Showa Univ J Med Sci **29**(2), 173～180, June 2017

Original

Association of Growth Hormone Receptor Gene Variants with Mandibular Form in an Egyptian Population

Mohamed ADEL*[1)], Tetsutaro YAMAGUCHI[1)], Daisuke TOMITA[1)], Takatoshi NAKAWAKI[1)], Yu HIKITA[1)], Shugo HAGA[1)], Masahiro TAKAHASHI[1)], Mohamed A. NADIM[2)], Walid El-KENANY[3)], Abbadi A. El-KADI[2)] and Koutaro MAKI[1)]

Abstract: A significant genetic component is involved in the development of the craniofacial form. Recently, the growth hormone receptor gene (*GHR*) variants rs6180 and rs6184 were associated with variations in mandibular form. To confirm these findings we evaluated the relationship between these *GHR* variants and mandibular form in a cohort of Egyptian individuals with a normal skull form. The patients consisted of 191 unrelated Egyptian adults (92 males, 99 females; 18–55 years). Allele frequencies of the rs6180 and rs6184 variants were determined from genomic DNA extracted from saliva using the Taqman genotyping assay. Lateral and posteroanterior cephalometric tracings were used to obtain 19 mandibular measurements. The association between the *GHR* variants and the mandibular measurements was examined using regression analysis. The frequency of the minor rs6184 variant was very low at 1.5%: therefore, association studies were not performed for this variant. The rs6180 variant was not associated with any of the measurements representing mandibular form in our study cohort. The frequency of the rs6184 variant was very low in our cohort of Egyptian subjects. We also found no association between the rs6180 variant and the measurements representing the mandibular form. By excluding the involvement of these *GHR* variants in influencing mandibular form, our results may help to identify the actual variant(s) affecting the mandibular form in the Egyptian population.

Key words: growth hormone receptor gene, craniofacial form, mandible, lower face, egyptians

Introduction

Development of the orofacial region occurs as a result of complex genetic and environmental interactions. The field of genetics has gained importance for many craniofacial specialists; it is a tool for understanding why a patient has a particular craniofacial form and for determining the prognosis of various treatments[1)]. Growth hormone (GH) and insulin-like growth factor I

[1)] *Department of Orthodontics, Showa University, School of Dentistry,* 2-1-1 Kitasenzoku, Oota-ku, Tokyo 145-8515, Japan.
[2)] *Department of Orthodontics, Suez Canal University, Ismailia, Egypt.*
[3)] *Department of Orthodontics, Alexandria University, Alexandria, Egypt.*
* To whom corresponding should be addressed.

(IGF-I) are important regulators of bone homeostasis and are central to the normal formation of longitudinal bone growth and bone mass. Both GH and IGF-I influence skeletal growth primarily by stimulating the growth of cartilage in areas of endochondral bone formation[2]. Cartilage-mediated growth in the mandibular condyle plays an important role in determining mandibular growth and form[3]. GH receptors (GHRs) are present in the mandibular condyle[4].

Growth-hormone insensitivity (Laron syndrome) and incomplete growth-hormone insensitivity, which causes idiopathic short stature, are examples of conditions associated with *GHR* mutations and polymorphisms[5–7]. These conditions are characterized by short stature : the individual's height is more than 2 standard deviations below the corresponding average height for other individuals of a given age, sex and population. Children with Laron syndrome grow at a subnormal rate and present a uniform type of growth retardation, which in turn might greatly affect their craniofacial form. Patients with Laron syndrome have a smaller skull size as measured by head circumference[8, 9]. Understanding how genetic mutations in the *GHR* contribute to the characteristics of these diseases would provide insight into the role of this gene in normal craniofacial development.

Previous studies have indicated that *GHR* mutations may affect craniofacial morphogenesis in populations from different geographical locations. Yamaguchi *et al* were the first to report an association between the rs6184 variant in exon 10 of *GHR* and mandibular ramus height in a Japanese cohort of 50 men and 50 women[10]. They found that normal Japanese individuals without the rs6184 variant had a significantly greater mandibular ramus length (condylion-gonion) than individuals with the rs6184 variant. Tomoyasu *et al* confirmed the association between the rs6182 (C440F) and rs6184 (P579T) variants and mandibular ramus height in 167 normal Japanese subjects[11]. A significant association between the rs6182 and rs6184 variants with mandibular ramus height was also confirmed in 159 Korean subjects[12]. Chinese Han individuals with the rs6180 (I544L) CC genotype also had a greater mandibular ramus length than those with the AC or AA genotypes[13]. Similarly, Sasaki *et al* studied the effect of the rs6184 variant on mandibular growth in 2 groups of Japanese children : those with mandibular prognathism and those with normal occlusion. This variant did not account for the difference between the 2 groups. However, the authors hypothesized that it may affect mandibular growth during early childhood, indicating that this *GHR* mutation may function as an inhibitory factor in the mandibular growth process[14].

Most of the above-mentioned studies were conducted on East Asian populations (Japanese, Koreans and Han Chinese) that have considerable similarities in their linkage disequilibrium patterns[12]. The relationship between *GHR* variants and craniofacial form in other populations has been reported. Allele and genotype frequencies of the *GHR* rs6182 and rs6184 variants and their relationship to mandibular prognathism were examined in a Turkish cohort consisting of 101 patients with Class Ⅲ mandibular prognathism and 99 individuals with normal occlusion. The rs6184 variant was associated with effective mandibular length (condylion-gnathion) and lower face height (anterior nasal spine-menton)[15].

Based on the literature, we concluded that the *GHR* variants are mainly related to mandibular

morphogenesis, particularly the vertical dimension, in East Asian populations. Therefore, we were interested to determine whether *GHR* variants also affect mandibular form in a population outside East Asia. It is essential to consider ethnicity before selecting variants for association studies, as there are differences in their biological structures and in the frequencies of haplotypes among different ethnic populations[16]. The Egyptian population, which has Caucasian origins, has a craniofacial form distinct from that of the East Asians, with a tendency toward skeletal Class II malocclusion and a more convex profile[17].

The present study aimed to determine the allele and genotype frequencies of the rs6180 and rs6184 variants and examined their association with 19 measurements representing the mandibular form in 191 adult Egyptian subjects.

Materials and methods

Genomic DNA and lateral and posteroanterior cephalograms were obtained from 191 Egyptian subjects who were either patients at the clinic at the Orthodontic Department, Faculty of Dentistry, Suez Canal University (Ismailia, Egypt) or volunteers comprising students and workers in the Faculty of Dentistry. The age range was 18 to 55 years. The study subjects consisted of 92 males (mean age = 22.37 years, SD = 4.27) and 99 females (mean age = 22.39 years, SD = 4.30) who met the following 3 inclusion criteria: 1) they were all unrelated; 2) had no congenital disorders such as cleft palate or general physical disease; and 3) had not undergone any previous orthodontic or orthopedic treatment. The Ethical Committee of Suez Canal University approved the protocol used in this study, and all patients signed a written informed consent form prior to participating in this study.

DNA was extracted from saliva from all subjects using the Oragene DNA kit (DNA Genotek, Ottawa, ON, Canada) according to the manufacturer's recommendations. The collected saliva was then stored at room temperature before DNA extraction. The *GHR* rs6184 and rs6180 loci were genotyped using the Taqman genotyping assay (Applied Biosystems assay number: C_27497202_10; Life Technologies, Carlsbad, CA, USA) and allele frequencies were determined. TaqMan assay, is a PCR-based assay for genotyping SNPs. The region flanking the SNP is amplified in the presence of two allele-specific fluorescent probes. The probes do not fluoresce in solution because of a quencher at the 3' end. The presence of two probes allows the detection of both alleles in a single tube. Moreover, because probes are included in the PCR, genotypes are determined without any post-PCR processing, a feature that is unavailable with most other genotyping methods. The rs6182 variant was not included in this study because it is in a strong linkage disequilibrium with the rs6184 variant in populations found in the International HapMap Project (hapmap.ncbi.nlm.nih.gov).

The mandibular form was measured using ImageJ software (ImageJ, version 1.48; Wayne Rasband, National Institutes of Health, Bethesda, MD, USA) at the Showa Dental Hospital (Tokyo, Japan) by plotting 11 points on the lateral cephalograms and 6 points on the posteroanterior cephalograms (Fig. 1). The distances between these facial landmarks were then measured. The geometric mean (GM) of these measurements was calculated to evaluate the

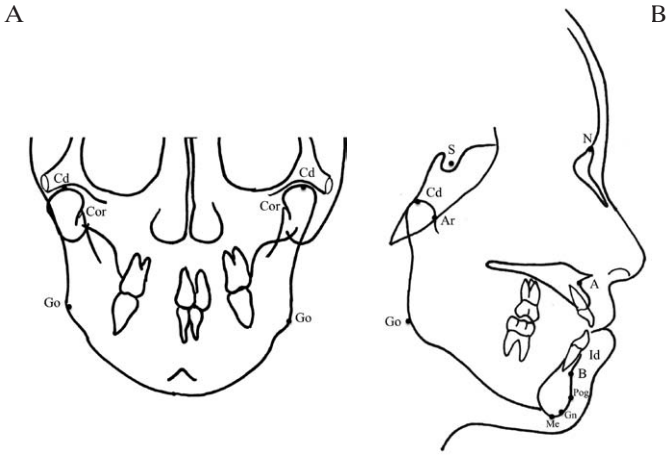

Fig. 1. Posteroanterior (A) and lateral (B) cephalometric tracings showing the facial landmarks used to obtain mandibular measurements. A, Point A (the most posterior point on the anterior contour of the upper alveolar process); Ar, articular; B, Point B (the most posterior point on the anterior contour of the lower alveolar process); Cd, condylion; Cor, coronoid; Gn, gnathion; Go, gonion; Id, infradentale; Me, menton; N, nasion; Pog, pogonion; S, sella turcica.

craniofacial size of the individual and to rule out allometric effects on the measurements.

Regression analysis was used to test the association between the rs6180 variants and the mandibular form measurements. The number of the derived allele was used as the explanatory variable. Regression analysis was performed using sex only as the covariate; sex and GM were then also included as covariates. The commercial software Statistical Package for the Social Sciences (SPSS, version 23.0; IBM Corporation, Armonk, NY, USA) was used to perform the regression analysis. A P value of less than 0.05 was considered statistically significant.

Results

Table 1 summarizes the measurements used to determine mandibular form, obtained from the facial landmarks on each subject's lateral and posteroanterior cephalograms. The allele frequencies of the rs6180 and rs6184 variants are presented in Table 2. The rs6184 variant was excluded from the association study as the frequency of the minor allele was very low (1.5%). Table 3 summarizes the regression analysis data for the association between the rs6180 variants and the mandibular form measurements. We did not find any significant correlation between the rs6180 variant and the measurements representing mandibular form in the Egyptian subjects.

Discussion

We investigated the association between the *GHR* variant rs6180 and mandibular measurements obtained from lateral and posteroanterior cephalograms of Egyptian adult subjects.

Table 1. Measurements from lateral and posteroanterior cephalograms of 191 Egyptian subjects

Trait	Male (n=92)		Female (n = 99)	
	Mean±SD	Range	Mean±SD	Range
Gn-Cd	130.18±6.68	115.84–150.87	119.85±5.21	106.40–135.23
Gn-Go	86.64±5.37	75.87–103.04	81.88±4.53	70.42–95.54
Cd-Go	65.45±6.35	52.11–78.91	56.32±4.98	42.20–66.75
Point B-(N-A)	–2.82±5.54	–16.36–14.04	–5.70±5.12	–18.64–8.85
Gn-(N-A)	–3.48±7.30	–23.69–18.88	–8.10±6.99	–27.37–9.15
Pog-Cd	127.48±6.64	112.46–149.62	117.41±5.05	103.39–132.63
Me-Go	83.23±5.44	71.93–99.80	78.39±4.31	66.44–90.94
Ar-Go	54.51±6.33	41.83–70.57	45.16±4.99	33.51–57.66
Pog-(N-A)	–1.81±7.08	–21.81–20.28	–5.91±6.68	–22.27–9.42
Id-(A-Pog)	2.23±2.54	–3.80–7.42	2.22±2.27	–3.19–7.96
Point B-(A-Pog)	–1.50±1.69	–6.73–3.51	–1.32±1.58	–5.22–2.67
Cd(R)-Cd(L)	131.76±5.45	113.51–145.87	131.76±5.45	113.51–145.87
Cor(R)-Cor(L)	118.60±6.11	101.09–133.15	118.60±6.11	101.09–133.15
Go(R)-Go(L)	120.19±7.22	105.82–140.28	120.19±7.22	105.82–140.28
A-N-Point B	1.51±2.94	–7.33–8.87	3.21±2.89	–5.12–11.41
S-N-Point B	81.23±4.26	72.87–91.10	79.07±4.01	69.41–88.34
Cd-Go-Me	120.81±6.98	104.67–136.25	122.98±7.24	102.80–140.60
Ar-Go-Me	126.10±7.07	109.75–145.22	128.55±7.44	107.96–146.74
Ar-Go-Gn	122.80±7.00	106.41–141.87	125.24±7.31	105.55–143.23

Abbreviations : A, point A ; Ar, articula ; Cd, condylion ; Cor, coronoid ; Gn, gnathion ; Go, gonion ; Id, infradentale ; L, left ; Me, menton ; N, nasion ; R, right ; SD, standard deviation ; S, sella turcica ; Pog, pogonion.

Table 2. Allele frequencies of the *GHR* variants examined in 191 Egyptian subjects

rs number	Chromosome position (GRCh38.p2)	mRNA position (forward to chr)	Alleles: ancestral/derived (forward to chr)	Derived allele frequency	A/A subjects	A/C subjects	C/C subjects
rs6180	chr5: 42719137	A1822C	A/C	48.0%	52	108	42
rs6184	chr5: 42719242	C1927A	C/A	1.50%	1	4	195

Previous studies examining the relationship between *GHR* variants and craniofacial form used lateral cephalograms only[10–15]. By using both lateral and posteroanterior cephalograms in our study, we were able to detect a third dimension of the mandible that could not be seen using lateral cephalograms only. This allowed us to analyze some important mandibular measurements, such as the condylar width. In previous association studies, the *GHR* variants rs6180, rs6182 and rs6184 were found to be related to mandibular form, with the rs6182 and rs6184 variants in complete linkage disequilibrium[10–15]. We also examined the rs6180 and rs6184 variants in this study, but the minor allele frequency of rs6184 was very low at 1.5% (Table 2). Our association

Table 3. Multiple regression analysis to test the association between rs6180 and mandibular morphology in 191 Egyptian subjects

Trait	B	SE	β	P
Gn-Cd	0.301	0.641	0.027	0.639
Gn-Go	0.13	0.546	0.016	0.812
Cd-Go	0.545	0.61	0.052	0.373
Point B-(N-A)	0.656	0.592	0.08	0.27
Gn-(N-A)	1.025	0.795	0.092	0.199
Pog-Cd	0.181	0.628	0.016	0.773
Me-Go	0.032	0.537	0.004	0.953
Ar-Go	0.781	0.609	0.073	0.201
Pog-(N-A)	1.043	0.763	0.098	0.174
Id-(A-Pog)	–0.024	0.268	–0.007	0.927
Point B-(A-Pog)	–0.067	0.184	–0.027	0.718
Cd(R)-Cd(L)	–0.733	1.213	–0.043	0.546
Cor(R)-Cor(L)	–0.998	1.163	–0.061	0.392
Go(R)-Go(L)	1.349	1.785	0.079	0.452
A-N-Point B	–0.367	0.325	–0.081	0.26
S-N-Point B	0.163	0.453	0.026	0.719
Cd-Go-Me	–0.428	0.815	–0.039	0.601
Ar-Go-Me	–0.541	0.831	–0.048	0.516
Ar-Go-Gn	–0.682	0.819	–0.061	0.406

The rs6180 genotypes were denoted by the number of derived alleles : AA = 0, AC = 1, and CC = 2. Sex and geometric mean were included as covariates in the regression analysis.

Abbreviations : A, point A ; Ar, articula ; β, standardized regression coefficient ; B, non-standardized regression coefficient ; Cd, condylion ; Cor, coronoid ; Gn, gnathion ; Go, gonion ; Id, infradentale ; L, left ; Me, menton ; N, nasion ; P, probability value ; R, right ; SE, standard error ; S, sella turcica ; Pog, pogonion.

study showed that there was no correlation between the rs6180 variant and the measurements representing mandibular form in our Egyptian cohort (Table 3).

Our results indicate that variations in mandibular form in the Egyptian population are unlikely to be linked to these *GHR* variants, even though they have been associated with mandibular form in East Asian populations. Further investigation is needed to identify the genetic factors that affect morphogenesis of the mandible in the Egyptian population. Moreover, the use of 2-dimensional evaluation techniques using cephalograms may present some limitations for analyzing the complex structure of the mandible[18]. Future association studies using recent and more standardized 3-dimensional imaging techniques, such as cone-beam computed tomography imaging, may allow more accurate evaluations of the variations in craniofacial form.

In conclusion, our findings revealed that the frequency of the minor rs6184 variant in our cohort of Egyptian subjects was very low, and the rs6180 variant was not associated with any of the measurements representing mandibular form. The present study suggests that both the rs6180 and rs6184 variants have no association with variations in the mandibular form in the

Egyptian population.

Acknowledgements

We thank all of the study participants and supporting medical and dental staff. We are also grateful to Professor Hajime Ishida, Dr. Ryosuke Kimura, Dr. Akira Kawaguchi and Dr. Mutsumi Isa from the Department of Human Biology and Anatomy, Graduate School of Medicine, University of the Ryukyus, Okinawa, Japan who provided expertise that greatly assisted the research. We would also like to thank Dr. Yong-Il Kim and Professor Soo-Byung Park from the Department of Orthodontics, Dental Research Institute, Pusan National University Dental Hospital, Yangsan, South Korea for supporting this research. This work was supported by JSPS KAKENHI (25670881 ; Grant-in-Aid for Challenging Exploratory Research to TY) and by the Ministry of Education, Culture, Sports, Science and Technology (MEXT) Supported Program for the Strategic Research Foundation at Private Universities, 2012-2017.

Conflict of interest disclosure

The authors declare no conflict of interest.

References

1) Hartsfield Jr JK, Morford LA, Otero LM. Genetic factors affecting facial growth. In *Bourzgui F, ed. Orthodontics-Basic Aspects and Clinical Considerations*. Rijeka, Croatia: InTech; 2012. pp125-152.
2) Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. *Endocr Rev*. 2008;**29**:535-559.
3) Proffit WR, Fields HW, Sarver DM, *et al*. Contemporary Orthodontics. 5th ed. St. Louis: Mosby Elsevier; 2013.
4) Litsas G. Growth hormone and craniofacial tissues. An update. *Open Dent J*. 2015;**9**:1-8.
5) Butler MG, Roberts J, Hayes J, *et al*. Growth hormone receptor (GHR) gene polymorphism and Prader-Willi syndrome. *Am J Med Genet A*. 2013;**161A**:1647-1653.
6) Berg MA, Argente J, Chernausek S, *et al*. Diverse growth hormone receptor gene mutations in Laron syndrome. *Am J Hum Genet*. 1993;**52**:998-1005.
7) Goddard AD, Covello R, Luoh SM, *et al*. Mutations of the growth hormone receptor in children with idiopathic short stature. The Growth Hormone Insensitivity Study Group. *N Engl J Med*. 1995;**333**:1093-1098.
8) Pedicelli S, Peschiaroli E, Violi E, *et al*. Controversies in the definition and treatment of idiopathic short stature (ISS). *J Clin Res Pediatr Endocrinol*. 2009;**1**:105-115.
9) Laron Z, Lilos P, Klinger B. Growth curves for Laron syndrome. *Arch Dis Child*. 1993;**68**:768-770.
10) 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**:650-653.
11) Tomoyasu Y, Yamaguchi T, Tajima A, *et al*. 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**:536-541.
12) Kang EH, Yamaguchi T, Tajima A, *et al*. Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population. *Arch Oral Biol*. 2009;**54**:556-562.
13) Zhou J, Lu Y, Gao XH, Chen YC, *et al*. The growth hormone receptor gene is associated with mandibular height in a Chinese population. *J Dent Res*. 2005;**84**:1052-1056.
14) Sasaki Y, Satoh K, Hayasaki H, *et al*. The P561T polymorphism of the growth hormone receptor gene has an inhibitory effect on mandibular growth in young children. *Eur J Orthod*. 2009;**31**:536-541.
15) Bayram S, Basciftci FA, Kurar E. Relationship between P561T and C422F polymorphisms in growth hormone receptor gene and mandibular prognathism. *Angle Orthod*. 2014;**84**:803-809.

16) Tomoyasu Y, Yamaguchi T, Tajima A, *et al*. External apical root resorption and the interleukin-1B gene polymorphism in the Japanese population. *Orthod Waves*. 2009;**68**:152-157.
17) Adel M, Yamaguchi T, Nadim M, *et al*. Evaluation of the craniofacial morphology of Egyptian adults undergoing orthodontic treatment. *Dentistry*. 2016;**6**:379-384.
18) Machado GL. CBCT imaging-A boon to orthodontics. *Saudi Dent J*. 2015;**27**:12-21.

[Received October 7, 2016 : Accepted January 23, 2017]