The GH receptor exon 3 deleted/full-length polymorphism is associated with central adiposity in the general population

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Abstract

Objective: To test the hypothesis that the GH receptor (*GHR*) exon 3 deleted (d3)/full-length (fl) polymorphism influences anthropometry and body composition in the general population.

Design and setting: The Swedish Obese Subjects (SOS) reference study is a cross-sectional population-based study, randomly selected from a population registry. A subgroup of the population-based Malmö Diet and Cancer study (MDC-CC) was used as a replication cohort.

Methods: The SOS reference study comprises 1135 subjects (46.2% men), with an average age of 49.5 years. The MDC-CC includes 5451 successfully genotyped subjects (41.5% men), with an average age of 57.5 years. *GHR* d3/fl genotypes were determined using TagSNP rs6873545. Linear regression analyses were used to test for genotype–phenotype associations.

Results: In the SOS reference study, subjects homozygous for the d3-*GHR* weighed ~4 kg more ($P=0.011$), and had larger waist-to-hip ratio (WHR, $P=0.036$), larger waist circumference ($P=0.016$), and more fat-free mass estimated from total body potassium ($P=0.026$) than grouped fl/d3 and fl/fl subjects (d3-recessive genetic model). The association with WHR was replicated in the MDC-CC ($P=0.002$), but not those with other anthropometric traits.

Conclusions: In this population-based study, the *GHR* d3/fl polymorphism was found to be of functional relevance and associated with central adiposity, such that subjects homozygous for the d3-*GHR* showed an increased abdominal obesity.

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Introduction

In higher organisms, traits of body composition and anthropometry (such as body height and body weight) are determined by a complex interplay of environmental and genetic factors. During the course of evolution, efficient parallel systems that monitor and regulate the metabolic state have emerged; systems that are composed of a collection of important proteins that ultimately sets the stage for interindividual variation in energy metabolism. As such, the growth hormone–insulin-like growth

factor 1 (GH–IGF1) axis is a major player in the regulation of metabolic traits in humans.

GH is secreted from the anterior pituitary gland in a pulsatile pattern that is influenced by factors such as age, sex, sleep, feeding, physical activity, and obesity (1). GH is an important stimulator of postnatal longitudinal growth and it has been shown to directly stimulate the cells in the growth plate to induce growth (2). In addition, GH has major effects on fuel metabolism by influencing muscle

(protein anabolism) and fat (lipolysis), and through regulation of glucose and lipid metabolism (1). In healthy subjects, GH secretion and visceral adipose tissue mass is strongly, and inversely, associated (3). Patients with obesity, particularly abdominal obesity, display a marked decrease in GH secretion (4). When subjects with abdominal obesity are treated with GH, the abdominal fat mass is reduced and the metabolic status is improved (5). Adults with severe GH deficiency (GHD) have abdominal adiposity, increased total body fat mass, and reduced muscle mass that is improved during GH replacement therapy (6), which further emphasizes the pronounced effects of GH on the regulation of anthropometric traits in humans.

At the molecular level, GH effects are mediated by the GH receptor (GHR), encoded by the *GHR* gene located on chromosome 5, region p13.1-p12 (7, 8). The GHR is a transmembrane glycoprotein belonging to the type 1 cytokine receptor superfamily (9, 10). A 22-amino acid truncated isoform of the GHR protein exists, due to a polymorphism within the *GHR* gene where the entire exon 3 sequence is missing (8). This genomic deletion is due to an early homologous recombination event and has been passed on through generations (11). The exon 3 deleted (d3)-GHR has been suggested to confer a higher responsiveness to GH compared with the full-length (fl)-GHR (12). Clinical data from GH treatment studies in adult populations have demonstrated conflicting results with reports of both increased (13) and decreased (14) responses to GH in carriers of the d3-GHR. In children, however, the d3-GHR has been confirmed to confer a larger growth response to GH in meta-analyses (15, 16).

Based on the proposed relation between the d3-GHR and increased GH sensitivity, we aimed to test the hypothesis that carriers of the d3-GHR in the general population would be taller, leaner, and have more fat-free mass (FFM), reflecting a more GH-sensitive phenotype.

Materials and methods

Subjects and samples

Ethics statement ► Regional ethic review boards at the University of Gothenburg and Lund University approved the studies, and all participants gave informed consent before the examinations.

The Swedish Obese Subjects reference study ► The Swedish Obese Subjects (SOS) reference study includes subjects from the Swedish cities Mölndal and Örebro, randomly selected from a population registry to constitute

a reference group to the SOS intervention study, presented elsewhere (17). The study includes 1135 subjects (46.2% men), with an average age of 49.5 ± 7.0 years and average BMI of 25.2 ± 3.8 kg/m² (18).

All anthropometric measurements were performed with the subjects dressed in underwear, as described by Sjöström *et al.* (17). The following circumferences were measured in the recumbent position. Waist circumference was measured at the level midway between the most caudal part of the lateral costal arch and the iliac crest at the end of a normal expiration. Hip circumference was measured at the symphysis–trochanter femoris level. BMI and waist-to-hip ratio (WHR) were calculated. Body weight was compartmentalized into FFM and body fat (BF) using the total body potassium (TBK) method. The TBK measurements were performed in the whole-body counter system II, at the Department of Radiation Physics at the Sahlgrenska University Hospital, Göteborg, Sweden. FFM (kg) was calculated from the TBK measurements using the constants 64.7 for men (19) and 62.0 for women (20). BF (kg) was then calculated as the FFM subtracted from the body weight. Blood samples were obtained after an overnight fast. IGF1 was measured in serum by a hydrochloric acid–ethanol extraction RIA using authentic IGF1 for labeling (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA), with an inter-assay coefficient of variation of 2.5 and 4.2% at serum concentrations of 125 and 345 µg/l respectively.

The Malmö Diet and Cancer study ► The Malmö Diet and Cancer study (MDC-CC) is a prospective population-based cohort study including 28 449 subjects recruited during the period of 1991–1996 (21). Subjects aged 45–69 years, living in the city of Malmö, Sweden were eligible for participation. Between November 1991 and February 1994, every other enrolled subject was also invited to take part in a substudy of the epidemiology of carotid artery disease (22). This MDC-CC cohort consists of 6103 subjects, 5540 of whom also agreed to have blood collected under standardized fasting conditions; 5451 subjects were successfully genotyped (41.5% men; average age 57.5 ± 5.9 years, average BMI 25.8 ± 4.0 kg/m²). Standardized anthropometrics measurements were performed. Waist circumference (cm) was measured in the standing position midway between the lower rib margin and the ileac crest.

DNA isolation and genotyping

The SOS reference study ► Genomic DNA was isolated using the Mag Maxi Plus Kit (AGOWA GmbH, Berlin,

Germany) and a semi-automated magnetic bead technique on a Microlab Star instrument (Hamilton Robotics, Reno, NV, USA). Genotyping was performed using TaqMan SNP genotyping of the *GHR* d3/fl TagSNP rs6873545, which was validated previously (23). Briefly, 10 ng genomic DNA was added to a reaction mix containing 1× TaqMan Genotyping PCR Master Mix (Applied Biosystems) and an rs6873545-specific genotyping assay (C_28966089_10; Applied Biosystems). All reactions were carried out in 5 µl reaction volumes on 384-well plates (Applied Biosystems). PCR amplification and allele detection were carried out in an ABI Prism 7900HT Sequence Detection System instrument (Applied Biosystems).

The MDC-CC ► DNA was extracted from whole-blood samples using Qiagen Maxipreps (Qiagen). SNP rs6873545 was genotyped using the Illumina Human OmniExpress-Exome BeadChip v1.0.

Statistical analyses

Linear regression analyses were used to investigate the impact of the *GHR* d3/fl polymorphism (in a d3-recessive model) on anthropometric measurements, body composition, and serum IGF1 concentrations using sex and age as other predictors. Deviations from the Hardy–Weinberg equilibrium (HWE) were tested by the goodness-of-fit χ^2 test. The Statistical Software R (version 2.12.1) was used for the statistical analyses.

Results

GHR d3/fl genotyping

Genotyping success rate was 99.1% (98.4%) and the frequency of the d3-GHR was 24.0% (fl/fl 57.6 (58.1)%, fl/d3 36.8 (36.6)%, and d3/d3 5.6 (5.4)% in the SOS reference and MDC-CC studies respectively (MDC-CC data presented within brackets)). No deviations from the HWE were found (χ^2 test, $P=0.8$ and 0.4 respectively).

Association analyses

The SOS reference study and the MDC-CC both contain unselected subjects from the general Swedish population, and hence these studies are unmatched for clinical parameters. Waist circumference and WHR tended to be higher in the SOS reference study, which also contained more men, than the MDC-CC. By contrast, both systolic and diastolic blood pressure tended to be higher in the

MDC-CC, and the subjects were also older than those in the SOS reference study. General baseline characteristics of the subjects are presented in Table 1. Clinical characteristics of the study subjects when divided into *GHR* d3/fl genotype groups and results from the linear regression analyses, adjusted for sex and age, are shown in Table 2.

The SOS reference study ► Homozygosity for the d3-GHR was associated with higher WHR ($P=0.036$), waist circumference ($P=0.016$), body weight ($P=0.011$), BMI ($P=0.049$), and FFM estimated from TBK ($P=0.026$). No associations were found between *GHR* d3/fl genotype and serum IGF1 levels ($P=0.4$), body height ($P=0.2$), fasting glucose ($P=0.5$), or plasma insulin ($P=0.9$) levels.

The MDC-CC ► In the MDC-CC, homozygosity for the d3-GHR was associated with a significantly higher WHR ($P=0.002$). Genotype was not associated with waist circumference ($P=0.1$), body weight ($P=0.6$), body height ($P=0.2$), fasting glucose ($P=0.5$), or plasma insulin ($P=0.9$) levels.

Discussion

In this study, we demonstrate for the first time an association of the *GHR* d3/fl polymorphism with central adiposity in two independent populations of Swedish middle-aged subjects. Specifically, subjects homozygous for the d3-GHR had larger WHR.

Based on previous findings, which suggest that subjects carrying the d3-GHR are more sensitive to GH,

Table 1 Clinical characteristics of the subjects in the SOS reference and the MDC-CC studies. Values are expressed as means $\pm$ s.d.

Clinical variables	SOS reference study	MDC-CC
<i>n</i>	1135	5451
Sex (men, %)	46.2	41.5
Age (years)	49.5 $\pm$ 7.0	57.5 $\pm$ 5.9
Body height (m)	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1
Body weight (kg)	75.1 $\pm$ 14.2	73.7 $\pm$ 13.7
BMI (kg/m ²)	25.2 $\pm$ 3.8	25.8 $\pm$ 4.0
Waist (cm)	87.9 $\pm$ 11.2	84.1 $\pm$ 13.0
WHR	0.88 $\pm$ 0.08	0.85 $\pm$ 0.09
SBP (mmHg)	125.2 $\pm$ 19.1	141.2 $\pm$ 18.9
DBP (mmHg)	76.6 $\pm$ 11.3	86.9 $\pm$ 9.4
Fat-free mass ^a (kg)	55.7 $\pm$ 11.5	NA
Body fat ^a (kg)	18.7 $\pm$ 8.6	NA
IGF1 (µg/l)	207.0 $\pm$ 64.9	NA

WHR, waist-to-hip ratio; SBP, systolic blood pressure; DBP, diastolic blood pressure.

^aEstimated from total body potassium (TBK) measurements.

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Table 2 Clinical characteristics of the study subjects when divided into genotype groups, and results (sex- and age-adjusted effect sizes and *P* values) from the regression analyses in the SOS reference and the MDC-CC studies, using a d3-GHR recessive model of inheritance.

Clinical variables	SOS reference study					MDC-CC				
	fl/fl	fl/d3	d3/d3	β	<i>P</i>	fl/fl	fl/d3	d3/d3	β	<i>P</i>
Body height (cm)	172.3	171.7	173.6	1.0	0.175	168.8	169.2	169.0	−0.5	0.2
Body weight (kg)	75.0	74.5	78.8	3.9	0.011	73.3	74.2	74.6	0.3	0.6
BMI (kg/m ²)	25.2	25.2	26.0	1.0	0.049	25.7	26.0	26.1	0.2	0.3
Waist (cm)	87.8	87.4	90.7	3.2	0.016	83.7	84.5	85.7	0.9	0.1
WHR	0.88	0.88	0.90	0.02	0.036	0.85	0.85	0.87	0.01	0.002
FFM (kg) ^a	56.2	54.4	58.7	2.2	0.021	NA	NA	NA	NA	NA
Body fat (kg) ^a	18.4	19.1	20.2	2.2	<i>0.065</i>	NA	NA	NA	NA	NA
IGF1 (μg/l)	207.1	206.5	203.8	−6.7	0.413	NA	NA	NA	NA	NA

WHR, waist-to-hip ratio. Significant *P* values are highlighted in bold and close-to-significant (*P* < 0.1) are shown in italics. β corresponds to average effect size. Positive effects size corresponds to a larger value in d3-GHR homozygotes, compared with the grouped fl/d3 and fl/fl subjects. Average (mean) observed values for selected clinical variables are shown.

^aEstimated from total body potassium (TBK) measurements.

we anticipated that d3-GHR carriers should be taller, have increased FFM and less body fat, and, in particular, reduced abdominal fat. By contrast, we found a metabolically adverse profile in subjects homozygous for the d3-GHR. However, the data suggesting increased GH sensitivity in carriers of the d3-GHR are based on treatment response to exogenous GH in patients with GHD, who exhibit severe disturbances in the normal regulation of the hypothalamic–pituitary axis (15, 16). Thus, our findings may reflect the complex relationship among GH sensitivity, GH secretion, and other adaptive metabolic mechanisms that occur throughout life in subjects with a normally functioning GH–IGF1 axis.

GH efficiently induces lipolysis. The lipolytic response to GH is more marked in visceral fat mass, which is demonstrated by a marked decrease in abdominal fat mass in response to GH treatment (24). Inversely, abdominal adiposity, particularly visceral adipose tissue mass, is a stronger negative determinant of GH secretion than age, sex, or BMI (3, 25, 26). In obesity, both spontaneous (27, 28) and stimulated (29) GH secretion is blunted. Specifically, the amount of GH secreted per burst is decreased, although burst frequency is unaffected (27, 28). The metabolic clearance rate of GH is accelerated in individuals with obesity (28), further decreasing the amount of bioavailable GH. Low endogenous GH secretion in the obese state has been associated with increased cardiovascular risk factors (30, 31, 32, 33), although a cause-and-effect relationship is yet to be established.

In the SOS reference study, homozygotes of the d3-GHR had larger FFM when compared with grouped fl/fl and d3/fl subjects, possibly indicating a higher GH sensitivity in muscle in this genotype group. It should be

noted, however, that the increased FFM in d3-GHR homozygotes could also be secondary to the higher body mass observed in these subjects, as subjects who weigh heavier generally require a larger muscle mass. Unfortunately, FFM was not measured in the replication cohort, and thus this finding could not be verified.

WHR is a strong predictor of cardiovascular risk (34). We found that subjects homozygous for the d3-GHR had larger WHR in two independent cohorts of adult Swedes. The d3-GHR and fl-GHR may have differential properties. Previously, the d3-GHR was shown to be associated with WHR in a cohort of healthy Chinese women and over-represented among subjects with polycystic ovary syndrome (35). Moreover, it has been shown that subjects with type 2 diabetes mellitus carrying the d3-GHR had a larger BMI, and a more adverse metabolic profile (36). In addition, in a study on Chinese children, the d3-GHR was over-represented among obese children (37). Although these studies do comprise strikingly different cohorts when compared with ours, these recent findings point in the same direction. The d3-GHR appears to be associated with a more obese, and specifically a more abdominally obese, phenotype than the fl-GHR. The implications of these novel data remain yet to be further elucidated, and more mechanistic studies are warranted. Clearly, most previous studies have been carried out in small, selected cohorts of patients with limited power to fully detect the possible significance of d3-GHR homozygosity. Furthermore, the treatment response to GHRT is in itself confounded by the subsequent GH dose titration regimen that may mask or skew the possible effects of this genetic variant. In our previous study on adult GHD subjects, fl-GHR homozygotes did respond better to GHRT in terms of the very rapid (1 week) percentage increase in

serum IGF1 levels (14). Hence, new data are emerging that challenge the concept of an increased GH sensitivity in d3-GHR carriers.

To our knowledge, this is the first study on the *GHR* d3/fl polymorphism in relation to anthropometry and body composition in large cohorts of randomly selected subjects. Our findings support the notion that the *GHR* d3/fl polymorphism is of functional relevance, and indicate an adverse influence of the d3-GHR on the regulation of central adiposity.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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