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NO ASSOCIATION BETWEEN A POLYMORPHIC VARIANT OF THE IRS-1 GENE AND PROSTATE CANCER RISK

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Abstract

Objective—Insulin receptor substrate-1 (IRS-1) acts as a docking protein between the insulin-like growth factor-1 (IGF-1) receptor and intracellular signaling molecules in the IGF-1 signaling pathway. Accumulating data support a role of IGF-1 in prostate carcinogenesis. We assessed the influence of the most common IRS-1 gene polymorphism (Gly972Arg) on prostate cancer risk, alone and in combination with IGF-1 and other components in the IGF-1 signaling pathway.

Material and Methods—In a nested case-control study within the Physicians' Health Study, the IRS-1 polymorphism was assayed from prospectively collected samples from 564 incident prostate cancer cases and 758 controls matched on age and smoking. We calculated relative risks (RR) and 95% confidence intervals (CI) using conditional logistic regression.

Results—Among the controls, 0.8% were homozygous (AA) and 12% were heterozygous (GA) for the polymorphic allele. There was no association between carriage of the A allele and total prostate cancer risk (RR=1.1 95% CI=0.8-1.5), advanced disease (stage C or D or lethal prostate cancer, RR=1.3 95% CI=0.8-2.3), or plasma IGF-1 levels. We explored possible interactions with body mass index and components in the IGF-1 pathway including IGFBP3, PI3k and PTEN but none of these factors influenced the relation between IRS-1 genotype and prostate cancer risk.

Conclusions—Our data do not support an association between carriage of the variant IRS-1 gene and prostate cancer risk.

Keywords

prostate cancer; IRS-1 genotype; IGF-1 pathway

INTRODUCTION

Accumulating data support a role of insulin-like growth factor 1 (IGF-1) in prostate carcinogenesis (1,2). Insulin receptor substrate-1 (IRS-1) acts as a docking protein between the insulin-like growth factor-1 receptor (IGF-1R) and intracellular signalling molecules in

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the IGF-1 signalling pathway. For example, IRS-1 plays a key role in activating the phosphoinositol-3-kinase (PI3K) cascade, which controls IGF-1 mediated cell growth and survival (3). This potentially cancer-promoting pathway is normally controlled by tumour inhibiting protein PTEN. *In vitro* studies have demonstrated that the most common polymorphism in the IRS-1 gene (Gly972Arg) imparts impaired insulin-stimulated signaling, especially along the PI 3-kinase pathway (4,5). This polymorphic allele is also related to higher fasting insulin levels (6) and type II diabetes (7). Based on these observations, one may hypothesize that carriage of IRS-1 Gly972Arg alters the risk for prostate cancer. However, prior studies are inconsistent; one demonstrated an almost 3-fold risk increase among carriers of the polymorphism compared to non-carriers (8), but another showed no association (9). The goal of the present study was to further investigate the IRS-1 polymorphism in a large nested case-control study and to explore the possible joint associations of several components in the complex IGF-1 pathway, including IGFBP3, and polymorphic variants of PI3k and PTEN.

MATERIAL AND METHODS

We performed a nested case-control study within the Physicians' Health Study (PHS) (10) (11), a randomized placebo-controlled trial of aspirin and beta carotene conducted among 22,071 male physicians, aged 40-84 years at study entry in 1982. For the present study, we identified all incident cases of prostate cancer that occurred among the 14,916 PHS participants for whom blood specimens were collected at baseline. All participants received blood kits with instructions to have their blood drawn into vacutainer tubes containing EDTA. The samples were centrifuged, sent over night in cryopreservation vials with cold packs, to the laboratory, where the samples were separated into aliquots and stored at -82°C . Precautions were taken to prevent thawing or warming of specimens during storage. All participant provided written consent and the Internal Review Board of the Brigham and Women's Hospital approved the PHS.

When a participant reported a new diagnosis of prostate cancer, medical records were requested and reviewed by an endpoints committee of physicians. We defined advanced-stage tumours as stage C (extraprostatic, but no evidence of distant metastases) or stage D (distant metastatic or fatal) at diagnosis; early stage tumours were defined as stage A (asymptomatic, incidentally detected lesions) or stage B (palpable tumour confined to the prostate gland). We also examined high-grade (Gleason score of ≥ 7) and low-grade (Gleason score of < 7) tumours separately.

For each prostate cancer case, we randomly selected one or two controls from the pool of men who gave blood at baseline who had (i) not undergone prostatectomy or (ii) did not report a diagnosis of prostate cancer at the time the diagnosis was reported by the case subject. The 758 controls were individually matched to the cases by age (± 1 year for men ages ≤ 55 and ± 5 years for men ages > 55 years) and smoking status (never, former, or current). Vital status was assessed for the participants at the end of follow-up in 2002. Coded whole blood samples from cases and matched controls were sent to the laboratory at the Dana Farber Cancer Institute, with the laboratory investigators blinded to the name and case/control status of each sample. Genomic DNA was obtained from 500 μL of the thawed whole blood using a commercially available kit (QIAamp DNA extraction kit, QIAGEN, Chatsworth, CA, USA). DNA concentration and purity were determined by UV absorbance on a Beckman DU640 spectrophotometer. Each sample was diluted to a final concentration of 20 ng/ μL and stored at -20°C until analysis.

We genotyped the G972R polymorphism for 564 prostate cancer cases that had accumulated during follow-up through 1995 and their matched cancer-free controls (1:3 ratio). The

G972R polymorphism was identified with a PCR amplification followed by digestion with BstN1. The PCR reaction mixture contained 1 μ M each primer (sense primer 5'-CTCACCTCCTCTGCAGCAATG-3', antisense primer 5'-AGGAGAGCACTGGGGTCGAGAT-3'), 0.25 μ M each dNTP, PCR buffer, 40 nG of genomic DNA, and 1 unit of Taq® polymerase (Perkin Elmer) in a volume of 22 μ l. Reactions were carried out using a Perkin Elmer 9600 thermocycler. The mutant allele introduces a BstN1 restriction site which can be used to rapidly genotype patients for this single nucleotide polymorphism. The genotyping methods for PTEN, and PI3K have been described previously (12,13). Plasma samples were assayed for IGF-1 and IGFBP3 by use of enzyme-linked immunosorbent assays (ELISA), with kits provided by Diagnostics Systems Laboratory (Webster, TX) (1).

To evaluate the association between IRS-1 Gly972Arg genotype and risk of prostate cancer, we used conditional logistic regression models to calculate odds ratios and 95% confidence intervals as estimates of relative risks. Because the very low frequency of the homozygous variant AA genotype (<1%), we used a dominant model of inheritance, and homozygous mutants were combined with the heterozygous group. We compared the mean values of IGF-1 and IGFBP3 by the A allele carriage status, adjusted for age (centered at 60 years) and assay batch. To evaluate potential joint effects of IRS-1 Gly972Arg and other selected factors involved in the IGF-1 signalling pathway (IGF-1, IGFBP3, PI3k and PTEN), we cross-classified IRS-1 genotype by tertiles of IGF-1, IGFBP3, and BMI, according to the control distribution, as well as by PI3K and PTEN genotypes. To assess statistical significance of the gene-gene and gene-plasma interactions, we used a likelihood ratio test to compare conditional logistic regression models that included IRS genotype indicator variables, indicator variables for the additional covariate of interest and the product terms of IRS genotype and covariate indicator variable, to models without the product terms. All statistical analyses were performed in SAS and a P <0.05 was considered statistically significant.

RESULTS

Selected characteristics of the study population are presented in Table 1. At time of diagnosis of prostate cancer, the mean age ($\pm$ SD) was 68.5 ($\pm$ 6.7) years. Among the controls, <1% were homozygous (AA) and 13% were heterozygous (GA) for the polymorphic allele (Table 1). The allele frequencies of all the studied genes are comparable to frequencies previously reported and conform to the Hardy-Weinberg equilibrium in the controls (Gly972Arg, p=0.14) (Table 1). Plasma levels of IGF-1 and IGFBP3, BMI, or height were not different by the IRS-1 genotype or (Table 2). There was further no significant difference of the IRS-1 genotype frequency by race, PTEN genotype, or PI3K genotype. Table 3 presents relative risks and 95% confidence intervals for the association between the variant IRS-1 allele and prostate cancer. There was no association of carriage of the GLy972Arg allele with risk of total prostate cancer (RR=1.1 95% CI=0.8-1.5), overall or by subgroup of the disease. Adjusting for age, IGF-1, IGFBP3 and BMI did not change the results. We also explored possible interactions with body mass index as well as with other components in the IGF-1 pathway including IGFBP3, PI3k and PTEN, but none of these factors affected the relation between IRS-1 genotype and prostate cancer risk. Restricting the analyses to Caucasians did furthermore not change the results.

DISCUSSION

The data from this nested case-control study suggest that the IRS-1 Gly972Arg polymorphism is not directly associated with prostate cancer risk. Further, there was no

evidence to support a joint association of the variant IRS-1 allele and any of the other key components in the IGF-1 signaling pathway on prostate carcinogenesis.

Our results confirm the earlier finding of no association between the polymorphic IRS-1 allele and overall (9) or advanced prostate cancer (Li *et al.*, personal communication). It is possible that other components in the IGF-1 signalling pathway may alter or even conceal potential effects of IRS-1 on prostate cancer risk. However, the results of our subgroup analyses do not support that hypothesis. The findings of a relatively strong positive association presented by Neuhasen *et al.* derives from a smaller data set, and the play of chance could possibly explain the diverging results (8). The variant IRS-1 allele has previously been associated with colon, but not rectal cancer (14), but its role in carcinogenesis in general is largely unclear.

Misclassification due to within-person variation in circulating hormone levels may be considerable. Although not explaining the lack of association between the IRS-1 polymorphism and prostate cancer risk, such misclassification may have obscured the influence of IGF-1 and IGFBP3. Another study weakness is that due to the small number of non-Caucasians, the generalizability to other populations may be limited. The strengths of the study include the prospective design, large number of prostate cancer cases, and the long-term follow-up with high participation rate. Because this case-control study was nested within a well-defined cohort, the validity should be comparable of that of a cohort study. With the relatively large sample size, we should have had sufficient power to detect any meaningful association between the IRS-1 polymorphism and prostate cancer. We had 80% power to detect an OR of 1.6 for the IRS-1 polymorphism. Therefore, we conclude that these data not support any direct or indirect links between IRS-1 genotype and prostate cancer risk.

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Table 1

Baseline characteristics of prostate cancer cases and controls

	Cases (N=564)	Controls (N=758)
Mean age at baseline ($\pm$ SD)	61.2 ($\pm$ 7.7)	61.2 ($\pm$ 7.6)
Mean age at diagnosis ($\pm$ SD)	68.5 ($\pm$ 6.7)	-
Mean BMI ($\pm$ SD)	24.7 ($\pm$ 2.4)	24.5 ($\pm$ 2.5)
Smoking status (%)		
Current	257 (45.6)	345 (45.5)
Former	259 (45.9)	346 (45.7)
Never	48 (8.5)	67 (8.8)
BMI		
<25	327 (58.0)	477 (62.9)
25 - <30	220 (39.0)	262 (34.6)
30+	17 (3.0)	19 (2.5)
Race		
Caucasian	541 (96.6)	717 (95.4)
Non-Caucasian	19 (3.4)	35 (4.6)
Gleason score (%)		
2-6	304 (60.7)	-
7-10	197 (39.3)	-
Tumor stage (%)		
A/B (localized)	343 (66.5)	-
C, D or lethal (advanced)	173 (33.5)	-
PSA era (%)		
$\leq$ 1991	387 (68.6)	-
>1992	177 (31.4)	-
PTEN gene polymorphism (%)		
No insertion	242 (43.3)	323 (42.9)
Heterozygous	266 (47.6)	349 (46.4)
Insertion	51 (9.1)	81 (10.8)
Pi3k gene polymorphism (%)		
GG	375 (68.6)	495 (67.4)
GA	161 (29.4)	225 (30.7)
AA	11 (2.0)	14 (1.9)
IRS-1 gene polymorphism (%)		
GG	489 (86.7)	662 (87.3)
GA	73 (12.9)	90 (11.9)
AA	2 (0.4)	6 (0.8)

Table 2

Distribution of covariates in relation to genotype among controls

	GG		GA/AA		P-value
	Mean*	Std Err	Mean*	Std Err	
Plasma IGF-I**	144.03	4.04	148.52	10.39	0.69
Plasma IGFBP-3, ng/ml**	2489.92	37.96	2451.77	97.69	0.72
BMI, kg/m ²	24.57	0.10	24.23	0.26	0.21
Height, inches	70.07	0.10	70.22	0.27	0.59
	GG		GA/AA		
	N	%	N	%	
Race					
White	624	95.0	93	97.9	NS
Non-White	33	5.0	2	2.1	
PTEN					
TT	287	43.7	36	37.5	0.43
TG	302	46.0	47	49.0	
GG	68	10.3	13	13.5	
PI3K					
GG	431	67.3	64	68.1	0.89
GA/AA	209	32.7	30	31.9	

* Means adjusted for age centered at 60 years

** Standardized to batch 3 mean

Table 3

Relative Risks (RR) and 95% confidence intervals (CI) for the association between IRS-1 Gly972Arg variant and prostate cancer

	Cases, n	RR (95% CI)
All prostate cancer		
GG	489	1.0 (Ref)
GA/AA	75	1.1 (0.8, 1.5)
Tumor stage		
A/B		
GG	266	1.00
GA/AA	37	0.9 (0.6, 1.4)
C, D or lethal		
GG	188	1.00
GA/AA	31 1.3	(0.8, 2.3)