BRIEF COMMUNICATION

Quantitative GSTP1 Methylation and the Detection of Prostate Adenocarcinoma in Sextant Biopsies

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Hypermethylation of the 5′ promoter region of the glutathione *S*-transferase π gene (GSTP1) occurs at a very high frequency in prostate adenocarcinoma. We compared the results of blinded histologic review of sextant biopsy samples from 72 excised prostates with those obtained using a quantitative methylation-specific polymerase chain reaction assay (QMSP) for GSTP1. Formal surgical pathologic review of the resected prostates was used to determine the number of patients with (n = 61) and without (n = 11) prostate cancer. Histology alone detected prostate carcinoma with 64% sensitivity (95% confidence interval [CI] = 51% to 76%) and 100% specificity (95% CI = 72% to 100%), whereas the combination of histology and GSTP1 QMSP at an assay threshold greater than 10 detected prostate carcinoma with 75% sensitivity (95% CI = 63% to 86%) and 100% specificity (95% CI = 72% to 100%), an 11% improvement (95% CI = 5% to 22%) in sensitivity over histology alone. The combination of histology and GSTP1 QMSP at an assay threshold greater than 5 detected prostate adenocarcinoma with 79% sensitivity (95% CI = 68% to 89%), a 15% improvement (95% CI = 7% to 26%) over histology alone. Thus, GSTP1 QMSP improved the sensitivity of histologic review of random needle biopsies for prostate cancer diagnosis. Further studies should determine whether detection of GSTP1 hypermethylation in a biopsy sample with normal histology indicates the need for an early repeat biopsy at the same site. [J Natl Cancer Inst 2003;95: 1634–7]

Prostatic adenocarcinoma is the most commonly diagnosed non-cutaneous cancer among men in the United States *(1)*. Men diagnosed with early-stage, small-volume prostate disease have the best outcomes following curative treatment *(2)*. Therefore, the aim of early-detection programs is to diagnose prostate cancer when it is at an early and curable stage. Prostate cancer diagnosis currently entails a digital rectal examination and the measurement of serum prostate-specific antigen (PSA) levels, which are frequently elevated in men with prostate cancer, followed by a transrectal prostatic needle biopsy. However, serum PSA levels can also be elevated in men who have benign conditions of the prostate, and needle biopsies may fail to identify even clinically significant amounts of cancer because of sampling error. Therefore, additional diagnostic tests are needed to improve the sensitivity of prostate cancer diagnosis.

Hypermethylation of the 5′ promoter region of the gene encoding glutathione *S*-transferase π (GSTP1) occurs at a very high frequency (i.e., 90%–96%) in prostate adenocarcinoma *(3,4)*. GSTP1 methylation can be detected with the use of methylation-specific polymerase chain reaction (PCR) assays *(5–7)*. We have developed a quantitative methylation-specific PCR (QMSP) method to detect GSTP1 hypermethylation and have used it to accurately distinguish between benign and neoplastic prostate biopsy specimens *(8)*. In a larger blinded study, we successfully used the GSTP1 QMSP assay to detect tiny foci of prostate adenocarcinoma in archived biopsy samples *(9)*.

In this study, we directly compared the diagnostic results obtained using the GSTP1 QMSP assay with those obtained using standard histologic review of needle biopsy specimens, with the goal of improving the sensitivity of prostate cancer diagnosis. Included in this study were 56 patients who were undergoing prostatectomy for prostate adenocarcinoma (median PSA level = 5.0 ng/mL, range = 0.5–25.8 ng/mL) and 16 patients who were undergoing cystoprostatectomy for bladder carcinoma at The Johns Hopkins Hospital between November 2001 and May 2002. Immediately after resection, sextant needle biopsy samples were taken from the left and right apex, middle, and base of all 72 prostate specimens and promptly frozen at −80 °C. All needle biopsy samples were sectioned, and DNA was then extracted from each sample as previously described *(10)*. In addition, a 5-μm frozen section was taken after every 10 sections from the needle biopsy samples, stained with hematoxylin–eosin, and examined by light microscopy, in a blinded fashion, by an expert uropathologist (J. I. Epstein). All of the resected prostates were then serially sectioned, and the complete series of sections from each prostate was submitted for formal surgical pathologic review, which is considered the gold standard for determining the presence of prostatic carcinoma. To examine the GSTP1 gene methylation status of each biopsy sample, we performed sodium bisulfite conversion of the DNA isolated from each sample *(8)*. We then used fluorogenic real-time QMSP to quantitate GSTP1 methylation in the modified DNA samples *(8,9)*. The relative level of methylated GSTP1 DNA in each sample was determined as a ratio of methylation-specific PCR-amplified GSTP1 to MYOD1 or ACTB (reference genes) and then multiplied by 1000 for easier tabulation. All statistical tests and 95% confidence intervals (CIs) took into account the within-sample pairing of diagnostic tests; all statistical tests were two-sided. The sensitivity and specificity for

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See "Notes" following "References."

DOI: 10.1093/jnci/djg082

Journal of the National Cancer Institute, Vol. 95, No. 21,

a range of threshold values were determined, and an ideal threshold was identified that distinguished true-positive cases from true-negative cases.

The final surgical pathology review detected clinically undiagnosed prostate adenocarcinoma in five (31%) of the 16 patients who underwent cystoprostatectomy for bladder carcinoma. Thus, when these five patients were added to the 56 patients known to have prostate adenocarcinoma, our study included 61 true-positive cases of prostate cancer and 11 true-negative cases. The pathologic stages and grades *(11,12)* of the 61 true-positive cases of prostate cancer were stage T2a, Gleason scores 4 through 7 (n = 19); stage T2b, Gleason scores 6 and 7 (n = 29), stage T3a, Gleason scores 6 and 7 (n = 11); and stage T3b, one Gleason score of 7 and one ductal carcinoma (n = 2).

We calculated the sensitivities and specificities of histologic review and GSTP1 QMSP as diagnostic tests, both separately and in combination, requiring that only one of the six biopsy samples from each case be called positive for the case to be positive (Table 1). Blinded histologic assessment of the biopsy samples detected prostate adenocarcinoma in 39 of the 61 true-positive cases, corresponding to a sensitivity of 64% (95% CI = 51% to 76%). The median size of carcinoma detected in the biopsy samples for these cases was 1 mm (range = 0–20 mm). All 11 true-negative cases were found to be negative in the blinded histology assessment, corresponding to a specificity of 100% (95% CI = 72% to 100%). GSTP1 QMSP alone, using a threshold value of 10 for GSTP1/MYOD1 ratios that we previously found distinguished between benign and malignant prostate tissue with optimal sensitivity and specificity *(8)*, detected cancer in 43 of the 61 true-positive cases (median GSTP1/MYOD1 ratio = 41.3, range = 0–791.4), corresponding to a sensitivity of 70% (95% CI = 57% to 81%) (Fig. 1 and Table 1). All 11 true-negative cases were negative by GSTP1 QMSP (median GSTP1/MYOD1 ratio = 0, range = 0–2.5), corresponding to a specificity of 100% (95% CI = 72% to 100%). GSTP1 QMSP in combination with histologic review (defining positivity in either test as a positive test) detected cancer in 46 of the 61 true-positive cases, corresponding to a sensitivity of 75% (95% CI = 63% to 86%), which was an 11% improvement (95% CI = 5% to 22%) over histology alone, and a specificity of 100% (95% CI = 72% to 100%).

We also examined the sensitivity and specificity of the GSTP1 QMSP assay at a less stringent threshold value of 5 for the GSTP1/MYOD1 ratio. At this threshold, the specificity of GSTP1 QMSP alone remained at 100% (95% CI = 72% to 100%), whereas sensitivity was 75% (95% CI = 63% to 86%), not statistically significantly different from that of histology alone ($P = .06$). Moreover, at this threshold, the GSTP1 QMSP assay in combination with histology detected 48 of the 61 true-positive cases, corresponding to a sensitivity of 79% (95% CI = 68% to 89%), a 15% improvement (95% CI = 7% to 26%) over histology alone.

We used receiver operating characteristic (ROC) curves to display and contrast the performance of histology alone, GSTP1 QMSP alone, and GSTP1 QMSP in combination with histology (Fig. 1, B). At the optimum threshold of 5 (the point on the ROC curve closest to 100% sensitivity and corresponding to the highest specificity), GSTP1 QMSP detected nine cases of prostate cancer that were missed by histology, and histology detected two cases that were missed by GSTP1 QMSP, suggesting the importance of combining both methods in prostate cancer detection. Only one of the nine cases detected by GSTP1 QMSP but missed by histology consisted of small foci of intermediate-grade cancer that were considered "potentially insignificant" by the reviewing uropathologist; however, the natural history of these foci, if left untreated, is unknown.

We found that a substantial number of small, intermediate-grade cancers (22 of the 61 true-positive cases [36%]) were not detected by histologic analysis of frozen sections of sextant biopsy samples. A possible limitation of this finding is the fact that histologic review of frozen sections is technically more difficult than histologic review of paraffin sections, and a comparison of results of histologic review of formalin-fixed paraffin-embedded sections with those of GSTP1 QMSP analysis on the same needle biopsy specimens may have resulted in more favorable results for histology.

GSTP1 QMSP might improve prostate cancer diagnosis by overcoming sampling error because it can detect as few as four cancer cells and can take advantage of the recognized field effect in many cancers *(8)*. In this latter sce-

Table 1. Sensitivity and specificity of histology and the gene encoding glutathione *S*-transferase π (GSTP1) quantitative methylation-specific polymerase chain reaction (QMSP) assay in prostate cancer detection*

	Patients with prostate cancer					Patients without prostate cancer	
	Histology alone or GSTP1 QMSP combined with histology			GSTP1 QMSP alone		Histology alone or GSTP1 QMSP combined with histology	
Test (threshold)	No. test-positive/ No. true-positive	% sensitivity (95% CI)	Sensitivity increment† (95% CI)	No. test-positive/ No. true-positive	% Sensitivity (95% CI)	No. test-negative/ No. true-negative	% specificity (95% CI)
Histology	39/61	64 (51 to 76)	NA	NA	NA	11/11	100 (72 to 100)
GSTP1 QMSP (>10)	46/61	75 (63 to 86)	11 (5 to 22)	43/61	70 (57 to 81)	11/11	100 (72 to 100)
GSTP1 QMSP (>5)	48/61	79 (68 to 89)	15 (7 to 26)	46/61	75 (63 to 86)	11/11	100 (72 to 100)
GSTP1 QMSP (>2)	52/61	85 (74 to 93)	21 (12 to 34)	50/61	82 (70 to 91)	10/11	91 (58 to 99)
GSTP1 QMSP (>1)	54/61	89 (78 to 95)	25 (15 to 37)	54/61	89 (78 to 95)	7/11	64 (31 to 89)

*All statistical tests and confidence intervals took into account the within-sample pairing of diagnostic tests. Relative level of methylated GSTP1 DNA in each sample was determined as a ratio of methylation-specific polymerase chain reaction–amplified GSTP1 to the reference gene and then multiplied by 1000 for easier tabulation. Sensitivity and specificity for a range of threshold values for GSTP1 positivity as a diagnostic test were determined, and an ideal threshold was identified that distinguished cancer cases from controls. CI = confidence interval; NA = not applicable.

†Sensitivity increment is the increase in sensitivity over histology alone (sensitivity = 64%) produced by combination with GSTP1 QMSP, with positivity on the combined test defined as a positive test on either individual test alone.

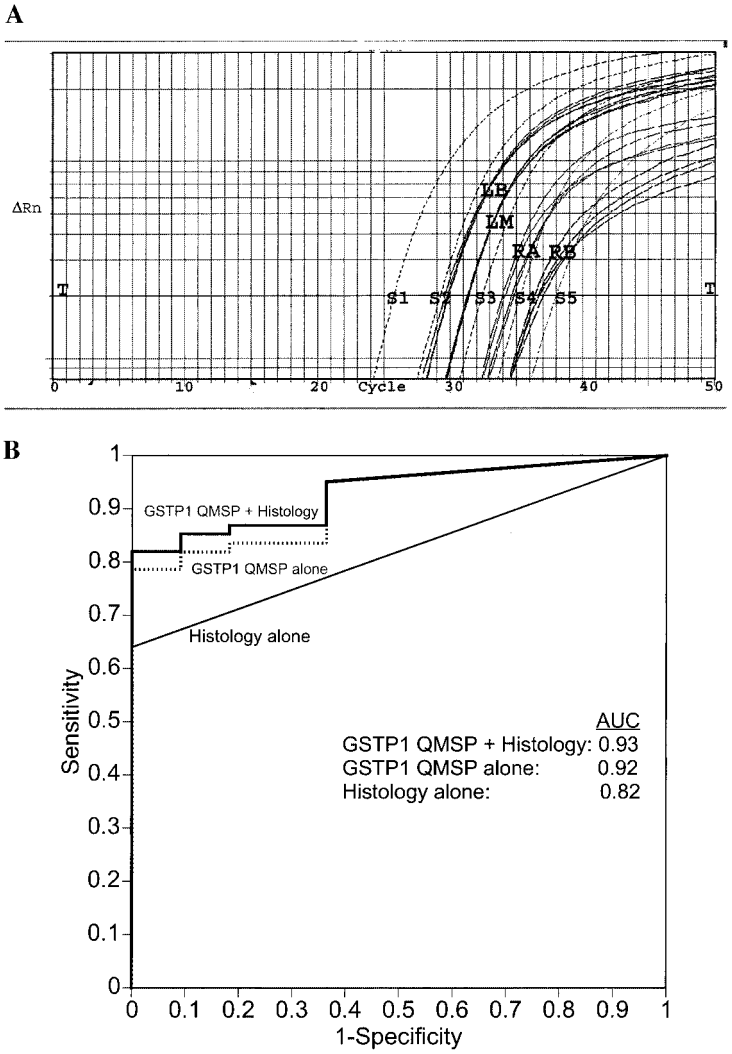

Fig. 1. Amplification curves for a patient who was positive for prostate carcinoma by GSTP1 quantitative methylation-specific polymerase chain reaction (QMSP) on four of six biopsy samples (**A**) and receiver operating characteristic (ROC) curves for histology, GSTP1 QMSP, and the two tests combined (**B). A**) DNA from each prostate biopsy sample (LB = left base, LM = left mid, RA = right apex, RB = right base), in quadruplicate, and standard dilutions (S1–S5 = 100 ng, 10 ng, 1 ng, 100 pg, and 10 pg, respectively) of DNA from the positive controls (human prostate cancer LNCaP cells and *in vitro*–methylated DNA from human leukocytes) were subjected to GSTP1 QMSP. *X*-axis is the polymerase chain reaction cycle number; *y*-axis (ΔRn) is defined as the cycle-to-cycle change in reporter fluorescence signal normalized to a passive fluorescence signal measured on a log scale. A biopsy sample was considered to be positive for prostate carcinoma if three of the four replicates showed amplification. A standard curve was generated for the dilutions of the positive control on the basis of the cycle number at which each amplification curve crossed the horizontal threshold line, T. All biopsy samples were then quantitated relative to their position on the curve *(13)*. The relative level of methylated GSTP1 in a particular sample was calculated using the ratio of the averaged GSTP1 value to that of the corresponding internal reference genes MYOD1 and ACTB (curves not shown). This ratio was then multiplied by 1000 for easier tabulation. This case was also positive for carcinoma by histology on two of the six biopsy samples (i.e., the LB sample had a 2-mm carcinoma and the LM sample had a 1-mm carcinoma). The extent of tumor seen histologically corresponded to the levels of GSTP1 methylation detected. **B**) The ROC curves display the estimated sensitivity and specificity at various thresholds for defining a positive test for GSTP1 QMSP (histology had only one threshold). For the combined tests, positivity in either test was considered a positive test. The greater the area under the ROC curve (AUC), the more discriminating the test. At all thresholds with perfect specificity, GSTP1 QMSP had greater sensitivity than histology alone.

nario, cells surrounding the neoplasm harbor some, but not all, of the genetic alterations in the primary tumor and thus do not always display the morphologic characteristics of the neoplasm. Our finding, that more biopsy samples from each true-positive case were positive by GSTP1 QMSP (mode = three positive/six total samples) than by histology (mode = one positive/six total samples), supports the benefit of performing this molecular analysis for every biopsy taken, particularly for the diagnosis of very small low- or intermediate-grade cancers that can be difficult to detect histologically on biopsy.

High specificity is very important for any diagnostic test, particularly when treatment options for the diagnosed disease include major surgery. In our study, the 95% confidence intervals for the specificities of both tests were wide because of the small number of true-negative samples analyzed. However, adding GSTP1 QMSP analysis to histology substantially improved diagnostic sensitivity for prostate cancer detection, even when we used a very conservative threshold ratio (i.e., GSTP1/MYOD1 ratio >10) that was associated with a stringent specificity. Thus, quantitation of GSTP1 methylation may provide an effective way to improve the diagnostic sensitivity of histologic review for prostate cancer on needle biopsy. At the very least, patients whose needle biopsy samples are negative by histologic review but have elevated levels of GSTP1 methylation may be at high risk for prostate cancer and thus should be prioritized for an early repeat biopsy to improve their chances of having the earliest possible diagnosis of prostate cancer. Moreover, biopsy samples that are positive by GSTP1 QMSP may correctly predict the location of the cancer, potentially identifying the best sites for repeat biopsies. Our results suggest that the addition of GSTP1 QMSP to routine histologic analysis of paraffin-embedded biopsy samples is likely to improve the sensitivity of diagnostic needle biopsies. However, our results require further testing in a large prospective trial of patients undergoing diagnostic transrectal needle biopsies.

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NOTES

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Funding was provided by Public Health Service grant 1U01CA84986 (D. Sidransky) from the National Cancer Institute, National Institutes of Health, Department of Health and Human Services and by OncoMethylome Sciences.

Manuscript received November 22, 2002; revised August 19, 2003; accepted September 4, 2003.