This inconsistency might be due to ethnic differences and higher prevalence of class III Angle in Asian populations compared to Middle Easters.

It has been demonstrated that Iranians have a relatively steeper mandibular plane resulting in higher lower facial height.<sup>27,28</sup> On the other hand, Nakawaki *et al.*,<sup>20</sup> used CBCT images to measure mandible dimensions and reported that there was no association between the P561T polymorphism and mandible size.

One of the limitations of this study was small sample size relative to the prevalence of the polymorphism in the target population. This study could not find a significant correlation between MP and P561T polymorphism, which might be due to the study power rather than a real lack of correlation.

Clinical significance of the results includes pediatric

patients with a class III tendency, in whom the orthodontist should estimate mandibular growth potential and decide whether to start orthodontic treatment and class III camouflage or defer treatment until adolescence for orthognathic surgery. In future genetic tests in conjugation with other indices could be used to estimate mandibular growth potential.<sup>29,30</sup> For this purpose, longitudinal studies evaluating the presence polymorphisms in related genes and mandibular growth pattern are required.

## CONCLUSION.

Within the limitations of this study, it could be concluded that there was no difference on the incidence of heterozygous mutation of GHR gene at codon 561 between MP and control groups.

However, the P561T polymorphism was significantly associated with ramus and lower facial height in MP patients and with ramus height in orthognathic patients. No mutation at codon 422 of the GHR gene was found.

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**Conflict of interests:** The authors declare no conflict of interest of any kind.

**Ethics approval:** Study protocol was approved by ethical committee of Shahid Beheshti University of Medical Sciences and an informed consent in accordance with Helsinki Declaration was taken from all participants.

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## REFERENCES.

1. Ngan P, Moon W. Evolution of Class III treatment in orthodontics. *Am J Orthod Dentofacial Orthop.* 2015;148(1):22-36.
2. Zere E, Chaudhari PK, Sharan J, Dhingra K, Tiwari N. Developing Class III malocclusions: challenges and solutions. *Clin Cosmet Investig Dent.* 2018;10:99-116.
3. Rongo R, D'Anto V, Bucci R. Skeletal and dental effects of Class III orthopaedic treatment: a systematic review and meta-analysis. *J Oral Rehabil.* 2017;44(7):545-62.
4. Zhou Y, Li Z, Wang X, Zou B, Zhou Y. Progressive changes in patients with skeletal Class III malocclusion treated by 2-jaw surgery with minimal and conventional presurgical orthodontics: A comparative study. *Am J Orthod Dentofacial Orthop.* 2016;149(2):244-52.
5. Palomares NB, Celeste RK, Miguel JA. Impact of orthosurgical treatment phases on oral health-related quality of life. *Am J Orthod Dentofacial Orthop.* 2016;149(2):171-81.
6. Grewal H, Sapawat P, Modi P, Aggarwal S. Psychological impact of orthodontic treatment on quality of life - A longitudinal study. *Int Orthod.* 2019;17(2):269-76.
7. de Frutos-Valle L, Martin C, Alarcon JA, Palma-Fernandez JC, Iglesias-Linares A. Subclustering in Skeletal Class III Phenotypes of Different Ethnic Origins: A Systematic Review. *J Evid Based Dent Pract.* 2019;19(1):34-52.
8. Alhammadi MS, Halboub E. Global distribution of malocclusion traits: A systematic review. *Dental Press J Orthod.* 2018;23(6):40.e1-e10.
9. Akbari M, Lankarani KB, Honarvar B, Tabrizi R, Mirhadi H, Moosazadeh M. Prevalence of malocclusion among Iranian children: A systematic review and meta-analysis. *Dent Res J.* 2016;13(5):387-95.
10. Doraczynska-Kowalik A, Nelke KH, Pawlak W, Sasiadek MM, Gerber H. Genetic Factors Involved in Mandibular Prognathism. *J Craniofac Surg.* 2017;28(5):e422-e31.
11. Liu H, Wu C, Lin J, Shao J, Chen Q, Luo E. Genetic Etiology in Nonsyndromic Mandibular Prognathism. *J Craniofac Surg.* 2017;28(1):161-9.
12. Ramirez-Yanez GO, Smid JR, Young WG, Waters MJ. Influence of growth hormone on the craniofacial complex of transgenic mice. *Eur J Orthod.* 2005;27(5):494-500.
13. Pilecka I, Whatmore A, Hooft van Huijsduijnen R, Destenaves B, Clayton P. Growth hormone signalling: sprouting links between pathways, human genetics and therapeutic options. *Trends Endocrinol Metab.* 2007;18(1):12-8.
14. Simmons KE. Growth hormone and craniofacial changes: preliminary data from studies in Turner's syndrome. *Pediatrics.* 1999;104(4 Pt 2):1021-4.
15. Stenson PD, Mort M, Ball EV, Shaw K, Phillips AD, Cooper DN. The Human Gene Mutation Database: building a comprehensive mutation repository for clinical and molecular genetics, diagnostic testing and personalized genomic medicine. *Hum Genet.* 2014;133(1):1-9.
16. Yamaguchi T, Maki K, Shibasaki Y. Growth hormone receptor gene variant and mandibular height in the normal Japanese population. *Am J Orthod Dentofacial Orthop.* 2001;119(6):650-3.
17. Bayram S, Basciftci FA, Kurar E. Relationship between P561T and C422F polymorphisms in growth hormone receptor gene and mandibular prognathism. *Angle Orthod.* 2014;84(5):803-9.
18. Tomoyasu Y, Yamaguchi T, Tajima A, Nakajima T, Inoue I, Maki K. Further evidence for an association between mandibular height and the growth hormone receptor gene in a Japanese population. *Am J Orthod Dentofacial Orthop.* 2009;136(4):536-41.
19. Kang EH, Yamaguchi T, Tajima A, Nakajima T, Tomoyasu Y, Watanabe M, Yamaguchi M, Park SB, Maki K, Inoue I. Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population. *Arch Oral Biol.* 2009;54(6):556-62.
20. Nakawaki T, Yamaguchi T, Isa M, Kawaguchi A, Tomita D, Hikita Y, Suzuki-Tomoyasu Y, Adel M, Ishida H, Maki K, Kimura R. Growth hormone receptor gene variant and three-dimensional mandibular morphology. *Angle Orthod.* 2016;87(1):68-73.
21. Sasaki Y, Satoh K, Hayasaki H, Fukumoto S, Fujiwara T, Nonaka K. The P561T polymorphism of the growth hormone receptor gene has an inhibitory effect on mandibular growth in young children. *Eur J Orthod.* 2009;31(5):536-41.
22. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res.* 1988;16(3):1215.
23. Laron Z. Laron syndrome (primary growth hormone resistance or insensitivity): the personal experience 1958-2003. *J Clin Endocrinol Metab.* 2004;89(3):1031-44.
24. Schaefer GB, Rosenbloom AL, Guevara-Aguirre J, Campbell EA, Ullrich F, Patil K, Frias JL. Facial morphometry of Ecuadorian patients with growth hormone receptor deficiency/Laron syndrome. *J Med Genet.* 1994;31(8):635-9.
25. Chujo S, Kaji H, Takahashi Y, Okimura Y, Abe H, Chihara K. No correlation of growth hormone receptor gene mutation P561T with body height. *Eur J Endocrinol.* 1996;134(5):560-2.
26. Zhou J, Lu Y, Gao XH, Chen YC, Lu JJ, Bai YX, Shen Y, Wang BK. The growth hormone receptor gene is associated with mandibular height in a Chinese population. *J Dent Res.* 2005;84(11):1052-6.
27. Rakhshan V, Ghorbanyjavdpour F. Anteroposterior and vertical soft tissue cephalometric norms of Iranians, interethnic comparisons, sex dimorphism, and the effect of age on cephalometric variables. *Oral Maxillofac Surg.* 2019;23(2):167-78.
28. Azarbayejani S, Omrani A, Kalaantar-Motamedi A, Abdollahi M, Taalebi V, Teimoori F. Cephalometric norms for 6-17 year-old Iranians with normal occlusion and well-balanced faces. *Dent Res J.* 2014;11(3):327-35.
29. Tehranchi A, Saedi S, Motamedian SR, Rohani K. Radiographic Evaluation of Frontal Sinus Dimensions and Anatomic Variations. *Br J Med Med Res.* 2015;8(5):454-62.
30. Tehranchi A, Motamedian SR, Saedi S, Kabiri S, Shidfar S. Correlation between frontal sinus dimensions and cephalometric indices: A cross-sectional study. *Eur J Dent.* 2017;11(1):64-70.