

The Use of a Whole-Blood RNA Transcript-Based Diagnostic Test to Improve the Diagnosis of Prostate Cancer (CaP) Compared with Prostate-Specific Antigen (PSA) Alone

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Abstract

Introduction: Screening for prostate cancer (CaP) with PSA testing is limited by a high number of false negatives as well as false positives, particularly in the setting of benign prostatic hypertrophy (BPH). The goal of this study was to develop a whole blood RNA transcript-based test that improves the diagnosis of untreated, localized CaP over PSA alone.

Methods: From August 2006 to October 2008, three prospective cohorts of 484 men consented to the collection of whole blood in PAXgene™ Blood RNA tubes for gene expression analysis: men with newly diagnosed, localized, untreated CaP (N=204), healthy normal men (N=170), and otherwise healthy men with BPH (N=110). The cohorts were split into age-matched (median age 61 yrs) Training and Validation sets. 174 inflammation and CaP-related genes (Source MDx Precision Profiles™) were assayed using optimized Q-PCR technology for 182 subject samples ($N_{\text{CaP}}=76$, $N_{\text{normals}}=76$, $N_{\text{BPH}}=30$) in the Training set. Logistic regression methods were used to develop a 6-gene model (with and without the inclusion of PSA) for discriminating between CaP patients and Normal subjects. This 6-gene model (with and without the inclusion of PSA) was validated in an independent sample set of 302 subjects ($N_{\text{CaP}}=128$, $N_{\text{normals}}=94$, $N_{\text{BPH}}=30$).

Abstract, continued

Results: A 6-gene model (RP51077B9.4, CD97, CDKN2A, SP1, S100A6, IQGAP1) developed in a Training set of CaP patients (N=76) and Normal healthy subjects (N=76) was found to diagnose CaP with a high degree of sensitivity (CaP, 88.2%) and specificity (Normals, 85.5%). Successful validation of this 6-gene model was achieved in an independent set of CaP patients (N=128) and Normal healthy subjects (N=94) using pre-specified coefficients and cut points established in the Training set. Validation in this independent set had an observed sensitivity of 85.9% and specificity of 83.0%.

Sensitivity and specificity was significantly improved (97.4% and 96.1%, respectively) in the Training set with the incorporation of PSA in the 6-gene model. Successful validation of this 6-gene + PSA model was again achieved in the independent set of CaP patients (N=128) and Normal healthy subjects (N=94) using pre-specified coefficients and cut points established in the Training set. Validation in this independent set had an observed sensitivity of 87.2% and specificity of 92.6%. Inclusion of BPH subjects in the Training and independent validation sets reduced the specificity to 91.5% and 91.4% respectively.

Conclusions: Development of a 6-gene model in a Training set of samples that is further validated in an independent dataset strongly suggest that specific whole blood RNA transcript levels can assess abnormal gene expression levels associated with untreated, localized CaP. Validation of such a model with and without the inclusion of PSA supports its potential value as a diagnostic tool in the management of early stage prostate cancer with economic benefits to the healthcare system.

Introduction

Each year in the U.S., over one million men undergo the anxiety and pain of prostate biopsies that are negative for cancer at a considerable psychological and social cost to the patients as well as a cost to the healthcare system of >\$2 billion ¹.

Two recent studies published in the *New England Journal of Medicine*² draw into question the value of PSA-based methods for prostate cancer screening, concluding that for every one life saved by PSA screening, 49 men suffer some harm from treatment. *An important limitation of PSA-based screening for prostate cancer is the high incidence of benign prostatic hypertrophy (BPH) in older men.*

The goal of this study was to develop a diagnostic test based on whole blood RNA transcript-based biomarkers to improve the diagnosis of CaP over the standard age-adjusted PSA criterion.

¹ Beck, Melinda. March 31, 2009, "The Man, the Gland, the Dilemmas", The Wall Street Journal.

²G. L. Andriole and others. Volume 360, March 26, 2009, Number 13, "Mortality Results from a Randomized Prostate-Cancer Screening Trial" New England Journal of Medicine.

²F. H. Schröder and others. Volume 360, March 26, 2009, Number 13, "Screening and Prostate-Cancer Mortality in a Randomized European Study" New England Journal of Medicine.

Patient Population

Three prospective cohorts of men were consented and enrolled contemporaneously:

- Prostate cancer cohort accrued men with newly diagnosed, untreated prostate cancer treated at Dana Farber Cancer Institute (DFCI)
- Healthy, age-matched male “normals” accrued in Sarasota, FL, Plantation, FL and Charleston, SC. at health fairs and primary care offices
- Otherwise healthy men with BPH accrued in a Charleston, SC urology clinic

Sample Sizes of Training and Validation Subjects by Age and Group									
Age	PSA Cut off by Age	Number of Training Subjects by Age				Number of Validation Subjects by Age			
		BPH	Normals	CaP	Total	BPH	Normals	CaP	Total
40-49	2.0 ng/mL	0	9	4	13	0	25	7	32
50-59	3.0 ng/mL	5	29	31	65	11	39	47	97
60-69	4.0 ng/mL	10	28	32	70	32	10	55	97
70-79	7.0 ng/mL	14	9	8	31	30	14	17	61
80+	7.0 ng/mL	1	1	1	3	7	6	2	15
Total		30	76	76	182	80	94	128	302
Median Age =		69.5	59.5	60	61	69	55	61	61.5
Median PSA Value (ng/mL) by Group									
Median PSA of Training Subjects					Median PSA of Validation Subjects				
		BPH	Normals	CaP	Total	BPH	Normals	CaP	Total
Median PSA =		2.4	1.2	4.7	2.8	1.6	0.8	4.6	2.2

Methods

Men in all three cohorts consented to blood draw for whole blood RNA transcription profiling. In brief, the method is summarized below:

- ***Sample Processing***

Whole blood samples collected in PAXgene™ Blood RNA tubes were manually processed to total RNA. RNA quality and quantity was assessed on the Agilent Bioanalyzer 2100.

- ***First strand cDNA synthesis***

RNA was converted to cDNA in a random hexamer primed reaction with reverse transcriptase. cDNA was quality checked and used as the template in a quantitative PCR assay optimized for precision and calibration.

- ***Precision Profile™ Assay***

Source MDx proprietary primer probe sets were used for target gene specific amplification. Individual target genes were multiplexed with 18s rRNA endogenous control. Assays are configured in a 384-well plate formatted for triplicate measures and run on the ABI Prism® 7900HT Sequence Detection System.

- ***Data Analysis***

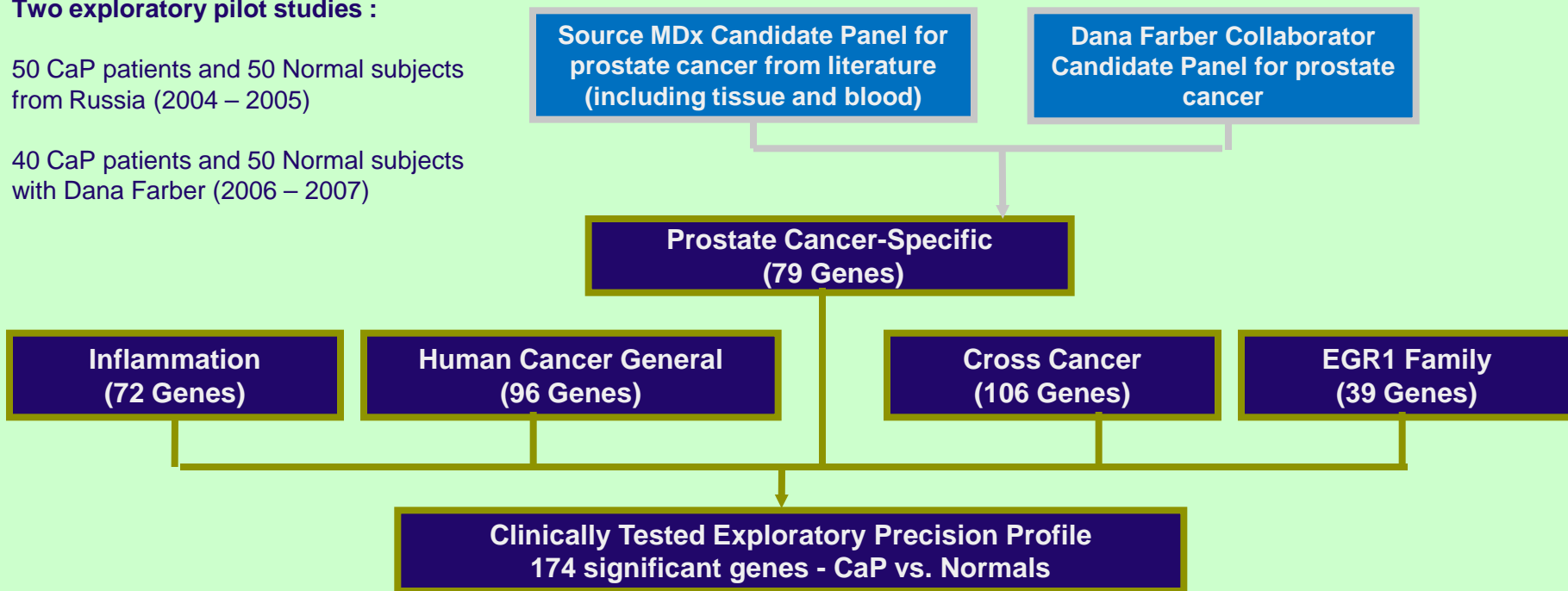
Data files are “filtered-by-rule” to ensure all replicate values meet predefined metrics. Normalized gene expression values (delta CT values) for each amplified target gene are calculated (target gene CT – endogenous control CT). Logistic regression methodology is used to obtain all possible 1, 2 and 3-gene models. Top qualifying 3-gene models are used to develop higher order models (4-6 gene) through stepwise regression technique.

Source MDx Prostate Cancer Candidate Diagnostic Test Development Involved 671 Subject Whole Blood Samples with 392 Genes Assayed

Two exploratory pilot studies :

50 CaP patients and 50 Normal subjects
from Russia (2004 – 2005)

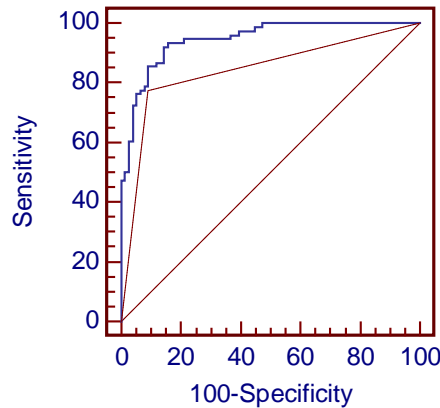
40 CaP patients and 50 Normal subjects
with Dana Farber (2006 – 2007)



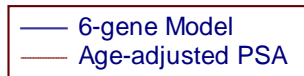
A 6-Gene Model Significantly Improves Prediction of Prostate Cancer Compared with Age-adjusted PSA

Training Set Results: CaP (N=76) vs. Normals (N=76)

Comparison of 6-gene Model vs. Age-adjusted PSA
ROC based on Training Data



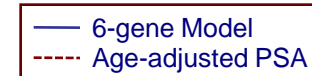
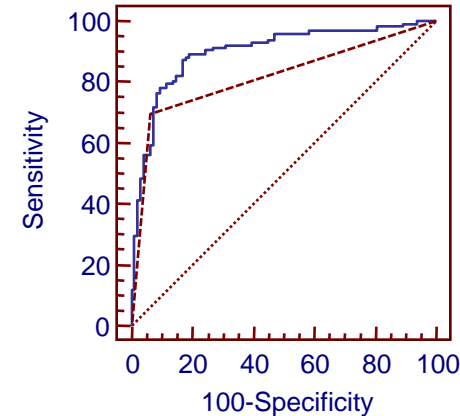
Predictors	beta	p-value
Intercept	-48.84	
SP1	-9.47	1.3E-06
CD97	3.67	4.0E-05
RP51077B9.4	5.19	7.2E-05
CDKN2A	1.89	1.4E-04
IQGAP1	3.44	6.4E-03
S100A6	-1.43	0.011



	6-gene	Age-adjusted PSA
Sensitivity	88.2%	77.6%
Specificity	85.5%	90.8%
AUC	0.946	0.842
AUC difference = .104 (p-value = .005)		

Validation Set Results: CaP (N=128) vs. Normals (N=94) Validation P-value = 1.3E-26

Comparison of 6-gene Model vs. Age-adjusted PSA
ROC based on Validation Data

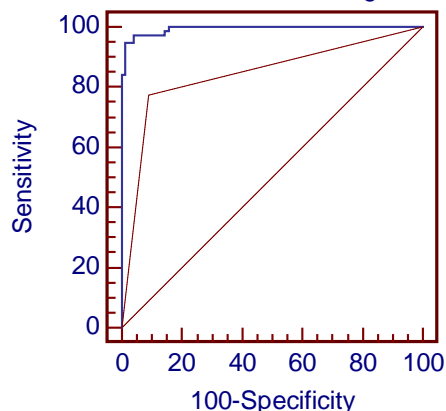


	6-gene	Age-adjusted PSA
Sensitivity	85.9%	69.5%
Specificity	83.0%	93.6%
AUC	0.898	0.816
AUC difference = .082 (p-value = .014)		

The Addition of PSA to the 6-Gene Model Further Improves the Diagnosis of Prostate Cancer

Training Set Results: CaP (N=76) vs. Normals (N=76)

'6-gene + PSA' Model vs. Age-adjusted PSA
ROC based on Training Data



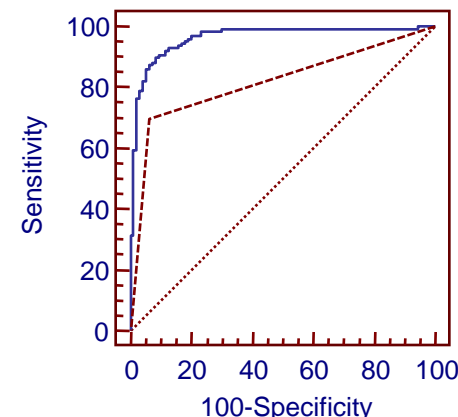
— '6-gene + PSA' Model
— Age-adjusted PSA

Predictors	beta	p-value
Intercept	-50.66	
plnPSA	4.50	4.4E-05
SP1	-15.11	2.8E-04
CD97	6.31	9.3E-04
RP51077B9.4	7.65	1.9E-03
CDKN2A	2.94	4.1E-03
S100A6	-2.63	0.014
IQGAP1	4.03	0.024

	6-gene + PSA	Age-adjusted PSA
Sensitivity	97.4%	77.6%
Specificity	96.1%	90.8%
AUC	0.994	0.842
AUC difference = .152 (p-value = 2.0E-6)		

Validation Set Results: CaP (N=128) vs. Normals (N=94) Validation P-value = 9.6E-37

'6-gene + PSA' Model vs. Age-adjusted PSA
ROC based on Validation Data



— '6-gene + PSA' Model
- - - Age-adjusted PSA

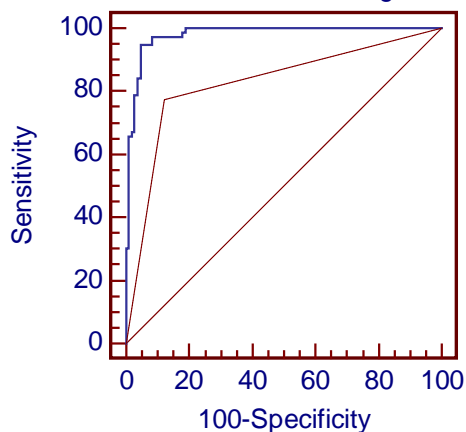
	6-gene + PSA	Age-adjusted PSA
Sensitivity	87.5%	69.5%
Specificity	92.6%	93.6%
AUC	0.962	0.816
AUC difference = .146 (p-value = 1.5E-7)		

6-Gene Model Plus PSA Retains its Superiority over Age-adjusted PSA Alone When BPH Subjects are Included with Normal Subjects

Training Set Results:

CaP (N=76) vs. Normals (N=76), BPH (N=30)

'6-gene + PSA' Model vs. Age-adjusted PSA
ROC based on Training Data



Predictors	beta	p-value
Intercept	-50.66	
pInPSA	4.50	4.4E-05
SP1	-15.11	2.8E-04
CD97	6.31	9.3E-04
RP51077B9.4	7.65	1.9E-03
CDKN2A	2.94	4.1E-03
S100A6	-2.63	0.014
IQGAP1	4.03	0.024

— '6-gene + PSA' Model
— Age-adjusted PSA

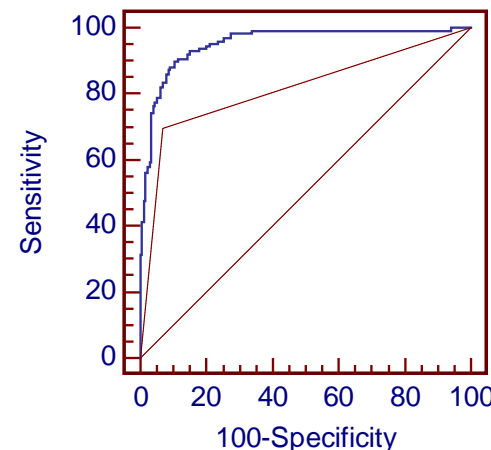
	6-gene + PSA	Age-adjusted PSA
Sensitivity	97.4%	77.6%
Specificity	91.5%	87.7%
AUC	0.979	0.827
AUC difference = .152 (p-value = 2.1E-6)		

Validation Set Results:

CaP (N=128) vs. Normals (N=94), BPH (N=80)

Validation P-value = 3.2E-47

'6-gene + PSA' Model vs. Age-adjusted PSA
ROC based on Validation Data

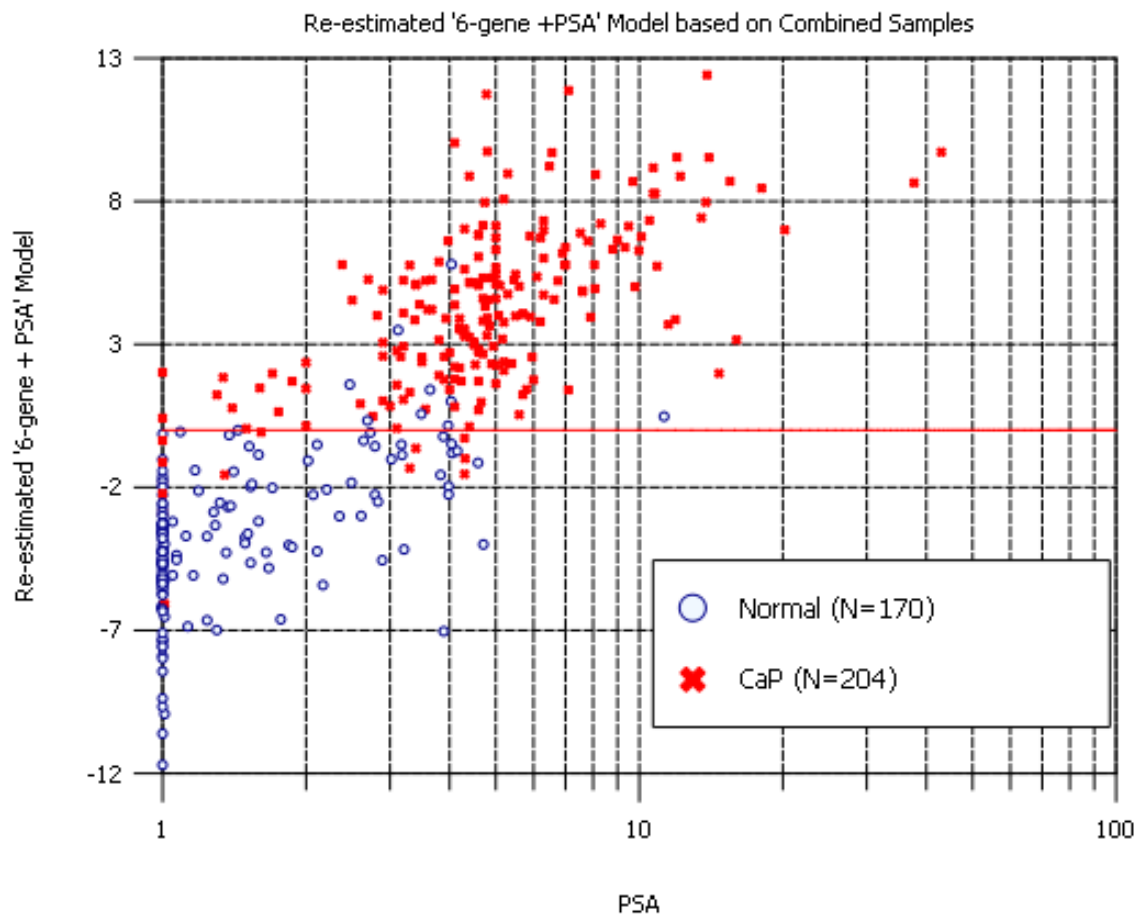


— '6-gene + PSA' Model
— Age-adjusted PSA

p-val = 9.0E-8	6-gene + PSA	Age-adjusted PSA
Sensitivity	87.5%	69.5%
Specificity	91.4%	93.1%
AUC	0.953	0.813
AUC difference = .140 (p-value = 9.0E-8)		

A Model Based on Combined Training and Validation Datasets Improves Sensitivity (93.6%) and Specificity (94.7%) of the 6-Gene + PSA Model

Combined Training and Validation Datasets: 6-gene + PSA model



Predictors	beta	p-value
Intercept	-25.34	
plnPSA	3.09	6.0E-15
SP1	-7.08	2.3E-09
CD97	4.37	4.1E-07
RP51077B9.4	2.98	4.1E-04
CDKN2A	1.16	0.002
S100A6	-0.78	0.07
IQGAP1	1.24	0.15

93.6% of the CaP subjects are correctly predicted by the model (above the red line)

94.7% of the Normal subjects are correctly predicted by the model (below the red line)

The Functional Consequences of the Differences Observed Suggest a Decrease in Immune System Activity in CaP vs. Normals

Gene Symbol	Gene Name	Gene Expression CaP vs. Normals	Hypothesized Functional Consequence
CD97	CD97 molecule	↓	Decreased Monocyte and T-cell Activation
CDKN2A	Cyclin-dependent kinase inhibitor 2A	↓	Decreased T-cell Activation
IQGAP1	IQ motif containing GTPase activating protein 1	↓	Decreased Phagocytic Activity (PMN / Monocytes)
RP5-1077B9.4 (MIIP)	Migration and invasion inhibitory protein	↓	Decreased T-cell Activation
S100A6	S100 calcium binding protein A6	↑	Increased Apoptosis (Decreased T-cell and Monocyte Survival)
SP1	Sp1 transcription factor	↓	Decreased Monocyte and T-cell Activation

Conclusions

- **Successful validation of a 6-gene model, with and without the inclusion of PSA, in an independent dataset demonstrates a significant improvement over the predictive value of the current age-adjusted PSA criterion alone.**
- **Specific whole blood RNA transcript levels can assess abnormal gene expression levels associated with untreated, localized CaP.**
- **A 6-gene, whole blood-based diagnostic test can be a powerful tool in the management of CaP to both reduce unnecessary biopsies in patients without CaP and detect CaP in patients with PSA values below the current cutoff.**
- **Further validation of this model will continue in multi-center studies in the near future.**

Acknowledgements

- **We would like to acknowledge the patients who consented to participate in this study.**
- **Funded by Source MDx, Dana-Farber/Harvard Cancer Center Prostate Cancer SPORE, Gelb Center and the Bing Sound Wong Fund.**