Growth hormone receptor gene variant and three-dimensional mandibular morphology

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ABSTRACT

Objective: To examine the relationship between three-dimensional mandibular morphology and growth hormone receptor (GHR) gene variants in a healthy Japanese population.

Materials and Methods: The subjects, who were unrelated Japanese orthodontic patients, consisted of 64 men and 114 women. Using the Taqman genotyping assay, GHR gene rs6184 and rs6180 variants were detected in genomic DNA extracted from saliva. Mandibular volume and length were measured from cone-beam computed tomography images that were analyzed using Analyze image-processing software. The relationship between GHR gene variants and three-dimensional mandibular morphology was statistically examined.

Results: Statistical significance for the relationship between the distance between the left and right coronoid processes and rs6180 was noted ($P < .05$).

Conclusion: Our results indicate that the GHR variant rs6180 is associated with the distance between the left and right coronoid process in the Japanese subjects. (Angle Orthod. 2017;87:68–73)

KEY WORDS: GHR; Three-dimensional mandibular morphology

INTRODUCTION

Genetic and environmental factors are involved in craniofacial size and the characteristics of craniofacial morphology\textsuperscript{1,2}; in particular, the results of studies investigating facial similarities between relatives suggest that genetic factors play an important role in determining craniofacial morphology.\textsuperscript{3,4} Comparisons between monozygotic and dizygotic twins have revealed even stronger relationships between genetic factors and craniofacial morphology.\textsuperscript{5,6}

Growth hormone (GH), a peptide hormone produced in the anterior pituitary, plays a major role in regulating growth and development of the maxillofacial complex.\textsuperscript{7} GH binds growth hormone receptors (GHRs) located on the cell surface in order to activate the intracellular signaling pathways involved in these processes.\textsuperscript{8} GH plays an important role in the growth of cartilage, and GHRs are especially present in the mandibular condyle,\textsuperscript{9,10} which plays a significant role in the growth and development of craniofacial morphology by regulating the angle and size of the morphology.\textsuperscript{7} Dysfunctional mutations in the GHR gene cause Laron syndrome (GH insensitivity syndrome), which is associated with characteristic craniofacial morphology and short stature.\textsuperscript{11} Interestingly, patients with GHR defi-
ciency exhibit craniofacial morphology characterized by poor vertical growth.12

The relationship between GHR gene variants in the general population and craniofacial morphology has been examined in previous studies. The relationship between rs6184 in exon 10 of GHR and mandibular ramus height in a Japanese population, as well as similar findings in Korean and Turkish populations, have been previously reported.13–15 Mandibular ramus length was significantly shorter in a Japanese population with genotype CC of variants rs6184 or genotype GG of rs6182.16 Furthermore, a relationship between the rs6180 variant and mandibular ramus length has been reported in a Chinese population.17 In young Japanese children, the rs6184 variant of the GHR gene has an inhibitory effect on mandibular growth.18

Previous studies examining the relationship between craniofacial morphology and GHR3–18 have used lateral cephalograms to measure craniofacial morphology. However, there are limitations associated with the application of two-dimensional data from lateral cephalograms to the evaluation of craniofacial morphology, which has a complex structure.7 In the present study, we examined the relationship between three-dimensional mandibular morphology and the rs6184 and rs6180 variants in exon 10 of the GHR gene in a sample of 178 Japanese subjects.

MATERIALS AND METHODS

Genomic DNA and cone-beam computed tomography (CBCT) images of the mandible were obtained from 178 Japanese adults: 64 men (aged 18–50 years; average age: 26.9 years) and 114 women (aged 19–57 years; average age: 27.3 years). All subjects were free of congenital and systemic diseases, such as cleft lip and palate or tooth agenesis, excluding third molars. The subjects were patients who visited the Department of Orthodontics, Showa University Dental Hospital and underwent CBCT imaging and lateral cephalometric radiography. The study was approved by the Ethics Committee of Showa University Dental Hospital and related committees, and all subjects provided written informed consent to participate.

Genotyping

Saliva was collected from the subjects using an Oragene DNA self-collection kit (DNA Genotek, Ottawa, Ontario, Canada) and stored at room temperature. Genomic DNA was extracted from the saliva samples. The GHR rs6184 and rs6180 loci were genotyped using the Taqman genotyping assay (Applied Biosystems assay number: C_27497202_10; Life Technologies, Carlsbad, Calif).

Mandibular Measurements

Images were acquired using a dental cone-beam X-ray CT scanner (CB MercuRay, Hitachi Medical Technology, Tokyo, Japan) and KaVo 3DeXam (KaVo, Biberach, Germany) at the Department of Radiology of the university hospital. Volume was measured following the method reported by Katayama et al.19 The mandibular bone region was segmented from the image data and analyzed using Analyze 3D reconstruction software (Biomedical Imaging Resource, Mayo Clinic and Foundation, Rochester, Minn). Mandibular volume and length were measured by autotracing the outer circumference of the cortical bone in all slides using Analyze. These autotraces were superimposed to prepare an object map for volume and length measurement. Dental crown data were extracted separately from those of the mandible as these may be affected by artifacts, such as the presence of prostheses. The measurement item is shown in Figures 1a,b. It was not possible to measure A’-PTM’ using the method of creating an object map from CBCT images obtained by measuring the mandibular bones in this study. Therefore, we measured A’-PTM’ using lateral cephalograms.

Statistical Analysis

The difference between the number of male and female subjects represented a potential limitation of this study that may have affected the statistical reliability of the results. Therefore, data were not analyzed using sex; instead, multiple regression analyses were used to test the association between the trait and each single nucleotide polymorphism (SNP), with the addition of sex as a covariate. A’-PTM’ was also added as a covariate to make a correction by the maxillary size. Statistical analyses were performed using Statcel3 software (OMS Publishing, Saitama, Japan) with significance level at 5%.

RESULTS

The details of each variant of the GHR gene are shown in Table 1.

The mean values and standard deviations of each measurement item are shown in Table 2. The results of multiple regression analysis of the two variants of the GHR gene (rs6180 AA = 0, AC = 1, CC = 2, rs6184 CC = 0, AC = 1, AA = 2) and craniofacial morphology measurement values are shown in Table 3. The derived allele frequency for each SNP was 45% and 7% for rs6180 and rs6184, respectively. The GHR variant rs6180 was significantly associated with the distance between the left and right coronoid processes (RCP-LCP) (P < .05). In multiple regression analyses
in which only sex was added as a covariate, $P = 0.008$; however, when sex and A’-PTM’ were added as covariates, $P = 0.016$.

**DISCUSSION**

In the present study, we examined the relationship between GHR gene variants rs6180 and rs6184 and three-dimensional mandibular morphology using CBCT imaging in Japanese orthodontic patients. In previous reports concerning the relationship between GHR gene variants and mandibular morphology, analysis by two-dimensional lateral cephalograms\(^{13–18}\) revealed relationships between mandibular ramus length and variants rs6184 and rs6182.\(^{13,16}\) However, owing to the complex structure of the mandible, two-dimensional evaluation using cephalograms cannot be used for precise measurement of the mandible.\(^{19}\) In the present study, we examined the association between the actual length of the mandibular bone and GHR gene variants using three-dimensional analysis. In previous reports, GHR gene SNPs (rs6180, rs6182, and rs6184) were described to be related to mandibular morphology\(^{13–18}\) with SNPs rs6182 and rs6184 in complete linkage disequilibrium. In this study, we examined two SNPs (rs6180 and rs6184). In the present study, which used three-dimensional imaging, similar results were found; however, the differences were not significant. Interestingly, we found a relationship between the distance between the coronoid processes and the GHR gene variant rs6180, which has not been observed using conventional imaging techniques such as cephalometry.

The mandible is a membranous bone that is affected by the growth of attached muscles.\(^{20}\) The temporal muscle is attached to the coronoid process, and negative correlations have been observed between temporal muscle activity and mandibular plane and gonial angle and overjet.\(^{21,22}\) A previous study examining the relationship between maxillofacial morphology and coronoid process morphology\(^{23}\) revealed that the morphology of the coronoid process influences not only mandibular position but also maxillary position and dentition. A previous report describing the measurement of the coronoid processes using CBCT imaging\(^{24}\) revealed that, in comparison with skeletal pattern differences, differences related to gender were greater in terms of craniofacial morphology and the volume and height of the coronoid processes. In addition, a relationship between rs6180 and body height has been previously reported.\(^{25}\) However, in the present study, the correlation between rs6180 and RCP-LCP was weakened on adding the covariate of A’-PTM’ to the correction value. As craniofacial size is strongly correlated with body height,\(^{26}\) we estimated a relationship between rs6180 and height by using A’-PTM’ data as an indicator for the craniofacial size.

To date, studies have focused on the analysis of human genes, other than the GHR gene, in order to identify associations between gene variants and facial morphology in the general population. Moreover, these studies have used magnetic resonance imaging and

![Figure 1. (a and b) Mandibular measurements performed using CBCT images: volume (mm$^3$); CD-GO (mm), GO-GN (mm), GN (mm), RCD-LCD (mm), RGO-LGO (mm), RCP-LCP (mm), and gonial angle ($\gamma$).](http://meridian.allenpress.com/angle-orthodontist/article-pdf/87/1/68/1399277/02316-154_1.pdf)
laser scans to measure facial morphology. The present study, to our knowledge, is the first to report a relationship between GHR gene variants and three-dimensional mandibular morphology using CBCT imaging, which accurately measures not only soft tissues but also bone morphology, and is therefore useful for examining the complex structure of the mandible.

The prediction of mandibular growth is challenging. In previous studies of families and twins with mandibular prognathism, a strong association between mandibular prognathism and genetic factors has been reported. Class III malocclusion is caused by multiple factors that interact during morphogenesis of the mandibular bone. Therefore, elucidation of the genetic factors that contribute to Class III malocclusion may enable early selection of optimal preventive strategies to address this condition.

### Table 2. Means and Standard Deviations (SDs) of the Measurements From Cone-Beam Computed Tomography

<table>
<thead>
<tr>
<th></th>
<th>Males (n = 64)</th>
<th></th>
<th>Females (n = 114)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>Volume (mm³)</td>
<td>68,539.86 ± 19,527.26</td>
<td>40,048.25–96,340.34</td>
<td>56,978.22 ± 8938.95</td>
</tr>
<tr>
<td>CD-GO (mm)</td>
<td>103.86 ± 9.22</td>
<td>87.44–127.32</td>
<td>91.38 ± 7.76</td>
</tr>
<tr>
<td>GO-GN (mm)</td>
<td>143.02 ± 10.61</td>
<td>119.32–183.98</td>
<td>132.01 ± 8.47</td>
</tr>
<tr>
<td>CD-GN (mm)</td>
<td>216.57 ± 14.87</td>
<td>190.04–273.15</td>
<td>198.02 ± 11.63</td>
</tr>
<tr>
<td>RCD-LCD (mm)</td>
<td>208.68 ± 12.85</td>
<td>186.63–268.67</td>
<td>197.76 ± 9.82</td>
</tr>
<tr>
<td>RGO-LGO (mm)</td>
<td>162.31 ± 11.67</td>
<td>144.40–203.27</td>
<td>147.73 ± 9.47</td>
</tr>
<tr>
<td>RCP-LCP (mm)</td>
<td>165.07 ± 10.82</td>
<td>146.58–210.08</td>
<td>154.88 ± 7.04</td>
</tr>
<tr>
<td>Gonial angle (°)</td>
<td>122.11 ± 5.87</td>
<td>110.30–137.60</td>
<td>124.13 ± 5.93</td>
</tr>
</tbody>
</table>

### Table 3. Association Tests Using Multiple Regression Analyses

<table>
<thead>
<tr>
<th>Traits</th>
<th>Single-Nucleotide Polymorphism</th>
<th>B</th>
<th>Standard Error</th>
<th>β</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Volume (mm³)</td>
<td>rs6180</td>
<td>1142.567</td>
<td>1382.061</td>
<td>0.059</td>
<td>.41</td>
</tr>
<tr>
<td>CD-GO (mm)</td>
<td>rs6180</td>
<td>1645.603</td>
<td>2614.246</td>
<td>0.044</td>
<td>.53</td>
</tr>
<tr>
<td>GO-GN (mm)</td>
<td>rs6180</td>
<td>0.336</td>
<td>1.323</td>
<td>0.016</td>
<td>.80</td>
</tr>
<tr>
<td>CD-GN (mm)</td>
<td>rs6180</td>
<td>−2.931</td>
<td>2.491</td>
<td>−0.071</td>
<td>.24</td>
</tr>
<tr>
<td>RCD-LCD (mm)</td>
<td>rs6180</td>
<td>−3.349</td>
<td>3.366</td>
<td>−0.068</td>
<td>.32</td>
</tr>
<tr>
<td>RGO-LGO (mm)</td>
<td>rs6180</td>
<td>−0.252</td>
<td>1.643</td>
<td>−0.010</td>
<td>.88</td>
</tr>
<tr>
<td>RCP-LCP (mm)</td>
<td>rs6180</td>
<td>1.680</td>
<td>3.104</td>
<td>0.034</td>
<td>.59</td>
</tr>
<tr>
<td>Gonial angle (°)</td>
<td>rs6180</td>
<td>0.326</td>
<td>0.791</td>
<td>0.031</td>
<td>.68</td>
</tr>
</tbody>
</table>

Model using sex and A'-PTM' as covariates

| Volume (mm³)          | rs6180                         | 1430.777  | 1393.655       | 0.073 | .31 |
| CD-GO (mm)            | rs6180                         | 1532.706  | 2793.652       | 0.039 | .58 |
| GO-GN (mm)            | rs6180                         | 0.516     | 1.377          | 0.024 | .71 |
| CD-GN (mm)            | rs6180                         | −3.341    | 2.743          | −0.076 | .22 |
| RCD-LCD (mm)          | rs6180                         | 1.927     | 1.780          | 0.074 | .28 |
| RGO-LGO (mm)          | rs6180                         | −3.349    | 3.366          | −0.068 | .32 |
| RCP-LCP (mm)          | rs6180                         | 1.680     | 3.104          | 0.034 | .59 |
| Gonial angle (°)      | rs6180                         | 0.326     | 0.791          | 0.031 | .68 |

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The allele frequency represents the incidence of a gene variant in a population, and the relationship between GHR gene variants and craniofacial morphology is dependent on ethnicity. In comparison with European Americans and whites, mandibular size tends to be slightly smaller in the Japanese population. This suggests the need for investigation of the relationship between craniofacial morphology and GHR gene variants in other populations.

CONCLUSION

- Our findings revealed that the rs6180 variant of the GHR gene is correlated with the distance between the left and right coronoid processes in the population studied.

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