SUPPLEMENTARY MATERIALS

Supplementary Methods

Study Design

From January 2015 to March 2017, consecutive patients who were scheduled to undergo prostate needle biopsy for the first time at each institution were enrolled after written consent was obtained. DNA was extracted from the patient’s blood drawn before biopsy. Genotyping of common variants and target sequencing of the eight prostate cancer-associated genes was performed. PRS was calculated and combined with known clinical parameters to evaluate the predictive performance of the combined models in PCa diagnosis. The cohort was followed up until October 2019, and final follow-up data, including data on re-biopsy after initial negative biopsy, were collected. After data clean-up, the data were finalized in December 2019.

Clinical data acquisition

Pre-biopsy and follow-up patient data were entered into the electronic data capture (EDC) system specifically designed for the study. The clinical data collected included digital rectal exam findings, PSA, prostate volume, family history of prostate cancer, number of prostate biopsy cores, number of PCa-positive cores, total PCa-positive core length, Gleason score, clinical stage, and MRI findings for those who underwent pre-biopsy MRI. Prostate volume was measured either by ultrasound or MRI, and PSA density (PSAD) was calculated
by dividing PSA value by prostate volume. MRI was evaluated by local radiologists, and only the findings that were highly suspicious for PCa, which translates into PI-RADS 4 or 5 in the Prostate Imaging Reporting and Data System version 2 (PIRADS-v2), were considered positive findings. All the input data were inspected at the data center at the Institute for Advancement of Clinical Translational Science (iACT), Kyoto University Hospital, and any missing data were queried.

**DNA extraction**

Two milliliters of blood were drawn before the prostate needle biopsy. The blood samples were anonymized and collected by Bio Medical Laboratories, Inc., and DNA was extracted using standard procedures. The extracted DNA was stored at −20 °C at the Laboratory for Genotype Development, RIKEN Center for Integrative Medical Sciences.

**Genotyping of common variants**

The list of the common variants genotyped can be found in Supplementary Table 8. We used a two-step PCR method to construct DNA libraries. Multiplex PCR (25 cycles) was performed using primers targeting each region followed by the 2nd PCR (4 cycles) where 8-bp barcodes and adapter sequences were added using primers targeting shared 5’ overhangs introduced during the 1st PCR. The pooled libraries were sequenced by 2 × 150-bp paired-end reads on a HiSeq 2500 (Illumina, San Diego, CA, USA) instrument. Sequence reads allocated
to each individual were aligned to the human reference sequence (hg19) using Burrows-Wheeler Aligner (version.0.7.12)[1] and processed using the Genome Analysis Tool kit (GATK, ver. 3.4-46)[2]. For quality control, we selected individuals in which more than 98% of the target region was covered with 20 or more sequencing reads. Genotypes of all individuals were jointly determined for each variant based on the sequencing read ratios of the reference and alternative alleles. We assigned homozygotes of the reference allele, heterozygotes, or homozygotes of the alternative allele, when the alternative allele frequency fell in the range of 0–0.15, 0.25–0.75, or 0.85–1, respectively. The SNPs that could not be analyzed by multi-index sequencing were genotyped separately using multiplex PCR-based invader assay[3].

**Target genome sequencing**

All transcripts registered in Consensus CDS (CCDS) release 152 for each gene were analyzed. The total length of the target region was 37,982 bp. Single nucleotide variants (SNVs) and insertions or deletions (INDELs) of each individual were called separately using UnifiredGenotyper and HaplotypeCaller of GATK, as described previously[4]. Variants with call rates <98% were excluded. Finally, we identified 328 genetic variants in 1387 samples, and 99.8% of the target region was covered by ≥20 sequence reads.

**Age-specific absolute risk estimation**
The age-specific absolute risk[5, 6] was estimated for the percentile categories of the PRS distribution: [0–10%], (10–20%], (30–40%], (40–60%], (60–70%], (70–80%], (80–90%], (90–99%], and (99–100%]. Note that “(”, “[”, and “]” indicate “greater than”, “greater than or equal to”, and “less than or equal to”, respectively. The absolute risk values at each age were calculated recursively as in Olama et al.[7] and Conti D et al.[8]. Japanese age-specific prostate cancer incidence data were extracted from the Cancer Registry and Statistics (2016–2017) (Cancer Information Service, National Cancer Center, Japan), and age-specific mortality rates in 2017 were available from the website of the Ministry of Health, Labour and Welfare, Japan.

References

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Nat Genet 2021;53(1):65-75.
## Supplementary Tables

### Supplementary Table 1. Case control analysis of the 16 SNPs included in polygenic risk score

| Chr | rs number | Position   | Gene      | Allele (risk/no-risk) | Case Risk homo | Case Hetero | Non-risk homo | Control Risk homo | Control Hetero | Non-risk homo | Total | Armitage's trend $P^a$ | OR (95% CI) |
|-----|-----------|------------|-----------|-----------------------|----------------|-------------|-------------|--------------------|----------------|-------------|-------|------------------------|-------------|
| 2   | rs13385191| 20888265   | C2orf43   | G/A                   | 259            | 396         | 146         | 163                | 269            | 103         | 1336  | 0.46                   | 1.06 (0.91 to 1.24) |
| 2   | rs11693801| 43590329   | THADA     | C/T                   | 394            | 335         | 72          | 257                | 221            | 57          | 1336  | 0.44                   | 1.07 (0.90 to 1.26) |
| 3   | rs9284813 | 87152169   | 3p12      | G/A                   | 62             | 301         | 437         | 28                 | 199            | 308         | 1335  | 0.12                   | 1.16 (0.97 to 1.38) |
| 5   | rs12653946| 1895829    | IRX4      | T/C                   | 204            | 417         | 180         | 115                | 255            | 165         | 1336  | <0.001 $^b$             | 1.28 (1.10 to 1.50) |
| 6   | rs1983891 | 41536427   | FOXP4     | T/C                   | 161            | 389         | 251         | 79                 | 262            | 194         | 1336  | 0.009                  | 1.23 (1.06 to 1.45) |
| 6   | rs339331  | 117210052  | RFX6/GPRC6A| T/C                  | 332            | 366         | 103         | 212                | 242            | 81          | 1336  | 0.29                   | 1.09 (0.93 to 1.28) |
| 8   | rs1512268 | 23526463   | NNX3.1    | T/C                   | 143            | 382         | 276         | 70                 | 257            | 208         | 1336  | 0.02                   | 1.21 (1.03 to 1.42) |
| 8   | rs10086908| 128011937  | 8q24(block1)| T/C                  | 550            | 225         | 26          | 352                | 161            | 22          | 1336  | 0.22                   | 1.13 (0.93 to 1.38) |
| 8   | rs1456315 | 128103937  | 8q24(block2/region2)| T/C             | 444            | 308         | 48          | 243                | 233            | 58          | 1334  | <0.001 $^b$             | 1.44 (1.21 to 1.70) |
| 8   | rs620861  | 128335673  | 8q24(block3/region3)| G/A             | 255            | 366         | 180         | 147                | 275            | 113         | 1336  | 0.45                   | 1.06 (0.91 to 1.24) |
| 8   | rs6983267 | 128413305  | 8q24(block4/region3)| G/T             | 110            | 384         | 307         | 71                 | 235            | 229         | 1336  | 0.20                   | 1.11 (0.95 to 1.31) |
| 8   | rs7837688 | 128539360  | 8q24(block5/region1)| T/G             | 39             | 251         | 511         | 14                 | 130            | 391         | 1336  | <0.001 $^b$             | 1.49 (1.21 to 1.84) |
| 10  | rs10993994| 51549496   | MSMB      | T/C                   | 236            | 366         | 199         | 127                | 266            | 142         | 1336  | 0.07                   | 1.16 (0.99 to 1.35) |
| 13  | rs9600079 | 73728139   | 13q22     | T/G                   | 135            | 387         | 279         | 74                 | 266            | 195         | 1336  | 0.23                   | 1.10 (0.94 to 1.29) |
| 17  | rs7501939 | 36101156   | HNF1B     | C/T                   | 439            | 309         | 53          | 261                | 225            | 49          | 1336  | 0.02                   | 1.24 (1.04 to 1.47) |
| 22  | rs5759167 | 43500212   | TTLL1/BIK | G/T                  | 359            | 358         | 84          | 224                | 241            | 70          | 1336  | 0.14                   | 1.13 (0.96 to 1.33) |

*a All statistical tests were 2-sided. Chr = chromosome, rs = reference single nucleotide polymorphism, A = adenine, T = thymine, C = cytosine, G = guanine, homo = homozygous, hetero = heterozygous, OR = odds ratio, CI = confidence interval
Statistically significant after Bonferroni correction ($P < 0.003$)
Supplementary Table 2. Patient characteristics and the results of initial biopsy $^a$

| Characteristic                     | PCa (+)     | PCa (-)     | Total       |
|------------------------------------|-------------|-------------|-------------|
| Total No. of patients (%)          | 778 (58.2)  | 558 (41.8)  | 1336 (100.0)|
| Age, years                         |             |             |             |
| Mean (SD)                          | 70.6 (7.20) | 66.1 (8.10) | 68.7 (7.91) |
| Median (Min , Max)                 | 71.0 (47, 90)| 67.0 (37, 91)| 69.0 (37, 91)|
| Digital rectal exam, No. (%)       |             |             |             |
| PCa suspected                      | 279 (36.0)  | 44 (7.9)    | 323 (24.2)  |
| PCa not suspected                  | 497 (64.0)  | 513 (92.1)  | 1010 (75.6)|
| N/A                                | 2           | 1           | 3           |
| PSA, ng/ml                         |             |             |             |
| Mean (SD)                          | 82.5 (583.8)| 7.4 (4.7)   | 51.1 (446.9)|
| Median (Min , Max)                 | 8.8 (0.034, 14426)| 6.0 (0.7, 42.7)| 7.3 (0.034, 14426)|
| Prostate volume, m$^3$             |             |             |             |
| Mean (SD)                          | 32.0 (17.9) | 41.0 (19.8) | 35.8 (19.2)|
| Median (Min , Max)                 | 28.00 (7.3, 175)| 37.1 (5.7, 186)| 31.00 (5.7, 186)|
| PSA density                        |             |             |             |
| Mean (SD)                          | 2.04 (11.11)| 0.21 (0.16) | 1.28 (8.98)|
| Median (Min , Max)                 | 0.341 (0.002, 265.672)| 0.173 (0.027, 1.640)| 0.247 (0.002, 265.672)|
| Family history, No. (%)            |             |             |             |
| Yes                                | 42 (5.7)    | 40 (7.6)    | 82 (6.5)    |
| No                                 | 698 (94.3)  | 486 (92.4)  | 1184 (93.5) |
| N/A                                | 38          | 32          | 70          |
| Suspicion of PCa on MRI, No. (%)   |             |             |             |
| Yes                                | 395 (87.6)  | 171 (57.8)  | 566 (75.8)  |
| No                                 | 56 (12.4)   | 125 (42.2)  | 181 (24.2)  |
| N/A                                | 327         | 262         | 589         |
| No. of biopsy cores                |             |             |             |
| Median (Min , Max)                 | 12.0 (2, 20)| 12.0 (8, 22)| 12.0 (2, 22)|

$^a$ PCa = prostate cancer; PSA = prostate specific antigen; MRI = magnetic resonance imaging; N/A = not available; SD = standard deviation.
### Supplementary Table 3. Patient characteristics of the patients with PSA 2-10 ng/ml

| Characteristic                                      | PCa (+) | PCa (−) | Total   |
|-----------------------------------------------------|---------|---------|---------|
| Total No. of patients (%)                          | 446 (50.2) | 443 (49.8) | 889 (100) |
| **Age, years**                                      |         |         |         |
| Mean (SD)                                           | 69.1 (6.5) | 65.6 (8.0) | 67.3 (7.5) |
| Median (Min, Max)                                   | 69 (47, 84) | 67 (37, 91) | 68 (37, 91) |
| **Digital rectal exam, No. (%)**                    |         |         |         |
| PCa suspected                                       | 96 (21.6) | 36 (8.1) | 132 (14.9) |
| PCa not suspected                                   | 349 (78.4) | 407 (91.9) | 756 (85.1) |
| N/A                                                 | 1       | 0       | 1       |
| **PSA, ng/ml**                                      |         |         |         |
| Mean (SD)                                           | 6.3 (1.7) | 5.9 (1.7) | 6.1 (1.7) |
| Median (Min, Max)                                   | 6.1 (2.7, 10.0) | 5.5 (2.1, 10.0) | 5.8 (2.1, 10.0) |
| **Prostate volume, ml³**                            |         |         |         |
| Mean (SD)                                           | 29.2 (14.5) | 40.1 (19.1) | 34.6 (17.8) |
| Median (Min, Max)                                   | 26 (8, 175) | 36.4 (5.7, 186) | 30.0 (5.7, 186) |
| **PSA density**                                     |         |         |         |
| Mean (SD)                                           | 0.25 (0.12) | 0.17 (0.09) | 0.21 (0.11) |
| Median (Min, Max)                                   | 0.24 (0.04, 0.98) | 0.16 (0.03, 0.70) | 0.19 (0.03, 0.98) |
| **Family history, No. (%)**                         |         |         |         |
| Yes                                                 | 32 (7.6) | 33 (8.0) | 65 (7.8) |
| No                                                  | 390 (92.4) | 382 (92.0) | 772 (92.2) |
| N/A                                                 | 24       | 28       | 52       |
| **Suspicion of PCa on MRI, No. (%)**                |         |         |         |
| Yes                                                 | 205 (85.4) | 130 (57.0) | 335 (71.6) |
| No                                                  | 35 (14.6) | 98 (43.0) | 133 (28.4) |
| N/A                                                 | 206      | 215      | 421      |
| **No. of biopsy core**                              |         |         |         |
| Median (Min, Max)                                   | 12 (8,20) | 12 (8,22) | 12 (8,22) |

*PCa = prostate cancer; PSA = prostate specific antigen; MRI = magnetic resonance imaging; N/A = not available; SD = standard deviation.*
Supplementary Table 4. Odds ratio by polygenic risk score category (model validation cohort in the previous study: number of cases = 3,294, number of controls = 6,281)

| PRS category | OR\(^a\) (95% CI) |
|--------------|-------------------|
| 0-10%        | 0.39 (0.32-0.47)  |
| 10-20%       | 0.59 (0.49-0.71)  |
| 20-30%       | 0.57 (0.48-0.69)  |
| 30-40%       | 0.72 (0.60-0.86)  |
| 40-60%       | 1.00 (reference)  |
| 60-70%       | 1.37 (1.16-1.62)  |
| 70-80%       | 1.47 (1.24-1.73)  |
| 80-90%       | 1.83 (1.56-2.16)  |
| 90-100%      | 3.22 (2.73-3.81)  |
| 99-100%      | 3.43 (2.21-5.31)  |

\(^a\)Odds ratio (OR) is calculated by logistic regression analysis with presence of prostate cancer as the objective variable and polygenic risk score (PRS) category and age as explanatory variables. CI = confidence interval. PRS = polygenic risk score.
Supplementary Table 5. Logistic regression analysis incorporating PSAD, age, PRS, and DRE

| Parameter                                | Log worth effect | OR\(^a\) (95% CI)          |
|------------------------------------------|------------------|-----------------------------|
| PSAD as a continuous variable            |                  |                             |
| PSAD (continuous variable)               | 24.46            | 3289.16 (583.75 to 18532.8) |
| age (continuous variable)                | 11.05            | 1.07 (1.05 to 1.10)         |
| PRS (continuous variable)                | 8.96             | 1.89 (1.52 to 2.34)         |
| DRE positive (negative as reference)     | 2.48             | 1.96 (1.24 to 3.08)         |
| PSAD as a categorical variable           |                  |                             |
| PSAD >0.2 (≦ 0.2 as reference)           | 17.00            | 3.60 (2.66 to 4.86)         |
| age (continuous variable)                | 10.87            | 1.07 (1.05 to 1.10)         |
| PRS (continuous variable)                | 9.72             | 1.93 (1.56 to 2.39)         |
| DRE positive (negative as reference)     | 3.24             | 2.15 (1.38 to 3.35)         |

\(^a\) Odds ratio (OR) is calculated by logistic regression analysis with presence of prostate cancer as the objective variable and PSAD, MRI, PRS, DRE findings and age as explanatory variables. PSAD = prostate specific antigen density; PRS = polygenic risk score; DRE = digital rectal exam; CI = confidence interval.
Supplementary Table 6. Logistic regression analysis incorporating MRI, PSAD, age, PRS, and DRE

| Parameter                                      | Log worth effect size | OR\(^a\) (95% CI)                  |
|------------------------------------------------|-----------------------|------------------------------------|
| **PSAD as a continuous variable**              |                       |                                    |
| PSAD (continuous variable)                     | 13.6                  | 3765.2 (343.0 to 41334.3)          |
| MRI positive (negative as reference)           | 4.54                  | 2.90 (1.74 to 4.83)                |
| age (continuous variable)                      | 4.48                  | 1.06 (1.03 to 1.10)                |
| PRS (continuous variable)                      | 4.3                   | 23.64 (4.78 to 116.9)              |
| DRE positive (negative as reference)           | 1.59                  | 1.93 (1.07 to 3.49)                |
| **PSAD as a categorical variable**             |                       |                                    |
| PSAD >0.2 (≤0.2 as reference)                  | 9.01                  | 3.70 (2.41 to 5.68)                |
| MRI positive (negative as reference)           | 4.51                  | 2.83 (1.72 to 4.65)                |
| age (continuous variable)                      | 4.27                  | 1.06 (1.03 to 1.09)                |
| PRS (continuous variable)                      | 4.38                  | 1.77 (1.33 to 2.35)                |
| DRE positive (negative as reference)           | 2.00                  | 2.10 (1.18 to 3.73)                |

\(^a\) Odds ratio (OR) is calculated by logistic regression analysis with presence of prostate cancer as the objective variable and PSAD, MRI, PRS, DRE findings and age as explanatory variables. 

PSAD = prostate specific antigen density; PRS = polygenic risk score; DRE = digital rectal exam; MRI = magnetic resonance imaging; CI = confidence interval.
Supplementary Table 7. Distribution of high-risk patients by different PRS cutoffs in the PCSSNP and model creation cohort

| Cohort                  | Total No. of patients | PRS $\geq$ 3 |   | PRS $\geq$ 2.5 |   | PRS $\geq$ 2.0 |   |
|-------------------------|-----------------------|---------------|---|----------------|---|----------------|---|
|                         |                       | No. of patients | % of all samples | No. of patients | % of all samples | No. of patients | % of all samples |
| PCSSNP cohort           | 1333                  | 48            | 3.6| 82             | 6.2| 175            | 13.1|
| Model creation cohort   | 11013                 | 371           | 3.4| 600            | 5.4| 1073           | 9.7 |

$^a$PRS = polygenic risk score; PCSSNP = Prostate Cancer Susceptibility Single Nucleotide Polymorphism.
Supplementary Table 8. PRS risk and age at diagnosis<sup>a</sup>

| PRS cutoff for defining high risk | PCa (+) | PCa (-) | Mean age for cases with PCa (SE), y |
|-----------------------------------|---------|---------|-----------------------------------|
|                                   | Median age, y | No. of patients | Median age, y | No. of patients |                                      |
| Cutpoint 2                        |          |         |                                  |                      |
| PRS ≥ 2                          | 70       | 138     | 67                               | 37                   | 69.5 (0.67)                          |
| PRS < 2                          | 71       | 663     | 67                               | 498                  | 70.8 (0.27)                          |
| Cutpoint 2.5                     |          |         |                                  |                      |
| PRS ≥ 2.5                        | 68.5     | 70      | 66                               | 12                   | 68.6 (0.96)                          |
| PRS < 2.5                        | 71       | 731     | 67                               | 523                  | 70.7 (0.26)                          |
| Cutpoint 3                        |          |         |                                  |                      |
| PRS ≥ 3                          | 68       | 43      | 68                               | 5                    | 68.8 (1.20)                          |
| PRS < 3                          | 71       | 758     | 67                               | 530                  | 70.7 (0.25)                          |

<sup>a</sup>PRS = polygenic risk score; PCa = prostate cancer; SE = standard error.
Supplementary Table 9. Prevalence of pathogenic rare variants in the eight genes

| Gene  | PCSSNP cohort, No. (%) | BioBank Japan\textsuperscript{a}, No. (%) |
|-------|-------------------------|------------------------------------------|
|       | All patients (N=1336)   | PCa (+) (n=801) PCa (-) (n=535) PCa (+) (n=7636) PCa (-) (n=12366) |
| ATM   | 4 (0.30) 3 (0.37) 1 (0.19) 37 (0.5) 21 (0.2) |
| BRCA2 | 10 (0.75) 7 (0.87) 3 (0.56) 83 (1.1) 24 (0.2) |
| BRIP1 | 3 (0.22) 2 (0.25) 1 (0.19) 6 (0.1) 7 (0.1) |
| CHEK2 | 1 (0.07) 1 (0.12) 0 (0) 12 (0.2) 8 (0.1) |
| HOXB13| 6 (0.45) 5 (0.62) 1 (0.19) 61 (0.8) 21 (0.2) |
| NBN   | 2 (0.15) 1 (0.12) 1 (0.19) 3 (0.0) 4 (0.0) |
| PALB2 | 1 (0.07) 1 (0.12) 0 (0) 4 (0.1) 4 (0.0) |
| All (excluding duplicate) | 26 (1.95) 19 (2.37) 7 (1.31) 219 (2.9) 99 (0.8) |

\textsuperscript{a}Momozawa Y et al. 2019 (4). PCa = prostate cancer; PCSSNP = Prostate Cancer Susceptibility Single Nucleotide Polymorphism
Supplementary Figures

Patient cohort

registered: 1394 patients
  ↓
failed quality check or no DNA sample: 7 patients
  ↓
genotyped: 1387 samples
  ↓
no biopsy: 41 patients
  ↓
1346 patients
  ↓
duplicate registration: 10 patients
  ↓
complete clinical and genomic data: 1336 patients
(3 patients had missing genomic data in one of the 16 SNPs)

Supplementary Figure 1. Schema explaining the PCSSNP cohort. SNP = single nucleotide polymorphism; PCSSNP = Prostate Cancer Susceptibility Single Nucleotide Polymorphism.
Supplementary Figure 2. Diagnostic performance of PRS for predicting biopsy positivity in all patients (right) and in patients with PSA 2–10 ng/mL (left) evaluated by ROC analysis. AUC is presented along with 95% confidence intervals in parentheses. AUC = area under the curve; PSA = prostate specific antigen.
Supplementary Figure 3. Box plots showing distribution of PRS in non-clinically significant and clinically significant PCa (A), and non-high risk PCa and high risk PCa (C). The upper and lower error bars in the box plots in (A) and (C) represent 1.5 x 1st and 3rd quartile, respectively. The ROC curves for discriminating between non-clinically significant and clinically significant PCa (B), and non-high risk PCa and high risk PCa (D) by PRS are drawn in the right with AUC (95% confidence interval). PRS = polygenic risk score; AUC = area under the curve.
Supplementary Figure 4. Sensitivity analysis focusing on the result of initial biopsy. Diagnostic performance of logistic regression models incorporating clinical parameters and PRS. ROC analysis was performed for each combination of clinical parameters and PRS, and AUC (95% confidence interval) were calculated. Panels B–D can be directly compared to those in Figure 1. PSA = prostate specific antigen; PRS = polygenic risk score; DRE = digital rectal exam; AUC = area under the curve.
Supplementary Figure 5. Diagnostic performance of logistic regression models incorporating clinical parameters and PRS. ROC analysis was performed for each combination of clinical parameters and PRS, and AUC (95% confidence interval) were calculated. The panels can be directly compared to those in Figure 1. PSA = prostate specific antigen; PRS = polygenic risk score; DRE = digital rectal exam; AUC = area under the curve.
Supplementary Figure 6. Absolute risk for a given age for each PRS category based on the age-specific prostate cancer incidence and age-specific mortality rates of Japanese. Y axis shows the absolute risk, and X axis shows age in years. The different colored lines show PRS categories. PRS = polygenic risk score.