Null Results in Brief

Insulin Resistance–Related Gene Polymorphisms and Risk of Prostate Cancer

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Introduction

Insulin resistance and hyperinsulinemia have been hypothesized to increase the risk of prostate cancer (1-3). Insulin has mitogenic and antiapoptotic activity and may exert these properties directly on prostate epithelial cells (4). In addition, hyperinsulinemia may affect prostate cancer risk by increasing levels of free (bioactive) insulin-like growth factor-I (IGF-I) or testosterone (3). In previous investigations, prostate cancer risk was positively related to serum insulin levels (2) and also to the metabolic syndrome (5, 6).

Several genetic variants within the insulin signaling pathway have been associated with insulin resistance and hyperinsulinemia (7-10). Polymorphisms of the insulin (*INS*) and insulin receptor substrate-1 (*IRS1*) genes have also been associated with prostate cancer (11-13), but data are inconsistent. In addition, no studies have evaluated prostate cancer risk in relation to genetic variation of the insulin receptor (*INSR*)—a pivotal component of the insulin signaling pathway. We therefore examined the association of prostate cancer with common variants of the *INS, INSR, IRS1*, and *IRS2* genes in a large cohort of Caucasian men.

Materials and Methods

Study Population. The study sample comprised 1,053 case/control pairs from the Alpha-Tocopherol, Beta-Carotene (ATBC) Cancer Prevention Study, a cohort of 29,133 men aged 50 to 69 years residing in southwestern Finland who smoked at least 5 cigarettes/day and gave informed consent (14, 15). The study was approved by the institutional review boards of the National Public Health Institute of Finland and the National Cancer Institute.

Cases were individuals with incident prostate cancer (International Classification of Diseases 9, code 185) diagnosed by April 30, 2003, and identified through the Finnish Cancer Registry, which provides almost 100% case coverage (16). DNA was successfully extracted from whole blood for 980 cases and 876 controls. For cases identified through April 1999, medical records were reviewed centrally by two oncologists for staging. Prostate cancer stage was available for 592 of the cases with successful DNA extraction; 408 cases (69%) were stages 0 to 2, and 184 cases (31%) were stages 3 and 4 (17). Controls were subjects alive at the time of case diagnosis and were matched to the cases on age (±5 years), treatment assignment, and date of baseline serum blood draw (±30 days).

Single Nucleotide Polymorphism Selection and Genotyping. Single nucleotide polymorphisms (SNP) were selected using the public databases dbSNP[7] and SNP-500[8] and via a literature search on *INS, INSR, IRS1*, and *IRS2*. Criteria for inclusion were a minor allele frequency >5% in Caucasian individuals and potential functionality, e.g., SNPs in exons, exon/intron boundaries, putative regulatory regions, or association with insulin resistance or related outcomes in previous studies (7). Polymorphic loci identified in this manner were verified in a panel of 102 individuals (SNP-500 population; ref. 18). This led to the selection of one SNP in *INS*, five SNPs in *INSR*, three SNPs in *IRS1*, and one SNP in *IRS2* (Table 2). Following this initial selection, we examined *INSR* more comprehensively by resequencing every 5 to 10-kb region around a SNP across the *INSR* gene region. This resulted in the identification of an additional 34 SNPs with minor allele frequencies exceeding 5% in Caucasians. Using the approach developed by Clayton et al. (htSNP2 software[9]), we identified 11 haplotype-tagging SNPs in *INSR* (including the original five) which predicted the 39 common SNPs among the SNP-500 Caucasian population with high probability ($R^2_H = 0.90$).

Genotyping was done at the Core Genotyping Facility of the Division of Cancer Epidemiology and Genetics, National Cancer Institute, using TaqMan (Applied Biosystems).[10] For validation purposes, TaqMan assays were initially applied to the 102 SNP-500 individuals (18) with sequence information; TaqMan results were 100% concordant. To assess quality control, duplicate masked specimens for 120 control samples

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[7] http://www.ncbi.nlm.nih.gov/SNP/
[8] http://snp500cancer.nci.nih.gov/
[9] http://www.gene.cimr.cam.ac.uk/clayton/software/
[10] Protocols for each specific assay are available at http://snp500cancer.nci.nih.gov (18).

were genotyped with 100% concordance. Departures from Hardy-Weinberg equilibrium were tested for each SNP using genotype distributions among the control participants.

Statistical Analysis. To assess the association of genotypes and haplotypes with prostate cancer risk, we used unconditional logistic regression, with the most common genotype/haplotype serving as reference. Tests of trend were calculated using ordinal values of 1, 2, and 3 assigned to genotypes in order of homozygous for common allele, heterozygous, and homozygous for the rare allele, respectively. Haplotype blocks were specified among controls according to the method of Gabriel et al. (19), and haplotype frequencies were estimated using the expectation-maximization (EM) algorithm (20). We found little phase ambiguity in the reconstruction of haplotypes. All analyses were adjusted for age and treatment assignment. We also conducted conditional logistic regression models but found no substantive differences from unconditional models. Separate analyses were conducted for advanced cancers (stages 3 and 4).

Results

Cases were slightly older and more likely to have a positive family history of prostate cancer than controls but were similar with respect to body mass index (BMI), physical activity, and smoking history (Table 1).

All genotypes were distributed in accordance with the Hardy-Weinberg equilibrium except for *INSR* Ex3+131C>T ($P = 0.02$). We found no association for any of the genotypes of *INS*, *INSR*, *IRS1*, and *IRS2* with risk of prostate cancer (Table 2). Haplotype analysis for the *INSR* and *IRS1* genes did not yield statistically significant findings (data not shown). In analyses restricted to advanced cases (stages 3 and 4), we found that carriers of the C allele at the *INSR* IVS7-126C>T locus had a 34% reduced risk of prostate cancer [OR, 0.69; 95% confidence interval (95% CI) 0.50, 0.96; $P = 0.03$] compared with men homozygous for the T allele. In a

Table 1. Baseline characteristics of the study population

Characteristic	Cases ($N = 975$)	Controls ($N = 871$)	P*
Age (y)	58.5	58.0	0.06
Family history of prostate cancer (%)	56 (6.6)	24 (3.1)	<0.01 †
BMI (kg/m^2)	26.1	26.2	0.72
Vigorous activity during leisure time (%)	193 (19.7)	202 (23.1)	0.07
Smoking status			
Years of smoking	36.3	36.6	0.67
Cigarettes per day	19.4	19.1	0.59 †
α-Tocopherol grp (%)	449 (45.8)	399 (46.1)	0.93 †
β-Carotene grp (%)	488 (50.1)	438 (50.3)	0.93 †
History of diabetes (%)	30 (3.1)	21 (2.4)	0.38 †
Histopathologic grade ‡			
1	140 (14.4)		
2	295 (30.3)		
3	135 (13.9)		
4	0 (0)		
Tumor-node-metastasis stage (%) ‡			
0	47 (4.8)		
1	138 (14.0)		
2	225 (22.9)		
3	79 (8.1)		
4	104 (10.7)		

*P values derived from the Wilcoxon signed-rank sum test unless otherwise indicated.
† P derived from the χ^2 test.
‡ Due to missing data, numbers may not add to 100%.

Table 2. Distribution of insulin-related genotypes and the OR of total prostate cancer

Genotype	Cases, n (%)	Controls, n (%)	OR* (95% CI)
Insulin gene (*INS*)			
IVS1-6A>T; rs689			
AA	588 (61.1)	525 (62.6)	1.0 (ref)
AT	325 (33.7)	278 (33.2)	1.04 (0.85-1.27)
TT	50 (5.2)	35 (4.2)	1.27 (0.81-1.99)
P for T carrier			0.50
P_{trend}			0.35
Insulin receptor gene (*INSR*)			
2,853 bp 3′ of STP G>T; rs1864193			
GG	708 (73.6)	598 (71.5)	1.0 (ref)
GT	223 (23.2)	220 (26.3)	0.85 (0.69-1.06)
TT	31 (3.2)	18 (2.2)	1.45 (0.80-2.62)
P for T carrier			0.31
P_{trend}			0.66
Ex22-326A>G; rs1051690			
GG	563 (59.2)	526 (62.7)	1.0 (ref)
AG	340 (35.8)	280 (33.4)	1.14 (0.93-1.39)
AA	48 (5.0)	33 (3.9)	1.36 (0.86-2.15)
P for A carrier			0.13
P_{trend}			0.09
Ex17-4C>T; rs1799817			
CC	624 (65.1)	547 (65.0)	1.0 (ref)
CT	288 (30.1)	261 (31.0)	0.96 (0.78-1.18)
TT	46 (4.8)	33 (3.9)	1.18 (0.74-1.87)
P for T carrier			0.89
P_{trend}			0.88
IVS14+88A>G; rs2860175			
GG	669 (70.8)	610 (72.6)	1.0 (ref)
GA	261 (27.6)	210 (25.0)	1.14 (0.92-1.41)
AA	15 (1.6)	20 (2.4)	0.69 (0.35-1.35)
P for A carrier			0.37
P_{trend}			0.64
IVS10+34A>G; rs3745548			
GG	855 (89.1)	759 (89.2)	1.0 (ref)
AG	102 (10.6)	90 (10.6)	1.00 (0.74-1.35)
AA	3 (0.3)	2 (0.2)	1.36 (0.23-8.19)
P for A carrier			0.94
P_{trend}			0.90
IVS8-20A>G; rs2245648			
AA	726 (76.2)	617 (72.3)	1.0 (ref)
AG	211 (22.1)	215 (25.2)	0.83 (0.67-1.03)
GG	16 (1.7)	21 (2.5)	0.65 (0.34-1.26)
P for G carrier			0.06
P_{trend}			0.04
Ex8+40G; rs2059806			
GG	493 (51.0)	446 (52.3)	1.0 (ref)
AG	391 (40.5)	323 (37.9)	1.09 (0.90-1.33)
AA	82 (8.5)	83 (9.7)	0.90 (0.65-1.26)
P for A carrier			0.57
P_{trend}			0.96
IVS7-126C>T; rs3815901			
TT	339 (35.6)	271 (32.1)	1.0 (ref)
TC	427 (44.9)	405 (48.0)	0.84 (0.68-1.04)
CC	185 (19.5)	167 (19.8)	0.89 (0.68-1.16)
P for C carrier			0.13
P_{trend}			0.27
Ex3+131C>T; rs891087			
CC	814 (84.2)	733 (85.8)	1.0 (ref)
CT	143 (14.8)	111 (13.0)	1.16 (0.89-1.51)
TT	10 (1.0)	10 (1.2)	0.91 (0.38-2.20)
P for T carrier			0.33
P_{trend}			0.42
IVS2-15330C>G; rs1035940			
CC	618 (64.6)	513 (60.9)	1.0 (ref)
CG	296 (31.0)	291 (34.6)	0.84 (0.69-1.03)
GG	42 (4.4)	38 (4.5)	0.91 (0.58-1.44)
P for G carrier			0.10
P_{trend}			0.15
IVS2+5915A>G; rs919275			
AA	449 (47.2)	398 (46.8)	1.0 (ref)
AG	423 (44.4)	358 (42.1)	1.05 (0.86-1.28)
GG	80 (8.4)	94 (11.1)	0.75 (0.54-1.04)
P for G carrier			0.90
P_{trend}			0.33

(*Continued on the following page*)

Table 2. Distribution of insulin-related genotypes and the OR of total prostate cancer (Cont'd)

Genotype	Cases, *n* (%)	Controls, *n* (%)	OR* (95% CI)
Insulin receptor substrate 1 gene (*IRS1*)			
IVS1+12345G>C; rs1366757			
GG	827 (85.6)	742 (86.8)	1.0 (ref)
GC	130 (13.5)	108 (12.6)	1.09 (0.83-1.43)
CC	9 (0.9)	5 (0.6)	1.59 (0.53-4.78)
P for C carrier			0.44
P_{trend}			0.37
IVS1+12245G>C; rs1820841			
CC	811 (84.5)	720 (85.4)	1.0 (ref)
CG	139 (14.5)	118 (14.0)	1.05 (0.81-1.37)
GG	10 (1.0)	5 (0.6)	1.78 (0.61-5.25)
P for G carrier			0.55
P_{trend}			0.43
IVS1+4357G>A; rs9282766			
GG	724 (77.4)	637 (76.3)	1.0 (ref)
AG	194 (20.7)	188 (22.5)	0.91 (0.73-1.15)
AA	18 (1.9)	10 (1.2)	1.50 (0.69-3.29)
P for A carrier			0.61
P_{trend}			0.86
Insulin receptor substrate 2 gene (*IRS2*)			
IVS1+11858A>G; rs2241745			
AA	701 (73.3)	616 (72.5)	1.0 (ref)
AG	236 (24.7)	215 (25.3)	0.97 (0.78-1.20)
GG	19 (2.0)	19 (2.2)	0.85 (0.44-1.62)
P for G carrier			0.68
P_{trend}			0.61

*Adjusted for age at randomization and treatment group.

secondary analysis, the reduction in advanced disease risk was primarily among men with a BMI > 25 kg/m^2 (OR, 0.47; 95% CI, 0.31, 0.72; $P = 0.0005$).

Discussion

Overall, our findings suggest that there is little association between the polymorphisms of the *INS*, *INSR*, *IRS1*, and *IRS2* genes studied here and the risk of prostate cancer. The statistical power was sufficient (>0.80) to detect an odds ratio (OR) of ≥1.5 for all SNPs under the assumption of a dominant mode of inheritance. Given that our study included a large, well-defined sample of prostate cancer cases and controls, it is unlikely that our null findings are due to chance, although we cannot exclude the possibility of undetected weaker associations (OR < 1.5).

One possible exception to our null findings is a lower risk of advanced prostate cancer among carriers of the C allele at the *INSR* IVS7-126C>T locus ($P = 0.03$). However, using either the false discovery rate (21) or the Bonferroni correction method and assuming two or more statistical tests, the *P* value for the association would need to have been 0.025 or lower to be considered statistically significant.

Insulin resistance and compensatory hyperinsulinemia have been hypothesized to promote prostate carcinogenesis through either the direct promitotic/antiapoptotic properties of insulin or via alterations in the IGF and/or sex hormone pathways. In prior studies, the *INS* IVS1-6T/T variant has been found to predict insulin levels and type II diabetes (7, 22) and also prostate cancer (11). However, our study and other studies did not confirm the latter association (12, 23). One investigation reported an association between the *IRS1* G972R polymorphism and prostate cancer (12), but a subsequent study was null (23). The *IRS1* IVS1+12245 SNP, which is in moderate linkage disequilibrium with *IRS1* G972R,[11] was not associated with prostate cancer risk in this population.

In conclusion, this large case-control study found little evidence for an association between allelic variants in insulin resistance–related genes and risk of prostate cancer.

[11] See http://snp500cancer.nci.nih.gov/.

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