Single Nucleotide Polymorphism of the Human Kallikrein-2 Gene Highly Correlates With Serum Human Kallikrein-2 Levels and in Combination Enhances Prostate Cancer Detection

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Purpose: **We examined the relationship between a mutant (T) for wild-type (C) allele substitution of the human kallikrein-2 gene (*KLK2*), circulating human kallikrein-2 (hK2) levels and prostate cancer risk.**

Patients and Methods: **We studied 1,287 consecutive men who underwent prostate biopsies because of an abnormal prostate-specific antigen level. Serum and DNA were obtained before biopsy. Cases were patients with cancer, and controls were patients with no cancer. The mutant and wild-type alleles of the *KLK2* gene were designated as the T and C alleles, respectively.**

Results: **Of the 1,287 men, 616 had cancer, and 671 had no cancer. The overall distribution of the CC, CT, and TT *KLK2* genotypes was 55.1%, 38.2%, and 6.8%, respectively. The median hK2 levels for men with the CC, CT, and TT genotypes were 0.24, 0.18, and 0.062 ng/mL and correlated with the genotypes, respectively (*P* = .0001). The adjusted odds ratios for prostate cancer for patients with the TT and CT genotypes compared with patients with the CC genotype, were 2.13 (95% confidence interval [CI], 1.3 to 3.5; *P* = .004) and 1.51 (95% CI, 1.2 to 2.0; *P* = .002), respectively. The adjusted odds ratio for prostate cancer for patients in the fourth quartile of hK2 compared with the first quartile was 4.33 (95% CI, 2.9 to 6.4; *P* = .0001). When combined, the adjusted odds ratio for having prostate cancer was 13.92 (95% CI, 6.6 to 29.2; *P* = .0001) for patients with high hK2 levels and at least one T allele.**

Conclusion: **The C/T polymorphism of the *KLK2* gene and circulating levels of hK2 are correlated and, in combination, are highly predictive for prostate cancer.**

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PROSTATE CANCER is the most commonly diagnosed malignancy in males and is the second leading cause of cancer death.[1,2] Screening for prostate cancer has been recommended by the American Cancer Society for men who are older than 50 years with at least a 10-year life expectancy.[3] Current screening tests include the measurement of serum levels of prostate-specific antigen (PSA) and digital rectal examination (DRE).[4] Two large clinical trials are underway to confirm whether screening reduces prostate cancer mortality.[5,6]

The sensitivity of PSA as a screening test is high, but the positive predictive value is relatively low for use in the general population.[7,8] Several benign conditions of the prostate gland can lead to elevated PSA levels.[9] Variants of the PSA test, including PSA density, and the free to total PSA ratio have been developed, but they do not significantly enhance the predictive value of the PSA test.[10,11]

A second problem related to PC screening is that the needle-core prostate biopsy may miss foci of cancer. With the aid of transrectal ultrasonography, a minimum of six needle-core samples are usually obtained from the prostate gland during the first biopsy session.[12] Because most tumors diagnosed through screening are microscopic and cannot be visualized radiographically, random samples are obtained in each anatomic zone of the prostate gland. Thus, malignant foci can be missed because of sampling. Patients who have no cancer detected on their first biopsy have a 15% to 30% of having cancer detected if a second biopsy is performed.[12,13] At present, there are no available tests to select patients who are at high risk for cancer on repeat prostatic biopsies.

Many new markers have been proposed to improve the detection of prostate cancer. Association studies have examined the significance of a number of candidate genes associated with prostate cancer. In particular, polymorphisms of the androgen receptor, vitamin D receptor, 5-alpha reductase enzyme, CYP17, and CYP3A4 genes have been associated with the presence of prostate cancer in one or more case-control studies.[14-18] Other candidate genes include the PSA and glutathione transferase genes, which have been linked to prostate cancer.[19,20] To date, no genetic test has been proven to be of clinical importance in diagnosing prostate cancer or in establishing high-risk groups.

We recently described the diagnostic value of the serum protein level of human kallikrein-2 (hK2) in screen-detected prostate cancer.[21] A single nucleotide polymorphism for the human kallikrein-2 gene (*KLK2*) encoding for the hK2 protein has been described, consisting of a nucleotide change from

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cytosine to thymine on exon 5.[22] It is not yet known whether this polymorphism is associated with a functional change in hK2 activity, with circulating levels of hK2, or with prostate cancer risk. In vitro, the two alleles code for an active and an inactive form of the protein product.[22] The common allele codes for Arg^{226}-hK2, which has trypsin-like activity; whereas the variant allele codes for Trp^{226}-hK2, which has no detectable activity.[22]

To determine whether the wild-type (C) for mutant (T) polymorphism predicts circulating levels of hK2 and prostate cancer risk, we examined serologic levels of hK2 and genotyped the C/T polymorphism of the *KLK2* gene in 1,287 men who are at risk for having prostate cancer.

PATIENTS AND METHODS

Study Subjects

Patients were drawn from a consecutive sample of 1,437 men who were referred to the Prostate Center of the University Health Network, between June 1998 and June 2000, because of either a PSA value of 4.0 ng/mL or greater or because of an abnormal DRE. No patient had a prior history of prostate cancer.

Of the 1,437 men, 23 patients were not capable of giving consent to participate in a research study. Of the remaining 1,414 men, 1,287 (91.0%) consented to participate. Blood samples were collected before clinical prostate examination. Plasma was separated from blood samples and was stored at −70°C. A urologic history was obtained, which was used to calculate the American Urological Association Symptom Score, which describes the severity of lower urinary tract voiding symptoms.[23] The results of DRE were recorded. A minimum of six ultrasound-guided needle biopsies was performed, with additional directed biopsies as needed, using an 18-gauge spring-loaded biopsy device (Bard Magnum, Murray Hill, NJ). The primary end point was the histologic presence of adenocarcinoma of the prostate. All research was conducted with informed consent and with the approval of the hospital research ethics board.

Repeat biopsies were offered to patients who did not have evidence of cancer on the initial prostate biopsy, but the decision to undergo repeat biopsy was made by the referring physician and patient. Of the 1,287 patients, 454 had cancer detected on the first biopsy session (35.3%). Of the remaining 833 men, 473 (56.8%) agreed to undergo one or more follow-up biopsies. An additional 153 cancers (32.4%) were detected on the second biopsy, and nine cancers (13.0%) were detected among 69 patients who had additional biopsies after a second negative biopsy.

Genetic Analysis

DNA samples from each patient were extracted from peripheral-blood leukocytes using standard protocols. We modified the technique described by Herrala et al[22] to detect the polymorphism on exon 5 of the *KLK2* gene, based on the complete sequence of the *KLK2* gene.[24] We used the forward primer 5′GAG CTG GGA ATT GCT CTC AGT-3′ and reverse primer 5′-TGC CAG AAC GTG AGG TGG AC-3′.

Genomic DNA (50 ng) was added to a 12.5-μL polymerase chain reaction (PCR) mix including 2 mmol/L of $MgCl_2$, 200 μmol/L of each deoxynucleotide triphosphate, 4% of dimethyl sulfoxide, 0.625 units of *Taq* DNA polymerase (Life Technologies, Rockville, MD), and 0.05 ng of forward and reverse primers. The PCR conditions included 32 cycles of 94°C for 30 seconds, 60°C for 30 seconds, 72°C 45 seconds, and 72°C for 10 minutes. Two units of *Msp1* restriction endonuclease (New England Biolabs Ltd, Beverly, MA) were added to 6 μL of the PCR product to digest at 37°C overnight. The digested product was then separated on 2% agarose gel. To ensure for quality control of the restriction digests, we randomly duplicated 25% of samples for comparison. All gel readers were blinded to the primary end point and covariates.

Serologic Analysis

Three plasma proteins were examined, including free PSA, total PSA, and hK2. Both free and total PSA levels were measured using commercially available kits, performed on the Immulite chemiluminescence immunoassay system (Diagnostic Products Corporation, San Diego, CA). Total hK2 levels were measured using a new, time-resolved immunofluorometric assay.[21,25] Approximately 90% of hK2 is in the free form, but the assay measures both free and total forms of hK2.[25] The hK2 assay has a detection limit of 0.006 ng/mL for both plasma and serum samples and has less than 0.2% cross-reactivity to PSA.[25] hK2 levels were analyzed in five batches. Because the concentration of reagents and the calibration of the equipment were not consistently the same between the five batches, the hK2 levels were adjusted according to their calibration settings and to the overall distribution of all the hK2 levels.[26] All adjustments were performed in a blinded fashion to the outcome and to the hK2 genotype and were independently performed by Hybritech, Inc, a subsidiary of Beckman Coulter (San Diego, CA).

Data Analysis

We compared the frequencies of the *KLK2* polymorphism between prostate cancer cases and controls and correlated the levels of hK2 with each genotype. Cases were patients who had adenocarcinoma of the prostate from any biopsy, and controls were patients who had no evidence of cancer in any of the biopsy samples. The distribution of the hK2 levels was compared between cases and controls. The odds ratios of the polymorphic alleles in predicting prostate cancer were estimated using multivariate, unconditional logistic regression modeling, controlling for age, serum PSA level, DRE, ethnic background, and the presence of lower urinary tract voiding symptoms. The mutant and wild-type alleles of the *KLK2* gene were designated as the T allele and C allele, respectively.

RESULTS

Of the 1,287 men, the mean age at first biopsy was 65.5 years (range, 41.4 to 93.8 years). The mean PSA level was 12.3 ng/mL (range, 0.4 to 498.8 ng/mL). The majority of the patients were white (84.0%). Of the other patients, 8.2% were black, 5.4% were Asian, and 2.3% were from other ethnic backgrounds. Eleven percent of patients had at least one relative with prostate cancer.

Of the 1,287 men, 616 (48.9%) were found to have adenocarcinoma of the prostate on the initial or subsequent biopsy. Of the 671 men with no evidence of invasive cancer, 360 (53.7%) had a single biopsy, 251 (37.4%) had one repeat biopsy, and 60 (8.9%) had two or more repeat biopsies. Of the men with no cancer, 52 had normal prostate tissue, 465 had inflammation/benign prostatic hyperplasia, 31 had atypical small acinar–cell proliferation, and 123 had prostatic intraepithelial neoplasia.

Based on all biopsies, cases were defined as patients with cancer, and controls were men without any cancer. The mean age at biopsy of the cases (66.7 years) was higher than controls (64.4 years, $P = .0001$). Cases were more likely to have had an abnormal DRE and, on average, had a higher PSA level (Table 1). Asians had the lowest probability for prostate cancer (Table 1).

Cancer Risk by hK2 Based on Any Biopsy

The overall distribution for the CC, CT, and TT *KLK2* genotypes was 55.1% (709 men), 38.2% (491 men), and 6.8% (87 men), respectively. The C/T polymorphism was correlated significantly with serum hK2 levels (Table 2). The median hK2 levels for men with the CC, CT, and TT genotypes were 0.24, 0.18, and 0.062 ng/mL, respectively ($P = .0001$, Table 2). The

Table 1. Distribution of Known Established Risk Factors for Prostate Cancer Between Cases and Controls Based on All Prostate Biopsy Results

Subgroup	Cases		Controls		*P*
	No.	%	No.	%	
Age					
≤ 50 years	12	31.6	26	68.4	.0001
51-60 years	139	44.8	171	55.2	
61-70 years	245	43.4	319	56.6	
> 70 years	220	58.7	155	41.3	
PSA					
≤ 4.0 ng/mL	18	18.0	82	82.0	.0001
4.1-10.0 ng/mL	345	47.5	381	52.5	
10.1-20.0 ng/mL	172	50.4	169	49.6	
20.0 ng/mL	81	67.5	39	32.5	
Digital rectal examination					
Nonpalpable	310	42.0	429	58.0	.0001
Asymmetry	137	49.6	139	50.4	
Nodule	169	62.1	103	37.9	
Ethnic background					
White	536	49.6	545	50.4	.0001
Black	54	50.9	52	49.1	
Asian	17	24.3	53	75.7	
Other	9	30.0	21	70.0	
Family history of PC					
Negative	543	47.6	599	52.5	.53
Positive	73	50.3	72	49.7	
Obstructive urinary symptoms					
Absent	132	50.8	128	49.2	.29
Present	484	47.1	543	52.9	

NOTE. The percent figure to the right of the number of cases can be interpreted as the probability of prostate cancer detected for men in their respective group.

Abbreviations: PSA, prostate-specific antigen; PC, prostate cancer.

likelihood of having prostate cancer was significantly greater for patients with one or more T allele than for those with no T allele. Patients with the CC, CT, and TT genotype had a 44.4%, 51.1%, and 57.5% probability for having prostate cancer detected, respectively ($\chi^2 = 8.66$, $P = .01$, Table 3). The *KLK2* variant was in Hardy-Weinberg equilibrium in the case and control groups.

For each genotype, the hK2 levels were higher for cases than controls (Table 2). After adjusting for age, PSA, free to total PSA ratio, DRE, ethnic background, family history, and obstructive voiding symptoms, the odds ratio for having prostate cancer according to the *KLK2* genotypes and circulating hK2 levels did not significantly change from the crude estimates (Table 4).

To determine the combined effect of the *KLK2* genotypes and levels, we calculated the odds ratio for having prostate cancer based on the combinations of the *KLK2* alleles and the quartile categories of the hK2 levels. Because of the relatively small number of patients in the TT group (n = 87), we combined patients with the CT and TT genotypes into one group. The group with the highest risk for having prostate cancer was patients who had the T allele and the highest quartile level of hK2 (adjusted odds ratio, 13.9; $P = .0001$; Table 5). The risk for having prostate cancer increased with higher hK2 quartiles for each allelic group. Also, patients with the T allele had a higher risk of having prostate cancer compared with patients with the C allele, and the risk increased with higher hK2 quartiles (Table 5).

When comparing the distribution of the *KLK2* genotypes with the established risk factors for prostate cancer, a significant difference was observed between ethnic background and the

Table 2. Distribution of Circulating hK2 Levels According to Each *KLK2* Genotype

KLK2 Genotype	hK2 Levels (ng/mL)						*P* for Cases and Controls
	All Patients (n = 1,287)		Cases (n = 616)		Controls (n = 671)		
	Median	Mean ± SD	Median	Mean ± SD	Median	Mean ± SD	
All patients	—	—	0.24	0.54 ± 1.92	0.17	0.22 ± 0.21	.0001*
CC, n = 709	0.24	0.41 ± 1.02	0.29	0.61 ± 1.5	0.19	0.25 ± 0.21	.0001*
CT, n = 491	0.18	0.37 ± 1.81	0.22	0.53 ± 2.51	0.15	0.21 ± 0.21	.0001*
TT, n = 87	0.062	0.095 ± 0.11	0.065	0.11 ± 0.12	0.059	0.077 ± 0.082	0.17†

Abbreviations: *KLK2*, human kallikrein-2 gene; hK2, human kallikrein-2; C, wild-type allele; T, mutant allele.

*Based on both parametric and nonparametric tests.

†Kruskal-Wallis test of significance.

Table 3. Probabilities for Prostate Cancer Detection for Each *KLK2* Genotype Based on the Number of Prostatic Biopsies Performed

KLK2 Genotype	Cases (n = 616) No.	Cases (n = 616) %	Controls (n = 671) No.	Controls (n = 671) %	*P*
All Patients, N = 1,287					
CC	315	44.4	394	55.6	.01
CT	251	51.1	240	48.9	
TT	50	57.5	37	42.5	
Patients after first biopsy, n = 1,287					
CC	223	31.5	486	68.5	.002
CT	190	38.7	301	61.3	
TT	41	47.1	46	52.9	
Patients undergoing one or more biopsies after initial negative biopsy, n = 473					
CC	86	30.3	198	69.7	.40
CT	59	34.7	111	65.3	
TT	8	42.1	11	57.9	

Abbreviations: *KLK2*, human kallikrein-2 gene; C, wild-type allele; T, mutant allele.

genotypes (Table 6). Fewer Asians than whites had the T allele, whereas blacks had a higher proportion than whites. When examining the effect of the *KLK2* polymorphism among white men, the *KLK2* polymorphism was predictive for prostate cancer but not among Asians or blacks.

Total PSA levels and age at biopsy positively correlated with hK2 levels (Spearman correlation coefficients: 0.50 for PSA, $P = .0001$; 0.32 for age, $P = .0001$). There were no significant differences in circulating hK2 levels among ethnic groups. The median hK2 levels for whites, blacks, Asians, and other ethnic groups were 0.21, 0.17, 0.19, and 0.18 mg/mL, respectively ($P = .25$). The median hK2 levels for patients with no nodule, asymmetric firmness, and a palpable nodule on DRE were 0.21, 0.18, and 0.22 ng/mL, respectively ($P = .16$).

To determine whether the combination of the *KLK2* genotype and circulating hK2 levels provided important information in the clinical setting, we examined how the *KLK2* genotypes and circulating levels affect the likelihood of detecting prostate cancer for patients who present for their first and second biopsies. We did not examine how it affected the likelihood for patients who received two or more repeat biopsies because of limited sample size (n = 69).

Cancer Risk by hK2 Based on First Biopsy

The probability of having cancer detected at the first prostate biopsy was 35.3% (454 of 1,287 patients). Patients with the CC, CT, and TT genotype had a 31.5%, 38.7%, and 47.1% probability for having prostate cancer detected, respectively ($\chi^2 = 12.41$, $P = .002$, Table 3). The median hK2 level was significantly higher for cases (0.24 ng/mL) than for controls (0.18 ng/mL, $P = .0001$). The adjusted odds ratio for prostate cancer detection by each *KLK2* genotype and quartile ranged from 2.6 to 10.3 (Table 5).

Cancer Risk by hK2 Based on Second Biopsy (After an Initial Negative Biopsy)

A total of 833 men had a negative first biopsy, and of them, 473 (56.8%) had a second biopsy. The probability for having cancer at repeat biopsy was 32.4% (153 of 473 patients). Patients with the CC, CT, and TT genotype had a 30.3%, 34.7%, and 42.1% probability for having prostate cancer detected, respectively ($P = .40$) (Table 3). The median hK2 level was significantly higher for cases (0.25 ng/mL) than for controls (0.20 ng/mL, $P = .0006$). When combining the *KLK2* genotypes and levels, the adjusted odds ratios for prostate cancer detection were also important predictors (Table 5).

Positive Predictive Value of hK2 and KLK2 *Gene*

To determine whether serum hK2 levels and the *KLK2* genotype enhance prostate cancer detection, we calculated the changes in positive predictive values for subgroups of patients based on the combination of age, PSA level, and DRE results. To establish the ideal cutoff value for serum hK2, we used receiver operating characteristic curves for optimal sensitivity and specificity. Among the 1,287 patients, the subgroup of patients who had the lowest positive predictive value for prostate cancer detection (38.5%) were patients whose age was less than 70 years old, who had a PSA of less than 10 ng/mL, and who had a nonpalpable nodule on DRE (n = 494). For patients with an hK2 level of more than 0.175 ng/mL and who have at least one T allele of the *KLK2* genotype compared with patients with an hK2 of 0.175 ng/mL or less or the CC *KLK2* genotype, the positive predictive value increased from 36.2% to 53.0%, respectively ($P = .008$, Table 7). Further, even for patients at higher risk for prostate cancer (age $\geq$ 70 years old, PSA $\geq$ 10 ng/mL, or a palpable nodule on DRE), the positive predictive value significantly increased by the hK2 level and *KLK2* genotype (Table 7).

DISCUSSION

Among unselected men who were screened with PSA and DRE for prostate cancer, we found a strong positive association between the *KLK2* gene, hK2 serum levels, and prostate cancer risk. Both the *KLK2* genotype and serum levels independently predicted the presence of prostate cancer, with the hK2 level having the strongest association. In combination, the hK2 profile demonstrated the highest odds ratio for prostate cancer detection and increased the positive predictive value for prostate cancer detection.

Previous studies that have examined the significance of polymorphisms of other candidate genes have been case-case studies or case-control studies and have demonstrated little clinical utility. [14,15,17-20,27] The two prostate cancer susceptibility genes identified to date by linkage analysis have not been shown to provide predictive value for prostate cancer in the general population. Further studies will be required to evaluate the significance of the newly identified tumor suppressor gene (*HPC1* or 2′-5′-oligoadenylate (2–5A)-dependent Rnase L).[28] However, the clinical impact of *HPC1* is uncertain given that the observed frequency of a common mutation was found to be

Table 4. Crude and Adjusted Odds Ratio for Prostate Cancer Detection Based on All Prostate Biopsies for All Established and Putative Risk Factors for Prostate Cancer

Covariate	Crude Odds Ratio	95% CI	*P*	Adjusted Odds Ratio*	95% CI	*P*
KLK2 genotype						
CC	1.00			1.00		
CT	1.31	1.0 to 1.6	.02	1.51	1.2 to 2.0	.002
TT	1.69	1.1 to 2.7	.02	2.13	1.3 to 3.5	.004
hK2 levels						
< 0.099 ng/mL	1.00			1.00		
0.099-0.166 ng/mL	1.20	0.8 to 1.7	.33	1.36	0.9 to 2.0	.13
0.166-0.284 ng/mL	2.36	1.7 to 3.3	.0001	2.80	1.9 to 4.1	.0001
> 0.284 ng/mL	3.09	2.2 to 4.3	.0001	4.33	2.9 to 6.4	.0001
Age						
≤ 50 years	1.00			1.00		
51-60 years	1.76	0.8 to 3.6	.12	2.20	1.0 to 4.7	.01
61-70 years	1.66	0.8 to 3.3	.16	2.53	1.2 to 5.3	.0001
> 70 years	3.08	1.5 to 6.3	.002	5.09	2.4 to 9.9	.0001
PSA level						
< 10.0 ng/mL	1.00	—	—	—	—	—
10.1-20.0 ng/mL	1.30	1.0 to 1.7	.04	—	—	—
> 20.0 ng/mL	2.65	1.8 to 4.0	.0001	—	—	—
Free:total PSA ratio						
> 0.17	1.00			1.00		
0.12-0.17	2.43	1.6 to 3.7	.0001	2.81	1.8 to 4.3	.0001
0.07-0.11	4.74	3.2 to 7.0	.0001	6.11	4.0 to 9.3	.0001
< 0.07	6.54	4.4 to 9.6	.0001	8.93	5.9 to 13	.0001
Digital rectal examination						
Normal	1.00			1.00		
Asymmetry	1.36	1.0 to 1.8	.03	1.42	1.0 to 1.9	.02
Nodule	2.27	1.7 to 3.0	.0001	2.34	1.7 to 3.2	.0001
Ethnic background						
White	1.00			1.00		
Black	1.08	0.7 to 1.6	.71	0.87	0.6 to 1.3	.53
Asian	0.33	0.2 to 0.6	.0001	0.33	0.2 to 0.6	.0004
Family history						
Absent	1.00			1.00		
Present	1.12	0.8 to 1.6	.53	1.13	0.8 to 1.6	.53
Obstructive voiding symptoms						
Absent	1.00			1.00		
Present	0.86	0.7 to 1.4	.29	0.93	0.7 to 1.3	.64

Abbreviations: *KLK2*, human kallikrein-2 gene; hK2, human kallikrein-2; CI, confidence interval; PSA, prostate-specific antigen.

*Based on a multivariate model including age, free to total PSA, digital rectal examination, ethnic background, family history, and obstructive voiding symptoms. Total PSA was excluded from the model because of its colinearity with free:total PSA.

higher in noncancer controls than prostate cancer cases.[28] We and others examined missense variant alleles of the second prostate cancer gene, *HPC2*, in a smaller subset of patients in the present study and failed to find an association with prostate cancer risk.[29-31]

Production of both PSA and hK2 is stimulated by androgens.[32-34] The genes encoding these proteins are located on chromosome 19, and the promoter regions of these genes contain androgen-response elements.[34,35] PSA has predominantly chymotrypsin-like protease activity, whereas hK2 has predominantly trypsin-like protease activity.[24,36] The primary function of the protease activity of PSA is to cleave semen proteins,[37] but the target for the protease activity of hK2 is unknown.[36]

Several studies have now shown that patients with prostate cancer have significantly higher serum hK2 levels than patients without prostate cancer.[21,38-41] At present, no standardized cutoff value for hK2 has been established, and the distributions of the hK2 levels in cases and controls vary widely between each study. For example, Becker et al[39] reported mean hK2 levels of 0.079 ng/mL for patients with prostate cancer (n = 144), whereas the mean value for our cancer patients was 0.54 ng/mL (n = 616). The reason for this wide disparity has been attributed to differences in the reagent concentrations, the monoclonal antibodies used, calibration of the methods, and storage length.[26] Magklara et al[41] reported a six-fold higher signal in the assay developed by Hybritech, Inc (a subsidiary of Beckman Coulter) compared with the assay we used. These potential factors remained consistent within the current study for cases and controls.

The observation that the inactive T allele of the *KLK2* gene is associated with lower hK2 levels but with a higher prostate cancer risk seems to be paradoxical. However, men with the T allele might have an inherent predisposition to produce less hK2 in normal and malignant cells. Thus, increased hK2 production by prostate cancer cells would have a more pronounced associ-

Table 5. Adjusted Odds Ratios for Prostate Cancer Detection for Each Combination of the *KLK2* Genotype and Circulating hK2 Levels Based on the Number of Prostate Biopsies Performed

	KLK2 Genotype					
	CC			CT or TT		
hK2 Level*	Adjusted Odds Ratio†	95% CI	*P*	Adjusted Odds Ratio†	95% CI	*P*
All patients, N = 1,287						
< 0.099 ng/mL	1.00	—	—	3.54	1.8 to 7.1	.0004
0.099-0.166 ng/mL	1.87	0.9 to 3.9	.09	5.52	2.7 to 11.4	.0001
0.166-0.284 ng/mL	4.92	2.5 to 9.6	.0001	10.68	5.2 to 22.0	.0001
> 0.284 ng/mL	8.76	4.5 to 17.2	.0001	13.92	6.6 to 29.2	.0001
Patients after first biopsy, n = 1,287						
< 0.106 ng/mL	1.00	—	—	4.58	2.2 to 9.4	.0001
0.106-0.181 ng/mL	2.56	1.2 to 5.5	.02	5.52	2.6 to 11.7	.0001
0.181-0.309 ng/mL	4.44	2.2 to 9.1	.0001	8.34	3.9 to 17.8	.0001
> 0.309 ng/mL	7.14	3.5 to 14.5	.0001	10.25	4.8 to 22.0	.0001
Patients Undergoing One or More Biopsies After Initial Negative Biopsy (n = 473)						
< 0.119 ng/mL	1.00	—	—	2.59	0.9 to 7.8	.09
0.119-0.199 ng/mL	2.22	0.7 to 6.8	.16	3.55	1.1 to 11.1	.03
0.199-0.340 ng/mL	4.34	1.6 to 12.1	.005	14.54	4.5 to 47.3	.0001
> 0.340 ng/mL	6.20	2.2 to 17.8	.0007	9.17	2.7 to 30.7	.0003

Abbreviations: *KLK2*, human kallikrein-2 gene; hK2, human kallikrein-2; CI, confidence interval; C, wild-type allele; T, mutant allele.
*hK2 quartile distribution based on controls of each group.
†Odds ratio for prostate cancer detection adjusting for age, ethnicity, digital rectal examination, and free:total prostate-specific antigen ratio.

ation with prostate cancer risk for patients who have low baseline production of serum hK2 levels (ie, patients with the TT genotype) compared with patients who have high baseline hK2 levels (ie, patients with the CC genotype).

However, the variant allele itself may be related to prostate cancer development. Patients with the variant allele had a two-fold increase in risk for having prostate cancer, independent of serum hK2 levels. Herrala et al[22] showed that in vitro, Arg^{226}-hK2 (CC genotype) had only trypsin-like activity, whereas Trp^{226}-hK2 (TT genotype) had no detectable enzymatic activity. The in vivo effects of a nucleotide change from C to T on exon 5 of the *KLK2* gene are unknown. It is possible that the inactive protein may not be sensitive to the current monoclonal antibodies used by our hK2 assay, which would be consistent with our current findings.

In addition to the trypsin-like activity of hK2, other functions of hK2 have been linked to possible pathways of carcinogenesis. Several proteases are implicated in the invasion of tissues by

Table 6. Correlation Between the Distribution of the *KLK2* Genotype and the Distributions of Established Risk Factors for Prostate Cancer

	KLK2 Genotype						
	CC		CT		TT		
Risk Factor	No.	%	No.	%	No.	%	*P*
Age at biopsy							
< 50 years	18	2.6	18	3.7	2	2.3	.13
51-60 years	161	22.7	122	24.8	27	31.0	
61-70 years	308	43.4	226	46.0	30	34.5	
> 70 years	222	31.3	125	25.5	28	32.2	
PSA level							
< 10.0 ng/mL	460	64.9	306	62.3	60	69.0	.74
10.1-20.0 ng/mL	182	25.7	139	28.3	20	23.0	
> 20.0 ng/mL	67	9.4	46	9.4	7	8.0	
Digital rectal examination							
Normal	424	59.8	274	55.8	41	47.1	.05
Abnormal	285	40.2	217	44.2	46	52.9	
Ethnic background							
Asian	52	7.3	18	3.7	0	0.0	.0001
White	601	84.8	414	84.3	66	75.9	
Black	35	4.9	52	10.6	19	21.8	
Other	21	3.0	7	1.4	2	2.3	

Abbreviations: *KLK2*, human kallikrein-2 gene; C, wild-type allele; T, mutant allele; PSA, prostate-specific antigen.

Table 7. Positive Predictive Values for Prostate Cancer Detection by the Combination of hK2 Serum Level and *KLK2* Genotype by Subgroups of Patients According to Age, PSA Level, and DRE Results

	Low-Risk Group: Age < 70 Years Old, PSA < 10 ng/mL, and Nonpalpable Nodule on DRE (n = 494)	High-Risk Group: Age ≥ 70 Years Old, PSA ≥ 10 ng/mL, or Palpable Nodule on DRE (n = 793)
hK2 level ≤ 0.175 ng/mL or CC *KLK2* genotype, n = 1,022, %	36.2	49.3
hK2 level > 0.175 ng/mL and at least one T allele of *KLK2* gene, n = 265, %	53.0	67.0
P	.008	.0001
χ^2	7.0	18.6

Abbreviations: *KLK2*, human kallikrein-2 gene; hK2, human kallikrein-2; PSA, prostate-specific antigen; DRE, digital rectal examination; C, wild-type allele; T, mutant allele.

tumor cells.[42] Frenette et al[42] showed that hK2 has plasmin-like activity and hypothesized that this could be the initiator of a proteolytic cascade leading to prostate cancer invasion. Also, hK2 may have indirect antiangiogenic properties similar to PSA.[43] Because hK2 has been shown to convert proPSA to an enzymatically active form of PSA,[36] hK2 may have effects in these pathways. Further study will be required to define the function of the Trp^{226}-hK2 and Arg^{226}-hK2 proteins.

Because patients have approximately a 30% chance of having cancer after an initial negative prostate biopsy,[12,13] patients with an initial negative biopsy were offered a repeat biopsy. This was done to reduce misclassification. At the time of analysis, 360 patients who had an initial negative biopsy did not undergo a repeat examination. The reasons for this included patient refusal, referring physician's refusal, and loss to follow-up. Nevertheless, the effect of misclassification seemed to be minimal because the established risk factors of age, PSA level, and DRE were found to be strong predictors for prostate cancer in our model. To further determine whether misclassification of the controls adversely affected our results, we used a second control group in our study. This group consisted of patients with no evidence of cancer from two or more biopsies. Using this second control group, the adjusted odds ratio for prostate cancer for patients with the CT and TT genotype compared with patients with the CC genotype was 1.67 for the CT genotype (95% CI, 1.2 to 2.3; $P = .002$) and 3.93 for the TT genotype (95% CI, 1.8 to 8.5; $P = .0005$). Similar findings were present for the hK2 level. Thus, using a control group who had the least likelihood of having prostate cancer, the odds ratios for having prostate cancer increased, which makes it unlikely that potential misclassification of the cases and controls were significant.

We did not find a family history of prostate cancer to be a significant factor for our patients because our cohort was already prescreened with PSA and DRE. Family history of prostate cancer is an important risk factor for the general population but may not be as important among this group.[44] There were significant relationships between the *KLK2* genotypes and ethnic background. No Asians had the TT genotype, whereas there seemed to be a higher proportion of blacks with the TT genotype. It is possible that differences in ethnic makeup of the cases and controls will lead to spurious effects. However, when the analysis was restricted to whites, the association between the *KLK2* genotypes and prostate cancer risk was still significantly present.

The combination of the *KLK2* C/T polymorphism and serum hK2 levels may help in the selection of patients for prostatic biopsy and further distinguish which patients are candidates for a second biopsy if the first biopsy is negative. The adjusted odds ratios for cancer after a repeat biopsy ranged from 2.2 to 14.5. These are among the highest odds ratios reported to date for any prostate cancer risk factor (Table 5).

Further, the combination of the *KLK2* gene and serum hK2 levels enhanced the positive predictive value in addition to the established current risk factors for prostate cancer, including age, PSA level, and DRE results (Table 7). In particular, among patients who had a low positive predictive value for prostate cancer based on these factors (age < 70 years old, PSA < 10 ng/mL, and nonpalpable nodule on DRE), the effect of the *KLK2* gene and serum hK2 level almost doubled the positive predictive value. This could be used to identify subgroups of men who are at high and low risk for having prostate cancer, which, in turn, could be used to aid in further management of these patients. Further confirmatory data will be required to validate these findings.

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