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14. ABSTRACT

Background: The *P582S C->T* and *A588T G->A* polymorphisms in the Hypoxia-inducible factor-1α (HIF-1α) gene have been associated with enhanced stability of the protein and androgen-independent prostate cancer (CaP). **Methods:** We examined the association of these polymorphisms with CaP among 1,072 incident cases and 1,271 controls, and further explored their joint associations with various prediagnostic plasma hormone levels.**Results:** The *A588T* polymorphism was too rare to provide meaningful conclusions and the *P582S* polymorphism was not associated with CaP. We observed a significant interaction of the *P582S* genotype with insulin-like growth factor binding protein (IGFBP)-3 in modifying CaP risk such that higher IGFBP-3 levels (>= versus <median) were associated with a reduced risk only among men with the wildtype (OR, 95% CI = 0.74, 0.57-0.97; $P_{interaction}$ = 0.01).
Conclusions: We found no association between these HIF-1α gene polymorphisms with CaP, but the interaction between the *P582S* polymorphism and the IGF axis merits further evaluation in mechanistic studies

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Manuscript included by Li, Bubley, Ba1k et al.

Introduction:

The goals of this proposal were to assess the frequency of 2 specific polymorphisms to assess the effect of these on the severity and incidence of prostate cancer. This was accomplished using a data set from the Men's Physician's Health Study here at Harvard. These data can be analyzed in the accompanying manuscript IN PRESS in the *Prostate*. I am co first author. This was the only aim that was approved in the study. Aim 2 was not funded.

This is a supplemental report based on the fact that the first report that was filed did not conforming to the appropriate format, and I am making changes before the deadline of may 12th.

It is possible that the reviewer referred to an old SOW, in that the reviewer refers to three tasks. However, this proposal was awarded with only one Specific Aim and cut back from three to two years.

Please see the revised SOW below:

Revised Statement of Work for PC040277

G. Bubley. PI

Year 1

1,) Collect DNA samples from the Physicians Health Study and the Harvard School of Public Health African American cohort. Samples will be obtained without identifiers, but will be coded as affected (having prostate cancer) or unaffected; and the race of the individual.

2,) Begin sample analysis for P582S polymorphism. In year one we will determine if we can rapidly analyze samples by screening for resistance to Tsp1 digestion (this will require sequencing to confirm the polymorphism the first approximate 100 samples resistant to Tsp 1 digestion. If this is not efficacious we will begin the analysis by other methods as outlined in the proposal

Year 2

1.) Continue analysis of specimens for P582S polymorphisms by the method chosen
2.) Meet with Dr. Regan (statistician) to go over these results.
3.) Preparation of manuscript

In the past two years we have made progress towards the goal above. We have detected one polymorphism with sufficient frequency on the Physician's Health Study (PHS) Survey such that we can assess whether the P582S polymorphism in the HIF 1 alpha gene is associated with a increased or poor prognosis with respect to prostate cancer.

The work performed is best assessed in the accompanying manuscript included in the APPENDIX, but will be included in the body below and the key research accomplishments. Please note that this is IN PRESS in *The Prostate*

Body:

The goal of the this research proposal was to assess whether or not one of two specific polymorphisms in the HIF 1 alpha gene was associated with disease progression. Genetic variations in the gene encoding HIF-1α may also influence its function. Two germline single-nucleotide polymorphisms within the oxygen-dependent degradation (ODD)/pVHL binding domain (in exon 12) of the HIF-1α gene were identified. *P582S* causes a change from proline to serine at codon 582, and *A588T* causes a change from alanine to threoine at codon 588. Although the functionality of these polymorphisms are not completely clear, both the *P582S* (1-3) and *A588T* (1) variants (versus wild-type) yielded significantly higher transcription activity *in vitro.*

Using a nested case-control design within the Physicians' Health Study, we investigated the associations of the HIF-1α *P582S* and *A588T* polymorphisms with prostate cancer incidence and survival. We hypothesized that the HIF-1α *P582S* (*CC or CT*) and *A588T* (*GA*) variant genotype may be associated with increased risk, especially with aggressive disease, and poorer survival after diagnosis. We further explored the relationship between these HIF-1α polymorphisms and pre-diagnostic plasma levels of IGF-I, IGFBP-3, and VEGF in relation to prostate cancer incidence

DNA was extracted from baseline blood specimens for these men. With the laboratory personnel blinded to the case-control status, all samples were genotyped using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) at the Dana Farber/Harvard Cancer Center Genotyping Core. The HIF-1α-RFLP (*P582S* and *A588T*) genotypes were determined by the PCR amplification, followed by restriction enzyme digestion, as described previously (4). The lab personnel were unaware of case-control status. To assess genotyping reproducibility, they repeated a random 10% selection of the samples; all genotypes matched initial designated genotypes.

Samples from cases and their matched controls were assayed in the same batch to minimize interassay variability, and aliquots from a pool of quality control plasma (10% of total sample) were inserted randomly. Laboratory personnel were unable to distinguish among case, control, and quality control samples. Plasma levels of IGF-I and IGFBP-3 were assayed by enzyme-linked immunosorbent assay with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory of M. N. Pollak at the Lady Davis Research Institute of the Jewish General Hospital and McGill University. The median intra-assay coefficients of variation (CV) for IGF-I, and IGFBP-3 from the blinded quality control samples were 4.5% and 4.0%, respectively. Plasma concentrations of VEGF for each man were assayed in duplicate using a microplate luminescence detection system (Dynex Technologies, Chantilly, VA) and a human VEGF immunoassay Quantiglo kit (R&D Systems, Minneapolis, MN). The median CV for the quality control samples was 22.8%.

A more complete description of the methodology is included in the appended manuscript in the materials and methods section.

Key Research Accomplishments:

In the PHS on average, men were 69 years of age when prostate cancer was diagnosed. The median interval from baseline in 1982 to diagnosis was 11.0 years for these cancer patients, and the average follow-up duration after diagnosis was 9.2 years. Among control subjects, the genotype frequencies were 80.6% (*CC*), 17.9% (*CT*) and 1.5% (*TT*) for the *P582S* polymorphism and 98.7% (*GG*), 1.3% (*GA*) for the *A588T* polymorphism (no instances for the *A588T AA* variant genotype). The genotype distributions were in Hardy-Weinberg equilibrium. The genotype frequencies were similar to previous reports for Caucasian populations; however, compared with a report in a Japanese population the *A588T* polymorphism in these men was rare (1.3% in our controls versus 8.2% in Japanese controls). Genotype frequencies for the HIF-1α *P582S* and *A588T* polymorphisms did not differ significantly between cases and controls. These data are demonstrated in Table 1 in the appended manuscript IN PRESS.

The *P582S* polymorphism was not associated with risk of overall prostate cancer, regardless of age at diagnosis or calendar time (by PSA era), and was not related to prostate cancer survival (HR, 95% CI for *P582S CT* or *TT* vs. *CC* = 0.96, 0.63-1.46). The HIF-1α *P582S* polymorphism did not affect risk for advanced-stage, metastatic and fatal disease. We also found no association of the *A588T* polymorphism with prostate cancer risk and survival; however, this polymorphism was too rare in this population to draw meaningful conclusions. These data are demonstrated in Table 2 in the appended manuscript IN PRESS.

Plasma levels of IGF-I, IGFBP-3, and VEGF measured from blood samples collected at the baseline, an average of 11 years before cancer diagnosis, were not significantly different between cases and controls or by the HIF-1α *P582S* genotype. We found no interaction of the HIF-1α *P582S* polymorphism with baseline levels of VEGF or IGF-I. Men with the *CT* or *TT* genotypes and higher plasma IGF-I levels consistently had the greatest risk of overall, advanced-stage, high-grade, metastatic and fatal prostate cancer. This polymorphism appeared to modify the association between plasma IGFBP-3 level and prostate cancer. Higher IGFBP-3 level (>= versus <median) was significantly associated with a reduced risk of overall prostate cancer only among those with the wildtype genotype (RR, 95% CI = 0.74, 0.57-0.97; $P_{interaction}$ = 0.01). The patterns were similar for advanced-stage or metastatic and fatal disease, and the risk reduction associated with higher level of IGFBP-3 was greater than 50% among the *CC* carriers. The results related to the IGF axis remained similar when levels of IGF-I and IGFBP-3 were categorized into tertiles. **These data are summarized in Tables 3 and 4 in the appended manuscript.**

Reportable Outcomes

The *P582S* polymorphism was not associated with risk of overall prostate cancer , regardless of age at diagnosis or calendar time (by PSA era), and was not related to prostate cancer survival (HR, 95% CI for *P582S CT* or *TT* vs. *CC* = 0.96, 0.63-1.46). The HIF-1α *P582S* polymorphism did not affect risk for advanced-stage, metastatic and fatal disease. We also found no association of the *A588T* polymorphism with prostate cancer risk and survival; however, this polymorphism was too rare in this population to draw meaningful conclusions.

Plasma levels of IGF-I, IGFBP-3, and VEGF measured from blood samples collected at the baseline, an average of 11 years before cancer diagnosis, were not significantly different between cases and controls or by the HIF-1α *P582S* genotype. We found no interaction of the HIF-1α *P582S* polymorphism with baseline levels of VEGF or IGF-I. Men with the *CT* or *TT* genotypes and higher plasma IGF-I levels consistently had the greatest risk of overall, advanced-stage, high-grade, metastatic and fatal prostate cancer. This polymorphism appeared to modify the association between plasma IGFBP-3 level and prostate cancer. Higher IGFBP-3 level (>= versus <median) was significantly associated with a reduced risk of overall prostate cancer only among those with the wildtype genotype (RR, 95% CI = 0.74, 0.57-0.97; $P_{interaction}$ = 0.01). The patterns were similar for advanced-stage or metastatic and fatal disease, and the risk reduction associated with higher level of IGFBP-3 was greater than 50% among the *CC* carriers. The results related to the IGF axis remained similar when levels of IGF-I and IGFBP-3 were categorized into tertiles**. These data are exhibited in TABLES 3 and 4 in the appended manuscript IN PRESS**.

Conclusion

We found no association between these HIF-1α gene polymorphisms with CaP, but the interaction between the *P582S* polymorphism and the IGF axis merits further evaluation. The relationship between higher IGFBP-3 levels (expected to be protective for prostate cancer) and the wild type genotype for HIF 1 alpha was somewhat striking. These two factors were associated with both a reduced risk of prostate cancer and a better outcome for affected patients. We are planning in vitro studies to assess this relationship

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Hypoxia-inducible factor-1α (HIF-1α) gene polymorphisms, prediagnostic hormone levels and prostate cancer

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ABSTRACT

Background: The *P582S C->T* and *A588T G->A* polymorphisms in the Hypoxia-inducible factor-1α (HIF-1α) gene have been associated with enhanced stability of the protein and androgen-independent prostate cancer (CaP).

Methods: We examined the association of these polymorphisms with CaP among 1,072 incident cases and 1,271 controls, and further explored their joint associations with various prediagnostic plasma hormone levels.

Results: The *A588T* polymorphism was too rare to provide meaningful conclusions and the *P582S* polymorphism was not associated with CaP. We observed a significant interaction of the *P582S* genotype with insulin-like growth factor binding protein (IGFBP)-3 in modifying CaP risk such that higher IGFBP-3 levels (>= versus <median) were associated with a reduced risk only among men with the wildtype (OR, 95% CI = 0.74, 0.57-0.97; $P_{interaction} = 0.01$).

Conclusions: We found no association between these HIF-1α gene polymorphisms with CaP, but the interaction between the *P582S* polymorphism and the IGF axis merits further evaluation in mechanistic studies.

Keywords (not in title): insulin-like growth factor (IGF)-I, insulin-like growth factor binding protein (IGFBP)-3, vascular endothelial growth factor (VEGF), prospective, nested case-control study.

INTRODUCTION

Hypoxia-inducible factor-1 (HIF-1), composed of an α and a β subunit (1), is a pivotal regulator of cellular response to hypoxia (2). The transcriptional activity of HIF-1 is determined by the oxygen-regulated expression of the HIF-1α subunit (3), which is hydroxylated and degraded rapidly under normoxia through von Hippel-Lindau (VHL) mediated ubiquitination whereas it becomes stabilized or even induced in response to hypoxia (3). HIF-1α is overexpressed in many tumors (4,5) and a significant association between such overexpression and mortality has been reported for many cancer types (2). In prostate cancer cell lines, expression of HIF-1α protein is positively associated with cell growth rates and metastatic potential (6,7). In humans, expression levels of HIF-1α are up-regulated in high-grade prostate intraepithelial neoplasia (PIN) lesions (versus adjacent normal tissue) and are further enhanced in primary and metastatic prostate cancer (4,8,9).

HIF-1 plays a major role in tumor progression and metastasis through activation of various genes that are linked to regulation of angiogenesis, cell survival, energy metabolism and apoptotic and proliferative responses (2,10). Under hypoxic conditions, HIF-1 activates the vascular endothelial growth factor (VEGF) gene, leading to increased production of VEGF, a potent stimulator of angiogenesis (11,12). Treatment of cultured cells with insulin, insulin-like growth factor-I (IGF-I), or IGF-2 induces HIF-1α protein expression, which is in turn required for expression of IGF-2 and the IGF binding proteins (IGFBP-2 and IGFBP-3) (13-16). These activities may promote cell

proliferation and survival. For these reasons, HIF-1 has become an attractive target for the development of anti-cancer drugs (2,17).

Genetic variations in the gene encoding HIF-1α may also influence its function. Two germline single-nucleotide polymorphisms within the oxygen-dependent degradation (ODD)/pVHL binding domain (in exon 12) of the HIF-1α gene were identified (18). *P582S* causes a change from proline to serine at codon 582, and *A588T* causes a change from alanine to threoine at codon 588. Although the functionality of these polymorphisms are not completely clear, both the *P582S* (19-21) and *A588T* (19) variants (versus wild-type) yielded significantly higher transcription activity. These polymorphisms may confer susceptibility to renal cell carcinoma (22,23); and patients with head and neck squamous cell carcinoma who carry these variants have tumors with significantly increased microvessels ($P = 0.02$) (19). One study on colorectal cancer incidence reported null associations for these variants (24). Our group recently identified the *P582S* mutation in bone marrow metastatic biopsies from men with androgen-independent prostate cancers (21). We observed significantly higher transcription activity, which reflected increased HIF-1α protein expression, associated with this mutation under normoxic conditions. Another group also found the same mutation in prostate tumors (25). One case-control study found that the *P582S* variant genotype was more common among men with androgen-independent prostate cancer (26). Taken together, these data suggest that the HIF-1α polymorphisms might be related to risk of prostate cancer, especially the more aggressive form, and provide the rationale for the current investigation.

Using a nested case-control design within the Physicians' Health Study, we investigated the associations of the HIF-1α *P582S* and *A588T* polymorphisms with prostate cancer incidence and survival. We hypothesized that the HIF-1α *P582S* (*CC or CT*) and *A588T* (*GA*) variant genotype may be associated with increased risk, especially with aggressive disease, and poorer survival after diagnosis. We and other previously showed that circulating levels of IGF-I are positively (whereas levels of IGFBP-3 are mostly inversely) associated with risk of prostate cancer (27-29), especially advanced-stage disease (27,28), therefore and aggressiveness. We further explored the relationship between these HIF-1α polymorphisms and prediagnostic plasma levels of IGF-I, IGFBP-3, and VEGF in relation to prostate cancer incidence

MATERIALS AND METHODS

Study Population

The Physicians' Health Study (PHS) was a randomized, double-blind, placebo-controlled trial of aspirin and β-carotene among 22,071 healthy U.S. male physicians, aged 40-84 years, that began in 1982 (30). During 1982 and 1984, 14,916 (68%) provided baseline blood samples prior to randomization (31), and more than 70% of the specimens were received between September and November in 1982. Through mailed baseline and follow-up questionnaires, we collected information on diet, lifestyle behaviors and medical history. Follow-up of the participants for morbidity and mortality is 97% complete to March 2005. Since 2003, information regarding disease progression and metastases was obtained from men with prostate cancer cases via additional follow-

up questionnaires. Thus far, 87% of the eligible participants have responded, and less than 2% of participants have refused to participate or cannot because of cognitive problems.

Prostate cancer cases for the current study were drawn from participants who provided blood specimens at baseline and reported a diagnosis of prostate cancer between 1982 and 2000. Study investigators, unaware of the questionnaire or assay data, verified the reports of prostate cancer by participants and reviewed medical records and pathological reports to determine the tumor Gleason score, grade, and stage, according to the modified Whitmore-Jewett classification scheme (32). The corresponding TNM staging to the Whitmore-Jewett system is as: stage A (T1N0M0), B (T2N0M0), C (T3N0M0), and D (TxN0M1, TxN1M0 or TxN1M1). We defined advanced-stage cancer as stage C and D diseases, and defined high-grade cancer as those with Gleason score of 7 to 10 and those that were poorly-differentiated. For each case, we selected one or two controls at random from those who had provided blood, were alive, had not had a prostatectomy, and had not reported a diagnosis of cancer (except for non-melanoma skin cancer) at the time the diagnosis was reported by the case subject. Controls were individually matched to cases by age (±1 year, ±5 years for elderly participants) and smoking status (never, former, or current).

Laboratory Assessment

The HIF-1α gene polymorphisms

DNA was extracted from baseline blood specimens for these men. With the laboratory personnel blinded to the case-control status, all samples were genotyped using

the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA) at the Dana Farber/Harvard Cancer Center Genotyping Core. The HIF-1α-RFLP (*P582S* and *A588T*) genotypes were determined by the PCR amplification, followed by restriction enzyme digestion, as described previously (18). The lab personnel were unaware of case-control status. To assess genotyping reproducibility, they repeated a random 10% selection of the samples; all genotypes matched initial designated genotypes.

Plasma concentrations of IGF-I, IGFBP-3 and VEGF

Samples from cases and their matched controls were assayed in the same batch to minimize interassay variability, and aliquots from a pool of quality control plasma (10% of total sample) were inserted randomly. Laboratory personnel were unable to distinguish among case, control, and quality control samples. Plasma levels of IGF-I and IGFBP-3 were assayed by enzyme-linked immunosorbent assay with reagents from Diagnostic Systems Laboratory (Webster, TX) in the laboratory of M. N. Pollak at the Lady Davis Research Institute of the Jewish General Hospital and McGill University. The median intra-assay coefficients of variation (CV) for IGF-I, and IGFBP-3 from the blinded quality control samples were 4.5% and 4.0%, respectively. Plasma concentrations of VEGF for each man were assayed in duplicate using a microplate luminescence detection system (Dynex Technologies, Chantilly, VA) and a human VEGF immunoassay Quantiglo kit (R&D Systems, Minneapolis, MN). The median CV for the quality control samples was 22.8%. Additional details of the VEGF assay are described elsewhere (33).

Statistical Analysis

We studied 1,072 men who developed prostate cancer during 18 years of follow-up and 1,281 controls. Of these, 1,041 cases (and 1,243 controls) were assayed for the HIF-1α *P582S* polymorphism and 1,066 cases (and 1,274 controls) for the HIF-1α *A588T* polymorphism. Because the majority (94%) of men were Caucasians and analyses with and without the non-Caucasian men yielded similar results, we included men of all ethnic groups in this study to maximize the sample size.

We compared allele and genotype frequencies between cases and controls with the χ^2 test. We examined the association of HIF-1α gene polymorphisms with risk of developing prostate cancer, then refitted models for men who developed metastases or died from prostate cancer during the follow-up, and within subgroups based on disease aggressiveness (by stage and grade), age at diagnosis (< 65 years or >= 65 years), or PSA-era (before or after 1990). Because very few men (< 2%) carried the HIF-1α *P582S TT* genotype, we combined men with the *CT* or the *TT* genotypes into a single group and compared them with the reference group (the wild-type) for all analyses. The results were similar from both conditional (matched) and unconditional (unmatched) logistic regression analyses, so we primarily report estimates (relative risks, RR and 95% confidence intervals, 95% CI) from unconditional logistic regression, adjusted for matching factors of age, smoking status, and duration of follow-up. The unconditional logistic regression model permits all control subjects to be included in each model for maximizing statistical power, which is especially important for the subgroup analyses. To assess the influence of these polymorphisms on prostate cancer survival, with survival time calculated from date of diagnosis to date of death from prostate cancer or other

causes, or the end of the follow-up in March 2005, hazard ratios (HRs) and 95% CIs were estimated using Cox proportional hazards models, adjusting for age at diagnosis and smoking status.

The joint associations of HIF-1α polymorphisms with plasma levels (categorized by median) of IGF-I, IGFBP-3, and VEGF on prostate cancer were examined for the HIF-1α P582S genotype only. The frequency of HIF-1α *A588T* (*GA*) variant was too rare (< 2%) to examine gene-hormone interactions. Conditional logistic regression models were implemented. Included in these analyses were 661 cases and 661 matched controls for IGF-I and IGFBP-3 and 397 cases and 398 matched controls for VEGF. Because levels of IGF-I and IGFBP-3 were correlated ($r = 0.46$, $P<0.0001$) and these two biomarkers have opposite effects on risk, it was necessary to adjust for them simultaneously. We further compared the age-, smoking status-, and batch-adjusted levels of IGF-I and IGFBP-3 and age- and smoking status-adjusted VEGF levels with the presence or absence of the HIF-1α *P582S* variant (*CT* or *TT*) genotype, using general linear regression models. All statistics were calculated using SAS (version 8.12; SAS institute Inc, Cary, NC) with a two-sided significance level of 0.05.

RESULTS

On average, men were 69 years of age when prostate cancer was diagnosed. The median interval from baseline in 1982 to diagnosis was 11.0 years for these cancer patients, and the average follow-up duration after diagnosis was 9.2 years. Among control subjects, the genotype frequencies were 80.6% (*CC*), 17.9% (*CT*) and 1.5% (*TT*) for the *P582S* polymorphism and 98.7% (*GG*), 1.3% (*GA*) for the *A588T* polymorphism

(no instances for the *A588T AA* variant genotype). The genotype distributions were in Hardy-Weinberg equilibrium. The genotype frequencies were similar to previous reports for Caucasian populations (18,26); however, compared with a report in a Japanese population (19), the *A588T* polymorphism in these men was rare (1.3% in our controls versus 8.2% in Japanese controls). Genotype frequencies for the HIF-1α *P582S* and *A588T* polymorphisms did not differ significantly between cases and controls (Table 1).

The *P582S* polymorphism was not associated with risk of overall prostate cancer (Table 2), regardless of age at diagnosis or calendar time (by PSA era), and was not related to prostate cancer survival (HR, 95% CI for *P582S CT* or *TT* vs. *CC* = 0.96, 0.63-1.46). The HIF-1α *P582S* polymorphism did not affect risk for advanced-stage, metastatic and fatal disease. We also found no association of the *A588T* polymorphism with prostate cancer risk and survival; however, this polymorphism was too rare in this population to draw meaningful conclusions.

Plasma levels of IGF-I, IGFBP-3, and VEGF measured from blood samples collected at the baseline, an average of 11 years before cancer diagnosis, were not significantly different between cases and controls or by the HIF-1α *P582S* genotype (Table 3). We found no interaction of the HIF-1α *P582S* polymorphism with baseline levels of VEGF or IGF-I. Men with the *CT* or *TT* genotypes and higher plasma IGF-I levels consistently had the greatest risk of overall, advanced-stage, high-grade, metastatic and fatal prostate cancer. This polymorphism appeared to modify the association between plasma IGFBP-3 level and prostate cancer (Table 4). Higher IGFBP-3 level (>= versus <median) was significantly associated with a reduced risk of overall prostate cancer only among those with the wildtype genotype (RR, 95% CI = 0.74, 0.57-0.97;

$P_{interaction}$ = 0.01). The patterns were similar for advanced-stage or metastatic and fatal disease, and the risk reduction associated with higher level of IGFBP-3 was greater than 50% among the *CC* carriers. The results related to the IGF axis remained similar when levels of IGF-I and IGFBP-3 were categorized into tertiles.

DISCUSSION

In this population of primarily Caucasian U.S. physicians, we found no overall association between the HIF-1α *P582S* or *A588T* polymorphism and prostate cancer. The *A588T* polymorphism was rare; only 13 cases and 17 controls (<2%) carried the *GA* variant genotype. The very low frequency of this polymorphism precludes definitive conclusions regarding its effect on prostate cancer incidence, aggressivenesss or survivorship.

The lack of significant associations of this polymorphism with aggressive prostate cancer in our study is somewhat at odds given previous observations. We detected a somatic *P582S* mutation in an androgen-independent prostate cancer case with metastatsis to the bone (21) as did another group in prostate tumor (25). A case-control study observed higher frequencies of the *P582S* variant genotypes among 196 androgen-independent prostate cancer cases compared with 196 controls (26). Although proline 582 has not been identified as a hydroxylation site necessary to mediate VHL binding (34), the *P582S* variant genotype (versus wild-type) has been associated with increased number of microvessels (19) and significantly higher transcription activity under both normoxic and hypoxic conditions (19-21).

VEGF is a pleiotrophic growth factor that promotes endothelial cell proliferation, vascular permeability, and angiogenesis (35,36) and is overexpressed in prostate cancer (37). Because it is shown that the binding of HIF-1α to the VEGF promoter is required for maximum transcription of VEGF mRNA following hypoxia (11,12), we had hypothesized that the HIF-1α *P582S* variant carriers may have higher circulating VEGF levels and therefore higher risk of prostate cancer. The large CV of the VEGF measurement, the null association between plasma VEGF level and prostate cancer that we described previously (33) as well as the lack of the interaction with the *P582S* polymorphism in the current study might reflect the difficulty for precise measurements of VEGF in frozen plasma. Improving assays for circulating VEGF levels or its tissue expression would be worthwhile to further evaluate the potential role of VEGF in both carcinogenesis and angiogenesis.

Our data suggest that the HIF-1α *P582S* polymorphism might potentially interact with the IGF axis (Table 4), and the associations of the IGF-I axis observed with prostate cancer were in agreement with our previous findings (27). High levels of IGFBP-3 were significantly associated with reduced the risk of overall prostate cancer, advanced-stage, metastatic and fatal prostate cancer primarily among those with the *CC* (but not *CT* or *TT*) wild-type. The interaction was significant for overall prostate cancer, but not for the aggressive subgroups probably due to smaller sample sizes. Feldser *et al.* (15) demonstrated that insulin, IGF-I, and IGF-2 induce expression of HIF-1α, which is required for expression of genes encoding IGF-2, IGFBP-2 and IGFBP-3. Recent studies further confirmed that IGF-I transcriptional activation by HIF-1α is a metabolic adaptive responses to hypoxia (38); IGF-I stimulates HIF-1α accumulation, HIF-1α nuclear

translocation, and HIF-1 activity, resulting in increased VEGF expression (13,16). Given these prior observations, the possible interaction between the HIF-1α P582S polymorphism and the IGF axis observed in this study provides new evidence that the growth hormone/insulin/IGF-axis may play an important role in prostate cancer development and angiogenesis.

Strengths of the current study include its prospective design, large sample size and complete long-term follow-up. Prospectively collected plasma enabled us to assess the levels of IGF-I, IGFBP3 and their interaction with the HIF-1α polymorphism. Although a single assessment of hormone levels from the baseline blood is an imperfect indicator of long-term exposures, a pilot study with blood drawn from the same individuals at a five-year interval showed a high correlation (r = 0.75) for IGF-1 (Ma, J, unpublished data). Furthermore, significant associations of plasma levels of IGF-I and IGFBP-3 with prostate cancer risk (27) also provide assurance that these hormone data are valid in reflecting long-term status in our study population.

To summarize, we found no association between either the HIF-1α *P582S* or *A588T* polymorphism with overall risk of prostate cancer. However, our data suggest a possible interaction between the *P582S* polymorphism and the IGF axis in modifying prostate cancer risk.

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Table 1. Baseline characteristics of prostate cancer cases and controls in the Physicians' Health Study

	Cases (n = 1072)	Controls (n = 1281)
Age at baseline (yr) [a]	58.9 ± 8.3	59.0 ± 8.1
Age at diagnosis (yr)	69.4 ± 7.3	NA
Smoking status (%) [a]		
Current	9.5	8.9
Former	42.7	42.6
Disease aggressiveness, N (%)		
Stage AB	581 (54.2)	
CD	233 (21.7)	NA
Unknown	258 (24.1)	
Grade [b]		
Low	661 (61.7)	
High	384 (35.8)	NA
Unknown	27 (2.5)	
Metastatic/Fatal prostate cancer [c]	177 (16.5)	NA
HIF-1α gene polymorphism, N (%) [d]		
P582S _CC	818 (78.6)	1002 (80.6)
P582S _CT	209 (20.1)	223 (17.9)
P582S _TT	14 (1.3)	18 (1.5)
A588T_GG	1053 (98.8)	1257 (98.7)
A588T_GA	13 (1.2)	17 (1.3)

[a] Matching variable.

[b] High-grade cancer included Gleason 7-10 or poorly differentiated tumors;

[c] Metastatic/Fatal prostate cancer, cases died of prostate cancer and those who developed metastasis during the follow-up.

[d] Data availability: HIF-1α *P582S* genotype, 1,041 cases and 1,243 controls; HIF-1α *A588T* genotype, 1,066 cases and 1,274 controls.

Table 2. HIF-1α *P582S* gene polymorphism and risk of prostate cancer [a]

	HIF-1α *P582S* polymorphism			
	CC		*CT or TT*	
	N	RR (Ref.)	N	RR (95% CI)
Number of controls	1002		241	
Overall prostate cancer	818	1.00	223	1.14 (0.93-1.39)
By tumor stage [c]				
Stage AB	436	1.00	129	1.23 (0.96-1.56)
Stage CD	178	1.00	49	1.14 (0.81-1.62)
By tumor grade [bc]				
Low	506	1.00	133	1.09 (0.86-1.39)
High	288	1.00	88	1.28 (0.97-1.69)
Metastatic/Fatal prostate cancer [d]	133	1.00	37	1.17 (0.79-1.74)
By age at diagnosis				
<65 yrs	227	1.00	65	1.11 (0.77-1.58)
>= 65 yrs	591	1.00	158	1.14 (0.90-1.44)
By PSA era				
Pre-PSA (1982-1990)	232	1.00	55	0.92 (0.64-1.34)
Post-PSA (1991-2000)	586	1.00	168	1.20 (0.95-1.52)

[a] Risk ratio (RR) and 95% confidence interval (95% CI); unconditional logistic regression, adjusting for age at study onset and smoking status at baseline (never, past and current) and duration of follow-up.

[b] High grade was defined as Gleason 7-10 or poorly differentiated;

[c] Cases with unknown stage or Gleason sum were excluded from the analyses.

[d] Metastatic/Fatal prostate cancer, cases died of prostate cancer and those who developed metastasis during the follow-up.

Table 3. Prediagnostic plasma hormone levels by HIF-1α *P582S* genotype [a]

	HIF-1α *P582S CC*		HIF-1α *P582S CT or TT*	
	N	Mean (SE)	N	Mean (SE)
IGF-I[a]				
Cases	577	192.6 (2.9)	155	203.1 (4.9)
Controls	561	191.2 (2.9)	130	191.1 (5.4)
IGFBP-3 [b]				
Cases	578	3145 (33)	155	3261 (56)
Controls	561	3187 (33)	130	3081 (61)
VEGF [c]				
Cases	377	134.3 (34.4)	104	182.0 (58.8)
Controls	339	189.6 (36.5)	88	143.0 (63.8)

[a] Levels by genotype, P >0.05.

[b] Age-, smoking status- and batch-adjusted levels.

[c] Age- and smoking status-adjusted levels.

Table 4. Joint association of the HIF-1α *P582S* polymorphism and plasma levels of IGF-I and IGFBP-3 with prostate cancer [a]

	Plasma IGF-I level				Plasma IGFBP-3 level			
	Low		High		Low		High	
	No. of ca/co	RR	No. of ca/co	RR (95% CI)	No. of ca/co	RR (95% CI)	No. of ca/co	RR (95% CI)
Overall prostate cancer *(n = 661 cases and 661 controls)*								
HIF-1α *CC*	267/264	1.00 (Ref.)	250/271	0.99	280/253	1.00 (Ref.)	237/282	0.74 (0.57-0.97)
CT or TT	66/64	1.03	78/62	1.35 (0.91-2.01)	72/74	0.90	72/52	1.24
$P_{interaction}$			0.74				0.01	
Advanced-stage prostate cancer [b] *(n = 155 cases and 155 controls)*								
HIF-1α *CC*	63/72	1.00 (Ref.)	59/54	1.70	68/54	1.00 (Ref.)	54/72	0.45 (0.25-0.82)
CT or TT	14/16	0.89	19/13	2.01 (0.87-4.64)	18/19	0.66	15/10	0.92
$P_{interaction}$			0.50				0.14	
High-grade prostate cancer [c] *(n = 221 cases and 221 controls)*								
HIF-1α *CC*	95/96	1.00 (Ref.)	73/91	0.80	91/91	1.00 (Ref.)	77/96	0.86 (0.54-1.38)
CT or TT	25/16	1.67	28/18	1.69 (0.84-3.37)	31/21	1.58	22/13	2.25
$P_{interaction}$			0.80				0.77	
Metastatic/Fatal prostate cancer [d] *(n = 123 cases and 123 controls)*								
HIF-1α *CC*	55/48	1.00 (Ref.)	41/54	0.81	58/43	1.00 (Ref.)	38/59	0.47 (0.25-0.90)
CT or TT	12/12	0.84	15/9	1.57 (0.60-4.07)	17/15	0.87	10/6	1.02
$P_{interaction}$			0.51				0.10	

[a] Conditional logistic regression, levels of IGF-I and IGFBP-3 were mutually adjusted in all models.

[b] Advanced-stage prostate cancer: stage C or D cancer at diagnosis; cases with unknown stage were excluded from the analyses.

[c] High grade was defined as Gleason 7-10 or poorly differentiated at diagnosis; cases with unknown grade were excluded from the analyses.

[d] Metastatic/Fatal prostate cancer, cases died of prostate cancer and those who developed metastasis during the follow-up