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Impact of the SPOP Mutant Subtype on the Interpretation of Clinical Parameters in Prostate Cancer.

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Impact of the SPOP Mutant Subtype on the Interpretation of Clinical Parameters in Prostate Cancer

original report

abstract

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Purpose Molecular characterization of prostate cancer, including The Cancer Genome Atlas, has revealed distinct subtypes with underlying genomic alterations. One of these core subtypes, *SPOP* (speckle-type POZ protein) mutant prostate cancer, has previously only been identifiable via DNA sequencing, which has made the impact on prognosis and routinely used risk stratification parameters unclear.

Methods We have developed a novel gene expression signature, classifier (Subclass Predictor Based on Transcriptional Data), and decision tree to predict the *SPOP* mutant subclass from RNA gene expression data and classify common prostate cancer molecular subtypes. We then validated and further interrogated the association of prostate cancer molecular subtypes with pathologic and clinical outcomes in retrospective and prospective cohorts of 8,158 patients.

Results The subclass predictor based on transcriptional data model showed high sensitivity and specificity in multiple cohorts across both RNA sequencing and microarray gene expression platforms. We predicted approximately 8% to 9% of cases to be *SPOP* mutant from both retrospective and prospective cohorts. We found that the *SPOP* mutant subclass was associated with lower frequency of positive margins, extraprostatic extension, and seminal vesicle invasion at prostatectomy; however, *SPOP* mutant cancers were associated with higher pretreatment serum prostate-specific antigen (PSA). The association between *SPOP* mutant status and higher PSA level was validated in three independent cohorts. Despite high pretreatment PSA, the *SPOP* mutant subtype was associated with a favorable prognosis with improved metastasis-free survival, particularly in patients with high-risk preoperative PSA levels.

Conclusion Using a novel gene expression model and a decision tree algorithm to define prostate cancer molecular subclasses, we found that the *SPOP* mutant subclass is associated with higher preoperative PSA, less adverse pathologic features, and favorable prognosis. These findings suggest a paradigm in which the interpretation of common risk stratification parameters, particularly PSA, may be influenced by the underlying molecular subtype of prostate cancer.

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INTRODUCTION

Prostate cancer is a clinically and molecularly heterogeneous disease.¹⁻⁴ Current risk stratification guidelines, such as those from the National Comprehensive Cancer Network,⁵ the American Urological Association/American Society for Therapeutic Radiology and Oncology,⁶ and the European Association of Urology–European Society for Radiotherapy–Oncology–International Society of Geriatric Oncology⁷ use clinical and pathologic parameters, including the level

of prostate-specific antigen (PSA), to guide management decisions for clinically localized disease. PSA is also used in a number of other clinical scenarios in prostate cancer, including initial diagnosis, monitoring for recurrence after primary therapy, and monitoring disease burden and treatment response for metastatic disease.

The emerging next-generation DNA and RNA sequencing data point toward distinct molecular subclasses of prostate cancer,^{2-4,8,9} but their clinical impact remains unclear. The Cancer

Genome Atlas (TCGA) study identified seven core prostate cancer subtypes, which were defined by underlying genomic alterations.⁴ One key molecular subclass, which represents approximately 10% of prostate cancers, harbors recurrent missense mutations in the E3 ubiquitin ligase component *SPOP*.^{2-4,10-13} To date, identification of *SPOP* mutations has required mutational analysis using genomic (DNA) sequencing. These cohorts typically have limited follow-up,^{3,4,10} thus limiting definitive conclusions about the clinical effect of the molecular subtypes. In contrast, gene expression (RNA) data are widely available from a variety of prostate cancer cohorts, often with the long follow-up necessary to define prognostic impact.¹⁴⁻²¹

Here, we reported the first development and validation of the Subclass Predictor Based on Transcriptional Data (SCaPT) model, which we used to define key prostate cancer molecular subclasses, including *SPOP* mutant cancer, using gene expression data. We used the SCaPT model to predict the molecular subclass from a retrospective cohort that included 1,626 patient samples and a prospective cohort that included 6,532 samples using genome-wide microarray gene expression data from a clinically available prognostic assay (Decipher; GenomeDx Biosciences, Vancouver, BC, Canada), and we explored the clinicopathologic and prognostic associations of key molecular subclasses of prostate cancer.

METHODS

Prostate Cancer Tumor Samples and Microarray Data

We used a total of 8,559 radical prostatectomy (RP) tumor expression profiles for training, testing, and validation. For training and testing, we used RNA sequencing expression and DNA mutation data from TCGA prostate cancer project (n = 333)⁴ and the Weill Cornell Medicine (WCM) sequencing (n = 68) cohort. For validation, expression profiles of retrospective (n = 1,626) and prospective (n = 6,532) cohorts were derived from the Decipher Genomics Resource Information Database (GRID) registry (ClinicalTrials.gov identifier: NCT02609269). The retrospective GRID cohort was pooled from seven published microarray studies: Cleveland Clinic,²² Erasmus MC,²³ Johns Hopkins,¹⁵ Memorial Sloan Kettering Cancer Center,¹ Mayo Clinic (Mayo I and Mayo II),^{20,24} and

Thomas Jefferson University.²¹ The prospective GRID cohort was from clinical use of the Decipher test. DNA and RNA from the TCGA and WCM cohorts were extracted from fresh frozen RP tumor tissue, as previously described.⁴ RNA from the GRID cohorts was extracted from routine formalin-fixed, paraffin-embedded RP tumor tissues, amplified, and hybridized to Human Exon 1.0 ST microarrays (Thermo Fisher Scientific, Waltham, MA).^{24,25}

SPOP Mutant Transcriptional Signature

We developed the *SPOP* mutant transcriptional signature, which includes 212 genes differentially expressed between *SPOP* mutant and wild-type samples from TCGA prostate cancer RNA sequencing data⁴ (Fig 1). Low-expressed genes (mean \log_2 [RNA-seq by expectation-maximization] < 1) were filtered before the analysis. Specifically, we identified significantly differentially expressed genes by comparing *SPOP* mutant and wild-type cases as determined from DNA mutational analyses among TCGA samples that lacked *ETS* family gene fusions (*ERG*, *ETV1*, *ETV4*, and *FLII*) using Wilcoxon rank-sum test,²⁶ and controlled for false discovery using Benjamini-Hochberg adjustment (false-discovery rate \leq 0.0001).²⁷ By performing DESeq2 (DESeq2 v1.20.0; <https://bioconductor.org/packages/release/bioc/html/DESeq2.html>) on *SPOP* mutant and wild-type cases (the same approach used initially), we found 300 differentially expressed genes at a false discovery rate of < 0.05 via DESeq2, and 105 genes that overlapped with the 212-gene list. The overlap between two methods was significant ($P < 2.2 \times 10^{-16}$), which confirmed similar results when applying these methods.

SCaPT Development on the Basis of *SPOP* Mutant Transcriptional Signature and the Support Vector Machine Model

To predict tumors in the *SPOP* mutant subclass in the absence of DNA sequencing data—that is, microarray data sets—we developed the SCaPT model on the basis of the support vector machine (SVM) model.²⁸⁻³⁰ Given a set of training data marked with two categories, SVM builds a model that assigns testing data into one category or the other, which makes it a nonprobabilistic binary linear classifier (Fig 2A). In our SCaPT model, training data were defined as the transcriptional

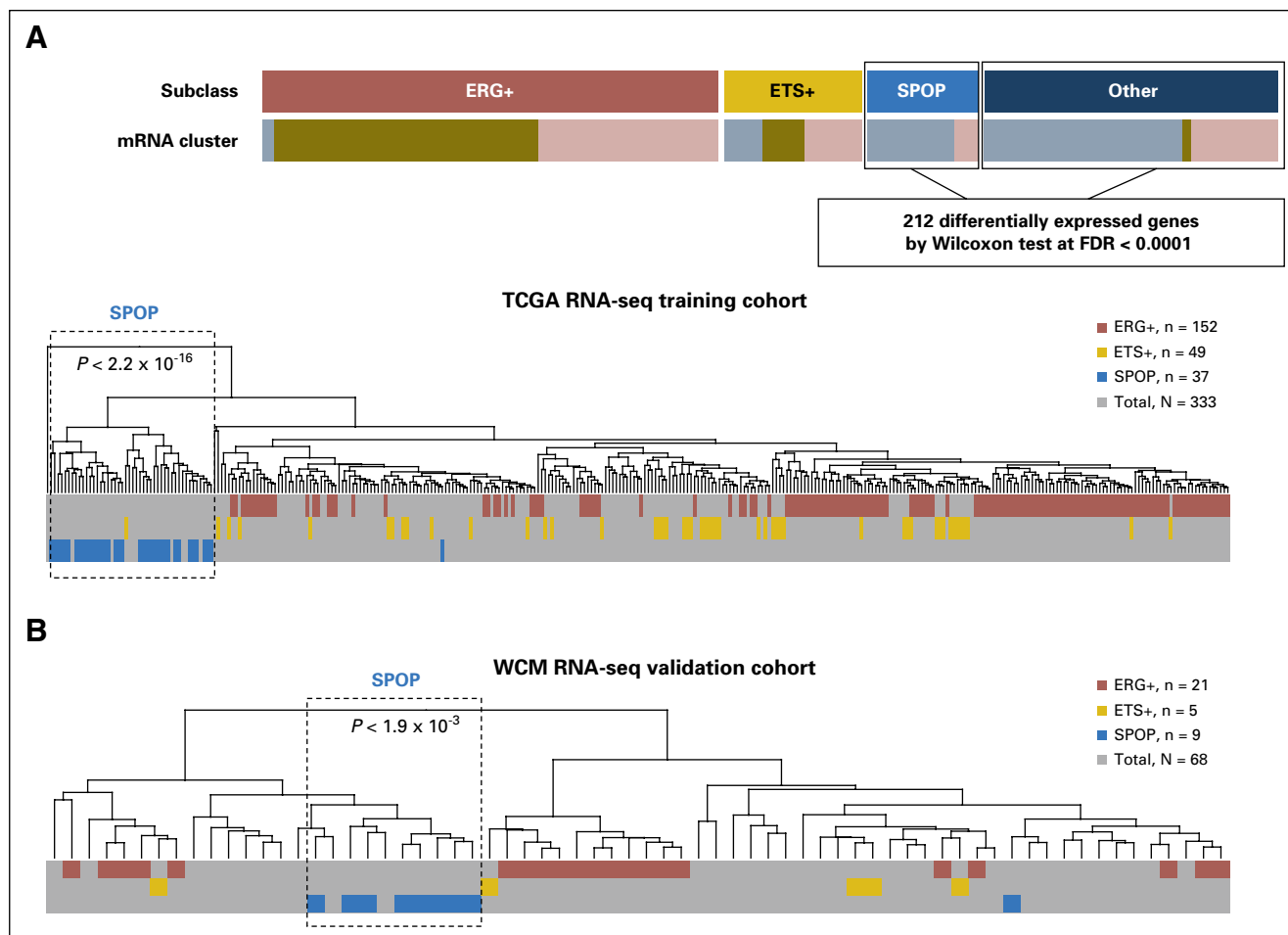


Fig 1. *SPOP* mutant transcriptional signature. (A) *SPOP* mutant transcriptional signature that included 212 differentially expressed genes between *SPOP* mutant and wild-type samples from The Cancer Genome Atlas (TCGA) non-ETS fusion RNA sequencing (RNA-seq) data. The signature was generated from the TCGA study and tested back in the TCGA training cohort. Significant enrichment of *SPOP* mutant cases was based on hierarchical clustering of 333 TCGA prostate cancer samples. Different colors represent molecular subclasses from genomic and transcriptomic annotations. (B) Significant enrichment of *SPOP* mutant case from 68 Weill Cornell Medicine (WCM) prostate cancer samples with *SPOP* mutant transcriptional signature on the basis of hierarchical clustering. *ERG*, *ERG*-fusion position; *ETS*: other *ETS* fusion positive; FDR, false-discovery rate.

z-scores of *SPOP* mutant signature from TCGA cohort. Testing data would be the transcriptional *z*-scores from RNA sequencing or microarray expression data of *SPOP* mutant signature. By performing 10-fold cross-validation on the TCGA training data set, we found cost—cost of constraints violation—equal to 0.04 to yield the model with the highest sensitivity and specificity. In the following analysis, we used cost equal to 0.04 to predict *SPOP* mutant subclass.

Prostate Cancer Molecular Subclass Prediction by Decision Tree

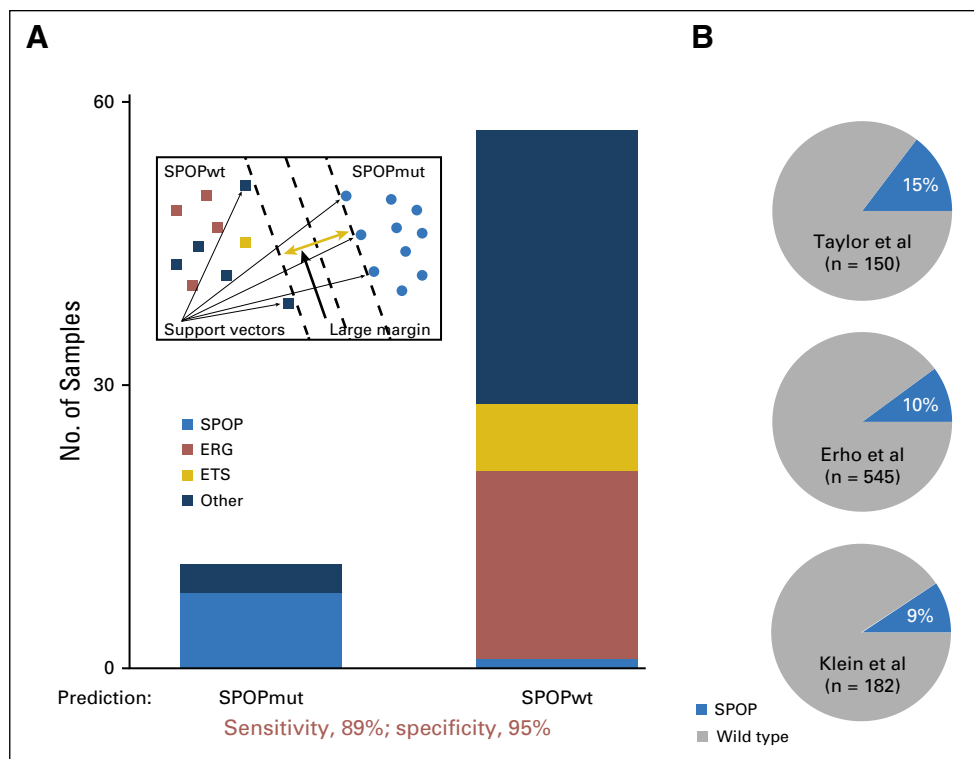
In each individual study of the retrospective and prospective GRID cohorts, the *SPOP* mutant subclass was first predicted using the SCaPT

model. Next, using a decision tree and previously developed microarray-based classifiers for the *ERG*-positive and *ETS*-positive subtypes,²⁵ we classified the remaining cases in each cohort. Some cases with both predicted *SPOP* mutant and *ERG*-positive/*ETS*-positive status were classified as conflict subclass, and the rest without *SPOP* mutant calling and outlier expression were considered as other subclass (Data Supplement).

Statistical Analysis

Statistical analyses were performed in R (version 3.1.2; <https://www.r-project.org/>). All statistical tests were two sided, with the significance level set at $P < .05$. Univariable logistic regression analyses were performed on the

Fig 2. High accuracy and confidence of *SPOP* mutant (*SPOP*mut) subclass prediction on Weill Cornell Medicine (WCM) and Gene Expression Omnibus (GEO) data set by the SCaPT (SubClass Predictor Based on Transcriptional Data) model. (A) SCaPT model example and its *SPOP*mut prediction on WCM prostate cancer RNA sequencing (RNA-seq) data. Different colors represent molecular subclasses from genomic and transcriptomic annotations. (B) The *SPOP*mut prediction of the SCaPT model on three independent exon array data downloaded from the GEO database. Data from Taylor et al,¹ Erho et al,²⁴ and Keln et al.²² wt, wild type.



combined cohort to test the statistical association between *SPOP* mutant status and clinical variables, including age, race, preoperative PSA, Gleason score, lymph node invasion, surgical margin status, extracapsular extension, and seminal vesicle invasion. We evaluated the associations between *SPOP* mutant status and patient outcomes, including biochemical recurrence, metastasis, and prostate cancer-specific mortality, on the basis of Kaplan-Meier analysis. Preoperative PSA from TCGA and Taylor cohorts were downloaded from cBioPortal (<http://www.cbioportal.org/>).^{31,32}

RESULTS

Development and Validation of a Transcriptional Signature for *SPOP* Mutant Prostate Cancer

To build an *SPOP* mutant prediction model that could be applied to RNA expression data, we first developed a transcriptional signature of *SPOP* mutant tumors. As *SPOP* mutations are mutually exclusive with *ERG* and other *ETS* rearrangements,¹³ we excluded prostate cancer samples with *ETS* fusions to define *SPOP* mutant-specific gene expression effects (Fig 1A). Using TCGA RNA sequencing data, we identified 212 differentially expressed genes between *SPOP* mutant and wild-type samples

(Data Supplement). Among those 212 genes, we found that upregulated genes from the *SPOP* mutant subgroup were enriched in transport vesicle membrane and oxidoreductase activity, but that there was no significant enriched function in downregulated genes from the *SPOP* mutant subgroup (Data Supplement). Applying the *SPOP* mutant transcriptional signature on the training data of 333 TCGA prostate cancer samples, we found significant enrichment ($P < 2.2 \times 10^{-16}$) of *SPOP* mutant cases on the basis of unsupervised clustering (Fig 1A and Data Supplement).

To test the *SPOP* mutant transcriptional signature, we used the WCM RNA sequencing cohort with *SPOP* mutant annotations based on whole-exome DNA sequencing (Data Supplement). In this independent cohort, we found significant enrichment ($P < 1.9 \times 10^{-3}$) of *SPOP* mutant tumors in one subcluster based on unsupervised clustering (Fig 1B and Data Supplement). We next used the *SPOP* mutant transcriptional signature in a locked SVM model—that is, with fixed parameters on the basis of the training step using the TCGA cohort (see Methods)—to generate scores for each sample in the WCM cohort. We found 89% sensitivity and 95% specificity of *SPOP* mutant prediction compared with DNA mutation annotation (Fig 2A). Finally, we applied the SCaPT model to

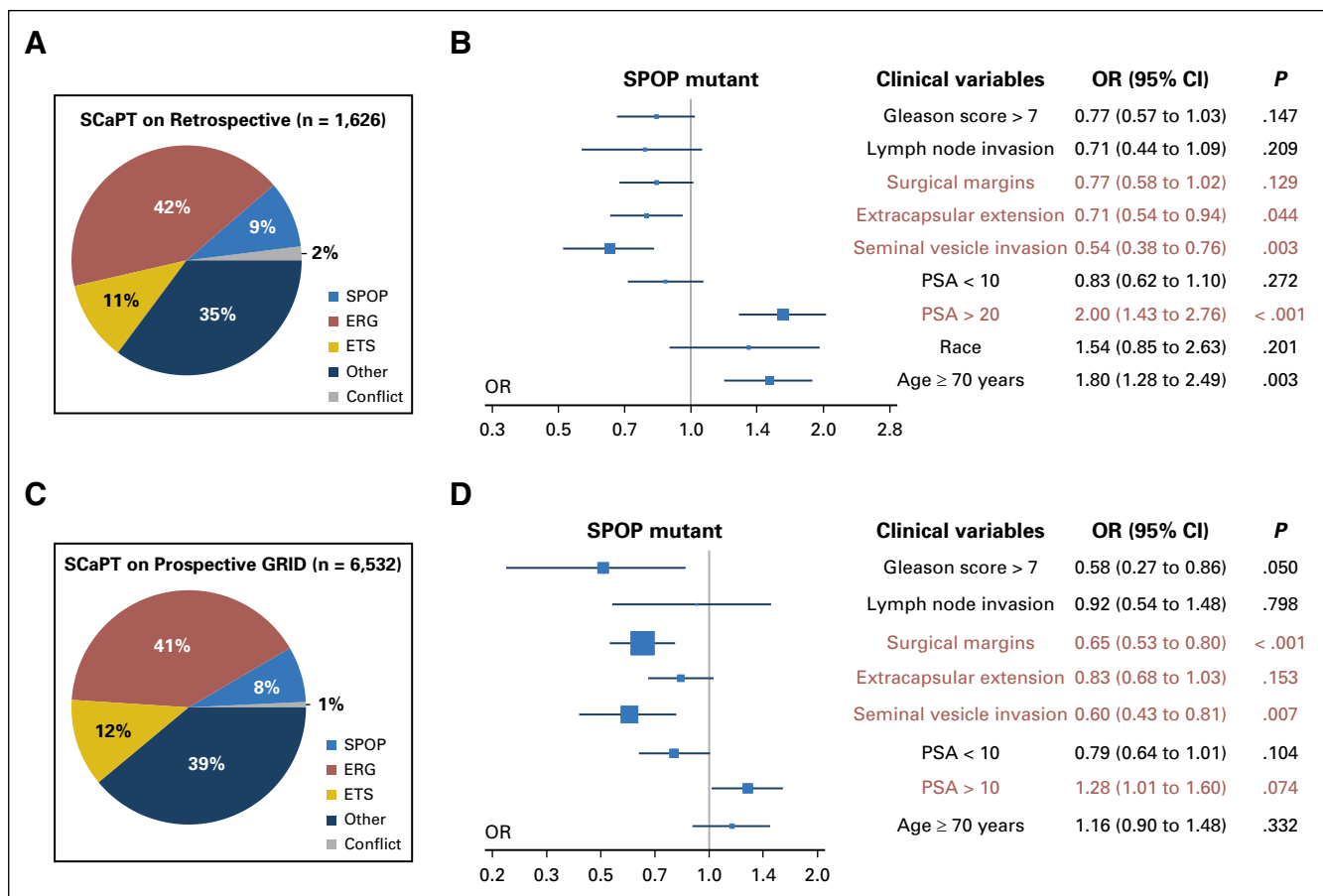


Fig 3. The *SPOP* mutant prediction and its impacts on clinical and prognostic outcomes from retrospective (n = 1,626) and prospective GRID (n = 6,532) cohorts. (A) The pie chart of predicted molecular subclasses from the retrospective cohort with 1,626 samples, on the basis of the SCaPT (SubClass Predictor based on Transcriptional data) model and decision tree. Different colors represent molecular subclasses. (B) Associations between predicted *SPOP* mutant status and clinical variables via univariable analysis in the retrospective cohort, with *SPOP* wild type as reference. Box size indicates the significance from univariable analysis. (C) The pie chart of predicted molecular subclasses from the prospective GRID cohort with 6,532 samples, on the basis of the SCaPT model and decision tree. Different colors represent molecular subclasses. (D) Associations between predicted *SPOP* mutant status and clinical variables via univariable analysis in the prospective GRID cohort, with *SPOP* wild type as reference. Box size indicates the significance from univariable analysis. *ERG*, *ERG*-fusion positive; *ETS*, other *ETS* fusion positive; OR, odds ratio; PSA, prostate-specific antigen.

the GRID microarray expression data, which do not have *SPOP* mutation annotation from DNA analysis. Between 9% and 15% of samples in the cohorts were predicted as *SPOP* mutant subclass, which is consistent with the known prevalence of *SPOP* mutations at the genomic level in previous prostate cancer studies^{3,4,10,12} (Fig 2B). Overall, these results demonstrated that the SCaPT model predicted *SPOP* mutant subclass on the basis of the transcriptional data with high accuracy and confidence.

Molecular Subtyping of 8,158 Patients Using the SCaPT and Decision Tree

We applied the SCaPT model and decision tree to 8,158 patients from retrospective and

prospective GRID cohorts. Among the retrospective cohort with 1,626 RP specimens, we predicted 9% (range, 2% to 13%) of samples to be *SPOP* mutant subclass (Data Supplement). Previously defined expression thresholds²⁵ classified 42% (35% to 68%) as *ERG* positive and 11% (8% to 13%) as non-*ERG* *ETS* positive, as well as 35% without outlier expression, which we defined as an “other” subtype (Fig 3A and Data Supplement). Approximately 2% of samples with both predicted *SPOP* mutant and *ERG*-positive/*ETS*-positive status were classified as conflict cases. Among the prospective cohort with 6,532 RP specimens, we predicted 8% of cases to be *SPOP* mutant subclass, 41% as *ERG* positive, 12% as *ETS* positive, 39% as other subtype, and 1% as conflict cases (Fig 3C).

The percentage of each molecular subclass was consistent with that reported in previous prostate cancer studies,^{1-4,14,25} which supports the validity of our approach.

***SPOP* Mutant Subclass Associated With Favorable Pathology at RP**

We used binominal univariable analysis to compare clinical and pathologic characteristics between *SPOP* mutant and wild-type subclasses (Figs 3B and 3D). *SPOP* mutant subclass was less likely to harbor adverse pathologic features, such as positive surgical margins, extraprostatic extension, and seminal vesicle invasion, compared with wild-type subclass in both retrospective and prospective cohorts (Figs 3B and 3D). Surprisingly, *SPOP* mutant cancers were associated with higher preoperative PSA in both retrospective (odds ratio [OR], 2.00; 95% CI, 1.43 to 2.76; $P < .001$) and prospective cohorts (OR, 1.28; 95% CI, 1.01 to 1.60; $P = .074$).

In contrast, consistent with prior reports,^{25,33-36} tumors in the *ERG*-positive subclass were associated with lower preoperative PSA in both retrospective (OR, 1.51; 95% CI, 1.27 to 1.79; $P < .001$) and prospective cohorts (OR, 1.47; 95% CI, 1.29 to 1.68; $P < .001$), but were more likely to have extraprostatic extension in both retrospective (OR, 1.37; 95% CI, 1.16 to 1.62; $P = .002$) and prospective cohorts (OR, 1.43; 95% CI, 1.28 to 1.60; $P < .001$; Data Supplement). These data suggest that the *SPOP* mutant subclass was associated with more favorable pathologic outcomes at RP, but expresses higher levels of PSA and was associated with tumors from older men, whereas the opposite was true in the *ERG*-positive subclass of prostate cancer.

Consistent Association Between *SPOP* Mutation and Higher PSA in Multiple Cohorts

The inverse association of PSA and prognosis in specific prostate cancer subtypes has potential clinical implications. To independently validate the association of *SPOP* mutant status and higher preoperative PSA, we examined multiple distinct RP cohorts (GRID, TCGA, Taylor, and WCM). We observed a similar trend of higher preoperative PSA in *SPOP* mutant cases, and the *SPOP* mutant subclass was more enriched in the higher PSA subgroups (PSA > 10; Fig 4A). All cohorts demonstrated significantly higher PSA in *SPOP* mutant than in the *ERG*-positive

subclass via Kolmogorov-Smirnov test. On univariable analysis across cohorts, *SPOP* mutant status was significantly associated with higher preoperative PSA (Fig 4B), whereas *ERG* fusion status was significantly associated with lower preoperative PSA (Fig 4C).

***SPOP* Mutation Is Associated With Favorable Clinical Outcomes After RP**

On Kaplan-Meier analysis, the *SPOP* mutant subclass had the highest biochemical-free, metastasis-free, and lowest prostate cancer-specific mortality compared with *ERG*-positive, *ETS*-positive, and other subtypes in the retrospective GRID cohort (Fig 5B and Data Supplement). Whereas long-term outcomes were not available for the prospective GRID cohort, we evaluated the association with metastasis risk using the Decipher score, a validated metric for prostate cancer metastatic potential.^{22,24,37,38} We found fewer *SPOP* mutant tumors in the Decipher high-risk score (> 0.6) group compared with the low- and average-risk groups (Data Supplement), which again is consistent with a favorable prognosis subtype. Together, these data support that *SPOP* mutant prostate cancer had favorable prognosis after RP, despite its association with higher preoperative PSA.

Favorable Prognosis in High-Risk PSA Subgroup in the *SPOP* Mutant Subclass

Pretreatment PSA level is a standard component of risk stratification for prostate cancer, with a PSA level > 20 ng/mL considered to be high risk by a number of risk assessment methods.³⁹⁻⁴¹ Consistent with this, higher PSA was associated with worse metastasis-free survival and prostate cancer-specific mortality in the retrospective cohorts (Fig 5A); however, *SPOP* mutant status had a dramatic effect on prognosis within the subgroup of patients with high PSA. Among patients with a PSA level > 20 ng/mL, *SPOP* mutant tumors were associated with better clinical outcomes, which were comparable to the lowest PSA subgroup (PSA < 10 ng/mL; Fig 5D). These data suggest that, among patients with high-risk PSA levels, the *SPOP* mutant subtype was associated with favorable prognosis in patients who underwent RP. More broadly, these data establish the principle that the identification of molecular subtype may impact the

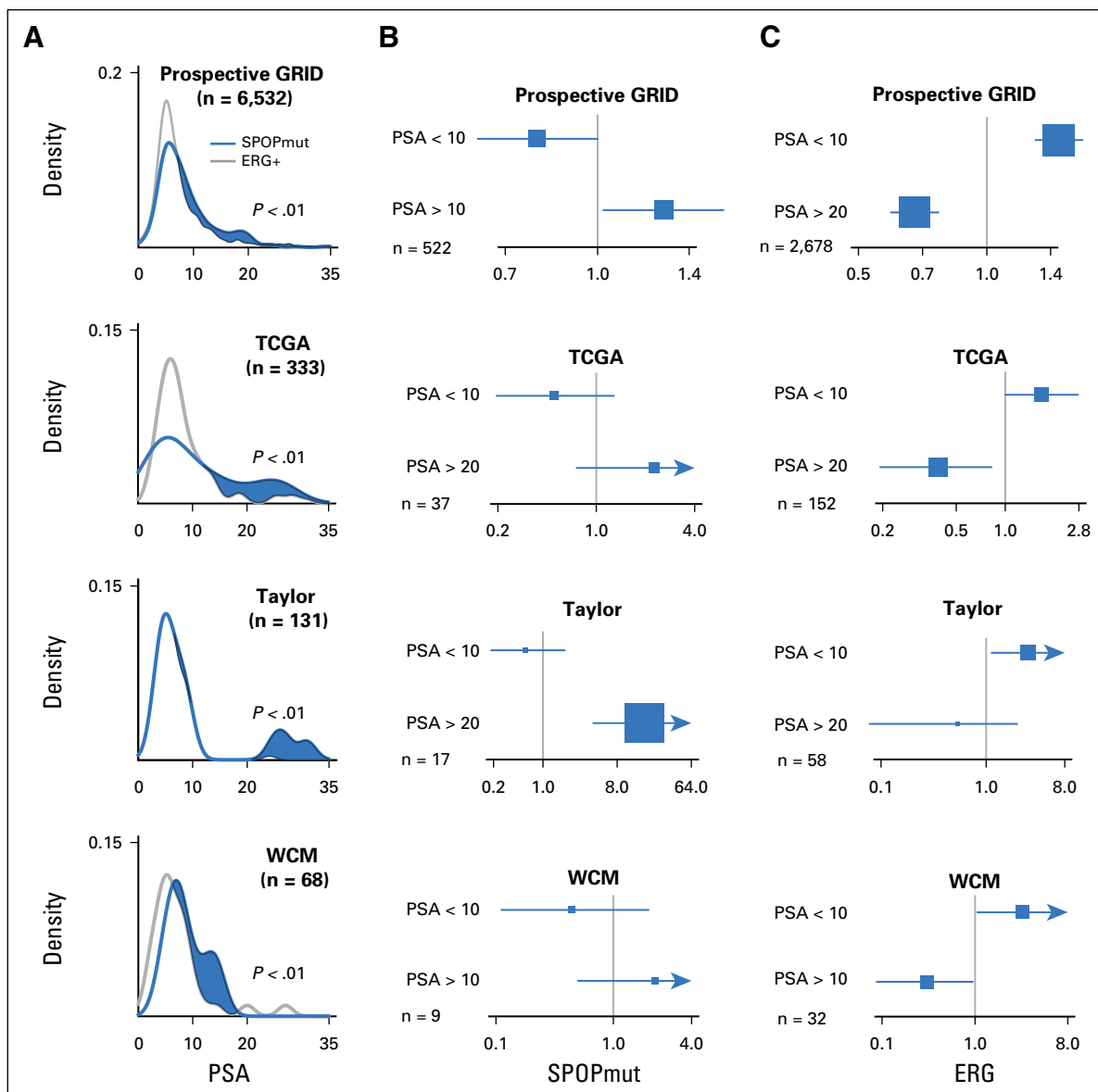


Fig 4. Association of *SPOP* mutant (*SPOPmut*) status and higher prostate-specific antigen (PSA) from four independent studies. (A) Enrichment of *SPOPmut* cases among higher PSA subgroups from prospective GRID, The Cancer Genome Atlas (TCGA), Taylor, and Weill Cornell Medicine (WCM) cohorts. *P* value indicates the significant difference between *SPOPmut* and *ERG*-positive cases via Kolmogorov-Smirnov test in each cohort. (B) Positive association between *SPOPmut* status and higher PSA via univariable analysis. The number of cases is shown in each cohort. (C) Positive association between *ERG* fusion status and lower PSA via univariable analysis. The number of cases is shown in each cohort.

interpretation of PSA-based risk stratification in a variety of clinical scenarios.

DISCUSSION

PSA is a critical component of risk assessment systems in multiple clinical scenarios: risk of prostate cancer before diagnosis, stratification of newly diagnosed disease, monitoring for recurrence after initial therapy, and as a marker for response to therapy and prognosis in metastatic

disease. Despite some controversy surrounding its clinical utility,⁴²⁻⁴⁴ PSA remains a key prostate cancer biomarker for the foreseeable future. The current work provides a framework for precision PSA interpretation by prostate cancer molecular subtype.

National Comprehensive Cancer Network, American Urological Association/American Society for Therapeutic Radiology and Oncology, and European Association of Urology–European Society for Radiotherapy and Oncology–International

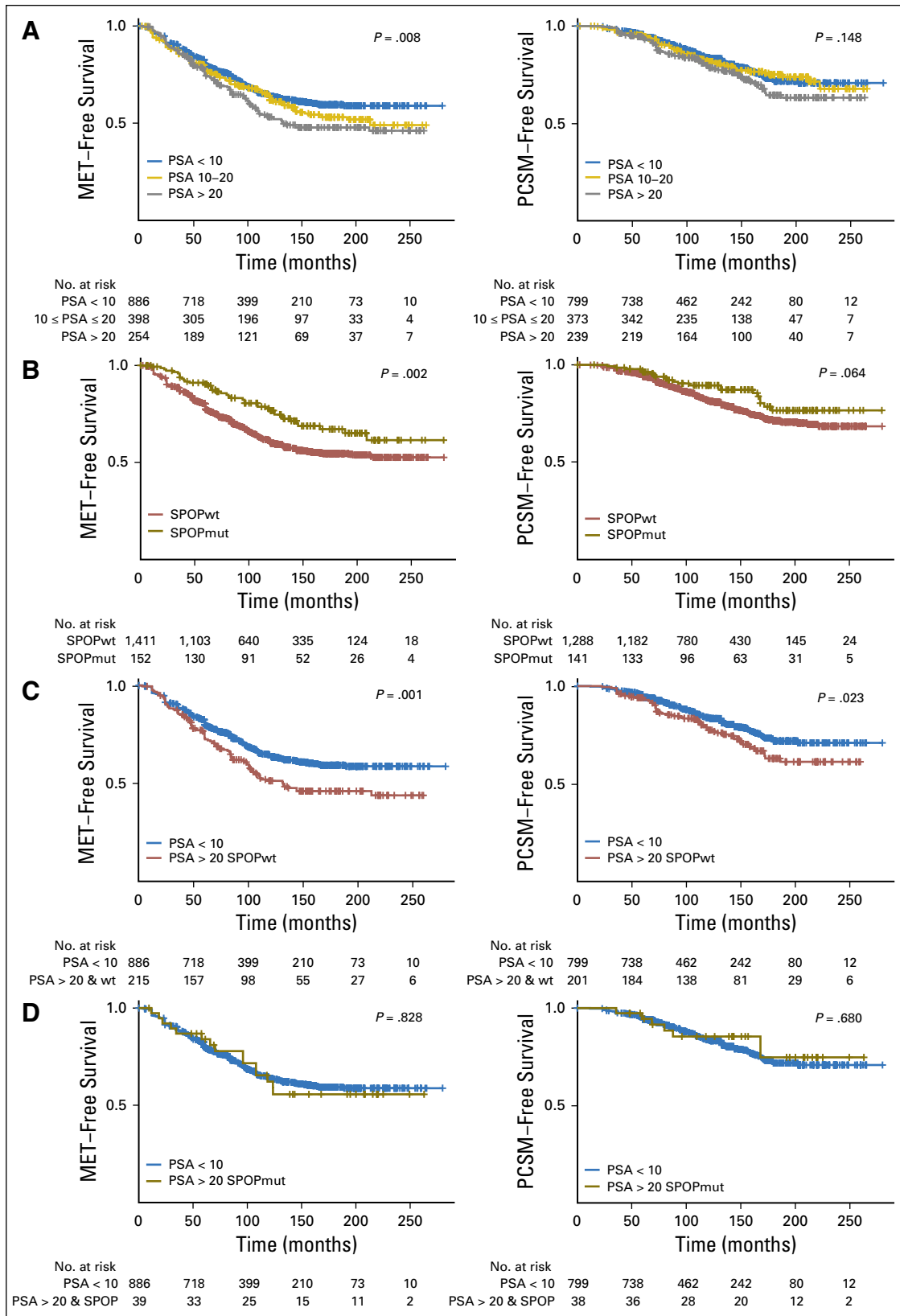


Fig 5. Favorable prognosis in high-risk prostate-specific antigen (PSA) subgroup in the *SPOP* mutant (*SPOP*mut) subclass. (A) Clinical outcome difference between lower, average, and higher PSA groups via Kaplan-Meier analyses for metastasis (MET) and prostate cancer-specific mortality (PCSM)–free survival rates. (B) Significant clinical outcome difference between *SPOP*mut and wild-type (wt) subclasses via Kaplan-Meier analysis of MET- and PCSM-free survival rates. (C) Significant clinical outcome difference between lower

Society of Geriatric Oncology guidelines for clinically localized prostate cancer⁵⁻⁷ classify patients with a pretreatment PSA level of > 20 as high risk, even in the absence of other adverse prognostic features. This results in increased treatment burden, and it is recommended that patients classified as high risk who opt for radiotherapy undergo 2 to 3 years of concurrent androgen-deprivation therapy compared with a duration of 4 to 6 months for patients with intermediate risk disease. Knowledge of the molecular subtype of prostate cancer and its impact on PSA level could therefore improve risk stratification, sparing unnecessary treatment burden and directing higher-intensity therapy to patients who are truly at higher risk; however, whether molecular subtype will actually add clinical value to the current risk stratification tools—or if it adds additional information compared with such tests as Decipher—remains unclear. PSA is heavily used in many other settings as well. The data presented here have implications for defining subtype-specific thresholds for PSA recurrence after local therapy and for monitoring PSA responses in metastatic disease. Additional studies will be necessary to optimally deploy these strategies clinically, but it is clear that molecular subtyping should be considered in future clinical trial designs.

Recent advances in technology have increased our understanding of molecular subclasses of prostate cancer, but clinical and biologic differences among the key *ERG*-positive, *ETS*-positive, and *SPOP* mutant subclasses remain poorly understood. Our finding, that there are distinct associations with PSA and pathologic stage among subclasses, has both biologic and clinical implications. Biologically, prostate cancer cells that harbor mutant *SPOP* may produce more PSA on a per-cell basis as a result of enhanced androgen transcription, essentially leading to higher PSA from fewer cancer cells, whereas the opposite

may be true from *ERG*-positive tumors. Clinically, this may lead to earlier detection of *SPOP* mutant cancers with lower pathologic stage because of lead-time bias. Alternatively, the underlying biology of these tumors may lead to different rates of progression or other impacts on patient outcomes. These hypotheses need to be rigorously tested in future functional and clinical studies.

As a result of the retrospective nature of the cohorts and small sample size of case cohort studies, survival analysis was inevitably affected by baseline risk. We grouped all individual studies from retrospective cohorts and performed survival analyses to study the clinical outcomes of the *SPOP* mutant subclass. Although the favorable prognosis was consistent with improved clinical outcomes in the *SPOP* mutant subclass, these survival results need to be independently validated in additional clinical trials to be generalizable.

In conclusion, we have developed the SCaPT model to predict *SPOP* mutant subclass purely on the basis of transcriptional data with high confidence and accuracy. The *SPOP* mutant subclass was associated with higher PSA but fewer adverse pathologic features and favorable prognosis. We believe this work not only builds a prediction model for *SPOP* mutant prostate cancers and expands the data types usable for the interrogation of clinical outcomes, but it also reinforces the concept that molecular subtyping of prostate cancer can alter the interpretation of the current standard of care risk stratification methods. More broadly, these data suggest a paradigm in which the interpretation of cancer biomarkers may be influenced by underlying molecular subtype.

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Fig 5. (Continued).

PSA (PSA < 10 ng/mL) and *SPOP* wild type (*SPOP*wt) subclass within higher PSA (PSA > 20 ng/mL) groups via Kaplan-Meier analysis of MET- and PCSM-free survival rates. (D) No clinical outcome difference between lower PSA (PSA < 10 ng/mL) and *SPOP*mut subclass within higher PSA (PSA > 20 ng/mL) groups via Kaplan-Meier analysis of MET- and PCSM-free survival rates.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

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Coinventor on a patent application filed regarding SPOP mutations in prostate cancer by Weill Cornell Medicine

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