Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

Title: Genetic and Protein Biomarkers for the Diagnosis and Cancer Risk Assessment of Prostate Cancer

See Also: Gene Expression Profiling and Protein Biomarkers for Prostate Cancer Management

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DESCRIPTION
There are a variety of genetic and protein biomarkers associated with prostate cancer. These tests have the potential to improve the accuracy of differentiating which men should undergo prostate biopsy or rebiopsy after a prior negative biopsy. This policy will address these types of tests, as well as single nucleotide polymorphisms (SNPs) testing for cancer risk assessment. Testing to determine cancer aggressiveness after a tissue
diagnosis of cancer has been made is addressed in Gene Expression Analysis for Prostate Cancer Management.

**Background**

Conventional decision-making tools for identifying men who should undergo prostate biopsy include serum prostate-specific antigen (PSA), digital rectal exam (DRE) and patient risk factors such as age, race, and family history of prostate cancer. However, these screening tools lead to unnecessary prostate biopsies because of their lack of specificity and inability to discriminate low- from high-risk prostate cancer.

Prostate cancer is a complex, heterogeneous disease in which numerous genetic alterations have been described, with the potential for use of these molecular markers to improve decision making as to whom should undergo prostate biopsy or rebiopsy after an initial negative biopsy.

For assessing future prostate cancer risk, numerous studies have demonstrated the association of many different SNPs with prostate cancer, and these studies generally show a modest degree of association with future risk for prostate cancer.

Commercially available tests include:

- **4Kscore Test (OPKO Lab)**, a blood test that measures 4 prostate-specific kallikreins, which are combined into an algorithm to decide whether a patient should proceed to prostate biopsy.
- **Prostarix (Metabolon/Bostwick Laboratories)** is a post-DRE urine test based on several metabolites and an algorithm to decide whether a patient should proceed to prostate biopsy or undergo repeat biopsy after an initial negative biopsy.
- **The PCA3 test** is offered in the United States by a number of reference laboratories including ARUP, Mayo Medical Laboratories, and LabCorp. Reagents used in testing are developed by Gen-Probe.
- **Prostate Core Mitomics Test (Mitomics [formerly Genesis Genomics])**, which measures mitochondrial DNA mutations in a negative prostate biopsy to determine whether a patient should undergo repeat biopsy.
- **ConfirmMDx (MDxHealth)** measures hypermethylation of 3 genes in a negative prostate biopsy to determine whether a patient should undergo repeat biopsy.
- **SNP testing** as part of genome-scanning tests for prostate cancer risk assessment are offered by a variety of laboratories, such as Navigenics (now Life Technologies), LabCorp (23andme), and ARUP (deCode), as laboratory-developed tests.

**Regulatory Status**

Only 1 **PCA3** test has been submitted to the U.S. Food and Drug Administration (FDA) for premarket approval. The Gen-Probe Progensa® **PCA3** Assay was approved by FDA on February 15, 2012 through the premarket approval process. According to the company’s press release, this assay is “indicated for use in conjunction with other patient information to aid in the decision for repeat biopsy in men 50 years of age or older who...”
have had 1 or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on the current standard of care, before consideration of Progensa PCA3 assay results.” FDA product code: OYM

Other tests mentioned in this policy, if available, are offered as laboratory-developed tests under the Clinical Laboratory Improvement Amendments (CLIA) licensed laboratories. Clinical laboratories may develop and validate tests in-house and market them as a laboratory service; laboratories offering such tests as a clinical service must meet general regulatory standards of the Clinical Laboratory Improvement Act and must be licensed by CLIA for high-complexity testing.

**POLICY**

A. The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered **experimental / investigational**:
   1. Kallikrein markers (eg, 4Kscore™ Test)
   2. Metabolomic profiles (eg, Prostarix™)
   3. PCA3 testing
   4. TMPRSS fusion genes
   5. Candidate gene panels
   6. Mitochondrial DNA mutation testing (eg, Prostate Core Mitomics Test™)
   7. Gene hypermethylation testing (eg, ConfirmMDx®)

B. Single nucleotide polymorphisms (SNPs) testing for cancer risk assessment of prostate cancer is considered **experimental / investigational**.

**RATIONALE**

The most recent period of literature review covers the period through March 16, 2015. The following text is a summary of current evidence.

In general, the evidence for genetic tests related to prostate cancer diagnosis addresses preliminary clinical associations between genetic tests and disease states. In some cases, the clinical validity of these tests, ie, the association of the test result with outcomes of interest, is expressed in terms of sensitivity, specificity, predictive value, and/or comparisons to current standards using receiver operating curve (ROC) analysis. There is no direct evidence of clinical utility, ie, that using a test will change treatment decisions and improve subsequent outcomes, such as mortality, morbidity, or quality of life.
Diagnosis

Whether to Perform a Prostate Biopsy or Rebiopsy

4Kscore Test (OPKO Lab)
The 4Kscore Test is a blood test that generates a risk score for the probability for finding high-grade prostate cancer (defined as a Gleason score ≥7) if a prostate biopsy were performed. The intended use of the test is to aid in the decision of whether or not to proceed with a prostate biopsy. The test algorithm combines the measurement of 4 prostate-specific kallikreins (total prostate-specific antigen [tPSA], free PSA [fPSA], intact PSA [iPSA], and human kallikrein 2 [hK2]), which are combined in an algorithm with patient age, digital rectal exam (DRE) (nodules or no nodules), and whether the patient has had a prior negative prostate biopsy. A kallikrein is a subgroup of enzymes that cleave peptide bonds in proteins. The iPSA and hK2 tests are immunoassays that employ distinct mouse monoclonal antibodies.

The test is not intended to be used in patients with a previous diagnosis of prostate cancer, a patient who has had a DRE in the previous 4 days, a patient who has received 5-alpha reductase inhibitor therapy in the previous 6 months, or a patient who has undergone any procedure or therapy to treat symptomatic benign prostatic hypertrophy in the previous 6 months.

The performance of the 4Kscore Test was validated in a total of 1012 patients who were enrolled from October 2013 to April 2014 in a blinded, prospective study at 26 urology centers in the United States. Enrollment into the study was open to all men who were scheduled for a prostate biopsy, regardless of age, PSA level, DRE, or prior prostate biopsy. Each patient underwent a transrectal ultrasound (TRUS)–guided prostate biopsy of at least 10 cores. A blinded blood sample that was collected before biopsy was sent to OPKO Lab for the 4 kallikrein markers. The results of the kallikrein markers, prostate biopsy histopathology, patient age, DRE, and prior biopsy status were unblinded and analyzed.

The biopsy was negative in 54% of cases (n=542), showed low-grade (all Gleason grade 6) prostatic cancer in 24% (n=239) and high-grade cancer in 23% (n=231). The statistical analysis of the 4Kscore Test clinical data had an area under the curve (AUC) of the receiver operator curve (ROC) of 0.82 for the detection of high-grade prostate cancer; the AUC for all patients using tPSA, age, DRE and prior biopsy was 0.76.

The authors have also conducted multiple studies predicting the use of the test in patient cohorts from the European Randomized Study of Prostate Cancer.

Prostarix (Metabolon/Bostwick Laboratories)
Prostarix™ is a post-DRE urine test that is based on a panel of biomarkers and is used in the early detection of prostate cancer. The results are intended to aid in clinical decision making as to whether to biopsy or repeat biopsy the prostate, particularly in patients who have a suspicious DRE and modestly elevated PSA (2.5-10 ng/mL). The test addresses metabolic abnormalities that have been associated with prostate cancer. Prostarix measures the concentration of several metabolites: sarcosine, alanine, glycine, and glutamate, and these quantitative measurements are combined in a logistic regression algorithm to generate a Prostarix Risk Score. If PSA level and TRUS-determined prostate volume are available, they can be used along with the metabolite measurements to generate the Prostarix-PLUS Risk Score. The test claims to have increased sensitivity and specificity over standard assessment tools to predict the likelihood of a positive prostate biopsy.
Two studies, described next, correlated the level of sarcosine in urine of prostate biopsy-positive and -negative patients, and found increased levels of sarcosine in the urine of patients with prostate cancer; however, is not clear in which patient population a test measuring urine sarcosine would be used, or what level of sarcosine would warrant a prostate biopsy. In addition, other studies done by different authors have shown conflicting results from those performed by the authors from Metabolon.

In their initial study of the potential role of metabolomic profiles to delineate the role of sarcosine in prostate cancer progression, Sreekumar et al profiled 1126 metabolites across 262 prostate-derived clinical samples (42 tissue samples and 110 matched specimens of plasma and post-DRE urine from biopsy-positive cancer patients \([n=59]\) and biopsy-negative control patients \([n=51]\)).\(^3\) The authors reported that levels of sarcosine increased progressively in benign, localized prostate cancer, and metastatic disease.

Subsequently, the investigators used benign prostate tissue and localized prostate cancer obtained from a radical prostatectomy series from one university’s hospital.\(^4\) Urine specimens were collected from patients who were being screened for prostate cancer with PSA levels considered clinically significant \((8.59\pm6.30)\). Urine was collected post-DRE but before prostate biopsy. Urine collected from patients undergoing prostatectomy was collected before surgery and used as a positive control. In total, 211 biopsy-positive and 134 biopsy-negative urine sediments were used. Using a logistic regression model, sarcosine levels were elevated in prostate cancer urine sediments compared with controls, with an area under the receiver operating curve of 0.71.

**PCA3**

PCA3 is overexpressed in prostate cancer, and PCA3 mRNA can be detected in urine samples collected after prostate massage. When normalized using PSA to account for prostate cells released into the urine (PCA3 score), the test has significantly improved specificity compared with serum PSA and may better discriminate patients with benign findings on (first or second) biopsy from those with malignant biopsy results. In particular, the test may be especially helpful for identifying patients with elevated PSA levels but negative first biopsy results, who need a follow-up biopsy. Based on several studies,\(^5-10\) average PCA3 score sensitivity and specificity for a positive prostate biopsy result is about 61% and 74%, respectively.

Ankerst et al (2008) reported that incorporating PCA3 score into the Prostate Cancer Prevention Trial risk calculator improved diagnostic accuracy of the calculator (from AUC of 0.653 to 0.696).\(^11\) Chun et al (2009), using a multivariate nomogram, demonstrated a 5% gain in predictive accuracy when PCA3 was incorporated with other predictive variables such as age, DRE results, PSA levels, prostate volume, and past biopsy history.\(^12\) In a 2011 study of 218 patients with PSA values of 10 ng/mL or less, Perdona et al performed a head-to-head comparison of these 2 risk assessment tools and suggested both might have value in clinical decision making.\(^13\)

Several studies have focused on evaluating PCA3 score as a tool for distinguishing between patients with indolent cancers who may need only active surveillance and patients with aggressive cancers who warrant aggressive therapy. Three studies from 2008—Haese et al,\(^9\) Nakanishi et al,\(^14\) and Whitman et al\(^15\)—demonstrated an association between PCA3 scores and evidence of tumor aggressiveness. However, these findings were not confirmed in a 2005 study by Bostwick et al\(^16\) or a 2008 study by vans Gils et al.\(^17\) Auprich et al (2010) reported that PCA3
scores appeared to enhance identification of indolent disease but not pathologically advanced or aggressive cancer.\textsuperscript{18}

A 2010 meta-analysis by Ruiz-Aragon and Marquez-Pelaez reviewed 14 studies of \textit{PCA3} for use in predicting prostate biopsy results.\textsuperscript{19} Sensitivity of testing ranged from 47\% to 82\% and specificity from 56\% to 89\%. Global results yielded sensitivity of 85\% (confidence interval [CI], 84 to 87) and specificity of 96\% (CI, 96 to 97). No publications on how this information affected decision making or either short- or long-term outcomes has been published.

Tosoian et al (2010) reported on a short-term prospective cohort study evaluating \textit{PCA3} in relation to outcomes in an active surveillance program involving 294 patients.\textsuperscript{20} \textit{PCA3} did not distinguish patients who had stable disease from those who developed more aggressive features. Durand et al (2012) found that \textit{PCA3} score offered some predictive prognostic accuracy in a cohort of 160 men.\textsuperscript{21} \textit{PCA3} scores were significantly associated with increased tumor volume and positive surgical margins. However, in multivariate analysis, \textit{PCA3} score and Gleason score (≥7) did not emerge as independent predictors of pathologic stage.

In 2013, the Agency for Healthcare Quality and Research published a comparative effectiveness review entitled, \textit{PCA3 Testing for the Diagnosis and Management of Prostate Cancer.}\textsuperscript{22} Literature was searched and updated through May 15, 2012. Forty-three studies were included; all were rated poor quality. In their conclusion, the authors stated, “For diagnostic accuracy, there was a low strength of evidence that \textit{PCA3} had better diagnostic accuracy for positive biopsy results than [serum] total PSA elevations, but insufficient evidence that this led to improved intermediate or long-term health outcomes.” This finding appeared to apply to both initial and repeat biopsies. Evidence was insufficient to assess the use of \textit{PCA3} in treatment decision making for men with positive biopsy.

Several studies published in 2013 and 2014 reported positive associations between \textit{PCA3} levels and prostate cancer diagnosis.\textsuperscript{23-28} Predictive value was increased when \textit{PCA3} testing was combined with PSA level and other clinical information.\textsuperscript{29,30} Other groups reported moderate diagnostic accuracy of \textit{PCA3} testing. Among men with PSA level greater than 3 ng/mL, AUC of \textit{PCA3} was 0.74.\textsuperscript{31} Conversely, in men with \textit{PCA3} scores of 100 or greater, positive predictive value was 39\%.\textsuperscript{32} In a Japanese study of 647 men, sensitivity and specificity were 67\% and 72\%, respectively; AUC was 0.742.\textsuperscript{33} Two studies compared \textit{PCA3} with multiparametric magnetic resonance imaging (MRI); MRI was more accurate than \textit{PCA3},\textsuperscript{34} but the combination was better than either alone.\textsuperscript{35}

A 2014, the National Cancer Institute conducted a prospective trial to validate the diagnostic use of \textit{PCA3} to complement PSA-based detection of prostate cancer.\textsuperscript{36} The target population included men who had been screened for prostate cancer, primarily with a PSA test, some of whom had undergone a previous prostate biopsy. The study included 859 men from 11 centers in the United States. The primary study end point was the diagnosis of prostate cancer on biopsy and the secondary study end point was diagnosis of high-grade prostate cancer, defined as a Gleason score greater than 6. The primary analyses, including \textit{PCA3} thresholds, were determined a priori, and statistical power was based on independent analyses of prevalidation data from similar cohorts. Of the men in the study, 562 were presenting for their initial prostate biopsy. Positive predictive value was 80\% (95\% CI, 72\% to 86\%), and using a \textit{PCA3} score of more than 60, diagnostic sensitivity and specificity of \textit{PCA3} was 0.42 (95\% CI, 0.36 to 0.48) and 0.91 (95\% CI,
0.87 to 0.94), respectively. For patients who underwent a repeat biopsy, the negative predictive value was 88% (95% CI, 81% to 93%), and by using a PCA3 score of less than 20, sensitivity and specificity were 0.76 (95% CI, 0.64 to 0.86) and 0.52 (95% CI, 0.45 to 0.58), respectively. For the detection of high-grade cancer, PCA3 performance in combination with Prostate Cancer Prevention Trial’s (PCPT) risk calculator was improved by the addition of PCA3 to the PCPT risk calculator factors with an AUC improvement of 0.74 to 0.78 for initial biopsy and 0.74 to 0.79 on repeat biopsy (p≤0.003).

Clinical utility studies using assay results for decision making for initial biopsy, repeat biopsy, or treatment have not been reported. One group reported potential reductions in unnecessary biopsies of 48% to 52% with attendant increases in missed prostate cancers of 6% to 15% using either a PCA3-based nomogram or PCA3 level corrected for prostate volume (PCA3 density). Although both studies were prospective, neither assessed utility of the test for clinical decision making because all patients underwent biopsy. Also, recurrence or survival outcomes were not evaluated.

In summary, studies of PCA3 as a diagnostic test for prostate cancer have reported sensitivities and specificities in the moderate range. In general, these studies are preliminary and report on clinical performance characteristics in different populations and with various assay cutoff values, reflecting the lack of standardization in performance and interpretation of PCA3 results. The clinical utility of this test is uncertain, as there is no evidence that the use of PCA3 can be used to change management in ways that improve outcomes.

**TMPRSS Fusion Genes for Diagnosis and Prognosis**

*TMPRSS2* is an androgen-regulated transmembrane serine protease that is preferentially expressed in normal prostate tissue. In prostate cancer, it may be fused to an ETS (E26 transformation-specific) family transcription factor (ERG, ETV1, ETV4, or ETV5), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis. The result of gene fusion with an ETS transcription gene is that the androgen-responsive promoter of *TMPRSS2* upregulates expression of the ETS gene, suggesting a mechanism for neoplastic transformation. Fusion genes may be detected in tissue, serum, or urine.

*TMPRSS2:ERG* gene rearrangements have been reported in 50% or more of primary prostate cancer samples. Although *ERG* appears to be the most common ETS family transcription factor involved in the development of fusion genes, not all are associated with *TMPRSS2*. About 6% of observed rearrangements are seen with *SLC45A3*, and about 5% appear to involve other types or rearrangement.

Attention has been directed at using post-DRE urine samples to look for fusion genes as markers of prostate cancer. Laxman et al (2006) developed an assay to measure ERG and *TMPRSS2:ERG* transcripts in urine samples after prostatic massage in 19 patients with prostate cancer. The authors observed a strong concordance between the presence of these transcripts and prostate cancer. In a subsequent study of 234 patients presenting for biopsy or radical prostatectomy (138 with cancer; 86 with benign disease), Laxman et al (2008) confirmed the association between cancer and *TMPRSS2:ERG* but did not demonstrate a significant association between cancer and ERG transcripts. An algorithm was created using 7 candidate biomarkers including SPINK1, PCA3, GOLPH2, and *TMPRSS2:ERG*. The AUC for this multiplex model was 0.785; sensitivity was 66%, and specificity was 76%. Because the study was performed on a population...
enriched for cancer, external validation would be critical in properly defining and understanding test performance.

Rice et al (2010) developed an assay directed at evaluation of ERG RNA in urine normalized for PSA RNA. In a study of 237 men scheduled for prostate biopsy, this assay was found to identify cancer with an AUC of 0.592, sensitivity of 31%, and specificity of 84%. Higher urine ERG values were significantly associated with positive biopsy, although these did not correlate with clinical stage or biopsy Gleason scores. Performance of the test was noted to be particularly good in white males with a PSA value of 4 ng/mL or less. Adding ERG to PSA results and other clinical parameters in a multivariate logistic regression model did not significantly improve performance in predicting biopsy results. The authors concluded that “further studies examining the long-term prognostic significance of these markers will show their full potential in augmenting the appropriate diagnosis and treatment of prostate cancer.”

In a prospective, multicenter study, Leyten et al (2014) investigated the predictive value of PCA3 and TMPRSS2 as individual biomarkers and as part of a panel in a prospective, multicenter study of 443 men. TMPRSS2 was found to be highly specific (93%) for predicting clinically significant prostate cancer on biopsy. Because of this high specificity, the authors suggested that rebiopsy or MRI be performed in TMPRSS2:ERG-positive patients who do not have prostate cancer detected on initial biopsy. The authors stated that if PCA3 in combination with TMPRSS2 data had been used to select men for prostate biopsy, 35% of biopsies could have been avoided.

In 2013, Yao et al published a systematic review with meta-analysis of TMPRSS2:ERG for the detection of prostate cancer. Literature was searched through July 30, 2013, and 32 articles were included. Pooled sensitivity, specificity, positive likelihood ratio, and negative likelihood ratio were 47% (95% CI, 46 to 49), 93% (95% CI, 92 to 94), 8.9 (95% CI, 5.7 to 14.1), and 0.49 (95% CI, 0.43 to 0.55), respectively. Statistical heterogeneity was high (I²>85%). It was unclear whether studies in screening populations were pooled with enriched patient samples, e.g., elevated PSA and/or biopsy-negative. There also was variability in the type of tissue samples analyzed (urine, prostatic secretions, biopsy, surgical specimens); the type of TMPRSS2:ERG assays used (fluorescence in situ hybridization [FISH], immunohistochemistry, real-time reverse transcriptase polymerase chain reaction, transcription-mediated amplification); and in TMPRSS2:ERG threshold cutoff values.

In summary, limited evidence reports that measurement of TMPRSS:ERG may improve the ability to predict prostate cancer. However, results are inconsistent and available studies differ as to the accuracy of TMPRSS:ERG for this purpose. Additionally, clinical utility of this test is uncertain, i.e., no studies report that the test leads to changes in management that result in improved health outcomes.

TMPRSS2:ERG in Combination With PCA3
Tomlins et al (2011) have recently developed a transcription-mediated amplification assay to measure TMPRSS2:ERG fusion transcripts in parallel with PCA3. Combining results from these 2 tests and incorporating them into the multivariate Prostate Cancer Prevention Trial risk calculator appeared to improve identification of patients with clinically significant cancer by Epstein criteria and high-grade cancer on biopsy. Although the study was large (1312 men at multiple centers), it was confounded by assay modifications during the course of the study and by the use of cross-
validation rather than independent validation, using independent training and testing sets. Further studies are warranted.

In 2014, this same group evaluated 45 men using a multivariable algorithm that included serum PSA plus urine TMPSS2:ERG and PCA3 from a post-DRE sample. Samples were collected before prostate biopsy at 2 centers. For cancer prediction, sensitivity and specificity were 80% and 90%, respectively. AUC was 0.88.

Robert et al (2013) retrospectively examined tissue levels of TMPRSS2:ERG and PCA3 in 48 men with benign prostatic hypertrophy, 32 men with normal prostate tissue sampled next to prostate cancer, and 48 men with prostate cancer. Sensitivity, specificity, and positive and negative predictive values for the tests in combination were 94%, 98%, 96%, and 96%, respectively.

In summary, concomitant detection of TMPRSS2:ERG and PCA3 may more accurately identify men with prostate cancer. However, current evidence is insufficient to support its use. Estimated accuracy varies across available studies, and comparative studies, demonstrating improvements in health outcomes with the test compared with no testing, are lacking.

**Candidate Gene Panels for Prostate Cancer Diagnosis**

Because no single gene marker that is both highly sensitive and highly specific for diagnosing prostate cancer has been found, particularly in men already known to have elevated PSA levels, some investigators are combining several markers into a single diagnostic panel. Although promising in concept, only single studies of various panels have been published, and none apparently is offered as a clinical service.

Ma et al (2014) examined various algorithms for cancer diagnosis and prognosis using urine and plasma levels of multiple genes, including PCA3, PSA, TMPRSS2, and ERG. One algorithm distinguished prostate cancer from benign prostatic hypertrophy with AUC 0.78. Another algorithm distinguished men with Gleason score 7 or higher from men with Gleason score less than 7 (AUC=0.88). Combination of these 2 algorithms into a scoring system predicted the presence of Gleason score 7 or higher in 75% of men. Qu et al (2013) reported preliminary results of a 3-gene panel (androgen receptor [AR], PTEN, and TMPRSS2:ERG) analyzed by FISH. Thirty-one percent of 110 archived primary tumor samples and 97 metastatic tumor samples from a separate cohort of patients were analyzable. Chromosomal abnormalities were detected in 53% of primary prostate cancers and 87% of metastatic tumors (p<0.001).

In summary, gene panels for prostate cancer diagnosis and prognosis are in an investigational phase of development.

**Diagnosis**

**Whether to Perform a Prostate Rebiopsy**

*Prostate Core Mitomics Test™ (Mitomics [Formerly Genesis Genomics])*  
The Prostate Core Mitomics Test (PCMT) is a proprietary test that is intended to determine whether a patient has prostate cancer, despite a negative prostate biopsy, by analyzing deletions in mitochondrial DNA by polymerase chain reaction (PCR) to detect “tumor field effect.” The test is performed on the initial negative prostate biopsy tissue. According to the company website, a negative PCMT result confirms the results of the negative biopsy (ie, the patient doesn’t have...
prostate cancer) and that the patient can avoid a second biopsy, but that a positive PCMT means that the patient is at high risk of undiagnosed prostate cancer. The company website states that the sensitivity of the test is 85% and has a negative predictive value of 92%.

Published literature from Genesis Genomics on the use of mitochondrial DNA mutations in prostate is described next.

A 2006 study retrospectively analyzed mitochondrial DNA mutations from 3 tissue types from 24 prostatectomy specimens: prostate cancer, adjacent benign tissue, and benign tissue distant to the tumor (defined as tissue from a nondiseased lobe or at least 10-cell diameters from the tumor if in the same lobe). Prostate needle biopsy tissue (from 12 individuals referred for biopsy) that were histologically benign were used as controls. Results from the prostatectomy tissue analysis showed that 16 of 24 (66.7%) had mutations in all three tissue types, 22 of 24 (91.7%) had mutations in malignant samples, 19 of 24 (79.2%) in adjacent benign samples, and 22 of 24 in distant benign glands. Overall, 273 somatic mutations were observed in this sample set. In the control group, 7 (58.3%) patients were found to have between 1 and 5 alterations, mainly in noncoding regions. The authors concluded that the mutations found in the malignant group versus the control group were significantly different and that mitochondrial DNA mutations are an indicator of malignant transformation in prostate tissue.

In 2008, Maki et al reported the discovery and characterization of a 3.4-kb mitochondrial genome deletion and its association with prostate cancer. A pilot study analyzed 38 benign biopsy specimens from 22 patients, 41 malignant biopsy specimens from 24 patients, and 29 proximal to malignant (PTM) biopsy specimens from 22 patients. All of the patients with malignant biopsies had a subsequent prostatectomy, and the diagnosis of cancer was confirmed. The PTM biopsy samples were negative for cancer and were from the cohort that underwent prostatectomy. A confirmation study used 98 benign biopsy specimens from 22 patients, 75 malignant biopsy specimens from 65 patients, and 123 PTM biopsy specimens from 96 patients. In the confirmation study, patients were required to have at least 2 successive negative biopsies; the first negative biopsy was used for analyses. For both the pilot and confirmation studies, samples for analysis were selected based on review of pathology reports. The levels of the mutation were measured by quantitative PCR and using PCR cycle threshold data were used to calculate a score for each biopsy sample. In the pilot study, the scores were statistically significant between benign and malignant (p<0.000) and benign and proximal (p<0.003) samples. The PTM samples closely resembled the malignant sample, with no statistical significant resolution between the scores (p<0.833), to which the authors attributed as a field cancerization phenomenon. Results from the larger confirmation study were similar. Compared with histopathologic examination of the benign and malignant samples, the sensitivity and specificity were 80% and 71%, respectively, and the area under a ROC curve was 0.83 for the deletion. A blinded, external validation study showed a sensitivity and specificity of 83% and 79% and the area under the ROC curve 0.87.

In 2010, Robinson et al. assessed the clinical value of the 3.4-kb deletion described in the Maki study in predicting re-biopsy outcomes. Levels of the deletion were measured by quantitative PCR in prostate biopsies negative for cancer from 101 patients who underwent repeat biopsy within 1 year and had known outcomes. Of the 101 first biopsies, the diagnosis was normal in 8, atypical and/or had prostatic intraepithelial neoplasia in 50, and hyperplasia or inflammation in 43. Using an empirically established cycle threshold cutoff, the lowest cycle threshold as diagnostic of prostate cancer, and the histopathologic diagnosis on second biopsy, the clinical
performance of the deletion was calculated. The final data were based on 94 patients, who on second biopsy had 20 malignant and 74 benign diagnoses. The cycle cutoff gave a sensitivity and specificity of 84% and 54%, respectively, with the area under a ROC curve of 0.75. Negative predictive value was 91%.

**Gene Hypermethylation for Diagnosis**

Epigenetic changes—chromatin protein modifications that do not involve changes to the underlying DNA sequence but can result in changes in gene expression—have been identified in specific genes. An extensive literature reports significant associations between epigenetic DNA modifications and prostate cancer. Studies are primarily small, retrospective pilot evaluations of the hypermethylation status of various candidate genes for discriminating prostate cancer from benign conditions (diagnosis) or for predicting disease recurrence and association with clinicopathologic predictors of aggressive disease (prognosis). A 2008 TEC Special Report noted that the best markers for diagnosis had not yet been identified. Research gaps included nonstandardized assays, interpretation criteria, and sample types for measuring potential biomarkers. Consistency and comparison of results across studies is therefore lacking.

**GSTP1** is the most widely studied methylation marker for prostate cancer, usually as a diagnostic application. Two studies of GSTP1 hypermethylation using tissue samples reported significant results for identifying cancer with sensitivity of 92%, specificity of 85%, and AUC of about 0.9.53,54 However, 2 other studies did not find significant associations with disease.55,56 In spite of these contradictory results, several investigators have evaluated detection of hypermethylation products in biological fluids for early detection of prostate cancer. Suh et al studied the ejaculates of patients with prostate cancer and observed methylated GSTP1 in 4 of 9 patients.57 Goessl et al confirmed the presence of the methylated biomarker in ejaculates (50%) and demonstrated an association with cancer using serum (82% of cancer patients), urine (36%), and urine after prostatic massage (73%).

Subsequently, Ellinger et al studied hypermethylation of GSTP1 with additional genes (T1G1, Reprimo, PTGS2) in 226 patients (168 with prostate cancer) in an effort to provide a more consistent yield of positive results.59 Detection of aberrant methylation in serum DNA had high specificity (92%) but variable and more modest sensitivity (42 to 47%) for cancer. Sunami et al assayed blood from 40 healthy individuals and 83 men with prostate cancer using a 3-gene cohort (GSTP1, RASSF1, RARβ2) and demonstrated sensitivity of 28% for cancer patients.

In a (2011) meta-analysis of 30 peer reviewed studies evaluating hypermethylation of GSTP1 and other genes in prostate tissue, Van Neste et al suggested that a valuable first step in diagnostic use might be to test for methylated genes to select patients undergoing prostate biopsy who might not require repeat biopsy.61

Trock et al (2011) conducted a small (86-patient) diagnostic exploratory cohort study and showed that hypermethylation of adenomatous polyposis coli (APC) was associated with high sensitivity and high specificity for cancer on repeat biopsy.62 There was no evidence suggesting how this test should be used to change management.

Stewart et al (2013) investigated a quantitative methylation assay (including GSTP1, APC, RASSF1) as a predictive test for occult prostate cancer.63 The study retrospectively assayed 498 prostate biopsy tissue samples from patients who had negative histopathologic findings on first
biopsy but received follow-up biopsy within 30 months. The authors reported sensitivity of 68% (95% CI, 57 to 77) and specificity of 64% (95% CI, 59 to 69) for the assay to predict occult cancer. Negative predictive value was 90% (95% CI, 87 to 93), which offered a significant improvement compared with histologic diagnosis alone (negative predictive value, 70%). On multivariate analysis, assay score was a significant predictor of prostate cancer on second biopsy, with odds ratio of 3.17 (95% CI, 1.81 to 5.5; p<0.001).

In 2013, several studies reported associations between DNA hypermethylation at various gene loci (RASSF1A, APC, GSTP1, PTGS2, RAR-beta, TIG1, AOX1, C1orf114, GAS6, HAPLN3, KLF8, MOB3B) and prostate cancer. In contrast, Kachakova et al (2013) found that HIST1H4K hypermethylation was more likely due to aging than to prostate carcinogenesis.

A commercially available test for gene methylation is ConfirmMDx, described next.

**ConfirmMDx® (MDxHealth)**

ConfirmMDx is intended to distinguish true from false negative prostate biopsies to avoid the need for repeat biopsy in cases of a true negative and to identify men who may need a repeat biopsy. The test measures methylation of the genes GSTP1, APC, and RASSF1. The company's website states that the test has a negative predictive value of 90% in confirming a negative biopsy.

Published literature on the validation and clinical use of ConfirmMDx is described next.

In 2014, Wojno et al reported a field observation study in which practicing urologists at 5 centers had used the ConfirmMDx test to evaluate at least 40 men with previous cancer-negative biopsies who were considered to be at risk for prostate cancer. Centers reported whether patients who had a negative test assay result had undergone a repeat biopsy at the time of the analysis. Median patient follow-up time after the assay results were received was 9 months. A total of 138 patients were included in the analysis. The current median PSA level was 4.7 ng/mL. Repeat biopsies had been performed in 6 of the 138 men (4.3%) with a negative ConfirmMDx test, in which no cancer was identified.

Two blinded multicenter validation studies of the ConfirmMDx test have been performed. One evaluated archived, cancer-negative prostate biopsy core tissue samples from 350 men from a total of 5 U.S. urological centers. All of the patients underwent repeat biopsy within 24 months. The ConfirmMDx test, performed on the first biopsy, resulted in a negative predictive value of 88% (95% CI, 85 to 91). Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for age, PSA, DRE, first biopsy histopathology characteristics and race, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome (odds ratio [OR], 2.69; 95% CI, 1.60 to 4.51).

The other validation study tested archived cancer-negative prostate biopsy needle core tissue samples from 498 men from the United Kingdom and Belgium. Patients underwent repeat biopsy within 30 months. The ConfirmMDx test, performed on the first biopsy, resulted in a negative predictive value of 90% (95% CI, 87 to 93). Multivariate analysis of potential predictors of cancer on repeat biopsy, corrected for patient age, PSA, DRE, and first biopsy histopathology characteristics, showed that the ConfirmMDx test was the most significant independent predictor of patient outcome (OR=3.17; 95% CI, 1.81 to 5.53).
Single Nucleotide Polymorphisms Testing for Cancer Risk Assessment

Single Nucleotide Polymorphisms
Single nucleotide polymorphisms (SNPs) occur when a single nucleotide is replaced with another, and they are the most common type of genetic variation in humans. They occur normally throughout the genome and can act as biological markers for disease association. Genome-wide association studies have identified associations between prostate cancer risk and specific SNPs. However, it is generally accepted that individually, SNP-associated disease risk is low and of no value in screening for disease, although multiple SNPs in combination may account for a higher proportion of prostate cancer. Investigators have begun to explore the use of algorithms incorporating information from multiple SNPs to increase the clinical value of testing.

A 2012 Agency for Healthcare Research and Quality report on multigene panels in prostate cancer risk assessment reviewed the literature on SNP panel tests for assessing risk of prostate cancer.70 All of the studies included in the review had poor discriminative ability for predicting risk of prostate cancer, had moderate risk of bias, and none of the panels had been evaluated in routine clinical settings. The conclusions of the review were that the evidence on currently available SNP panels does not permit meaningful assessment of analytic validity, the limited evidence on clinical validity is insufficient to conclude that SNP panels would perform adequately as a screening test and that there is no evidence available on the clinical utility of current panels.

Kader et al (2012) evaluated a panel of 33 prostate cancer-associated SNPs that were identified from genome-wide association studies in 1654 men.71 Genetic score was a significant (p<0.001) independent predictor of prostate cancer, with an OR of 1.72 (95% CI, 1.44 to 2.09) after adjustment for clinical variables and family history. Addition of genetic markers to the classification of prostate cancer risk resulted in 33% of men reclassified into a different risk quartile. Approximately half of these (n=267) were downgraded to a lower risk quartile, and the other half (n=265) were upgraded into a higher risk quartile. The net reclassification benefit was 10% (p=0.002). The authors concluded that with the additional information of genetic score, the same number of cancers could be detected by using 15% fewer biopsies.

In a 2010 review by Ioannidis et al,72 27 gene variants across a large range of chromosomal locations were identified that increased risk for prostate cancer, although in all cases, the observed incremental risk was modest (OR ≤1.36).

Lindstrom et al (2011), in a study of 10,501 cases of prostate cancer and 10,831 controls, identified 36 SNPs showing association with prostate cancer risk including 2 (rs2735893, rs266849) that showed differential association with Gleason grade.73 Per allele ORs ranged from 1.07 to 1.44.

Ishaak and Giri (2011) reviewed 11 replication studies involving 30 SNPs (19 in men of African descent, 10 in men with familial prostate cancer).74 ORs were positively associated with prostate cancer, although the magnitude of association was generally small (range, 1.11-2.63).

In summary, numerous studies have demonstrated the association of many different SNPs with prostate cancer. These studies generally show a modest degree of association with future risk for prostate cancer in patients with prostate cancer. The clinical utility of these tests is uncertain;
there is no evidence that information obtained from SNPs testing can be used to change management in ways that will improve outcomes.

**Ongoing and Unpublished Clinical Trials**
Some currently unpublished trials that might influence this policy are listed in Table 1.

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<th>NCT No.</th>
<th>Trial Name</th>
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<td>Medical Economics of Urinary PCSA3 Test for Prostate Cancer Diagnosis</td>
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<td>Prostate Cancer Risk Assessment Using Genetic Markers in General Practice</td>
<td>1298</td>
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NCT: national clinical trial.

**Summary of Evidence**
Evidence on the clinical validity of genetic and protein biomarker tests related to prostate cancer is variable and incomplete, leaving considerable uncertainty regarding clinical performance characteristics such as sensitivity, specificity, and predictive value. Some tests show evidence for predictive ability in the diagnosis of prostate cancer; however, incremental accuracy in comparison with currently available tests has not been consistently demonstrated. In addition, these data do not demonstrate clinical utility, ie, that using a test will change treatment decisions and improve subsequent outcomes.

Numerous studies have demonstrated the association of many different single nucleotide polymorphisms (SNPs) with prostate cancer, and these studies generally show a modest degree of association with future risk for prostate cancer. However, the clinical utility of these tests is uncertain; there is no evidence that information obtained from SNP testing can be used to change management in ways that will improve outcomes.

**Practice Guidelines and Position Statements**

**American Urological Association**
In 2013, American Urological Association published guidelines for the early detection of prostate cancer. Based on systematic review of the literature to 2013, the guideline panel recognized that novel urinary markers, such as *PCA3* and *TMPRSS2:ERG*, may be “used as adjuncts for informing decisions about the need for a prostate biopsy—or repeat biopsy—after PSA [prostate-specific antigen] screening,” but emphasized the lack of evidence “that these tests will increase the ratio of benefit to harm.”
Evaluation of Genomic Applications in Practice and Prevention

In 2013, the Evaluation of Genomic Applications in Practice and Prevention Working Group published the following recommendations for PCA3 testing in prostate cancer, based on the Agency for Healthcare Quality and Research comparative effectiveness review\textsuperscript{22} summarized earlier\textsuperscript{76}:

- Evidence was insufficient to recommend PCA3 testing to inform decisions for when to re-biopsy previously biopsy-negative patients for prostate cancer, or to inform decisions to conduct initial biopsies for prostate cancer in at-risk men (eg, previous elevated PSA or suspicious digital rectal examination).
- Evidence was insufficient to recommend PCA3 testing in men with cancer-positive biopsies to determine if the disease is indolent or aggressive in order to develop an optimal treatment plan.
- The overall certainty of clinical validity to predict the diagnosis of prostate cancer using PCA3 is deemed “low.” Clinical use for diagnosis is discouraged unless further evidence supports improved clinical validity.
- The overall certainty of net health benefit is deemed “low.” Clinical use is discouraged unless further evidence supports improved clinical outcomes.

National Comprehensive Cancer Network

Current National Comprehensive Cancer Network guidelines\textsuperscript{77} recommend PCA3 testing in men with a suspicious digital rectal exam, PSA greater than 3.0 ng/mL, or excess risk based on multiple factors (eg, accelerated PSA velocity or elevated risk using a risk calculator tool) who have not undergone a transrectal ultrasound-guided biopsy.\textsuperscript{77} PCA3 is recommended as one of several tests to consider for following patients who have had a negative biopsy. Guideline authors note:

“Biomarkers that improve the specificity of detection are not recommended as first-line screening tests, but are reserved mostly for selecting for repeat biopsy, those who have undergone at least one negative biopsy...PCA3 score greater than 35 is strongly suspicious for prostate cancer.”

U.S. Preventive Services Task Force Recommendations

The U.S. Preventive Services Task Force published recommendations for Prostate Cancer Screening on May 2012. Genetic and protein biomarkers addressed in this policy, including PCA3, were not mentioned.

CODING

The following codes for treatment and procedures applicable to this policy are included below for informational purposes. Inclusion or exclusion of a procedure, diagnosis or device code(s) does not constitute or imply member coverage or provider reimbursement. Please refer to the member’s contract benefits in effect at the time of service to determine coverage or non-coverage of these services as it applies to an individual member.

CPT/HCPCS

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<tr>
<td>81313</td>
<td>PCA3/KLK3 (prostate cancer antigen 3 [non-protein coding]/kallikrein-related peptidase 3 [prostate specific antigen]) ratio (eg, prostate cancer)</td>
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<tr>
<td>81479</td>
<td>Unlisted molecular pathology procedure</td>
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<tr>
<td>81599</td>
<td>Unlisted multianalyte assay with algorithmic analysis</td>
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0010M Oncology (High-Grade Prostate Cancer), biochemical assay of four proteins (Total PSA, Free PSA, Intact PSA and human kallikrein 2 [hK2]) plus patient age, digital rectal examination status, and no history of positive prostate biopsy, utilizing plasma, prognostic algorithm reported as a probability score

- Effective July 1, 2015, there is a specific CPT code for 4Kscore™ Test: 0010M.
- Effective January 1, 2015, there is a specific CPT code for PCA3 testing: 81313.
- Prior to 2015, for PCA3 testing and currently for the other types of testing mentioned above, there are no specific CPT codes. The unlisted molecular pathology code 81479 would be used. If the test includes multiple assays, utilizes an algorithmic analysis and is reported as a numeric score or a probability, the unlisted multianalyte assay with algorithmic analysis (MAAA) code 81599 would be reported.

DIAGNOSES
Experimental / investigational for all diagnoses related to this policy.

REVISIONS

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In Policy section:
- In Item A removed "Genetic tests for the screening, detection, and management" and "This includes, but is not limited to the following:" and added "The following genetic and protein biomarkers for the diagnosis" to read "The following genetic and protein biomarkers for the diagnosis of prostate cancer are considered experimental / investigational."
- In Item A added the following E/I protein biomarkers:
  1. Kallikrein markers (eg, 4Kscore™ Test)
  2. Metabolomic profiles (eg, Prostarix™)
  6. Mitochondrial DNA mutation testing (eg, Prostate Core Mitomics Test™)
- In Item A 3 added "testing" and removed "for disease diagnosis and prognosis" to read "PCA3 testing"
- In Item A 4 removed "for diagnosis and prognosis" to read "TMPRSS fusion genes"
- In Item A 5 added "Candidate" and removed "multiple gene tests" and "for prostate cancer diagnosis" to read "Candidate gene panels"
- In Item A 7 added "testing (eg, ConfirmMDx®)" and removed "for diagnosis and prognosis" to read "Gene hypermethylation testing (eg, ConfirmMDx®)"
- In Item A relocated "single-nucleotide polymorphisms (SNPs) for risk assessment" to stand-alone Item B to read "Single nucleotide polymorphisms (SNPs) testing for cancer risk assessment of prostate cancer is considered experimental / investigational."

Rationale section updated

In Coding section:
- Added CPT Codes: 81599, 0010M
- Updated Coding notations.

References updated

REFERENCES


76. Recommendations from the EGAPP Working Group: does PCA3 testing for the diagnosis and management of prostate cancer improve patient health outcomes? Genet Med. Sep 26 2013. PMID 24071797