

Association between the Growth Hormone Receptor Exon 3 Polymorphism and Metabolic Factors in Korean Patients with Acromegaly

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Background: This study investigated the association between the frequency of growth hormone receptor (GHR) exon 3 polymorphism (exon 3 deletion; d3-GHR) and metabolic factors in patients with acromegaly in Korea.

Methods: DNA was extracted from the peripheral blood of 30 unrelated patients with acromegaly. GHR genotypes were evaluated by polymerase chain reaction and correlated with demographic data and laboratory parameters.

Results: No patient had the d3/d3 genotype, while four (13.3%) had the d3/fl genotype, and 26 (86.7%) had the fl/fl genotype. Body mass index (BMI) in patients with the d3/fl genotype was significantly higher than in those with the fl/fl genotype ($P=0.001$). Age, gender, blood pressure, insulin-like growth factor-1, growth hormone, fasting plasma glucose, triglycerides, high density lipoprotein cholesterol, and low density lipoprotein cholesterol levels showed no significant differences between the two genotypes.

Conclusion: The d3-GHR polymorphism may be associated with high BMI but not with other demographic characteristics or laboratory parameters.

Keywords: Growth hormone receptor exon 3 deletion; Polymorphism; Acromegaly; Body mass index; Fasting plasma glucose

INTRODUCTION

Acromegaly is a rare chronic pathology that results from excessive secretion of growth hormone (GH) [1]. The most common cause of acromegaly is a somatotroph (growth hormone-secreting) adenoma of the anterior pituitary that accounts for about one-third of all hormone-secreting pituitary adenomas [2]. The clinical features of acromegaly are attributable to high serum concentrations of both pituitary-derived GH and liver-derived insulin-like growth factor-1 (IGF-1), which are GH

dependent. The clinical spectrum of acromegaly ranges from a relatively asymptomatic condition with slowly evolving acral changes to a severe incapacitating disorder that includes headaches, arthropathy, nerve entrapment syndrome, diabetes, hypertension, and cardiomyopathy [3].

Several recent studies have investigated between clinicopathological characteristics in patients with acromegaly and polymorphisms in a number of genes, including the growth hormone receptor (GHR) gene. The GHR gene is located on the short arm of chromosome 5 (p13.1-p12) and contains nine

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coding exons [4,5]. The GHR protein is a single polypeptide consisting of an intracellular domain, transmembrane portion and an extracellular, ligand-binding domain encoded by exons 3 to 7 [4,5]. Several GHR isoforms have been identified; one of these lacks a 22-amino-acid sequence encoded by exon 3 to create two isoforms: one containing exon 3, or a full-length GHR (fl-GHR), and one missing exon 3 (d3-GHR) [6,7]. Pantel and colleagues [8] recently hypothesized that these two isoforms are the consequence of an in-frame deletion derived from an intrachromosomal recombination event between two similar primate-specific retro-elements (DNA sequences derived from a retrovirus) that flank exon 3, which occurred late during primate evolution. During cell division, these flanking sequences may undergo recombination, which results in a 2.7-kb genomic deletion that spans exon 3 [8]. This polymorphism enhances signal transduction by GH although its binding to the GHR is not altered [9-11]. In most studies investigating the frequency of these GHR isoforms in healthy control subjects, approximately half are homozygous for the fl/fl-GHR genotype, while the fl/d3-GHR genotype is present in 30% to 40% of individuals; the d3/d3 genotype is present in 10% to 20% of subjects [8,11,12]. About one-half of patients with acromegaly have at least one d3 allele [13]. In addition, patients with acromegaly carrying a d3-GHR allele may be at increased risk of more severe complications and may achieve biochemical control with great difficulty [7].

Acromegaly is potentially a very severe condition because chronic GH hypersecretion lead to cause cardiometabolic complications that reduce life expectancy and increase morbidity. While several studies have attempt to correlate cardiometabolic complications with the presence of the d3-GHR polymorphism, the role of two isoforms remains controversial. While the presence of the d3-GHR allele has been related to clinical manifestation of acromegaly in some studies, these results are controversial [7,13,14].

The present study investigated the association between the d3-GHR polymorphism and demographic and laboratory parameters in Korean patients with acromegaly.

METHODS

Study cohort

Thirty unrelated patients with acromegaly were recruited for the present cross-sectional study among those attending Kyungpook National University Hospital, Daegu, South Korea, from January 2008 to June 2012. The inclusion criteria for

this study was described as following; first, a diagnosis of acromegaly was established by documenting an elevated IGF-1 level in combination with failure of GH to suppress after oral glucose to below 0.3 µg/L [15] and second, patients was confirmed by pathological examination of surgically resected tissues. We excluded patients with any medication which can affect their obese state (e.g., oral pills, herbal medication, lipid lowering agents). All patients agreed to participate in the Korean Genomic Cohort Study and provided written informed consent for the investigation. The study protocol was approved by the Institutional Review Board of Kyungpook National University Hospital.

Demographical and laboratory assessments

Demographic data from 30 patients with acromegaly were reviewed. Demographic parameters (age, gender, height, weight, and systolic blood pressure [SBP] and diastolic blood pressure [DBP]) were collected from the medical charts when they visited our hospital at first visit. Body mass index (BMI, kg/m²) was also calculated based on their height and weight.

Blood samples were collected in the morning after overnight fasting. Fasting plasma glucose (FPG) was measured by the hexokinase method (Roche Diagnostics, Basel, Switzerland). Low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) were measured using a selective enzymatic protection assay. Triglycerides were measured by enzymatic assay without a glycerol blank using a Hitachi Molecular Analytics D2400 apparatus (Roche, Tokyo, Japan). Serum GH and IGF-1 were measured by radioimmunoassay using SR-300 (STRATEC, Birkenfeld, Germany).

Genetic analysis

DNA was extracted from the leukocyte of peripheral blood by using the Puregene DNA isolation kit was used according to the manufacturer's instructions. Genotyping for the d3-GHR gene was performed using polymerase chain reaction (PCR) with the following primers: G1 5'-TGT GCT GGT CTG TTG GTC TG-3', G2 5'-AGT CGT TCC TGG GAC AGA GA-3', and G3 5'-CCT GGA TTA ACA CTT TGC AGA CTC-3' [14]. Amplification was performed for 40 cycles of 94°C for 30 seconds, 57°C for 30 seconds, and 68°C for 30 seconds, followed by a final extension time at 68°C for 10 minutes. The polymorphism detected by PCR was evident as a 592 bp product in the presence of the deletion (d3/d3) and a 935 bp fragment in the presence of the full length fragment (fl/fl). Each sample was geno-typed as d3/d3, d3/fl, or fl/fl.

Table 1. Demographic and Laboratory Parameters of Korean Patients with Acromegaly ($n=30$)

| Parameter | Value |
|------------------------|--------------|
| Age, yr | 47.5±9.32 |
| Sex, male:female | 12:18 |
| BMI, kg/m ² | 25.3±2.94 |
| SBP, mm Hg | 130.2±12.65 |
| DBP, mm Hg | 78.8±12.35 |
| FPG, mg/dL | 133.2±71.70 |
| Triglycerides, mg/dL | 144.8±56.84 |
| LDL-C, mg/dL | 126.1±28.19 |
| HDL-C, mg/dL | 44.9±12.92 |
| GH, ng/mL | 33.9±35.63 |
| IGF-1, ng/mL | 888.3±413.71 |

Values are expressed as mean±SD.

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol; GH, growth hormone; IGF-1, insulin like growth factor-1.

Statistical analysis

Continuous variables were expressed as mean±standard deviation (SD). Independent sample *t* test and Mann-Whitney *U* test were used to analyze differences in continuous variables between the different genotypes. A chi-square test was used for comparison of nominal variables between groups. *P* values <0.05 were considered statistically significant. Statistical significance of genotypic frequencies was evaluated according to the Hardy-Weinberg rule considering the expected genotypic frequencies and evaluated by the chi-square test. All analyses were carried out using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA).

RESULTS

Thirty patients with acromegaly met the inclusion criteria. Selected demographic characteristics are shown in Table 1. The d3-GHR genotypes were distributed as follows: four patients (13.3%) had the d3/fl genotype and 26 (86.7%) had the fl/fl

Table 2. Comparison of d3-GHR Genotypes with Demographic Characteristics and Laboratory Parameters in Korean Patients with Acromegaly

| | d3/d3 | d3/fl | fl/fl | <i>P</i> <0.05 |
|-------------------------|-------|---------------|--------------|---|
| All patients ($n=30$) | 0 | 4 (13.3) | 26 (86.7) | |
| Age, yr | | 41.25±7.805 | 48.50±9.283 | NS |
| Sex | | | | |
| Male | | 3 | 9 | NS ^a |
| Female | | 1 | 17 | NS ^a |
| Non-DM | | 3 | 22 | NS ^a |
| DM | | 1 | 4 | NS ^a |
| BMI, kg/m ² | | 28.5±0.80 | 24.7±2.87 | 0.001 ^b , 0.045 ^c |
| SBP, mm Hg | | 137.3±6.43 | 129.3±13.10 | NS |
| DBP, mm Hg | | 83.0±5.196 | 78.24±13.034 | NS |
| GH, ng/mL | | 22.8±20.01 | 34.9±36.85 | NS |
| IGF-1, ng/mL | | 1017.9±192.64 | 869.8±436.16 | NS |
| FPG, mg/dL | | 196.7±165.71 | 123.7±48.27 | NS |
| Triglycerides, mg/dL | | 88 | 147.7±56.76 | NS |
| LDL-C, mg/dL | | 99 | 127.5±28.20 | NS |
| HDL-C, mg/dL | | 48 | 44.7±13.26 | NS |

Values are expressed as number (%) or mean±SD.

d3-GHR, growth hormone receptor exon 3 deletion; fl, full length; DM, diabetes mellitus; BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; GH, growth hormone; IGF-1, insulin like growth factor-1; FPG, fasting plasma glucose; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

^aChi-square test; ^bIndependent *t* test; ^cMann-Whitney *U* test.

genotype while none had the d3/d3 genotype. These frequencies are different from previous reports in other population. For example, Montefusco et al. [14] reported a distribution of 9.2% for d3/d3 and 31.6% for d3/fl in 76 patients with acromegaly in Italy, while Mercado et al. [7] documented 32.4% of d3/d3 and 21.6% of d3/fl in 148 patients with acromegaly in Mexico.

Demographic and laboratory parameters were compared between the different genotypes. No significant differences were seen between age, gender, SBP, DBP, IGF-1, GH, FPG, triglycerides, and HDL-C and LDL-C between the d3/fl and fl/fl genotypes (Mann-Whitney *U* test) (Table 2). BMI was significantly higher in patients with the d3/fl genotype compared to fl/fl ($P=0.001$).

DISCUSSION

In this study of Korean patients of acromegaly, the distribution of the d3-GHR genotype was somewhat dissimilar to that reported in previous studies shown in Table 3. In particular, in patients with acromegaly from Italy, France, and Mexico, the d3/d3 genotype was present 14.3% to 32.4% of cases, compared to no cases herein. On the other hand, comparing with healthy controls in same ethnic groups, especially Korea and China, the d3/d3 genotype was present lower (3.2% to 7.2% of cases) than that of other ethnic groups spoken above. In distribution of genotype with d3 allele (d3/d3+d3/fl), patients with acromegaly from Italy, France, Mexico were higher as 45.7% to 54% than the present study (13.3%) and the study with healthy controls in Korea (27.4%) and China (25.4%) [7,14,16-19]. While this may suggest the existence of ethnic differences in the frequency of d3-GHR genotypes, some limitations to the present study must be considered. First, our study was conducted a local area of South Korea, and thus reflects only this ethnic group. Sec-

ond, this study enrolled a small number of patients, and no patient was homozygous for d3-GHR. Obviously, the heterozygote d3-GHR cannot represent all d3 GHR allele carriers.

None of the parameters assessed showed any significant differences between d3-GHR genotypes with the exception of BMI, in agreement with previous studies [7,14,16,17]. Cinar et al. [20] showed that the d3-GHR variant genotype did not have an effect on clinical features or comorbidity in patients with acromegaly, but it might play a role in GH/IGF-1 level discordance in acromegaly. There are also some previous studies reported that basal levels of GH were lower in patients bearing the fl/fl genotype [7,14,16,17], while no significant differences between genotypes were seen in the present analysis. Likewise, IGF levels are reported to be higher in patients with the d3/fl genotype, but no significant difference was seen in this cohort of Korean patients. SBP was somewhat higher in

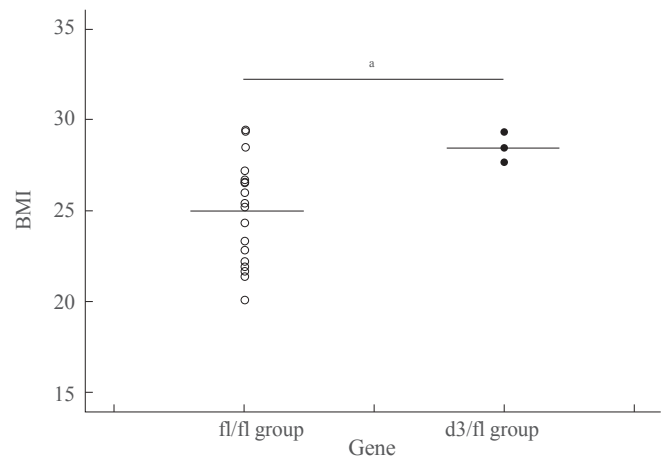


Fig. 1. Scatter plot of growth hormone receptor exon 3 deletion gene polymorphism and body mass index (BMI). *BMI was significantly higher in patients with the d3/fl genotype compared to fl/fl.

Table 3. d3-GHR Gene Polymorphism Genotype of Variable Populations

| Study | Population | State of disease | Number | Genotype, % | | | |
|------------------------|------------|------------------|--------|-------------|-------|-------|-------------|
| | | | | fl/fl | d3/fl | d3/d3 | d3/fl+d3/d3 |
| This study | Korean | Acromegaly | 30 | 86.7 | 13.3 | 0 | 13.3 |
| Mercado et al. [7] | Mexican | Acromegaly | 148 | 45.9 | 21.6 | 32.4 | 54.0 |
| Kamenicky et al. [17] | French | Acromegaly | 105 | 51.4 | 29.5 | 19.0 | 48.5 |
| Bianchi et al. [16] | Italy | Acromegaly | 84 | 52.4 | 29.8 | 17.9 | 47.7 |
| Montefusco et al. [14] | Italy | Acromegaly | 76 | 59.2 | 31.4 | 14.3 | 45.7 |
| Kang et al. [18] | Korean | Healthy control | 125 | 73.6 | 19.2 | 7.2 | 27.4 |
| Shen et al. [19] | Chinese | Healthy control | 441 | 74.6 | 22.2 | 3.2 | 25.4 |

d3-GHR, growth hormone receptor exon 3 deletion; fl, full length.

d3/fl heterozygotes, but these levels were under 140 mm Hg in both genotypes, no significant difference was observed between genotypes. The same is true of DBP, which was under 85 mm Hg in both groups.

Considering the lipid profile, triglycerides and LDL-C were lower in the d3/fl genotype, but with no significant differences between groups. One limitation in interpreting these results that data on lipids was not available for all patients, further decreasing the sample size, because we only could collect laboratory results of lipid profile depending their medical records and some were missed from their initial laboratory test when patients diagnosed acromegaly.

BMI was the only parameter showing significant differences between groups, and was higher in patients with the d3/fl genotype ($P=0.001$) as shown in Table 2, Fig. 1. Interestingly, most previous studies reported no significant difference between GHR genotypes and BMI [13,16,17], although one found a significant relationship between lower BMI and patients bearing the d3 GHR allele compared to fl/fl carriers [14]. Turgut et al. [21] reported that BMI was significantly higher in individuals with the d3/fl genotype, similar to the present study.

Some previous reports have documented slightly higher prevalence of type 2 diabetes in patients with acromegaly bearing the d3-GHR genotype, along with possible differences in glucose metabolism in individuals with the d3/d3 [7,21]. On the basis of these studies, we presumed the higher levels of FPG may be associated with the higher BMI in patients with the d3-GHR genotype, at least to some degree. Nonetheless, FPG seen herein did not reach statistical significance ($P=0.526$). Thus, the d3-GHR genotype is unlikely to affect glucose metabolism in this study.

In conclusion, the d3-GHR polymorphism may be associated with higher BMI. Although this study has some inherent limitation, it is our belief that further investigations are warranted on large cohorts of patients with acromegaly to validate these findings.

CONFLICTS OF INTEREST

No potential conflict of interest relevant to this article was reported.

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