IMPORTANT REMINDER

Medical Policies are developed to provide guidance for members and providers regarding coverage in accordance with contract terms. Benefit determinations are based in all cases on the applicable contract language. To the extent there may be any conflict between the Medical Policy and contract language, the contract language takes precedence.

PLEASE NOTE: Contracts exclude from coverage, among other things, services or procedures that are considered investigational or cosmetic. Providers may bill members for services or procedures that are considered investigational or cosmetic. Providers are encouraged to inform members before rendering such services that the members are likely to be financially responsible for the cost of these services.

DESCRIPTION

There are a variety of gene-based biomarkers that have been associated with prostate cancer. These tests have the potential to improve the accuracy of risk prediction, diagnosis, staging, or prognosis of prostate cancer.

Prostate cancer is a complex, heterogeneous disease. At the extremes of the spectrum, if left untreated, some prostate cancers behave aggressively, metastasize quickly, and cause mortality, while others are indolent and never progress to cause harm. Current challenges in prostate cancer care are assessing risk; providing early and accurate detection; monitoring low-risk patients undergoing surveillance only; predicting recurrence after initial treatment; detecting recurrence after treatment; and assessing efficacy of treatment for advanced disease.

In response to the need for better biomarkers for risk assessment, diagnosis, and prognosis, a variety of exploratory research is ongoing. Some products of this work have already been translated or are in the process of being translated into commercially available tests, including:

- Single-nucleotide polymorphisms (SNPs) for risk assessment
- The Gen-Probe PROGENSA® PCA3 Assay (PCA3) for diagnosis
- TMPRSS fusion genes for diagnosis and prognosis
• Gene hypermethylation for diagnosis and prognosis
• Mitochondrial DNA mutation testing for diagnosis

While studies using these tests generate information that may help elucidate the biologic mechanisms of prostate cancer and eventually help design treatments, the above-mentioned tests are currently in a developmental phase, without evidence of clinical utility for diagnosis, prognosis, or risk assessment. Many of the tests listed above have not been submitted to the U.S. Food and Drug Administration (FDA) for marketing clearance but, if available, are offered as laboratory-developed tests by Clinical Laboratory Improvement Amendments (CLIA) licensed laboratories.

SNP testing as part of genome-scanning tests with risk assessment for prostate cancer are offered by a variety of laboratories including Navigenics, LabCorp (23andme), and ARUP (deCode) as laboratory-developed tests. The PCA3 test is offered in the U.S. by a number of reference laboratories including ARUP, Mayo Medical Laboratories, and LabCorp. The reagents used in testing are developed by Gen-Probe. A test for hypermethylation of GSTP1 was available from LabCorp ("Glutathione S-transferase Gene [GSTP1, pi-class] Methylation Assay"); but as of January 2015 this test is no longer offered. Epigenomics AG has entered licensing agreements with one U.S. laboratory (Quest Diagnostics) to establish and commercialize laboratory-developed tests for its proprietary methylation biomarker GSTP1. This test is not yet available, and it is unclear what matrices will be used.

**Regulatory Status**

One mitochondrial DNA test, Mitomics (Broomfield, CO), is currently available. Mitomics offers the Prostate Core Mitomics Test which measures mitochondrial DNA mutations in a negative prostate biopsy to determine whether a patient should undergo repeat biopsy. The test is performed on the initial negative prostate biopsy tissue.

The PCA3 Assay was approved by the FDA on February 15, 2012 through the premarket approval process. According to the approval granted by the FDA,[1]

> “The PROGENSA PCA3 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 one or more previous negative prostate biopsies and for whom a repeat biopsy would be recommended by a urologist based on current standard of care, before consideration of PROGENSA PCA3 assay results.”

The other tests mentioned in this policy, if available, are offered as laboratory-developed tests under the Clinical Laboratory Improvement Amendments (CLIA) licensed laboratories.

**MEDICAL POLICY CRITERIA**

Genetic tests for the screening, detection, and management of prostate cancer are considered **investigational** including but not limited to the following:

A. Single-nucleotide polymorphisms (SNPs) for risk assessment;
B. PCA3 for disease diagnosis;
C. TMPRSS fusion genes for diagnosis and prognosis;
D. Gene hypermethylation for diagnosis and prognosis;
E. Mitochondrial DNA mutation testing for diagnosis.

POLICY GUIDELINES

It is critical that the list of information below is submitted for review to determine if the policy criteria are met. If any of these items are not submitted, it could impact our review and decision outcome.

1. Name of the genetic test(s) or panel test
2. Name of the performing laboratory and/or genetic testing organization (more than one may be listed)
3. The exact gene(s) and/or mutations being tested
4. Relevant billing codes
5. Description of how the genetic test results will guide clinical decisions that would not otherwise be made in the absence testing?
6. Medical records related to this genetic test
   - History and physical exam
   - Conventional testing and outcomes
   - Conservative treatment provided, if any

SCIENTIFIC EVIDENCE

In general, the evidence for genetic tests related to prostate cancer screening, detection, and management addresses either preliminary clinical associations between genetic tests and disease states or, in some cases, the clinical validity of these tests i.e., the association of the test result with outcomes of interest, expressed in terms of clinical performance characteristics such as sensitivity, specificity, predictive value, and comparisons to current standards using receiver-operating curve (ROC) analysis and/or logistic regression. There is no published evidence demonstrating clinical utility (i.e., a test will change treatment decisions and improve patient important outcomes).

Gene-Based Tests in General

A 2009 BlueCross BlueShield Association (BCBSA) TEC Special report of recently published studies on gene-based tests (SNPs, PCA3, TMPRSS, gene panels, and gene hypermethylation) for prostate cancer risk assessment and diagnosis concluded that, in general, research on these tests is still in a “developmental phase, currently without evidence of clinical utility.”[2]

Single-Nucleotide Polymorphisms (SNPs) for Prostate Cancer Risk Assessment and Prognosis

There have been numerous large observational correlational studies focusing on the association of many different SNPs with prostate cancer, an example of which includes the study by Lindstrom and colleagues of 10,501 cases of prostate cancer and 10,831 controls, which identified 36 SNPs showing
association with prostate cancer risk including two (rs2735893 and rs266849) showing differential association with Gleason grade. Per allele odds ratios ranged from 1.07 to 1.44.\[3\]

Because the SNPs individually provide relatively modest incremental information on both the occurrence of cancer and its behavior, investigators have begun to explore use of algorithms incorporating information from multiple SNPs to increase the clinical value of testing. Several such recent studies focused on the development of testing algorithms incorporating SNPs.\[4-7\]

**Systematic Reviews**

A systematic review of multigene panels for prostate cancer risk assessment was published by Little et al. in 2016.\[8\] The authors included 21 studies that evaluated 18 individual panels. All studies were focused on clinical validity, moderate risk of bias, and had poor discriminative ability for predicting prostate cancer risk and/or distinguishing between aggressive and latent cancers. The authors noted that the current evidence is insufficient to assess analytic validity, and that “at best the panels assessed would add a small and clinically unimportant improvement” to current factors used for risk stratification, like age and family history. Additionally, they found no evidence on the clinical utility of these panels.

A 2012 AHRQ report on multigene panels in prostate cancer risk assessment, also by Little et al., reviewed the literature on SNP panel tests for assessing risk of prostate cancer.\[9\] 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.

Ishaak and Giri reviewed 11 replication studies involving 30 SNPs (19 in men of African descent and 10 in men with familial prostate cancer).\[10\] Odds ratios were positively associated with prostate cancer, although the magnitude of association was generally small (range, 1.11–2.63).

Al Olama et al. conducted a meta-analysis of 4 GWAS including 5,953 cases of aggressive prostate cancer (PCa) and 11,463 controls (men without PCa).\[11\] Authors computed association tests for approximately 2.6 million SNPs and followed up the most significant SNPs by genotyping 49,121 samples in 29 studies through the international PRACTICAL and BPC3 consortia. The authors confirmed the association of a PCa susceptibility locus, rs11672691 on chromosome 19, but also showed an association with aggressive PCa [odds ratio = 1.12 (95% confidence interval 1.03-1.21), P = 1.4 × 10(-8)]. The authors concluded their report described a genetic variant which is associated with aggressive PCa, and which is a type of PCa associated with a poorer prognosis.

**Nonrandomized Studies**

A pilot study by Castro et al. tested the use of a 71-SNP panel in 100 men with a family history of prostate cancer.\[12\] These men underwent a prostate biopsy regardless of PSA level, and 25 were diagnosed with prostate cancer. Age and PSA level were significantly associated with a cancer diagnosis, but the SNP risk score was not. While this study might not have been adequately powered to detect such an association, there was a clear relationship seen for age and PSA level (p = 0.00004 and 0.00037, respectively).
Kader et al. evaluated a panel of 33 SNPs identified from GWAS associated with prostate cancer in 1,654 men. Genetic score was a significant (p<.001) independent predictor of prostate cancer, with an odds ratio of 1.72 (95% CI, 1.44-2.09) 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. However, this study includes a limited sample size and there is no clear indication of how clinical management changed when patients were reclassified into lower risk groups.

Ren et al. calculated genetic scores for various combinations of 29 PCa risk-associated SNPs in 667 consecutive patients that underwent prostate biopsy. Performance of these genetic scores for discriminating prostate biopsy outcomes were compared using the area under a receiver operating characteristic curve (AUC). The discriminative performance of genetic score derived from a panel of all 29 SNPs (24 previous and 5 new) was similar to that derived from the 24 previously established SNPs, the AUC of which were 0.60 and 0.61, respectively (P = 0.72). Authors concluded that genetic score based on PCa risk-associated SNPs implicated to date is a significant predictor of biopsy outcome.

Tsuchiya et al. identified 14 SNPs in 6 genes (XRCC4, PMS1, GATA3, IL13, CASP8, and IGF1) that were statistically associated with cancer-specific survival. Using a subset of 6 SNPs, 3 subgroups of men with prostate cancer were defined by the number of SNP’s present (0 to 1, 2 to 3, or 4 to 6). Median cancer-specific survival in these subgroups was 13.3, 7.0, and 3.8 years, respectively (log-rank test, p<0.001).

Conclusion

The available evidence generally reports a modest association with future risk for prostate cancer and/or prognosis in patients with prostate cancer. The clinical utility of these tests is uncertain, there is no evidence that the information obtained from SNP testing can be used to change management in ways that will improve outcomes.

**PCA3 for Prostate Cancer Diagnosis**

*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 the amount of prostate cells released into the urine (*PCA3* Score), the test has been proposed for use in discriminating between patients with eventual benign findings on (first or second) biopsies from those with malignant biopsy results. In particular, the test may be especially helpful at identifying patients with elevated PSA levels but negative first biopsy results who need a follow-up biopsy.

Systematic Reviews

In 2013, the Agency for Healthcare Quality and Research (AHRQ) published a comparative effectiveness review entitled, “*PCA3* Testing for the Diagnosis and Management of Prostate Cancer.” 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 *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 PCA3 in treatment decision-making for men with positive biopsy.

In a 2012 systematic review, authors discuss the potential use of genetic markers to better define groups of men at high risk of developing prostate cancer, to improve screening techniques, discriminate indolent versus aggressive disease, and improve therapeutic strategies in patients with advanced disease.[17] Genetic tests for PCA3 and TMPRSS2-ERG genes were included. Authors concluded that most markers have not been prospectively validated for providing useful prognostic or predictive information or improvement upon clinicopathologic parameters already in use.

A meta-analysis by Ruiz-Aragon and Marquez-Pelaez reviewed 14 studies of PCA3 for use in predicting prostate biopsy results.[18] Sensitivity of testing ranged from 47% to 82% and specificity from 56% to 89%. Global results provided a sensitivity of 85% (confidence interval [CI]: 84 to 87) and a 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.

Nonrandomized Studies

Several studies published between 2013 and 2016 reported positive associations between PCA3 levels and prostate cancer diagnosis.[19-27] Predictive value was increased when PCA3 testing was combined with PSA level and other clinical information.[27-29] Other groups reported moderate diagnostic accuracy of PCA3 testing. Among men with PSA level greater than 3 ng/mL, AUC of PCA3 was 0.74.[30] Conversely, in men with PCA3 scores of 100 or greater, positive predictive value was 39%.[31] In a multicenter study of 647 men, sensitivity and specificity were 67% and 72%, respectively; AUC was 0.742.[32] Three studies compared PCA3 to multiparametric MRI; MRI was more accurate than PCA3,[33,34] but the combination was better than either alone.[35]

In 2014, the National Cancer Institute conducted a prospective trial to validate the diagnostic use of PCA3 to complement PSA-based detection of prostate cancer.[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 endpoint was the diagnosis of prostate cancer on biopsy and the secondary study endpoint was diagnosis of high grade prostate cancer, defined as a Gleason score greater than 6. The primary analyses, including 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 PCA3 score of more than 60, diagnostic sensitivity and specificity of 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 NPV 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≤.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 48-52% with attendant increases in missed prostate cancers of 6-15% using either a PCA3-based nomogram[37] or PCA3 level corrected for prostate volume (PCA3 density).[38] Although both studies were prospective, neither assessed utility of the test for clinical decision-making because all patients
underwent biopsy, and recurrence or survival outcomes were not evaluated. Another group evaluated the estimated long-term impact of using the \textit{PCA3} score to guide the decision to recommend a repeat biopsy for men with elevated PSA levels.\cite{39} Their models suggested that using a \textit{PCA3} score threshold of 25 would result in 55.4\% reduction in repeat biopsies for a base-case patient, while reducing the 10-year survival by 0.93\%. However these, estimates have not been validated in real patient populations.

\textbf{Conclusion}

Studies of \textit{PCA3} as a diagnostic test for prostate cancer 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 \textit{PCA3} results. Several studies have reported a modest incremental improvement in diagnostic accuracy when \textit{PCA3} was tested in combination with PSA level and other clinical findings. The clinical utility of this test is uncertain, as there is insufficient evidence that the use of \textit{PCA3} can be used to change management in ways that improve outcomes.

\textbf{TMPRSS Fusion Genes}

\textit{TMPRSS2} fusion gene detection has been studied for prognostic value (e.g., to identify aggressive disease or to predict disease recurrence). In prostate cancer, it may be fused to an ETS family transcription factor (ERG, ETV1, ETV4, or ETV5), which modulates transcription of target genes involved in cell growth, transformation, and apoptosis (\textit{TMPRSS2:ERG}).

No studies of clinical utility have been published to date; the evidence consists of correlational studies (association between a fusion gene and prostate cancer).\cite{40-43} However, the results of available studies differ as to the accuracy of \textit{TMPRSS2:ERG} in improving the ability to predict prostate cancer, and/or the ability to estimate prognosis for this purpose.

\textbf{Systematic Review}

In 2013, Yao et al published a systematic review with meta-analysis of \textit{TMPRSS2:ERG} for the detection of prostate cancer.\cite{44} Literature was searched and 32 articles were identified. 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, eg, elevated PSA and/or biopsy-negative. There also was variability in the type of tissue samples analyzed (urine, prostatic secretions, biopsy or surgical specimens); the type of \textit{TMPRSS2:ERG} assays used (fluorescence in situ hybridization [FISH], immunohistochemistry [IHC], real-time reverse transcriptase polymerase chain reaction [RT-PCR], and transcription-mediated amplification); and in \textit{TMPRSS2:ERG} threshold cutoff values.

\textbf{Nonrandomized Studies}

Leyton and others investigated the predictive value of \textit{PCA3} and \textit{TMPRSS2} as individual biomarkers and as part of a panel in a prospective, multicenter study of 443 men.\cite{45} \textit{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 re-biopsy or magnetic resonance imaging (MRI) be performed in \textit{TMPRSS2:ERG}-positive patients who do not have prostate cancer detected on initial biopsy. The authors stated that if \textit{PCA3} in combination with \textit{TMPRSS2} data had been used to select men for prostate biopsy,
35% of biopsies could have been avoided. However, the clinical utility of this test is uncertain, as there are no studies that report the test leads to changes in management that result in improved health outcomes.

Whelan et al (2014) compared 2 multivariate models to assess up-staging in 216 patients meeting National Comprehensive Cancer Network (NCCN) criteria for active surveillance.[46] (65) One model included TMPRSS2:ERG plus serum PSA; the other model included serum PSA, total RNA in expressed prostatic secretion (EPS, collected by milking the urethra after prostatic massage), and total EPS volume. AUCs were similar (0.80 [95% CI, 0.75 to 0.85] and 0.79 [95% CI, 0.73 to 0.84], respectively). However, the second model was more accurate for detecting patients who were up-staged, or up-staged and up-graded, by NCCN criteria. Specifically, the second model decreased the risk of up-staging in patients with a negative test approximately 8-fold (from 7% to 1%); decreased the risk of up-staging plus up-grading approximately 5-fold (from 5% to 1%); and doubled the prevalence of up-staging in the positive test group. In comparison, the TMPRSS2:ERG model decreased up-staging 2.4-fold (from 7% to 3%) and decreased upstaging and upgrading approximately 3-fold (from 5% to 2%).

A modeling study by Merdan et al. estimated that using TMPRSS2:ERG score to guide repeat biopsy decisions in men with elevated PSA could avoid 64.7% of these biopsies, but also reduce the 10-year survival rate by 1.4%. These estimations have not been validated in real-world trials.

A number of studies have reported positive associations between TMPRSS2 fusion gene levels and prostate cancer diagnosis.[23,24,47,48] One study reported a lack of association between TMPRSS2:ERG status and biochemical relapse-free rate in 244 men treated with image-guided radiotherapy (IGRT) for prostate cancer.[49] The authors concluded that “TMPRSS2-ERG is therefore unlikely to be a predictive factor for IGRT response.”

Conclusion

Limited evidence reports that the measurement of TMPRSS2:ERG may improve the ability to predict prostate cancer, and/or the ability to estimate prognosis. However, the results of available studies differ as to the accuracy of TMPRSS2:ERG for this purpose. In addition, the clinical utility of this test is uncertain.

TMPRSS2:ERG in Combination With PCA3

The evidence of increased accuracy following simultaneous detection of TMPRSS2:ERG and PCA3 is limited. Three studies that address TMPRSS2:ERG and PCA3 are described below.

Nonrandomized Studies

Tomlins et al. developed a transcription-mediated amplification assay to measure TMPRSS2:ERG fusion transcripts in parallel with PCA3.[43] Combining results from these 2 tests and incorporating them into the multivariate Prostate Cancer Prevention Trial risk calculator improved the identification of patients with clinically significant cancer by Epstein criteria and high-grade cancer on biopsy. Though the study was large (1312 men at multiple centers), results were confounded by assay modifications during the course of the study, by the use of cross-validation rather than independent validation, and the use of independent training and testing sets. A validation study by the same group evaluated this risk prediction model (termed Mi-Prostate Score or MiPS), which incorporated serum PSA with urine TMPRSS2:ERG, and PCA3, in a group of 1244 men presenting for biopsy.[50] They reported that it improved on PSA
alone for predicting prostate cancer and high-grade prostate cancer, but did not assess the clinical utility of this risk score.

A study by Feibus et al. evaluated the clinical use of PCA3 and TMPRSS2:ERG in African-American men undergoing prostate biopsy.[26] This study included 182 African-American and 139 nonAfrican-American patients. They found that PCA3 and TMPRSS2:ERG scores were associated with prostate cancer, and adding PCA3 to a standard of care plus PSA model improved concordance statistics for the detection of any prostate cancer in both groups. However, PCA3 score was only predictive of high grade prostate cancer in African-Americans and not in nonAfrican-Americans, while TMPRSS2:ERG did not improve these measures in either group.

In a pilot study, Salami et al. evaluated 45 men using a multivariable algorithm that included serum PSA plus urine TMPSS2:ERG and PCA3 from a post-DRE sample.[51] Samples were collected before prostate biopsy at 2 centers. For cancer prediction, sensitivity and specificity were 80% and 90%, respectively. AUC was 0.88. Limitations in this study included the small number of patients and authors used a quantitative RT-PCR assay to measure PCA3 in urine sediment, and evaluation of this model with the commercially available TMA assay of whole urine would help inform broader clinical applicability. Authors concluded a larger validation study could determine whether a multiplex model combining PCA3, TMPRSS2:ERG and serum PSA can predict aggressive versus indolent prostate cancer than any of these biomarkers alone.

Robert et al. 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.[52] Sensitivity, specificity, and positive and negative predictive values for the tests in combination were 94%, 98%, 96%, and 96%, respectively. The limitations of the study included that not all samples were confirmed with a diagnosis, there was a lack of urinary data, and the study results are not generalizable to clinical practice.

Conclusion

Concomitant detection of TMPRSS2:ERG and PCA3 may accurately identify men with prostate cancer. However, estimated accuracy varies across the available studies and clinical utility has not been established.

Gene Hypermethylation for Prostate Cancer Diagnosis and Prognosis

Epigenetic changes, chromatin protein modifications that do not involve changes to the underlying DNA sequence but which can result in changes in gene expression (particularly expression of genes associated with prostate cancer), have been identified in specific genes. An extensive literature reports significant associations between epigenetic DNA modifications and prostate cancer. Studies of genetic associations aim to test whether single-locus alleles or genotype frequencies differ between two groups of individuals (usually diseased subjects and healthy controls). Association studies cannot test causality. The identified studies are primarily small, retrospective pilot evaluations of 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). Research gaps included nonstandardized assays, interpretation criteria, and sample types for measuring potential biomarkers. Consistency and comparison of results across studies is therefore lacking. No published studies were identified that demonstrated the clinical utility of testing.
Several studies reported associations between DNA hypermethylation at various gene loci (RASSF1A, APC, GSTP1, PTGS2, RAR-beta, TIG1, AOX1, C1orf114, GAS6, HAPLN3, KLF8, and MOB3B) and prostate cancer.\textsuperscript{136,43,53-64} In contrast, several studies have not found evidence of an association.\textsuperscript{65,66} Further, Kachakova et al. concluded that HIST1H4K hypermethylation was more likely due to aging than to prostate carcinogenesis.\textsuperscript{67} Nevertheless, no standardized assays and interpretation criteria have been established to enable consistency and comparison of results across studies.

Conclusion

Studies reporting the diagnostic accuracy and predictive ability of gene hypermethylation report differing results regarding the accuracy of hypermethylation. These inconsistent results make it difficult to determine whether hypermethylation is a useful parameter for diagnosis and/or prognosis of prostate cancer. Further research is needed to elucidate the clinical validity of this test.

Mitochondrial DNA Mutation Testing

Mutations in the mitochondrial genome (mtDNA) are emerging as tools for the diagnosis of prostate cancer. A growing body of literature is reporting significant associations between both single nucleotide changes and large scale deletions in mtDNA and prostate cancer, however, the identified studies are of small to medium sample size and do not address clinical utility. A laboratory developed assay, offered by Mitomics (formerly Genesis Genomics), called the Prostate Core Mitomics Test\textsuperscript{TM} (PCMT) is a proprietary test which is intended to determine whether a patient has prostate cancer, despite a negative prostate biopsy, by analyzing deletions in mitochondrial DNA by 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%.

Nonrandomized Studies

A trial published in 2016 examined the role of the mitochondrial genome in prostate cancer risk in 4,086 prostate cancer cases and 3,698 controls from the Multiethnic Cohort.\textsuperscript{68} In this study, 350 mitochondrial SNPs were tested in five racial/ethnic populations: Asian Americans, Africans, Europeans, Latinos, and Native Hawaiians. No significant associations were found.

Published literature from Genesis Genomics on the use of mitochondrial DNA mutations in prostate includes several studies. A 2006 study retrospectively analyzed mitochondrial DNA mutations from three 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).\textsuperscript{69} 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, seven (58.3%) patients were found to have between one and five alterations, mainly in non-coding 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[70]. 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 who 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 upon 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<.0001) and benign and proximal (p<.003) samples. The PTM samples closely resembled the malignant sample, with no statistical significant resolution between their scores (p<.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[70]. Levels of the deletion were measured by quantitative PCR in prostate biopsies negative for cancer from 101 patients who underwent repeat biopsy within a year and had known outcomes. Of the 101 first biopsies, the diagnosis was normal in 8, atypical and/or had prostatic intraepithelial neoplasia (PIN) 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 was 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%.

Conclusion

Studies using the PCMT test for the diagnosis of prostate cancer reported sensitivities and specificities in the moderate range. In general, these studies are preliminary and only report on clinical validity. There is a lack of standardization in methodology and the clinical utility of this test was not addressed.

Clinical Practice Guidelines

American Urological Association (AUA)

In 2013, the AUA published guidelines for the early detection of prostate cancer.[71] Based on a systematic review of the literature, 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 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 (EGAPP)
In 2013, the EGAPP Working Group published the following recommendations for PCA3 testing in prostate cancer, based on an AHRQ comparative effectiveness systematic review summarized above:\[72\]

- 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 (e.g., 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 (NCCN)

The NCCN guidelines suggest considering tests that improve specificity in higher risk patients after negative biopsy, including 4Kscore, percent free PSA, PHI, PCA3, and ConfirmMDx.\[73\] PCA3 is not recommended for use in the initial biopsy setting. Guideline authors note:

“Biomarkers that improve the specificity of detection are not recommended as first-line screening tests. However, there may be some patients who meet PSA standards for consideration of prostate biopsy, but for whom the patient and/or the physician wish to further define the probability of high-grade cancer. A percent free PSA <10%, PHI >35 or 4Kscore (which provides an estimate of the probability of high-grade prostate cancer) are potentially informative in patients who have never undergone biopsy or after a negative biopsy; a PCA3 score >35 is potentially informative after a negative biopsy.”

U.S. Preventive Services Task Force Recommendations

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

Summary

There is not enough research to recommend using gene-based tests for prostate cancer screening, detection and management, as many important characteristics of these tests have not yet been determined. Some research shows that they might help predict the diagnosis or prognosis of prostate cancer, but it is not yet known how much information they add to currently available tests. More research is needed to demonstrate how these tests can improve outcomes for patients. Therefore, use of gene-based testing for screening, detection, and management of prostate cancer is considered investigational.

REFERENCES


22. Capoluongo, E, Zambon, CF, Basso, D, et al. PCA3 score of 20 could improve prostate cancer detection: results obtained on 734 Italian individuals. *Clinica chimica acta; international journal of clinical chemistry.* 2014 Feb 15;429:46-50. PMID: 24269853


### CROSS REFERENCES

**Genetic and Molecular Diagnostic Testing**, Genetic Testing, Policy No. 20

**Gene Expression Analysis for Prostate Cancer Management**, Genetic Testing, Policy No. 71

**Protein Biomarkers for Screening, Detection, and/or Management of Prostate Cancer**, Laboratory, Policy No. 69

### CODES

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