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A descriptive study on selected growth parameters and growth hormone receptor gene in healthy young adults from the American Midwest

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ABSTRACT

Context: The first study of growth hormone receptor (*GHR*) genotypes in healthy young adults in the United States attending a Midwestern university and impact on selected growth parameters.
Objective: To describe the frequency of *GHR* genotypes in a sample of healthy young adults from the United States attending a university in the Midwest and analyze the relationship between *GHR* genotypes and selected growth parameters.
Design: Saliva was collected from 459 healthy young adults (237 females, 222 males; age range = 18–25 y) and DNA isolated for genotyping of *GHR* alleles (*fl/fl*, *fl/d3*, *or d3/d3*). Selected growth parameters were collected and *GHR* genotype data examined for previously reported associations (e.g., height, weight or bone mass density) or novel findings (e.g., % body water and index finger length).
Results: We found 219 participants (48%) homozygous for *fl/fl*, 203 (44%), heterozygous *fl/d3* and 37 (8%) homozygous *d3/d3*. The distribution of *GHR* genotypes in our participants was consistent with previous reports of non-US populations. Several anthropometric measures differed by sex. The distribution of *GHR* genotypes did not significantly differ by sex, weight, or other anthropometric measures. However, the *fl/d3* genotype was more common among African-Americans.
Conclusions: Our study of growth and anthropometric parameters in relationship to *GHR* genotypes found no association with height, weight, right index finger length, BMI, bone mass density, % body fat or % body water in healthy young adults. We did identify sex differences with increased body fat, decreased bone density, body water and index finger length in females.

1. Introduction

The growth hormone receptor (*GHR*) is a transmembrane protein belonging to the cytokine receptor superfamily [1]. When growth hormone (*GH*) binds to *GHR*, the receptor dimerizes and activates intra- and intercellular signal transduction pathways. The *GHR* gene encodes for two isoforms, the full-length allele, *GHRfl* (27%–60% of Europeans) and the deletion allele, *GHRd3* (exon 3 deleted, 4%–17% of Europeans) [2]. The *GHRd3* isoform is the result of a retroviral insertion event that leads to the deletion of 22 residues near the n-terminus [1]. In vitro studies have shown that the binding of growth hormone to either *GHR* isoform is similar. However, the presence of *GHRd3* leads to higher transcriptional activity of *GH* and increased sensitivity to recombinant *GH* in individuals with *GH* deficiency [1,3–5].

The *GHR* gene has been found to play an important role in growth regulation of individuals as supported by studies on *GHR* deficiency in Laron syndrome, a rare genetic disorder that causes impaired growth and severe short stature [6]. Further studies with the *GHRd3* isoform have shown a 1.8 to 2 fold accelerated growth rate that directly impacts birth and placental weight, stature, skeletal growth, body composition and bone density prior to adulthood in the general population [3,7,8]. Similarly, this accelerated growth rate correlates with the *GHRd3*

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genotype in those with Prader-Willi syndrome, a genetic obesity-related disorder with growth hormone deficiency requiring treatment due to errors in genomic imprinting [9–12]. Other studies have shown that the exon 3 deletion in the *GHR* gene appeared not to alter the interaction with *GH* in those with severe growth hormone deficiency or other genetic syndromes with short stature [4,13–15]. A combination of genetic and environmental factors appears to control human body composition by utilizing *GH* pathways throughout life [16]. However, the amount that *GH* contributes to physical traits during a lifetime appears to change over time. Studies on *GHR* isoforms have been conducted in European, Canadian and South American populations and clinical phenotypes such as adult height and bone mineral density appear not to be linked to the *GHR exon 3* deletion genotype in healthy young adults of different racial or ethnic backgrounds [2–4,17,18].

The objective of our study was to analyze the frequency of *GHR* genotypes in 459 healthy young adults attending a public university in the American Midwest by using traditional PCR based methods and DNA sequencing. We then collected measures of selected growth parameters and compared with the literature including height, weight, hand size (right index finger length), % body fat, % body water and bone mass density for each participant with their *GHR* genotype. Our study population included both healthy female and male adults with ages ranging from 18 to 25 years, representing multiple racial and ethnic backgrounds and residing in the United States.

2. Methods

2.1. Participants

The study protocol was approved by the affiliated Institutional Review Board and all participants gave written informed consent before examinations were undertaken. Participants were recruited using SONA (http://www.sona-systems.com), an experiment tracking software system that allows students to participate in active and available studies at the university. The participants received three credits for their undergraduate psychology course and received a gift card for their time and effort. The participants were asked to refrain from eating one hour before as well as smoking, taking drugs (including prescription), caffeine, and alcohol at least three hours before appointment time. Participants began by rinsing their mouths with water and then provided 2 mL of saliva via passive drool at 10 min after rinsing for genetic analysis. Participants then completed an online survey using Qualtrics software, which was completed in approximately 1 h. They were given a unique ID number and no identifying information was collected, keeping the identity of all participants anonymous. The study sample was comprised of 459 healthy young adults (n = 222 M; n = 237 F; ages 18–25 years old; average BMI 23.77 kg/m^2 ± 4.08) enrolled at a mid-sized university of ~28,000 students located in the American Midwest. The racial demographic was primarily non-Hispanic Caucasian (72%; see Table 1) with 10% Asian, 6% Hispanic, 6% Mixed Race, 4% African-American, 2% Other and 1% Native American. Race/ethnicity was collected by self-report based upon subject identification with no further characterization of parent or grandparent racial/ethnic affiliation.

Table 1
Growth hormone receptor (*GHR*) genotypes by race and ethnicity.

Race	n (%)	*fl/fl*	*fl/d3*	*d3/d3*
Caucasian	329 (71.7%)	161 (48.9%)	141 (42.9%)	27 (8.2%)
Asian	47 (10.2%)	25 (53.2%)	20 (42.6%)	2 (4.3%)
Hispanic/Latino	29 (6.3%)	13 (44.8%)	14 (48.3%)	2 (6.9%)
Mixed	25 (5.5%)	13 (52.0%)	8 (32.0%)	4 (16.0%)
African-American	17 (3.7%)	4 (23.5%)[a]	12 (70.6%)	1 (5.9%)
Other	7 (1.5%)	1 (14.3%)	5 (71.4%)	1 (14.3%)
Native American	5 (1.1%)	2 (40.0%)	3 (60.0%)	0 (0%)

[a] Chi-Square test of *fl/fl GHR* genotype compared to the combined *fl/d3* and *d3/d3* genotypes for African-Americans relative to all races, $p < 0.05$. No other statistically significant associations were found.

2.2. Phenotypic data collection

Phenotypic data were collected by trained research staff. Standing height was measured to the nearest 0.254 cm using a standard stadiometer (SECA model number 213, Chino, California). Weight was measured to the nearest 0.045 kg using the Tanita InnerScan Body Composition Monitor-Model BC-533 (Arlington Heights, Illinois) [19,20]. Body composition (% body fat, % body water and bone mass density) were determined using the Tanita monitor following manufacturer's instructions and as reported in published literature [21]. Height, weight and body composition measures were collected with the participants wearing one layer of clothing and no footwear. The Tanita BC-533 model uses standard bioelectric impedance technology, cleared by the Food and Drug Administration and provides highly accurate and reproducible body composition data within 5% of DEXA (dual-energy X-ray absorptiometry). Finger measurements were taken from the participants' right index finger. Participants placed their right hand on a flat surface, palmar side up. Using a plastic ruler, measurements were taken from the basal crease to the tip of the finger.

2.3. Growth hormone receptor (GHR) genotypes

The *GHR* genotypes were identified using PCR with primers G1, G2, and G3 obtained from Integrated DNA Technologies (Coralville, Iowa. GenBank, accession number AF155912). Genotyping was performed on each participant sample to identify *GHRfl* and *GHRd3* genotypes, using two separate PCR procedures with primers G1/G2 and G1/G3 run as separate reactions. The following PCR cycle parameters were used for G1/G2: the initial denaturation for five minutes at 94 °C followed by 35 cycles of 30 s at 94 °C, 60 s at 60 °C; and for 90 s at 72 °C, followed by a final extension period for 7 min at 72 °C. The following PCR cycle parameters were used for G1/G3: the initial denaturation for five minutes at 94 °C followed by 40 cycles of 30 s at 94 °C, 60 s at 60 °C; and for 90 s at 72 °C, followed by a final extension period for 7 min at 72 °C. Electrophoresis of the PCR fragments was performed with 2% agarose. Primers for G1 and G3 generated a PCR fragment of 935 bp indicating the wild type full length (*fl*) allele while primers G1 and G2 generated a PCR fragment of 532 bp representing the deletion (*d3*) allele indicating that exon 3 contains the deletion. Participants were retested if the initial results were inconclusive.

2.4. GHR SNP rs6873545 sequencing

The rs6873545 SNP of *GHR* previously reported in complete linkage disequilibrium with the exon 3 deletion polymorphism was amplified and sequenced to confirm our primary genomic analysis in a subset of *d3/d3* and *fl/d3* participants [22,23]. The C/T SNP polymorphism is located in the third intron of the *GHR* gene, near one of the two retroviral repeated sequences that create the *d3* allele. PCR amplification was carried out as follows: initial denaturation for five minutes at 95 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C; and for 90 s at 72 °C, followed by a final extension period for 7 min at 72 °C. This genotyping method used two primers: *GHR* rs6873545 forward (CCGTCAGATTCA GTTTGGTTC) and the G2 primer from above. This reaction generated a 266 bp fragment that was sent to GeneWiz (South Plainfield, New Jersey) for sequencing, a commercially available sequencing laboratory for a fee for service activity. Control samples for each genotype that had been previously validated by DNA gel electrophoresis and fragment analysis were used to confirm the SNP sequencing.