Association between GH receptor polymorphism (exon 3 deletion), serum IGF1, semen quality, and reproductive hormone levels in 838 healthy young men

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Abstract

Introduction: GH activity may be involved in male reproductive function. A common genetic polymorphism in the gene encoding the GH receptor (*GHR*) results in deletion of the entire exon 3 sequence (*GHRd3* isoform). The short *GHRd3/d3* isoform seems more sensitive compared with full-length receptors (*GHRfl/fl*).
Aim: To investigate the associations between GH activity, evaluated by exon 3 GHR polymorphism, and serum IGF1 vs reproductive hormones, semen quality, and pre- and postnatal growth in healthy young males ($n=838$, mean age: 19.4 years).
Results: Compared with *GHRfl/fl* homozygous individuals ($n=467$) *GHRd3/d3* homozygous individuals ($n=69$) tended to have larger semen volume (3.2 (2.4–4.3) vs 3.6 (2.6–4.7) ml, $P=0.053$) and higher serum inhibin-B levels (208 pg/ml (158–257) vs 227 pg/ml (185–264), $P=0.050$). Semen quality, levels of gonadotropins, testosterone, estradiol, sex hormone-binding globulin, and IGF1 were not associated with *GHRd3* genotype.
A twofold increase in serum IGF1 was associated with a 13% (4–23) increase in calculated free testosterone ($P=0.004$). By contrast IGF1 was inversely associated with serum inhibin-B ($P=0.027$), but showed no associations to semen quality. *GHR* genotype and serum IGF1 were not associated with size at birth or final height.
Conclusions: *GHRd3* polymorphism seemed only to have a weak influence on male reproductive function of borderline significance. The sensitive *GHRd3/d3* genotype may slightly increase testicular function, as evaluated by semen volume and levels of inhibin-B, but does not seem to influence Leydig cell steroidogenesis. *GHR* genotype did not influence pre- and postnatal growth.

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Introduction

A growing body of evidence has pointed to the possibility that growth hormone (GH) and its primary downstream mediator insulin-like growth factor 1 (IGF1) may be important for normal male reproductive function (1). The influence of GH/IGF1 activity seems to involve reproductive hormone levels (2, 3), semen characteristics (2, 4) and phenotype (e.g. penile size and risk of cryptorchidism) (3, 5, 6, 7, 8). The clinical observations are supported by histological studies identifying GH and IGF1 receptors in Leydig, Sertoli, and germ cells as well as in the accessory reproductive glands (1, 9, 10). A central hypothalamic/pituitary effect of GH has also been suggested including the stimulation of gonadotropin secretion (1, 11, 12, 13). Finally, GH/IGF1 activity may increase the anabolic effects of androgens (5, 14).

GH action is mediated upon binding to the GH receptor (GHR) (15). The *GHR* gene is located on chromosome 5 and consists of nine coding exons.

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A common genetic polymorphism is deletion of the entire exon 3 sequence (*GHRd3* isoform). Exon 3 is one of five exons (exon 3–7) encoding the ligand binding extracellular domain of the GHR. The *GHRd3* isoform seems to be more sensitive with increased downstream GH signaling compared with full-length receptors (*GHRfl/fl*) (15). A vast majority of clinical studies have focused on the influence of *GHRd3*-genotype on growth velocity in children and the effect of GH therapy. The results have been conflicting, but a meta-analysis concluded that *GHRd3*-genotype seems to account for a modest increase in the therapeutic effect of GH therapy (16). By contrast, a recently published large study in adults with GH deficiency has found that carriers of *GHRfl*-genotype had a better IGF1 response to GH therapy (17). In relation to reproductive function, we have shown that GHR*d3/d3* homozygosity is associated with earlier pubertal onset, thus supporting that this common exon 3 genetic variant may be of physiological significance also in relation to gonadal function (18).

In this population-based study, we investigated the associations between GH/IGF1 activity, as evaluated by the common exon 3 GHR polymorphism, circulating levels of IGF1, and its major binding protein IGF-binding protein 3 (IGFBP3) vs reproductive hormones, semen quality, and pre- and postnatal growth in a large cohort of healthy young men. The purpose of this study was to elucidate the hypothesis that high IGF1 activity judged by presence of the sensitive *GHRd3*-genotype and high IGF levels have a stimulating effect on male reproductive function.

Subjects and methods

Participants

The study population was part of an ongoing cross-sectional study of young Danish men who were included in collaboration with the Danish military health board (19). In Denmark, all young men, except those with chronic severe diseases, are required to undergo a compulsory medical examination before they may be considered for military service. The overall study population was recruited from 1996 to 2010 and consisted of 4867 individuals (average participation rate 24%) (19). This study consists of 999 consecutively included individuals. In these participants, semen analyses and biochemical measurements of serum IGF1, IGFBP3, and reproductive hormones were carried out. Additional *GHR* genotyping was done in a subgroup consisting of 838 individuals.

Detailed data concerning the study design, data acquisition, quality control of data, etc. have been published previously (19). In brief, on the day of medical examination at the military barrack, the potential participants were offered verbal as well as written information about the study. They were permitted either to provide immediate acceptance or to forward written acceptance at a later date. Those who agreed to participate had to complete an extensive questionnaire and to attend a physical examination at our hospital outpatient clinic. On the day of physical examination semen and blood samples were collected.

Questionnaire

The questionnaire included information on gestational age, length and weight at birth, current life style factors, history of fertility, and previous and current diseases. If possible, the participants were asked to complete the questionnaire in collaboration with their parents.

Physical examination

Height and weight were measured and an evaluation of external genitalia was carried out. Testicular size was assessed by a wooden orchidometer (Pharmacia and Upjohn, Hillerød, Denmark) and by ultrasonographic evaluation.

Semen samples

The participants had been instructed to abstain from ejaculation for at least 48 h before attendance at the clinic. The semen samples were obtained by masturbation into a clean tube at a room adjacent to the laboratory. The samples were maintained at 37 °C until analysis. The semen analyses were performed based on the WHO guidelines, 1992, with modifications as described previously (19). Ejaculation volume was estimated through weighing of the collecting tubes. Fresh semen samples were submitted to phase-contrast microscopy (×400 magnification) for the assessment of sperm motility. The samples were classified as either motile (WHO motility classes A+B+C) or not progressive motile (WHO motility class D). Sperm concentration in semen samples diluted in a solution of $NaHCO_3$, formaldehyde, and distilled water was assessed using a hemocytometer (Bürker-Türk). The smears were prepared and sperm morphology was assessed according to strict criteria by a trained laboratory technician.

Blood samples

Blood samples were withdrawn from an antecubital vein in the nonfasting state. Serum was separated and frozen at −20 °C until analyses. Genomic DNA was extracted from blood lymphocytes.

Serum IGF1 and IGFBP3 were determined by immunoassays (IMMULITE 2000, Siemens Healthcare Diagnostics, Los Angeles, CA, USA) on automated IMMULITE 2000 (Siemens). Intra- and interassay coefficients of variation (CV) were <4 and <9% respectively. Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), sex hormone-binding globulin (SHBG), testosterone, and estradiol were measured by time-resolved immunofluorometric assays (Delfia, Perkin-Elmer, Turku, Finland). Intra- and interassay CV for FSH and LH were 3 and 5% respectively. Intra- and interassay CV for both SHBG and testosterone were <8 and <5% respectively. For estradiol, intra- and interassay CV were <4 and 8%. Serum inhibin-B was measured by a specific two-sided enzyme immunometric assay (Serotec, Kidlington, UK), intra- and interassay CV 15 and 18%. Free testosterone was calculated from the testosterone and SHBG concentrations using the method reported by Vermeulen *et al.* (20), with the assumption of an average serum albumin concentration of 43.8 g/l.

The frequency of GHR transcript variation with retention or exclusion of exon 3 was tested by the multiplex PCR assay originally described by Pantel *et al.* (21). This assay was carried out with primers G1, G2, and G3 (GenBank association no. AF155912) as follows: initial step of denaturation of 3 min at 95 °C followed by 25 cycles consisting of 30 s at 95 °C, 1 min at 64 °C, 1 min at 72 °C, followed by an extension period of 72 °C for 5 min. Application of DNA fragments was analyzed by electrophoresis on a 1% agarose gel stained with ethidium bromide. A 935-bp band represented the *GHRfl* allele and a 532-bp fragment represented the *GHRd3* allele. We have previously described this methodology in more detail (22). The distribution of the *GHR* genotypes did not deviate significantly from Hardy–Weinberg equilibrium.

Statistical analyses

The associations between *GHR* genotype and serum levels of IGF1 and IGFBP3 vs different variables reflecting growth and reproductive function were assessed by general linear models. Unadjusted *P* values as well as *P* values adjusted for possible confounding variables were calculated. Data are presented as median (IQR) or as mean ± 1 SD. The influence of *GHR* genotype was evaluated as *GHRfl/fl* vs *GHRd3/d3*.

To determine the approximate normal distribution of the residuals, reproductive hormone levels and percentage of motile and morphological normal spermatozoa were log (ln x)-transformed. To facilitate the interpretation of data, changes in reproductive hormone levels were quantified as changes (95% CI) per twofold increase in serum IGF1 and IGFBP3 respectively (log 2 transformed). A twofold increase represents a physiologically relevant change; for IGF1 from e.g. ~10th percentile (212 ng/ml) to 90th percentile (433 ng/ml) and for IGFBP3 from e.g. ~2.5th percentile (3540 ng/ml) to 97.5th percentile (6570 ng/ml). Semen volume and all data involving sperm counts were cubic root transformed.

In the adjusted models, semen volume, concentration of spermatozoa, and all variables containing total numbers of spermatozoa were adjusted for ejaculation abstinence. Data involving sperm motility were adjusted for time for processing of semen samples. Serum testosterone, SHBG, calculated free testosterone, and estradiol were adjusted for BMI. Inhibin-B was adjusted for time on the day the samples were collected (23). We observed a slight unequal age distribution between *GHRfl/fl* individuals compared with *GHRd3/d3* individuals (19.5 vs 19.1 years, $P=0.06$). To avoid any unadjusted influence of age, all follow-up analyses comparing these two groups (Table 1) were further adjusted for age. A *P* value <0.05 is considered significant.

Ethical aspects

The study was approved by the local ethics committee in Copenhagen, Denmark (Reference numbers KF 01-117/96 and H-KF-289428). A written informed consent was obtained from all participants.

Results

Clinical characteristics and data on semen quality and serum levels of reproductive hormones according to the *GHR* genotype (*fl/fl, fl/d3,* and *d3/d3*) are given in Table 1. About 56% were homozygous for the *GHRfl/fl* allele, 36% were heterozygous, and 8% were homozygous for the *GHRd3/d3* allele.

The GH and IGF1 axis vs semen quality

As adjusted for age and ejaculation abstinence, *GHRd3/d3* homozygous individuals tended to have larger semen volume compared with *GHRfl/fl* homozygous individuals (3.6 (2.6–4.7) vs 3.2 (2.4–4.3) ml, $P=0.053$) (Table 1).

Table 1 Clinical characteristics, semen quality, and hormone levels according to GHR genotype. In the adjusted models, semen volume, concentration of spermatozoa, and all variables containing total numbers of spermatozoa were adjusted for ejaculation abstinence. Data involving motility were further adjusted for time for processing of semen samples. Serum testosterone, SHBG, calculated free testosterone, and estradiol were adjusted for BMI. Inhibin-B was adjusted for time on the day the samples were collected. All follow-up data were further adjusted for age.

				P value	*P* value adj
GHR	**fl/fl** ($n=467$)	**fl/d3** ($n=302$)	**d3/d3** ($n=69$)	fl/fl vs d3/d3	fl/fl vs d3/d3
At birth					
Birth weight (g) ($n=718$)	3533±600	3521±633	3651±647	0.21	
Birth length (cm) ($n=683$)	52.2±2.8	52.0±2.6	52.2±2.9	0.95	
GA (weeks) ($n=558$)	39.0±2.1	39.3±1.9	39.0±1.6	0.86	
Birth weight (SDS) ($n=488$)	0.15±1.3	0.01±1.4	0.55±1.6	0.09	
Cryptorchidism, no. (%)	43 (10.4%)	18 (6.6%)	6 (9.8%)	0.56	
Follow-up					
Age (years)	19.5±1.3	19.4±1.2	19.1±0.82	0.06	
Height (cm)	180.4±10.6	180.9±6.2	181.1±6.5	0.59	0.63
Weight (kg)	74.3±13.2	74.6±12.4	76.1±10.3	0.29	0.25
BMI (kg/m^2)	22.4 (21.0–24.7)	22.3 (20.4–24.5)	22.8 (20.9–24.5)	0.73	0.15
Testes size (palp) (cm^2)	21.1±4.6	20.8±4.8	20.4±4.3	0.24	0.39
Testes size (US) (cm^2)	14.0±3.9	14.2±4.2	14.0±3.8	0.89	0.77
Semen analyses					
Semen volume (ml)	3.2 (2.4–4.2)	3.3 (2.5–4.3)	3.6 (2.6–4.7)	0.040	0.053
Spermatozoa conc. (mill/ml)	43 (21–77)	42 (20–80)	40 (16–80)	0.49	0.50
Total no. (mill)	136 (62–247)	140 (54–256)	156 (69–255)	0.62	0.67
Motile spermatozoa (%)	65 (55–73)	65 (55–73)	67 (55–75)	0.33	0.24
Total no. (mill)	83 (37–158)	87 (36–157)	88 (45–188)	0.47	0.47
Morphol. normal sperma. (%)	7.0 (4.0–11.0)	6.0 (3.0–10.5)	6.5 (3.6–11.5)	0.73	0.96
Total no. (mill)	8.6 (2.4–22.6)	9.1 (1.8–22.4)	9.3 (2.3–24.9)	0.81	0.85
Hormone analyses					
IGF1 (ng/ml)	310 (250–371)	313 (252–382)	316 (257–376)	0.79	0.84
IGFBP3 (ng/ml)	4930 (4420–5430)	4870 (4408–5453)	4860 (4430–5260)	0.49	0.25
IGF1:IGFBP3 ratio	0.23 (0.20–0.26)	0.23 (0.20–0.27)	0.24 (0.20–0.26)	0.52	0.68
Inhibin B (pg/ml)	208 (158–257)	204 (151–257)	227 (185–264)	0.068	0.050
FSH (U/l)	2.66 (1.58–3.82)	2.38 (1.56–3.50)	2.20 (1.67–3.31)	0.63	0.66
Testosterone (nmol/l)	19.5 (15.6–23.6)	20.2 (16.0–24.3)	20.7 (16.2–25.7)	0.31	0.20
LH (U/l)	3.41 (2.52–4.36)	3.37 (2.40–4.43)	3.44 (2.43–4.15)	0.94	0.66
SHBG (nmol/l)	30 (22–40)	30 (24–38)	30 (23–38)	0.86	0.75
Calculated free testosterone (pmol/l)	430 (349–533)	441 (355–533)	469 (359–576)	0.19	0.09
Estradiol (pmol/l)	69 (59–87)	71 (56–86)	74 (60–93)	0.43	0.25

The highest total numbers of spermatozoa, motile spermatozoa, and morphological normal spermatozoa were also found in *GHRd3/d3* homozygotes and the lowest numbers in *GHRfl/fl* homozygotes; however, the numbers were nonsignificant. Testicular size was not influenced by GHR genotype (Table 1).

Associations between circulating levels of IGF1 and IGFBP3 vs concentrations of spermatozoa, total numbers of spermatozoa, and semen volume are presented in Table 2. There were no significant associations in either the univariate analyses or the adjusted models.

The GH and IGF1 axis vs reproductive hormone levels

The increased levels of inhibin-B of borderline significance could be identified in individuals homozygous for the *GHRd3* allele (*GHRd3/d3* vs *GHRfl/fl*: 227 (185–264) pg/ml vs 208 (158–257) pg/ml $P=0.050$) (Table 1). Serum levels of IGF1, testosterone, calculated free testosterone, and estradiol were also higher in *GHRd3/d3* homozygous individuals compared with both *GHRfl/fl* homozygous individuals and heterozygous individuals. However, for all these variables the results were nonsignificant (Table 1).

Unadjusted serum IGF1 was inversely associated to serum SHBG ($P=0.042$) (Table 3, Model I). Adjustment for levels of IGFBP3, testosterone, and BMI strengthened the inverse association between levels of IGF1 and SHBG ($P<0.001$) and showed that IGFBP3 was also independently (positively) associated to SHBG ($P<0.001$) (Table 3, Model II).

Adjusted for BMI and levels of SHBG, serum IGF1 was positively ($P=0.003$) and IGFBP3 was inversely ($P=0.019$)

Table 2 Associations between serum IGF1 and IGFBP3 vs semen quality. Model I provides unadjusted associations between IGF1 vs the three variables obtained by general linear models. In Model II, IGFBP3 and ejaculation abstinence time were added to the statistical analyses.

	Spermatozoa (mil/ml)		**Total spermatozoa** (mil)		**Semen volume** (ml)	
	β-coefficient	*P* value	β-coefficient	*P* value	β-coefficient	*P* value
Model I						
IGF1	0.002	0.96	−0.021	0.51	−0.02	0.54
Model II						
IGF1	−0.021	0.62	−0.058	0.18	−0.064	0.13
IGFBP3	0.037	0.41	0.068	0.11	0.073	0.09

associated with serum testosterone. The same was observed for calculated free testosterone. A twofold increase in serum IGF was associated with a 13% (4–23) ($P=0.004$) increase in calculated free testosterone. Further adjustment for serum LH did not change the associations between serum IGF1 and IGFBP3 vs testosterone or free testosterone (data not shown).

Serum IGF1 was positively associated with serum estradiol ($P=0.009$) but when serum IGFBP3 was included in the statistical model there was no longer any significant association (data not shown). Further adjustments for BMI and testosterone levels did not change this result (Table 2, Model II).

In contrast to the other reproductive hormones, serum inhibin-B was inversely associated with serum IGF ($P=0.027$) (Table 3, Model II). Adjustment for levels of FSH strengthened the inverse relationship between levels of inhibin-B and IGF1 ($P=0.01$).

Associations between levels of IGF1 vs IGFBP3 ($P<0.001$), LH vs testosterone ($P<0.001$), testosterone vs SHBG ($P<0.001$), and FSH vs inhibin-B ($P<0.001$) are shown in Fig. 1. As shown in the figure, the three different GHR genotypes did not influence the association between the different hormone levels.

Pre- and postnatal growth

GHR genotype was not significantly associated with birth length or birth weight. When corrected for gestational age, there was a trend toward higher birth weight (SDS) among *GHRd3/d3* homozygotes (*GHRfl/fl* vs *GHRd3/d3*, $P=0.09$, Table 1). Frequency of cryptorchidism did not seem to be influenced by *GHR* genotype. For details see Table 1.

Postnatal growth was also not significantly associated with the *GHR* genotype (*GHRd3/d3* vs *GHRfl/fl* homozygotes; 181.1±6.5 vs 180.4±10.6 cm, $P=0.63$).

Discussion

In this large cross-sectional study of 838 thoroughly examined healthy young men from the general population, we evaluated *GHR* genotype and the circulating levels of IGF1/IGFBP3 in relation to reproductive hormone levels and semen quality. The sensitive *GHRd3* genotype seemed only to have a minor influence of borderline significance on the measured variables. We observed slightly larger semen volume and higher levels of serum inhibin-B, providing some support to a masculinizing effect of *GHRd3/d3* homozygosity analogous to that observed in our previous study in healthy boys (18). We acknowledge that the results are at risk of being a chance finding.

The stimulating effect of the *GHRd3* genotype on inhibin-B secretion and seminal fluid volume was present despite unchanged levels of circulating total and estimated free testosterone. As one possibility, GH or local-secreted IGF1 might exert a direct action on Sertoli cells and on the secretory epithelium in accessory reproductive glands, where GH and IGF1 and their respective receptors have been identified (1, 9, 10, 24). Alternatively, a sensitive GHR might potentiate the stimulating effects of androgens on Sertoli cells and seminal fluid volume. The mechanisms are not clarified, but in a case report it was suggested that GH action may influence 5α-reductase activity (5). The differences in inhibin-B levels between the two homozygous genotypes did not translate into significant changes in sperm counts. Thus, based on our observations, the effect of GH action could be restricted to Sertoli cells and not germ cells.

In accordance with the results obtained by *GHR* genotyping, one clinical study found reduced semen volume in patients with untreated isolated GHD (4), whereas another study reported increased semen volume during GH replacement therapy (2). As concerns the

Table 3 Associations between serum IGF1 and IGFBP3 vs reproductive hormone levels. The table provides associations between IGF1 vs reproductive hormones quantified as change in percent (95% CI) pr. twofold increase in serum IGF1 and IGFBP3.

	SHBG		Testosterone		Calculated free testosterone		Estradiol		Inhibin-B	
	%-change	*P* value	%-change	*P* value	%-change	*P* value	%-change	*P* value	%-change	*P* value
Model I										
%-change pr. twofold increase in IGF1	−6 (−12 to 0)	0.042	3 (−4 to 9)	0.43	7 (0 to 14)	0.039	7 (2 to 12)	0.009	−7 (−14 to 0)	0.059
Model II										
%-change pr. twofold increase in IGF1	−16 (−21 to −9)	[1]<0.001	12 (4 to 20)	[1]0.003	13 (4 to 23)	[2]0.004	3 (−3 to 9)	[3]0.35	−11 (−20 to −1)	[4]0.027
%-change pr. 2-fold increase in IGFBP3	31 (15 to 48)	[1]<0.001	−14 (−25 to −3)	[1]0.019	−14 (−26 to −1)	[2]0.037	6 (−4 to 18)	[3]0.23	12 (−7 to 34)	[4]0.23

Model 1: unadjusted associations between IGF1 vs reproductive hormones. Model 2: [1]IGF1, IGFBP3, SHBG, testosterone, and BMI are included in the statistical model. [2]IGF1, IGFBP3, calculated free testosterone (Testo), and BMI are included in the statistical model. [3]IGF1, IGFBP3, estradiol, testosterone, and BMI are included in the statistical model. [4]IGF1, IGFBP3, inhibin-B, and time of day are included in the statistical model.

relationship between GH/IGF1 signaling and spermatogenesis, clinical data are limited and conflicting: in Laron-type dwarfism (primary GH resistance), no specific data on semen quality have been reported, but these patients are not infertile, indicating that GH action is not essential for sperm production (25). Reduced total sperm numbers have been reported in a group of patients with isolated GHD (4), whereas two other studies did not report any influence on spermatogenesis during GH replacement therapy (2, 26). Experimental GH therapy has also been applied to infertile men without pituitary disease (27, 28) or as adjuvant therapy during gonadotropin therapy of hypogonadotropic hypogonadism (29). The results have been conflicting but in general disappointing and GH treatment has not been recommended for treatment of infertility.

In contrast to the information obtained by *GHR* genotyping, high-systemic IGF1 action (high serum IGF1, low IGFBP3) was associated with reduced inhibin-B levels, but not with changes in sperm parameters. In acromegalic patients, improvement of semen quality has been reported during short-term treatment of GH/IGF1 excess, pointing to the possibility that high levels of circulating IGF1 might actually have a detrimental effect on spermatogenesis (30).

Gonadal steroidogenesis and SHBG secretion

As concerns Leydig cell function, information obtained by *GHR* genotype and circulating IGF1 activity points in the same direction, although the stimulating effect of a sensitive GHR was weak and nonsignificant. The inverse association between serum IGF1 vs SHBG is a confirmation of previous population-based cross-sectional studies (31, 32, 33). The underlying mechanisms are not fully understood. There might be a causal relationship between high IGF1 activity and reduced SHBG formation (34). Alternatively, insulin has been suggested as a possible confounding factor, reducing SHBG secretion (35). An important observation was that the decrease in serum SHBG associated with high IGF1 activity seemed not to be accompanied by a compensatory decrease in total serum testosterone. Consequently, calculated free testosterone was statistically and quantitatively strongly associated with IGF1 levels and inversely with IGFBP3. The relationship between IGF1 activity vs serum testosterone was independent of LH levels, suggesting that the effect is not mediated through increased levels of gonadotropins.

One clinical study of patients with isolated GHD showed an improved Leydig cell function in response to

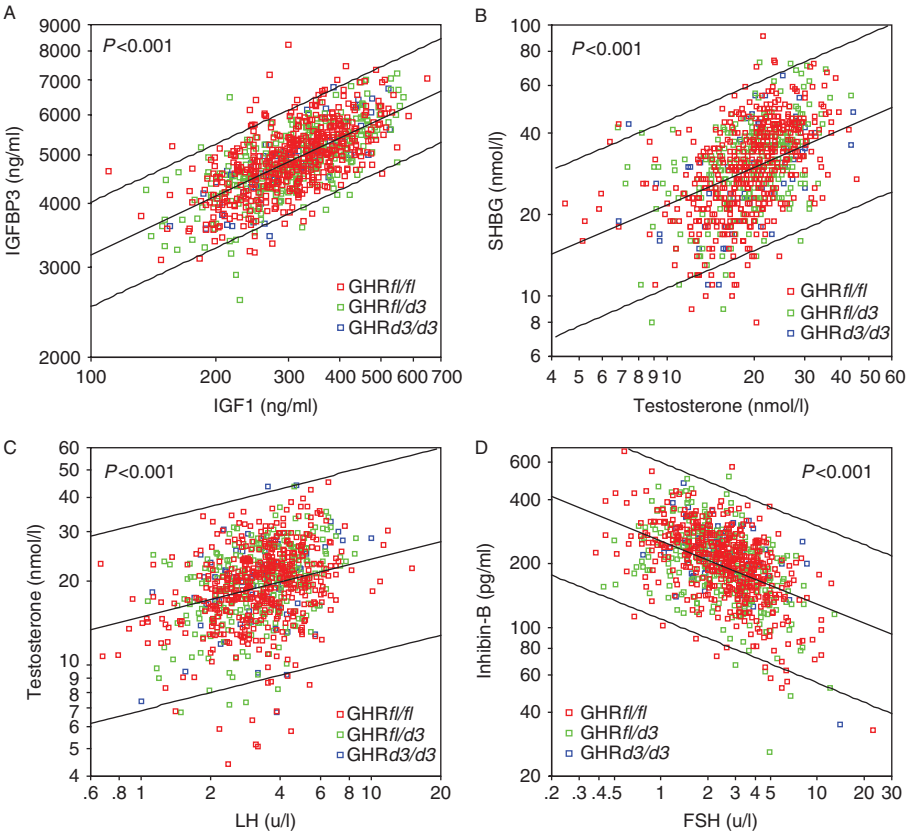

Figure 1

Associations between IGF1 vs IGFBP3 (A), testosterone vs SHBG (B), LH vs testosterone (C), and FSH vs inhibin-B (D). Homozygous carriers of the full-length allele are presented in red, heterozygous carriers in green, and homozygous carriers of the exon 3-deleted allele in blue.

GH treatment, supporting a causal role for GH/IGF1 action on Leydig cell steroidogenesis (2). In analogy, patients with Laron dwarfism have delayed and prolonged puberty, and in some cases IGF1 treatment has led to a marked increase in levels of androgens (3). In accordance with the biochemical observations, decreased penile size at birth and cryptorchidism have been observed in patients with isolated GH deficiency/GH insensitivity, supporting that GH action is important for full masculinization (3, 5, 6, 7, 8).

Pre- and postnatal growth

There were no significant associations between GHR genotype and size at birth. A study from 2006 found that homozygosity for GHR*fl* was associated with increased risk of being born small for gestational age (36). However, other investigations performed by us (18, 22) and others (37) found evidence of the opposite scenario with reduced prenatal growth in carriers of *GHRd3* alleles. We have no explanation for the discrepant results. Concerning our own observations there were no major differences in year of birth, geographic data, and maternal factors between the two previous cohorts (18, 22) and the present one.

The data on postnatal growth are supported by two population-based studies, where no significant association between *GHR* polymorphism and final height was observed (36, 38). On the other hand, observations of spontaneous growth in adolescence as well as observations in children treated with GH support indicate that presence of the short-GHR isoform might have a favorable impact on postnatal growth velocity (15, 16, 39). Taken together, the influence of *GHR* genotype on pre- and postnatal growth is an area of research where controversy exists and where no consensus has been reached.

As limitations to the present study, data on postnatal growth were based on questionnaires, and both semen and biochemical analyses were based only on single

measurements. Moreover, total testosterone levels were measured by an immunoassay and not by the more accurate mass spectrometry method. The free fraction of testosterone was based on calculations using the equation by Vermeulen *et al*. (20). Calculated free testosterone may not correspond exactly to free testosterone measured by the gold standard method equilibrium dialysis, but overall there is a good correlation between these two methods (20).

The use of serum IGF1 as a marker for pulsatile secreted GH should always be interpreted with caution because not only GH but also factors such as nutritional status, insulin levels, liver function, and age influence IGF1 formation (40). Moreover, IGF1 is produced in an autocrine/paracrine fashion in virtually all tissues and organs giving rise to local effects (40). Finally, GH has multiple effects independent of IGF1, and in some cases as e.g. glucose homeostasis, there are opposing effects of GH action, as compared to IGF1 action (41). Thus, evaluation of the GH/IGF1 axis is complicated and new methods to evaluate the GH/IGF1 system are therefore highly warranted. In this perspective, more studies are needed to further elucidate the significance of the different *GHR* polymorphisms on different GH target tissues.

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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