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Association of the growth hormone receptor gene polymorphisms with mandibular height in a Korean population

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ARTICLE INFO

Article history:
Accepted 6 March 2009

Keywords:
Growth hormone receptor gene
Mandible
Korean
Ethnicity

ABSTRACT

Growth hormone receptor gene (*GHR*) is one of the likely candidates for determining morphological traits, because GH is a key regulator of bone growth. The genetic association of *GHR* in exon 10 with mandibular ramus height has been found in different populations, Japanese and Chinese. On the other hand, two common isoforms of *GHR*, one full-length (*fl-GHR*) and the other lacking the extracellular domain encoded by exon 3 (*d3-GHR*), are associated with differences in responsiveness to GH. The purpose of this study involving 159 Korean subjects was to study the associations between a *GHR* polymorphism (*d3/fl-GHR*) that results in genomic deletion of exon 3 and craniofacial morphology, and to study the associations between *GHR* genotypes in exon 10 and craniofacial morphology. Moreover, the allelic frequencies in a multi-ethnic population (24 Han Chinese, 24 African-Americans, 24 European-Americans, and 24 Hispanics) in a *GHR* polymorphism (*d3/fl-GHR*) were compared in this study. The five craniofacial linear measurements (cranial base length, maxillary length, overall mandibular length, mandibular corpus length, and mandibular ramus height) obtained from lateral cephalograms were examined as craniofacial morphology. We found that the *d3/fl-GHR* polymorphism had no association for any measurements, and a statistically significant association ($P = 0.024$) between the *GHR* polymorphisms P561T and C422F in exon 10 and mandibular ramus height. Neither SNPs besides P561T and C422F polymorphisms in exon 10 nor the measurements besides mandibular ramus height have statistically significances. Both derived alleles at P561T and C422F SNPs were highly associated with only one haplotype, haplotype-4 in Korean population. As quantitative haplotype association, the results showed a significant difference in mandibular ramus height between individuals having one haplotype-4 and others without haplotype-4 ($P = 0.028$). Moreover, we found that the *d3/fl-GHR* polymorphism showed diverse frequency in different population. Regarding *GHR* genotypes in exon 10, the present study mostly

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0003–9969/$ – see front matter
doi:10.1016/j.archoralbio.2009.03.002

reflected the results obtained for a Japanese population, although our current study does not replicate the correlation between the I526L polymorphism of *GHR* and mandibular ramus height as was reported in a previous study of Han Chinese. The results of the present study suggest that the *GHR* exon 10 SNPs, not *d3/fl-GHR*, contribute to changes in the mandibular ramus height of Koreans.

1. Introduction

Craniofacial morphology is a polygenic, quantitative trait that is determined by genetic and environmental factors.[1] Studies on similarities in craniofacial morphology between close relatives have clarified that the genetic factors play an important role in the determination of craniofacial morphology.[2,3] Furthermore, a comparison of monozygous and dizygous twins has revealed a clear genetic influence on craniofacial morphology.[4] Identifying the genetic susceptibility for specific craniofacial phenotypes would enable more effective diagnosis and treatment of cranial malformations such as mandibular prognathism.[5]

Recent advances in clinical genetics have increased the fund of knowledge on genetic susceptibilities for craniofacial phenotypes.[5–7] It has recently been reported that single nucleotide polymorphisms (SNPs) of the growth hormone receptor (*GHR*) gene are associated with mandibular height in Japanese and Chinese populations.[8,9] It has been reported that the *GHR* polymorphism P561T in exon 10 is associated with mandibular height in the Japanese population.[8] Zhou et al. found that the *GHR* polymorphism I526L in exon 10 is associated with mandibular height in the Chinese population,[9] and they speculated that the discordance between Chinese and Japanese populations is due to either a lack of power in their experiments or a real difference between these populations. Considerable similarities have been observed in the linkage disequilibrium (LD) pattern between the East Asian populations (Korean, Japanese, and Han Chinese). The patterns of haplotype structure and the haplotype frequencies in the Korean population are very similar to those in the Japanese and Han Chinese populations. In particular, the LD patterns and haplotype frequencies of the Korean population show high degrees of similarity with those of the Japanese population.[10]

A polymorphism of *GHR* (*d3/fl-GHR*) that results in genomic deletion of exon 3 is associated with increased responsiveness to growth hormone; i.e., children carrying at least one *d3-GHR* allele show 1.7–2 times greater response to growth hormone than do *fl-GHR/fl-GHR* homozygotes.[11] This common polymorphism of human *GHR* that results in genomic deletion of exon 3 has recently been associated with the degree of height increase in response to GH therapy in French short children who were born small for gestational age or with idiopathic short stature[11], as well as in German Turner syndrome patients[12] and Brazilian GH-deficient children[13]. It may be a clue to understanding the ethnic difference of the association between *GHR* and mandibular ramus height to determine the allelic frequencies in a multiethnic population.

The purpose of this study is to characterise further the roles of the *d3/fl-GHR* SNP of *GHR* and five SNPs in exon 10 of *GHR* in 159 Korean subjects with regard to craniofacial morphology, and to define the allelic frequencies of *d3/fl-GHR* in a multiethnic population, which consisted of Han Chinese, African-Americans, European-Americans, and Hispanics.

2. Material and methods

2.1. Subjects

Genomic DNA samples and lateral cephalograms were obtained for the group of 159 Korean subjects, which comprised 100 men (age range, 20–49 years; mean age, 25.24 years) and 59 women (age range, 18–58 years; mean age, 24.39 years). The subjects were patients at dental hospitals or volunteers from the Pusan area. All the individuals were unrelated. Subjects who had congenital disorders, such as cleft palate or general physical disease, were excluded from the study. None of the subjects had received orthodontic or orthopaedic treatment. The 159 Korean subjects were comprised of 87 Class I, 44 Class II, and 28 Class III subjects.

In addition, DNA samples from 24 Han Chinese, 24 African-Americans, 24 European-Americans, and 24 Hispanics without craniofacial measurement data were obtained from the Coriell Cell Repository (Camden, NJ, USA), and used only as reference populations for the allelic frequencies of the exon 3-deleted/full-length (*d3/fl-GHR*) polymorphism.[14]

The protocol used in this study was approved by the Ethical Committee of Pusan National University, and all patients gave their written informed consent to participate in the study before DNA samples were taken.

2.2. Genotyping and sequencing

For DNA sample collection, the inside of the mouth was scraped with 10 strokes of a brush (MasterAmp™ Buccal Swab DNA Extraction Kit; AR Brown Co. Ltd., Tokyo, Japan). Four samples were collected from each subject. Genomic DNA was obtained from these samples.

We screened the *GHR* coding region for the five known SNPs of exon 10, [9] and for a polymorphic deletion of exon 3.[14] The analysed SNPs were C422F (dbSNP ID; rs6182), S473S (rs6176), P477T (rs6183), I526L (rs6180), P561T (rs6184), and *d3/fl-GHR*. The dbSNP numbers are taken from the dbSNP database at the NCBI (http://www.ncbi.nlm.nih.gov/SNP/).

Polymerase chain reaction (PCR) was performed according to a standard protocol. To determine the *GHR* exon 10