Short Communication

Variant in Sex Hormone-Binding Globulin Gene and the Risk of Prostate Cancer


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Abstract

Sex hormones have been implicated in prostate carcinogenesis and are thought to modulate cell proliferation and growth. To investigate the association between polymorphisms in hormone-related genes and prostate cancer risk, we conducted a two-stage, case-control study within the screening arm of the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial. Using DNA extracted from blood specimens, we initially genotyped 14 single nucleotide polymorphisms in genes involved in hormone regulation or metabolism (AKR1C3, CYP1A1, CYP1B1, CYP3A4, ESR1, GNRH1, HSD173B, HSD3B2, SHBG, and SRD5A2) in 488 prostate cancer cases and 617 matched controls. Heterozygotes at SHBG D356N were found to be associated with an increased risk of prostate cancer compared with the homozygous wild type, particularly among non-Hispanic whites (odds ratio, 1.54; 95% confidence interval, 1.13-2.09; P = 0.006). No significant associations were observed with the other polymorphisms. The SHBG D356N polymorphism, which has potential functional significance, was subsequently genotyped in additional 769 cases and 1,168 controls. Overall, SHBG D356N heterozygotes were found to have an increased risk of prostate cancer among whites (odds ratio, 1.34; 95% confidence interval, 1.10-1.63; P = 0.0007). This study suggests that genetic variation in SHBG may influence prostate cancer susceptibility. (Cancer Epidemiol Biomarkers Prev 2007;16(1):165–8)

Introduction

Sex hormones are thought to play a role in prostate carcinogenesis by altering the balance between cell proliferation and apoptosis. Although androgens are essential for the normal development and growth of the prostate, prolonged administration of testosterone has been shown to generate prostate tumors in rodents (1, 2), and androgens enhance the growth of several prostate cancer cell lines (3). The regulation and relative balance of sex hormones may be important in prostate carcinogenesis as estrogens modulate the effects of testosterone on prostate cancer in rodents (4, 5) and androgen ablation serves as a palliative treatment for advanced prostate cancer (6). However, epidemiologic studies investigating the association between hormone levels and prostate cancer risk have been inconclusive (reviewed in ref. 7), suggesting that the role of hormones in prostate cancer is more complex than originally thought.

Genetic factors are thought to influence the regulation of sex hormones with estimates of heritability between 12% and 76% for most sex hormones (8-10), raising interest in single-nucleotide polymorphisms suggested to alter hormone regulation or metabolism. Several polymorphisms have been reported to alter hormone metabolism (11-13). For example, the SRD5A2 89L variant has been shown to reduce steroid 5α-reductase activity both in vitro and in vivo (11). Other polymorphisms may affect hormone activity or distribution. A common polymorphism in the sex hormone-binding globulin (SHBG) gene, D356N, encodes for an additional N-glycosylation consensus site, which may reduce its clearance from circulation and/or alter its binding to membrane receptors (14).

Epidemiologic studies investigating polymorphisms in hormone-related genes and prostate cancer have yielded mixed results (reviewed in ref. 15). To further evaluate the association between prostate cancer risk and genetic variation in hormone metabolism and regulation, we conducted a two-stage, case-control study within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial.

Materials and Methods

The Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial is a multicenter trial designed to evaluate screening methods for the early detection of these cancers (16, 17). Briefly, >75,000 men, ages 55 to 74 years, were recruited from 10 centers between 1993 and 2001 and randomized to receive either prostate screening (serum prostate-specific antigen testing and digital rectal exam) annually or standard care. Participants completed a risk factor questionnaire at baseline, and blood specimens were collected at each screening visit. Men with abnormal screening results were referred to a physician for diagnostic workup, and information about cancer diagnosis was abstracted from medical records. The study was approved by the institutional review boards at the National Cancer Institute and the screening centers, and all participants provided informed consent.

A total of 1,320 prostate cancer cases and 1,842 matched controls from the screening arm of the trial were selected for etiologic studies of prostate cancer. Cases included non-Hispanic whites (n = 1,154) and blacks (n = 103) diagnosed...
with prostate cancer from 1993 through 2001. A total of 453 cases were diagnosed within 1 year of the baseline screen and 867 were diagnosed on longer follow-up. Controls were randomly selected from among men without evidence of prostate cancer at the time of case selection and were frequency matched to cases on age (± 5 years), fiscal year at randomization, study year of trial, and race (in a ratio of 1:2.1 for whites and 4:1 for blacks). The cases and their matched controls were then randomly divided into two samples: phase 1 (n = 503 cases and 652 controls) and phase 2 (n = 817 cases and 1,190 controls), with phase 1 cases being oversampled for advanced disease.

DNA was extracted from stored blood samples using QIAamp DNA Blood Midi or Maxi Kits. From genes involved in hormone regulation or metabolism, we selected 14 single-nucleotide polymorphisms that may alter function (11-14, 18-20), encoded a nonsynonymous amino acid change, or were located in the 3' untranslated region of the gene and thus could alter mRNA stability. The 14 single-nucleotide polymorphisms [AKR1C3 H5Q (rs12529), CYP1A1 T461N (rs1799814), CYP1A1 H42V (rs1048943), CYP1B1 R48G (rs10012), CYP1B1 L432V (rs1056836), CYP1B1 N453S (rs1800440), CYP3A4 –391A>G (a.k.a., CYP3A4*1B; rs2740574), ESR1 IVS1-397C>T (a.k.a., the PvuII restriction site; rs2234693), GNRH1 W16S (rs6185), HSD17B3 G289S (rs2066479), HSD3B2 Ex4-133C>T in the 3' untranslated region (rs1819698), SHBG D356N (rs6259), SHBG –67C>A (rs1799941), and SRD5A2 V89L (rs253349)] were genotyped initially in the phase 1 cases and controls. Phase 2 genotyping was reserved for single-nucleotide polymorphisms that showed results suggestive of an association in phase 1 (P < 0.05).

All genotyping was done at the National Cancer Institute Core Genotyping Facility using TaqMan or MGB Eclipse (21). Laboratory personnel were blinded to case-control status and replicate quality control samples (48 individuals assayed three to seven times per genotype) were interspersed in the plates. Replicate samples displayed 100% concordance for all single-nucleotide polymorphisms, except ESR1 IVS1-397C>T (98.8%), CYP1A1 T461N (97.8%), and CYP1B1 R48G (95.7%). Approximately 4% of the men were found to have insufficient DNA for genotyping and were excluded. Of those remaining, genotyping was successfully completed for 95% to 99% of subjects.

The genotype frequencies in the controls were consistent with Hardy-Weinberg proportions (P > 0.05) using a goodness-of-fit χ² test (or exact test if cell counts were small) for all single-nucleotide polymorphisms, except CYP1B1 L432V (P = 0.04 in whites) and SHBG D356N (P = 0.03 in phase 2 whites). Although statistically significant, the observed genotype frequencies were not much different than expected under Hardy-Weinberg equilibrium, especially for SHBG D356N (DD, 80% versus 80%; DN, 18% versus 19%; NN, 2% versus 1%), and no discrepancies were observed in the replicate samples.

Conditional logistic regression was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for the association between each polymorphism and prostate cancer. The results were similar when using unconditional logistic regression and adjusting for matching factors. The global statistical significance of the locus was assessed by comparing the replication and metabolizing enzymes were found to have insufficient DNA for genotyping and were excluded. Of those remaining, genotyping was successfully completed for 95% to 99% of subjects.

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a high school education (OR, 0.73; 95% CI, 0.56-0.94) or be obese (OR, 0.80; 95% CI, 0.65-1.00) and more likely to have a first-degree relative with prostate cancer than controls (OR, 1.62; 95% CI, 1.24-2.09). Among the phase 1 participants, SHBG D356N genotype variants were associated with prostate cancer risk ($P_{\text{global}} = 0.01$) with heterozygotes showing an increased risk of prostate cancer compared with the homozygous wild type, especially among non-Hispanic whites (OR, 1.54; 95% CI, 1.13-2.09; Table 2). None of the other polymorphisms examined in phase 1 were significantly associated with prostate cancer ($P > 0.05$; Supplementary Table A).

Because SHBG 0356N was found to be associated with prostate cancer in phase 1, both SHBG -67G>A and D356N were genotyped in the phase 2 cases and controls. The association between the SHBG D356N polymorphism and prostate cancer risk was weaker and not statistically significant in phase 2 ($P_{\text{global}} = 0.27$), but the point estimate for the heterozygotes remained >1.0 (Table 2), and overall, combining the phases, the global test remained statistically significant ($P_{\text{global}} = 0.004$) with SHBG D356N heterozygotes showing increased risk of prostate cancer compared with the homozygous wild type (OR, 1.32; 95% CI, 1.09-1.59). When the results were stratified by ethnicity, the SHBG D356N polymorphism was associated with prostate cancer in non-Hispanic whites but not in the smaller sample of non-Hispanic blacks (Table 2). The SHBG -67G>A and SHBG D356N polymorphisms were in strong linkage disequilibrium ($D^2 = 1.0$) but weakly correlated ($r^2 = 0.04$), and no clear association was observed between the SHBG -67G>A polymorphism and prostate cancer risk (AA versus GG: OR, 1.12; 95% CI, 0.82-1.53). Adjustment for the SHBG -67G>A polymorphism did not significantly alter the association between the SHBG D356N heterozygote and prostate cancer (OR, 1.33; 95% CI, 1.09-1.62).

The risk of prostate cancer for the SHBG D356N heterozygotes seemed to be slightly greater among men <65 years of age (OR, 1.51; 95% CI, 1.11-2.05) than older men (OR, 1.22; 95% CI, 0.96-1.54). However, no differences were observed when the results were stratified by prostate cancer stage (I/II versus III/IV), Gleason score (<7 versus ≥7), or body mass index, and the effect of the SHBG D356N heterozygote on prostate cancer risk was similar between incident and prevalent cases.

### Discussion

Historically, SHBG was thought to regulate the bioavailability of sex hormones simply by binding them and preventing their diffusion across cell membranes; however, recent evidence suggests that SHBG plays a more complex role in regulating hormone activity. Although controversial, studies have reported that the binding of the SHBG-hormone complex to its membrane receptor increases the production of intracellular cyclic AMP (23, 24), suggesting an alternative signaling pathway. In addition, Hammes et al. (25) showed that physiologically relevant amounts of sex hormones bound to SHBG are taken up by cells in target tissues through receptor-mediated endocytosis. By binding to the megalin receptor, SHBG acts as a shuttle for the endocytic uptake of androgens and estrogens in target tissues.

In our study, we found that SHBG D356N heterozygotes were associated with an increased risk of prostate cancer. The SHBG 356N variant introduces an additional site for N-glycosylation (14). Although the extra carbohydrate chain encoded by the 356N variant is unlikely to alter the affinity of SHBG for testosterone or other hormones (26, 27), it may affect the binding of SHBG to other proteins or receptors, as glycosylation is thought to be important in cell membrane signal transduction for other glycoprotein hormones (28) and for the attachment of SHBG to cell membranes (29). The extent of glycosylation is reported to affect the ability of SHBG to interact with matrix-associated proteins (30). Thus, it is possible that the SHBG 356N variant may increase prostate cancer risk by altering protein or receptor binding.

SHBG 356N homozygosity was not associated with an increased risk of prostate cancer in our study. This is likely due to low statistical power; however, it is notable that a borderline protective association was observed in this group. The plasma half-life of SHBG is reported to be significantly longer in 356N homozygotes compared with wild-type carriers (31). Studies in postmenopausal and hirsute women have shown that the 356N allele, particularly the NN genotype, is associated with increased plasma SHBG levels (20, 32, 33). Although not consistent across published studies (reviewed in ref. 7), two prospective studies reported an inverse association between high levels of SHBG and prostate cancer risk (34, 35). Thus, the lack of increased cancer risk among the homozygotes may be partially due to associated elevated SHBG plasma levels.

Alternatively, the association between the SHBG D356N polymorphism and prostate cancer risk may be due to linkage disequilibrium with another unobserved variant in the region. In Caucasians, but not African Americans, the SHBG D356N is in linkage disequilibrium with a (TAAAA)$_n$ pentanucleotide repeat polymorphism, located at the 5′ boundary of the SHBG promoter (33). Although the data are not entirely consistent, the (TAAAA)$_n$ repeat polymorphism has been reported to alter

| Table 2. ORs and 95% CIs of prostate cancer for SHBG D356N in the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Phase 1 cases  |                 | **OR** (95% CI) | **P**           | Phase 2 cases  |                 | **OR** (95% CI) | **P**           | Phases 1 and 2 combined cases  |                 | **OR** (95% CI) | **P**           |
| All men         | 362            | 501             | 1.0            | 0.01            | 610            | 973             | 1.0            | 0.22            | 972             | 1,474           | 1.0            | 0.0007          |
| DD              | 362            | 501             | 1.0            | 0.01            | 610            | 973             | 1.0            | 0.22            | 972             | 1,474           | 1.0            | 0.0007          |
| DN              | 118            | 100             | 1.48 (1.10-1.99)| 0.01            | 150            | 173             | 1.17 (0.91-1.49)| 0.22            | 268             | 273             | 1.32 (1.09-1.59)| 0.0001         |
| NN              | 3              | 9               | 0.46 (0.08-1.87)| 0.38            | 8              | 17              | 0.65 (0.28-1.54)| 0.33            | 11              | 26              | 0.56 (0.28-1.14)| 0.06           |
| Non-Hispanic whites |            |                 |                |                 |                |                 |                |                 |                |                |                 |                 |
| DD              | 342            | 432             | 1.0            | 0.01            | 536            | 679             | 1.0            | 0.11            | 878             | 1,111           | 1.0            | 0.0000         |
| DN              | 116            | 91              | 1.54 (1.32-2.09)| 0.006           | 144            | 151             | 1.17 (0.91-1.52)| 0.22            | 260             | 242             | 1.34 (1.01-1.63)| 0.0007         |
| NN              | 3              | 9               | 0.42 (0.07-1.71)| 0.25            | 7              | 16              | 0.57 (0.23-1.39)| 0.21            | 10              | 25              | 0.51 (0.24-1.06)| 0.04           |
| Non-Hispanic blacks |            |                 |                |                 |                |                 |                |                 |                |                |                 |                 |
| DD              | 20             | 69              | 1.0            | 0.21            | 74             | 294             | 1.0            | 0.71            | 94              | 363             | 1.0            | 0.0003         |
| DN              | 2              | 9               | 0.77 (0.07-4.17)| 1.0             | 6              | 22              | 1.05 (0.42-2.66)| 0.92            | 8               | 31              | 0.98 (0.44-2.18)| 0.96           |
| NN              | 0              | 0               | 1.0            | 1.0             | 1              | 1               | 3.97 (0.05-312.9)| 0.36            | 1               | 1               | 3.86 (0.05-303.9)| 0.37           |

*ORs and 95% CIs were estimated using conditional logistic regression.*

*P values reported for the combined phases are adjusted for the two-stage study design.*

*Exact OR, 95% CI, and P value are reported.*
transcriptional activity (36) and differences in plasma SHBG levels have been noted (32, 33, 37). SHBG is also located near the T P53 tumor suppressor gene on chromosome 17. Thus, the observed association with SHBG D356N may also be due to linkage disequilibrium with a variant in TP53.

In summary, we found that the SHBG D356N polymorphism was associated with an altered risk of prostate cancer. However, our findings could be due to chance, and additional studies are needed to confirm these results and to further characterize the biological effects of genetic variation in SHBG.

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