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Interaction Among Variant Vascular Endothelial Growth Factor (VEGF) and Its Receptor in Relation to Prostate Cancer Risk

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Abstract

BACKGROUND—Prostate cancer (PCa) incidence and mortality are disproportionately high among African-American (AA) men. Its detection and perhaps its disparities could be improved through the identification of genetic susceptibility biomarkers within essential biological pathways. Interactions among highly variant genes, central to angiogenesis, may modulate susceptibility for prostate cancer, as previous demonstrated. This study evaluates the interplay among three highly variant genes (i.e., IL-10, TGF β R-1, VEGF), their receptors and their influence on PCa within a case-control study consisting of an under-served population.

METHODS—This study evaluated single gene and joint modifying effects on PCa risk in a case-control study comprised of 859 AA men (193 cases and 666 controls) using TaqMan qPCR. Interaction among polymorphic IL-10, TGF β R-1 and VEGF was analyzed using conventional logistic regression analysis (LR) models, multi-dimensionality reduction (MDR) and interaction entropy graphs. Symbolic modeling allowed validation of gene–gene interaction findings identified by MDR.

RESULTS—No significant single gene effects were demonstrated in relation to PCa risk. However, carriers of the VEGF 2482T allele had a threefold increase in the risk of developing aggressive PCa. The presence of VEGF 2482T combined with VEGFR IVS6 + 54 loci were highly significant for the risk of PCa based on MDR and symbolic modeling analyses. These findings were substantiated by 1,000-fold cross validation permutation testing ($P = 0.04$), respectively.

CONCLUSION—These findings suggest the inheritance of VEGF and VEGFR IVS6 + 54 sequence variants may jointly modify PCa susceptibility through their influence on angiogenesis. Larger sub-population studies are needed to validate these findings and evaluate whether the VEGF-VEGR axis may serve as predictors of disease prognosis and ultimately clinical response to available treatment strategies.

Keywords

prostate cancer; angiogenesis; single nucleotide polymorphisms; cytokines; gene–gene interactions; multifactor dimensionality reduction; African-American

INTRODUCTION

The American Cancer Society estimates 186,320 men will be diagnosed with prostate cancer (PCa) and 28,660 will die from the disease in 2008. The risk of developing PCa increases with age, family history, and race/ethnicity. Other risk factors of this disease may include inheritance of susceptibilities in high and low penetrance genes. Notably, inheritance of high (e.g., HPCa1, HPCAX, BrCA1, BRCA2, CAPB, PCaP, ELAC2/HPCA2) and low penetrance susceptibility genes may account for as much as 42–50% of all PCa cases [1–8]. Common sequence variants in low penetrance genes involved in important biological pathways required for tumor progression are prevalent in sporadic PCa cases and may account for more of the public health burden. Pathways that play a role in PCa tumorigenesis include chronic inflammation, immunosuppression, and angiogenesis.

Angiogenesis is a biological process involving the division and migration of endothelial cells, leading to the formation of microvasculature [9,10]. This process facilitates tumor growth by providing oxygenation to the tumor through a series of interrelated steps, including endothelial cell proliferation, motility of endothelial cells through the extracellular matrix toward the angiogenic stimuli, and capillary differentiation. Tumor cells and their microenvironment mediate angiogenesis by altering the expression of angiostatic and angiogenic cytokines, which promote and inhibit angiogenesis, respectively [11–15].

In patients with prostate and other cancers, IL-10, VEGF, and TGF- β 1 levels are frequently elevated and correlate with invasive disease, poor clinical outcome, progression, and metastasis [12,16–26]. Therefore, therapeutic agents inhibiting cytokine mediated angiogenesis can inhibit tumor growth and improve clinical outcomes [27].

Interleukin-10 (IL-10) is an important cytokine that acts as a double-edged sword in cancer development; however, the exact mechanisms remain unclear. IL-10 suppresses tumor immunity and supports tolerance to cancer cells [28,29]. Conversely, IL-10 may suppress

tumor growth and metastasis by inhibiting macrophage-derived angiogenic factors (e.g., vascular endothelial growth factor) [30–33].

Similar to IL-10, TGF- β 1 can have divergent roles in cancer development [34]. In normal epithelial, endothelial, and hematopoietic cells, TGF- β 1 suppresses tumor growth by promoting differentiation and inhibiting proliferation. During tumorigenesis, defects in TGF- β 1 signaling pathways cause cancer cells to become resistant to TGF- β 1-mediated growth inhibition and enhances angiogenesis and immunosuppression, improving tumor invasion and metastatic potential.

VEGF, serves a pivotal function during angiogenesis [35–37]. Six VEGF isoforms have been identified with lengths ranging from 121 to 206 amino acids [35,36,38–41]. These isoforms bind two major receptors, namely VEGF-R1 and VEGF-R2 [42–44]. Although VEGF-R1 has a greater affinity for VEGF, VEGF-R2 is tyrosine phosphorylated more efficiently upon ligand binding, leading to mitogenesis, chemotaxis, and changes in cell morphology of endothelial cells [41,45,46]. Elevated VEGF levels have been associated with prostate tumor progression and survival rate [47,48].

To our knowledge, there are no published reports emphasizing the intricate interplay among various highly variant angiogenesis-related genes and their joint modifying effects on PCa risk. To further clarify the impact of sequence variants detected in *IL-10*, *TGF β 1*, *VEGF* and their corresponding receptors in relation to PCa, the current study evaluated the individual and joint modifying effects of 15 sequence variants in relation to PCa risk among 859 Men of African descent. Based on their role in angiogenesis, we hypothesize inheritance *IL-10* low-expressing, *TGF β 1* high-expressing and *VEGF* high-expressing alleles will increase the risk of PCa and high tumor grade, than compared to the other alleles. These SNPs were chosen, in part, based on their capacity to alter protein/mRNA expression or relationship with cancer outcomes or other malignancies (Table I). With the ultimate goal of improving clinical management practices, we anticipate the current study will provide a strong foundation for identifying and validating molecular tools capable of enhancing more appropriately cancer detection and individualized treatment strategies among prostate cancer patients.

MATERIALS AND METHODS

Study Population and Data Management

Unrelated male residents (n = 1,061) of Washington D.C. and Columbia SC, were considered for eligibility in the current PCA case control study. Study participants (n = 202) were not considered in the current study if they met one or more of the following exclusion criteria: (1) they were diagnosed with benign prostatic hyperplasia (n = 62); (2) had an abnormal PSA and DRE (n = 15); (3) had European ancestry based on a Global Ancestry score of <25% (n = 71) [49]; and (4) poor quality DNA (n = 62). Eligible men of African descent (i.e., self-identified African-Americans, East Africans, West Africans, and Afro-Caribbeans), including 193 patients (ages 41–91) and 666 healthy volunteers (ages 26–89), were recruited from the Howard University Hospital (HUH) Division of Urology PCA patient population, the HUH PCA screening program, and the South Carolina PCA

screening program (Table I). The PCA patients and controls were recruited between 2001 and 2005. Incident PCA cases in the current study were identified by an HUH urologist based on abnormal prostate-specific antigen (PSA) and/or digital rectal examination (DRE) as well as histological findings following a radical prostatectomy. Inclusion criteria of controls were men with PSA levels less than 4.0 ng/ml and/or normal DREs/biopsies. Clinical characteristics including Gleason score, PSA, and age at diagnosis for the study participants were obtained from medical records, as detailed in Table II. Tumor grade, ranging from 4 to 10, was collected for 60.6% of the cases (n = 117). Cases (n = 58) were classified with aggressive disease if they had a Gleason score ≥ 7 . Histopathological grade was recorded as the Gleason score. Specifically histological grades I/II and III were equivalent to Gleason scores 4–7 and 8–10, respectively. All study participants had available DNA extracted from whole blood and provided written informed consent for participation in genetic analysis studies under a protocol approved by the Howard University, the HUH Division of Urology, and the University of Louisville Institutional Review Board.

Allelic Discrimination of IL-10, IL-10R, TGF β R-1, TGF β -1, VEGF, and VEGFR Sequence Variants

Polymorphisms in six angiogenesis-related genes were ascertained using TaqMan Polymerase Chain Reaction (PCR) allelic discrimination assays [50]. The following 15 alleles were detected: (1) *IL-10* (G⁻¹⁰⁸²A, C⁻⁸¹⁹T, C⁻⁵⁹²A); (2) *IL-10R* (A⁻¹⁵³G, G⁻¹⁰⁹A, A²⁴¹G); (3) *TGF- β 1* (T⁸⁹⁶C, C⁻⁵⁰⁹T); (4) *TGF- β R1* (A¹⁹⁵G); (5) *VEGF* (C²⁴⁸²T, C²⁵⁷⁸A); and (6) *VEGFR2* (IVS6 A⁵⁴G, G⁸⁸⁹A, T¹⁴¹⁶T, IVS25 G⁻⁹²A). The sequences for the primers and probes for *IL-10* (G⁻¹⁰⁸²A) was obtained from ABI and those for, *IL-10R* Exon 7 (G⁻¹⁰⁹A), and *TGF- β R1* (Exon 9 A¹⁹⁵G) were found in the NCI SNP500 database. PrimerExpress 3.0 software (Applied Bio-systems, Foster City, CA) was used to design allelic discrimination primer and probes for: *IL-10* (C⁻⁸¹⁹T, C⁻⁵⁹²A); *IL-10R* (A⁻¹⁵³G, A²⁴¹G); *VEGF* (C²⁴⁸²T, C²⁵⁷⁸A); *VEGFR* (IVS6 + A⁵⁴G, G⁸⁸⁹A, IVS25G⁻⁹²A, and T¹⁴¹⁶A); and *TGF β -1* (C⁻⁵⁰⁹T, T⁸⁹⁶C). The discrimination assay contained approximately 40 ng of germ-line DNA, 1X Universal Master Mix (Applied Biosystems), 300 nM of each primer (forward and reverse), and 100 nM of each probe (FAM and VIC) to comprise a 10 μ l reaction. PCR reactions were carried out in an ABI Prism 7900HT Sequence Detection System (Applied Biosystems). The thermocycling settings consisted of two holds at 50°C for 2 min and 95°C for 10 min, followed by 40–42 cycles of 15 sec at 95°C, and 1 min at a specific temperature for each individual SNP. PCR reactions were completed and fluorescent intensity from the probes was measured using the ABI 7900 sequence detection system. Genotypes were assigned by SDS 2.2.1 software (Applied Biosystems). To minimize misclassification bias, laboratory technicians were blinded to the case status of subjects. Based on 24 non-DNA template controls per batch analysis, the percent cross-contamination during sample handling was minimal (4.7%). Duplicate genotyping was performed for 72 randomly selected samples for quality control purposes, resulting in concordance rates 97.5%. The genotype call rates ranged between 89.6 and 96.2% across the fifteen SNPs. Subjects (n = 62) who had 8 or more missing SNPs values across the 15 SNPs were removed from the final analysis (i.e., 27 cases and 35 controls). In addition, deviations from the Hardy–Weinberg equilibrium among controls were tested using a significance level of $P < 0.005$.

Ancestry markers—One hundred ancestry previously validate autosomal markers were included to account for potential population stratification among our admixed population of self-reported African-Americans, West African, East African, Afro-Caribbean, as previously described [51]. Study participants were grouped from lowest to highest genetic West African Ancestry, with scores ranging from 0% to 100%. These 100 markers were assembled using DNA from self-identified African-Americans (Coriell Institute for Medical Research, n = 96), Yoruban West Africans (HapMap, n = 60), West Africans (Bantu and Nilo Saharan speakers, n = 72), Europeans (New York City, n = 24), and CEPH Europeans (HapMap Panel, n 60), as previously reported [51]. Individuals (n = 859) with a high degree of West African Ancestry greater than or equal to 75% were considered in the final analysis.

Evaluation of single gene markers predictive of PCa risk using conventional logistic regression—To assess whether individuals possessing at least one variant angiogenesis-related allele have an elevated risk of developing PCa, we tested for significant differences in the distribution 15 cytokine genotypes between 193 cases and 666 controls using the chi-square test of homogeneity. Associations between PCa risk and candidate polymorphic genes, expressed as odds ratios (ORs) and corresponding 95% confidence intervals (CIs), were estimated using unconditional multivariate logistic regression (LR) models adjusted for potential confounders (age, PSA, and WAA). To account for population admixture, percentage of West African Ancestry was estimated using a panel of SNP markers and included as a covariate in all LR models. All reported risk estimates and 95% CIs for the selected polymorphic genes used the following as reference genotypes: *IL-10* -1082 G/G, *IL-10* -819 C/C, *IL-10* -592 C/C, *IL-10R* -153 G/G, *IL-10R* -109 G/G, *IL-10R* 241 G/G, *TGFβ-1* -896 T/T, *TGFβ-1* -509 C/C, *TGFβR-1* +195 A/A, *VEGF* 2482 C/C, *VEGF* 2578 A/A, *VEGFR* 889 G/G, *VEGFR* IVS25 -92 G/G, *VEGFR* IVS6 +54 A/A. For *VEGFR* 1416 the minor homozygous alleles (AA) were combined with the heterozygous (AT) genotype because of their small cell count. Test for trend included genotypes as ordinal variables. Statistical significance was assessed using a *P*-value <0.05. All chi-square test and LR analyses were conducted using SAS 9.1.3.

Evaluating gene combination effects using multifactor dimensionality reduction—To complement LR analyses, multifactor dimensionality reduction (MDR) was used to further evaluate gene–gene interactions associated with PCa risk. The details of MDR are detailed and reviewed elsewhere [52,53]. We used 10-fold cross-validation to estimate the average testing accuracy (ATA) and cross-validation consistency (CVC) of MDR models. The MDR model with the highest ATA and CVC was selected as the overall best model. Statistical significance was evaluated using a 1,000-fold permutation test. We considered MDR permutation results to be statistically significant at the 0.05 level. The MDR software is open-source and freely available online [54].

Interaction graphs—Interaction entropy was used as a third strategy to verify, visualize, and interpret combination effects identified by LR and MDR [55,56]. Interaction entropy uses information gain (IG) to gauge whether interactions between two or more variables provide more information about a class variable relative to each variable considered independently [55] and has been applied to several recent epidemiological studies [55,57–

59]. The colors range from red representing a high degree of synergy (positive information gain), orange a lesser degree, and gold representing independence and a midway point between synergy and redundancy. Blue represents the highest level of redundancy (negative information gain), followed by green.

Validate MDR higher order interaction models using symbolic modeling—

Symbolic modeling (SyMod) was used as a nonparametric and model-free approach to detect nonlinear gene–gene interactions. SyMod accepts a list of attributes (e.g., SNPs) along with a list of mathematical functions and then uses genetic programming as a stochastic search algorithm to identify an optimal model that can take any shape or form. An advantage of this approach is that it doesn't make any assumption about the functional form of the model beyond the basic mathematical functions that are provided as building blocks. A symbolic model is developed within the framework of discriminant analysis. The SyMod software has been previously described in detail by Moore et al. [60]. Configuration parameters included a population size of 100 models, 500 generations, a crossover rate of 0.9 and a mutation rate of 0.1. With these settings a maximum of 50,000 models were explored. A three-way cross-validation strategy was used to prevent overfitting as described by Moore et al. [60]. Expert knowledge in the form of ReliefF scores [61] were used in a multiobjective fitness function [62] and to help guide selection [63].

RESULTS

Patient and Tumor Characteristics

The patient and tumor characteristics in the current study are summarized in Table I. Cases were significantly older than controls and had higher PSA levels. Although there was a small portion of controls (5.4%) who had PSA levels that exceeded 4.0 ng/ml, these individuals did not have an abnormal DRE or irregular biopsy. There was no significant difference in median West African genetic ancestry estimates comparing cases and controls ($P = 0.065$).

Prevalence of Angiogenesis-Related Alleles Among Men of African Descent

Fifteen SNPs were detected within 6 highly variant angiogenesis-associated genes among ~91.5–95.9% of the study participants. The genotype frequencies among controls did not deviate from the Hardy–Weinberg equilibrium ($P = 0.056$) with the exception of marginal departures for *TGFβR1* 195 ($P = 0.005$). The reason for this deviation is unknown. Within the current study set, inheritance of at least one minor or “high-risk” (linked with reduced mRNA/protein expression) *IL-10*⁻¹⁰⁸²A (56.4%), *IL-10*⁻⁸¹⁹T (61.3%), *IL-10*⁻⁵⁹²A (61.4%), *IL-10RA*⁻¹⁵³G (69.8%), *IL-10R*⁻¹⁰⁹G (56.7%), *IL-10R*²⁴¹A (35.1%), *TGFβ1*⁻⁵⁰⁹T (38.5%), *TGFβ1*⁻⁸⁹⁶C (62.0%), *TGFβ-R1*⁺¹⁹⁵G (31.6%), *VEGF*²⁴⁸²T (27.1%), *VEGF*²⁵⁷⁸C (94.5%), *VEGFR*⁸⁸⁹A (40.7%), *VEGFR*¹⁴¹⁶A (16.9%), *VEGFR IVS25*⁻⁹²A (54.4%), and *VEGFR IVS6*⁺⁵⁴A (65.7%) was fairly common among controls (Table I). The genotype frequencies among controls were in agreement with the NCBI and NCI SNP500 databases [50,64].

Single Gene, Haplotype, and Combination Effects

The current study evaluated the independent effects of genetic variations in highly variant cytokines in relation to PCa susceptibility using unconditional LR models. No significant main effects were observed in relation to PCa risk among our study participants (Table III). Moreover, with the exception of *VEGF* C2482T, no significant relationships were revealed in relation to disease progression. Notably, individuals who possessed two *VEGF* 2482T alleles had a ~3-fold increase in PCa risk (OR = 3.11; 95% CI = 1.23–7.89) (Table IV). The capacity of single loci as well as complex interaction models, involving two or more highly variant bases and nucleotides, were also evaluated to predict cancer risk using the MDR data-mining tool. While *IL-10* –1082 was the best single factor to predict PCa risk, this loci did not reach statistical significance following MDR cross-validation or 1,000-fold cross validation (CVC = 60%; $P = 0.828$). There was no significant relationship between a commonly studied *IL10* haplotype (ordered as –1082, –819, –592) and PCa risk. Relative to 91 pairwise SNP combinations, permutation testing revealed both two- and four-factor models as the best predictors of PCa. Since two- and three-factor models shared comparable outcome measures (i.e., CVC, accuracy, sensitivity, and specificity), the two-factor model was selected as the overall best as well as parsimonious model. *VEGF* 2482 and *VEGFR* IVS 6 + 54 had the highest CVC (100%) and classification accuracy (58.9%) (P -value = 0.04 from permutation testing). Symbolic modeling confirmed these MDR findings with an overall accuracy of 0.584 (data not shown).

Interaction Entropy Graphs

Following identification of high-risk loci using MDR, the interpretation of the relationship among the selected variables was facilitated using interaction entropy algorithms. As indicated in the hierarchical interaction graph (Fig. 1), the *VEGF* 2482/*VEGFR* IVS 6 + 54 SNP pair had the highest degree of entropy percentage (0.99%) when compared to individual markers and pairwise SNP combinations.

DISCUSSION

Angiogenesis plays a fundamental role in neoplasm development and is essential for tumor progression [65] by promoting endothelial cell proliferation, motility and capillary differentiation.

In the current study, it was hypothesized that interactions among two or more variant angiogenesis-associated genes significantly modify PCa risk when compared to possession of referent alleles. Consistent with our hypothesis, it appears complex interactions between two sequence variants detected within the *VEGF* at position 2482 and its receptor (*VEGF* IVS 6+54) serve as predictors of PCa risk among our study participants based on MDR (permutation $P < 0.04$) and Symbolic modeling. While our findings substantiate the need for a multi-faceted statistical approach to identify markers as effective predictors of PCa risk, confirmation of these findings will require larger studies.

Interestingly, this is the first time these two angiogenic markers in combination have been evaluated in relation to PCa. While others have observed a relationship between commonly

studied angiogenesis-related cytokines and various cancer malignancies, sequence variations in *IL-10* (−1082, −819, −592), *TGFB1* (869, −509), and their corresponding receptors have not been individually linked with PCa. To our knowledge, this is the first published report to demonstrate a threefold increase in the risk of developing aggressive PCa (i.e., Gleason score 7) among carriers of the *VEGF* 2482 TT genotype.

The observation of *VEGF* 2482 sequence variant combined, with *VEGFR1* IVS6 + 54, serving as PCa detection tools may be explained by their synergistic effects in relation to neovascularization and tumorigenesis. Indeed, VEGF mediates angiogenesis [35–37] and supports, tumor growth [66] primarily through interaction with one of its receptors, namely VEGFR (Flk-1/KDR) [67]. PCa, both VEGF and VEGFR-1 are both over-expressed and correlate with malignant transformation [67]. Their co-expression provides additional evidence of their synergistic role in the progression of PCa [66].

Our findings beg the question what are the functional consequences of the *VEGFR* intronic and *VEGF* 3′ untranslated SNPs on mRNA expression and stability. The *VEGF* C>T change at position 2482 results in the loss of an important repressor element silencing transcription factor (REST/NRSF). Based on previously published studies [68,69] in silico analysis (<http://www.genomatix.de/cgi-bin/.eldorado/main.pl>) [70], we speculate the loss of the “C” allele may result in the loss of the REST/NRSF binding site. This would result in diminished capacity to repress *VEGF* transcription. It has also been suggested that dysregulated *VEGF* contributes to tumor progression through enhanced angiogenesis [71,72]. Similarly, the *VEGFR* A to G change at position IVS6+54 results in the gain of a CCAAT/enhancer binding protein (C/EBP) site [70]. The presence of a C/EBP site would enable leucine-zipper transcription factors to bind and promote the transcription of *VEGFR* [70,73]. There is emerging evidence that a C/EBPβ protein isoforms (i.e., LIP) disrupt normal growth, development and tumorigenesis. Interestingly, neither of the aforementioned SNPs alone significantly modified PCa risk; however, both MDR and symbolic modeling revealed a complex interaction between these two markers may contribute to PCa risk. Future studies will assess the functional consequence of these angiogenesis-associated SNPs. In addition, on-going studies will allow us to determine whether sequence variants in the VEGF-VEGR axis may serve as important predictors of disease prognosis and ultimately clinical response to angiogenesis inhibitors (e.g., bevacizumab or carboxyamidotriazole) [27,74]. In the later case, inheritance of susceptibilities these angiogenic markers may guide dosing regimens of selective therapies based on the genetic profile of angiogenic targets, resulting in more appropriate individualized treatment for prostate cancer patients.

The strengths and challenges were considered in the present study. It is plausible that the observed associations are not related to linkage disequilibrium of the studied loci with neighboring SNPs. Future studies will evaluate a parsimonious panel of angiogenesis-associated SNPs using available genomic databases (e.g., HapMap and NCBI) to identify other angiogenic factors that contribute to PCa risk. The controls, recruited from PCa screening programs, are subject to potential misclassification in that they may represent higher risk populations attributed to self-selection. Selection bias may stem from the fact that the groups under consideration may differ substantially in their reasons for screening. For instance, individuals who attend a cancer screening program may have a higher

proportion of 1st degree relatives with PCa than those who fail to participate. Consequently, screeners may have a worse prognosis, regardless of their inheritance of variant high penetrance genes. Self-selection bias cannot be completely ruled out, since the current study was not specifically designed to compare selected demographics and other baseline characteristics between screeners and non-screeners. Subsequent studies will attempt to prevent this type of selection bias by ensuring that the cancer screening decisions are not influenced by characteristics of the study population. To minimize control bias, men were excluded if they: (1) were diagnosed with benign prostate hyperplasia (BPH); or (2) had elevated prostate specific antigen (PSA) levels > 4ng/ml and/or an irregular digital rectal examination (DRE). Fortunately, the likelihood of genotype misclassification is limited because the concordance rate for 288 duplicate quality control samples was 97.5%.

In conclusion, our findings suggest the inheritance of *VEGF* C²⁴⁸²T/*KDR* IVS6A⁺⁵⁴G sequence variants serve as a predictor of PCa risk among African-American men. These findings require validation in larger observational studies. Such studies will analyze a comprehensive panel of genes involved in angiogenesis as well as tumor lymphangiogenesis [*VEGF*-A, -B, -C, -D, -E, and their corresponding receptors *VEGFR*-1, -2, -3]. By utilizing a pathway-wide approach, we hope to elucidate other genes in various pathways that will serve as prognostic as well as diagnostic indicators for PCa.

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Abbreviations

PCR	polymerase chain reaction
PCa	prostate cancer
AA	African-American
SNP	single nucleotide polymorphism
MDR	multifactor dimensionality reduction

References

1. Lichtenstein P, Holm NV, Verkasalo PK, Iliadou A, Kaprio J, Koskenvuo M, Pukkala E, Skytthe A, Hemminki K. Environmental and heritable factors in the causation of cancer—analyses of cohorts of twins from Sweden, Denmark, and Finland. *N Engl J Med*. 2000; 343:78–85. [PubMed: 10891514]
2. Michaud DS, Daugherty SE, Berndt SI, Platz EA, Yeager M, Crawford ED, Hsing A, Huang WY, Hayes RB. Genetic polymorphisms of interleukin-1B (IL-1B), IL-6, IL-8, and IL-10 and risk of prostate cancer. *Cancer Res*. 2006; 66:4525–4530. [PubMed: 16618781]
3. Tavtigian SV, Simard J, Teng DH, Abtin V, Baumgard M, Beck A, Camp NJ, Carillo AR, Chen Y, Dayananth P, Desrochers M, Dumont M, Farnham JM, Frank D, Frye C, Ghaffari S, Gupte JS, Hu R, Iliev D, Janecki T, Kort EN, Laity KE, Leavitt A, Leblanc G, McArthur-Morrison J, Pederson A, Penn B, Peterson KT, Reid JE, Richards S, Schroeder M, Smith R, Snyder SC, Swedlund B, Swensen J, Thomas A, Tranchant M, Woodland AM, Labrie F, Skolnick MH, Neuhausen S,

- Rommens J, Cannon-Albright LA. A candidate prostate cancer susceptibility gene at chromosome 17p. *Nat Genet.* 2001; 27:172–180. [PubMed: 11175785]
4. Gibbs M, Stanford JL, McIndoe RA, Jarvik GP, Kolb S, Goode EL, Chakrabarti L, Schuster EF, Buckley VA, Miller EL, Brandzel S, Li S, Hood L, Ostrander EA. Evidence for a rare prostate cancer-susceptibility locus at chromosome 1p36. *Am J Hum Genet.* 1999; 64:776–787. [PubMed: 10053012]
 5. Xu J, Meyers D, Freije D, Isaacs S, Wiley K, Nusskern D, Ewing C, Wilkens E, Bujnovszky P, Bova GS, Walsh P, Isaacs W, Schleutker J, Matikainen M, Tammela T, Visakorpi T, Kallioniemi OP, Berry R, Schaid D, French A, McDonnell S, Schroeder J, Blute M, Thibodeau S, Gronberg H, Emanuelsson M, Damber JE, Bergh A, Jonsson BA, Smith J, Bailey-Wilson J, Carpten J, Stephan D, Gillanders E, Amundson I, Kainu T, Freas-Lutz D, Baffoe-Bonnie A, Van AA, Sood R, Collins F, Brownstein M, Trent J. Evidence for a prostate cancer susceptibility locus on the X chromosome. *Nat Genet.* 1998; 20:175–179. [PubMed: 9771711]
 6. Berthon P, Valeri A, Cohen-Akenine A, Drelon E, Paiss T, Wöhr G, Latil A, Millasseau P, Mellah I, Cohen N, Blanche H, Bellane-Chantelot C, Demenais F, Teillac P, Le DA, de PR, Hautmann R, Chumakov I, Bachner L, Maitland NJ, Lidereau R, Vogel W, Fournier G, Mangin P, Cussenot O. Predisposing gene for early-onset prostate cancer, localized on chromosome 1q42.2–43. *Am J Hum Genet.* 1998; 62:1416–1424. [PubMed: 9585607]
 7. Smith JR, Freije D, Carpten JD, Gronberg H, Xu J, Isaacs SD, Brownstein MJ, Bova GS, Guo H, Bujnovszky P, Nusskern DR, Damber JE, Bergh A, Emanuelsson M, Kallioniemi OP, Walker-Daniels J, Bailey-Wilson JE, Beaty TH, Meyers DA, Walsh PC, Collins FS, Trent JM, Isaacs WB. Major susceptibility locus for prostate cancer on chromosome 1 suggested by a genome-wide search. *Science.* 1996; 274:1371–1374. [PubMed: 8910276]
 8. Ford D, Easton DF, Bishop DT, Narod SA, Goldgar DE. Risks of cancer in BRCA1-mutation carriers. Breast Cancer Linkage Consortium. *Lancet.* 1994; 343:692–695. [PubMed: 7907678]
 9. Folkman J. Angiogenesis in psoriasis: Therapeutic implications. *J Invest Dermatol.* 1972; 59:40–43. [PubMed: 5039962]
 10. Giavazzi R, Taraboletti G. Angiogenesis and angiogenesis inhibitors in cancer. *Forum (Genova).* 1999; 9:261–272. [PubMed: 10504172]
 11. Huang S, Ullrich SE, Bar-Eli M. Regulation of tumor growth and metastasis by interleukin-10: The melanoma experience. *J Interferon Cytokine Res.* 1999; 19:697–703. [PubMed: 10454339]
 12. Stearns ME, Rhim J, Wang M. Interleukin 10 (IL-10) inhibition of primary human prostate cell-induced angiogenesis: IL-10 stimulation of tissue inhibitor of metalloproteinase-1 and inhibition of matrix metalloproteinase (MMP)-2/MMP-9 secretion. *Clin Cancer Res.* 1999; 5:189–196. [PubMed: 9918218]
 13. Folkman J, Shing Y. Angiogenesis. *J Biol Chem.* 1992; 267:10931–10934. [PubMed: 1375931]
 14. Ek ET, Ojaimi J, Kitagawa Y, Choong PF. Outcome of patients with osteosarcoma over 40 years of age: Is angiogenesis a marker of survival? *Int Semin Surg Oncol.* 2006; 3:7. [PubMed: 16551370]
 15. Zetter BR. Angiogenesis and tumor metastasis. *Annu Rev Med.* 1998; 49:407–424. [PubMed: 9509272]
 16. Kong FM, Washington MK, Jirtle RL, Anscher MS. Plasma transforming growth factor-beta 1 reflects disease status in patients with lung cancer after radiotherapy: A possible tumor marker. *Lung Cancer.* 1996; 16:47–59. [PubMed: 9017584]
 17. Hasegawa Y, Takanashi S, Kanehira Y, Tsushima T, Imai T, Okumura K. Transforming growth factor-beta1 level correlates with angiogenesis, tumor progression, and prognosis in patients with nonsmall cell lung carcinoma. *Cancer.* 2001; 91:964–971. [PubMed: 11251948]
 18. Kong F, Jirtle RL, Huang DH, Clough RW, Anscher MS. Plasma transforming growth factor-beta1 level before radiotherapy correlates with long term outcome of patients with lung carcinoma. *Cancer.* 1999; 86:1712–1719. [PubMed: 10547543]
 19. Picon A, Gold LI, Wang J, Cohen A, Friedman E. A subset of metastatic human colon cancers expresses elevated levels of transforming growth factor beta1. *Cancer Epidemiol Biomarkers Prev.* 1998; 7:497–504. [PubMed: 9641494]

20. Ardizzoia A, Lissoni P, Brivio F, Tisi E, Perego MS, Grassi MG, Pittalis S, Crispino S, Barni S, Tancini G. Tumor necrosis factor in solid tumors: Increased blood levels in the metastatic disease. *J Biol Regul Homeost Agents*. 1992; 6:103–107. [PubMed: 1492596]
21. Dummer W, Becker JC, Schwaaf A, Leverkus M, Moll T, Brocker EB. Elevated serum levels of interleukin-10 in patients with metastatic malignant melanoma. *Melanoma Res*. 1995; 5:67–68. [PubMed: 7734958]
22. Derynck R, Goeddel DV, Ullrich A, Gutterman JU, Williams RD, Bringman TS, Berger WH. Synthesis of messenger RNAs for transforming growth factors alpha and beta and the epidermal growth factor receptor by human tumors. *Cancer Res*. 1987; 47:707–712. [PubMed: 3467839]
23. Dickson RB, Kasid A, Huff KK, Bates SE, Knabbe C, Bronzert D, Gelmann EP, Lippman ME. Activation of growth factor secretion in tumorigenic states of breast cancer induced by 17 beta-estradiol or v-Ha-ras oncogene. *Proc Natl Acad Sci U S A*. 1987; 84:837–841. [PubMed: 2880347]
24. Linderholm B, Lindh B, Tavelin B, Grankvist K, Henriksson R. p53 and vascular-endothelial-growth-factor (VEGF) expression predicts outcome in 833 patients with primary breast carcinoma. *Int J Cancer*. 2000; 89:51–62. [PubMed: 10719731]
25. Giatromanolaki A, Koukourakis MI, Sivridis E, O'Byrne K, Cox G, Thorpe PE, Gatter KC, Harris AL. Coexpression of MUC1 glycoprotein with multiple angiogenic factors in non-small cell lung cancer suggests coactivation of angiogenic and migration pathways. *Clin Cancer Res*. 2000; 6:1917–1921. [PubMed: 10815916]
26. Howell WM, Turner SJ, Bateman AC, Theaker JM. IL-10 promoter polymorphisms influence tumour development in cutaneous malignant melanoma. *Genes Immun*. 2001; 2:25–31. [PubMed: 11294564]
27. Tan WW. Novel agents and targets in managing patients with metastatic prostate cancer. *Cancer Control*. 2006; 13:194–198. [PubMed: 16885915]
28. Richter G, Kruger-Krasagakes S, Hein G, Huls C, Schmitt E, Diamantstein T, Blankenstein T. Interleukin 10 transfected into Chinese hamster ovary cells prevents tumor growth and macrophage infiltration. *Cancer Res*. 1993; 53:4134–4137. [PubMed: 8364905]
29. Nabioullin R, Sone S, Mizuno K, Yano S, Nishioka Y, Haku T, Ogura T. Interleukin-10 is a potent inhibitor of tumor cytotoxicity by human monocytes and alveolar macrophages. *J Leukoc Biol*. 1994; 55:437–442. [PubMed: 8145013]
30. Stearns ME, Wang M. Antimetastatic and antitumor activities of interleukin 10 in transfected human prostate PC-3 ML clones: Orthotopic growth in severe combined immunodeficient mice. *Clin Cancer Res*. 1998; 4:2257–2263. [PubMed: 9748147]
31. Huang S, Xie K, Bucana CD, Ullrich SE, Bar-Eli M. Interleukin 10 suppresses tumor growth and metastasis of human melanoma cells: Potential inhibition of angiogenesis. *Clin Cancer Res*. 1996; 2:1969–1979. [PubMed: 9816156]
32. Sato T, McCue P, Masuoka K, Salwen S, Lattime EC, Mastrangelo MJ, Berd D. Interleukin 10 production by human melanoma. *Clin Cancer Res*. 1996; 2:1383–1390. [PubMed: 9816311]
33. Balkwill F, Mantovani A. Inflammation and cancer: Back to Virchow? *Lancet*. 2001; 357:539–545. [PubMed: 11229684]
34. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. *Nat Genet*. 2001; 29:117–129. [PubMed: 11586292]
35. Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science*. 1989; 246:1306–1309. [PubMed: 2479986]
36. Tischer E, Mitchell R, Hartman T, Silva M, Gospodarowicz D, Fiddes JC, Abraham JA. The human gene for vascular endothelial growth factor. Multiple protein forms are encoded through alternative exon splicing. *J Biol Chem*. 1991; 266:11947–11954. [PubMed: 1711045]
37. Claffey KP, Robinson GS. Regulation of VEGF/VPF expression in tumor cells: Consequences for tumor growth and metastasis. *Cancer Metastasis Rev*. 1996; 15:165–176. [PubMed: 8842488]
38. Houck KA, Ferrara N, Winer J, Cachianes G, Li B, Leung DW. The vascular endothelial growth factor family: Identification of a fourth molecular species and characterization of alternative splicing of RNA. *Mol Endocrinol*. 1991; 5:1806–1814. [PubMed: 1791831]

39. Poltorak Z, Cohen T, Sivan R, Kandelis Y, Spira G, Vlodavsky I, Keshet E, Neufeld G. VEGF145, a secreted vascular endothelial growth factor isoform that binds to extracellular matrix. *J Biol Chem.* 1997; 272:7151–7158. [PubMed: 9054410]
40. Lei J, Jiang A, Pei D. Identification and characterization of a new splicing variant of vascular endothelial growth factor: VEGF183. *Biochim Biophys Acta.* 1998; 1443:400–406. [PubMed: 9878851]
41. Robinson CJ, Stringer SE. The splice variants of vascular endothelial growth factor (VEGF) and their receptors. *J Cell Sci.* 2001; 114:853–865. [PubMed: 11181169]
42. Lu H, Shu XO, Cui Y, Kataoka N, Wen W, Cai Q, Ruan ZX, Gao YT, Zheng W. Association of genetic polymorphisms in the VEGF gene with breast cancer survival. *Cancer Res.* 2005; 65:5015–5019. [PubMed: 15958542]
43. Ferrara N. VEGF and the quest for tumour angiogenesis factors. *Nat Rev Cancer.* 2002; 2:795–803. [PubMed: 12360282]
44. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med.* 2003; 9:669–676. [PubMed: 12778165]
45. Waltenberger J, Claesson-Welsh L, Siegbahn A, Shibuya M, Heldin CH. Different signal transduction properties of KDR and Flt1, two receptors for vascular endothelial growth factor. *J Biol Chem.* 1994; 269:26988–26995. [PubMed: 7929439]
46. Quinn TP, Peters KG, De VC, Ferrara N, Williams LT. Fetal liver kinase 1 is a receptor for vascular endothelial growth factor and is selectively expressed in vascular endothelium. *Proc Natl Acad Sci U S A.* 1993; 90:7533–7537. [PubMed: 8356051]
47. Peyromaure M, Camparo P, Badoual C, Descazeaud A, Dinh-Xuan AT. The expression of vascular endothelial growth factor is associated with the risk of cancer progression after radical prostatectomy. *BJU Int.* 2007; 99:1150–1153. [PubMed: 17437444]
48. Green MM, Hiley CT, Shanks JH, Bottomley IC, West CM, Cowan RA, Stratford IJ. Expression of vascular endothelial growth factor (VEGF) in locally invasive prostate cancer is prognostic for radiotherapy outcome. *Int J Radiat Oncol Biol Phys.* 2007; 67:84–90. [PubMed: 17189065]
49. Giri VN, Egleston B, Ruth K, Uzzo RG, Chen DY, Buyyounouski M, Raysor S, Hooker S, Torres JB, Ramike T, Mastalski K, Kim TY, Kittles R. Race, genetic West African ancestry, and prostate cancer prediction by prostate-specific antigen in prospectively screened high-risk men. *Cancer Prev Res (Phila Pa).* 2009; 2:244–250.
50. Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L, Sicotte H, Staats B, Acharya M, Crenshaw A, Eckert A, Puri V, Gerhard DS, Chanock SJ. SNP500Cancer: A public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. *Nucleic Acids Res.* 2006; 34:D617–D621. [PubMed: 16381944]
51. Tian C, Hinds DA, Shigeta R, Kittles R, Ballinger DG, Seldin MF. A genomewide single-nucleotide-polymorphism panel with high ancestry information for African American admixture mapping. *Am J Hum Genet.* 2006; 79:640–649. [PubMed: 16960800]
52. Ritchie MD, Hahn LW, Roodi N, Bailey LR, Dupont WD, Parl FF, Moore JH. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. *Am J Hum Genet.* 2001; 69:138–147. [PubMed: 11404819]
53. Moore JH. Computational analysis of gene-gene interactions using multifactor dimensionality reduction. *Expert Rev Mol Diagn.* 2004; 4:795–803. [PubMed: 15525222]
54. <http://www.epistasis.org>.
55. Jakulin, A.; Bratko, I. Analyzing attribute dependencies. In: Lavrac, N.; Gamberger, D.; Blockeel, H.; Todorovski, L., editors. *PKDD 2003*. Cavtat, Croatia: Springer-Verlag; 2003. p. 229-240.
56. Demosar, J.; Zupan, B. Orange: From experimental machine learning to interactive data mining. 2004. White Paper
57. Ritchie MD, Hahn LW, Moore JH. Power of multifactor dimensionality reduction for detecting gene-gene interactions in the presence of genotyping error, missing data, phenocopy, and genetic heterogeneity. *Genet Epidemiol.* 2003; 24:150–157. [PubMed: 12548676]
58. Andrew AS, Nelson HH, Kelsey KT, Moore JH, Meng AC, Casella DP, Tosteson TD, Schned AR, Karagas MR. Concordance of multiple analytical approaches demonstrates a complex relationship

- between DNA repair gene SNPs, smoking, and bladder cancer susceptibility. *Carcinogenesis*. 2005;1030–1037. [PubMed: 16311243]
59. Jakulin, A.; Bratko, I.; Smrike, D.; Demsar, J.; Zupan, B. Attribute interactions in medical data analysis. *Protarus: Cyprus*; 2003. p. 229-238.
 60. Moore, JH.; Barney, N.; White, BC. *Genetic Programming Theory and Practice V*. Springer US; 2008. Solving complex problems in human genetics using genetic programming: The importance of the theorist-practitioner-computer interaction; p. 69-85.
 61. Moore, JH.; White, BC. *Evolutionary Computation, Machine Learning and Data Mining in Bioinformatics*. Springer Berlin/Heidelberg; 2007. Tuning ReliefF for genome-wide genetic analysis; p. 166-175.
 62. Moore, JH.; White, BC. *Genetic Programming Theory and Practice IV*. Springer US; 2007. Genome-wide genetic analysis using genetic programming: The critical need for expert knowledge; p. 11-28.
 63. Moore, JH.; White, BC. *Parallel Problem Solving from Nature – PPSN IX*. Springer Berlin/Heidelberg; 2006. Exploiting expert knowledge in genetic programming for genome-wide genetic analysis; p. 969-977.
 64. National Center for Biotechnology Information (NCBI). <http://www.ncbi.nih.gov>.
 65. Kaya A, Ciledag A, Gulbay BE, Poyraz BM, Celik G, Sen E, Savas H, Savas I. The prognostic significance of vascular endothelial growth factor levels in sera of non-small cell lung cancer patients. *Respir Med*. 2004; 98:632–636. [PubMed: 15250229]
 66. Sfar S, Hassen E, Saad H, Mosbah F, Chouchane L. Association of VEGF genetic polymorphisms with prostate carcinoma risk and clinical outcome. *Cytokine*. 2006; 35:21–28. [PubMed: 16908180]
 67. Kollerma J, Helpap B. Expression of vascular endothelial growth factor (VEGF) and VEGF receptor Flk-1 in benign, premalignant, and malignant prostate tissue. *Am J Clin Pathol*. 2001; 116:115–121. [PubMed: 11447740]
 68. Plaisance V, Niederhauser G, Azzouz F, Lenain V, Haeffliger JA, Waeber G, Abderrahmani A. The repressor element silencing transcription factor (REST)-mediated transcriptional repression requires the inhibition of Sp1. *J Biol Chem*. 2005; 280:401–407. [PubMed: 15528196]
 69. Kim CS, Choi HS, Hwang CK, Song KY, Lee BK, Law PY, Wei LN, Loh HH. Evidence of the neuron-restrictive silencer factor (NRSF) interaction with Sp3 and its synergic repression to the mu opioid receptor (MOR) gene. *Nucleic Acids Res*. 2006; 34:6392–6403. [PubMed: 17130167]
 70. Genomatix. <http://www.genomatix.de/>
 71. Carmeliet P. Angiogenesis in life, disease and medicine. *Nature*. 2005; 438:932–936. [PubMed: 16355210]
 72. Carmeliet P, Jain RK. Angiogenesis in cancer and other diseases. *Nature*. 2000; 407:249–257. [PubMed: 11001068]
 73. Zahnow CA. CCAAT/enhancer-binding protein beta: Its role in breast cancer and associations with receptor tyrosine kinases. *Expert Rev Mol Med*. 2009; 11:e12. [PubMed: 19351437]
 74. Figg WD, Kruger EA, Price DK, Kim S, Dahut WD. Inhibition of angiogenesis: Treatment options for patients with metastatic prostate cancer. *Invest New Drugs*. 2002; 20:183–194. [PubMed: 12099578]
 75. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. *Eur J Immunogenet*. 1997; 24:1–8. [PubMed: 9043871]
 76. Dunning AM, Ellis PD, McBride S, Kirschenlohr HL, Healey CS, Kemp PR, Luben RN, Chang-Claude J, Mannermaa A, Kataja V, Pharoah PD, Easton DF, Ponder BA, Metcalfe JC. A transforming growth factor-beta1 signal peptide variant increases secretion in vitro and is associated with increased incidence of invasive breast cancer. *Cancer Res*. 2003; 63:2610–2615. [PubMed: 12750287]
 77. Yokota M, Ichihara S, Lin TL, Nakashima N, Yamada Y. Association of a T29!C polymorphism of the transforming growth factor-beta1 gene with genetic susceptibility to myocardial infarction in Japanese. *Circulation*. 2000; 101:2783–2787. [PubMed: 10859282]

78. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD. Genetic control of the circulating concentration of transforming growth factor type beta1. *Hum Mol Genet.* 1999; 8:93–97. [PubMed: 9887336]
79. Ewart-Toland A, Chan JM, Yuan J, Balmain A, Ma J. A gain of function TGFB1 polymorphism may be associated with late stage prostate cancer. *Cancer Epidemiol Biomarkers Prev.* 2004; 13:759–764. [PubMed: 15159307]
80. Lambrechts D, Storkebaum E, Morimoto M, Del-Favero J, Desmet F, Marklund SL, Wyns S, Thijs V, Andersson J, van M I, Al-Chalabi A, Bornes S, Musson R, Hansen V, Beckman L, Adolfsson R, Pall HS, Prats H, Vermeire S, Rutgeerts P, Katayama S, Awata T, Leigh N, Lang-Lazdunski L, Dewerchin M, Shaw C, Moons L, Vlietinck R, Morrison KE, Robberecht W, Van BC, Collen D, Andersen PM, Carmeliet P. VEGF is a modifier of amyotrophic lateral sclerosis in mice and humans and protects motoneurons against ischemic death. *Nat Genet.* 2003; 34:383–394. [PubMed: 12847526]
81. Shahbazi M, Fryer AA, Pravica V, Brogan IJ, Ramsay HM, Hutchinson IV, Harden PN. Vascular endothelial growth factor gene polymorphisms are associated with acute renal allograft rejection. *J Am Soc Nephrol.* 2002; 13:260–264. [PubMed: 11752046]
82. Park HW, Lee JE, Shin ES, Lee JY, Bahn JW, Oh HB, Oh SY, Cho SH, Moon HB, Min KU, Elias JA, Kim YY, Kim YK. Association between genetic variations of vascular endothelial growth factor receptor 2 and atopy in the Korean population. *J Allergy Clin Immunol.* 2006; 117:774–779. [PubMed: 16630933]
83. Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. *J Vasc Res.* 2000; 37:443–448. [PubMed: 11146397]
84. Krippel P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Haas J, Samonigg H. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. *Int J Cancer.* 2003; 106:468–471. [PubMed: 12845639]
85. Krippel P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, Paulweber B, Bahadori B, Samonigg H. The L10P polymorphism of the transforming growth factor-beta 1 gene is not associated with breast cancer risk. *Cancer Lett.* 2003; 201:181–184. [PubMed: 14607332]

Fig. 1.

Interaction Entropy model. This graphical model, describes the percent entropy that is explained by each Angiogenesis-Related SNP or a combination of two loci within our study population. Positive percent entropy indicates information gain or synergy. However, negative percent indicates redundancy or lack of information gain. Schematic coloration used in the visualization tools represents a continuum from synergy (i.e., non-additive) to redundancy. The colors range from red representing a high degree of synergy (positive information gain), orange a lesser degree, and gold representing independence and a midway point between synergy and redundancy. On the other hand, green represents redundancy. Notably, VEGF combined with VEGFR IVS6 + 54 provide more information in relation to prostate cancer risk relative to other individual loci or SNP pairs.

Functional Consequences of Selected SNPs in the Angiogenesis Pathway and their Potential Role in Prostate Cancer Susceptibility

TABLE I

Gene	rs Number	Nucleotide change	Amino acid change	Impact on mRNA/protein stability/expression	Proposed influence on PCa risk	References
<i>IL10</i> -1082	rs1800896	G>A	Promoter	Low expressor = AA	Increase	[26,50,75]
<i>IL10</i> -819	rs1800871	C>T	Promoter	Low expressor = TT	Increase	[50,75]
<i>IL10</i> -592	rs1800872	C>A	Promoter	Low expressor = AA	Increase	[50,75]
<i>IL-10R</i> -153	rs2256111	A>G	Promoter			[64]
<i>IL-10R</i> Ex7 -109	rs9610	G>A				[50]
<i>IL-10R</i> Ex7 241	rs22229113	A>G	R ³⁵¹ G			[50]
<i>TGFB1</i> -509	rs1800469	C>T	Promoter	Low expressor = CC	Decrease	[50,76-78]
<i>TGFB1</i> 896	rs1982073	T>C	Leu ¹⁰ Pro	High expressor = CC	Increase	[50,77,79]
<i>TGFβRI</i> Ex9 + 195	rs868	A>G	3'UTR			[50,80,81]
<i>VEGF</i> 2482	rs3025040	C>T	3'UTR			
<i>VEGF</i> 2578	rs699947	C>A				
<i>VEGFR</i> +889	rs2305948	G>A	V ²⁹⁷ I	High expressor = CC	Increase	[81]
<i>VEGFR</i> +1416	rs1870377	T>A	H ⁴⁷² Q	Essential for maintaining the association rate with VEGF and retention of the receptor; may alter VEGF signaling pathways		[82]
<i>VEGFR</i> IVS25 -92	rs1531289	G>A	Intronic	Essential for maintaining the association rate with VEGF and retention of the receptor; may alter VEGF signaling pathways		[82]
<i>VEGFR</i> IVS6+54	rs7692791	A>G	Intronic			[82]
						[50,83-85]

TABLE II**Patient and Tumor Characteristics**

Characteristics	Cases	Controls	<i>P</i>-value^a
No. of participants, n	193	666	–
Age (years) median (range)	65.0 (41–91)	52.0 (26–89)	<0.0001
PSA in ng/ml median (range)	7.0 (0–5,000)	0.9 (0–108)	<0.0001
Gleason score, n (%)			
<8	173 (89.6)	–	–
8	20 (10.4)	–	–
Global West African Ancestry Mean (SD)	0.73 (0.18)	0.72 (0.16)	0.3375

PSA, prostate specific antigen.

^aDifferences in frequencies were tested by a Chi-square test of heterogeneity (i.e., Gleason score); Differences in PSA levels between cases and controls was tested using the Wilcoxon sum Rank test for continuous variables (PSA); Variations in age and Global West African Ancestry was tested using the ANOVA.

TABLE III

Relationship between Cytokine Polymorphisms and Prostate Cancer Risk

Genotype	Case, n (%)	Control, n (%)	OR (95% CI)	Adj OR (95% CI) ^a	P-value ^b	P-trend ^c
<i>IL-10 G⁻¹⁰⁸²A</i>						
GG	75 (39.0)	288 (43.6)	1.00	1.00	0.213	0.711
GA	95 (49.5)	280 (42.4)	1.30 (0.92, 1.84)	1.40 (0.86, 2.29)		
AA	22 (11.5)	92 (14.0)	0.92 (0.54, 1.56)	1.36 (0.68, 2.69)		
GA+AA	117 (61.0)	372 (56.4)	1.21 (0.87, 1.68)	1.39 (0.88, 2.21)		
<i>IL-10C⁻⁸¹⁹T</i>						
CC	76 (39.8)	246 (38.7)	1.00	1.00	0.848	0.633
TC	85 (44.5)	278 (43.8)	0.99 (0.70, 1.41)	0.98 (0.61, 1.56)		
TT	30 (15.7)	111 (17.5)	0.88 (0.54, 1.41)	0.58 (0.29, 1.13)		
TC+TT	115 (60.2)	389 (61.3)	0.96 (0.69, 1.33)	0.85 (0.55, 1.32)		
<i>IL-10C⁻⁵⁹²A</i>						
CC	72 (38.1)	251 (38.6)	1.00	1.00	0.875	0.882
CA	87 (46.0)	288 (44.2)	1.05 (0.74, 1.50)	1.04 (0.65, 1.65)		
AA	30 (15.9)	112 (17.2)	0.93 (0.58, 1.51)	0.53 (0.27, 1.07)		
CA+AA	117 (61.9)	400 (61.4)	1.02 (0.73, 1.42)	0.88 (0.57, 1.37)		
<i>IL-10R A⁻¹⁵³G</i>						
AA	63 (32.8)	199 (30.2)	1.00	1.00	0.253	0.162
AG	95 (49.5)	307 (46.5)	0.98 (0.68, 1.41)	1.20 (0.71, 2.01)		
GG	34 (17.7)	154 (23.3)	0.70 (0.44, 1.11)	1.01 (0.53, 1.92)		
AG+GG	129 (67.2)	461 (69.8)	0.88 (0.63, 1.25)	1.14 (0.70, 1.85)		
<i>IL-10R G⁻¹⁰⁹A</i>						
AA	78 (41.0)	283 (43.3)	1.00	1.00	0.599	0.930
GA	90 (47.4)	284 (43.4)	1.15 (0.81, 1.62)	0.92 (0.57, 1.48)		
GG	22 (11.6)	87 (13.3)	0.92 (0.54, 1.56)	0.58 (0.27, 1.24)		
GA + AA	112 (59.0)	371 (56.7)	1.10 (0.79, 1.52)	0.83 (0.53, 1.31)		
<i>IL-10R G⁻²⁴¹A</i>						
GG	125 (66.1)	427 (64.9)	1.00	1.00	0.705	0.575
AG	58 (30.7)	201 (30.5)	0.99 (0.69, 1.40)	0.82 (0.51, 1.32)		

Genotype	Case, n (%)	Control, n (%)	OR (95% CI)	Adj OR (95% CI) ^a	P-value ^b	P-trend ^c
AA	6 (3.2)	30 (4.6)	0.68 (0.28, 1.68)	0.41 (0.11, 1.48)		
AG+AA	64 (33.9)	231 (35.1)	0.95 (0.67, 1.33)	0.76 (0.48, 1.21)		
<i>TGFB1</i> C ⁻⁵⁰⁹ T						
CC	114 (60.3)	398 (61.5)	1.00	1.00	0.297	0.398
CT	59 (31.2)	214 (33.1)	0.96 (0.68, 1.37)	1.05 (0.66, 1.69)		
TT	16 (8.5)	35 (5.4)	1.60 (0.85, 2.99)	1.94 (0.82, 4.58)		
CT+TT	75 (39.7)	249 (38.5)	1.05 (0.76, 1.46)	1.16 (0.75, 1.81)		
<i>TGFB1</i> T ⁻⁸⁹⁶ C						
TT	62 (33.3)	244 (38.0)	1.00	1.00	0.516	0.338
CT	84 (45.2)	269 (41.8)	1.23 (0.85, 1.78)	1.11 (0.67, 1.86)		
CC	40 (21.5)	130 (20.2)	1.21 (0.77, 1.90)	1.22 (0.66, 2.27)		
CT+CC	124 (66.7)	399 (62.0)	1.22 (0.87, 1.72)	1.15 (0.72, 1.84)		
<i>TGFB-RI</i> A ⁻¹⁹⁵ G						
AA	141 (73.4)	443 (68.4)	1.00	1.00	0.400	0.233
GA	43 (22.4)	175 (27.0)	0.77 (0.53, 1.13)	0.78 (0.47, 1.28)		
GG	8 (4.2)	30 (4.6)	0.84 (0.38, 1.87)	0.79 (0.24, 2.54)		
GA+GG	51 (26.6)	205 (31.6)	0.78 (0.54, 1.12)	0.78 (0.48, 1.26)		
<i>VEGF</i> C ²⁴⁸² T						
CC	148 (77.5)	480 (72.9)	1.00	1.00	0.331	0.154
CT	41 (21.5)	163 (24.8)	0.82 (0.55, 1.20)	0.78 (0.45, 1.34)		
TT	2 (1.0)	15 (2.3)	0.43 (0.10, 1.91)	0.72 (0.12, 4.30)		
CT+TT	46 (22.5)	178 (27.1)	0.78 (0.54, 1.15)	0.77 (0.46, 1.31)		
<i>VEGF</i> C ²⁵⁷⁸ A						
AA	12 (6.3)	35 (5.5)	1.00	1.00	0.662	0.734
CA	53 (27.9)	198 (31.2)	0.78 (0.38, 1.61)	0.99 (0.34, 2.88)		
CC	125 (65.8)	402 (63.3)	0.91 (0.46, 1.80)	1.44 (0.52, 3.99)		
CA+CC	178 (93.7)	600 (94.5)	0.86 (0.44, 1.70)	1.28 (0.47, 3.50)		
<i>VEGFR</i> G ⁸⁸⁹ A						
GG	104 (54.4)	388 (59.3)	1.00	1.00	0.463	0.315
GA	76 (39.8)	229 (35.0)	1.24 (0.88, 1.74)	1.08 (0.69, 1.70)		
AA	11 (5.8)	37 (5.7)	1.11 (0.55, 2.25)	0.98 (0.38, 2.53)		

Genotype	Case, n (%)	Control, n (%)	OR (95% CI)	Adj OR (95% CI) ^a	P-value ^b	P-trend ^c
GA+AA	87 (45.6)	266 (40.7)	1.22 (0.88, 1.69)	1.07 (0.69, 1.65)		
<i>VEGFR</i> T ¹⁴¹⁶ A						
TT	154 (81.5)	545 (83.1)	1.00	1.00	0.857	0.645
TA	34 (18.0)	107 (16.3)	–	–		
AA	1 (0.5)	4 (0.6)	–	–		
TA+AA	35 (18.5)	111 (16.9)	1.12 (0.73, 1.70)	0.54 (0.28, 1.05)		
<i>VEGFR</i> G ^{1VS25-92} A						
GG	87 (46.0)	293 (45.6)	1.00	1.00	0.068	0.332
AG	68 (36.0)	272 (42.4)	0.84 (0.59, 1.20)	0.82 (0.50, 1.35)		
AA	34 (18.0)	77 (12.0)	1.49 (0.93, 2.38)	1.44 (0.77, 2.71)		
AG+AA	102 (54.0)	349 (54.4)	0.98 (0.71, 1.36)	0.97 (0.62, 1.53)		
<i>VEGFR</i> ^{1VS6+54}						
CC	65 (34.8)	225 (34.3)	1.00	1.00	0.338	0.392
CT	93 (49.7)	299 (45.6)	1.08 (0.75, 1.54)	1.08 (0.67, 1.75)		
TT	29 (15.5)	132 (20.1)	0.76 (0.47, 1.24)	0.73 (0.38, 1.40)		
CT+TT	122 (65.2)	431 (65.7)	0.98 (0.70, 1.38)	0.98 (0.62, 1.54)		

^a Associations were determined using multivariate LR models to estimate the risk of developing PCA using IL10 (–1082 G/G, –819CC, –592CC), *IL-10R* (–153 G/G, –109 G/G, 241 G/G), *TGFβ-1* (–896 T/T, 509 C/C), *TGFβR-1* +195 A/A, *VEGF* (2482 C/C, 2578 A/A), *VEGFR* (889 G/G, IVS25 –92 G/G, IVS6 + 54 A/A) as the reference genotypes.

^b Risk estimates adjusted for age (continuous variable) and prostate specific antigen (continuous variable).

^c Differences in the frequency of variant and referent genotypes between cases and controls were determined using the chi-square test of association and a significance level of 0.05.

TABLE IVRelationship Between *VEGF* C²⁴⁸²T and Disease Progression

Gene/SNP	Unadjusted OR (95% CI) ^a	Adjusted OR (95% CI) ^b	P-value ^c
<i>VEGF</i> C ²⁴⁸² T			
CC	1.00	1.00	0.017
CT+TT	2.12 (0.92–5.10)	3.12 (1.23–7.92)	

^a Associations were determined using multivariate LR models to estimate the risk of developing aggressive prostate cancer (tumor grade >8) PCA using *VEGF* 2482 C/C as the reference genotypes.

^b Risk estimates adjusted for age (continuous variable) and prostate specific antigen (continuous variable).

^c P-value adjusted for age and PSA were calculated using logistic regression modeling comparing the genotype profile of men with aggressive disease to those with non-aggressive disease with a significance level of <0.05.

TABLE V

Multi-Dimensionality Reduction Models for Angiogenesis-Related Markers

Best model	Cross validation consistency (CVC)	Average testing accuracy	Permutation testing <i>P</i> -value
One factor			
<i>IL-10</i> -1082 6/	6/10	0.504	>0.10
Two factor			
<i>VEGF</i> 2482	10/10	0.584	0.04
<i>VEGFR</i> _54			