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14. ABSTRACT

Purpose and scope: A large genetic association study was conducted to examine relationships of prostate cancer risk with polymorphic variation in a series of selected candidate genes that are involved in pathways determining the synthesis of IGF-I and IGF-binding proteins, as well as biological response to IGF-I. The study is being performed within a large Swedish case-control study ("CAPS"). Progress report: We have completed the selection of DNA and haplotype tagging SNPs to be analyzed for all candidate genes, and have completed about three quarters thirds of all genotyping assays for the prostate cancer cases and control subjects. We have completed analysis of complete genetic variation in the IGF1, IGFBP3, IGFBP1, IGFALS, SST, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5 and GHR genes, as well as selected polymorphisms in the GHRL and GHSR using the linked database, containing data on tumour grade, stage and serum PSA levels, for all prostate cancer cases. Plasma assays of IGF-I and IGFBP-3 were performed and statistical analysis between circulating plasma levels and SNPs as well as prognostic factors, has been completed. Evidence was identified for associations between SNPs in IGF1, IGFBP3 and SSTR5 and circulating plasma levels. Evidence for association between genetic variation in the IGF1 gene and prostate cancer risk was identified. Conclusions: Concerning the construction of the study databases, the plasma measures, the genetic component of our project and statistical analysis, we have completed the project relatively on schedule. The delay in the genotyping aspect of the project was mainly due non-receipt of funds, a substantial amount is remains outstanding at this time.

15. SUBJECT TERMS
Insulin-like growth factor-I (IGF-I), Genetic Polymorphisms, Epidemiology, Prostate Cancer Risk

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Introduction

Evidence is rapidly accumulating that insulin-like growth factor-I (IGF-I) can enhance the development of tumors in different organs. Studies *in vitro* have shown that IGF-I inhibits apoptosis and stimulates cell proliferation in a wide variety of cell types. Furthermore, tumor development can be strongly enhanced in animals or organs that have been genetically or otherwise manipulated to either over express IGF-I or the IGF-I receptor, whereas animals made deficient in IGF-I are protected. Experiments with IGF-I$^{-/-}$ null mice have shown that normal IGF-I levels are required for prostate gland development, and transgenic mice expressing human *IGF1* in basal epithelial cells of the prostate have a high spontaneous incidence of prostatic tumors. In men, several prospective cohort studies and case-control studies have shown an increased prostate cancer risk among men who have elevated plasma IGF-I levels – expressed either as absolute concentrations, or relative to levels of IGFBP-3, IGF's major plasmatic binding protein.

Most of IGF-I and IGF-binding proteins in the circulation originates from the liver, but all peptides are also formed in other organs, including the prostate, where they exert paracrine and autocrine effects. Circulating IGF-I, as an endocrine factor, can diffuse towards its target tissues. In addition, IGF-I synthesis by the liver and many other organs is very much controlled by the same endocrine and nutritional factors. The major endocrine stimulus to IGF-I synthesis, in liver and many other tissues, is provided by growth hormone (GH). Thus, elevated IGF-I in blood most likely reflects an elevated pituitary GH secretion, and most likely indicates also elevated levels in other tissues where GH also provides the principal stimulus to IGF-I synthesis.

Given the increasing evidence that elevated IGF-I may enhance cancer development, it is important to understand what factors can lead to elevated IGF-I in the circulation and tissues. Besides nutritional status (Kaaks & Lukanova, 2001), heritability studies have shown that, at least in western, well-nourished populations, a large part (40-60 %) of variation in IGF-I is (co-) determined by genetic factors (Hong et al., 1996; Harrela et al., 1996; Verhaeghe et al., 1996). So far, however, no studies have been published, reporting a comprehensive search for polymorphisms in a full panel of genes involved in regulating IGF-I synthesis, and correlating such a panel with inter-subject variations in IGF-I and IGFBP-3 levels.

Besides the genes for IGF-I (*IGF1*) and IGFBP-3 (*IGFBP3*), major candidate genes to be examined are those involved in the pituitary release or biological action of growth hormone — the primary physiological stimulus for the synthesis of both IGF-I and IGFBP-3. This latter includes the genes for somatostatin (*SST*) and its receptors (*SSTR1-5*), pituitary-specific transcription factor (or POU-domain class 1 transcription factor 1 (*POU1F1*); growth hormone (*GH1*) and its receptor (*GHR*), growth hormone releasing hormone (*GHRH*), and the GHRH receptor (*GHRHR*). Ghrelin (*GHRL*), a recently identified new peptide hormone produced by endocrine cells in the stomach, also stimulates growth hormone secretion. It is the first identified natural ligand for a previously cloned growth hormone secretagogue receptor (*GHSR*) which is present in the pituitary gland and the hypothalamic region of the brain. In the circulation, IGF-I and a large percentage of IGFBP-3 are bound to a third peptide, referred to as Acid Labile Subunit (*IGFALS*) which has a key role in stabilizing the circulating pool of these peptides, and in regulating IGF-I release towards tissues. For each of these genes, polymorphisms that change gene expression or protein function can be expected to result in a relative increase or decrease in circulating IGF-I or IGFBP-3 levels.

The specific aims of our project are the following:

- to examine associations of prostate cancer risk with polymorphic variants (single nucleotide polymorphisms [SNPs] or their haplotypes) of selected candidate genes that may determine the synthesis and circulating levels of IGF-I, or and biological response to IGF-I;
- to confirm that elevated IGF-I levels, as absolute concentrations or expressed relative to concentrations of IFBP-3, are associated with an increased risk of prostate cancer; and
- examine whether associations of prostate cancer risk with polymorphic gene variants can be explained, at least in part, by associations of the same gene variants with circulating IGF-I or IGFBP-3 levels.

Table 1. Candidate genes for studies of association with plasma IGF-I, IGFBP-3, and prostate cancer risk.

Gene	**Name and Function of gene product**
IGF-I	Insulin-like growth factor-I
GH1	Growth hormone: Main stimulus for synthesis of IGF-I and IGFBP-3
GHR	Growth hormone receptor: mediates GH effects
GHRH	Growth hormone releasing hormone: stimulates pituitary GH release
GHRHR	Growth hormone releasing hormone receptor; Mediates GHRH effects
SST	Somatostatin; inhibits pituitary GH release
SSTR1 – SSTR5	Somatostatin receptors, types 1 - 5; mediate SST effects on pituitary GH release
POU1F1	pituitary-specific transcription factor; crucial for pituitary GH synthesis
IGF1R	IGF-I receptor
GHRL	Ghrelin
GHSR	Growth hormone secretagogue receptor
IGFALS	IGF binding protein, acid labile subunit
IGFBP1 - 6	IGF-binding proteins 1 to 6

For year 1, our tasks, as in the "Statement of Work" of our original grant application, were the following:

Task 1: Completion of the recruitment of prostate cancer cases and control subjects into the Swedish "CAPS" (CAncer of the Prostate Sweden) project, using suitable matching and selection criteria for the controls subjects; Storage of blood samples (plasma and buffy coats) in the Medical Biobank at Umeå University;

This objective was entirely achieved, and actually even exceeded: A total of 2831 prostate cancer cases (57 percent of which were localized tumors, and 43 percent locally advanced tumors) and 1784 control subjects were recruited into the CAPS project, and from these subjects questionnaire data and blood samples were collected as planned. Blood samples were fractionated into plasma and buffy coats, and stored in the Umeå Medical Biobank. The increase in numbers of prostate cancer cases and control subjects was motivated by the fact that the speed of subject recruitment could be accelerated (thus allowing a cost-effective extension of study size), plus the consideration that the sample size initially foreseen (1200 cases and 1200 control subjects) might have provided insufficient statistical power to examine associations of genetic polymorphisms with prostate cancer risk, by subsets of different tumor grade and stage (e.g., local *vs*. advanced tumors).

Task 2: Retrieval of plasma samples from the Medical Biobank, assembly of plasma samples into batches of case-control sets for immunoassay of IGF-I and IGFBP-3;

This task was completed in year 2 of the project, not in year 1, because of some changes in the agenda of the Hormones and cancer Laboratory where the assays of IGF-I and IGFBP-3 will be performed, and because we have to liberate freezer space at IARC where to store the plasma aliquots.

Task 3: DNA extraction from buffy coat samples of all prostate cancer cases and control subjects (including a total of 2400 subjects originally foreseen); and

This task was fully completed: DNA was extracted from the buffy coats of all 2831 prostate cancer cases, and 1784 controls.

Task 4: Preparation of microwell plates with DNA aliquots for genotyping of genetic polymorphisms, at IARC.

This task was also entirely completed: 500 ng. aliquots of DNA were distributed into microtiter plates and shipped to the IARC.

For year 2 and 3, our tasks, as in the "Statement of Work" of our original grant application, were the following:

Task 5: Measurement of assays of IGF-I and IGFBP-3 in plasma of prostate cancer cases (n=1000) and controls (1200). Plasma samples have been shipped in year 2, from the central CAPS biobank in Umeå (Sweden) to the hormone assay laboratory at IARC (Lyon, France). The assays were completed at the hormone assay laboratory at IARC.

Task 6: Genotyping of cases and controls for polymorphisms in genes related to the IGF system. An extensive search was made in the now publicly accessible "HapMap" database, which provides very detailed information about the presence of genetic variants and their linkage disequilibrium patterns, in genes throughout the genome. This search, combined with our own previous work for the identification of SNPs, has allowed us to make a more exhaustive screen of the genetic and haplotypic that exists in the candidate genes of the IGF1 pathway than initially envisaged. By following the 'haplotype tagging' SNP approach, discussed in the year one and two progress reports, allows greater efficiency in our genotyping strategy. One clear example is the *GHR* gene in which, following the protocol outlined by Stram et al. (2003), a total of 113 SNPs can be tested by only 19 htSNPs, with only minimal loss of information (due to the fact that SNPs are often in linkage disequilibrium, making measurement one SNP a measurement of others by proxy) (Stram et al., 2003) (**Table 2**).

Table 2. htSNPs' selected and genotyping completed in the CAPS study.

Genes	Genome size (kb)[Ψ]	SNP's in gene region[t]	htSNPs selected*	SNPs genotyped
IGF1	128	44	11	11
IGFBP1	24	10	6	6
IGFBP3	70	18	6	6
IGFALS	9	6	3	3
GHR	447	113	18	18

SST	46	18	4	2
SSTR1	18	6	4	3
SSTR2	16	8	6	6
SSTR3	40	29	6	4
SSTR4	8	3	5	5
SSTR5	19	9	5	4
GHRL	10			1
GHSR	37			1

[Ψ]Genomic size including blocks of LD (defined by Gabriel et al., 2002 method) that may partially overlap with genomic sequence

[t]Number of confirmed polymorphic SNPs contained in the gene region (and in LD blocks that cover the gene) identified from the HapMap initiative and IARC SNP discovery work

*Selected on the basis of an R2h>0.8 for SNPs inside haplotype blocks (defined by Gabriel et al., 2002 method) and R2s>0.8 for SNPs falling in-between or just outside haplotype blocks if that distance is less than 10kB (Stram et al., 2003).

We have completed all htSNPs that represent all the common genetic variation in the *IGF1, IGFBP3, IGFBP1, IGFALS, SST, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5* and *GHR* genes, as well as selected polymorphisms in the *GHRL* and *GHSR*. QC analysis have been completed for all and found to be satisfactory (concordance >99%).

Due to serious administrative errors in the transfer of funds from the DoD to IARC (see further comments at the end of this report) we have not yet been able to complete genotyping testing for the remaining genes (*IGFBP2*, *IGFBP4*, *IGFBP5*, *IGFBP6*, *GHRH*, *PouF1*, *GHRHR*).

Task 7: Linkage of study set to primary registry of four regions to obtain data on tumor grade, stage and serum PSA levels as well as date and type of cancer treatment. This task has been completed, and data (additional variables) have been added to the central CAPS database.

Tasks 8 and 9: Statistical analysis of associations between genetic polymorphisms, plasma levels of IGF-I, IGFBP-3 and risk of prostate cancer. We have completed statistical analyses on the relationship of prostate cancer risk with polymorphic variants in the *IGF1*, *IGFBP1*, *IGFBP3*, *IGFALS, SST*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR4*, *SSTR5* and *GHR* genes. Notable associations were identified between genetic variation in *IGF1* and *GHR* and risk of prostate cancer risk. We have also completed statistical analysis of associations between genetic polymorphisms in *IGF1*, *IGFBP1*, *IGFBP3*, *IGFALS*, *GHR*, *SST*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR4*, *SSTR5* in relation to plasma levels of IGF-I and IGFBP-3; with statistically significant associations being identified with genetic variation in *IGF1*, *IGFBP3* and *SSTR5*. A list of papers, published and submitted/in preparation, is given below, and copies of the published manuscripts are attached to this report. Statistical analysis of associations between genetic polymorphisms, plasma levels of IGF-I, IGFBP-3 and different outcomes in the prostate cancer disease progression and response to treatment is complete and a manuscript is in preparation.

Task 10: Interpretation of data, writing of reports. Three scientific papers have been published in peer reviewed journals to date. The two papers by Johansson et al. demonstrated that genetic variation in the *IGF1* gene increases prostate cancer risk by affecting the concentrations of circulating IGF1 hormone levels. These two studies also give further support for the causal link between elevated levels of IGF1 in the circulation and increased prostate cancer risk along

the lines of Mendelian randomization. In the paper by McKay et al. we found one genetic variant affecting both prostate cancer risk in older subjects, as well as body mass index. One manuscript (McKay et al.) is currently under review and in this study we demonstrated that genetic variation within the *SSTR5* gene affects circulating levels of IGF1 and IGFBP3 hormone. One manuscript is currently in preparation. In this study we investigate genetic variation within *IGFBP1*, *IGFBP3* and *IGFALS* genes in relation to prostate cancer risk, survival and influence on circulating hormone levels of IGF1 and IGFBP3. In addition we investigate the impact of circulating hormone levels on prostate cancer specific survival.

Key research accomplishments

Accomplishments of this project include include:

- Selection of haplotype tagging SNPs (htSNPs) for genes to be genotyped. Optimization of TAQMAN assays for the htSNPs
- Completion of genotyping in the case/control series at IARC in 4865 individuals for the genes *IGF1, IGFBP3, IGFBP1, IGFALS, SST, SSTR1, SSTR2, SSTR3, SSTR4, SSTR5* and *GHR* genes, as well as selected polymorphisms in the *GHRL* and *GHSR* (in excess of 300,000 genotypes have been completed).
- Completion of a linked database containing data on tumour grade, stage and serum PSA levels has been assembled and distributed among the collaborating partners.
- The plasma levels of IGF-I and IGFBP-3 for the CAPS samples have been analyzed at the hormone assay laboratory at IARC.
- Statistical analysis has been completed for *IGF1*, *IGFBP3*, *IGFALS*, *GHR*, *SST*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR4*, *SSTR5*; with statistically significant associations being found with haplotypes in *IGF1* and *GHR* and increased prostate cancer.
- Statistical analysis has been completed for polymorphic gene variants in *IGF1*, *IGFBP1*, *IGFBP3*, *IGFALS*, *GHR*, *SST*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR4*, *SSTR5* in relation to plasma levels of IGF-I and IGFBP-3. Statistically significant associations being identified in *IGF1* (rs6220), *IGFBP3* (SNP rs2854744) and *SSTR5* (SNP rs4988483). The associations between rs2854744 and rs4988483 and circulating hormone levels were replicated in a second study independent to the CAPS series.

Reportable outcomes

Scientific Papers for peer reviewed scientific journals have been published for three key results where interesting associations have been found.

Johansson M, McKay JD, Wiklund F, Rinaldi S, Verheus M, van Gils C, Hallmans G, Bälter K, Adami H, Grönberg H, Stattin P, Kaaks R, Genetic variation in the *IGF1* gene – in relation to circulating levels of IGF1 - Implications for prostate cancer. *J Clin Endo Metab* Epub ahead of print. Oct 2007

McKay JD, Kaaks R, Johansson M, Biessy C, Wiklund F, Balter K, Adami HO, Boillot C, Gioia-Patricola L, Canzian F, Stattin P, Gronberg H. Haplotype-based analysis of common variation in the growth hormone receptor gene and prostate cancer risk. Cancer Epidemiol Biomarkers

Prev. 2007;16(1):169-73.

Johansson M, McKay JD, Stattin P, Canzian F, Boillot C, Wiklund F, Adami HO, Balter K, Gronberg H, Kaaks R. Comprehensive evaluation of genetic variation in the IGF1 gene and risk of prostate cancer. Int J Cancer. 2007;120(3):539-42.

An additional paper for the Samatostatin genes has been submitted.
McKay JD, Johansson M, Wiklund F, Rinaldi S, Gioia L, Charbier A, Gilibert I, Hallmans G, Bälter K, Adami H, Grönberg H, Stattin P, Kaaks R. Genetic variation in the SST gene and its receptors – in relation to circulating levels of IGF1, IGFBP3 and prostate cancer risk. Submitted . *J Clin Endo Metab* 2007

Copies of all 4 are attached as appendices and contain due acknowledgement of the DoD.

A complete financial report and comments about the administrative errors is attached as a separate document.

Conclusions

We have completed the project relatively on schedule. We have constructed the biological samples and databases required for the CAPS studies investigation of the genes of the *IGF1* pathway. For all candidate genes we have performed "tagging" analyses, covering all common haplotypic variations in these genes. For the genes *IGF1*, *IGFBP1*, *IGFBP3*, *IGFALS*, *GHR*, *SST*, *SSTR1*, *SSTR2*, *SSTR3*, *SSTR4*, *SSTR5* all genotyping assays have been designed, optimized and completed. Assays of plasma IGF-I and IGFBP-3 were completed for all CAPS prostate cancer cases and controls. Statistical analysis of associations between all genetic polymorphisms, plasma levels of IGF-I, IGFBP-3 and risk of prostate cancer has been completed. Four publications have been completed, with three publications have been published (copies attached), a fourth is submitted (copy attached) and a fifth is in preparation.
For the remaining genes (*IGFBP2*, *IGFBP4*, *IGFBP5*, *IGFBP6*, *GHRH*, *PouF1*, *GHRHR*), genotyping could not be completed, because of errors/delays in the transfer of the funds from the US Dept of defense to IARC.

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Hong Y, Pedersen NL, Brismar K, Hall K, de Faire U. 1996; Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. J Clin Endocrinol Metab. 81:1791-7.

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Jernstrom H, Deal C, Wilkin F, Chu W, Tao Y, Majeed N, Hudson T, Narod SA, Pollak M. 2001; Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth

factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. Cancer Epidemiol Biomarkers Prev. 10:377-84.

Kaaks R & Lukanova A. 2001; Energy balance and cancer: the role of insulin and IGF-I. Proc Nutr Soc. 60: 91-106.

Verhaeghe J, Loos R, Vlietinck R, Herck EV, van Bree R, Schutter AM. 1996; C-peptide, insulin-like growth factors I and II, and insulin-like growth factor binding protein-1 in cord serum of twins: genetic versus environmental regulation. Am J Obstet Gynecol. 175:1180-8.

Stram DO, Haiman CA, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Pike MC. 2003; Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered. 55:27-36.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D. 2002. The structure of haplotype blocks in the human genome. Science :296(5576):2225-9.

Appendices

Appendix 1. Johansson et al. 2006
Appendix 2. McKay et al. 2006
Appendix 3. Johansson et al. 2007
Appendix 4. McKay et al. Submitted

Int. J. Cancer: **120,** 539–542 (2006)

Comprehensive evaluation of genetic variation in the *IGF1* gene and risk of prostate cancer

Mattias Johansson[1], James D. McKay[2,3], Pär Stattin[1], Federico Canzian[2,4], Catherine Boillot[2], Fredrik Wiklund[5], Hans-Olov Adami[5,6], Katarina Bälter[5], Henrik Grönberg[5] and Rudolf Kaaks[2,7]*

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Insulin-like growth factor-I (IGF1) stimulates cell proliferation, decreases apoptosis, and has been implicated in cancer development. Epidemiological studies have shown elevated levels of circulating IGF1 to be associated with increased risk of prostate cancer. To what extent genetic variation in the *IGF1* gene is related to prostate cancer risk is largely unknown. We performed a comprehensive haplotype tagging (HT) assessment of single nucleotide polymorphisms (SNPs) representing the common haplotype variation in the *IGF1* gene. We genotyped 10 SNPs (9 haplotype tagging SNPs (htSNPs)) within Cancer Prostate in Sweden (CAPS), a case–control study of 2,863 cases and 1,737 controls, in order to investigate if genetic variation in the *IGF1* gene is associated with prostate cancer risk. Three haplotype blocks were identified across the *IGF1* gene and 9 SNPs were selected as haplotype tagging SNPs. Common haplotypes in the block covering the 3′ region of the *IGF1* gene showed significant global association with prostate cancer risk ($p = 0.004$), with one particular haplotype giving an odds ratio of 1.46 (95% CI = 1.15–1.84, $p = 0.002$). This haplotype had a prevalence of 5% in the study population. Our results indicate that common variation in the *IGF1* gene, particularly in the 3′ region, may affect prostate cancer risk. Further studies on genetic variations in the *IGF1* gene in relation to prostate cancer risk as well as to circulating levels of IGF1 are needed to confirm this novel finding.

Key words: *IGF1*; prostate cancer; single nucleotide polymorphism; haplotype; block

Insulin-like growth factor-1 (IGF1) stimulates proliferation, decreases apoptosis, and has been implicated in cancer development by results from *in vitro* and *in vivo* studies.[1–3]

Prospective studies have consistently shown elevated circulating levels of IGF1 to be associated with several types of cancer, including prostate cancer.[4–6] Although nutrition is an important determinant of circulating IGF1, twin studies have shown that a large part of the variation in IGF1 levels is due to genetic variations,[7–9] and several single nucleotide polymorphisms (SNPs) in the *IGF1* gene have been found associated with elevated plasma levels of IGF1.[10,11] A recent nested case–control study assessed genetic variation across the *IGF1* gene and found significant association between common haplotypes and prostate cancer risk.[12]

In the present study, we used a haplotype tagging approach in order to make a comprehensive evaluation of genetic variation in the *IGF1* gene in relation to prostate cancer. Haplotype tagging SNPs were genotyped in a large case–control study, the CAPS study (Cancer Prostate in Sweden),[13] with 2,863 cases and 1,737 controls, to investigate whether common genetic variation in the *IGF1* gene influence the development of prostate cancer.

Material and methods

Study population

Cancer Prostate in Sweden (CAPS) is a population-based case–control study that has been extensively described previously.[13] In brief, cases of prostate cancer were recruited to the study in two rounds, CAPS1 and CAPS2, from 4 out of 6 regions in Sweden through a rapid ascertainment scheme at each regional oncological center between March 2001 and October 2003.

In total, 3,648 cases of prostate cancer were identified and invited to participate in the study, out of whom 3,013 (83%) agreed to participate and for 2,975 of those, a blood sample and a questionnaire concerning demographic, medical and lifestyle data were obtained.

Clinical characteristics of the tumour including local tumour stage, lymph node stage, metastasis at bone scan, tumour differentiation assessed by Gleason score and serum prostate specific antigen (PSA) level at time of diagnosis were obtained from the National Prostate Cancer Register.[14] Advanced disease was defined as local tumour stage T3 or T4, lymph node metastasis, bone metastasis or serum PSA levels above 50 ng/ml, as in previous studies from CAPS.[13]

Controls were randomly selected from the Swedish population register within groups of men matching the case distribution for age (groups of 5-year interval) and region. A total of 3,153 controls were invited to the study and 1,896 out of these men (60%) agreed to participate.

In total, there were 2,863 cases (CAPS1: 1,412, CAPS2: 1,451, mean age: 65.9) and 1,737 controls (CAPS1: 831, CAPS2: 906, mean age: 67.2) available for genotyping in this study. 1,215 cases (mean age: 67.5) were defined as having advanced disease.

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

SNP selection and haplotype block definition

As the *IGF1* gene displays a well-defined block structure with all SNPs located in regions of high linkage disequilibrium, we adopted a haplotype tagging approach based on haplotype blocks.

Abbreviations: CAPS, Cancer Prostate in Sweden; CEPH, Centre d'Etude du Polymorphisme Humain; htSNPs, haplotype tagging SNPs; IGF1, insulin-like growth factor-I; kb, kilo base pairs; LD, linkage disequilibrium; MAF, minor allele frequency; PCR, polymerase chain reaction; PSA, prostate specific antigen; SNP, single nucleotide polymorphism; UTR, untranslated region.

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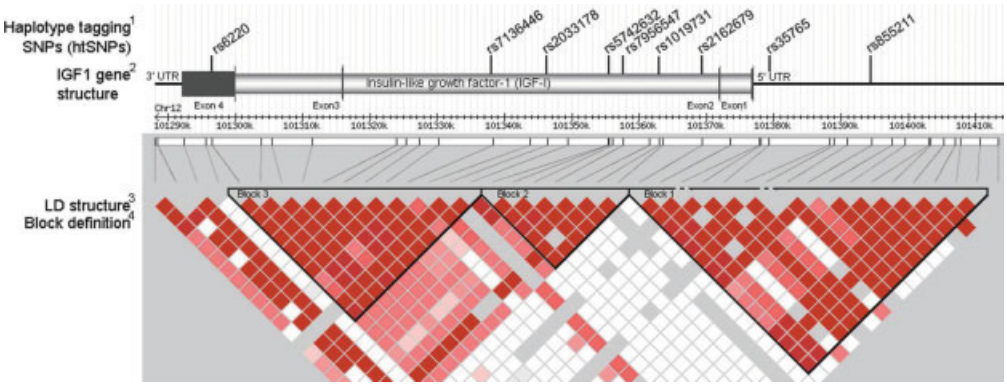

FIGURE 1 – [1]Haplotype tagging SNPs (htSNPs), see Table I for details. [2]IGF1 gene structure. Dark grey vertical lines/boxes represent exons, with the thicker lines/boxes representing translated regions. [3]LD structure of the IGF1 gene assessed with the use of HapMap phase I data. Colour intensity is proportional to LD strength between pairs of SNPs. Gray diamonds represent noninformative pairs. [4]Haplotype blocks were defined according to the criteria defined by Gabriel *et al*.,[16] using the "Haploview" software,[17] by decreasing the default maximum of strong linkage disequilibrium (LD) in informative comparisons to be a minimum of 0.9 or greater, and the confidence interval minima for strong LD to 0.6–0.9. Blocks covering regions larger than 10 kb outside genes were kept intact when they overlapped parts of the gene or the promoter region.

TABLE I – SELECTION OF HAPLOTYPE TAGGING SNPS FOR THE THREE BLOCKS COVERING THE *IGF1* GENE

SNP (rs#)	Position[1] (chromome position)	Block	MAF[2]	Global *p*-value[3,4]
rs855211	−36651 (chr 12: 101413277)	1	0.15	0.44
rs35765	−7537 (chr 12: 101384163)	1	0.1	0.26
rs2162679	2900 (chr 12: 101373726)	1	0.14	0.53
rs1019731	9734 (chr 12: 101366892)	2	0.17	0.28
rs7956547	15343 (chr 12: 101361283)	2	0.26	0.64
rs5742632	17685 (chr 12: 101358941)	2	0.25	0.78
rs2033178	27083 (chr 12: 101349543)	3	0.04	0.011
rs7136446	35644 (chr 12: 101340982)	3	0.33	0.031
rs6220	79644 (chr 12: 101296982)	3	0.27	0.17

[1]Positions (bp) are based on the initiation codone (ATG) from IGF1 genomic DNA.–[2]Minor allele frequency.–[3]Test for association between SNP variation and prostate cancer risk.–[4]Likelihood ratio test with two degrees of freedom based on conditional logistic regression.

TABLE II – FREQUENCIES FOR ALL COMMON HAPLOTYPES IN THE CAPS AND CEPH POPULATIONS

	Haplotype	CAPS[1,2]	Hapmap[1]
Block 1	GCA	0.83	0.87
	AAG	0.10	0.07
Block 2	CAA	0.55	0.57
	CGG	0.23	0.19
	AAA	0.16	0.17
	CGA	0.03	0.06
Block 3	CTT	0.57	0.54
	CCC	0.18	0.17
	CCT	0.12	0.20
	CTC	0.05	0.04
	TCC	0.05	0.05

[1]Haplotype frequencies have been estimated with the Haploview software.[15]–[2]Haplotype frequencies for the CAPS population have been estimated using the whole study population.

We identified regions with limited haplotype diversity, *i.e*. haplotype blocks, by downloading genotype data for 30 Caucasian Centre d'Etude du Polymorphisme Humain (CEPH) trios from the HapMap Phase 1 data base,[15] covering the *IGF1* gene and including 10 kb upstream and 5 kb downstream to the gene. The HapMap data was augmented with a SNP (rs6220) previously studied in our lab[11] by genotyping DNA from the same 30 Caucasian CEPH trios that were used in the HapMap project. Haplotype blocks were then identified according to the criteria defined by Gabriel *et al*.[16] using the "Haploview" software,[17] by decreasing the default maximum of strong linkage disequilibrium (LD) in informative comparisons to a minimum of 0.9 or greater and the confidence interval minima for strong LD to 0.6–0.9. Blocks covering regions larger than 10 kb outside genes were kept intact, when they overlapped parts of the gene or promoter region.

Individual haplotype frequencies were reconstructed using a partition–ligation maximum likelihood method implemented in the "tagSNPs" software.[18] The same software was also used to select haplotype tagging SNPs with the criteria $R_h^2 > 0.8$.

Genotyping

Genotyping was performed by the 5′ nuclease assay (TaqMan). Cases and controls were randomized on the polymerase chain reaction (PCR) plates in order to ensure the same study conditions for all samples. TaqMan probes were synthesized by either Applied Biosystems (MGB chemistry) or Proligo (LNA chemistry, France). Primer and probe sequences are available on request. The reaction mix included 10 ng genomic DNA, 5 pmol of each primer, 1 pmol of each probe, and 2.5 µl of 2× master mix (Applied Biosystems) in a final volume of 5 µl. The thermocycling included 50 cycles with 30 sec at 95°C followed by 60 sec at 60°C. PCR plates were read on an ABI PRISM 7900HT instrument (Applied Biosystems). Laboratory personnel were blinded to case–control status throughout the study. Genotyping call rates ranged between 95 and 99% and duplicate concordance rates were higher than 99.7%.

Statistical tests

All SNPs and haplotypes used conformed to Hardy-Weinberg equilibrium (HWE). Risk estimates were assessed with the use of conditional logistic regression,[19] conditioning on age group and geographical region, using a codominant inheritance model. As the controls were not matched on a one to one basis to cases, the 'TIES=DISCRETE option' was used in the PHREG procedure (SAS Base®) according to Allison (1999).[20]

Risk estimates for haplotypes were assessed only 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, testing all haplotypes in each block simultaneously. Homozygote carriers of the most common haplotype were held as reference and haplotypes with a frequency below 5% were grouped together. Global *p*-values for each haplotype block were assessed with the likelihood-ratio test. *p*-values were adjusted for multiple

TABLE III – *IGF1* HAPLOTYPE ASSOCIATION TESTS

Haplotype			Control frequency[1]	Case frequency[1]	OR (95% CI)[2]	*p*-value	Global *p*-value[3]
Block 1	GCA	(2 copies)	0.705	0.69	1.00 (reference)		
	GCA	(1 copy)	0.263	0.277	0.96 (0.72–1.27)	0.76	0.77
	AAG	(1 copy)	0.171	0.185	1.17 (0.88–1.56)	0.29	
	AAG	(2 copies)	0.012	0.013	1.08 (0.61–1.93)	0.79	
	<5%	(1 copy)	0.126	0.126	1.01 (0.77–1.31)	0.97	
	<5%	(2 copies)	0.003	0.003	1.33 (0.44–4.07)	0.61	
Block 2	CAA	(2 copies)	0.3	0.31	1.00 (reference)		
	CAA	(1 copy)	0.498	0.495	0.95 (0.83–1.1)	0.52	0.60
	CGG	(1 copy)	0.336	0.355	1.04 (0.9–1.2)	0.59	
	CGG	(2 copies)	0.057	0.053	0.85 (0.64–1.14)	0.28	
	AAA	(1 copy)	0.278	0.267	0.95 (0.81–1.1)	0.48	
	AAA	(2 copies)	0.029	0.022	0.72 (0.48–1.09)	0.12	
	<5%	(1 copy)	0.109	0.111	0.94 (0.76–1.16)	0.57	
	<5%	(2 copies)	0.004	0.002	0.53 (0.16–1.75)	0.3	
Block 3	CTT	(2 copies)	0.382	0.35	1.00 (reference)		
	CTT	(1 copy)	0.462	0.474	0.9 (0.77–1.06)	0.2	0.0036
	CCC	(1 copy)	0.271	0.3	1.19 (1.02–1.4)	0.03	
	CCC	(2 copies)	0.04	0.032	0.78 (0.55–1.11)	0.16	
	CCT	(1 copy)	0.202	0.218	1.19 (0.99–1.43)	0.065	
	CCT	(2 copies)	0.014	0.015	1.18 (0.68–2.05)	0.56	
	CTC	(1 copy)	0.094	0.096	1.07 (0.83–1.38)	0.59	
	CTC	(2 copies)	0.006	0.002	0.3 (0.1–0.94)	0.038	
	TCC	(1 copy)	0.078	0.106	1.46 (1.15–1.84)	0.0015	
	TCC	(2 copies)	0.003	0.002	0.82 (0.25–2.71)	0.74	
	<5%	(1 copy)	0.002	0.004	2.88 (0.59–14.04)	0.2	
	<5%	(2 copies)	0	0	–	–	

[1]Dosage estimates from the "tagSNPs" software, see statistical tests for details.–[2]Risk estimates were assessed by performing conditional logistic regression, conditioning on age and region.–[3]Global *p*-value for entire block assessed with likelihood ratio test.

testing by permutation testing, considering haplotypes from all blocks.[12] Heterogeneity in strength of effect between subgroups of localized and advanced cases was tested by performing χ^2 tests. All *p*-values are from 2-sided tests.

Results

IGF1 *haplotype and block structure*

Using the HapMap phase I data, in total 31 SNPs with a minor allele frequency (MAF) of more than 0.03, the *IGF1* gene exhibited three haplotype blocks. Block 1 covered 43 kb and contained the promoter region (5′UTR), Exons 1 and 2; Block 2 covered 8 kb of Intron 2; Block 3 contained 55 kb and Exons 3 and 4 (Fig. 1). Three SNPs per block were sufficient to represent the haplotypes predicted by HapMap genotyping by the criteria $R_h^2 > 0.8$ and were chosen as haplotypes tagging SNPs (Table I). All common haplotypes predicted using HapMap data were observed in the CAPS population (Table II). By analysing the HapMap Phase II data, with about three times the initial number of markers, we concluded that all common haplotypes were characterized by our initial SNP selection based on the HapMap phase I data, with the exception of the haplotype CAA in Block 2 that was split into two haplotypes by the SNP rs12821878.

Prostate cancer risk according to genetic variation in the IGF1 *gene*

In the haplotype risk association tests, Blocks 1 and 2 showed no association with risk, but haplotype variation in Block 3 was found to be significantly associated with prostate cancer risk (global $p = 0.004$, Table III). The TCC haplotype, with a frequency of 5%, showed an increased risk of 1.46 (95% CI = 1.15–1.84, $p = 0.002$, Table III) for heterozygote carriers. Homozygote TCC carriers were not associated with increased risk; however, homozygote carriers were rare (freq. = 0.003, Table III), and the odds ratio estimate displayed a wide confidence interval (0.25–2.71, Table III). Another, more common haplotype, CCC, also showed association with prostate cancer risk (odds ratio = 1.19, 95% CI = 1.02–1.40, Table III) but had a borderline statistical significance ($p = 0.03$, Table III). The rarer TCC haplotype may have arisen from the more common CCC haplotype; however, grouping those into a single clade did not strengthen the significance (data not shown). After adjustment for multiple testing, only the global test for haplotypes in Block 3 and the specific test for the TCC haplotype remained significant, both with *p*-values of 0.02. No evidence for heterogeneity in strength of effect was observed between subgroups of localized and advanced cases (data not shown).

One additional SNP (rs6214) was genotyped to account for some of the variation downstream of Block 3. No significant association with prostate cancer risk was observed for this SNP (data not shown).

Cheng *et al*. recently reported a significant association for several haplotypes across the *IGF1* gene with prostate cancer risk.[12] These haplotypes appeared to be correlated which indicates a single gene wide haplotype for which the SNPs rs7978742 and rs7965399 act as strong proxies. We reconstructed haplotypes across the entire *IGF1* gene using our haplotype tagging SNPs (htSNPs) and identified the haplotype tagged by rs7978742 but it was not significantly associated with prostate cancer risk (odds ratio = 1.06, 95% CI = 0.77–1.45); however, this haplotype was rare in the CAPS population with a frequency of 2%, thus limiting our power to detect a true association.

Discussion

We investigated the association between genetic variation in the *IGF1* gene and prostate cancer risk with the use of a comprehensive haplotype tagging approach in a large case control study. Haplotype variation in a block covering the 3′ region of the *IGF1* gene was significantly associated with prostate cancer risk and one specific haplotype, TCC, displayed a significant odds ratio of 1.46 for heterozygote haplotype carriers. The corresponding haplotype reconstructed from HapMap phase I data, exhibited the rare alleles on all loci and had a frequency of 5% in the study population. Although the increase in risk was only observed among heterozygote carriers, homozygote carriers were very rare, and we interpreted a dominant effect associated with the TCC haplotype. The more frequent and possibly ancestral form of the TCC haplotype,

CCC, also showed a similar tendency towards an increased risk, although the association was not as strong as for the TCC haplotypes. Both the global test for Block 3 and the haplotype specific test for the TCC haplotype remained significant after adjustment for multiple testing.

Most studies analysing genetic variation in the *IGF1* gene have not shown consistent association with prostate cancer risk in a Caucasian population, although none of these studies assessed gene wide variation.[21–24] However, a recent nested case–control study from the multiethnic cohort (MEC) assessed haplotype variation across the entire *IGF1* gene and found several haplotypes to be associated with prostate cancer risk.[12] Although the selection of htSNPs did vary between the studies making direct comparison difficult, we do not appear to replicate the specific haplotype findings from the MEC study. However, it should be noted that the gene wide risk haplotype found in their study was rare in our population, thus limiting our power to detect a true association. In parallel, the TCC haplotype found in our study was not observed with a prevalence of at least 5% in Caucasians in the MEC study. The differences in risk haplotypes may reflect the quite different nature of the study populations and/or that the underlying causative risk allele was not directly measured by either study. Nevertheless, the fact that two amply powered and comprehensive studies find associations with increased risk strengthens the importance of the *IGF1* gene in prostate cancer aetiology.

High plasma levels of IGF1 have consistently been associated with increased prostate cancer risk.[4–6] A recent breast cancer study found 5 SNPs to be associated with elevated plasma levels of IGF1,[10] and 4 of those SNPs were positioned in the region of Block 3, as defined in our study. Interestingly one of those 4 SNPs was rs6220, whose minor allele is the final C allele in the TCC and CCC risk haplotypes (Table III). In addition, we studied the SNP rs6220, in a breast cancer study[11] and also observed a significant increase in IGF1 serum levels for heterozygote allele carriers. Most recently, we have again observed this association with increased circulating IGF1 levels and rs6220 in Dutch women (Verheus, in preparation). Although these observations between SNPs and IGF1 levels have been in women, by assuming that our observed risk increase is caused by genetic effects on serum IGF1 levels, our result is compatible with the previous prospective studies' observations between prostate cancer and IGF1 level.

In conclusion, our observation of an almost 50% increase in risk of prostate cancer for a common haplotype indicate that genetic variation in the *IGF1* gene affects prostate cancer risk. The nature of the precise genetic variant driving this association is ambiguous and requires further genetic and functional studies.

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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 or in GHR knockout animal models, the consequence is 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

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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

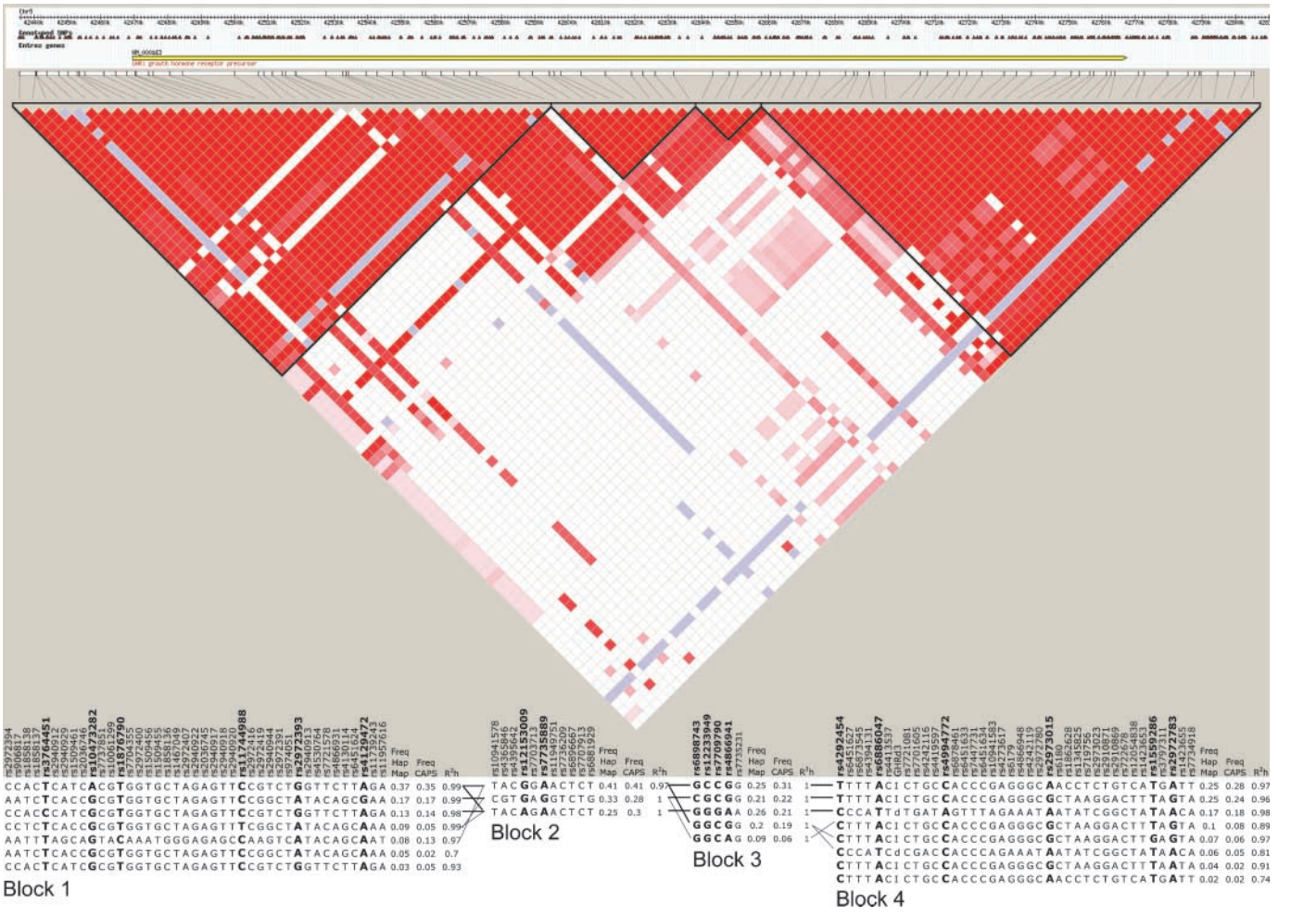

Figure 1. LD structure across the *GHR* gene. Haploview (http://www.broad.mit.edu/mpg/haploview/) display of *GHR* showing, from top to bottom: the location in base pairs of chromosome 5, the genomic structure of *GHR*, the location of 92 SNPs typed by HapMap, graphical representation of LD and block structure, the haplotypes predicted inside the blocks, with the position of htSNPs typed in this study marked in bold and the R^2_h 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*).

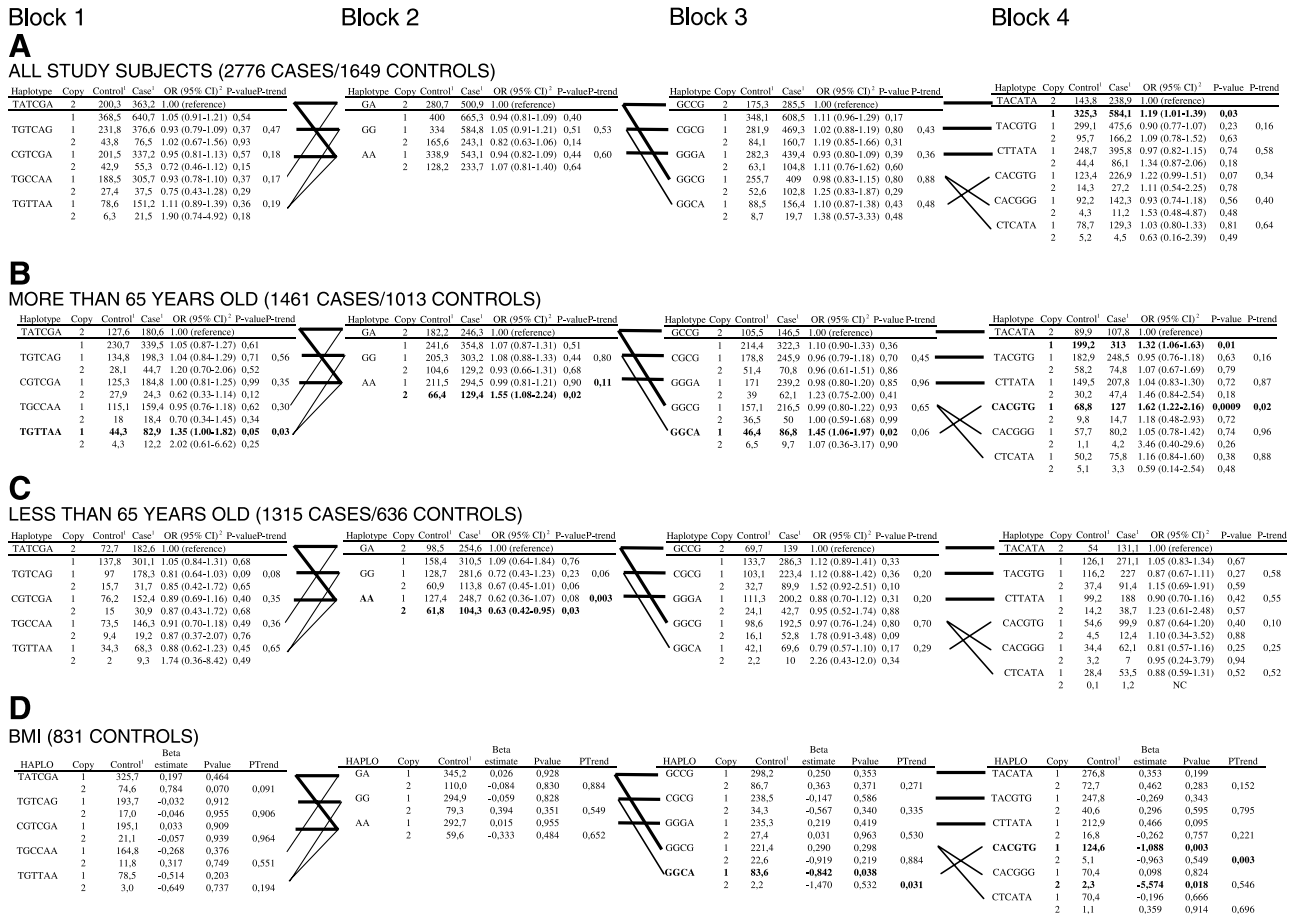

Figure 2. Haplotypic variation in the four haplotype blocks in the GHR genomic region and associations with prostate cancer risk (**A**), prostate cancer risk stratified by age (**B** and **C**), and BMI (**D**). Results that achieved an uncorrected $P < 0.05$ (*bold*). Relative degree of correlation between the haplotype blocks (*lines between the haplotypes*); haplotypes that were transmitted in >10% of cases (*bold lines*) and in >5% of cases (*thin lines*). Summed dosage estimates from the tagSNPs software (1); β estimates for BMI for a one unit change in haplotype adjusted for age (2).

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 χ^2 statistics. Relationships between haplotypic 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.

As the *GHR* gene displayed a large region with high LD and a distinct block structure, we adopted a haplotype-based tagging approach to represent genetic variation in the *GHR* gene. We selected 18 htSNPs that were able to represent

Table 1. Corrected *P* values for associations showing a *P* < 0.05 and as estimated by permutation testing

Group	Block	Haplotype (copy)	ORs	*P*	Corrected
All	4	TACATA (one copy)	1.19 (1.01-1.39)	0.03	0.74
MT65	1	TGTTAA (one copy)	1.35 (1.00-1.82)	0.05	0.88
		TGTTAA (trend)	1.34 (1.03-1.75)	0.03	0.52
	2	AA (two copies)	1.55 (1.08-2.24)	0.02	0.54
	3	GGCA (one copy)	1.45 (1.06-1.97)	0.02	0.52
	4	CACGTG (one copy)	1.62 (1.22-2.16)	0.0009	0.03
		CACGTG (trend)	1.32 (1.04-1.68)	0.02	0.36
LT65	2	AA (two copies)	0.63 (0.42-0.95)	0.03	0.52
		AA (trend)	0.75 (0.62-0.91)	0.003	0.06
BMI	3	GGCA (one copy)	−0.842	0.04	0.44
		GGCA (trend)	−0.831	0.03	0.37
	4	CACGTG (one copy)	−1.088	0.003	0.05
		CACGTG (trend)	−0.988	0.003	0.05
		CACGGG (two copies)	−5.574	0.02	0.25

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 ($D' = 1.0$; $R^2 = 1.0$), 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 (OR_{het}), 1.62; 95% confidence interval (CI), 1.21-2.16; $P_{het} = 0.0009$, $P_{trend} = 0.02$]. 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 of prostate cancer (OR_{het}, 2.74; 95% CI, 1.45-5.20; $P_{het} = 0.002$, $P_{trend} = 0.008$).

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 ($P_{trend} = 0.003$; Fig. 2). Similar with prostate cancer risk, the correlation between haplotypes in the four blocks gave an association with a decrease in BMI observed with the other haplotypes in blocks 1 (TGTTAA) and 2 (GGCA; see Fig. 2).

When analyzing the htSNPs as individual SNPs rather than haplotypes, the only result of note was rs11744988, in which carriers of the T allele had an increased risk of prostate cancer (OR, 1.25; 95% CI, 1.01-1.54). This association was again strongest in elderly cases (OR, 1.43; 95% CI, 1.08-1.89; $P_{trend} = 0.007$). rs11744988 effectively tags the TGTTAA haplotype in block 1, so this effect is also likely to be correlated with the CACGTG haplotype results in block 4 discussed above. The GHRd3 deletion proxy, rs6886047, was not associated with prostate cancer risk, or with BMI (data not shown).

We assessed the chance of obtaining these results by chance through permutation testing (see Table 1). Of 10,000 simulations, a *P* value of 0.0009 (as observed with the CACGTG haplotype risk observation) was observed 300 times ($P = 0.03$). Similarly, of 10,000 simulations, a *P* 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; $P_{het} = 0.0009$). When further stratifying the elderly cases for family history of prostate cancer, the risk associated with CACGTG increased (OR_{het}, 2.74; 95% CI, 1.45-5.20; $P_{het} = 0.002$, $P_{trend} = 0.008$), 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

(33, 34). 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 prostate intraepithelial 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.

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Implications for Prostate Cancer of *IGF1* Genetic Variation and Circulating IGF1 Levels

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Abstract

Background: Elevated levels of circulating IGF1 have consistently been associated with increased prostate cancer risk. We recently found a haplotype in the 3' region of the *IGF1* gene associated with increased risk of prostate cancer and we hypothesised that the observed association is mediated by circulating IGF1.

Material and methods: We analyzed haplotypes and 3 haplotype tagging SNPs (htSNPs) in the 3´ region of the *IGF1* gene in relation to circulating levels IGF1 in 698 control subjects from the CAPS study and 599 cases and controls from the prospective NSHDC study. We also performed a meta-analysis of these two and four other association studies on genetic variation in the 3' region of the *IGF1* gene in relation to circulating IGF1 levels.

Results: The *IGF1* haplotype previously associated with prostate cancer risk, labelled "TCC", was associated with elevated levels of IGF1 in CAPS ($p = 0.02$), but not in NSHDC. In contrast, two of the three *IGF1* htSNPs tagging this haplotype, rs6220 and rs7136446, were associated with elevated levels of IGF1 in NSHDC ($p = 0.03$ and 0.04, respectively), but not in CAPS. In the meta-analysis, the TCC haplotype and the rs6220 SNP were associated with elevated levels of circulating IGF1 ($p = 0.001$ and <0.0001, respectively).

Conclusions: Genetic variation in the 3´ region of the *IGF1* gene seems to influence circulating levels of IGF1. This observation is consistent with the hypothesis that variation in the *IGF1* gene plays a role in prostate cancer susceptibility by influencing circulating levels of IGF1.

Introduction

Insulin-like growth factor-1 (IGF1) stimulates proliferation, decreases apoptosis and has been implicated in cancer development by results from *in vitro* and *in vivo* studies (1-3). In prospective and case-control studies, elevated levels of IGF1 in circulation have consistently been associated with some malignancies, including prostate cancer (4-7). We recently found several variants in the 3' region of the *IGF1* gene associated with increased risk of prostate cancer (8). In particular one haplotype carrying the rare allele on all loci gave an increased risk of 46%. We hypothesized that a germline genetic variant causes increased *IGF1* expression and thereby elevated circulating levels of IGF1, which ultimately leads to increased prostate cancer risk. In order to investigate this hypothesis, we related the genotypes of the 3' region to plasma levels of IGF1. In addition, we performed a meta-analysis of this study and four other studies on the association between genetic variants in the 3' region of the *IGF1* gene and circulating levels of IGF1.

Subjects and methods

The Prostate CAncer in Sweden study (CAPS)

CAncer Prostate in Sweden (CAPS) is a population–based case-control study extensively described previously (9). In brief, cases of prostate cancer were recruited, in two rounds (CAPS1 and CAPS2) from four out of six regions in Sweden through a rapid ascertainment scheme at each regional oncological center between March 2001 and October 2003. In total, 2,975 cases donated a blood sample and filled out a questionnaire concerning demographic, medical and lifestyle data. Control subjects were randomly selected from the Swedish population register within groups of men matching the case distribution for age (groups of five-year interval) and residency. A total of 3,153 control subjects were invited to the study. Out of these men 1,896 (60%) agreed to participate and answered the same questionnaire as the cases. All study participants were asked to donate blood at the nearest health clinic or hospital. The samples were mailed overnight to the Medical Biobank at

Umeå University. Leukocytes, erythrocytes, plasma, and serum were separated and stored at -70°C. At the time of this study, plasma was available for the first part of the CAPS study, CAPS1, including 698 control subjects (mean age at blood draw: 69.7 years) available for plasma analysis of IGF1.

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

The Northern Sweden Health and Disease Cohort (NSHDC)

The Northern Sweden Health and Disease Cohort (NSHDC) is a long-term population-based study earlier described in detail (6). In short, since 1985, all residents of the Västerbotten County are invited to a health survey at the age of 40, 50 and 60 years. The health examination includes measurement of weight, height and blood pressure followed by a blood draw. The blood sample is fractioned into plasma, buffy coat and erythrocyte aliquots and cryopreserved at -80°C. Subjects included in the present study were originally included in a case-control study of prostate cancer nested within this cohort (6). The original study included measurements of plasma IGF1 in 281 prospective prostate cancer cases and 560 controls matched on age (6 months) and date of blood draw (2 months). In total 575 subjects were available for genotyping in the present study (mean age at blood draw: 57.8 years for cases and 58.6 years for controls) of which 239 had been included as cases in the original study.

All participants signed an informed consent form and the study was approved by the Ethical committee of Umeå University Hospital.

SNP selection, genotyping and hormone measurements

SNPs were selected using a haplotype tagging approach as previously described (8).

Genotyping was performed by the 5' nuclease assay (TaqMan) (10). Primer and probe sequences are available on request. Genotyping call rates ranged between 95% and 99% and duplicate concordance rates were higher than 99.7%. All SNPs conformed to Hardy-Weinberg equilibrium.

Measurements of plasma levels of IGF1 in subjects from CAPS were performed by an enzyme-linked immunosorbent assay (ELISA) by DSL (Diagnostic Systems Laboratories, Webster, Texas) as described previously (10). IGF1 measurements from prevalent cases were not included in the present study. The mean intra-batch coefficients of variation was 4.1%. IGF1 measurements in NSHDC were performed using double-antibody, immunoradiometric assays from Immunotech (Marseille, France) as described by Stattin et al. (6). The intra-assay coefficient of variation was 13.5%. All hormone analyses were performed at the International Agency for Research on Cancer (Nutrition and Hormones Group).

Statistical analysis of CAPS and NSHDC data.

We analyzed the relationship between IGF1 levels and polymorphic variants using linear regression models. For each SNP, a variable indicating the number of rare alleles carried by an individual was included as a covariate in the regression model. For each haplotype, two variables ("dosage variables"), ranging from 0 to 1.0, indicating the probability for carrying one or two copies of the haplotype (heterozygosity or homozygosity), were calculated using the "tagSNPs" software (11). The dosage variables were then included as covariates in the linear regression model, testing all haplotypes simultaneously, using the homozygote carriers of the most common haplotype as reference category. These statistical analyses were performed in Statistical Analysis System software (SAS Institute, Cary, USA) (12).

Meta-analysis of results from CAPS, NSHDC, including other studies

We conducted a meta-analysis to estimate the combined effect in this and previous studies on the relationship between genetic variation and IGF1 levels. Epidemiological studies published before February 2007 assessing the relationship between SNPs in the 3' region of the *IGF1* gene and circulating levels of IGF1 were included in the analysis. We searched the PubMed data base and identified two prospective breast cancer studies where the relevant SNPs had been investigated in relation to IGF1 levels (10, 13). In the first study, Al-Zahrani et al. analyzed 9 tagging SNPs within the *IGF1* gene in relation to breast cancer risk and circulating levels of IGF1 in 600 men and women within the MRC Ely study (13). In the second study, Canzian et al. (10) analyzed 5 SNPs within the *IGF1* gene and circulating levels of IGF1 in 807 cases and 1588 controls participating in the European Prospective Investigation into Cancer and Nutrition (EPIC). Canzian et al. selected SNPs because of potentially functional roles, i.e. SNPs in exons, exon-intron junctions etc. Through personal communication we also included a third study, analyzing haplotype tagging SNPs in relation to mammographic breast density as well as to circulating levels of IGF1 among 656 women participating in Prospect-EPIC, a Dutch breast cancer screening cohort, which is part of the EPIC study (Verheus et al., manuscript). Verheus et al. selected haplotype tagging SNPs with the criteria $R^2_h \geq 0.95$, and also included three additional SNPs. This resulted in 18 SNPs of which 7 were located in the region of the 3' block.

Because absolute levels of IGF1 differed substantially between studies, we calculated the within study mean difference in IGF1 levels between wild type homozygotes and heterozygotes, and between wild type homozygotes and rare type homozygotes, respectively. In the meta-analysis the estimated differences were then used to calculate the combined genotype specific effect on IGF1 levels. To investigate heterogeneity between studies Cochran's Q tests were performed. We used

the random effects model when heterogeneity was significant, otherwise the fixed effects model. To assess global significance we estimated study specific beta coefficients with corresponding confidence intervals based on the genotype specific level differences. The beta coefficients were then included in meta-analysis as described above and the resulting p-values are hereafter referred to as p-trend.

All reported p-values are two-sided. All meta-analyses were performed using the "StatsDirect" software (Cheshire, UK) (14).

Results

Haplotype tagging

In total 9 SNPs from three linkage disequilibrium (LD) blocks were selected as haplotype tagging SNPs. In the present study, only the three SNPs tagging block 3 were used as variation in this particular block was previously shown to be associated to prostate cancer (8). Details concerning *IGF1* gene structure, LD-pattern, htSNPs and haplotypes in block 3 can be seen in figure 1. We also included one additional SNP (rs2946834), located downstream of block 3, as this SNP previously has been related to elevated levels of IGF1 (13).

CAPS and NSHDC

The "TCC" haplotype, previously shown by us to be associated with increased risk of prostate cancer (8), was associated with elevated levels of IGF1 for heterozygote carriers in CAPS ($p = 0.02$), but not in NSHDC ($p = 0.12$, Table 1). The mean increase in IGF1 levels for heterozygote TCC carriers in CAPS was 25.6 ng/mL (95% CI: 4.5 - 46.8) and 18.8 ng/mL (95% CI: -4.7 – 42.2) in NSHDC.

At the level of individual SNPs, the minor alleles of SNPs rs6220, rs7136446 and rs2946834 were each significantly associated with elevated levels of IGF1 (p_{trend} = 0.03, 0.04 and 0.02) in subjects from NSHDC (Table 2). In CAPS however, only rs2946834 was significantly associated with IGF1 levels (p_{trend} = 0.02).

Meta-analysis

In the Dutch Prospect-EPIC study, Verheus et al. selected 18 SNPs of which 7 were located in the region of block 3, although they extended the block to include the SNP rs2946834 as well. The haplotype corresponding to the TCC haplotype, previously demonstrated by us to be associated with prostate cancer risk (8), was identified as TTCAGCC with a frequency of 6% in the Dutch population, compared to a frequency of 5% in the CAPS study (see Table 3). Verheus et al. also identified an additional haplotype which was not tagged by our three htSNPs, defined by the SNPs rs1520220 and rs5742714.

Verheus et al. found the TTCAGCC haplotype associated with levels of IGF1 with borderline statistical significance in comparison with all other haplotypes (p = 0.05). We also acquired data on *IGF1* genotypes and IGF1 levels from the Verheus et al. study in order to investigate the relation with the same regression model as in our study, i.e. using the most common haplotype as reference. We found a significant association for the heterozygote carriers of the TCC haplotype to levels of IGF1 consistent with our result in the CAPS study (p = 0.03). A meta-analysis of the heterozygote carriers of the TCC haplotype including the Verheus et al. result yielded a significant association with a mean increase in IGF1 levels of 11.1 ng/mL (95% CI: 4.3 – 18.0, p-value: 0.001, Figure 2). In analyses at the SNP level, Verheus et al. found, under the trend model, three SNPs significantly associated with elevated levels of IGF1, see figure 3.

Al-Zahrani et al. found five SNPs significantly associated with elevated levels of IGF1 in women, but no corresponding associations were found in men, see figure 3. Of particular interest is that four of these SNPs were located in the region of the block spanning the 3' region of the *IGF1* gene. Canzian et al. analyzed only one SNP (rs6220) within the 3' block and found it to be associated with elevated levels of IGF1 under a dominant model ($p = 0.03$), but not under a trend model ($P_{trend} = 0.17$).

Only one SNP (rs6220) was analyzed in all studies and a meta-analysis of the genotype specific IGF1 mean differences compared to the major homozygotes gave a highly significant combined result of 5.6 ng/mL increase for the heterozygotes (95% CI: 1.4 – 9.9) and 11.1 ng/mL for the minor homozygotes (95% CI: 4.3 – 17.9, $P_{trend} = <0.0001$, Figure 3). This result was similar using both random and fixed effects models. All other SNPs analyzed in more than two studies are presented in figure 3. Combining the other SNPs that were analyzed in more than two studies, yielded modest but significant associations for all SNPs in the meta-analysis. There were no evidence of heterogeneity between studies apart from the SNP rs2946834 ($p_{het} = 0.05$).

Overall we found significant associations with elevated IGF1 levels in the meta- analysis for the SNPs rs6220 ($P_{trend} = <0.0001$), rs1520220 ($P_{trend} = 0.04$), rs2033178 ($P_{trend} = 0.02$), rs7136446 ($P_{trend} = 0.01$) and rs2946834 ($P_{trend} = 0.005$), as well as for the TCC haplotype ($P = 0.001$).

Discussion

In this study, the TCC haplotype, previously related to increase prostate cancer risk, displayed a significant association with elevated levels of IGF1 within the case control study CAPS, but not in the prospective study NSHDC. In contrast, in SNP analysis three out of four SNPs displayed significant association in NSHDC, whereas only one SNP showed significant association in CAPS. In a meta-analysis of all published studies on 3' genotypes and IGF1 circulating levels, the TCC

haplotype and 5 SNPs were modestly but significantly associated with elevated levels of IGF1. These results are consistent with the hypothesis that rare alleles in the 3' region of the *IGF1* gene affects prostate cancer risk by increasing levels of circulating IGF1.

Elevated levels of circulating IGF1 have consistently been associated with increased risk of prostate cancer in prospective and case-control studies (4-7). Although nutrition strongly influences levels of IGF1 in the circulation (15, 16), twin studies have suggested that a large part of the variation is due to hereditary factors (17-19). Recently we reported a 46% increase in prostate cancer risk associated with a haplotype in a LD block spanning the 3' region of the *IGF1* gene (8). We hypothesized that the risk increase may be due to an increase in IGF1 levels caused by rare genetic variants in that region of the gene.

When we analyzed the TCC haplotype in relation to levels of IGF1, the association was significant for heterozygote carriers in both the CAPS and the Verheus et al. studies but not in the NSHDC study. Combining all three studies in meta-analysis gave a significant result overall. However, homozygote carriers did not show any association with increased levels of IGF1 in this study, nor with prostate cancer risk as reported in our previous study. Because a dose response effect on levels usually would be anticipated for increasing number of rare alleles, the lack of association among the homozygotes may indicate that the association among the heterozygotes is due to chance, or that the TCC haplotype has to be inherited with another genetic variant to produce elevated IGF1 levels. Still, no firm conclusion can be drawn regarding the homozygote TCC carriers because they were rare (0.5%) and estimates of the association displayed a wide confidence interval.

In our previous study, all three SNPs in block 3 showed borderline association with increased prostate cancer risk depending on analyzed subgroup (8). Odds ratios for individual htSNPs, previously not reported, can be seen in Table 4. Both rs2033178 and rs7136446 were significantly

associated with overall risk while rs6220 was only significantly associated with risk in advanced cases. In analysis of the SNPs in relation to IGF1 levels, none of the block 3 htSNPs showed significant association in CAPS. In contrast, both rs6220 and rs7136446 showed significant association with elevated IGF1 levels in NSHDC. In the Verheus et al. study both rs6220 and rs2033178 were significantly associated with elevated IGF1 levels. After combining the three studies in our meta-analysis, all SNPs showed a modest but statistically significant association with elevated levels of IGF1. The only SNP analyzed in all five studies, rs6220, gave a highly significant result in meta-analysis and displayed a dose response trend in IGF1 levels for increasing number of rare alleles.

Overall, the most noteworthy findings of the present study were the strong associations of the TCC haplotype and rs6220 SNP with elevated IGF1 levels. The TCC haplotype was associated with increased risk of prostate cancer in our previous study, but the rs6220 SNP was only significantly associated with prostate cancer risk when considering individuals with advanced prostate cancer.

One potential limitation in the present study is that the study groups have been selected. As noted by Reilly et al., this selection may introduce bias when estimating the genotype effect on an intermediate phenotypes – such as circulating levels of IGF1 - that the study was not originally design to investigate (20). Another important limitation is the possible existence of additional unpublished studies that we were unaware of. In addition, the inclusion of both sexes in the meta-analysis may also cause some concern since there are systematic differences between men and women in the regulation of circulating IGF1. To a large part, this can be attributed to differences in growth hormone secretion patterns, i.e. due to sexual dimorphism (21). The question is if these differences modify the effect that a germline genetic polymorphism may have on circulating levels of IGF1. The association noted in our study could be the result of increased expression/transcription of the gene, or to a coding variant that leads to a protein with longer half-life in the circulation for example. It is reasonable to assume that such an effect is present in both genders. The association between IGF1 levels and polymorphisms in the 3' region of *IGF1* gene noted in both men and women supports this assumption.

In conclusion, our study support the hypothesis that genetic variation in the 3' region of the *IGF1* gene influences levels of circulating IGF1 and therefore prostate cancer risk. Because none of the genetic variants that we investigated outshines the others in strength of association, we are unlikely to have tested the causative polymorphism. Functional studies are required to identify the causal genetic variant. This study also gives further support for the causal link between high levels of IGF1 in circulation and increased prostate cancer risk along the lines of Mendelian randomization (22).

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Figure legends

Figure 1. Structure of the IGF1 gene - Dark grey vertical lines/boxes represent exons, with the thicker lines/boxes representing translated regions - LD-pattern and definition of haplotype blocks Color intensity is proportional to LD strength between pairs of SNPs. Blue diamonds represent non-informative pairs. - Common haplotypes and frequencies based HapMap data and block 3 htSNPs used in the CAPS and NSHDC studies

Figure 2. Meta analysis of differences in IGF1 levels per 1 unit increase in dosage for heterozygote carriers of the TCC haplotype compared to homozygote carriers of the most common haplotype - 95% CI indicated by horizontal line - The size of the squares represents the weight that the corresponding study exerts in the meta-analysis. - The combined estimate is marked with an unfilled diamond that has an ascending dotted line from its upper point.

Figure 3. Meta analysis of differences in IGF1 levels per genotype compared to major homozygote for SNPs in the 3' region of the IGF1 gene - 95% CI indicated by horizontal line - The size of the squares represents the weight that the corresponding study exerts in the meta-analysis. - The combined estimate is marked with an unfilled diamond that has an ascending dotted line from its upper point.

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Tables

Table 1. Association between haplotypes and levels of IGF1

Haplotype		CAPS			NSHDC		
		Frequency[1]	Mean difference[2]	P-value	Frequency[1]	Mean difference[2]	P-value
CTT	(2 copies)	39.2%	Reference		39.3%	Reference	
CTT	(1 copy)	44.5%	-5.3 (-20.5 - 9.9)	0.50	47.2%	10.8 (-6.1 - 27.7)	0.21
CCC	(1 copy)	26.1%	3.3 (-11.6 - 18.2)	0.66	23.7%	14.7 (-2.7 - 32.2)	0.10
CCC	(2 copies)	3.4%	23.4 (-10.6 - 57.4)	0.18	1.7%	21.7 (-34.8 - 78.3)	0.45
CCT	(1 copy)	21.1%	12.9 (-4.7 - 30.5)	0.15	24.6%	-7.1 (-25.9 - 11.7)	0.46
CCT	(2 copies)	2.1%	-20.9 (-63.2 - 21.3)	0.33	1.3%	20.2 (-44.1 - 84.6)	0.54
TCC	(1 copy)	9.1%	25.6 (4.5 - 46.8)	0.02	9.9%	18.8 (-4.7 - 42.2)	0.12
TCC	(2 copies)	0.5%	-63.3 (-149.8 - 23.2)	0.15	0.6%	1.6 (-90.1 - 93.3)	0.97
CTC	(1 copy)	8.1%	7.6 (-19.4 - 34.6)	0.58	8.3%	-13.2 (-42.0 - 15.6)	0.37
CTC	(2 copies)	0.3%	20.8 (-84.8 - 126.4)	0.70	0.2%	33.8 (-124.6 - 192.1)	0.68

1) Mean haplotype dosage, 2) Mean difference in IGF1 levels [ng/mL] for 1 unit increase in haplotype dosage compared to the homozygote carriers of the most common haplotype (CTT)

Table 2. Associations between SNPs and IGF1 levels in CAPS and NSHDC

Study	SNP	Genotype			P-trend
		Homozygous major[1]	Heterozygous[1]	Homozygouse minor[1]	
NSHDC[2]	rs6220	205.4 (196.7 - 214.2)	221.4 (210.2 - 232.5)	221.2 (192 - 250.4)	0.03
	n	323	199	29	
	rs2033178	208.5 (201.3 - 215.7)	229.3 (207.2 - 251.3)	204 (124.7 - 283.4)	0.14
	n	485	52	4	
	rs7136446	202.3 (192.3 - 212.4)	217.9 (207.8 - 228.1)	218.7 (198 - 239.4)	0.04
	n	252	246	59	
	rs2946834	204.7 (195.7 - 213.8)	218.6 (208 - 229.2)	226.1 (202.9 - 249.3)	0.02
	n	305	221	46	
CAPS[3]	rs6220	186 (178.4 - 193.7)	187.6 (178.1 - 197.1)	205.7 (185.7 - 225.7)	0.16
	n	370	242	55	
	rs2033178	186.7 (180.7 - 192.7)	205.5 (186.9 - 224.1)	121.3 (35.5 - 207.2)	0.26
	n	616	64	3	
	rs7136446	183.3 (174.6 - 192)	194.1 (184.7 - 203.5)	190.9 (174.7 - 207.2)	0.19
	n	298	257	86	
	rs2946834	180.9 (172.8 - 189)	195.2 (185.7 - 204.7)	197.8 (175.3 - 220.4)	0.02
	n	325	235	42	

1) IGF1 mean values for each genotype (95% CI) [ng/mL], 2) 599 subjects, cases and controls combined, 3) 698 subjects, controls only

Table 3. Haplotype frequencies defined by htSNPs

	Haplotype ID[1]	rs9989002	rs2033178	rs7136446	rs978458	rs1520220	rs6220	rs5742714	Haplotype frequency[2]
	SNP location[3]	-23937	-27084	-35645	-71921	-77638	-79645	-84308	
Johansson et al.	CTT		C	T			T		57% (57%)
	CCC		C	C			C		18% (18%)
	CCT		C	C			T		12% (15%)
	TCC		T	C			C		5% (6%)
Verheus et al.	CTT	C	C	T	G	C	T	C	53%
	CCC[4]	T	C	C	A	G	C	G	10%
	CCC[4]	T	C	C	A	C	C	C	7%
	CCT	C	C	C	G	C	T	C	15%
	TCC	T	T	C	A	G	C	C	6%

1) Haplotype ID based on htSNP in CAPS, 2) Haplotype frequencies for the CAPS study and NSHDC study within brackets, 3) SNP location relative ATG at chromosome 12, 101376626 base pairs, 4) CAPS CCC haplotype split by rs5742714 and rs1520220

Table 4. Logistic regression of SNPs within CAPS

	all cases				localized cases				advanced cases			
SNP	cases	controls	OR[1]	P_{trend}[1]	cases	controls	OR[1]	P_{trend}[1]	cases	controls	OR[1]	P_{trend}
rs2033178				0.01				0.02				0.11
Homozygous major	2293	1509	1.00 (reference)		1264	1509	1.00 (reference)		983	1509	1.00 (reference)	
Heterozygous	278	131	1.39 (1.12-1.74)		157	131	1.44 (1.12-1.85)		117	131	1.33 (1.02-1.74)	
Homozygous minor	8	5	1.02 (0.33-3.16)		4	5	0.87 (0.23-3.34)		4	5	1.11 (0.3-4.17)	
rs6220				0.17				0.50				0.03
Homozygous major	1315	881	1.00 (reference)		745	881	1.00 (reference)		539	881	1.00 (reference)	
Heterozygous	1062	615	1.12 (0.98-1.28)		571	615	1.04 (0.89-1.22)		477	615	1.25 (1.06-1.47)	
Homozygous minor	209	136	0.96 (0.76-1.22)		108	136	0.88 (0.66-1.16)		97	136	1.11 (0.84-1.48)	
rs7136446				0.03				0.25				0.01
Homozygous major	1034	727	1.00 (reference)		581	727	1.00 (reference)		429	727	1.00 (reference)	
Heterozygous	1150	670	1.18 (1.03-1.36)		627	670	1.13 (0.97-1.33)		505	670	1.26 (1.06-1.49)	
Homozygous minor	332	186	1.21 (0.98-1.48)		175	186	1.14 (0.9-1.45)		149	186	1.32 (1.02-1.69)	

1) Odds ratios for prostate cancer risk calculated by conditional logistic regression, conditioning on age category and recidency

Figures

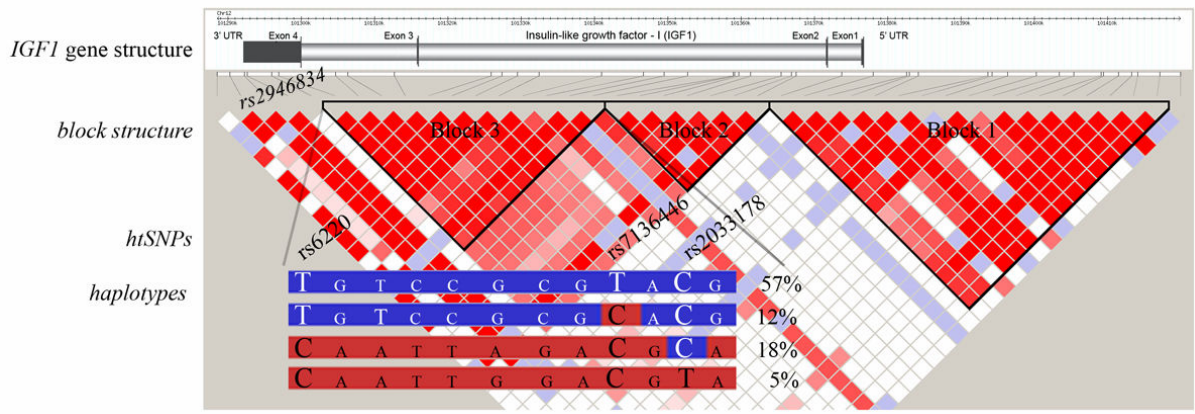


Figure 1

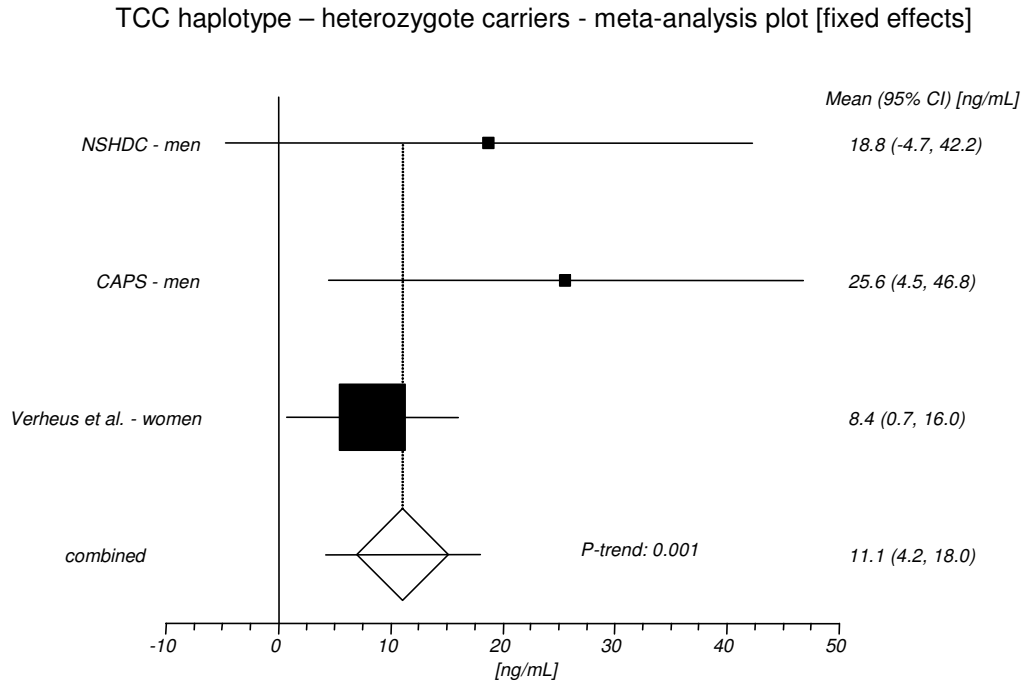
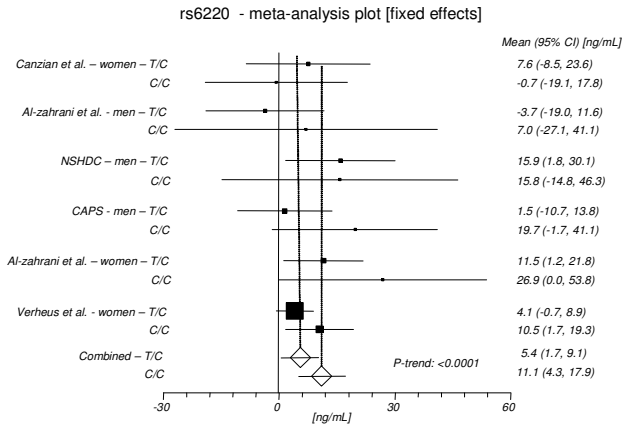
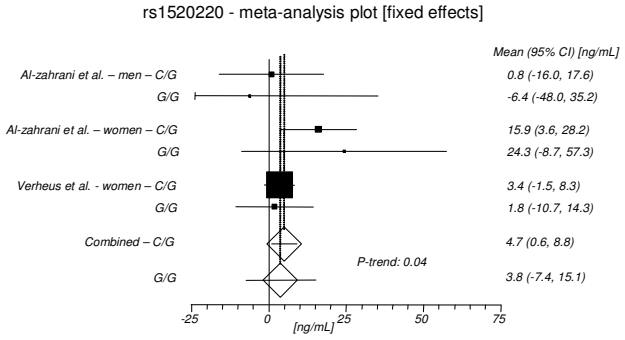
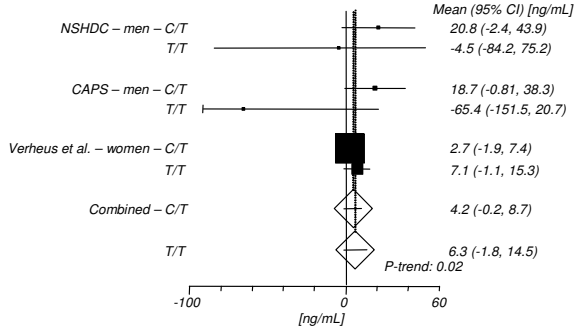
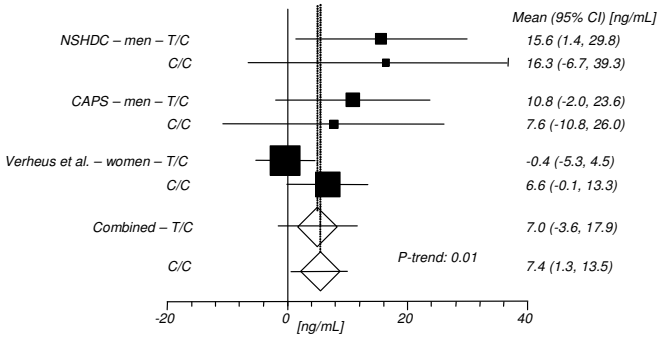


Figure 2


rs6220 - meta-analysis plot [fixed effects]
Mean (95% CI) [ng/mL]
Canzian et al. – women – T/C
7.6 (-8.5, 23.6)
C/C
-0.7 (-19.1, 17.8)
Al-zahrani et al. - men – T/C
-3.7 (-19.0, 11.6)
C/C
7.0 (-27.1, 41.1)
NSHDC – men – T/C
15.9 (1.8, 30.1)
C/C
15.8 (-14.8, 46.3)
CAPS - men – T/C
1.5 (-10.7, 13.8)
C/C
19.7 (-1.7, 41.1)
Al-zahrani et al. – women – T/C
11.5 (1.2, 21.8)
C/C
26.9 (0.0, 53.8)
Verheus et al. - women – T/C
4.1 (-0.7, 8.9)
C/C
10.5 (1.7, 19.3)
Combined – T/C
5.4 (1.7, 9.1)
C/C
11.1 (4.3, 17.9)
P-trend: <0.0001
-30
0
30
60
[ng/mL]


rs1520220 - meta-analysis plot [fixed effects]
Mean (95% CI) [ng/mL]
Al-zahrani et al. – men – C/G
0.8 (-16.0, 17.6)
G/G
-6.4 (-48.0, 35.2)
Al-zahrani et al. – women – C/G
15.9 (3.6, 28.2)
G/G
24.3 (-8.7, 57.3)
Verheus et al. - women – C/G
3.4 (-1.5, 8.3)
G/G
1.8 (-10.7, 14.3)
Combined – C/G
4.7 (0.6, 8.8)
G/G
3.8 (-7.4, 15.1)
P-trend: 0.04
-25
0
25
50
75
[ng/mL]


rs2033178 - Summary meta-analysis plot [fixed effects]
Mean (95% CI) [ng/mL]
NSHDC – men – C/T
20.8 (-2.4, 43.9)
T/T
-4.5 (-84.2, 75.2)
CAPS – men – C/T
18.7 (-0.81, 38.3)
T/T
-65.4 (-151.5, 20.7)
Verheus et al. – women – C/T
2.7 (-1.9, 7.4)
T/T
7.1 (-1.1, 15.3)
Combined – C/T
4.2 (-0.2, 8.7)
T/T
6.3 (-1.8, 14.5)
P-trend: 0.02
-100
0
60
[ng/mL]


rs7136446 - meta-analysis plot [fixed effects]
Mean (95% CI) [ng/mL]
NSHDC – men – T/C
15.6 (1.4, 29.8)
C/C
16.3 (-6.7, 39.3)
CAPS – men – T/C
10.8 (-2.0, 23.6)
C/C
7.6 (-10.8, 26.0)
Verheus et al. – women – T/C
-0.4 (-5.3, 4.5)
C/C
6.6 (-0.1, 13.3)
Combined – T/C
7.0 (-3.6, 17.9)
C/C
7.4 (1.3, 13.5)
P-trend: 0.01
-20
0
20
40
[ng/mL]

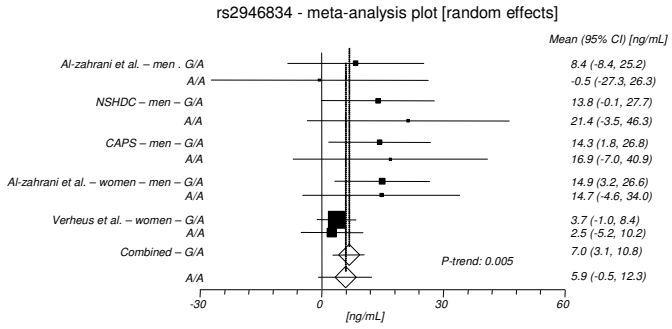

rs2946834 - meta-analysis plot [random effects]
Mean (95% CI) [ng/mL]
Al-zahrani et al. – men . G/A
8.4 (-8.4, 25.2)
A/A
-0.5 (-27.3, 26.3)
NSHDC – men – G/A
13.8 (-0.1, 27.7)
A/A
21.4 (-3.5, 46.3)
CAPS – men – G/A
14.3 (1.8, 26.8)
A/A
16.9 (-7.0, 40.9)
Al-zahrani et al. – women – men – G/A
14.9 (3.2, 26.6)
A/A
14.7 (-4.6, 34.0)
Verheus et al. – women – G/A
3.7 (-1.0, 8.4)
A/A
2.5 (-5.2, 10.2)
Combined – G/A
7.0 (3.1, 10.8)
A/A
5.9 (-0.5, 12.3)
P-trend: 0.005
-30
0
30
60
[ng/mL]

Figure 3

Genetic variation in the *SST* gene and its receptors in relation to circulating levels of IGF1, IGFBP3 and prostate cancer risk

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Key Words: *SST*, *IGF1*, IGFBP3, plasma levels, single nucleotide polymorphism, haplotype, block

Abbreviations: SST Somatostatin, SSTR Somatostatin receptor. LD linkage disequilibrium, IGF1 Insulin like growth factor GH1 growth Hormone.

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Abstract

Background: The *SST* gene and its receptors (*SSTR1-5*) may have a role in prostate cancer by influencing the IGF1 hormone axis or through direct effects on prostate epithelia. We have investigated if genetic variation in the *SST* gene or *SSTR1-5*, influences prostate cancer risk and/or circulating IGF1 or IGFBP3 hormone levels.

Material and methods: We analyzed 28 *SST* and *SSTR1-5* haplotype tagging SNPs (htSNPs) in 2863 cases and 1737 controls from the Cancer Prostate in Sweden (CAPS) study in order to investigate the association between genetic variation and prostate cancer risk using conditional logistic regression models. To investigate the genetic influence on circulating hormone levels, plasma concentrations of IGF1 and IGFBP3 were analyzed in 852 controls of the CAPS study using linear regression models and in 550 male subjects from the Northern Sweden Health and Disease Cohort (NSHDC) subjects.

Results: While no clear association between prostate cancer risk and *SST* and *SSTR* genetic variation was identified, the *SSTR5* missense SNP rs4988483 was associated with circulating IGF1 ($p=0.002$) and IGFBP3 ($p=0.0003$) hormone levels in CAPS controls under a codominant model, with a per allele decrease of approximately 8%. This decrease was replicated in NSHDC for circulating IGFBP3 ($p=0.01$), but not for IGF1 ($p=0.09$), hormone levels. Combining CAPS and NSHDC subjects indicated evidence of association between rs4988483 and both IGFBP3 ($p=2X10^{-5}$) and IGF1 ($p=0.0004$) hormone levels.

Conclusions: Our results suggest that genetic variation in the *SSTR5* gene, and particularly the rs4988483 SNP, may influence circulating IGF1 and IGFBP3 hormone levels.

Introduction

Insulin-like growth factor-1 (IGF1) stimulates proliferation, decreases apoptosis and has been implicated in cancer development by results from *in vitro* and *in vivo* studies (1-3). In prospective studies, elevated levels of IGF1 in circulation have consistently been associated with several types of cancer, including prostate cancer (4-6). We recently identified several variants in the 3' region of the gene associated with prostate risk (7). Consistent with the association between IGF1 levels and prostate cancer risk observed in prospective studies, the variants associated with prostate cancer were also associated with increased circulating plasma levels of IGF1, suggesting that the risk increase related to this variant is mediated by a genetic effect on circulating IGF1 levels (7).

Somatostatin (SST) acting through its receptors (*SSTR1-5*), has a clear role in the regulation of the Growth Hormone (GH)/IGF1 axis. *SST* expression appears to result in suppression levels of both IGF1 and GH1 (8). The *SST* and its *receptors* may therefore play a role in prostate cancer development by affecting GH and IGF1 circulating hormone levels. SST may also have a more direct role in tumorigeneis, with its normal function to directly suppress growth in normal and neoplastic cells (9).

In the present study we have investigated if genetic variation in *SST* and *SSTR1-5* influences circulating hormone plasma levels of IGF1, IGFBP3 and also prostate cancer risk.

Material and methods

Study population

CAncer Prostate in Sweden (CAPS) is a population–based case-control study that has been extensively described previously (7,10). In brief, from regional oncological centres 2,975 case patients donated a blood sample and filled out a questionnaire concerning demographic, medical and lifestyle data. Altogether, 1,896 control subjects were randomly selected from the Swedish population register within groups of men matching the case distribution for age (groups of five-year interval) and residency with a participation rate of approximately 60%. Plasma was available for the first part of the CAPS study, CAPS1, including 698 control subjects (mean age at blood draw: 69.7 years) available for plasma analysis of IGF1. Written informed consent was obtained

from all participants and the research ethical committee at the Karolinska Institutet and Umeå University Hospital approved of the study.

The Northern Sweden Health and Disease Cohort (NSHDC) is a long-term population-based study which has been extensively described before (6). Subjects included in the present study were originally used in a nested case-control study of prostate cancer (6). In total 575 subjects collected prospectively were available for genotyping in which plasma IGF1 levels have been measured in the present study (mean age at blood draw: 57.8 years for cases and 58.6 years for controls). All participants signed an informed consent form and the study was approved by the Ethical committee of Umeå University Hospital.

SNP selection, hormone measurements and genotyping

SNPs were selected using a haplotype tagging approach as described previously (7,11). Genotype data on SNPs genotyped by the HapMap consortium across the *SST* and *SSTR1 - 5* loci were obtained. Additional SNPs previously studied by our group (12), were genotyped in HapMap (CEU-CEPH) DNA and the LD-structure was analyzed together with the HapMap data. Haplotype blocks were defined using a slightly relaxed criteria compared to those described by Gabriel et al. (13). Genes *SST*, *SSTR1*, *SSTR4, SSTR5* were contained in single haplotype blocks, whereas *SSTR2* and *SSTR3* were covered by two blocks. Haplotype tagging SNPs (htSNPs) were then selected by the criteria $R^2_h > 0.8$ in each block using tagSNPs (14). *SSTR1*, *SSTR2* and *SSTR5* had "singleton" SNPs that positioned outside the haplotype blocks, and were not correlated ($r^2_S<0.8$), with any SNP inside the blocks and were thus genotyped independently. In total 34 htSNPs were selected but we were unable to design taqman assays for two SNPs in *SSTR4* (rs11696609, rs3991894) and one SNP in *SSTR5* (rs619698) due to DNA sequence complexity.

Genotyping was carried out as described previously (12) with cases and controls distributed randomly on genotyping plates and technicians were blinded to cases/control status. Genotyping call rates ranged between 92% and 99% and genotype concordance rates were higher than 99.7% in duplicate samples.

Measurements of plasma levels of IGF1 in subjects from CAPS were performed at the International Agency for Research on Cancer by an enzyme-linked immunosorbent assay (ELISA) by DSL (Diagnostic Systems Laboratories, Webster, Texas) as described previously (12). The mean intra-batch coefficient of variation was 4.1%.

IGF1 measurements in NSHDC were performed using double-antibody, immunoradiometric assays from Immunotech (Marseille, France) as described previously (6). The intra-assay coefficient of variation was 13.5%.

Statistical analysis of CAPS and NSHDC data.

We investigated the relationship between polymorphic variants and hormone levels using linear regression models adjusting for age. In analyses of prostate cancer risk, conditional logistic regression models were used. For each SNP, a variable indicating the number of rare alleles carried by an individual was included as a covariate in the appropriate regression model, thus creating a co-dominant model. Haplotype dosages were calculated using tagSNPs (14) to indicate each subjects probability of being heterozygote (B-statistic) or homozygote (C-statistic) for each haplotype. The tagSNPs dosage variables were then implemented as covariates in the appropriate regression model keeping homozygotes for the most common haplotype as reference category. These statistical analyses were performed in Statistical Analysis System software (SAS Institute, Cary, USA). All tests were two tailed and statistical significance was considered at 0.05.

Because the recruitment of subjects in the CAPS and NSHDC studies differed, we combined the results between genetic variants and hormone levels by pooling the estimates from each study group. The pooled estimate was assessed as a weighted mean with weights calculated as the inverse of the study specific variance. To investigate heterogeneity between studies, Cochran's Q tests were performed. We used the random effects model when heterogeneity was significant, otherwise the fixed effects model. This analysis was performed using the "StatsDirect" software (Cheshire, UK).

Results

The genotype distributions for three SNPs rs10513817 (*SST*), rs3746726 (*SSTR4*) and rs213654 (*SSTR5*) deviated significantly (p=0.008, 0.002, 0.0004, respectively) from that expected by Hardy-Weinberg equilibrium (HWE) in the control population, but not in cases. These deviations from HWE complicates interpretation of the results of these SNPs and are not discussed further here.

Genetic variation and circulating of IGF1 and IGFBP3 hormone levels

Associations between genetic variation in the *SST* gene and its receptors and hormone levels in CAPS controls are outlined in Table 1. In the *SSTR5* gene, the rs4988483 SNP was associated with a decrease in both circulating IGF1 and IGFBP3 hormone levels among CAPS controls. This effect appeared to be most consistent with a codominant mode of inheritance, with an approximate 8% per allele decrease in IGFBP3 levels (p=0.0003) and IGF1 levels (p=0.002). We attempted to confirm this observation in the independent NSHDC study. In NSHDC rs4988483 was again significantly associated with a decrease in circulating IGFBP3 levels (p=0.01), but not with IGF1 hormone levels (p=0.09) (Figure 1). Combining the CAPS (controls) and NSHDC studies indicated consistent evidence for association between rs4988483 and circulating IGFBP3 levels ($p=2x10^{-5}$) and IGF1 levels (p=0.0004) (Figure 1).

Genetic variation and prostate cancer risk

Overall, no clear associations between genetic variants of the *SST* and *SSTR* genes and prostate cancer risk were observed (see Table 1). The rs4988483 SNP that was associated with hormone levels, was not associated with prostate cancer risk. In *SSTR2*, two haplotypes were modestly associated with prostate cancer risk, with heterozygote carriers of the GGT haplotype in block 1 (located directly proximal to *SSTR2)* displaying an OR of 1.29 (95% CI: 1.07-1.50) and for a second haplotype, AGC, located in block 2 (covering the coding region of *SSTR2),* heterozygotes had an OR of 0.77 (95% CI: 0.62-0.95). These associations were unchanged when adjusting one for the other, implying independence. The associations between these *SSTR2* haplotypes were both more prominent in individuals with younger age of diagnosis (less than 65 years of age), with heterozygote carriers of the GGT and AGC haplotypes displaying OR's of 1.58 (95% CI: 1.18-2.12, p=0.002) and 0.54 (95% CI: 0.39-0.76, p=0.0003), respectively. Evidence for heterogeneity between the risk estimates for the <65 and >65 years age of diagnosis' was present for the AGC (p=0.004) but not GGT (p=0.09). One *SSTR1* haplotype was associated with increased risk, with homozygote carriers having an RR of 1.41 (95% CI: 1.07-1.87, p=0.02), although this association was not more prominent in any sub analysis.

Discussion

Heritability studies suggest that 40-60% of the variation in circulating levels of IGF1 and IGFBP3 hormones is genetically determined (15,16). While the *IGF1* and *IGFBP3* genes are the most obvious candidates to account for this variability (7,17), other members of the GH1/IGF1 axis are also candidates to influence circulating IGF1 and IGFBP3 hormone levels.

Increased *SST* expression appears to result in suppression of both GH1 and IGF1 circulating levels (9), suggesting that genetic variation causing over transmission of the SST signal would also lower IGF1 and IGFBP3 hormone levels. In this a large scale investigation of common genetic variation in the *SST* and the *SST receptor* genes in the homogenous Swedish population, one SNP in the *SSTR5* gene resulted in a per allele decrease of approximately 8% in circulating IGFBP3 hormone levels. It is not clear if this particular SNP is causative or if it is in LD with a true causative allele. The rs4988483 SNP encodes a missense change in the SSTR5 protein (M48L), however the consequence of this change is not predicted to be damaging to function based on evolutionary conservation (by SIFT (http://blocks.fhcrc.org/sift/SIFT.html) (18). The literature on the relation between *SSTR5* and circulating IGF1 and IGFBP3 hormone levels are limited. In a previous study on women, we found no significant association for the rs4988483 SNP in relation to levels of IGFBP3 (12), possibly indicating sexual dimorphism.

Overall, there were no striking association between genetic variation in the *SST* or the *SSTR's* genes and prostate cancer risk. Prospective studies suggest positive association between cancer risk (including prostate cancer) and circulating IGF1 hormone levels, and perhaps IGFBP3 hormone levels (6,19). Along the lines of mendelian randomization (20), a SNP associated with a decrease in circulating IGF1/IGFBP3 levels may translate into a decreased risk of developing prostate cancer. We found no significant association between rs4988483 and prostate cancer risk, although our statistical power to detect an effect from a relative modest change in hormone levels was limited. Some evidence for association was present in *SSTR2*. The modest nature of these associations with prostate cancer suggests that they may have arisen by chance. Nevertheless, it is interesting that the associations of both haplotypes associated with risk appear independent from one another, and that they are pronounced in cases with earlier age of diagnosis, consistent with the notion of genetic susceptibility being more relevant to cancer of an earlier age at diagnosis.

In conclusion, genetic variation in the *SSTR5* gene may explain some of the inherited variability of circulating IGF1 and IGFBP3 hormone levels, but these associations does not seem to translate into prostate cancer risk. Further independent studies of the rs4988483 SNP in relation to IGF1 and IGFBP3 hormone levels are warranted to confirm this finding.

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