Prostate Cancer Polygenic Risk Score Associated with Upgrading in Patients on Active Surveillance

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Abstract

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Epidemiology

Active surveillance (AS) is the preferred management strategy for patients with low- or favorable intermediate-risk prostate cancer (PCa). However, AS requires frequent and costly healthcare visits. In a prospective cohort study of 1,220 PCa patients on AS in the Canary Prostate Active Surveillance Study (PASS), we investigated whether polygenic risk scores (PRS) could identify patients who may benefit from intensive vs passive surveillance to reduce these burdens. We constructed a PRS of 451 PCa risk variants (PRS_{451}) in PASS, along with a PRS of 400 variants, excluding 51 prostate-specific antigen (PSA)-associated variants (PRS_{400}) and tested PRS associations with upgrading and extreme upgrading at follow up and prostate volume, PSA, percent of biopsy cores with cancer, and Gleason grade at diagnosis. In AS patients, high PRS was associated with risk of upgrading and possibly tumor multifocality. Excluding PSA variants from the PRS enhanced these associations and revealed an association with smaller prostate size, which has been previously associated with more aggressive tumors. Our findings suggest that PRS may inform intensity of surveillance.

Introduction

Prostate cancer (PCa) is the most diagnosed cancer and second leading cause of cancer death in US men.[1] Over 70% of PCa diagnoses are localized,[1] with localized and regional patients having >99% five-year survival rates.[2] As such, active surveillance (AS) is now the preferred treatment strategy for the >50% of patients diagnosed with low- or favorable intermediate-risk PCa.[2,3] While this drastically reduces the number of patients unnecessarily undergoing surgical and radiation treatments that have significant side effects, AS requires frequent and potentially costly healthcare visits, involving prostate-specific antigen (PSA) testing, digital rectal exams, magnetic resonance imaging, and prostate biopsies. Identifying patients who may benefit from more intensive versus passive surveillance could reduce these burdens.

Recent attention has been drawn to the potential clinical utility of PCa polygenic risk scores (PRS). Large-scale and diverse PCa genome-wide association studies (GWAS) have led to the development of a multi-ancestry PRS that was highly predictive of PCa risk and moderately distinguished aggressive from non-aggressive disease across diverse populations.[4,5] An earlier multi-ancestry PCa PRS[4] was reportedly associated with conversion from AS to treatment.[6] However, the association with risk of upgrading has not been explicitly investigated and the association with prostate tumor features is not well characterized, which could inform PRS interpretation. Other prognostic variables, including PSA level, have been identified as informative early markers of PCa risk but have limited ability to differentiate advanced from localized disease.[7] Differential performance of a prior PRS and PSA in the years leading up to diagnosis, suggests that PRS provides complementary information and that together, along with additional prognostic variables, could contribute to nomograms for predicting PCa upgrading.

Therefore, in a prospective cohort study of PCa patients on AS, we investigated the association of PRS with prostate tumor features, particularly risk of upgrading.

Methods

This observational investigation was reported following the guidelines in the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement.

Participants

Patients were from the Canary Prostate Active Surveillance Study (PASS), a cohort study that prospectively enrolled 1455 patients with clinically localized PCa (cT1-cT2) between July 2008 to November 2017 across 10 national sites to undergo AS.[8] Our primary outcomes of interest were upgrading (any grade increase) and extreme upgrading (increase from GG1/GG2 to GG3+). Gleason grade was collected from biopsies at diagnosis, 6-12 months and 24 months after diagnosis, then every 2 years, with ongoing follow-up. For this investigation, follow-up was available through August 2022. Secondary outcomes included prostate volume, PSA, PSA density (PSAD), AUA symptom score, AUA quality of life urinary score, percent of biopsy cores with cancer, and Gleason grade group at diagnosis (**Supplementary Methods**). Written informed consent was obtained from all participants, and study protocols were approved by the respective Institutional Review Board at each study site.

Polygenic Risk Scores

A total of 1220 participants had GWAS data used to calculate a previously developed multi-ancestry PRS of 451 PCa risk variants (PRS_{451}).[5] A PRS of 400 variants, excluding 51 PSA-

associated variants,[9] was also calculated (PRS_{400}; **Supplementary Methods**), as this was previously shown to strengthen associations with aggressive versus non-aggressive PCa.[5,9] PRS were mean standardized and evaluated as continuous variables. PRS were also evaluated categorically as quintile risk groups, comparing each PRS quintile to the lowest 0-20% PRS quintile (**Supplementary Methods**).

Statistical Analyses

PRS associations with upgrading were investigated with Cox proportional hazards regression adjusting for age at diagnosis and the top 10 principal components (PCs) of ancestry, with date of diagnosis as the entry time and date at upgrading as the exit time. For censored patients who were not upgraded, exit time was the earliest of either treatment date, date of last contact, or 2 years after the last biopsy. Associations with extreme upgrading were performed with a competing risk analysis, where the competing event was treatment in the absence of extreme upgrading. Discriminative ability was evaluated with Harrell's C-index for four models: 1) covariates only (age at diagnosis and 10 PCs), 2) covariates and PRS, 3) covariates and clinical risk factors (BMI, GG2/GG3 vs GG1, cT2 vs cT1, PSA, prostate volume, and percentage of biopsy cores with cancer), and 4) covariates, clinical risk factors, and PRS. A decision curve analysis was used to evaluate the net benefit of these models relative to biopsying all or biopsying no patients (**Supplementary Methods**).

To better account for population structure (**Figure S1**), we also fit mixed models with fixed effects for PRS, age at diagnosis, and 10 PCs, with a random effect for a genetic relationship matrix (**Supplementary Methods**). Continuous outcomes were fit using Gaussian distributions,

while binary outcomes were fit with binomial distributions. PRS discriminative ability for upgrading based on logistic regression was evaluated with area under the curve (AUC) with the four comparisons described above.

Statistical significance was defined as $P<0.05$.

Results

Of the 1220 study participants, 470 had upgrading and 152 had extreme upgrading during the median 5.3 years (range: 3.0-7.6 years) of follow-up in event-free patients (**Table S1**). Age at diagnosis, BMI, race, and ethnicity were comparable between those who did and did not upgrade, with 85% (n=1041) of participants self-reporting as non-Hispanic White (**Table S1**). The 2- and 5-year risks of upgrading were 17.7% (95% CI=15.5-19.9%) and 33.3% (95% CI=30.5-36.3%), respectively, while the 2- and 5-year risks of extreme upgrading were 5.0% (95% CI=3.8-6.3%) and 11.1% (95% CI=9.2-13.2%), respectively.

In time-to-event analyses, each SD unit increase in PRS_{451} was associated with 23% increased probability of upgrading (95% CI=1.11–1.35, $P=4.2x10^{-5}$), while PRS_{400} was associated with 27% increased probability (95% CI=1.15–1.39, $P=1.6x10^{-6}$; **Table 1**) at any point in time during follow-up. PRS_{451} and PRS_{400} were associated with 19% (95% CI=1.01–1.41, P=0.04) and 22% (95% CI=1.03–1.45, P=0.02) increased probability of extreme upgrading, respectively. Patients in the top 80-100% PRS_{451} and 80-100% PRS_{400} categories had 72% (95% CI=1.28-2.31, $P=3.22x10^{-4}$) and 113% (95% CI=1.57-2.89, $P=1.24x10^{-6}$) increased probabilities, respectively, of upgrading compared to patients in the lowest 0-20% PRS categories (**Table S2**). The 80-100%

PRS_{451} and PRS_{400} categories were also associated with 68% (95% CI=1.02-2.80, P=0.04) and 100% (95% CI=1.18-3.40, P=0.01) increased probabilities of extreme upgrading, respectively, compared to the lowest 0-20% PRS categories. The top PRS quintile identified 26.0% (PRS_{451}) and 26.6% (PRS_{400}) of patients that upgraded (**Table S2**). Mixed model results were highly consistent (**Table S2** and **Table S4**). Compared to a model with age at diagnosis, PCs, and clinical risk factors (C-index=0.645, 95% CI=0.618-0.672), including PRS_{400} led to a slight but not significant improvement in detecting upgrading (C-index=0.653, 95% CI=0.626-0.68; **Figure S2**). At a 20% risk threshold, compared to the best default strategy of biopsying none, a model with age at diagnosis, PCs, clinical risk factors, and PRS_{400} had 4 more net detected upgraders per 100 patients, which corresponds to using the model on 25 patients to detect one instance of upgrading (**Figure S3** and **Table S3**). However, the net benefit was similar when including or excluding PRS (**Figure S3** and **Table S3**).

Each SD unit increase in PRS_{400} was associated with 1.38 higher percentage points of biopsy cores with cancer (95% CI=0.76–2.00, $P=1.1x10^{-5}$), 1.48 cc smaller prostate volume (95% CI=-2.82, -0.14, P=0.03), 0.18 ng/mL higher PSA (95% CI=0.01–0.35, P=0.05), and higher PSAD (beta=0.011; 95% CI=0.006–0.016, $P=4.7x10^{-5}$) at diagnosis (**Table 1** and **Table S2**). Except for PSA, associations were similar or weaker for PRS_{451} (**Table 1** and **Table S2**).

Discussion

In PCa patients on AS, higher PRS was associated with increased probability of upgrading and tumor features that could indicate risk of more aggressive disease, including smaller prostate volume, higher PSAD, and a greater percentage of positive biopsy cores, the latter implicating

tumor multifocality. Our findings demonstrate the potential utility of PRS beyond risk-stratified screening of unaffected patients. Incorporating information on PCa genetic susceptibility into disease management could help identify AS patients who may benefit from more intensive versus passive surveillance, reducing burdens for patients with lower risk of disease progression.

Higher PRS was associated with smaller prostate volume, which has been associated with more aggressive tumors among patients with comparable serum PSA levels and is reflected in higher PSAD.[10] Higher PRS was also associated with more biopsy cores with cancer, which has been associated with adverse pathological outcomes at radical prostatectomy[11] and corroborates previous findings.[12] Further, excluding PSA-associated variants slightly enhanced associations with upgrading, prostate volume, and percentage of biopsy cores with cancer, consistent with findings showing that excluding or adjusting for PSA-associated variants strengthened associations between PRS and aggressive disease.[5,9] These findings collectively demonstrate the ability of PRS to help identify patients at risk of developing more severe disease while underscoring the need to disentangle variants influencing PCa risk from those elevating PSA to optimize risk prediction models. Further, understanding how the PCa PRS, initially developed to assess risk of developing PCa, relates to markers of disease aggressiveness is important for understanding the utility and implications of PCa PRS.

While 60% of low-risk PCa patients undergo AS, AS varies widely between practices and practitioners,[13] likely due to limited monitoring and surveillance intensity guidelines.[2] Prior Canary PASS models have found that AS intensity can be safely modulated based on patients' clinical risk factors.[14] Our findings and previous rare variant studies[15,16] suggest that

incorporating genetic information into these models could guide the standardization of AS protocols, with PRS modulating risk prediction across more patients.

Limitations

It will be important to validate our findings in independent studies to ensure that they are generalizable, particularly across diverse populations, as our investigation included a limited number of patients from non-European ancestry populations. Additionally, it will be important to evaluate progression to outcomes such as metastatic and lethal PCa, which will require a large long-term cohort of AS patients given the low proportion of low-risk patients who advance to lethal disease. Our study was not powered to assess these severe outcomes. Further, our findings on the association between PRS and tumor multifocality should be investigated using histologic data to directly measure tumor multifocality. Inferring multifocality from percentage of biopsy cores with cancer likely misestimates the number of clonally distinct tumor foci.

Conclusion

In AS patients, high PRS was associated with increased probability of upgrading, smaller prostate volume, and possibly tumor multifocality. As such, PRS could inform surveillance intensity for low-risk PCa patients, reducing the burden of AS for patients with decreased risk of upgrading.

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Data Access: Burcu Darst had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Data Sharing Statement: Genotype data used in this investigation are available through the database of genotypes and phenotypes (dbGaP, accession number: phs002056.v1.p1).

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Tables

Table 1. PRS association with risk of PCa upgrading and tumor features at diagnosis. Participants were patients with clinically localized PCa on AS undergoing scheduled prostate biopsies in the Canary PASS cohort enrolled from July 2008 to November 2017, with follow-up through August 2022. PRS_{400} excludes 51 variants previously associated with PSA. β reflects the mean difference in the outcome associated with one SD unit increase in PRS.

Outcomes measured at follow-up						
	PRS_{400}			**PRS_{451}**		
	HR	**95% CI**	**p-value**	**HR**	**95% CI**	**p-value**
Upgrading[a]	1.27	[1.15, 1.39]	1.55E-06	1.23	[1.11, 1.35]	4.22E-05
Extreme upgrading[b]	1.22	[1.03, 1.45]	0.02	1.19	[1.01, 1.41]	0.04
Outcomes measured at diagnosis						
	PRS_{400}			**PRS_{451}**		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Grade Group[c]	1.05	[0.85, 1.30]	0.63	1.03	[0.83, 1.28]	0.80
	β	**95% CI**	**p-value**	**β**	**95% CI**	**p-value**
Prostate volume (cc)	-1.48	[-2.82, -0.14]	0.03	-0.85	[-2.29, 0.52]	0.22
PSA (ng/ml)	0.18	[0.0038, 0.35]	0.045	0.26	[0.079, 0.43]	4.71E-03
PSAD (ng/ml^2)	0.011	[0.0056, 0.016]	4.74E-05	0.012	[0.0069, 0.018]	7.67E-06
Total cancer cores	0.18	[0.10, 0.25]	2.33E-06	0.18	[0.11, 0.26]	2.46E-06
Biopsy cores with cancer (%)	1.38	[0.76, 1.99]	1.09E-05	1.37	[0.74, 2.00]	2.08E-05
AUA symptom score[d]	-0.32	[-0.69, 0.048]	0.09	-0.32	[-0.70, 0.056]	0.10
AUA quality of life urinary score[e]	-0.035	[-0.109, 0.0391]	0.35	-0.004	[-0.081, 0.072]	0.91

[a] Any increase in Gleason grade in biopsies collected at 6 to 12 and 24 months after diagnosis, then every 2 years (n=470) versus did not upgrade (n=750).
[b] Increase in grade group from GG1 or GG2 to GG3+ (n=152) versus did not have extreme upgrading (n=1068).
[c] GG2/GG3 (n=110) versus GG1 (n=1110) at diagnosis.
[d] 0-7 = mild, 8-19 = moderate, 20-35 = severe.
[e] 0 = delighted, 1 = pleased, 2 = mostly satisfied, 3 = mixed, 4 = mostly dissatisfied, 5 = unhappy, 6 = terrible.

Supplement

Supplementary Methods

Supplementary Methods

Participants

Cancer upgrading was determined from biopsies collected per protocol at diagnosis and 6 to 12 and 24 months after diagnosis, then every 2 years. Upgrading for this study was defined as an increase in the overall Gleason score (a value between 2 and 10) and/or an increase in the corresponding primary grade group (a value between 1 and 5), considering only the first upgrading event. Time to upgrading was recorded as years between diagnosis and the earliest upgrading date of biopsy (for participants who were censored/did not upgrade, it was the time between diagnosis to the earliest date from either the first treatment date, date of last contact, or 2-years after the last non-upgrading biopsy). Extreme upgrading was defined as an increase from GG1 or GG2 to GG3+ and considered second-time reclassification events (e.g., GG1 to GG2 to GG3+). Given that many patients who reclassify from GG1 to GG2 get treated and are therefore not 'available' to reclassify to GG3, treatment in the absence of extreme upgrading was included as a competing event. Of the 530 PCa patients that received treatment, 270 underwent radical prostatectomy (51%), 229 received radiation (43%), 11 were treated with androgen deprivation therapy (2%), and 20 patients received other treatment (4%). Time to event for competing risk analyses was recorded either as years between diagnosis to extreme upgrading, diagnosis to PCa treatment (after upgrading to GG2 or without any upgrading), or for censored participants, diagnosis to the earliest of time to date of last contact or 2-years after last non-extreme-upgrading biopsy.

As a proxy for tumor multifocality, the percentage of biopsy cores with cancer was calculated as the percentage of biopsies taken at diagnosis with cancer for participants with a minimum of 6 biopsy cores. The American Urological Association (AUA) symptom score was calculated from the AUA worksheet for participants at enrollment. Scores can be interpreted as 0-7=Mild, 8-19=Moderate, and 20-35=Severe symptoms. Descriptions of a participant's AUA quality of life due to urinary symptoms at enrollment were converted to a score as defined by the AUA Symptoms Score document: 0 = Delighted, 1 = Pleased, 2 = Mostly satisfied, 3 = Mixed, 4 = Mostly dissatisfied, 5 = Unhappy, and 6 = Terrible. Gleason grade group at diagnosis was evaluated as GG2/GG3 vs GG1, due to the limited number of participants with GG2 or GG3.

Information on race and ethnicity was based on self-reported data with investigator-defined options. This information is provided in **Table S1** along with other participant characteristics for generalizability but was not considered in any analyses.

Genetic Data and Polygenic Risk Score

Genotyping of the 1,220 PASS participants was conducted as part of a previous AS investigation, with a subset of these participants included in analyses showing that a previous PCa PRS was associated with conversion from AS to treatment.[6] As previously described, genotyping was performed using the Illumina Infinium Multi-Ethnic Global Array (MEGA) at the NIH Center for Inherited Disease Research (CIDR) at Johns Hopkins University. Genotypes were called using GenomeStudio version 2011.1, genotyping module version 1.9.4, and GenTrain version 1.0 on build 38. The full array consisted of 1,760,143 genotyped variants. Unmeasured genetic variants were imputed using the Trans-Omics for Precision Medicine (TOPMed) imputation server[17] (pipeline=michigan-imputationserver-1.3.3,

imputation=minimac4-1.0.2, phasing=eagle-2.4, r2Filter=0.0). The TOPMed (Version R2 on GRC38) reference panel included 97,256 reference samples and 308,107,085 variants. A principal component analysis was conducted to determine the genetic similarity of participants, and the top 10 principal components (PCs) were used in analyses to account for potential population stratification. Further details, including genotyping, imputation, and quality control, have been previously described.[6]

A previously developed multi-ancestry PRS included 451 PCa risk variants identified in a PCa GWAS of 122,188 cases and 604,640 controls of European ancestry, 19,391 cases and 61,608 controls of African ancestry, 10,809 cases and 95,790 controls of East Asian ancestry, and 3,931 cases and 26,405 controls of Hispanic ethnicity.[5] The resulting multi-ancestry PRS was highly predictive of PCa risk across populations. The PRS was calculated as a weighted sum of the number of risk alleles carried, using the previously established variant-specific weights.[5] PRS variants and weights can be found in the PGS Catalog under the accession code PGP000488. As previously described,[5] of the 51 variants excluded from the PRS_{451} to calculate PRS_{400}, 48 overlapped with one of 128 PSA-associated variants (being either the same variant or highly correlated) reported in a multi-ancestry PSA GWAS,[9] while three variants were near the *KLK3* gene that encodes the PSA protein. PRS were mean standardized and evaluated as continuous variables. PRS were also evaluated using an indicator variable, splitting into the following PRS categories: [0-20%], (20-40%], (40-60%], (60-80%], and (90-100%]. PRS category cutoffs were determined using PRS distributions in patients who did not upgrade. Effects for each PRS category were calculated using the 0-20% PRS category as the reference.

Statistical Analyses

We developed a genetic relatedness matrix (GRM) to better account for population structure. The GRM is a correlation matrix applied to a subset of variants that estimates the proportion of genomes of individuals that are identical by state. Variants included in the GRM were common autosomal variants (minor allele frequency [MAF] >5%), independent with an r^2 of <0.01% using a window size of 100 and step size of 25, and had high genotype imputation quality with an INFO score of >0.9. This led to 46,187 variants used to calculate the GRM.

Genetic relatedness was estimated among all 1220 individuals from the filtered variants using GCTA-GRM.[18] Subsequent analyses were performed using R version 4.1.2 (2021-11-01). The 'GENESIS' package (GENetic Estimation and Inference in Structured samples)[19] was used to fit the mixed models. The 'survival' package was used to estimate hazard ratios from Cox PH models to evaluate the probability of upgrading and cause-specific hazard ratios for competing risk regression models used to evaluate the probability of extreme upgrading. C-index[20] values were taken as the concordance output of Cox PH models with 95% confidence intervals calculated from SE. The 'pROC' package was used to plot ROC curves and calculate AUCs.

We estimated 2- and 5-year risks of upgrading by estimating the probability of non-upgrading through 2 and 5 years from a 'survfit' object (from the 'survival' R package) based on the above-mentioned Cox PH models and subtracting those probabilities from one. For extreme upgrading, we estimated 2- and 5-year risks by calculating cumulative incidence for competing risks at the specified time points using 'cuminc' function in the 'tidycmprsk' R package.

The net benefit (NB) of the four predictive models relative to treating all or treating no patients was evaluated using decision curve analysis (DCA). In this case, treatment refers to a biopsy. Risk thresholds were pre-specified (above which would be recommended biopsy) for both upgrading and extreme upgrading and informed by the predicted risk of those events; the thresholds were 0-30% and 0-20%, respectively. Additionally, test tradeoff (TT), which indicates the number of patients biopsied to detect one patient who upgraded, was calculated as one divided by the difference between the NB of the model and the NB of the best default strategy: biopsy all or biopsy none.

Table S1. Characteristics of Canary PASS patient cohort. Participants were patients with clinically localized PCa on AS undergoing scheduled prostate biopsies in the Canary PASS cohort enrolled from July 2008 to November 2017, with follow-up through August 2022.

	Overall (N=1220)	PRS_{400} 0% - 20% (N=219)	PRS_{400} 80% - 100% (N=275)
Characteristics at diagnosis			
Age at diagnosis (years)			
Median [IQR]	63.0 [58.0 - 67.0]	64.0 [60.0 - 68.0]	62.0 [56.0 - 67.0]
BMI			
Median [IQR]	27.3 [25.1 - 30.3]	26.9 [24.6 - 30.1]	27.7 [25.5 - 30.4]
Race			
American Indian	1 (0.1%)	0 (0%)	0 (0%)
Asian	24 (2.0%)	9 (4.1%)	3 (1.1%)
Black	78 (6.4%)	1 (0.5%)	48 (17.5%)
More than one race	13 (1.1%)	0 (0%)	7 (2.5%)
Pacific Islander	3 (0.2%)	0 (0%)	1 (0.4%)
Unknown	9 (0.7%)	2 (0.9%)	1 (0.4%)
White	1092 (89.5%)	207 (94.5%)	215 (78.2%)
Ethnicity			
Hispanic	54 (4.4%)	7 (3.2%)	15 (5.5%)
Non-Hispanic	1151 (94.3%)	208 (95.0%)	258 (93.8%)
Unknown	15 (1.2%)	4 (1.8%)	2 (0.7%)
PSA (ng/mL)			
Median [IQR]	4.94 [3.83 - 6.50]	4.70 [3.60 - 6.10]	5.36 [4.00 - 6.84]
Prostate volume (cc)			
Median [IQR]	43.0 [31.0 - 57.5]	44.0 [32.0 - 60.0]	41.0 [30.0 - 55.0]
PSAD (ng/mL^2)			
Median [IQR]	0.11 [0.08 - 0.16]	0.10 [0.07 - 0.14]	0.12 [0.09 - 0.18]
Biopsy Cores with Cancer			
1 core	655 (53.7%)	140 (63.9%)	126 (45.8%)
2 cores	324 (26.6%)	49 (22.4%)	72 (26.2%)
≥3 cores	223 (9.0%)	26 (11.9%)	75 (27.3%)
Missing	18 (1.5%)	4 (1.8%)	2 (0.7%)
Grade Group			
GG1	1110 (91.0%)	202 (92.2%)	250 (90.9%)
GG2	103 (8.4%)	16 (7.3%)	23 (8.4%)
GG3	7 (0.6%)	1 (0.5%)	2 (0.7%)

	Overall (N=1220)	PRS_{400} 0% - 20% (N=219)	PRS_{400} 80% - 100% (N=275)
Clinical T Stage			
T1	1097 (89.9%)	200 (91.3%)	256 (93.1%)
T2	123 (10.1%)	19 (8.7%)	19 (6.9%)
AUA Symptom Score			
Median [IQR]	7.00 [3.00 - 12.0]	8.00 [5.00 - 12.0]	7.00 [3.00 - 11.0]
Missing	7 (0.6%)	0 (0%)	3 (1.1%)
AUA QOL due to Urinary Symptoms			
Median [IQR]	1.00 [1.00 - 2.00]	2.00 [1.00 - 2.50]	1.00 [1.00 - 2.00]
Missing	3 (0.2%)	0 (0%)	1 (0.4%)
Follow-up characteristics			
Upgrading			
Yes	470 (38.5%)	69 (31.5%)	125 (45.5%)
No	750 (61.5%)	150 (68.5%)	150 (54.5%)
Extreme upgrading			
Yes	152 (12.5%)	24 (11.0%)	38 (13.8%)
No	1068 (87.5%)	195 (89.0%)	237 (86.2%)
Time to upgrading (Years)			
Median [IQR]	2.21 [1.09 – 4.73]	3.16 [1.19 – 6.25]	1.91 [1.02 – 4.31]
Follow-up time non-upgraders (Years)			
Median [IQR]	5.30 [3.03 – 7.61]	5.91 [3.30 – 8.64]	4.26 [2.47 – 6.49]
Time to extreme upgrading (Years)			
Median [IQR]	2.77 [1.28 – 5.52]	3.90 [1.26 – 5.98]	2.35 [1.24 – 4.33]
Time to competing event (treatment; n=398) (Years)			
Median [IQR]	2.66 [1.41 – 4.83]	3.81 [1.72 – 5.31]	2.15 [1.22 – 4.15]
Follow-up time non-extreme upgraders (n=670) (Years)			
Median [IQR]	6.21 [4.19 – 8.72]	6.87 [4.64 – 9.31]	5.36 [3.28 - 8.68]
PCa Treatment			
Yes	530 (43.4%)	67 (30.6%)	137 (49.8%)
No	690 (56.6%)	152 (69.4%)	138 (50.2%)

Table S2. Association between PRS categories and PCa upgrading and tumor features at diagnosis. PRS_{400} excludes 51 variants previously associated with PSA. β reflects the mean difference in the outcome associated with one SD unit increase in PRS. Results in red font indicate associations with p-value<0.05.

Time-to-event analyses										
	PRS_{400}					PRS_{451}				
	Co[a]	Ca[a]	HR	95% CI	p-value	Co[a]	Ca[a]	HR	95% CI	p-value
Upgrading										
0-20%	150	69	Ref	--	--	150	82	Ref	--	--
20-40%	150	88	1.39	[1.01, 1.91]	0.04	150	71	1.00	[0.73, 1.38]	1.00
40-60%	150	91	1.41	[1.03, 1.94]	0.03	150	101	1.32	[0.98, 1.77]	0.07
60-80%	150	97	1.47	[1.07, 2.01]	0.02	150	94	1.27	[0.94, 1.71]	0.13
80-100%	150	125	2.13	[1.57, 2.89]	1.24E-06	150	122	1.72	[1.28, 2.31]	3.22E-04
Extreme upgrading (CR)[b]										
0-20%	195	24	Ref	--	--	203	29	Ref	--	--
20-40%	202	36	1.68	[0.99, 2.83]	0.05	195	26	1.15	[0.67, 1.96]	0.62
40-60%	212	29	1.37	[0.79, 2.37]	0.26	223	28	1.08	[0.64, 1.82]	0.78
60-80%	222	25	1.14	[0.65, 2.01]	0.65	213	31	1.27	[0.76, 2.13]	0.36
80-100%	237	38	2.00	[1.18, 3.40]	0.01	234	38	1.68	[1.02, 2.80]	0.04
Mixed model analyses with a random effect for population structure										
	PRS_{400}					PRS_{451}				
	Co[a]	Ca[a]	OR	95% CI	p-value	Co[a]	Ca[a]	OR	95% CI	p-value
Upgrading										
0-20%	150	69	Ref	--	--	150	82	Ref	--	--
20-40%	150	88	1.29	[0.62, 1.36]	0.20	150	71	0.88	[0.61, 1.34]	0.54
40-60%	150	91	1.37	[0.60, 1.32]	0.12	150	101	1.27	[0.58, 1.23]	0.21
60-80%	150	97	1.47	[0.63, 1.39]	0.05	150	94	1.21	[0.68, 1.46]	0.34
80-100%	150	125	2.04	[0.61, 1.33]	3.33E-04	150	122	1.68	[0.67, 1.45]	7.88E-03
Extreme upgrading[c]										
0-20%	150	24	Ref	--	--	150	29	Ref	--	--
20-40%	150	36	1.61	[0.51, 1.63]	0.11	150	26	0.98	[0.50, 1.63]	0.93
40-60%	150	29	1.37	[0.49, 1.63]	0.30	150	28	1.05	[0.47, 1.52]	0.86
60-80%	150	25	1.15	[0.50, 1.75]	0.65	150	31	1.21	[0.56, 1.77]	0.51
80-100%	150	38	2.01	[0.50, 1.63]	0.02	150	38	1.63	[0.56, 1.75]	0.09
Grade Group										
0-20%	202	17	Ref	--	--	204	28	Ref	--	--
20-40%	213	25	1.40	[0.47, 1.77]	0.32	205	16	0.60	[0.46, 1.74]	0.13
40-60%	225	16	0.87	[0.43, 1.84]	0.70	231	20	0.65	[0.46, 1.58]	0.17
60-80%	220	27	1.51	[0.49, 1.81]	0.22	224	20	0.68	[0.53, 1.87]	0.23
80-100%	250	25	1.22	[0.45, 1.80]	0.57	246	26	0.76	[0.53, 1.85]	0.38
	β			95% CI	p-value	β			95% CI	p-value
Prostate volume (cc)										
0-20%	Ref			--	--	Ref			--	--
20-40%	-1.80			[-5.98, 2.38]	0.40	0.31			[-3.90, 4.51]	0.89
40-60%	-2.18			[-6.34, 1.98]	0.30	-1.16			[-5.23, 2.91]	0.57
60-80%	-3.40			[-7.56, 0.77]	0.11	-0.97			[-5.11, 3.17]	0.65
80-100%	-3.05			[-7.24, 1.14]	0.15	-1.08			[-5.26, 3.10]	0.61

PSA (ng/mL)						
0-20%	Ref	--	--	Ref	--	--
20-40%	0.36	[-0.18, 0.90]	0.19	0.50	[-0.041, 1.04]	0.07
40-60%	0.42	[-0.12, 0.96]	0.12	0.46	[-0.070, 0.98]	0.09
60-80%	0.40	[-0.14, 0.94]	0.15	0.41	[-0.13, 0.94]	0.14
80-100%	0.70	[0.16, 1.24]	0.01	0.95	[0.41, 1.49]	5.32E-04
PSAD (ng/ml^2)						
0-20%	Ref	--	--	Ref	--	--
20-40%	0.012	[-0.0048, 0.028]	0.16	0.014	[-0.0027, 0.030]	0.10
40-60%	0.014	[-0.0024, 0.030]	0.10	0.012	[-0.0034, 0.028]	0.12
60-80%	0.022	[0.0054, 0.038]	9.04E-03	0.019	[0.0026, 0.035]	0.02
80-100%	0.031	[0.015, 0.047]	1.98E-04	0.033	[0.016, 0.049]	8.32E-05
Total Cancer Cores						
0-20%	Ref	--	--	Ref	--	--
20-40%	0.17	[-0.062, 0.40]	0.15	0.16	[-0.068, 0.40]	0.17
40-60%	0.29	[0.060, 0.52]	0.01	0.20	[-0.027, 0.42]	0.08
60-80%	0.31	[0.080, 0.54]	8.28E-03	0.24	[0.0076, 0.46]	0.04
80-100%	0.53	[0.30, 0.76]	8.17E-06	0.58	[0.35, 0.81]	6.87E-07
Biopsy cores with cancer (%)						
0-20%	Ref	--	--	Ref	--	--
20-40%	1.20	[-0.72, 3.11]	0.22	1.41	[-0.52, 3.33]	0.15
40-60%	2.31	[0.40, 4.22]	0.02	1.64	[-0.23, 3.50]	0.09
60-80%	2.12	[0.20, 4.04]	0.03	2.08	[0.18, 3.97]	0.03
80-100%	4.01	[2.09, 5.93]	4.19E-05	4.29	[2.38, 6.20]	1.08E-05
AUA symptom score						
0-20%	Ref	--	--	Ref	--	--
20-40%	-0.48	[-1.63, 0.67]	0.41	-0.11	[-1.26, 1.05]	0.86
40-60%	-0.52	[-1.67, 0.63]	0.38	-0.91	[-2.04, 0.21]	0.11
60-80%	-1.29	[-2.44, -0.14]	0.03	-0.53	[-1.67, 0.61]	0.36
80-100%	-0.93	[-2.08, 0.23]	0.12	-0.58	[-1.73, 0.58]	0.33
AUA Quality of life urinary score						
0-20%	Ref	--	--	Ref	--	--
20-40%	-0.089	[-0.32, 0.14]	0.45	-0.11	[-0.34, 0.13]	0.37
40-60%	-0.12	[-0.35, 0.12]	0.33	-0.16	[-0.39, 0.061]	0.15
60-80%	-0.064	[-0.29, 0.17]	0.59	-0.0036	[-0.23, 0.23]	0.98
80-100%	-0.10	[-0.33, 0.13]	0.39	-0.011	[-0.24, 0.22]	0.93

[a] For the outcome of upgrading, Co and Ca refer to the number of non-upgraders and upgraders, respectively, in each PRS category. For the outcome of extreme upgrading, Co and Ca refer to the number of non-extreme upgraders and extreme upgraders, respectively, in each PRS category. For the outcome of Gleason grade group, Co and Ca refer to the number of GG1 and GG2/GG3 patients, respectively, in each PRS category.

[b] Competing risk regression of extreme upgraders (increase in grade group from GG1 or GG2 to GG3+, n=152) versus did not have extreme upgrading (n=1068).

[c] Extreme upgrading (n=152) versus did not upgrade (n=750; excludes participants that during study period only upgraded from GG1 to GG2; n=318)

Table S3. Decision curve analysis net benefit and test tradeoff for upgrading and extreme upgrading Cox PH models for relevant thresholds. Test tradeoff (TT) refers to the minimum number of tests performed to identify one true positive (i.e., the number of biopsies conducted to identify one patient who upgrades). TT is calculated as one divided by the difference between the net benefit (NB) of the model and the NB of the best default strategy: biopsy all or biopsy none (typically referred to as "treat" all or "treat" none in decision curve analysis). The NB for biopsy none is zero across all thresholds.

Upgrading												
Threshold Probability	**Biopsy all NB**	**Ref**		**Ref + PRS_{400}**		**Ref + PRS_{451}**		**Ref + Clinical**		**Ref + Clinical + PRS_{400}**		**Ref + Clinical + PRS_{451}**	
		NB	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**
5%	0.137	0.137	*NA*	0.137	*NA*	0.137	*NA*	0.137	*NA*	0.137	*NA*	0.137	*NA*
10%	0.0891	0.0891	*NA*	0.0888	*NA*	0.0888	*NA*	0.0886	*NA*	0.0902	909.1	0.0927	277.8
15%	0.0356	0.0335	*NA*	0.0423	149.3	0.0406	200.0	0.0569	47.0	0.0594	42.0	0.0587	43.3
20%	-0.0247	0.000163	6135.0	0.0165	60.6	0.0105	95.2	0.0473	21.1	0.0403	24.8	0.0412	24.3
25%	-0.093	0.00354	282.5	0.00831	120.3	-0.00292	*NA*	0.0283	35.3	0.035	28.6	0.0344	29.1
30%	-0.171	0.00147	680.3	0.00152	657.9	0.000924	1082.3	0.0149	67.1	0.0229	43.7	0.0176	56.8

Extreme Upgrading													
Threshold Probability	**Biopsy all NB**	**Ref**		**Ref + PRS_{400}**		**Ref + PRS_{451}**		**Ref + Clinical**		**Ref + Clinical + PRS_{400}**		**Ref + Clinical + PRS_{451}**	
		NB	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**	**NB**	**TT**
0%	0.0506	0.0506	*NA*	0.0506	*NA*	0.0506	*NA*	0.0506	*NA*	0.0506	*NA*	0.0506	*NA*
5%	0.000652	0.00922	116.7	0.00751	145.8	0.00698	158.0	0.0218	47.3	0.0205	50.4	0.0201	51.4
10%	-0.0549	-0.00283	*NA*	0.00157	636.9	0.000158	6329.1	0.00235	425.5	0.000302	3311.3	0.00157	636.9
15%	-0.117	-0.00118	*NA*	-0.0000633	*NA*	0.000131	7633.6	-0.00303	*NA*	-0.00399	*NA*	-0.00369	*NA*
20%	-0.187	-0.000418	*NA*	-0.000628	*NA*	-0.000418	*NA*	-0.0032	*NA*	-0.00302	*NA*	-0.00394	*NA*

Table S4. Mixed model results of the association between PRS and PCa upgrading and extreme upgrading. Models include a random effect for a genetic relationship matrix to further account for population structure.

	PRS_{400}			PRS_{451}		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
Upgrading	1.26	[1.11, 1.42]	3.00E-04	1.23	[1.08, 1.40]	1.47E-03
Extreme upgrading[a]	1.23	[1.02, 1.49]	0.03	1.19	[0.99, 1.44]	0.07

[a] Extreme upgrading (n=152) versus did not upgrade (n=750; excludes participants that during study period only upgraded from GG1 to GG2; n=318)

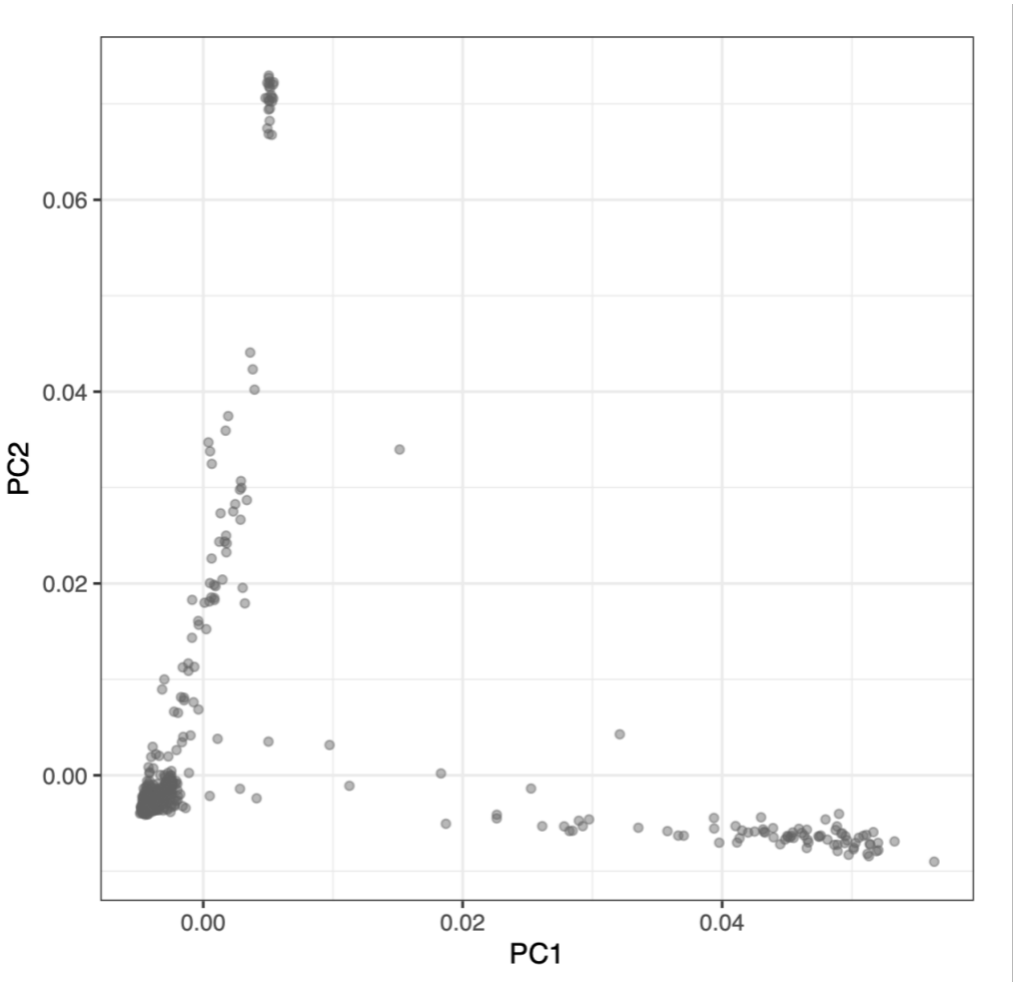

Figure S1. Genetic similarity of 1220 patients included in this investigation based on a principal component analysis. Population descriptors are not included in this figure as they do not capture the continuous nature of genetic similarity. The purpose of this figure is to convey the need for statistical analyses to use mixed models to account for the population structure that exists in the data.

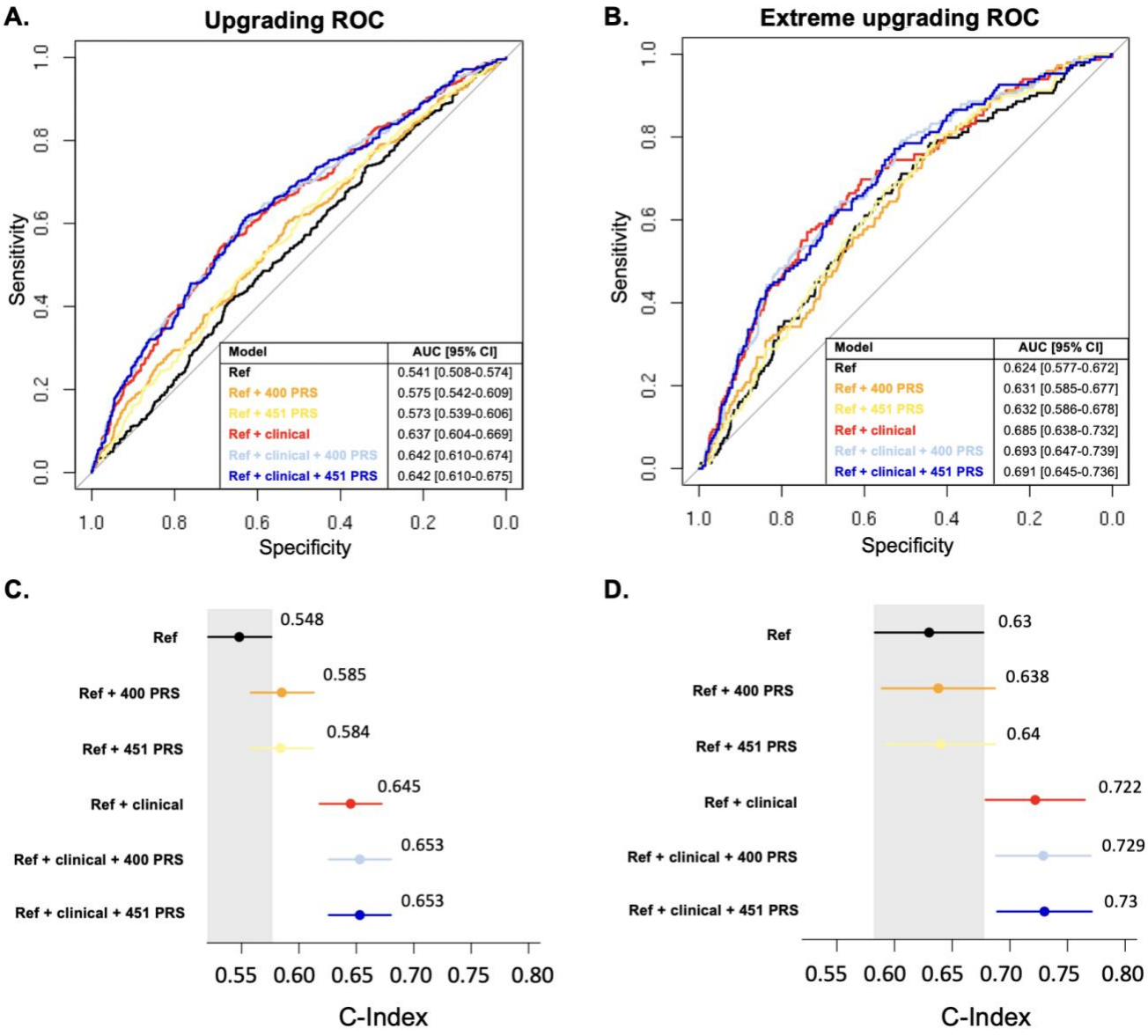

Figure S2. Discriminative ability of models for grade reclassification. AUCs using logistic regression models for A) upgrading (n=1195) and B) extreme upgrading (n=883). Harrell's C-index of Cox regression models of time to C) upgrading and D) extreme upgrading. The reference ("Ref") model included age at diagnosis and the top 10 principal components. Clinical risk factors were BMI, grade group (GG2+ vs GG1), clinical T stage (T2 vs T1), PSA, prostate volume, and the percentage of biopsy cores with cancer at diagnosis. Models were limited to participants with no missing data across variables included.

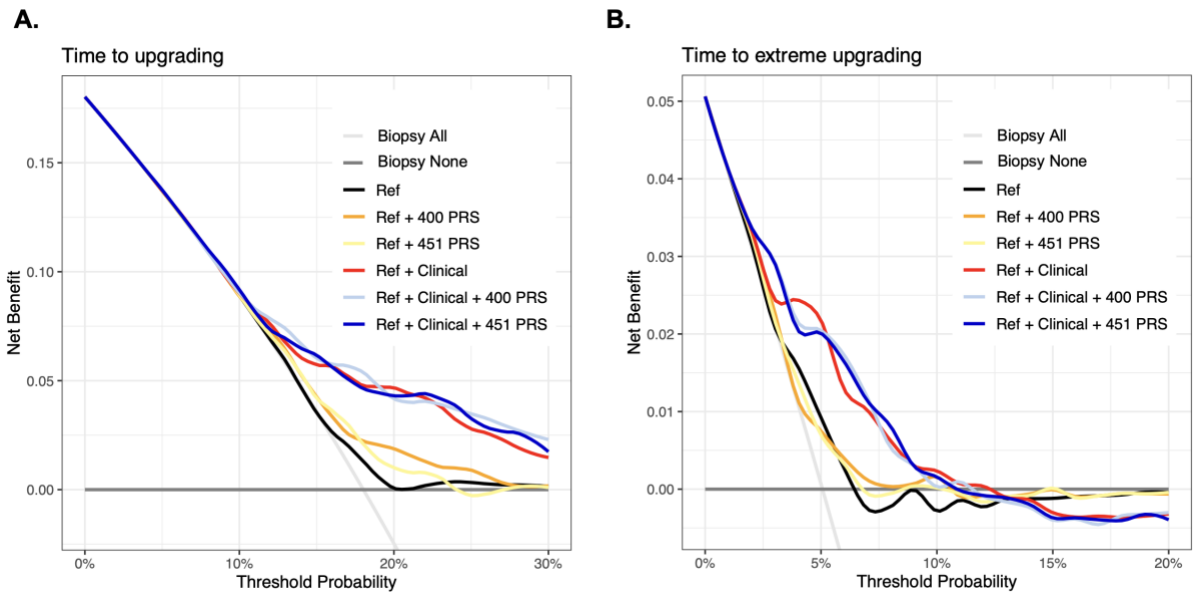

Figure S3. Decision curves for models predicting A) upgrading and B) extreme upgrading within 2 years to inform biopsy. The reference ("Ref") model included age at diagnosis and the top 10 principal components. Clinical risk factors were BMI, grade group (GG2+ vs GG1), clinical T stage (T2 vs T1), PSA, prostate volume, and the percentage of biopsy cores with cancer at diagnosis. Models were limited to participants with no missing data across variables included.