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

Nectaria Xita, Agathocles Tsatsoulis

► 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-00550177>

Submitted on 24 Dec 2010

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Accepted Manuscript

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

Authors: Nectaria Xita, Agathocles Tsatsoulis

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

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

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

Nectaria Xita and Agathocles Tsatsoulis

Department of Endocrinology, University of Ioannina, Ioannina, 45110, Greece.

Corresponding author: Agathocles Tsatsoulis, MD, PhD, FRCP

Professor of Medicine/Endocrinology

Department of Endocrinology

University of Ioannina,

45110, Ioannina, Greece

Tel: +3026510-98089

Fax: +3026510-98098

Email: atsatsou@uoi.gr

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.

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 (Kajala 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 (Ahrens 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 Ferk 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 Ferk 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 (Hogeveen 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.

References

- Abbott DH, Dumesic DA, Franks S. 2002 Developmental origin of polycystic ovary syndrome- a hypothesis. *J Endocrinol* 174:1-5.
- Ahrensens OD, Jensen HK, Johnsen SG. 1982 Sex-hormone-binding globulin deficiency. *Lancet* 2:377.
- Alevizaki M, Saltiki K, Xita N, Cimponeriu A, Stamatelopoulos K, Mantzou E, Doukas C, Georgiou I. 2008 The importance of the (TAAAA)n alleles at the SHBG gene promoter for the severity of coronary artery disease in postmenopausal women. *Menopause* 15:461-8.
- Anderson DC. 1974 Sex-hormone-binding globulin. *Clin Endocrinol* 3:69-96.
- An P, Rice T, Gagnon J, Borecki IB, Rankinen T, Gu C, Leon AS, Skinner JS, Wilmore JH, Bouchard C, Rao DC. 2001 Population differences in the pattern of familial aggregation for sex hormone-binding globulin and its response to exercise training: the HERITAGE Family Study. *Am J Hum Biol* 13:832-7.
- Becchis M, Frairia R, Ferrera P, Fazzari A, Ondei S, Alfarano A, Coluccia C, Biglia N, Sismondi P, Fortunati N. 1999 The additionally glycosylated variant of human sex hormone-binding globulin (SHBG) is linked to estrogen-dependence of breast cancer. *Breast Cancer Res Treat* 54:101-7.
- Bendlová B, Zavadilová J, Vanková M, Vejrazková D, Lukášová P, Vcelák J, Hill M, Cibula D, Vondra K, Stárka L, Vrbíková J. 2007 Role of D327N sex hormone-binding globulin gene polymorphism in the pathogenesis of polycystic ovary syndrome. *J Steroid Biochem Mol Biol* 104:68-74.
- Berndt SI, Chatterjee N, Huang WY, Chanock SJ, Welch R, Crawford ED, Hayes RB. 2007 Variant in sex hormone-binding globulin gene and the risk of prostate cancer. *Cancer Epidemiol Biomarkers Prev* 16:165-8.
- Bérubé D, Séralini GE, Gagné R, Hammond GL. 1990 Localization of the human sex hormone-binding globulin gene (SHBG) to the short arm of chromosome 17 (17p12----p13). *Cytogenet Cell Genet* 54:65-7.
- Bocchinfuso WP, Ma KL, Lee WM, Warmels-Rodenhiser S, Hammond GL. 1992 Selective removal of glycosylation sites from sex hormone-binding globulin by site-directed mutagenesis. *Endocrinology* 131:2331-6.
- Bogaert V, Taes Y, Konings P, Van Steen K, De Bacquer D, Goemaere S, Zmierzczak H, Crabbe P, Kaufman JM. 2008 Heritability of blood concentrations of sex-steroids in relation to body composition in young adult male sibling. *Clin Endocrinol* 69:129-35.
- Cargill M, Altshuler D, Ireland J, Sklar P, Ardlie K, Patil N, Shaw N, Lane CR, Lim EP, Kalyanaraman N, Nemesh J, Ziaugra L, Friedland L, Rolfe A, Warrington J, Lipshutz R, Daley GQ, Lander ES. 1999 Characterization of single-nucleotide polymorphisms in coding regions of human genes. *Nat Genet* 22:231 - 8.
- Costantino L, Catalano MG, Frairia R, Carmazzi CM, Barbero M, Coluccia C, Donadio M, Genta F, Drogo M, Boccuzzi G, Fortunati N. 2009 Molecular mechanisms of the D327N SHBG protective role on breast cancer development after estrogen exposure. *Breast Cancer Res Treat* 114:449-56.
- Cousin P, Déchaud H, Grenot C, Lejeune H, Pugeat M. 1998 Human variant sex hormone-binding globulin (SHBG) with an additional carbohydrate chain has a reduced clearance rate in rabbit. *J Clin Endocrinol Metab* 83:235-40.
- Cousin P, Calemard-Michel L, Lejeune H, Raverot G, Yessaad N, Emptoz-Bonneton A, Morel Y, Pugeat M. 2004 Influence of SHBG gene pentanucleotide TAAAA repeat and D327N polymorphism on serum sex hormone-binding globulin concentration in hirsute women. *J Clin Endocrinol Metab* 89:917-24.
- Cui Y, Shu XO, Cai Q, Jin F, Cheng JR, Cai H, Gao YT, Zheng W. 2005 Association of breast cancer risk with a common functional polymorphism (Asp327Asn) in the sex hormone-binding globulin gene. *Cancer Epidemiol Biomarkers Prev* 14:1096-101.
- Cunningham JM, Hebring SJ, McDonnell SK, Cicek MS, Christensen GB, Wang L, Jacobsen SJ, Cerhan JR, Blute ML, Schaid DJ, Thibodeau SN. 2007 Evaluation of genetic variations in the androgen and estrogen metabolic pathways as risk factors for sporadic and familial prostate cancer. *Cancer Epidemiol Biomarkers Prev* 16:969-78.

Dunning AM, Dowsett M, Healey CS, Tee L, Luben RN, Folkard E, Novik KL, Kelemen L, Ogata S, Pharoah PD, Easton DF, Day NE, Ponder BA. 2004 Polymorphisms associated with circulating sex hormone levels in postmenopausal women. *J Natl Cancer Inst* 96:936–45.

Eriksson AL, Lorentzon M, Mellström D, Vandenput L, Swanson C, Andersson N, Hammond GL, Jakobsson J, Rane A, Orwoll ES, Ljunggren O, Johnell O, Labrie F, Windahl SH, Ohlsson C. 2006 SHBG gene promoter polymorphisms in men are associated with serum sex hormone-binding globulin, androgen and androgen metabolite levels and hip bone mineral density. *J Clin Endocrinol Metab* 91:5029–37.

Ferk P, Teran N, Gersak K. 2007 The (TAAAA)_n microsatellite polymorphism in the SHBG gene influences serum SHBG levels in women with polycystic ovary syndrome. *Hum Reprod* 22:1031–6.

Försti A, Jin Q, Grzybowska E, Söderberg M, Zientek H, Sieminska M, Rogozinska-Szczepka J, Chmielik E, Utracka-Hutka B, Hemminki K. 2002 Sex hormone-binding globulin polymorphisms in familial and sporadic breast cancer. *Carcinogenesis* 23:1315–20.

Garcia-Closas M, Brinton LA, Lissowska J, Richesson D, Sherman ME, Szeszenia-Dabrowska N, Peplonska B, Welch R, Yeager M, Zatonski W, Chanock SJ. 2007 Ovarian cancer risk and common variation in the sex hormone-binding globulin gene: a population-based case-control study. *BMC Cancer* 7:60.

Gershagen S, Lundwall A, Fernlund P. 1989 Characterization of the human sex hormone binding globulin (SHBG) gene and demonstration of two transcripts in both liver and testis. *Nucleic Acids Res* 17:9245–58.

Goderie-Plomp HW, van der Klift M, de Ronde W, Hofman A, de Jong FH, Pols HA. 2004 Endogenous sex hormones, sex hormone-binding globulin, and the risk of incident vertebral fractures in elderly men and women: the Rotterdam Study. *J Clin Endocrinol Metab* 89:3261–9.

Haffner SM, Shaten J, Stern MP, Smith GD, Kuller L. 1996 Low levels of sex hormone-binding globulin and testosterone predict the development of non-insulin-dependent diabetes mellitus in men. MRFIT Research Group. Multiple Risk Factor Intervention Trial. *Am J Epidemiol* 143:889–97.

Haiman CA, Riley SE, Freedman ML, Setiawan VW, Conti DV, Le Marchand L. 2005 Common genetic variation in the sex steroid hormone-binding globulin (SHBG) gene and circulating SHBG levels among postmenopausal women: the Multiethnic Cohort. *J Clin Endocrinol Metab* 90:2198–204.

Hammond GL, Underhill DA, Smith CL, Goping IS, Harley MJ, Musto NA, Cheng CY, Bardin CW. 1987 The cDNA-deduced primary structure of human sex hormone-binding globulin and location of its steroid-binding domain. *FEBS Lett* 215:100–4.

Hammond GL, Underhill DA, Rykse HM, Smith CL. 1989 The human sex hormone-binding globulin gene contains exons for androgen-binding protein and two other testicular messenger RNAs. *Mol Endocrinol* 3:1869–76.

Hammond GL, Bocchinfuso WP. 1995 Sex hormone-binding globulin/androgen-binding protein: steroid-binding and dimerization domains. *J Steroid Biochem Mol Biol* 53:543–52.

Hammond GL, Bocchinfuso WP. 1996 Sex hormone-binding globulin: gene organization and structure/function analyses. *Horm Res* 45:197–201.

Herbert Z, Göthe S, Caldwell JD, Bernstein HG, Melle C, von Eggeling F, Lewis J, Jirikowski GF. 2005 Identification of sex hormone-binding globulin in the human hypothalamus. *Neuroendocrinology* 81:287–93.

Hogeveen KN, Talikka M, Hammond GL. 2001 Human sex hormone-binding globulin promoter activity is influenced by a (TAAAA)_n repeat element within an Alu sequence. *J Biol Chem* 276:36383–90.

Hogeveen KN, Cousin P, Pugeat M, Dewailly D, Soudan B, Hammond GL. 2002 Human sex hormone-binding globulin variants associated with hyperandrogenism and ovarian dysfunction. *J Clin Invest* 109:973–81.

Jaquish CE, Blangero J, Haffner SM, Stern MP, MacCluer JW. 1997 Quantitative genetics of serum sex hormone-binding globulin levels in participants in the San Antonio Family Heart Study. *Metabolism* 46:988–91.

- Kahn SM, Hryb DJ, Nakhla AM, Romas NA, Rosner W. 2002 Sex hormone-binding globulin is synthesized in target cells. *J Endocrinol* 175:113-20.
- Kajaia N, Binder H, Dittrich R, Oppelt PG, Flor B, Cupisti S, Beckmann MW, Mueller A. 2007 Low sex hormone-binding globulin as a predictive marker for insulin resistance in women with hyperandrogenic syndrome. *Eur J Endocrinol* 157:499-507.
- Kataoka N, Cai Q, Xu WH, Xiang YB, Cai H, Zheng W, Shu XO. 2007 Association of endometrial cancer risk with a functional polymorphism (Asp(327)Asn) in the sex hormone-binding globulin gene. *Cancer* 109:1296-302.
- Kuijper EA, Lambalk CB, Boomsma DI, van der Sluis S, Blankenstein MA, de Geus EJ, Posthuma D. 2007 Heritability of reproductive hormones in adult male twins. *Hum Reprod* 22:2153-9.
- Lapidus L, Lindstedt G, Lundberg PA, Bengtsson C, Gredmark T. 1986 Concentrations of sex hormone-binding globulin and corticosteroid-binding globulin in serum in relation to cardiovascular risk factors and to 12-year incidence of cardiovascular disease and overall mortality in postmenopausal women. *Clin Chem* 32:146-52.
- Larrea F, Díaz L, Cariño C, Larriva-Sahd J, Carrillo L, Orozco H, Ulloa-Aguirre A. 1993 Evidence that human placenta is a site of sex hormone-binding globulin gene expression. *J Steroid Biochem Mol Biol* 46:497-505.
- Lazaros L, Xita N, Kaponis A, Zikopoulos K, Sofikitis N, Georgiou I. 2008 Evidence for association of sex hormone-binding globulin and androgen receptor genes with semen quality. *Andrologia* 40:186-91.
- Lehrman MA, Goldstein JL, Russell DW, Brown MS. 1987 Duplication of seven exons in LDL receptor gene caused by Alu-Alu recombination in a subject with familial hypercholesterolemia. *Cell* 48:827-35.
- Li L, He S, Sun JM, Davie JR. 2004 Gene regulation by Sp1 and Sp3. *Biochem Cell Biol* 82:460-71.
- Lindstedt G, Lundberg PA, Lapidus L, Lundgren H, Bengtsson C, Bjorntorp P. 1991 Low sex-hormone-binding globulin concentration as independent risk factor for development of NIDDM: 12-yr follow-up of population study of women in Gothenburg, Sweden. *Diabetes* 40:123-8.
- Lorentzon M, Swanson C, Andersson N, Mellstrom D, Ohlsson C. 2005 Free testosterone is a positive, whereas free estradiol is a negative, predictor of cortical bone size in young Swedish men: the GOOD study. *J Bone Miner Res* 20:1334-41.
- Low YL, Taylor JI, Grace PB, Dowsett M, Folkard E, Doody D, Dunning AM, Scollen S, Mulligan AA, Welch AA, Luben RN, Khaw KT, Day NE, Wareham NJ, Bingham SA. 2005 Polymorphisms in the CYP19 gene may affect the positive correlations between serum and urine phytoestrogen metabolites and plasma androgen concentrations in men. *J Nutr* 135:2680-6.
- Low YL, Dunning AM, Dowsett M, Luben RN, Khaw KT, Wareham NJ, Bingham SA. 2006 Implications of gene-environment interaction in studies of gene variants in breast cancer: an example of dietary isoflavones and the D356N polymorphism in the sex hormone-binding globulin gene. *Cancer Res* 66:8980-3.
- Meikle AW, Stanish WM, Taylor N, Edwards CQ, Bishop CT. 1982 Familial effects on plasma sex-steroid content in man: testosterone, estradiol and sex hormone-binding globulin. *Metabolism* 31:6-9.
- Misao R, Fujimoto J, Itoh N, Nakanishi Y, Tamaya T. 1997 Localization of sex hormone-binding globulin mRNA expression in human uterine endometrium. *Gynecol Endocrinol* 11:1-4.
- Nestler JE. 1993 Sex hormone binding globulin: a marker for hyperinsulinemia and/or insulin resistance? *J Clin Endocrinol Metabol* 76:273-4.
- Potischman N, Hoover RN, Brinton LA, Siiteri P, Dorgan JF, Swanson CA, Berman ML, Mortel R, Twiggs LB, Barrett RJ, Wilbanks GD, Persky V, Lurain JR. 1996 Case-control study of endogenous steroid hormones and endometrial cancer. *J Natl Cancer Inst* 88:1127-35.

- Power SG, Bocchinfuso WP, Pallesen M, Warmels-Rodenhiser S, Van Baelen H, Hammond GL. 1992 Molecular analyses of a human sex hormone-binding globulin variant: evidence for an additional carbohydrate chain. *J Clin Endocrinol Metab* 75:1066-70.
- Que BG, Petra PH. 1987 Characterization of a cDNA coding for sex steroid-binding protein of human plasma. *FEBS Letters* 219:405-9.
- Riancho JA, Valero C, Zarrabeitia MT, García-Unzueta MT, Amado JA, González-Macías J. 2008 Genetic polymorphisms are associated with serum levels of sex hormone binding globulin in postmenopausal women *BMC Med Genet* 9:112.
- Ring HZ, Lessov CN, Reed T, Marcus R, Holloway L, Swan GE, Carmelli D. 2005 Heritability of plasma sex hormones and hormone binding globulin in adult male twins. *J Clin Endocrinol Metab* 90:3653-8.
- Selva DM, Hammond GL. 2006 Human sex hormone-binding globulin is expressed in testicular germ cells and not in sertoli cells. *Horm Metab Res* 38:230-5.
- Siiteri PK, Murai JT, Hammond GL, Nisker JA, Raymoure WJ, Kuhn RW. 1982 The serum transport of steroid hormones. *Recent Prog Horm Res* 38:457-510
- Su MT, Chen CH, Kuo PH, Hsu CC, Lee IW, Pan HA, Chen YT, Kuo PL. 2008 Polymorphisms of estrogen-related genes jointly confer susceptibility to human spermatogenic defect. *Fertil Steril* 2008 Nov 1. [Epub ahead of print]
- The Endogenous Hormones and Breast Cancer Collaborative Group. 2002 Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies. *J Natl Cancer Inst* 94:606-16.
- Thompson DJ, Healey CS, Baynes C, Kalmyrzaev B, Ahmed S, Dowsett M, Folkard E, Luben RN, Cox D, Ballinger D, Pharoah PD, Ponder BA, Dunning AM, Easton DF. 2008 Studies in Epidemiology and Risks of Cancer Heredity Team. Identification of common variants in the SHBG gene affecting sex hormone-binding globulin levels and breast cancer risk in postmenopausal women. *Cancer Epidemiol Biomarkers Prev* 17:3490-8.
- Toscano V, Balducci R, Bianchi P, Guglielmi R, Mangiantini A, Sciarra F. 1992 Steroidal and non-steroidal factors in plasma sex hormone-binding globulin regulation. *J Steroid Biochem Mol Biol* 43:431-7.
- Turk A, Kopp P, Colangelo LA, Urbanek M, Wood K, Liu K, Skinner HG, Gapstur SM. 2007 Associations of serum sex hormone binding globulin (SHBG) levels with SHBG gene polymorphisms in the CARDIA Male Hormone Study. *Am J Epidemiol* 167:412-8.
- Ukkola O, Rankinen T, Gagnon J, Leon AS, Skinner JS, Wilmore JH, Rao DC, Bouchard C. 2002 A genome-wide linkage scan for steroids and SHBG levels in black and white families: the HERITAGE Family Study. *J Clin Endocrinol Metab* 87:3708-20.
- Vanbillemont G, Bogaert V, De Bacquer D, Lapauw B, Goemaere S, Toye K, Van Steen K, Taes Y, Kaufman JM. 2009 Polymorphisms of the SHBG gene contribute to the interindividual variation of sex steroid hormone blood levels in young, middle-aged and elderly men. *Clin Endocrinol* 70:303-10.
- Vohl MC, Dionne FT, Dériaz O, Chagnon M, Bouchard C. 1994 Detection of a MspI restriction fragment length polymorphism for the human sex hormone-binding globulin (SHBG) gene. *Hum Genet* 93:84.
- Weiss JM, Huang WY, Rinaldi S, Fears TR, Chatterjee N, Hsing AW, Crawford ED, Andriole GL, Kaaks R, Hayes RB. 2008 Endogenous sex hormones and the risk of prostate cancer: a prospective study. *Int J Cancer* 122:2345-50.
- Xita N, Tsatsoulis A, Chatzikiyiakidou A, Georgiou I. 2003 Association of the (TAAAA)n repeat polymorphism in the sex hormone-binding globulin (SHBG) gene with polycystic ovary syndrome and relation to SHBG serum levels. *J Clin Endocrinol Metab* 88:5976-80.
- Xita N, Tsatsoulis A, Stavrou I, Georgiou I. 2005 Association of SHBG gene polymorphism with menarche. *Mol Hum Reprod* 11:459-62.
- Xita N, Tsatsoulis A. 2006 Fetal programming of polycystic ovary syndrome by androgen excess: evidence from experimental, clinical, and genetic association studies. *J Clin Endocrinol Metab* 91:1660-6.

Xita N, Georgiou I, Lazaros L, Psofaki V, Kolios G, Tsatsoulis A. 2008a The synergistic effect of sex hormone-binding globulin and aromatase genes on polycystic ovary syndrome phenotype. *Eur J Endocrinol* 158:861-5.

Xita N, Georgiou I, Lazaros L, Psofaki V, Kolios G, Tsatsoulis A. 2008b The role of sex hormone-binding globulin and androgen receptor gene variants in the development of polycystic ovary syndrome. *Hum Reprod*. 23:693-8.

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