Effectiveness of the Combined Evaluation of *KLK3* Genetics and Free-to-Total Prostate Specific Antigen Ratio for Prostate Cancer Diagnosis

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Abbreviations and Acronyms

DHT = dihydrotestosterone
f/tPSA = free-to-tPSA ratio
LR = likelihood ratio
NPV = negative predictive value
PCa = prostate cancer
PPV = positive predictive value
PSA = prostate specific antigen
SNP = single nucleotide polymorphism
tPSA = total PSA

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Purpose: Of serum prostate specific antigen variability 40% depends on inherited factors. We ascertained whether the knowledge of *KLK3* genetics would enhance prostate specific antigen diagnostic performance in patients with clinical suspicion of prostate cancer.

Materials and Methods: We studied 1,058 men who consecutively underwent prostate biopsy for clinical suspicion of prostate cancer. At histology prostate cancer was present in 401 cases and absent in 657. Serum total prostate specific antigen and the free-to-total prostate specific antigen ratio were determined. Four polymorphisms of the *KLK3* gene (rs2569733, rs2739448, rs925013 and rs2735839) and 1 polymorphism of the *SRD5A2* gene (rs523349) were studied. The influence of genetics on prostate specific antigen variability was evaluated by multivariate linear regression analysis. The performance of total prostate specific antigen and the free-to-total prostate specific antigen ratio alone or combined with a genetically based patient classification were defined by ROC curve analyses.

Results: For prostate cancer diagnosis the free-to-total prostate specific antigen ratio index alone (cutoff 11%) was superior to total prostate specific antigen (cutoff 4 ng/ml) and to free-to-total prostate specific antigen ratio reflex testing (positive predictive value 61%, 43% and 54%, respectively). Prostate specific antigen correlated with *KLK3* genetics (rs2735839 polymorphism $p = 0.001$, and rs2569733, rs2739448 and rs925013 haplotype combination $p = 0.003$). In patients with different *KLK3* genetics 2 optimal free-to-total prostate specific antigen ratio cutoffs (11% and 14.5%) were found. For free-to-total prostate specific antigen ratio values between 11% and 14.5% the prostate cancer probability ranged from 30.0% to 47.4% according to patient genetics.

Conclusions: The free-to-total prostate specific antigen ratio is superior to total prostate specific antigen for prostate cancer diagnosis, independent of total prostate specific antigen results. Free-to-total prostate specific antigen ratio findings below 11% are positively associated with prostate cancer and those above 14.5% are negatively associated with prostate cancer, while the interpretation of those between 11% and 14.5% is improved by patient *KLK3* genetic analysis.

Key Words: prostate; prostatic neoplasms; prostate-specific antigen; 3-oxo-5-alpha-steroid 4-dehydrogenase; polymorphism, genetic

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PROSTATE specific antigen serum determination for screening has increased the detection rate of resectable PCa, although its effect on the mortality rate, and the risk of over diagnosis and overtreatment is still widely debated.[1–3] PSA limitations are mainly in specificity and sensitivity.[4] Reflex testing of the noncomplexed PSA fraction and the derived f/tPSA index enhances specificity and sensitivity for tPSA values within the 4 to 10 ng/ml gray zone[5] and decreases over diagnosis and overtreatment rates.[5,6]

Of serum tPSA variability in the general population 40% depends on inherited factors.[7] Several polymorphisms of *KLK3* gene encoding for PSA are associated with tPSA serum level changes and/or PCa risk,[8–11] including a polymorphism (rs2735839) at the 3′ boundary of the *KLK3* locus[8,12–15] and 3 SNPs of the *KLK3* promoter region (rs2569733, rs2739448 and rs925013).[16] The latter 3 SNPs might affect tPSA mRNA levels in response to transcriptional stimuli as DHT. DHT is produced from testosterone by the enzymatic activity of 5α-reductase type II encoded by the *SRD5A2* gene.[17] Enzyme variants with different activity levels are associated with tPSA serum level changes, and PCa risk and recurrence, as described for the rs523349 *SRD5A2* polymorphism (Val89Leu variant).[18–21]

The current cross-sectional study was done to ascertain whether *KLK3* and *SRD5A2* genetic variability might influence tPSA and f/tPSA in healthy and diseased states, and identify whether a genetically based patient classification might enhance the clinical performance of tPSA and f/tPSA for PCa diagnosis.

MATERIALS AND METHODS

Patients

We studied 1,058 white Italian men with a median age of 67 years (range 45 to 88) who consecutively underwent transrectal ultrasound biopsy of the prostate with a 10 to 16-core template for clinical suspicion of PCa. At histology PCa was present in 401 patients with a median age of 69 years (range 45 to 88) and absent in 657 patients (reference group) with a median age of 65 years (range 47 to 85). Gleason score was G3 in 6 cases, G4 in 28, G5 in 30, G6 in 197, G7 in 83, G8 in 44 and G9 in 13. According to the Declaration of Helsinki, written informed consent was signed by each patient and the local ethical committee approved the research protocol.

Serum Markers

In serum collected before any prostate manipulation tPSA and f/tPSA were measured using the Immulite® 2000 system.

Genetic Analyses

The polymorphisms studied were rs2569733, rs2739448, rs925013, rs2735839 (*KLK3* locus) and rs523349 (*SRD5A2* gene). Due to limited quantities of DNA, the rs523349 polymorphism was analyzed in 762 of 1,058 patients. Genomic DNA was extracted from whole blood using the BioSprint® 15 DNA Blood Kit. Polymorphisms were studied by TaqMan® allelic discrimination assays using an ABI Prism® 7900 HT sequence detection system. The SNPs rs2569733 and rs2739448 were identified by a common primer pair, including PSApromF 5′CAGTCTCCTGACCTCRTGATCTG3′and PSApromR 5′GAGCCCCCCAAAAAATCTCT3′. For rs2569733 SNP the probes were 5429G 5′6-FAM-CTGGGATGACAGGCG-MGB3′and 5429T 5′6-VIC-CTGGGATTACAGGCGTG-MGB3′. For rs2739448 the probes were 5412C 5′6-FAM-TGAGCCACCGCGC-MGB3′and 5412T 5′6-VIC-TGAGCCACTGCGC-MGB3′. Assays were done separately using 80 ng DNA in a final volume of 25 μl containing 1× TaqMan Universal PCR Master Mix, 900 nM of each primer, 100 nM 5429G, 200 nM 5429T, 150 nM 5412C and 200 nM 5412T (Applied Biosystems®). Thermocycling conditions were 50C for 2 minutes and 95C for 10 minutes, followed by 50 cycles at 95C for 15 seconds and 54C for 1 minute for rs2569733, and at 95C for 15 seconds and 53C for 1 minute for rs2739448. The rs925013 polymorphism of the *KLK3* gene was analyzed using the TaqMan Drug Metabolism Assay (assay ID C_1531063_10) (Applied Biosystems) while the rs2735839 polymorphism was analyzed as previously described.[22]

The rs523349 SNP of the *SRD5A2* gene was analyzed by the assay (assay ID 001_1860) described at the National Cancer Institute Variant GPS site (http://variantgps.nci.nih.gov/cgfseq/pages/resultSubmit.do?method=getPlatform&assayLid=001_1860).

Statistical Analysis

We used Stata® 10 and SPSS® for Windows®, version 9. The Pearson chi-square and Fisher exact tests were used to assess differences between groups for proportions. The Kruskal-Wallis rank test was used to compare any differences between tPSA and f/tPSA values in diagnosis and genotypes. The Wilcoxon test for trend statistics was used to verify significant increases across ordered groups. ROC curve analysis was done to assess serum marker performance and establish optimal cutoffs, corresponding to the highest differential positive rate, [sensitivity – (1 – specificity)]. For multiple linear regression $\log_{10}$ tPSA and $\log_{10}$ f/tPSA transformations were used. Due to the strong influence of age and diagnosis on tPSA and f/tPSA, statistical models included these 2 variables. Heteroskedasticity was assessed with the Breusch-Pagan/Cook-Weisberg test. The Hardy-Weinberg equilibrium test, pairwise linkage disequilibrium calculation and haplotype analysis were done using Arlequin, version 2.000 (http://cmpg.unibe.ch/software/arlequin3/).

RESULTS

Serum Markers and PCa Diagnosis

In the PCa group tPSA was significantly higher than in the reference group (mean 12.72 ng/ml, median 6.61, IQR 4.68–10.40 vs mean 7.38, median 5.30 ng/ml, IQR 3.08–8.37, chi-square 41.2, $p < 0.0001$). However, f/tPSA was significantly lower in the PCa group than in the reference group (mean 12.9%,