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Sex Hormone Binding Globulin Deficiency Due to a Homozygous Missense Mutation

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Context: SHBG is known as the major sex steroid binding protein in plasma, and it regulates the bioavailability of both T and estradiol levels required for effects on target tissues. We identified a man with an undetectable SHBG concentration in combination with low total T. He presented with a 7-year history of muscle weakness, fatigue, and a low libido.

Objectives: To determine the cause of the SHBG deficiency, we employed both genetic analysis of the *SHBG* gene and transgene SHBG expression.

Results: Genetic analysis identified a novel homozygous missense mutation that was predicted to be deleterious for protein function. Transgene expression showed that the mutation resulted in a block in SHBG secretion accompanied by increased expression of the endoplasmic reticulum molecular chaperone HSPA5. The mutation results in accumulation of the mutant SHBG within the cell and failure to secrete the mutant protein. Screening of family members identified one sister who was also deficient for SHBG.

Conclusions: We have identified a family with a missense mutation within the *SHBG* gene, which results in a complete deficiency of plasma SHBG in the homozygous state. Although total T level was low in the male patient, it did not interfere with normal gonadal development and spermatogenesis, suggesting a limited role of SHBG in sexual maturation and male physiology. (*J Clin Endocrinol Metab* 99: E1798–E1802, 2014)

SHBG is a liver-secreted plasma glycoprotein consisting of two identical monomers encoded by a 4-kb gene on chromosome 17 spanning a total of eight exons. The *SHBG* translation product contains 402 amino acids that is cleaved to remove a 29-amino acid N-terminal sorting peptide (1). The SHBG dimer shows binding affinity for the sex steroids T and DHT, and to a lesser extent estradiol, and serves as a sex hormone transport protein and regulator of sex hormone bioavailability for responsive tissues (2). As such, SHBG is believed to orchestrate sexual characteristics and reproductive function in both men and women (3). A decreased SHBG plasma concentration increases bioavailable T levels, which contributes to reproductive and metabolic phenotypes in polycystic ovary syndrome (4). In addition, variations in SHBG concentration have been associated with cancer, dyslipidemia, and type

2 diabetes, suggesting a more elaborate role for SHBG in overall human metabolism (5–7).

Only two reports exist that describe very low SHBG levels associated with hyperandrogenism, hirsutism, and amenorrhea in women (8, 9). However, a complete absence of plasma SHBG with its associated effects on physiology has not been reported before. Here, we describe a novel homozygous missense mutation in exon 5 of the human *SHBG* gene in an adult male and female, resulting in a complete deficiency of plasma SHBG. Both siblings reported only mild symptoms, which could, however, not be directly related to the SHBG deficiency. Surprisingly, the absence of SHBG and the low sex hormone concentrations did not interfere with male gonadal differentiation or pubertal development and reproductive function of the gonads in both the male and the female.

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Abbreviations: eGFP, enhanced green fluorescent protein; ER, endoplasmic reticulum; HSPA5, heat shock protein A5.

Materials and Methods

A detailed description regarding materials and the methods used is available as [Supplemental Materials and Methods](#).

Results

Case description

A 27-year-old man was referred to our Internal Medicine department with a 7-year history of fatigue, muscle weakness, impaired exercise tolerance, and low body weight. In addition, he experienced trembling muscles during minor exercise. At the age of 3, he was diagnosed with paratyphoid fever and rickets. Physical examination showed a thin young man (height, 1.76 m; body weight, 58 kg; body mass index, 19 kg/m²). Cycle ergometry excluded cardiac or pulmonary pathology. Further examination was unremarkable. Laboratory tests including erythrocyte sedimentation rate, blood count, renal function, liver enzymes, ferritin, creatine kinase, thyroid function, vitamin D, and serological tests for celiac disease were normal, except for a low T (4.8 nmol/L, measured at 8:30 AM [10–30 nmol/L]). Further analysis showed LH and FSH levels of 3.0 U/L each (LH, 1.7–8.6 U/L; FSH, 1–12 U/L), with a normal response during a pituitary GnRH function test. Other pituitary hormones were normal (prolactin, 212 mU/L [<400 mU/L]; 8 AM cortisol, 0.52 μ mol/L [0.15–0.70 μ mol/L]; IGF-1, 182 ng/mL [89–350 ng/mL]). In addition, magnetic resonance imaging excluded pituitary pathology. Estradiol level was <20 pmol/L (28–156 pmol/L), indicating that increased aromatase activity was unlikely as a cause for the low T concentration. Dialyzable free T level was normal (174 pmol/L [120–750 pmol/L]), but plasma SHBG was repeatedly undetectable using two different assays (detection limit, 0.35 nmol/L). Despite normal free T levels, the patient had multiple signs that could be related to a decreased T availability: low libido, decreased spontaneous morning erections, fatigue, muscular weakness, decreased shaving frequency (once per 4 d), inability to concentrate, sleep disturbance, and depressed mood. Skeletal age was 19 years, and bone densitometry was normal (T-score and Z-score, -0.9). There were no signs of erectile dysfunction during sexual arousal, and testicular volume was normal (16 mL). Male Tanner stage was 4. His intelligence was rated good to excellent because he held a master's degree. Further analysis of the SHBG deficiency included *SHBG* sequence analysis and expression of SHBG in vitro.

Identification of a novel SHBG missense mutation

Initial laboratory results of the proband identified a low T concentration and the absence of SHBG (Supplemental

Table 1). Sequence analysis of the proband's *SHBG* gene identified a homozygous missense mutation in exon 5 (c.670G \rightarrow A [NCBI NM_001040.3], p.G224R [NCBI NP_001031.2]) (Figure 1A). This prompted us to analyze the proband's family; we identified one sister (daughter C) also deficient for plasma SHBG (Supplemental Table 1). *SHBG* sequence analysis of daughter C showed the same homozygous missense mutation. Sequence analysis of both parents identified them as heterozygous carriers of the *SHBG* c.670G \rightarrow A missense mutation, whereas the other children were not affected (Figure 1B). Both parents originate from a region within The Netherlands that is known for its high genetic kinship. A multispecies *SHBG* sequence comparison identified the substituted glycine as part of a region that is highly conserved between species (Figure 1C) and where the introduction of an arginine, harboring a polar side chain, could potentially interfere with normal folding, processing within the endoplasmic reticulum (ER), or secretion of SHBG. Indeed, *in silico* prediction of the p.G224R mutation on protein function using PredictSNP (10) classified the substitution as deleterious, with a confidence of 87%.

The p.G224R missense mutation results in a secretory defect of SHBG

For localization of both overexpressed wild-type SHBG and SHBG p.G224R in relation to the ER and Golgi, a HEK293 cell line with expression of ER and Golgi localized enhanced green fluorescent protein (eGFP) was used. Wild-type SHBG showed complete overlap with the eGFP signal (Figure 2A). Expression of SHBG p.G224R also showed complete overlap with the ER and Golgi localized eGFP signal (Figure 2B), which demonstrates that the mutation did not affect subcellular localization and that transfer from ER to Golgi of the mutant SHBG is maintained.

To study the consequences of the identified missense mutation on SHBG synthesis and secretion, we expressed either wild-type SHBG or SHBG p.G224R in HEK293-T cells. At the protein level, both wild-type and SHBG p.G224R were expressed, but SHBG p.G224R showed increased intracellular levels, possibly due to retention inside the ER and Golgi (Figure 2C). In addition, multiple bands were observed, which probably represent different SHBG glycosylation forms. An increase in unfolded proteins in the ER will generally lead to the induction of ER resident chaperones like heat shock protein A5 (HSPA5) to meet the increased folding demand (11). Indeed, overexpression of mutant SHBG was associated with higher HSPA5 levels compared with wild-type SHBG (Figure 2D), which suggests increased utilization of the ER quality control system due to either prolonged folding time or retention of the mutant protein within the ER. To assess whether SHBG p.G224R was indeed accumulating

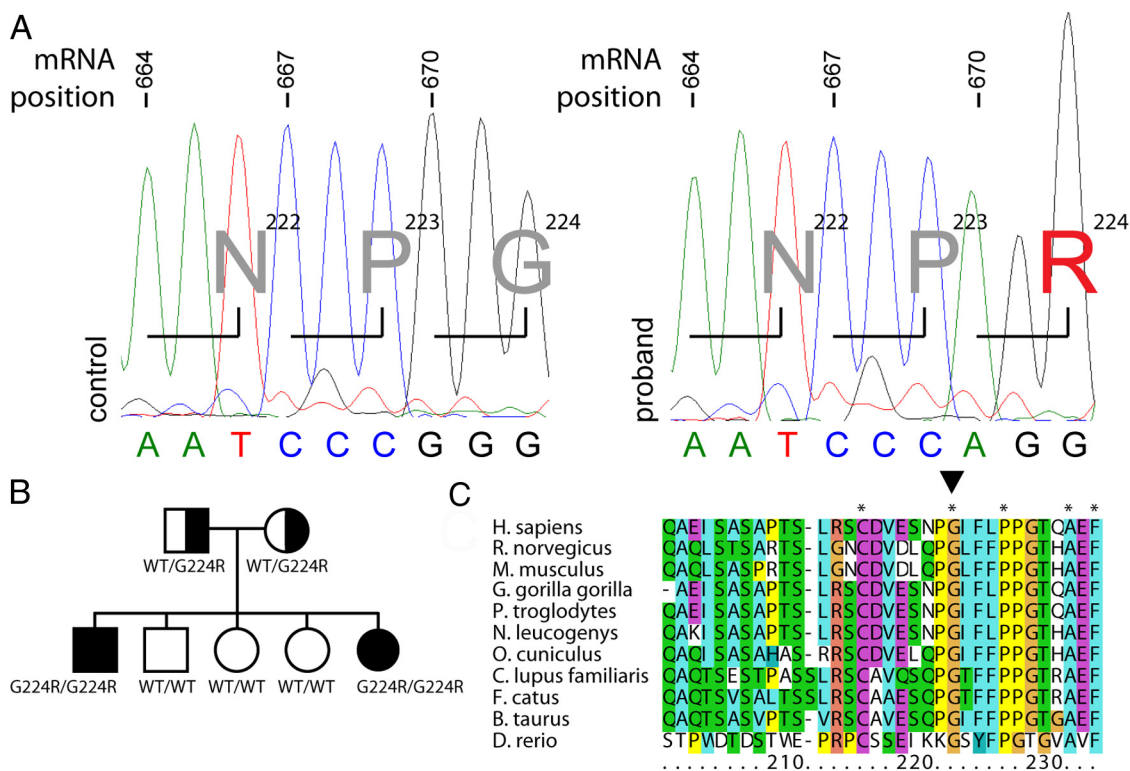


Figure 1. Identification of a novel SHBG mutation. A, Sequence analysis of the *SHBG* gene in the proband identified a missense mutation at cDNA position 670 (G → A). B, Graphical representation of *SHBG* sequence analysis within the proband's family (squares represent males, circles represent females, wild-type allele [WT]). C, Multispecies alignment of the SHBG protein sequence with color coding of related amino acids (triangle shows position of human SHBG p.224G). * indicate fully conserved amino acids.

inside the ER and Golgi without being secreted, we measured secreted SHBG in the cell culture medium. Whereas wild-type SHBG was secreted into the culture medium, medium of cells expressing SHBG p.G224R was devoid of SHBG (Figure 2E).

Plasma SHBG and the reproductive system

Many physiological roles have been reserved for SHBG, including sexual development in both males and females (12, 13). To address the question of whether plasma SHBG deficiency affects the reproductive system, we performed semen analysis in the proband and measured hormonal changes during a menstrual cycle in the SHBG-deficient female. Despite low total T and the absence of SHBG, semen analysis in the proband showed normal semen volume and sperm concentration and a favorable progressive motile fraction (Supplemental Table 2). Hormonal changes measured weekly during a menstrual cycle in the female (Tanner stage 4) showed an extended cycle of 6 weeks, with a moderate LH peak (20 U/L) followed by an increase in estradiol and progesterone, suggesting a functional menstrual cycle independent of plasma SHBG (data not shown).

Discussion

We identified an adult male and female with a complete plasma SHBG deficiency due to a novel homozygous mu-

tation in the *SHBG* gene. To our knowledge, this is the first description of a complete SHBG deficiency in both a male and a female. The mutant transgenic protein was present at higher levels within cells and was associated with a higher level of the ER chaperone HSPA5, providing evidence for retention of the mutant protein in the ER and subsequent failure to secrete the protein. Interestingly, both parents are heterozygous for the mutation and have normal SHBG concentration. This suggests that expression of SHBG p.G224R in vivo does not affect folding and secretion of wild-type SHBG, excluding a dominant negative effect of the mutation.

In the literature, only two previous reports exist describing females with decreased SHBG concentration. The first publication reports two siblings who had plasma SHBG concentrations below 10 nmol/L, the detection limit of the assay at that time (8). One patient presented with amenorrhea and hirsutism, but her sister was pregnant despite the very low SHBG. It is unclear, however, whether these patients actually had a complete deficiency of plasma SHBG due to the detection limit of the SHBG assay used. The other report describes the occurrence of two single nucleotide polymorphisms in the *SHBG* gene, leading to low SHBG levels, hyperandrogenism, and ovarian dysfunction (9). Our female patient only reported a late menarche at 16 years and showed a normal, although

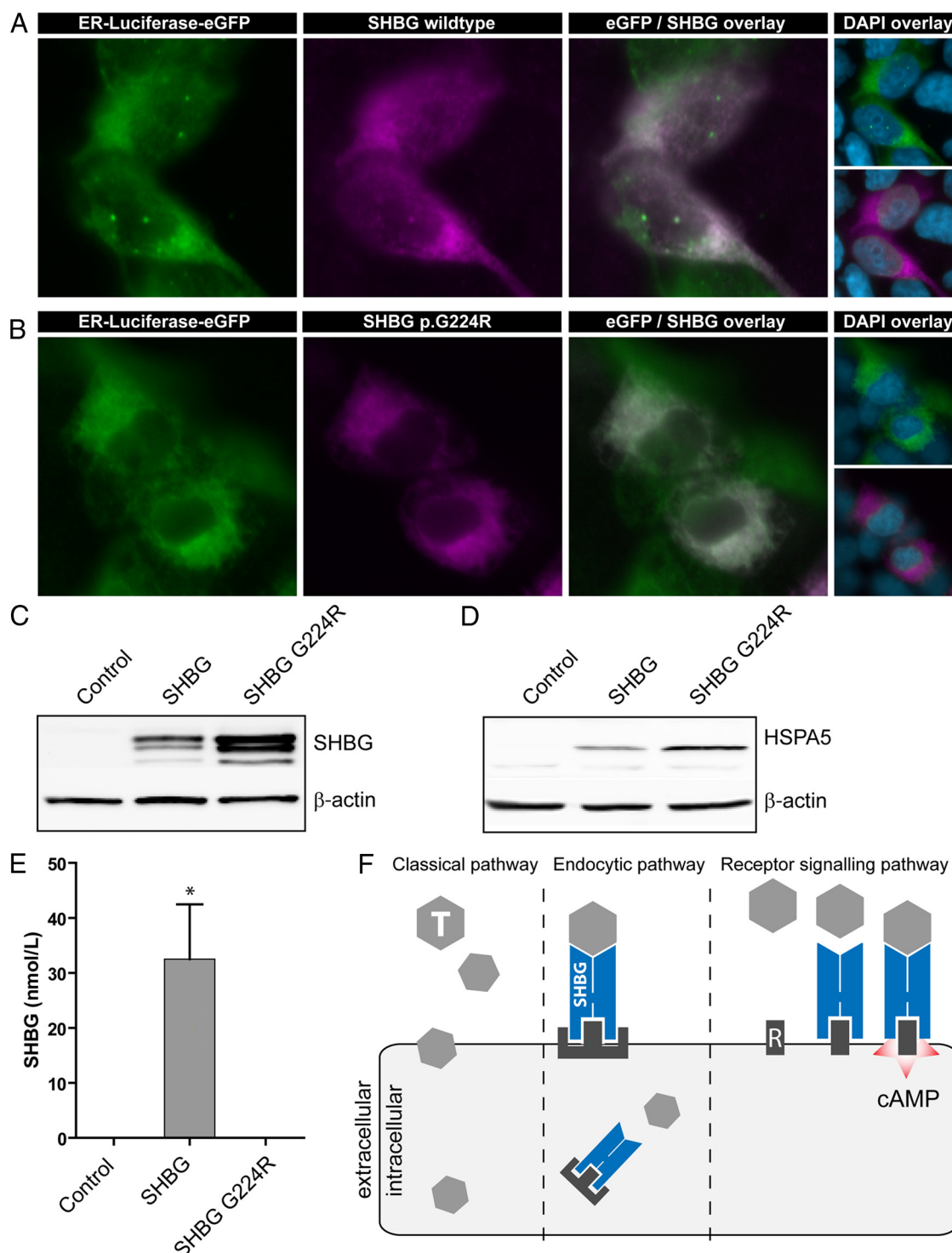


Figure 2. Microscopic and biochemical analysis of wild-type and mutant SHBG. Subcellular localization of wild-type SHBG (A) and p.G224R SHBG (B) (magenta signal) was analyzed in Flp-In T-Rex HEK293 cells constitutively expressing ER and Golgi localized luciferase-eGFP (green signal) by transfection of the indicated *SHBG* containing plasmids. SHBG was stained using specific antibodies, after which the cell nucleus was stained using 4',6-diamidino-2-phenylindole (DAPI). The white signal obtained in the overlay image indicates overlap of the eGFP and SHBG signal. C, Expression analysis of wild-type and p.G224R SHBG in transfected HEK293-T cells. D, Analysis of ER chaperone HSPA5 induction under conditions of overexpression of wild-type or p.G224R SHBG in HEK293-T cells. E, Secretion of wild-type and p.G224R SHBG by HEK293-T cells was analyzed 48 hours after transfection by diluting 250 μ L of cell culture medium in 250 μ L of PBS before SHBG measurement. *, *P* value < .05 using a Student's *t* test. F, Model of different T and SHBG uptake pathways: classical pathway, endocytic pathway, receptor signalling pathway. R, SHBG receptor.

irregular, menstrual cycle and no signs of hirsutism or hyperandrogenism. This suggests that a complete lack of plasma SHBG is not accompanied by severe physiological symptoms in the female and in addition does not interfere with gonadal function. In addition, the male gonads developed normally, a process that requires androgen availability. Furthermore, spermatogenesis was not affected by the low total T levels, indicating that intratesticular T concentrations are sufficient to maintain reproductive function. Non-elevated LH in the absence of pituitary pathology indicated normal pituitary perception of T availability. Our patient did show some symptoms that could be due to late-onset hypogonadism (14). However, these symptoms could not be solely explained by T deficiency because his free T level was within normal limits as used by our laboratory.

Androgens and estrogens can passively diffuse into cells by a nonspecific mechanism (the “free hormone hypothesis”) (Figure 2F, classical pathway). However, the discovery of a specific SHBG receptor located on membranes of sex steroid-responsive cells has led to the notion that SHBG is not only a transport protein but also actively regulates sex steroid action at the target cell level (15). Evidence of a specific SHBG signal transduction pathway is accumulating (Figure 2F, receptor signaling pathway) (16). In addition, megalin, a member of the low-density lipoprotein receptor family, has recently been identified as an endocytic receptor for the cellular uptake of SHBG-bound sex steroids (Figure 2F, endocytic pathway) (17, 18). Whether and how these findings could explain the symptoms experienced by the proband is far from conclusive. However, passive diffusion of T alone may be insufficient for target tissues requiring large amounts of sex steroids (17, 18). This might explain some of the proband's symptoms. The lower binding affinity of SHBG for estrogens in comparison to T may explain the milder symptomatology of plasma SHBG deficiency in the female. As a result, women may be less dependent on SHBG receptor-mediated signaling than men. The distribution of the SHBG receptor-mediated uptake of sex hormones in different tissues has yet to be studied to fully explain the possible symptomatology of SHBG deficiency.

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