Null Results in Brief

Association between the *Met326Ile* Polymorphism of the p85α Regulatory Subunit of Phosphatidylinositol 3-Kinase and Prostate Cancer Risk: A Prospective Study[1]

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Introduction

PI3-K[3] is an important mediator of cell-survival signals in CaP (1). PI3-K catalyzes the addition of phosphate to the phosphoinositides at the 3′-OH position of the inositol ring. These phosphorylated lipids target Akt kinase. When activated, Akt targets and inhibits a variety of proteins that are necessary for apoptosis. The tumor suppressor PTEN negatively regulates this pathway by dephosphorylating the PI3-K substrates (2). Loss of PTEN function is correlated with CaP progression, possibly as a result of constitutive PI3-K signaling (2). Numerous molecules signal through PI3-K, including the IGFs. Epidemiological studies have shown that increased circulating IGF-1 is associated with CaP risk (3, 4).

There is a missense polymorphism in codon 326 of the gene for p85α, the regulatory subunit of PI3-K. Methionine is replaced by isoleucine. This amino acid is six places away from the N-terminal SH2 domain coding region, an area crucial for the binding of receptor tyrosine kinases, which mediate the effects of many growth factors (reviewed in Ref. 5). IGF type 1 receptor (IGF-1R) is one such receptor tyrosine kinase. Some earlier studies suggest that the *Met326Ile* polymorphism is associated with decreased PI3-K activity (6, 7). However, recent *in vivo* and *in vitro* evidence suggests that the *Met326Ile* variant may be functionally normal (8, 9). We tested the hypothesis that the variant allele is associated with decreased risk of CaP.

Subjects and Methods

Subjects were selected from the Physician's Health Study, a randomized-double-blind trial of aspirin and β-carotene among United States male physicians. Study characteristics, including blood collection and processing, documentation of CaP, and tumor grade/stage, were described previously (10). Among the 14,916 men (94% Caucasian) who provided blood in 1982, we documented 590 cases of incident CaP for this analysis, confirmed and classified with medical records (10). These were matched by age and smoking status to one or two controls with no CaP.

Oligonucleotide primers CCAACAACGGTATGAATAACCATAT-3′ and 5′-CGAGATATCTCCCCAGTACC-3′ were selected to amplify a 65-bp fragment that included the site of the polymorphism. A one-base mismatch was inserted into the forward primer to create a restriction site for the enzyme *Nde*I (New England BioLabs, Beverly, MA). PCR amplification was carried out with 40 ng of DNA in 2.0 mM magnesium chloride, 1.5 units Taq DNA polymerase, 1.8 mM dNTPs, and 0.76 μM each primer in a total volume of 22 μl. Cycling conditions were 95°C for 4 min; 35 cycles of 95°C for 30 s, 54°C for 30 s, and 72°C for 30 s; and 72°C for 5 min. in an MJ Research PTC-200 thermal cycler (MJ Research, Waltham, MA). Digestion was with 20 units *Nde*I for 3 h at 37°C. Samples were separated on a 3% agarose gel stained with ethidium bromide.

Statistical Analysis. We determined the genotype frequency by case and control status, among low- or high-grade/stage cases as categorized by clinical stage and Gleason grade at diagnosis, and in cases of death caused by CaP. We calculated age- and smoking-adjusted OR as an estimate of relative risk and 95% CI from logistic regression models. Because of small numbers and limited statistical power in the subgroup analysis by tumor grade/stage and death, we calculated the adjusted OR and 95% CI comparing the subgroup cases with the overall controls. We also calculated the OR and 95% CI for the combined *Met/Ile* and *Ile/Ile* genotypes. Using the combined genotypes, we also examined whether there was a difference between men older or younger than 67 years, the median age at diagnosis. As a result of increased prostate-specific antigen (PSA) screening, CaP diagnoses, particularly of early-stage/grade disease, peaked nationwide from 1990 to 1993. We assessed whether this trend affected our analysis of the genotypes as categorized by stage and grade by performing an additional analysis of cases diagnosed before and after 1992.

Results and Discussion

Five hundred ninety cases and 781 controls were successfully genotyped, but only Caucasians were included in the analysis (555 cases and 738 controls). The *Met326Ile* polymorphism distribution fit the Hardy-Weinberg equilibrium. No appreciable difference in genotype frequency was observed between cases and controls (Table 1). Neither the *Met/Ile* nor the *Ile/Ile* genotype was associated with overall decreased risk of CaP. Likewise, these genotypes were not associated with low-grade/stage cases. The data suggest a trend of decreasing risk of high-grade/stage CaP with an increasing number of variant alleles among the three genotypes (*Met/Met*, referent; *Met/Ile*

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[3] PI3K, phosphatidylinositol 3-kinase; IGF, insulin-like growth factor; OR, odds ratio; CI, confidence interval; CaP, prostate cancer.

Table 1 Frequency of *PI3K-p85* genotype, age and smoking-adjusted OR and 95% CI of CaP in the Physicians' Health Study

	PI3K-p85 genotype			
	Met/Met	*Met/Ile*	*Ile/Ile*	*Met/Ile* and *Ile/Ile*
Controls (%)	495 (67.1)	230 (31.2)	13 (1.8)	243 (32.9)
Overall cases (%)	378 (68.1)	166 (29.9)	11 (2.0)	177 (31.9)
OR (95% CI)	1.00 (reference)	0.95 (0.74–1.20)	1.11 (0.49–2.50)	0.95 (0.75–1.21)
Low-grade/stage cases (%)	176 (65.7)	85 (31.7)	7 (2.6)	92 (34.3)
OR (95% CI)	1.00 (reference)	1.04 (0.77–1.40)	1.52 (0.59–3.87)	1.06 (0.79–1.43)
High-grade/stage cases (%)	183 (71.2)	71 (27.6)	3 (1.2)	74 (28.8)
OR (95% CI)	1.00 (reference)	0.84 (0.61–1.15)	0.61 (0.17–2.17)	0.82 (0.60–1.12)
Death caused by CaP	52 (72.2)	19 (26.4)	1 (1.4)	20 (27.8)
OR (95% CI)	1.00 (reference)	0.80 (0.46–1.38)	0.72 (0.09–5.69)	0.79 (0.46–1.36)

OR, 0.8, 95% CI 0.6–1.2; *Ile/Ile* OR, 0.6, 95% 0.2–2.2). The combined *Met/Ile* and *Ile/Ile* genotypes (Table 1) also suggest slightly decreased risk of high-grade/stage CaP (OR, 0.8; 95% CI 0.6–1.1). However, none of the ORs was statistically significant. There were no significant differences between patients older and younger than 67 years at diagnosis. Neither was there an appreciable difference between cases diagnosed before and after 1992.

This large, nested case-control study of United States Caucasian men did not support an overall association between possession of the isoleucine variant of codon 326 of the p85α subunit of PI3-K and decreased risk of CaP. However, the findings are compatible with a slightly decreased risk of high grade/stage CaP in carriers of the *Ile* allele, but our study has limited statistical power to detect weak associations. Although the impact of the homozygous *Ile/Ile* variant genotype on high risk CaP is probably minimal given its low frequency in the population, a larger study might be needed to further investigate its potential modification of the influence of the IGF/insulin pathway on cancer risk.

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