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► **To cite this version:**

Nectaria Xita, Agathocles Tsatsoulis. Genetic variants of sex hormone-binding globulin and their biological consequences. *Molecular and Cellular Endocrinology*, 2009, 316 (1), pp.60. <10.1016/j.mce.2009.08.025>. <hal-00550177>

HAL Id: hal-00550177

<https://hal.science/hal-00550177v1>

Submitted on 24 Dec 2010

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Accepted Manuscript

Title: Genetic variants of sex hormone-binding globulin and their biological consequences

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PII: S0303-7207(09)00458-4
DOI: doi:10.1016/j.mce.2009.08.025
Reference: MCE 7305

To appear in: *Molecular and Cellular Endocrinology*

Received date: 11-5-2009
Revised date: 18-8-2009
Accepted date: 30-8-2009

Please cite this article as: Xita, N., Tsatsoulis, A., Genetic variants of sex hormone-binding globulin and their biological consequences, *Molecular and Cellular Endocrinology* (2008), doi:10.1016/j.mce.2009.08.025

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Genetic variants of sex hormone-binding globulin and their biological consequences

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KEYWORDS ,

Sex hormone-binding globulin *SHBG* gene, polymorphisms

ABSTRACT

Several hormonal and metabolic factors have been found to influence the production of sex hormone-binding globulin (SHBG). In addition, twin studies have suggested that genetic factors may also contribute to variation in SHBG levels. Given the clinical significance of SHBG in regulating bioavailable sex steroid hormones, a number of studies examined the potential association between polymorphisms of *SHBG* gene and serum SHBG levels as well as their possible contribution in the pathogenesis of common diseases. Thus, polymorphisms of *SHBG*, altering either the production or the metabolism of the protein, may represent part of the genetic background of sex steroid hormone activity in humans. There is considerable heterogeneity in the results of these studies indicating the multiplicity of the factors influencing SHBG variation. However, the weight of evidence suggests that some common genetic variants of *SHBG* may influence SHBG levels and in part contribute to the phenotypic expression of human diseases.

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INTRODUCTION

In humans, sex hormone-binding globulin (SHBG) is synthesized mainly in the liver under the influence of various hormonal and metabolic factors (Anderson, 1974). In the circulation, SHBG binds androgens with high affinity and estrogens with lower affinity and regulates their access and possibly action in target tissues (Siiteri et al., 1982; Kahn et al., 2002). SHBG is also produced in other tissues including the placenta, testis, brain and endometrium (Larrea et al., 1993; Selva and Hammond, 2006; Herbert et al., 2005; Misao et al., 1997). The appropriate expression of SHBG by the placenta and the fetal liver during development may be critical in maintaining normal fetal exposures to androgens originating from the mother or produced by the fetus itself. As a consequence, variation in the levels of SHBG may influence sex steroid bioavailability and cellular effects with resultant clinical implications.

In overweight men and women, low SHBG levels are considered a biomarker of the metabolic/insulin resistance syndrome and may predict the development of type 2 diabetes and cardiovascular disease (Nestler 1993; Haffner et al., 1996; Lindstedt et al., 1991; Lapidus et al., 1986). In women with polycystic ovary syndrome (PCOS), levels of SHBG are frequently low and may contribute to the hyperandrogenic phenotype of the syndrome (Kajaja et al., 2007). Changes in SHBG levels are also implicated in the pathogenesis of osteoporosis and hormone-dependent cancers (Goderie-Plomp et al., 2004; Lorentzon et al., 2005; The Endogenous Hormones and Breast Cancer Collaborative Group, 2002; Potischman et al., 1996; Weiss et al., 2008)). Thus, it is important to understand the factors that modulate SHBG levels.

It is currently recognized that SHBG production is under multifactorial regulation with hormonal, nutritional and metabolic factors influencing SHBG levels (Toscano et al., 1992). In addition, genetic factors may also contribute to variation in SHBG levels (Ahrentsen et al., 1982; Meikle et al., 1982). Based on the San Antonio Family Heart study and twin studies, the heritability for SHBG is estimated to be 30-80% (Jaquish et al., 1997; Ring et al., 2005, Bogaert et al., 2008, An et al., 2001, Kuijper et al., 2007). This implies that not only environmental but also genetic factors play a significant role in SHBG variation.

Given the clinical significance of SHBG, a number of studies examined the potential association between polymorphisms of *SHBG* gene and serum SHBG levels that could be implicated in the pathogenesis of several clinical conditions. Thus, polymorphisms within the coding sequence and, potentially, in the regulatory sequence of the *SHBG* gene, altering either the production or the metabolism of the protein, may represent part of the genetic background of sex steroid hormone activity in humans.

In this review, the association of *SHBG* genetic variants with SHBG levels and their potential contribution in the phenotypic expression of common diseases are discussed

STRUCTURE OF *SHBG* GENE

Human SHBG is encoded by a 4-kb gene located on the short arm (p12-p13) of chromosome 17 and consists of eight exons (Berube et al., 1990). Exon 1 contains the coding sequence for the SHBG secretion signal polypeptide, as well as the first few amino-terminal residues of the mature secreted protein, while exons 2-8 encode two contiguous laminin G-like domains. The N-terminal G domain of SHBG is responsible for steroid transport while the functional significance of the C-terminal G domain of SHBG is less well established. This domain normally contains two sites for N-glycosylation (Hammond et al., 1987; Hammond et al., 1989; Hammond and Bocchinfuso, 1995; Hammond and Bocchinfuso, 1996)

Two major SHBG transcripts are known, each originating from a different promoter (Hammond et al., 1989; Gerhansen et al., 1989). The first major transcript encodes a precursor for the secreted (plasma) form of SHBG, and was originally described in the liver (Que and Petra, 1987), while the second encodes a protein of unknown function and was originally described in the testis (Hammond et al., 1989). The two transcripts differ in their 5' sequences and in the absence of exon 7 in the latter one.

Specific chromosomal abnormalities have not been associated with diseases characterized by low SHBG levels but the p12 region of chromosome 17 is considered as a

fragile site within the human genome containing multiple *Alu* sequences in the introns (Hammond et al., 1989). These sequences have been implicated as mediators of chromosomal recombination events and influence alternative exon splicing (Lehrman et al., 1987). Mutations and single nucleotide polymorphisms (SNPs) of *SHBG* have rarely been reported (Cargill et al., 1999). Only a few nucleotide variations in coding and regulatory sequences have been described as discussed below (Figure 1).

THE (TAAAA)_n REPEAT POLYMORPHISM IN *SHBG* PROMOTER

The pentanucleotide (TAAAA)_n repeat polymorphism [reference sequence (rs) 35785886] has been identified in the upstream region of human *SHBG* promoter within an *Alu* sequence. The number of TAAAA repeats is highly variable and six alleles with six to eleven TAAAA repeats have been described (Hogeveen et al., 2001). The distribution of the polymorphism varies widely across populations (Haiman et al., 2005). Using transient transfection experiments in human hepatoblastoma (HepG2) cells, it was found that the transcriptional activity of the *SHBG* promoter was directly related to the number of TAAAA repeats, but this relation was not linear with respect to TAAAA repeat number (Hogeveen et al., 2001). A reduction in transcription was found to be associated specifically with the six TAAAA sequence and this was attributed to the preferential binding of a 46kDa liver-enriched nuclear protein to six TAAAA repeats. Thus, the allele with six TAAAA repeats was found to have a silencing effect on *SHBG* transcription (Hogeveen et al., 2001).

The effect of the (TAAAA)_n repeat polymorphism on SHBG levels was also tested in clinical studies. Thus, in a multiethnic population of healthy postmenopausal women, carriers of the six TAAAA repeat allele were found to have modestly lower mean serum SHBG levels than non carriers (Haiman et al., 2005). However, other studies in women with PCOS, premenopausal women with hirsutism and in healthy men showed an opposite relation, with carriers of long alleles presenting with lower SHBG concentrations compared to carriers of short alleles. More particularly, in a Greek cohort of women with PCOS it was found that patients with longer repeats (more than eight TAAAA repeats) had lower SHBG levels and higher levels of free androgen index than those with shorter alleles (Xita et al., 2003). This observation was confirmed by Ferik et al (2007), who reported lower serum SHBG levels in women with longer TAAAA alleles not only in PCOS patients but also in healthy women. Cousin et al (2004) studied a population of French women with hirsutism and found that homozygous patients for the six TAAAA repeat allele had higher serum SHBG levels compared with patients being homozygous for the nine TAAAA repeat allele, while SHBG levels of homozygous patients for the eight TAAAA allele lay between the two. This association between the six TAAAA allele and elevated SHBG levels was also found in healthy men (Turk et al., 2007; Eriksson et al., 2006; Vanbillemont et al., 2009).

The discrepancy between the findings of clinical studies with the results of the *in vitro* experiments may be explained by the fact that the *in vitro* study examined the activity of *SHBG* promoter of naked DNA and the contributions of higher chromatin structures on transcriptional activity or the possible long-range contributions of other regulatory sequences were not taken into consideration. Furthermore, the silencing effect of the six TAAAA allele on *SHBG* transcription described *in vitro*, is dependent on the presence of an SP1 binding site downstream of the polymorphism (Hogeveen et al., 2001). Nuclear factors SP1 or SP3 may bind with a SP1 binding site and in cooperation with other proteins, these transcription factors may act as positive or negative regulators of transcription. Therefore, the net effect on the transcription depends on the abundance of factors forming a complex with SP1 (Li et al., 2004).

The (TAAAA)_n repeat polymorphism has been proposed to act as a susceptibility polymorphism for the development of PCOS (Xita et al., 2003). Women with PCOS were found to have alleles with longer repeats (more than eight TAAAA repeats) more frequently than normal women who had shorter alleles (less than eight TAAAA repeats) in a much higher frequency (Figure 2). It is hypothesized that those individuals with genetically determined low SHBG levels may be exposed to higher free androgen levels throughout life, but more importantly, during fetal life, when programming of the differentiating target tissues

takes place (Figure 1). Thus, genetically determined low SHBG levels may provide the genetic link for the developmental origin hypothesis of PCOS (Abbott et al., 2002, Xita and Tsatsoulis 2006). Further studies in the same cohort of women with PCOS, revealed that the combination of long SHBG(TAAAA)n repeat alleles with either a short microsatellite (TTTA)n repeat allele of aromatase gene or a short (CAG)n repeat allele of the androgen receptor gene has a synergistic effect on the hyperandrogenic phenotype of PCOS (Xita et al., 2008a; Xita et al., 2008b).

The (TAAAA)n repeat polymorphism was also studied in a group of Greek adolescent females, in order to evaluate whether variations in this pentanucleotide repeat are associated with age at menarche. It was shown that early menarche is associated with shorter TAAAA alleles, whereas late menarche (≥ 13 years) is associated with longer repeat alleles (Xita et al., 2005). Taken into consideration that the (TAAAA)n repeat polymorphism of *SHBG* gene has been associated with PCOS, it can be hypothesized that girls with long TAAAA alleles and later onset of menarche might be at risk of developing PCOS.

In postmenopausal women undergoing coronary angiography, an association between more severe coronary disease and the presence of the longer alleles of the (TAAAA)n polymorphism was found. Furthermore calculated free testosterone levels were higher, whereas SHBG levels tended to be lower in the carriers of long TAAAA alleles (Alevizaki et al., 2008). This association may reflect the lifelong adverse cardiovascular effect of androgenic exposure in this group of women.

The role of the (TAAAA)n repeat polymorphism on semen quality among normal men was also studied. It was found that men with short alleles (with less than eight TAAAA repeats) had higher sperm concentration compared with men with long alleles (Lazaros et al., 2008). Lastly among elderly men, the (TAAAA)n genotype was associated with androgen and androgen metabolite levels, and it was suggested as an independent but modest predictor of hip bone mineral density (Eriksson et al., 2006).

SINGLE NUCLEOTIDE POLYMORPHISMS OF *SHBG* GENE

The first reported variation of *SHBG* was the D327N polymorphism. It is a missense single nucleotide polymorphism (SNP) (G>A) at nucleotide 5790, in exon 8 of *SHBG* and results in an amino-acid substitution of asparagine for aspartic acid at residue 327 (rs6259). This polymorphism is distributed worldwide with different frequencies across ethnic groups (Haiman et al., 2005). In healthy Caucasians, the reported frequency of the D327N polymorphism is about 7.5–12% (Becchis et al., 1999; Forsti et al., 2002; Cousin et al., 2004; Haiman et al., 2005; Ferk et al., 2007; Bendlova et al., 2007; Garcia-Closas et al., 2007) The lowest frequency of the polymorphism was found in African-Americans (2%), while the highest frequency (18%) was observed in Chinese women [Haiman et al., 2005; Cui et al., 2005; Kataoka et al., 2007].

The D327N polymorphism is a functional polymorphism. It introduces an additional site for N-linked glycosylation within the carboxy-terminal globular laminin G-like domain (Power et al., 1992). Expression studies have shown that this polymorphism does not influence the steroid binding properties or the immunologic characteristics of SHBG protein (Bocchinfuso et al., 1992). In an animal model, it has been reported that the additional carbohydrate chain in SHBG protein, encoded by the Asn327 allele, decreases its metabolic clearance rate, and this allele is associated with an increased half-life of the protein and, in turn, with increased SHBG levels in variant allele carriers (Cousin et al., 1998).

Indeed, in postmenopausal women, the Asn327 allele was found to be significantly related with increased circulating SHBG levels and with reduced estradiol-to-SHBG ratio, suggesting that this genetic variant contributes to the bioavailability of estrogens (Dunning et al., 2004). The association of this polymorphism with SHBG levels was confirmed in a multiethnic cohort study of healthy postmenopausal women (Haiman et al., 2005). Higher SHBG levels were also found in hirsute women with the Asn327 allele compared with patients with the Asp/Asp genotype, independently of the PCOS status and their body mass index (Cousin et al., 2004). However, there are studies with conflicting results. Thus, no association between the D327N genotype and serum SHBG concentration was found in

healthy young men (Turk et al., 2007). The same conclusion was supported by Bendlova et al (2007), who studied this polymorphism in women with PCOS, while Ferik et al (2007) found a lowering effect of the Asn327 allele on serum SHBG levels in both women with PCOS and healthy women. Furthermore, both studies in women with PCOS found no difference in the distribution of this polymorphism when comparing patients with healthy controls.

The D327N polymorphism was also studied for its possible association with the risk of breast cancer. In a large case-control study of postmenopausal women, it was reported that the Asn327 allele of *SHBG* was significantly associated with a reduced risk of breast cancer by increasing serum SHBG levels (Cui et al., 2005). Moreover, a significantly higher frequency of this polymorphism was observed in postmenopausal patients with estrogen receptor-positive breast cancer than in estrogen receptor-negative suggesting a link between estrogen dependence of breast cancer and the presence of this *SHBG* variant (Becchis et al., 1999). The protective role of D327N polymorphism of *SHBG* in breast cancer was further supported by Costantino et al in postmenopausal women taking hormone replacement therapy (Costantino et al., 2009). The authors suggested that this effect was not due to modification of serum SHBG levels, since no difference in SHBG levels was observed (Costantino et al., 2009). They showed that the D327N polymorphism confers a higher capacity to SHBG to bind to breast cancer cells and trigger downstream signaling leading to inhibition of the estradiol effect, and thus making D327N SHBG protein much more effective than the wild type protein at the cell site (Costantino et al., 2009). However, there are studies supporting contradictory results. In Polish and Nordic populations of women with familial or sporadic breast cancer, carriers of the Asn327 allele presented an overall reduced breast cancer risk but statistical significance was not attained (Forsti et al., 2002). In addition, no significant association between the Asn327 variant and breast cancer risk was reported by Dunning et al (2004).

The D327N polymorphism was also studied in other hormone-sensitive cancers. It was found that the Asn327 allele was associated with a reduced endometrial cancer risk only in postmenopausal women but not in premenopausal women (Kataoka et al., 2007). Women with ovarian cancer were also found to have less frequent the Asn327 allele compared to healthy controls but the difference was not statistically significant (Garcia-Closas et al., 2007). Moreover, men being heterozygotes for this polymorphism were found to be at increased risk of developing prostate cancer (Berndt et al., 2007). Even though Cunningham et al (2007) found no difference in the distribution of the D327N polymorphism between patients with prostate cancer and healthy controls, they proposed that this polymorphism might be weakly associated with tumor aggressiveness.

A stronger association with serum SHBG levels has been shown for another polymorphism, the 5'UTR G-A polymorphism (rs1799941), located within the *SHBG* proximal promoter sequence, only eight nucleotides upstream of the transcription start point. The frequency of this polymorphism in Caucasians is 23.5%-27% (Dunning et al., 2004; Low et al., 2005; Eriksson et al., 2006; Riancho et al., 2008;). The AA genotype was found to be related to higher SHBG levels compared to GG genotype in both men and postmenopausal women (Low et al., 2005; Eriksson et al., 2006; Dunning et al., 2004; Riancho et al., 2008). Furthermore, the AA genotype was associated with higher bone mineral density and androgen levels in elderly men (Eriksson et al., 2006). However, no relation of this polymorphism with spine or hip bone mineral density among postmenopausal women was observed, despite its association with serum SHBG levels (Riancho et al., 2008). This is probably due to multiple factors influencing postmenopausal osteoporosis. The 5'UTR G-A polymorphism combined with the D327N polymorphism was also studied in oligospermatic/azoospermatic men and an association of their haplotype with spermatogenic defects was reported (Su et al., 2008).

Another *SHBG* polymorphism associated with SHBG levels is the C/T rs6257 polymorphism (Riancho et al., 2008). This polymorphism is located in intron 1, in a potential binding site for the transcription factor hepatocyte nuclear factor 3/Fox. Recently, the rs6257 has been proposed as a potential breast cancer susceptibility variant (Thompson et al., 2008).

Lastly, an *MspI* restriction fragment length polymorphism was described having an allele frequency of 0.04 in the French-Canadian population (Vohl et al., 1994). This

polymorphism encodes a missense mutation, the P156L located at the exon 4/intron 4 boundary and found to allow normal steroid ligand binding but causing abnormal glycosylation and inefficient secretion of SHBG. The combination of this polymorphism with a single nucleotide deletion in exon 8 producing a reading frame shift within the codon E326 was described in a woman with extremely low serum SHBG levels and severe hyperandrogenism. It was also identified in the heterozygous state in four other patients out of 482 women with either PCOS, idiopathic hirsutism or ovarian failure (Hogveen et al., 2002).

CONCLUSION

There is considerable heterogeneity in results of studies investigating the association of *SHBG* variants with serum SHBG levels and their possible role in the development of common diseases. This may indicate the multiplicity of the factors influencing SHBG variation and consequently its biological effects. First, the SNPs that are discussed above exhibit significant linkage disequilibrium. In particular, a strong linkage disequilibrium was found between the D327N variant allele and the allele with eight TAAAA repeats (Haiman et al., 2005; Cousin et al., 2004) as well as between the (TAAAA)_n repeat polymorphism and the rs1799941 SNP (Eriksson et al., 2006). Since these polymorphisms are in linkage disequilibrium, it cannot be established with certainty which one is truly responsible for the association with SHBG levels.

In addition there may be a possibility that other functional polymorphisms in *SHBG* or in a neighboring gene, in linkage with those reported so far, are actually responsible for the association with SHBG levels. Thompson et al (2008) found SNPs within or close to the *SHBG* to be significantly associated with SHBG levels. In a genome-wide linkage scan numerous candidate genetic loci exhibited significant linkages for serum SHBG levels, suggesting that SHBG levels may be regulated by many genes rather than a genetic marker. Thus, a strong association was found between SHBG levels and microsatellite markers located on the chromosomes 1q44, 5p13.3, 10q24.1 and 12q12 (Ukkola et al., 2002). Therefore, interactions between different genetic factors should be considered influencing the variations in SHBG levels.

Secondly, the impact of environmental factors and their interaction with genetic factors cannot be excluded. A significant diet-gene interaction between isoflavones and the D327N polymorphism of *SHBG* affecting SHBG levels has been reported (Low et al., 2006). More precisely, in women carrying the D327N variant, isoflavones stimulate SHBG levels in a dose response manner (Low et al., 2006). This finding shows that the effects of some genetic variants may be magnified and become detectable in interaction with certain environmental factors.

In conclusion, the biological role of *SHBG* genetic variants in influencing the risk of common diseases is not fully understood. However, the weight of evidence suggests that some common genetic variants of *SHBG* may influence SHBG levels and in part, contribute to the phenotypic expression of human diseases. Further studies are needed to substantiate this association and clarify its biological consequences. The possible interaction between environmental and genetic factors in influencing SHBG variation also requires further investigation.

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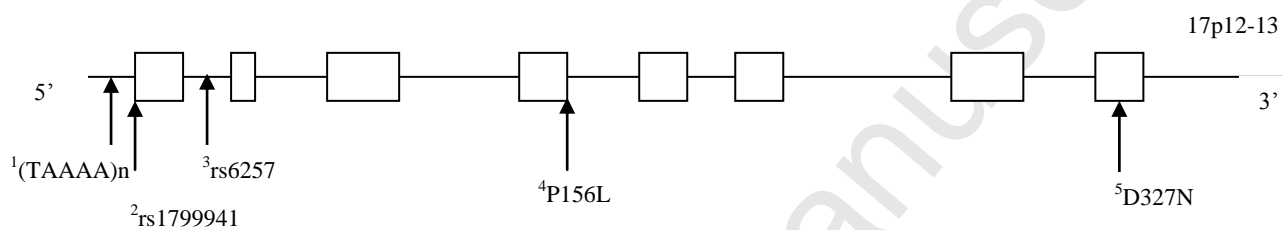
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Accepted Manuscript

Legends to Figures

Figure 1. Description of *SHBG* gene and location of polymorphisms

Figure 1.



¹Association with SHBG levels, PCOS, age at menarche, coronary disease in postmenopausal women, sperm concentration and hip bone mineral density in men.

²Association with SHBG levels, bone mineral density and androgen levels in elderly men, and spermatogenic defects.

³Association with SHBG levels and breast cancer susceptibility

⁴Association with extremely low SHBG levels and hyperandrogenism.

⁵Association with SHBG levels in postmenopausal women and risk of hormone-sensitive cancers.

Figure 2. Upper panel: Frequency of genotypes, grouped in short and long allele genotypes, show significant differences between PCOS and controls. Lower panel: Correlation of (TAAAA)n genotypes with SHBG levels. PCOS women with short allele genotypes had higher SHBG level compared to women with long allele genotypes. (modified from Ref. Xita et al, 2003)

Figure 2

