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# Upgrading of Grade Group 1 Prostate Cancer at Prostatectomy: Germline Risk Factors in a Prospective Cohort

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## ABSTRACT

**Background:** Localized prostate tumors show significant spatial heterogeneity, with regions of high-grade disease adjacent to lower grade disease. Consequently, prostate cancer biopsies are prone to sampling bias, potentially leading to underestimation of tumor grade. To study the clinical, epidemiologic, and molecular hallmarks of this phenomenon, we conducted a prospective study of grade upgrading: differences in detected prostate cancer grade between biopsy and surgery.

**Methods:** We established a prospective, multi-institutional cohort of men with grade group 1 (GG1) prostate cancer on biopsy who underwent radical prostatectomy. Upgrading was defined as detection of $GG2^{+}$ in the resected tumor. Germline DNA from 192 subjects was subjected to whole-genome sequencing to quantify ancestry, pathogenic variants in DNA damage response genes, and polygenic risk.

**Results:** Of 285 men, 67% upgraded at surgery. PSA density and percent of cancer in pre-prostatectomy positive biopsy cores were significantly associated with upgrading. No assessed genetic risk factor was predictive of upgrading, including polygenic risk scores for prostate cancer diagnosis.

**Conclusions:** In a cohort of patients with low-grade prostate cancer, a majority upgraded at radical prostatectomy. PSA density and percent of cancer in pre-prostatectomy positive biopsy cores portended the presence of higher-grade disease, while germline genetics was not informative in this setting. Patients with low-risk prostate cancer, but elevated PSA density or percent cancer in positive biopsy cores, may benefit from repeat biopsy, additional imaging or other approaches to complement active surveillance.

**Impact:** Further risk stratification of patients with low-risk prostate cancer may provide useful context for active surveillance decision-making.

## Introduction

When prostate cancer is low volume, localized to the prostate, low-grade, and producing little prostate-specific antigen (PSA), it is almost never lethal. Randomized clinical trials have clearly demonstrated that this disease, classified as either low risk or favorable intermediate risk (1), can be safely monitored for progression rather than actively treated (2, 3). This monitoring is termed active surveillance (AS) and can both increase patient quality-adjusted life years and reduce medical expenses (4, 5).

Unfortunately, the identification of patients with low-risk or favorable intermediate-risk disease for AS can be error prone. In some series, fully half of men diagnosed with low-risk cancer by needle biopsy, who then undergo prostatectomies, are found to have higher risk disease when the full prostate is examined (6–8). The

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underlying cause of many or most of these pathologic reclassifications (9) is the remarkable spatial heterogeneity of prostate cancer (10–13). Some guidelines have now removed the "preferred" designation for AS (14), replacing it with the wording that AS is "preferred for most" in recognition of clinical scenarios with elevated suspicion for high-risk disease in patients categorized as low risk (15). Patients on AS are often offered imaging and subsequent "confirmatory" biopsies to reduce sampling error (1, 16), with consequent financial expenses and clinical morbidities (17–19). Germline genetic features have also been explored in prostate cancer risk stratification, with *BRCA2* carriers having a higher risk of poor outcomes (20, 21) and germline mutations in at least one of *BRCA1*, *BCRA2*, or *ATM* associated with grade reclassification in patients on AS (22). Polygenic risk scores with 100 of component variants predict diagnosis and potentially disease-risk (23–25). There is an ongoing need to identify factors that can improve the accuracy of risk stratification when biopsy findings indicate low-risk prostate cancer.

To fill this gap, the NCI Early Detection Research Network (EDRN) conducted a prospective multicenter clinical study to collect clinical and epidemiologic measures as well as biospecimens for biomarker analysis for patients with low-risk prostate cancer and who were scheduled to undergo radical prostatectomy. The primary objective of this study was to evaluate potential biomarkers, including epidemiological, clinical, and genetic information, to predict upgrading at radical prostatectomy—an increase in tumor grade after surgical treatment compared to biopsied grade prior to treatment—in men undergoing surgery for low-risk prostate cancer.

Across eight sites, 431 patients were accrued and collected biospecimens established an EDRN Upgrading Reference Set (URS). PSA density and percent cancer in biopsy cores were statistically significantly associated with risk of upgrading at surgery. Neither *BRCA2* status nor polygenic risk were significantly associated with upgrading at prostatectomy. These results define the clinical hallmarks of prostate cancer upgrading in the context of low-grade biopsies with other higher risk features and provide the ideal sample set for ongoing blinded biomarker validation studies.

## Materials and Methods

### Study design

This EDRN study was compliant with PRoBE guidelines for biomarker validation studies (clinicaltrials.gov NCT02189486; refs. 26–28). We recruited men with International Society of Urologic (ISUP; ref. 29) grade group 1 (GG1) prostate cancer on biopsy who elected to undergo radical prostatectomy at eight separate clinical sites around the United States. The primary outcome was upgrading from ISUP GG1 prostate cancer at biopsy to ISUP GG2 or higher at prostatectomy. Enrollment occurred from February 2015 to July 2021. Patients were enrolled immediately prior to prostatectomy. Clinical study data analysis was performed on a data freeze instituted April 2021. Additional subjects enrolled after the data freeze were added to the EDRN reference set but were not included in analysis and are not reported. Race and ethnicity information was self-reported. Clinical information, biopsy slides, and biospecimens were collected following a standard operating protocol prior to radical prostatectomy (30). Urine and blood specimens were biobanked to create the EDRN URS. Outcomes of interest were uniformly assessed for all participants and samples were labeled with randomly generated identification numbers. The Data Management and Coordinating Center (DMCC) of the EDRN coordinated sample collection and ensured blinding to case (ISUP GG1 at prostatectomy) and control (ISUP GG2 or higher at prostatectomy) status and other clinical information for biomarker validation. The DMCC performed validation of genetic biomarkers against case-control status, with the groups doing genomic analyses and scoring individual patients being blinded to clinical outcomes.

### Population

We recruited men who had undergone needle biopsy, had a diagnosis of ISUP GG1 prostate cancer, and subsequently underwent prostatectomy. Men were approached for the study at the time of the decision to proceed with radical prostatectomy. Exclusion criteria included prostate cancer of > ISUP GG1 at biopsy and men undergoing surgery more than 2 years after diagnosis (see **Fig. 1A** for details). Institutional Review Board approval was obtained at each site and subjects provided written informed consent. Subjects were enrolled from eight sites: University of Texas Health Science Center at San Antonio, Eastern Virginia Medical School, University of Washington, Stanford University Medical Center, University of Michigan, Emory University, Glickman Urological and Kidney Institute at Cleveland Clinic, and Icahn School of Medicine at Mt. Sinai.

### Pathology

Pathology of the needle biopsy material and representative slides of final prostatectomy specimens were reviewed centrally by genitourinary pathologists at Weill Cornell Medicine. Pathology of ISUP GG1 disease in biopsied specimens and upgrading status in prostatectomy tissues was confirmed by review of biopsy tissue in formalin-fixed, paraffin-embedded with hematoxylin and eosin staining.

### Predictor variables

Initial analysis considered epidemiological and clinical variables including age, race, body mass index (BMI), serum PSA levels, PSA density, family history of prostate cancer, and the number of previous prostate biopsies. Prostate size for PSA density calculations was derived from measurements taken from ultrasound at biopsy. We also recorded whether magnetic resonance imaging (MRI) was performed. Data on final radiologic findings were not centrally reviewed and were not included in statistical models. Pathologic variables included atrophy, average of the percent cancer in each positive core (divided by the number of positive cores), percentage of positive cores (number of positive cores divided by the number of total cores obtained), perineural invasion, high-grade prostatic intraepithelial neoplasia, and atypical glands suspicious for carcinoma. An exploratory analysis of genetic risk of upgrading was performed in a subset of the cohort. Two genetic scores were considered, each incorporating carrier status of a pathogenic or likely pathogenic variant in the *BRCA2* gene and one of two published polygenic risk scores for diagnosis of any prostate cancer (23, 25).

### DNA sequencing and bioinformatics

A subset of 192 subjects consented to undergo whole-genome sequencing (WGS). DNA isolated from buffy coat biospecimens was sequenced at 60× target coverage with 150 bp paired reads. Reads were aligned to build GRCh38 of the human reference genome with decoys and without alternative haplotypes (not alt-aware) using BWA-MEM2 (v2.2.1; ref. 31). Indel realignment was performed using GATK Indel Realigner (v3.7.0) and all samples were evaluated for sequencing and alignment quality using SAMtools (v1.17; ref.

32) and picard tools (v3.0.0;Supplementary Fig. S1A–S1C). Variants were called with GATK Haplotype Caller, jointly re-genotyped using GATK Genotype GVCFs and filtered using GATK Variant Recalibrator (v4.2.0.0) according to GATK best practices (33).

### Genetic feature annotation

Variants were annotated for clinical significance using the tool SnpEff (v5.1) and the ClinVar database (GRCh38, release 20,211016) according to American College of Medical Genetics criteria (34–36). Variants in genes involved in DNA damage repair (DDR; ref. 37) were filtered for pathogenic or likely pathogenic (P/LP) clinical significance and a ClinVar review status of two or more stars. Genetic ancestry was annotated for each sample relative and defined as genetic similarity to the 1000 genomes (1KG) project ancestral super-continental populations (38). The PLINK toolset (v1.90b7, v2.00a3.6LM; ref. 39) was used to merge study sample genotypes with the 1KG reference panel, extract intersecting SNPs and prune SNPs in high linkage disequilibrium (LD) using a sliding window of 100 Kbp and a correlation $r^2$ threshold of 0.1. Principal component analysis (PCA) on genotype dosages (PLINK v2.00a3.6LM), followed by K-nearest-neighbors (KNN; R v4.2.2) trained on annotated 1KG samples was used to make a classification of categorical ancestry. Geography-based ancestral populations are defined by 1KG as European (EUR), African (AFR), East Asian (EAS), South Asian (SAS), and Admixed American (AMR).

### External pathogenic variant comparison

We conducted an external analysis of DDR P/LP carrier frequency in 302 germline whole genomes from patients with intermediate-risk prostate cancer from the International Cancer Genomic Consortium (project ICGC PRAD-CA). That cohort, along with sample processing and DNA sequencing were described previously (40, 41; Yamaguchi TN; submitted for publication). P/LP variant annotation was performed as described above.

### Polygenic risk scores

Two polygenic risk scores (PRS) for prostate cancer outcomes were chosen to form the basis of two genetic risk of upgrading scores. The Conti and colleagues PRS is a 269-variant multiethnic genetic risk score for diagnosis of any prostate cancer (23). As per Huynh-le and colleagues, PRS is a 290-variant polygenic hazard score for time to any prostate cancer diagnosis (25). PRSs were applied using a standard weighted sum formula (23) in R (v4.2.2) using published weights and dosages derived from called and imputed genotypes. Component SNPs that could not be genotyped or did not meet quality control criteria were imputed using the TopMed reference panel on the TopMed imputation server v1.7.3 (Supplementary Fig. S1D; ref. 42). Prior to submission to the imputation server, genotypes were preprocessed using PLINK2 v2.00a3.6LM. Briefly, SNPs were restricted to biallelic variants and filtered out if minor allele frequency <1%, missingness rate >5% and Hardy–Weinberg equilibrium test $P$ value $<1.0 \times 10^{-12}$. Then SNPs in high LD were pruned out using a sliding window of 100 Kbp and a correlation $r^2$ threshold of 0.9. Hard-called imputed genotypes with an imputation quality $R^2$ >0.3 were used for PRS calculation. Missing PRS component SNPs that could not be genotyped or imputed were handled as follows. The Conti and colleagues score excluded missing SNPs and was normalized by dividing each individual score by the total number of non-missing SNPs in that score. Due to the methods by which the Huynh-Le and colleagues score weights were originally derived, a complete set of SNPs is required for a valid calculation and missing SNPs cannot be excluded. We used the mean population dosage of each missing SNP as a replacement to not introduce bias in these cases.

A third 128-variant polygenic score for PSA ($PGS_{PSA}$) was calculated in the same manner as the Conti and colleagues score for genetically adjusted PSA analysis using published weights (43).

### Genetic risk of upgrading biomarkers

All genetic biomarkers were calculated blind to case-control status. The two categories of genetic information with the most evidence of biomarker utility in prostate cancer are rare pathogenic variants in the *BRCA2* gene and polygenic risk scores for diagnosis of prostate cancer. We designed a decision-tree strategy to combine PRS and *BRCA2* carrier status information into a genetic risk of upgrading (GRU) prediction score. PRSs calculated across the study cohort were scaled between 0 and 1 using min–max normalization. An individual's scaled PRS forms the basis of their GRU. Each individual was then evaluated for the presence of a P/LP variant in *BRCA2*. If a P/LP variant was not present, the scaled PRS becomes the final GRU for that individual. If a P/LP variant was present, the GRU was updated to the maximum PRS of the cohort.

GRU biomarker scores for each subject were sent the EDRN DMCC for validation, where prediction ability was assessed against case-control status known only to DMCC analysts.

### Genetically adjusted PSA density

Genetically adjusted PSA was calculated for each individual as described by Kachuri and colleagues (43):

$$PSA_G = \frac{PSA}{exp(PGS_{PSA})}$$

This is the equivalent of a normalization of measured PSA levels by the PSA levels predicted by an individual's genetics. Adjusted PSA density (PSAD) was computed using genetically adjusted PSA:

$$PSAD_G = \frac{PSA_G}{prostate\ volume} exp(K)$$

where the exponentiated constant K shifts the median of adjusted PSAD to match that of the unadjusted PSAD distribution.

### Sample size

The primary objective of the study was to create a cohort that would be sufficiently powered to validate biomarkers using all clinical, epidemiological, and molecular information to predict a lack of upgrading at radical prostatectomy with a >90% negative predictive value (NPV). A successful risk stratification tool in a clinical setting would require an operational performance criterion of ≥35% specificity at 98% sensitivity at the chosen threshold for avoiding radical prostatectomy. Under the assumption that ISUP GG upgrading at prostatectomy would occur among 60% of participants, these specificity and sensitivity thresholds correspond to an NPV of 92%. The sample size necessary to achieve 90% power when testing against the null hypothesis (25% specificity at 98% sensitivity) under these criteria is a total of 195 subjects (44). To account for various factors, including fewer than 60% of participants with upgraded ISUP GG, inadequate tumor tissue for assays, tissue blocks not retrievable, and missing radical prostatectomy outcome, the target minimum sample size was inflated by 40% to a total of 240 subjects.

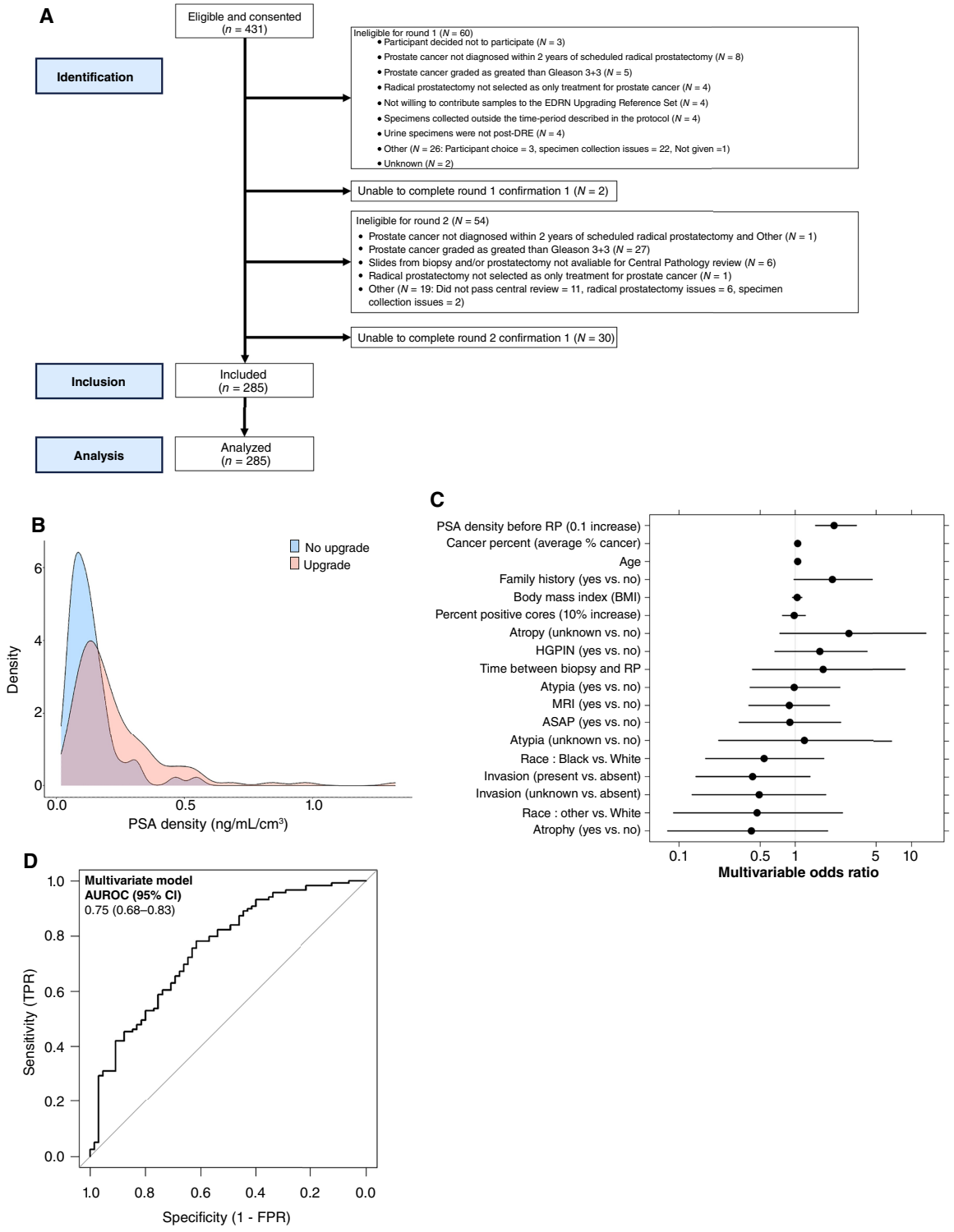


**Figure 1.**

Clinical predictors of upgrading at prostatectomy. Associations of clinical features with ISUP GG upgrading in 285 patients diagnosed with ISUP GG1 prostate cancer and treated with radical prostatectomy. **A,** CONSORT diagram of enrollment and exclusions in the URS. **B,** Histogram of preoperative PSA density distributions in patients. Colors indicate upgrading status at prostatectomy to > ISUP GG1. **C,** Forest plot indicating odds ratios and 95% confidence intervals from multivariable logistic regression of upgrading at prostatectomy in URS with complete data for all variables including all available clinico-epidemiologic predictors. **D,** Receiver–operator curve from 10-fold cross-validation of a two-predictor logistic regression model of upgrading at prostatectomy, including predictors: average cancer percent in positive biopsy cores and 0.1 ng/mL/cm$^3$ increase in PSA density.

### Statistical analyses

The primary outcome variable of upgrading was defined as an increase from ISUP GG assigned at previous biopsies compared to that assigned after radical prostatectomy. Men whose surgically resected tumors were graded as ISUP GG1 on final pathology were considered controls. Men whose surgically resected tumors were graded as ISUP GG2 or higher on final pathology were considered cases. Thus, controls were patients whose tumors were not upgraded between presurgery biopsy and surgery, while cases were patients whose tumors were upgraded in that interval. For initial comparisons of clinico-epidemiologic data, Wilcoxon rank sum tests were performed for continuous variables and Pearson's $\chi^2$ tests were performed for categorical variables.

A group of clinico-epidemiologic features was used to build univariable logistic regression models to assess individual clinical predictors of upgrading. These predictors were then assessed in combination in a multivariable logistic model within a subcohort with complete data. PSA density was multiplied by 10 for scaling and included as a continuous variable. Continuous PSA density regression coefficients are interpreted as log odds of upgrading for each 0.1 ng/mL/cm$^3$ increase in PSA density. A second multivariable logistic regression model was designed using the two significant predictors from the previous model: continuous PSA density and average cancer percent in positive biopsy cores. The two-predictor model was internally verified for predictive performance using 10-fold cross-validation. Sensitivity and specificity were calculated and plotted as a receiver–operator curve, and the area under the receiver–operator curve (AUROC) was calculated as a measure of predictive performance. Confidence intervals on AUROC values were calculated with 3,000 bootstrap samples. A third multivariable logistic regression model was designed using the two significant predictors from the full multivariable model but with PSA density encoded as a more clinically informative dichotomous variable with a cutoff threshold of 0.2 ng/mL/cm$^3$, with regression coefficients interpreted as log odds of upgrading with PSA density above the threshold. This model was verified for predictive performance in the same manner as the two-predictor model with PSA density encoded as a continuous variable. AUROC statistics were calculated for genetically adjusted PSA density and unadjusted PSA density as predictors of upgrading. In the exploratory genetic analyses, carrier frequencies of DDR P/LP variants in the URS and ICGC PRAD-CA cohorts were compared using a test of equality of proportions. The two genetic risk of upgrading biomarkers from the exploratory analysis were similarly evaluated for predictive performance with the calculation of AUROC statistics. Confidence intervals were obtained using 3,000 bootstrap samples.

All tests were two-sided and an alpha level of 0.05 was considered significant for all analyses, which were performed using R software, versions ranging between v4.0.2 and v4.2.3.

### Data visualization

Visualizations were generated in the R statistical environment (v4.2.2) using the packages lattice (v0.20-45), latticeExtra (v0.6-29), BPG (v7.0.5; ref. 45), and pROC (v.18.0).

### Data availability

Specimens from the URS are available from the EDRN through the specimen set application process https://edrn.nci.nih.gov/data-and-resources/specimen-reference-sets/specimen-set-request-form/. Open access data from the URS cohort generated by this study are available through the EDRN Laboratory Catalog and Archive Service (LabCAS) data repository. Raw sequencing data, aligned BAMs, and variant calls from the URS genetic cohort are available through the dbGaP data repository via accession phs003670.v1. Raw sequencing data for ICGC PRAD-CA are available on EGA via accession EGAD00001003706.

## Results

### Demographics and pathology

We enrolled 431 men across eight sites during the 6-year enrollment period from February 2015 to April 2021. A series of exclusions were made (**Fig. 1A**), including extensive time (exceeding 2 years) on active surveillance ($n = 8$), specimen collection issues ($n = 36$) and biopsies containing ISUP GG2 or higher tumor on centralized pathology review ($n = 32$). After all exclusions, 285 subjects with biopsied ISUP GG1 cancer underwent analysis, with collected biospecimens constituting the URS biorepository (**Fig. 1A**; **Table 1**). The majority of the cohort (87%) was between 50 and 69 years of age. Race was self-reported as 83% White (236/285) and 11% Black (32/285). Median PSA at diagnosis was 5.3 ng/mL (interquartile range; IQR: 4.2–7.6 ng/mL). PSA exceeding 10 ng/mL, a criterion of favorable intermediate NCCN risk, was present in 14% of subjects with available diagnostic PSA (38/268). Surgical pathology at prostatectomy identified ISUP GG2 or higher disease in 67% (191/285), mostly ISUP GG2 (**Table 2**). Lymph node dissection was performed in 51% of the cohort. Among these, one patient had lymph node metastasis; this patient also upgraded to ISUP GG2. Locally advanced prostate cancer with extraprostatic extension was identified in 22% (62/285) subjects, primarily in the group with ISUP GG2 or higher disease in the surgical specimen.

### Clinico-epidemiologic predictors of upgrading

We performed univariable comparisons of clinical and epidemiological features between subjects with and without upgrading to ISUP GG2 or higher disease. Age, race, and family history were not associated with risk of upgrading. PSA density was higher in upgraded patients ($\text{median}_{\text{upgraded}} = 0.18$ ng/mL/cm$^3$; $\text{median}_{\text{not-upgraded}} = 0.11$ ng/mL/cm$^3$; $P < 0.001$; Wilcoxon rank-sum test; **Fig. 1B**; **Table 1**). The average cancer percent in positive biopsy cores (cancer percent) was significantly higher in upgraded patients ($\text{median}_{\text{upgraded}} = 15\%$; $\text{median}_{\text{not-upgraded}} = 24\%$; $P = 0.002$; Wilcoxon rank-sum test; **Table 1**). Other metrics of cancer detected in biopsy cores (percent positive cores, max percent cancer in a core), followed a similar trend. Indicators of favorable intermediate NCCN risk (PSA ≥10, total positive cores) did not differ significantly (**Table 1**).

We investigated the association of clinical and epidemiological features with risk of upgrading by fitting a multivariable logistic regression to the 176 subjects without any missing covariate data. When controlling for all factors, PSA density, encoded as a continuous variable, was the most significant predictor of upgrading at time of prostatectomy (0.1 ng/mL/cm$^3$ increase in PSA density OR, 2.16; 95% CI, 1.5–3.36; $P < 0.001$). Cancer percent in positive biopsy cores was the second most significant predictor (OR, 1.05; 95% CI, 1.02–1.08; $P < 0.001$; **Fig. 1C**; **Table 3**; Supplementary Fig. S2).

A second multivariable logistic regression model was constructed using only continuous PSA density and cancer percent as predictors (Supplementary Table S1). To assess the predictive capacity of this model, we performed 10-fold cross-validation, yielding an AUROC of 0.75 (95% CI, 0.68–0.83; **Fig. 1D**). A third multivariable model, with dichotomized PSA density (at 0.2 ng/mL/cm$^3$) and cancer

**Table 1.** Preoperative demographics and clinical data.

| Characteristic | Overall ($N$ = 285)[a] | No upgrade ($N$ = 94)[a] | Upgrade ($N$ = 191)[a] | $P$-value[b] |
|---|---|---|---|---|
| Age group | | | | 0.8 |
| 40–49 | 19 (6.7) | 8 (8.5) | 11 (5.8) | |
| 50–59 | 117 (41) | 39 (41) | 78 (41) | |
| 60–69 | 130 (46) | 41 (44) | 89 (47) | |
| 70–79 | 19 (6.7) | 6 (6.4) | 13 (6.8) | |
| Race | | | | 0.4 |
| White | 236 (83) | 75 (80) | 161 (84) | |
| Black | 32 (11) | 14 (15) | 18 (9.4) | |
| Other | 17 (6.0) | 5 (5.3) | 12 (6.3) | |
| BMI | 27.89 (25.84–30.74) | 27.58 (25.82–29.79) | 28.12 (25.84–31.18) | 0.2 |
| Diagnostic PSA (ng/mL) | 5.30 (4.20–7.61) | 4.70 (3.81–6.55) | 5.80 (4.54–7.93) | 0.002 |
| Diagnostic PSA ≥10 ng/mL | 38 (14) | 9 (10) | 29 (16) | 0.2 |
| PSA closest to prostatectomy day (ng/mL) | 5.65 (4.20–8.24) | 4.80 (3.80–6.40) | 6.24 (4.60–9.13) | <0.001 |
| Ln (prostate size) | 3.65 (3.36–3.96) | 3.72 (3.43–4.13) | 3.56 (3.30–3.83) | 0.02 |
| PSA density closest to prostatectomy day (ng/mL/cm$^3$) | 0.14 (0.10–0.23) | 0.11 (0.07–0.16) | 0.18 (0.12–0.28) | <0.001 |
| Family history of prostate cancer | | | | 0.3 |
| No | 161 (56) | 59 (63) | 102 (53) | |
| Yes | 115 (40) | 33 (35) | 82 (43) | |
| Unknown | 9 (3.2) | 2 (2.1) | 7 (3.7) | |
| MRI performed | | | | 0.9 |
| No | 191 (67) | 62 (66) | 129 (68) | |
| Yes | 93 (33) | 32 (34) | 61 (32) | |
| Unknown | 1 (0.4) | 0 (0) | 1 (0.5) | |
| MRI performed on or before prostate biopsy | 69 (24) | 23 (25) | 46 (24) | 0.9 |
| MRI type | | | | 0.7 |
| MRI—no fusion biopsy | 33 (12) | 13 (14) | 20 (11) | |
| MRI—fusion biopsy | 58 (21) | 19 (20) | 39 (21) | |
| No MRI | 191 (68) | 62 (66) | 129 (69) | |
| Atrophy | | | | 0.2 |
| No | 220 (77) | 71 (76) | 149 (78) | |
| Yes | 22 (7.7) | 11 (12) | 11 (5.8) | |
| Unknown | 43 (15) | 12 (13) | 31 (16) | |
| Cancer percent (average % cancer)[c] | 21.00 (10.00–34.44) | 15.00 (10.00–30.00) | 23.75 (12.50–36.43) | 0.002 |
| Number of cores with cancer | | | | 0.4 |
| 1 | 47 (17) | 21 (22) | 26 (14) | |
| 2 | 49 (17) | 17 (18) | 32 (17) | |
| 3 | 37 (13) | 12 (13) | 25 (13) | |
| 4 | 50 (18) | 17 (18) | 33 (18) | |
| 5 | 31 (11) | 11 (12) | 20 (11) | |
| >5 | 67 (24) | 16 (17) | 51 (27) | |
| Percent positive cores | 28.57 (16.67–41.67) | 25.00 (15.38–38.33) | 33.33 (16.67–45.20) | 0.027 |
| Maximum % cancer in a biopsy core | 40 (15–60) | 26.5 (10–50) | 40 (18–70) | 0.001 |
| Perineural invasion | | | | >0.9 |
| Absent | 199 (70) | 65 (69) | 134 (70) | |
| Present | 32 (11) | 11 (12) | 21 (11) | |
| Unknown | 54 (19) | 18 (19) | 36 (19) | |
| Intraductal carcinoma (IDC-P) | | | | 0.6 |
| No | 53 (19) | 19 (20) | 34 (18) | |
| Unknown | 232 (81) | 75 (80) | 157 (82) | |
| HGPIN | 61 (21) | 18 (19) | 43 (23) | 0.5 |
| Atypical glands suspicious of carcinoma | 46 (16) | 14 (15) | 32 (17) | 0.7 |
| Atypia | | | | 0.4 |
| No | 200 (70) | 62 (66) | 138 (72) | |
| Yes | 60 (21) | 24 (26) | 36 (19) | |
| Unknown | 25 (8.8) | 8 (8.5) | 17 (8.9) | |
| Number of prior biopsies | 1.00 (1.00–2.00) | 1.00 (1.00–2.00) | 1.00 (1.00–2.00) | 0.3 |
| Number of positive biopsies | 1.00 (1.00–1.00) | 1.00 (1.00–1.00) | 1.00 (1.00–1.00) | 0.8 |

NOTE: Summary of demographic and clinical features in the URS controls (no upgrade at prostatectomy) and cases (upgrade at prostatectomy). Results of univariate comparisons between cases and controls. Biopsy-related variables are taken from the biopsy closest to consent date.

[a]$n$ (%); Median (25%–75%).

[b]Pearson's $\chi^2$ test; Wilcoxon rank sum test; Fisher's exact test.

[c]Cancer percent: Percent cancer = (Σ % cancer in each biopsy core/total number of cores).

**Table 2.** Postoperative pathology.

| Characteristic | Overall *N* = 285[a] | No upgrade *N* = 94[a] | Upgrade *N* = 191[a] | *P*-value[b] |
|---|---|---|---|---|
| Pathologic Gleason score | | | | <0.001 |
| Grade group 1 (3+3) | 94 (33) | 94 (100) | 0 (0) | |
| Grade group 2 (3+4) | 166 (58) | 0 (0) | 166 (87) | |
| Grade group 3 (4+3) | 24 (8.4) | 0 (0) | 24 (13) | |
| Grade group 4 (4+4) | 0 (0) | 0 (0) | 0 (0) | |
| Grade group 5 (4+5) | 1 (0.4) | 0 (0) | 1 (0.5) | |
| Pathologic T stage | | | | <0.001 |
| pT2 | 223 (78) | 89 (95) | 134 (70) | |
| pT3a | 58 (20) | 4 (4.3) | 54 (28) | |
| pT3b | 4 (1.4) | 1 (1.1) | 3 (1.6) | |
| Pathologic M stage | | | | 0.003 |
| M0 | 108 (38) | 24 (26) | 84 (44) | |
| MX | 177 (62) | 70 (74) | 107 (56) | |
| Pathologic N stage | | | | 0.004 |
| pN0 | 145 (51) | 36 (38) | 109 (57) | |
| pN1 | 1 (0.4) | 0 (0) | 1 (0.5) | |
| pNX | 139 (49) | 58 (62) | 81 (42) | |
| Time between baseline Bx and RP (years) | 0.30 (0.21–0.45) | 0.28 (0.20–0.44) | 0.30 (0.21–0.46) | 0.5 |

NOTE: Summary of pathologic findings in radical prostatectomy specimens in the URS controls (no upgrade at prostatectomy) and cases (upgrade at prostatectomy). Results of univariate comparisons between cases and controls.
[a]*n* (%); Median (25%–75%).
[b]Fisher's exact test; Pearson's $\chi^2$ test; Wilcoxon rank-sum test.

percent as predictors, yielded a comparable AUROC of 0.73 (95% CI, 0.66–0.81).

### Germline genetic characterization

To determine if genetic features were associated with upgrading to ISUP GG2 or higher disease, we performed WGS of blood samples from 192 URS patients representative of the full cohort (**Fig. 2A**; Supplementary Table S2). Sequencing quality was high for the majority the of samples (Supplementary Fig. S1A and S1B). Median coverage was between 20× and 60×, with one sample excluded for failed sequencing quality control, leaving a final 191-subject genetics subcohort (Supplementary Fig. S1B). The number of called variants was typical of human WGS (Supplementary Fig. S1C; ref. 38). Principal components of genetic ancestry depicted clear clustering of the individuals in our cohort with those in the 1KG reference panel (**Fig. 2B**). The majority of the cohort had highest genetic similarity to EUR-annotated reference individuals (38). KNN classification confirmed the cohort was primarily

**Table 3.** Clinical predictors of upgrading.

| | Univariable | | Multivariable adjusted | |
|---|---|---|---|---|
| **Predictors** | **OR (95% CI)** | ***P*-value** | **OR (95% CI)** | ***P*-value** |
| Age | 1.01 (0.97–1.05) | 0.6 | 1.05 (0.99–1.11) | 0.1 |
| Race: Black vs. White | 0.6 (0.28–1.29) | 0.18 | 0.54 (0.17–1.76) | 0.31 |
| Race: other vs. White | 1.12 (0.4–3.62) | 0.84 | 0.47 (0.09–2.55) | 0.37 |
| BMI | 1.03 (0.97–1.1) | 0.34 | 1.04 (0.95–1.14) | 0.37 |
| Family history (yes vs. no) | 1.44 (0.86–2.42) | 0.17 | 2.09 (0.98–4.62) | 0.06 |
| MRI (yes vs. no) | 0.92 (0.54–1.56) | 0.74 | 0.89 (0.4–1.97) | 0.77 |
| Atrophy (yes vs. no) | 0.48 (0.2–1.16) | 0.1 | 0.42 (0.08–1.9) | 0.27 |
| Atrophy (unknown vs. no) | 1.23 (0.61–2.62) | 0.57 | 2.9 (0.74–13.24) | 0.14 |
| PSA density before RP (0.1 increase) | 2.02 (1.45–2.98) | <0.001 | 2.16 (1.5–3.36) | <0.001 |
| Cancer percent (average % cancer) | 1.03 (1.01–1.05) | <0.001 | 1.05 (1.02–1.08) | <0.001 |
| Percent positive cores (10% increase) | 1.12 (0.98–1.28) | 0.1 | 0.98 (0.78–1.22) | 0.84 |
| Invasion (present vs. absent) | 0.93 (0.43–2.1) | 0.85 | 0.43 (0.14–1.34) | 0.14 |
| Invasion (unknown vs. absent) | 0.97 (0.52–1.87) | 0.93 | 0.49 (0.13–1.83) | 0.3 |
| HGPIN (yes vs. no) | 1.23 (0.67–2.31) | 0.52 | 1.63 (0.67–4.15) | 0.29 |
| ASAP (yes vs. no) | 1.15 (0.59–2.34) | 0.69 | 0.9 (0.33–2.46) | 0.83 |
| Atypia (yes vs. no) | 0.67 (0.37–1.23) | 0.2 | 0.98 (0.41–2.42) | 0.97 |
| Atypia (unknown vs. no) | 0.95 (0.4–2.45) | 0.92 | 1.2 (0.22–6.75) | 0.84 |
| Time between biopsy and RP | 1.41 (0.64–3.34) | 0.41 | 1.74 (0.43–8.78) | 0.46 |

NOTE: Results of univariate and full multivariable logistic regression of upgrading at prostatectomy in the URS. Multivariable regression performed on subcohort with complete data ($n = 176$) with all predictors measured prior to radical prostatectomy.
Abbreviation: RP, radical prostatectomy.

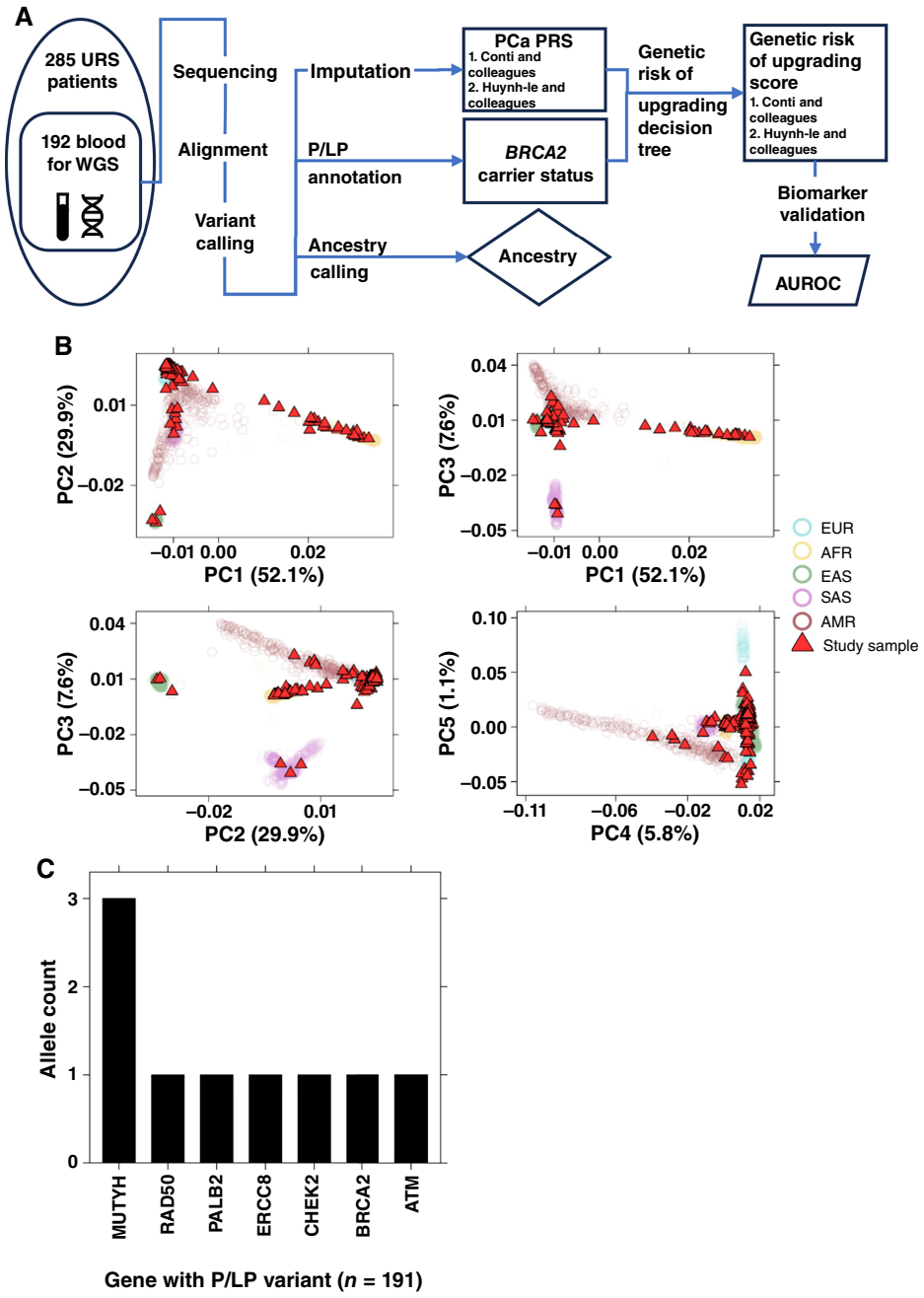


**Figure 2.**

Genetic characterization of the upgrading reference set. Germline genetic features in a set of 192 whole-genome sequenced patients diagnosed with ISUP GG1 prostate cancer and treated with radical prostatectomy. **A,** Schematic of genetic analysis study design. A subset of 192 URS blood samples undergoes whole genome sequencing and bioinformatics processing. Captured germline variants are used to infer genetic ancestry, are annotated for pathogenicity in DDR genes, and undergo imputation for the calculation of two polygenic risk scores of prostate cancer diagnosis. Pathogenic or likely pathogenic variants in the *BRCA2* gene are combined with PRSs to form two scores of genetic risk of upgrading using a decision tree. The putative biomarkers are validated with the AUROC statistic. **B,** Principal components of genetic ancestry in the 1000 Genomes reference panel population and URS genetic cohort. AFR, African; AMR, Admixed American; EAS, East Asian; EUR, European; PC, Principal component; PCa, prostate cancer; SAS, South Asian. **C,** Allele count of all DDR genes with at least one P/LP variant.

composed of the EUR (81%) and AFR (12%) subgroups (Supplementary Fig. S3A–S3C; Supplementary Table S3). Genetic ancestry was highly concordant with self-reported race (Supplementary Fig. S3B). All four subjects for whom self-reported race was not available were classified as EUR.

To explore the frequency of rare germline P/LP variants in DDR genes, we annotated variants for clinical significance using the ClinVar database. Nine P/LP variants were detected in seven DDR genes across nine subjects (**Fig. 2C**; Supplementary Table S3). Only a single *BRCA2* P/LP variant was detected (0.5% of the subcohort). To verify that the P/LP frequencies were reasonable, we re-analyzed a published cohort of 302 patients with intermediate-risk disease and similar WGS depth (Supplementary Fig. S4; ref. 40; Yamaguchi TN; submitted for publication). There was a statistically indistinguishable 1.7% (5/302) rate of *BRCA2* P/LP variants in that intermediate-risk cohort ($P = 0.49$; proportion test; Supplementary Table S4).

Substantial variability in PSA is associated with heritable factors in the general population (46). PSA measurements adjusted for an individual's genetic predisposition to baseline PSA levels (genetically adjusted PSA) may show improved prediction of prostate cancer risk by accounting for the confounding factor of variable

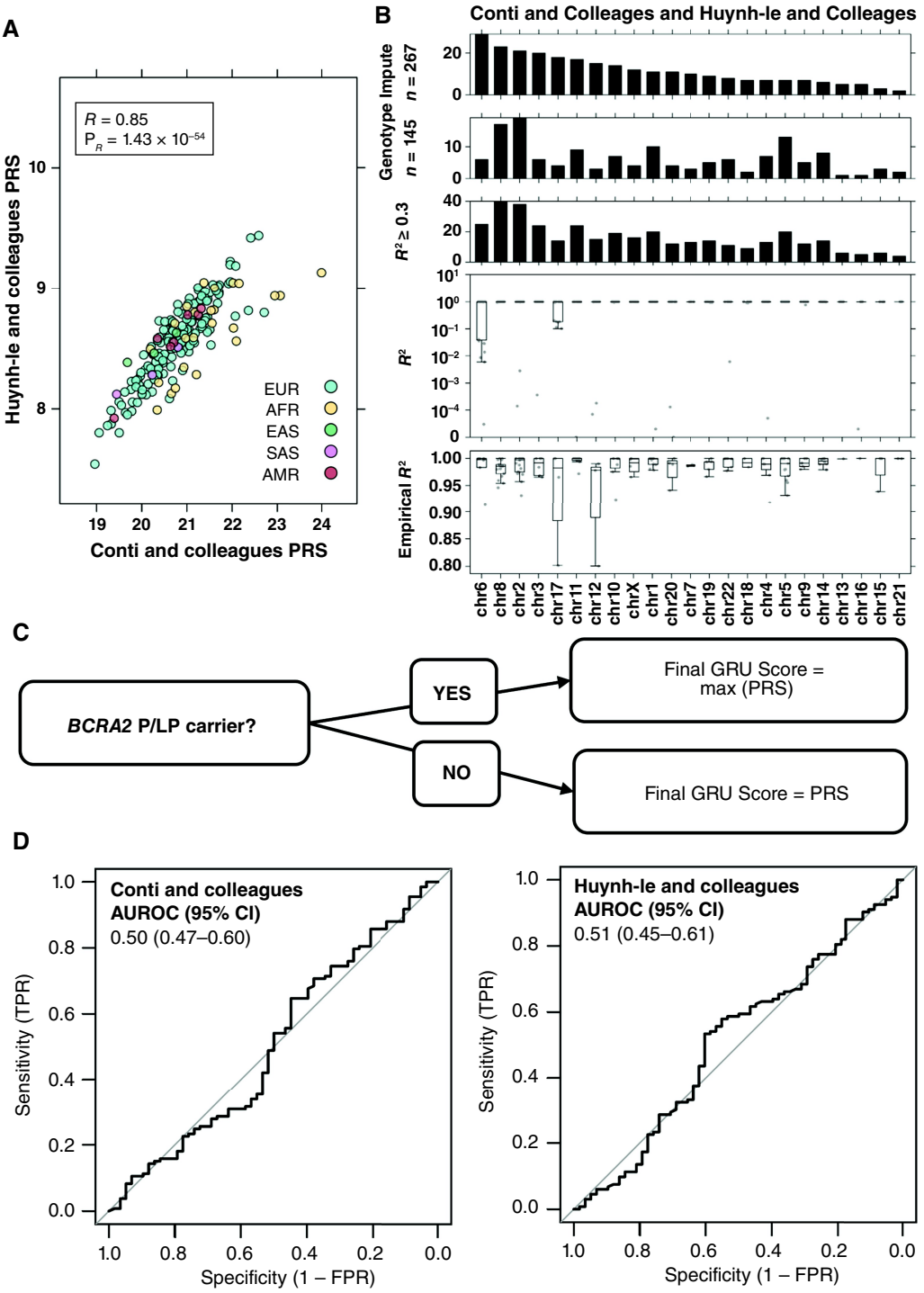


**Figure 3.**

Germline genetic biomarkers of upgrading at prostatectomy. **A,** Correlation of Conti and colleagues and Huynh-le and colleagues polygenic risk scores. Colors indicate ancestral super-populations from the 1,000 Genomes project. AFR, African; AMR, Admixed American; EAS, East Asian; EUR, European; PRS, Polygenic Risk Score; SAS, South Asian. **B,** By-chromosome statistics from imputation with the TopMed imputation server at Conti and colleagues and Huynh-le and colleagues PRS component SNP loci (multiallelic sites counted separately). Top to bottom: imputed SNP count, genotyped SNP count, count of SNPs imputed at imputation quality $R^2$ >0.3, distribution of empirical $R^2$. **C,** Decision tree for genetic risk of upgrading score calculation based on *BRCA2* P/LP variant carrier status. **D,** Receiver operator curve of Conti and colleagues (left) and Huynh-le and colleagues (right) PRS-derived genetic risk of upgrading score validation in the upgrading reference set.

baseline PSA (43). Given that PSA density derived from clinical measurements was the strongest clinical predictor of grade upgrading at prostatectomy, we considered genetically adjusted PSA density in the 136 patients with complete genetic and PSA density data. A polygenic score for PSA was calculated (Supplementary Table S3) and showed a modest correlation with measured PSA levels ($r = 0.21$; Supplementary Fig. S3D). AUROC for prediction of upgrading risk was unchanged whether genetically adjusted or unadjusted PSA density were considered, at 0.69 (Supplementary Fig. S3E).

### Genetic predictors of upgrading

To evaluate whether known germline genetics can predict grade upgrading at prostatectomy, we constructed two GRU scores (see "Materials and Methods"; Supplementary Table S3). These scores were based on PRS for the diagnosis of any prostate cancer published by Conti and colleagues (23) and Huynh-le and colleagues (**Fig. 3A**; Supplementary Fig. S3F; ref. 25). Extensive quality control was performed on PRS component variants (see "Materials and Methods"; **Fig. 3B**; Supplementary Figs. S1D and S3F). The two scores were highly correlated in the multi-ancestry cohort (Pearson $r = 0.85$; $P < 0.01$; **Fig. 3A**). We combined PRS and *BRCA2* carrier status into two final GRU scores using a decision tree (**Fig. 3C**). Validation of both GRU scores against case-control status of upgrading was performed by the DMCC of the EDRN, and the investigators generating the scores were blinded to case/control status. Neither GRU score showed predictive accuracy or discrimination ability for upgrading at radical prostatectomy (**Fig. 3D**; Supplementary Tables S2 and S5).

## Discussion

A cohort of 285 men with biopsied ISUP GG1 prostate cancer were evaluated for clinical and molecular predictors of upgrading to ISUP GG2 prostate cancer at prostatectomy. PSA density and cancer percent in biopsied cores were significant predictors of upgrading, while genetic biomarkers were not predictive.

It is well-established that the management of low-risk prostate cancer can include AS, significantly reducing the risk of side effects and complications of treatment. These side effects can substantially affect quality of life and include high rates of erectile dysfunction and voiding dysfunction. Nevertheless, AS rates for low-risk disease remain below 60% and continue to vary widely by practice and individual practitioner (47). As occult high-grade disease remains a concern, current guidelines allow for flexibility in the implementation of AS protocols, with tumor factors suggested to tailor the intensity of surveillance (14). Our findings add to the body of evidence (2, 48, 49) suggesting that PSA density and cancer volume indicate a higher risk of upgrading, and could act as biomarkers for more regimented AS protocols.

Our prospective cohort exhibits a high rate of upgrading from prostate biopsy to radical prostatectomy pathology. This is consistent with retrospective studies. For example, a study reported reclassification in 55.7% of 1,766 patients with D'Amico low-risk biopsies (50). It is not yet clear whether an upgrade to intermediate risk results in worse outcomes for patients. In another cohort of 676 patients, representing a range of initially diagnosed ISUP GGs (459 GG1), 36% of ISUP GG1 patients experienced upgrading, and upgrading from any initial ISUP GG was observed in 29.1% of patients (51). Upgrading from any ISUP GG was associated with higher rates of biochemical recurrence (BCR), however BCR is not an excellent surrogate of prostate cancer mortality (52). The same study found no difference in 5- and 10-year overall survival rates between patients with and without upgrading from any ISUP GG.

The strongest predictor of prostate cancer upgrading at prostatectomy was PSA density, long known as a predictor of progression in active surveillance and upgrading at prostatectomy (53–55). For every 0.1 ng/mL/cm$^3$ increase in PSA density, there was a two-fold increase in the risk of upgrading at the time of prostatectomy. The NCCN uses PSA density and percent of prostate cancer per biopsy core in differentiating very low-risk from low-risk prostate cancer (PSA density <0.15 ng/mL/cm$^3$; <3 positive prostate biopsy cores and <50% cancer per core; reflecting Epstein criteria; refs. 1, 53). The second most important predictor of upgrading was the average percent of cancer in biopsy cores, which essentially represents cancer volume (total % cancer in positive cores/total positive cores). Cancer percent has been associated with several other pathologic volume determinants, such as cancer core lengths (56).

Genetic features are predictive of prostate cancer diagnosis (20, 23, 25, 57). The two genetic biomarkers (GRU scores) derived from PRSs for diagnosis of any prostate cancer are not predictors of upgrading at prostatectomy, and there is a paucity of deleterious pathogenic variation in DDR genes. These results add to the body of evidence that PRSs of disease incidence do not accurately predict disease aggression (23, 58). However, this resource of high-quality whole genome sequences from 58 controls and 133 cases of upgrading at prostatectomy may be leveraged both to validate new biomarkers and in the context of future biomarker discovery efforts.

Limitations of this work include the relatively small sample size and preponderance of subjects of European descent. These limitations preclude answering questions regarding the safety of active surveillance in men of African or Asian descent, and the role of rare and common genetic variation in upgrading. Enrollment was slow, likely due to the reduction in PSA screening following the 2012 USPSTF guidelines (59), and the dramatic increase in use of active surveillance shortly after the study was opened (i.e., most eligible patients with ISUP GG1 tumors were managed with active surveillance at EDRN institutions). Our entry criteria included all men going to surgery with ISUP GG1 prostate cancer and may not reflect a typical active surveillance population. This cohort was surgically managed, but all patients would have been candidates for active surveillance protocols. The patient and provider choices that led to active treatment may include unreported clinically relevant observations, which may impact cohort representativeness. That two-thirds of the participants had ISUP GG upgrades on final pathology suggests that study investigators may have identified risk factors leading to a higher index of suspicion for high-grade disease in enrolled patients. We used cancer percent as we did not have the total length of cancer on each of the cores, which may be a better indicator of prostate cancer volume. This study began prior to the widespread adoption of MRI imaging, which was not part of our protocol, and no MRI findings are included in our statistical analysis. However, the increasingly widespread use of MRI imaging with accurate prostate volume measurements should increase the use and availability of PSA density for incorporation in clinical decision-making.

In conclusion, our multisite cohort study found a high risk of upgraded ISUP GG (67%) in men with low-grade tumors who subsequently underwent radical prostatectomy. PSA density and tumor core volume are closely associated with risk of upgrading. As part of our efforts, we have assembled a reference set of biologics

and genetic data for continued evaluation of existing and future biomarkers for improved predictions of upgrading.

## Authors' Disclosures

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## Authors' Contributions

**M.A. Liss:** Conceptualization, investigation, data curation, supervision, funding acquisition, writing original draft, writing review and editing. **N. Zeltser:** Software, formal analysis, funding acquisition, visualization, writing–original draft, writing–review and editing. **Y. Zheng:** Formal analysis, supervision, methodology, writing–review and editing. **C. Lopez:** Formal analysis, visualization. **M. Liu:** Formal analysis, visualization. **Y. Patel:** Formal analysis, visualization. **T.N. Yamaguchi:** Software, formal analysis. **S.E. Eng:** Software, formal analysis, writing–review and editing. **M. Tian:** Software, formal analysis. **O.J. Semmes:** Data curation, investigation, writing–review and editing. **D.W. Lin:** Data curation, investigation, writing–review and editing. **J.D. Brooks:** Data curation, investigation, writing–review and editing. **J.T. Wei:** Data curation, investigation. **E.A. Klein:** Data curation, investigation, writing–review and editing. **A.K. Tewari:** Data curation, investigation. **J.M. Mosquera:** Data curation, investigation, funding acquisition, writing–review and editing. **F. Khani:** Data curation, investigation, funding acquisition. **B.D. Robinson:** Data curation, investigation, funding acquisition. **M. Asad:** Data curation, investigation, funding acquisition. **D.A. Troyer:** Data curation, investigation, writing–review and editing. **J. Kagan:** Conceptualization. **M.G. Sanda:** Data curation, investigation, writing–review and editing. **I.M. Thompson:** Data curation, investigation. **P.C. Boutros:** Conceptualization, resources, funding acquisition, supervision, writing–original draft, writing–review and editing. **R.J. Leach:** Conceptualization, resources, funding acquisition, supervision, writing–original draft, writing–review and editing.

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## Note

Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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