**Short Communication**

**Haplotype-Based Analysis of Common Variation in the Growth Hormone Receptor Gene and Prostate Cancer Risk**

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**Abstract**

The growth hormone receptor (GHR) is potentially involved in prostate cancer through its role in stimulating insulin-like growth factor I production and its cellular effects on prostate epithelium. We have used a haplotype-based tagging approach within Cancer Prostate Sweden, a large retrospective case-control study of 2,863 cases and 1,737 controls to investigate if genetic variation in the GHR gene influences prostate cancer risk. One haplotype in the 3′ region of the GHR gene was found associated with prostate cancer risk in elderly men (>65 years old at the time of diagnosis), with heterozygote haplotype carriers having an odds ratio of 1.65 (95% confidence interval, 1.21-2.16; P = 0.0009, P corrected = 0.03). GHR function has been implicated in the determination of body mass index. Interestingly, the same haplotype associated with risk in the 3′ end of the GHR gene was also associated with a decrease in body mass index in controls (P = 0.003, P corrected = 0.05), possibly indicating some functionality with this haplotype. These results suggest that whereas genetic variation in the GHR gene does not seem to play a major role in prostate cancer etiology, one haplotype in the 3′ region may be potentially relevant to cases with later onset of prostate cancer. (Cancer Epidemiol Biomarkers Prev 2007;16(1):169–73)

**Introduction**

Insulin-like growth factor-I (IGF-I) stimulates proliferation, decreases apoptosis, and has been implicated in cancer development by *in vitro* and *in vivo* studies (1-3). Prospective studies have shown elevated levels of circulating IGF-I to be associated with several cancer types, including prostate cancer (4-6). Genetic variation in the *IGF1* gene seems to play a role in determining circulating levels (7, 8, 9) and may also influence prostate cancer risk (10, 11).

The main endocrine stimulus of hepatic and tissue production of IGF-I is growth hormone. The growth hormone receptor (GHR) acts as the cellular receptor of growth hormone and the growth hormone binding protein in the circulation. When the GHR is absent, e.g., in growth hormone–inhibitory syndrome (GHI) cases, IGF-I levels are markedly lower circulating IGF-I levels (12-14). Therefore, GHR seems to have a direct influence on circulating IGF-I levels.

In addition, the GHR gene is expressed in normal and neoplastic prostate epithelium (15), and increased GHR expression seems to be required for the progression of benign prostate intraepithelial neoplasia to prostate cancer (14). GHR maps to chromosome 5p12, a region highlighted by several independent prostate cancer family-based linkage analysis studies (16-20).

Capitalizing on the extended linkage disequilibrium (LD) at the GHR locus, we have performed a haplotype-based association study in the Cancer in Prostate in Sweden (CAPS) study to investigate if common haplotypes are associated with prostate cancer risk.

**Materials and Methods**

**Study Population.** The study subjects were selected from an existing prostate cancer case-control study collected in Sweden (21). CAPS is a large-scale, population-based case-control study in Sweden. Patients with prostate cancer were identified and recruited from four of the six regional cancer registries in Sweden (from two rounds, CAPS1 and CAPS2). The inclusion criteria for case subjects was pathologically or cytologically verified adenocarcinoma of the prostate, diagnosed between July 1, 2001 and October 31, 2003. Control subjects were randomly selected from the continuously updated Swedish Population Registry and frequency-matched according to age (within 5 years) and geographic origin of the case subjects. In total, 3,013 cases and 1,896 control subjects were recruited, representing a 92% and 60% participation rate among all eligible case and control subjects, respectively. In this study, samples from 2,863 cases (mean age, 65.9) and 1,737 controls (mean age, 67.2) were available for analyses. Clinical information such as tumor-node-metastasis stage, Gleason grade, and prostate-specific antigen levels at diagnosis were available (from the National Prostate Cancer Registry) for 94% of the
case subjects. The case subjects were classified as having advanced disease (i.e., prone to progressive disease) if they met any of the following criteria: \( T_{3/4}, N+, M+, \) grade 3, Gleason score sum of 8 to 10, or a prostate-specific antigen level of >20 ng/mL. All other case subjects were classified as having localized disease. In subgroup analyses, 1,215 cases were defined as having advanced disease, 269 cases had a family history of prostate cancer in first-degree relatives, 1,512 were at an "elderly" age at diagnosis (>65 years), and 1,351 cases were at a "young" age at diagnosis (<65 years).

Written informed consent was obtained from all participants and the research ethical committee at the Karolinska Institutet and Umeå University Hospital approved the study.

Genetic Variation Across the GHR Locus and Haplotype Tagging Single Nucleotide Polymorphism Selection. We obtained genotypes for 92 single nucleotide polymorphisms (SNP) with a minor allele frequency of >4% in the CEPH population (Utah residents with ancestry from Northern and Western Europe) from phase I of the HapMap consortium (http://www.hapmap.org), covering a total range of 30 kb upstream and 30 kb downstream of the GHR locus. We also included the common 3 kb deletion of exon 3 of the GHR gene (GHRd3; ref. 22) by genotyping this deletion in the HapMap CEPH individuals using multiplex PCR (22). Pairwise LD estimates were calculated and haplotype blocks were defined using a slightly relaxed criteria to those outlined by Gabriel et al. (23) by lowering the fraction of strong LD in informative comparisons to >0.85.

Haplotype tagging SNPs (htSNP) were selected to represent each common (>4%) haplotype inside the blocks, using the tagSNPs program (24) at a sufficient density to predict all common haplotypes with a coefficient of determination \( (R^2)_h \) of >0.70 (Fig. 1).

Genotyping. Genotyping was done blinded to case-control status by the 5'-nuclease assay (TaqMan) as described previously (25) or MGB eclipse (Nanogen Technologies, San Diego, CA). Sequences of primers and probes are available on request. Genotyping call rates ranged between 92.7% and 98.6%. Repeated quality control genotypes showed an average concordance of 99.9%. All htSNPs conformed to Hardy-Weinberg equilibrium in controls, except for rs1559286. The deviation from Hardy-Weinberg equilibrium in rs1559286 was caused by a slight excess of rare homozygotes. As this deviation was slight \( (P = 0.01)\), not significant after correction

![Figure 1. LD structure across the GHR gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of GHR showing, from top to bottom: the location in base pairs of chromosome 5, the genomic structure of GHR, the location of 92 SNPs typed by HapMap, graphical representation of LD and block structure, the haplotypes predicted inside the blocks, with the position of htSNPs typed in this study marked in bold and the \( R^2 \) for each haplotype, and the haplotypic frequencies based on the CEU hapmap population (HapMap) and the CAPS control group (CAPS). Relative degree of correlation between the haplotype blocks \( (lines \ between \ the \ haplotypes) \), haplotypes that are transmitted in >10% of cases \( (bold \ lines) \); and in >5% of cases \( (thin \ lines) \). The color code shows the confidence boundaries of LD estimations: with the depth of color showing degree evidence of LD; evidence of recombination \( (white) \) or higher correlation \( (darker) \).](http://www.broad.mit.edu/mpg/haploview/)
for multiple testing ($P = 0.14$ using Bonferroni correction), and TaqMan regenotyping of the CEPH CEU individuals showed 100% concordance when compared with the HapMap genotyping, this htSNP was included in subsequent analyses.

**Statistical Analysis.** Risk estimates were assessed with the use of conditional logistic regression (26), matched by each combination of age group (in five age groups) and region using a codominant inheritance model. As the controls were not matched on a one-to-one basis with cases, the "TIES = DISCRETE option" was used in the PHREG procedure (SAS Base) according to Allison (27).

Risk estimates for haplotypes (frequency of $>4\%$ in CAPS), were assessed within blocks. For each specific haplotype, two dummy variables, ranging from 0 to 1.0, were calculated using the "tagSNPs" software, indicating one or two copies ("dosages") of the haplotype, i.e., heterozygosity or homozygosity. The dosage dummy variables were then implemented as covariates in the conditional logistic regression model, thus creating a codominant inheritance model. The most common haplotype was held as the reference category. Three $P$ values were calculated, one indicating significance for heterozygote haplotype carriers ($P_{het}$), one indicating significance for homozygote haplotype carriers ($P_{hom}$), and one indicating significance for a codominant model ($P_{trend}$). We examined the heterogeneity of relative risk estimates by age at diagnosis (>65 or <65 years old), based on $\chi^2$ statistics. Relationships between haplotype variation and anthropometric measurements were estimated in 831 controls from CAPS1 (measurements from CAPS2 were not available) by standard regression models. Only controls were assessed as CAPS in the retrospective case-control study, and therefore, body mass index (BMI) in cases may have been influenced by treatment and diagnosis. The additive model was used to test if BMI or height were linearly related to the number of copies an allele carried (0, 1, or 2 for SNP alleles, or dosages from 0 to 2 for haplotypes). Due to the high LD across GHR, significant $P$ values were adjusted for multiple testing by determining the number of times a $P$ value of that magnitude or less is observed in 10,000 permutations of the data. All $P$ values were from two-sided tests.

**Results**

**GHR Haplotype Block Structure.** Based on the 92 SNPs genotyped by the HapMap project, the GHR genomic region exhibiting extended LD could be divided into four large haplotype blocks (Fig. 1): block 1 contains the putative regulatory regions, the 5\' untranslated region, and exon 1; block 2 contains exon 2, including the translation start codon ATG; block 3 contains an intronic region; and block 4 contains the majority of the coding region and the 3\' untranslated region.
common haplotypes in the four blocks (six, two, four, and six htSNPs for blocks 1, 2, 3 and 4, respectively; Fig. 1). The GHRd3 deletion polymorphism was highly correlated with the surrounding SNPs in block 4 and had several “perfect” proxies (D’ = 1.0; R² = 1.0), including the htSNP, rs6886047.

Haplotype Variation in Relation to Prostate Cancer Risk. We assessed risk estimates for common haplotypes in each block in the whole study population as well as in subgroup analyses according to age, tumor characteristics, and family history. We also assessed haplotypic variation in the context of changes in BMI as BMI may be surrogate markers for GHR expression. The prostate cancer risk estimates for the overall analysis, stratified by age of onset and BMI, are displayed in Fig. 2.

The most significant effect was noted in block 4, in which heterozygote carriers of the CACGTG haplotype were associated with increased prostate cancer risk in cases >65 years at diagnosis [odds ratio for heterozygote haplotype carriers (ORhet), 1.62; 95% confidence interval (CI), 1.21-2.16; \( P_{\text{het}} = 0.0009, P_{\text{trend}} = 0.02 \)]. This risk effect seemed to be isolated to this subgroup, with a test for heterogeneity that showed a significant difference in risk estimates between the subgroups at <65 or >65 years at diagnosis (\( P = 0.01 \)). The association for the CACGTG haplotype was strengthened when considering men with both elderly age at diagnosis and with a family history. We also assessed haplotypic variation in the context of changes in BMI as BMI may be surrogate markers for GHR expression. The prostate cancer risk estimates for the overall analysis, stratified by age of onset and BMI, are displayed in Fig. 2.

In analyses of BMI in relation to genetic variation, interestingly, the same haplotype in block 4 (CACGTG) was also the most clearly associated, with carriers observed to have a decreased BMI (\( P_{\text{trend}} = 0.003 \); Fig. 2). Similar with prostate cancer risk, the correlation between haplotypes in the other blocks gave similar associations for haplotypes in the other blocks. The main association noted was in a block covering the majority of the coding region of the GHR gene, in which heterozygote carriers of the haplotype CACGTG among elderly patients with prostate cancer (age at diagnosis, >65 years) displayed an OR of 1.62 (95% CI, 1.21-2.16; \( P_{\text{het}} = 0.002, P_{\text{trend}} = 0.008 \)).

Haplotypes in blocks 1 (TGTTAA) and 3 (GGCA) also showed an increased risk in elderly onset cases of approximately the same magnitude as the risk observed for CACGTG in block 4, although at borderline significance (see Fig. 2). These three haplotypes are correlated (TGTTAA, GGCA, and CACGTG) and are often inherited together in >5% of instances (see Figs. 1 and 2). Therefore, these associations are not independent.

In analyses of BMI in relation to genetic variation, interestingly, the same haplotype in block 4 (CACGTG) was also the most clearly associated, with carriers observed to have a decreased BMI (\( P_{\text{trend}} = 0.003 \); Fig. 2). Similar with prostate cancer risk, the correlation between haplotypes in the four blocks gave an association with a decrease in BMI observed in 500 of 10,000 simulations, a value of 0.003 (as observed with the BMI results) was observed in 500 of 10,000 simulations.

Discussion

The HapMap genotyping data indicated that the GHR gene has a simple LD structure, which can be defined into four correlated haplotype blocks. This simple LD structure, consistent with the generally lower recombination rates at centromeres, lent itself towards a block-based haplotype-tagging approach, as employed elsewhere (28, 29). The use of HapMap data for the selection of haplotype-tagging SNPs relies on the assumption that the block structures are approximately equal in the HapMap data and in the Swedish population. As we found that the haplotype frequencies within each block as well as the LD measures between the htSNPs were very similar between the Swedish and HapMap populations, that assumption seems reasonable.

Using haplotype-tagging SNPs in a genetically homogenous study population, we investigated if common genetic variations across the GHR gene contribute to prostate cancer risk. The main association noted was in a block covering the majority of the coding region of the GHR gene, in which heterozygote carriers of the haplotype CACGTG among elderly patients with prostate cancer (age at diagnosis, >65 years) displayed an OR of 1.62 (95% CI, 1.21-2.16; \( P_{\text{het}} = 0.002, P_{\text{trend}} = 0.008 \)), a finding consistent with the prostate cancer 5p12-q12 linkage evidence from the Swedish population, which is also strongest among patients with elderly age at diagnosis (17). The association was strongest and most significant in the 3’ block, although the highly correlated blocks in this gene also gave similar associations for haplotypes in the other blocks.

The central hypothesis tested by this study is that aberrant GHR function caused by genetic variation in the GHR gene may result in prostate cancer risk. Aberrant GHR function may manifest as other traits related to GHR function in addition to prostate cancer risk. Both studies on mice and humans have reported changes in body mass and size with changes in the expressions of growth hormone and growth hormone receptor (13, 30-32), and genetic variation in other members of the growth hormone pathway have been implicated in BMI
We hypothesized that if the GHR haplotypes showing association with risk were truly involved in prostate cancer etiology, they may also bring changes in BMI (as it may be a surrogate marker for GHR expression levels). We therefore find it intriguing that whereas the associations noted by our study were modest when considering multiple testing, the most significant associations identified with prostate cancer risk and BMI overlapped, as expected by our hypothesis. The direction of the association is consistent, as a decrease in BMI could be related to an increase in GHR expression (growth hormone exerts lipolytic actions, favoring leanness) and increased GHR expression would also be consistent with the observation that GHR expression is required for progression from BPH to intraductal neoplasia to prostate cancer. To confirm these speculations, however, more detailed experimental data would be needed on the associations of haplotypes or specific SNP alleles with GHR expression.

In conclusion, our results suggest that whereas common genetic variation in the GHR gene does not play a major role in prostate cancer susceptibility, it may have relevance in patients with a more elderly age at diagnosis. Furthermore, the association with changes in the BMI suggests that genetic variation in the GHR could also possibly be involved in the regulation of adiposity and associated metabolic alterations such as insulin resistance. Nevertheless, these findings require confirmation in other large epidemiologic studies and in experimental studies on allelic variants in GHR on gene function.

Acknowledgments

We thank Björn-Anders Jonsson for DNA logistics; all the study participants in the CAPS study; Ulrika Undén for coordinating the study center at Karolinska Institute; Lotta Spängberg, Berit Andersson, and Karin Andersson for help in data collection and analyses; and all the patients following the patients in CAPS; Karin Andersson, Susan Lindh, Gabriella Thörén-Berglund, and Margareta Åsvard at the Regional Cancer Registries in Umeå, Uppsala, Stockholm-Gotland, and Linköping, respectively. In addition, we thank Sören Holmgren and the personnel at the Medical Biobank in Umeå and Stephanie Monnier at IARC for her assistance in haplotype tagging.

References
