Evidence of Novel Susceptibility Variants for Prostate Cancer and a Multi-ancestry Polygenic Risk Score Associated with Aggressive Disease in Men of African Ancestry

Fei Chen[1], Ravi K. Madduri[2], Alex A. Rodriguez[2], Burcu F. Darst[1,3], Alisha Chou[1], Xin Sheng[1], Anqi Wang[1], Jiayi Shen[1], Edward J. Saunders[4], Suhn K. Rhie[5], Jeannette T. Bensen[6,7], Sue A. Ingles[1], Rick A. Kittles[8], Sara S. Strom[9], Benjamin A. Rybicki[10], Barbara Nemesure[11], William B. Isaacs[12], Janet L. Stanford[3], Wei Zheng[13], Maureen Sanderson[14], Esther M. John[15], Jong Y. Park[16], Jianfeng Xu[17], Ying Wang[18], Sonja I. Berndt[19], Chad D. Huff[9], Edward D. Yeboah[20], Yao Tettey[21,22], Joseph Lachance[23], Wei Tang[24], Christopher T. Rentsch[25,26,27], Kelly Cho[28,29], Benjamin H. Mcmahon[30], Richard B. Biritwum[22], Andrew A. Adjei[31], Evelyn Tay[22], Ann Truelove[32], Shelley Niwa[32], Thomas A. Sellers[16], Kosj Yamoah[33,16], Adam B. Murphy[34], Dana C. Crawford[35], Alpa V. Patel[18], William S. Bush[35], Melinda C. Aldrich[36], Olivier Cussenot[37,38], Gyorgy Petrovics[39], Jennifer Cullen[39,35], Christine M. Neslund-Dudas[10], Mariana C. Stern[1], Zsofia Kote-Jarai[4], Koveela Govindasami[4], Michael B. Cook[19], Anand P. Chokkalingam[40], Ann W. Hsing[15], Phyllis J. Goodman[41], Thomas J. Hoffmann[42], Bettina F. Drake[43], Jennifer J. Hu[44], Jacob M. Keaton[13,45], Jacklyn N. Hellwege[13,46], Peter E. Clark[47], Mohamed Jalloh[48], Serigne M. Gueye[48], Lamine Niang[48], Olufemi Ogunbiyi[49], Michael O. Idowu[49], Olufemi Popoola[49], Akindele O. Adebiyi[49], Oseremen I. Aisuodionoe-Shadrach[50], Hafees O. Ajibola[50], Mustapha A. Jamda[50], Olabode P. Oluwole[50], Maxwell Nwegbu[50], Ben Adusei[51], Sunny Mante[51], Afua Darkwa-Abrahams[22], James E. Mensah[22], Halimatou Diop[52], Stephen K. Van Den Eeden[53,54], Pascal Blanchet[55], Jay H. Fowke[56], Graham Casey[57], Anselm J. Hennis[11], Alexander Lubwama[58], Ian M. Thompson Jr.[59], Robin Leach[60], Douglas F. Easton[61], Michael H. Preuss[62], Ruth J. Loos[62], Susan M. Gundell[1], Peggy Wan[1], James L. Mohler[7,63], Elizabeth T. Fontham[64], Gary J. Smith[63], Jack A. Taylor[65,66], Shiv Srivastava[67], Rosaline A. Eeles[4,68], John D. Carpten[69], Adam S. Kibel[70], Luc Multigner[71], Marie-Élise Parent[72], Florence Menegaux[73,74], Geraldine Cancel-Tassin[37,38], Eric A. Klein[75], Caroline Andrews[76,77], Timothy R. Rebbeck[76], Laurent Brureau[55], Stefan Ambs[24], Todd L. Edwards[13], Stephen Watya[58], Stephen J.

Chanock[19], John S. Witte[78,79], William J. Blot[13,80], J. Michael Gaziano[28,81], Amy C. Justice[25,26], David V. Conti[1], Christopher A. Haiman[1*]

[1]Department of Population and Public Health Sciences, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [2]Argonne National Laboratory, Lemont, IL, USA; [3]Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [4]The Institute of Cancer Research, London, UK; [5]Department of Biochemistry and Molecular Medicine, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [6]Department of Epidemiology, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [7]Lineberger Comprehensive Cancer Center, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA; [8]Department of Population Sciences, City of Hope Comprehensive Cancer Center, Duarte, CA, USA; [9]Department of Epidemiology, University of Texas M.D. Anderson Cancer Center, Houston, TX, USA; [10]Department of Public Health Sciences, Henry Ford Hospital, Detroit, MI, USA; [11]Department of Family, Population and Preventive Medicine, Stony Brook University, Stony Brook, NY, USA; [12]James Buchanan Brady Urological Institute, Johns Hopkins Hospital and Medical Institution, Baltimore, MD, USA; [13]Division of Epidemiology, Department of Medicine, Vanderbilt University Medical Center, Nashville, TN, USA; [14]Department of Family and Community Medicine, Meharry Medical College, Nashville, TN, USA; [15]Department of Medicine, Stanford Cancer Institute, Stanford University School of Medicine, Stanford, CA, USA; [16]Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, FL, USA; [17]Program for Personalized Cancer Care and Department of Surgery, NorthShore University HealthSystem, Evanston, IL, USA; [18]Department of Population Science, American Cancer Society, Kennesaw, GA, USA; [19]Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, MD, USA; [20]University of Ghana Medical School, Accra, Ghana; [21]Department of Pathology, University of Ghana, Accra, Ghana; [22]Korle Bu Teaching Hospital, Accra, Ghana; [23]School of Biological Sciences, Georgia Institute of Technology, Atlanta, GA, USA; [24]Laboratory of Human Carcinogenesis, Center for Cancer Research, National Cancer Institute, Bethesda, MD, USA; [25]Yale School of Medicine, New

Haven, CT, USA; [26]VA Connecticut Healthcare System, West Haven, CT, USA; [27]Faculty of Epidemiology and Population Health, London School of Hygiene & Tropical Medicine, London, UK; [28]Division of Aging, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [29]VA Boston Healthcare System, Jamaica Plain, MA, USA; [30]Theoretical Biology Division, Los Alamos National Lab, Los Alamos, NM, USA; [31]Department of Pathology, University of Ghana Medical School, Accra, Ghana; [32]Westat, Rockville, MD, USA; [33]Department of Radiation Oncology, Moffitt Cancer Center, Tampa, FL, USA; [34]Department of Urology, Northwestern University, Chicago, IL, USA; [35]Department of Population and Quantitative Health Sciences, Cleveland Institute for Computational Biology, Case Western Reserve University, Cleveland, OH, USA; [36]Division of Epidemiology, Department of Thoracic Surgery, Vanderbilt University Medical Center, Nashville, TN, USA; [37]Department of Urology and Predictive Onco-Urology Group, Sorbonne Université, GRC 5 Predictive Onco-Urology, APHP-Sorbonne Université, Paris, France; [38]CeRePP, Tenon Hospital, Paris, France; [39]Department of Surgery, Center for Prostate Disease Research, Uniformed Services University of the Health Sciences, Bethesda, MD, USA; [40]School of Public Health, University of California, Berkeley, Berkeley, CA, USA; [41]SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, WA, USA; [42]Department of Epidemiology and Biostatistics, University of California, San Francisco, San Francisco, CA, USA; [43]Division of Public Health Sciences, Washington University School of Medicine, St. Louis, MO, USA; [44]The University of Miami School of Medicine, Sylvester Comprehensive Cancer Center, Miami, FL, USA; [45]Center for Precision Health Research, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA; [46]Division of Genetic Medicine, Department of Medicine, Vanderbilt Genetics Institute, Nashville, TN, USA; [47]Atrium Health/Levine Cancer Institute, Charlotte, NC, USA; [48]Hôpital Général Idrissa Pouye, Dakar, Senegal; [49]College of Medicine, University of Ibadan and University College Hospital, Ibadan, Nigeria; [50]College of Health Sciences, University of Abuja, University of Abuja Teaching Hospital and Cancer Science Center, Abuja, Nigeria; [51]37 Military Hospital, Accra, Ghana; [52]Laboratoires Bacteriologie et Virologie, Hôpital Aristide Le Dantec, Dakar, Senegal; [53]Division of Research, Kaiser Permanente, Northern California, Oakland, CA, USA;

[54]Department of Urology, University of California, San Francisco, San Francisco, CA, USA; [55]CHU de Pointe-à-Pitre, Univ Antilles, Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), Pointe-à-Pitre, Guadeloupe, France; [56]Department of Preventive Medicine, Division of Epidemiology, The University of Tennessee Health Science Center, Memphis, TN, USA; [57]Department of Public Health Science, Center for Public Health Genomics, University of Virginia, Charlottesville, VA, USA; [58]Uro Care, Kampala, Uganda; [59]CHRISTUS Santa Rosa Medical Center Hospital, San Antonio, TX, USA; [60]Department of Urology, Cancer Therapy and Research Center, University of Texas Health Science Center at San Antonio, San Antonio, TX, USA; [61]Department of Public Health and Primary Care, Centre for Cancer Genetic Epidemiology,, University of Cambridge, Strangeways Research Laboratory, Cambridge, UK; [62]The Charles Bronfman Institute for Personalized Medicine, Icahn School of Medicine at Mount Sinai, New York, NY, USA; [63]Department of Urology, Roswell Park Cancer Institute, Buffalo, NY, USA; [64]School of Public Health, Louisiana State University Health Sciences Center, New Orleans, LA, USA; [65]Epidemiology Branch, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [66]Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, Research Triangle Park, NC, USA; [67]Department of Biochemistry and Molecular & Cellular Biology, Georgetown University Medical Center, Washington, DC, USA; [68]Royal Marsden NHS Foundation Trust, London, UK; [69]Department of Translational Genomics, Keck School of Medicine, University of Southern California, Los Angeles, CA, USA; [70]Department of Urology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA; [71]Univ Rennes, Inserm, EHESP, Irset (Institut de recherche en santé, environnement et travail), Rennes, France; [72]Epidemiology and Biostatistics Unit, Centre Armand-Frappier Santé Biotechnologie, Institut national de la recherche scientifique, Laval, QC, Canada; [73]Cancer & Environment Group, Center for Research in Epidemiology and Population Health (CESP), INSERM, University Paris-Sud, University Paris-Saclay, Villejuif Cédex, France; [74]Paris-Sud University, Villejuif Cédex, France; [75]Cleveland Clinic Lerner Research Institute, Cleveland, OH, USA; [76]Harvard TH Chan School of Public Health and Division of Population Sciences, Dana Farber Cancer Institute, Boston, MA, USA; [77]Glickman Urological

& Kidney Institute, Cleveland, OH, USA; [78]Department of Epidemiology and Population Health, Stanford University, Stanford, CA, USA; [79]Stanford Cancer Institute, Stanford University, Stanford, CA, USA; [80]International Epidemiology Institute, Rockville, MD, USA; [81]VA Boston Healthcare System, Boston, MA, USA

***Corresponding Author:**

Christopher A. Haiman

1450 Biggy Street

Los Angeles, CA 90033 USA

1-323-442-7755

haiman@usc.edu

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ABSTRACT

Background: Genetic factors play an important role in prostate cancer (PCa) susceptibility.

Objective: To discover common genetic variants contributing to the risk of PCa in men of African ancestry.

Design, Setting, and Participants: We conducted a meta-analysis of ten genome-wide association studies (GWAS) consisting of 19,378 cases and 61,620 controls of African ancestry.

Outcome measurements and Statistical Analysis: Common genotyped and imputed variants were tested for association with PCa risk. Novel susceptibility loci were identified and

incorporated into a multi-ancestry polygenic score (PRS). The PRS was evaluated for association with PCa risk and disease aggressiveness.

Results and Limitations: Nine novel susceptibility loci for PCa were identified, of which seven were only found or substantially more common in men of African ancestry, including an African-specific stop-gain variant in the prostate-specific gene anoctamin 7 (*ANO7*). A multi-ancestry PRS of 278 risk variants conferred strong associations with PCa risk in African ancestry studies (ORs>3 and >5 for men in the top PRS decile and percentile, respectively). More importantly, compared to men in the 40-60% PRS category, men in the top PRS decile had a significantly higher risk of aggressive PCa (OR=1.23, 95% CI=1.10-1.38, $P=4.4\times10^{-4}$).

Conclusions: This study demonstrates the importance of large-scale genetic studies in men of African ancestry for a better understanding of PCa susceptibility in this high-risk population and suggests a potential clinical utility for PRS in differentiating risk of developing aggressive versus non-aggressive disease in men of African ancestry.

Patient Summary: In this large genetic study in men of African ancestry, we discovered nine novel PCa risk variants. We also showed that a PRS was effective in stratifying PCa risk and was able to differentiate the aggressive and non-aggressive disease.

INTRODUCTION

Genetic susceptibility plays a major role in prostate cancer (PCa) risk[1–5], with many established risk variants found at a higher frequency in African ancestry men [1,6–11]. While genome-wide association studies (GWAS) of PCa have been focused predominately on men of European ancestry[1–5], smaller GWAS of African ancestry are successful in identifying African ancestry-specific risk variants that are not found in other populations [6,7,9,11,12], underscoring the importance of including greater diversity in genetic studies. Trans-ancestry and ancestry-specific GWAS have also revealed variants that substantially improve risk prediction in non-European ancestry populations and highlighted both shared and ancestry-specific allelic architecture of PCa across populations[1].

To discover PCa risk variants that are important for men of African ancestry, we conduct the largest genetic analysis to date combining GWAS results from ten consortia and biobanks. We also evaluated the performance of a multi-ancestry polygenic risk score (PRS) composed of known and novel risk variants in association with PCa risk and disease aggressiveness.

METHODS

The GWAS meta-analysis included 19,378 PCa cases and 61,620 controls of African ancestry from AAPC Consortium[10], ELLIPSE/PRACTICAL Onco-Array Consortium (ELLIPSE)[6], Ghana Prostate Study (Ghana)[13], ProHealth Kaiser GWAS (Kaiser)[14], Electronic Medical Records and Genomics (eMERGE) Network[15], BioVU Biobank[16], BioMe Biobank[17], California and Uganda Prostate Cancer Study (CA UG)[18], VA Million Veteran Program (MVP)[18], and Maryland Prostate Cancer Case-Control Study (NCI-MD)[19]. Of all studies contributed samples and/or summary statistics, 9,011 cases and 50,634 controls from CA UG, eMERGE, BioVU, BioMe, NCI-MD, and MVP were not part of any previous PCa

GWAS (**Figure S1**). An overview of each study is provided in **Table S1** and information on genotyping and imputation is described in **Table S2** and **Supplementary Materials**.

Per-allele odds ratios (ORs) and standard errors were combined in a fixed-effects inverse-variance-weighted meta-analysis. For genome-wide significant variants ($P<5.0\times10^{-8}$), Joint Analysis of Marginal summary statistics (JAM) was used to obtain conditional effects and P values, conditioning on all known risk variants in the same region[1]. Associations with a conditional $P<5.0\times10^{-8}$ were considered novel. Credible set variants were identified using JAM from all variants within ± 800 kb of each index variant. The nine novel variants and their 95% credible sets were annotated for putative evidence of biological functionality using publicly available datasets according to the framework described previously[1].

A PRS was constructed by summing variant-specific weighted allelic dosages from 269 known and nine novel risk variants using the multi-ancestry weights from a previous trans-ancestry GWAS[1]. We also constructed a PRS using the African ancestry-specific effects estimated from African ancestry men(10,367 cases and 10,986 controls)[1]. The PRS association with PCa risk was assessed in six studies included in the GWAS ("Discovery Sample") and evaluated for replication in an independent sample from Men of African Descent and Carcinoma of the Prostate (MADCaP) Network ("Replication Sample"; **Table S3**)[20,21].

In all studies, PCa was considered aggressive if one or more of the following criteria was met: tumor stage T3/T4, regional lymph node involvement, metastatic disease (M1), Gleason score ≥ 8.0, prostate-specific antigen (PSA) level ≥ 20 ng/mL or PCa as the underlying cause of death. Non-aggressive PCa was defined as men with no aggressive features meeting one or more of the following criteria: Gleason score ≤ 7.0, PSA < 20 ng/mL, and stage ≤ T2 (**Table S3**).

We further tested the PRS for association with PCa risk stratified by age (age ≤ 55 years vs. age > 55 years) and geographic area (African countries vs non-African countries), and with disease aggressiveness. P for heterogeneity was determined using a Q statistic[22]. More details on statistical analysis are provided in **Supplementary Materials**.

RESULTS

Novel Susceptibility Loci

A total of 27,753,840 genotyped and imputed single-nucleotide variants (SNVs) and small insertion/deletion variants with minor allele frequency (MAF) ≥ 1% in African populations were tested for association with PCa risk. The inflation factor (λ) was estimated to be 1.12 (**Figure S2**), which is equivalent to 1.005 for a study with 1,000 cases and 1,000 controls ($\lambda_{1,000}$)[23].

In the meta-analysis, 3,510 variants were genome-wide significant ($P<5\times10^{-8}$; **Figure 1** and **Figure S2**). These variants are located in 37 known risk regions and two novel risk regions >1.4 Mb from known risk regions on chromosomes 3q13.31 (rs72960383/*ZBTB20*) and 4q21.1 (rs144842076/-). Within known risk regions, 7 novel associations were detected on 2p21 (rs73923570/*THADA*), 2q37.3 (rs60985508/*ANO7*), 5p15.33 (rs13172201/*TERT*), 14q23.2 (rs114053368/*SYNE2*), 17p13.1 (rs9895704/*CHD3*), 17q11.2 (rs73991216/-) and 20q13.33 (rs150947563/*ZBTB46*; **Table 1, Figure S3, Figure S4**). The associations with these variants remained genome-wide significant in analysis conditioning on the known risk variants in the same region (**Table S4**).

The minor alleles for five of the nine novel risk variants (MAFs, 12%-40%) were positively associated with PCa risk with per-allele ORs ranging from 1.09 to 1.12 (**Table 1**). Four of these variants were substantially more common in African ancestry populations than in

other populations, with three being rare in European and Asian populations (≤2%; rs73923570, rs60985508, and rs72960383). The major alleles for the other four risk variants (RAFs, 89%-98%) were positively associated with PCa risk, of which three variants (rs9895704, rs73991216, and rs150947563) were only polymorphic in African ancestry populations (**Table 1**). For all novel risk variants except rs144842076, MAFs were greater in men with higher proportions of African ancestry (AFR%; **Table S5**). Only rs144842076 was not associated with African ancestry.

Based on a familial risk estimate for PCa ranging from 2.0 to 3.0, the 278 PCa variants (269 previously known plus nine novel) are estimated to capture 37% to 59% of the total familial relative risk (FRR). The nine novel risk variants explain 0.83% to 1.3% of the FRR, accounting for ~ 2.3% of the FRR explained by the 278 variants (**Table S6**).

For each novel risk variant, a 95% credible set defined potentially causal variants (**Table S7, Figure S3**). At 2q37.3, the lead variant rs60985508) introduces a stop-gain in exon 24 of the long isoform of *ANO7*(NP_001357623.1:pSer860>*). The association at 14q23 is represented by rs114053368 and comprises a credible set of 20 variants adjacent to the *ESR2* and *SYNE2* genes. This credible set contains three potential enhancer variants (rs17101673, rs8022302, and rs8007874) that intersect varying combinations of AR, CTCF, ERG, FOXA1, GABPA, GATA2, or NKX3.1 transcription factor binding peaks identified through chromatin immunoprecipitation sequencing (ChIP-seq) in PCa cell lines, in addition to chromatin marks indicative of regulatory element[1]. Similarly, the lead variant rs9896704 at 17p13/*CHD3* and rs59249234 in the credible set may affect the transcription factor binding of AR, CTCF, FOXA1, GATA2, or NKX3.1. The remaining six lead variants included four intronic variants within the genes *THADA, ZBTB20*, *TERT,* and *ZBTB46* and two intergenic variants at 4q21.1 and 17q11.2.

PRS Association with PCa Risk

Of the 269 known PCa risk variants, 246 were polymorphic in African ancestry populations (MAF ≥ 1%), 236 had a directionally consistent association with PCa risk as previously reported, of which 163 were nominally significant($P<0.05$) and 35 were genome-wide significant (**Table S8**). The multi-ancestry PRS of 278 variants conferred a 3.19-fold (95% CI=3.00–3.40) risk of PCa for men in the top 10% (90%-100% category) and 5.75-fold (95% CI=5.06-6.53) for men in the top 1% (99%-100% category), compared to men with average genetic risk (40%-60% category; **Table 2, Figure S5**). PRS associations were replicated in an independent sample of African ancestry from the MADCaP Network, with an OR of 3.52 (95% CI=2.12–5.84) for men in the top 10% and 7.55 (95% CI=2.42–23.6) for men in the top 1% of the PRS (**Table 2, Figure S5**). The OR per one standard deviation (SD) increase in PRS was 1.91 (95% CI=1.87-1.95) in the discovery studies and 1.68 (95% CI=1.45-1.94) in the replication study (**Figure S6**). Comparing to the PRS of 269 known risk variants (per SD OR=1.87, 95% CI=1.83-1.91), the inclusion of the nine novel risk variants did not lead to statistically significant improvement in the PRS associations (P-heterogeneity = 0.17) [18]. PRS associations with PCa risk in studies from African countries (average AFR% 92-97%) were similar to those from non-African countries (average AFR% 76-79%; **Table S9, Figure S6**). Similar results were also observed for a PRS based on African ancestry-specific weights (**Table S9, Table S10**). All subsequent PRS analyses were performed using the multi-ancestry PRS. In the MVP study, adding the PRS to a base model of age and principal components of ancestry led to an increase of 0.148 in the area under the curve (AUC; **Table S11**).

The PRS association with PCa risk was stronger in younger men. Compared to men in the 40%-60% PRS category, for men in the top PRS decile, the OR was 4.13 (95% CI=3.53-4.84) in

men aged ≤55 years and 2.96 (95% CI=2.76-3.17) in men >55 years (P-heterogeneity=1.4×10^{-4}; **Table S12**). The difference in ORs between younger and older men was even greater for those in the top PRS percentile (OR of 8.95 vs. 4.76, P-heterogeneity=1.2×10^{-4}). The OR per one SD increase in PRS was also greater in men aged ≤55 years (OR=2.19, 95% CI=2.08-2.30) than in men >55 years (OR=1.84, 95% CI=1.80-1.88, P-heterogeneity=1.1×10^{-9}; **Figure S6**).

The PRS showed a stronger association with aggressive disease (OR=3.95, 95% CI=3.55-4.39) than non-aggressive disease (OR=3.08, 95% CI=2.87-3.31) for men in the top PRS decile compared to men in the 40%-60% PRS category (P-heterogeneity=1.5×10^{-4}; **Figure 2**, **Table S13**). This greater association with aggressive than non-aggressive disease was similar across individual studies from African and non-African countries (**Figure S7**, **Table S14**). Consistent with the case-control analysis, in the case-case analysis being in the top PRS decile was associated with a 1.23-fold (95% CI=1.10-1.38, P=4.4×10^{-4}) risk of aggressive PCa compared to the 40% - 60% PRS category. The ORs per one SD increase in PRS in both case-control and case-case analyses supported these positive associations with aggressive prostate cancer (**Figure S6, Table S15**). In the subgroup analyses by tumor stage, Gleason score, metastasis, and PCa death (see **Supplementary Materials**), the multi-ancestry PRS was also positively associated with high-grade (Gleason score ≥ 8), advanced (stage of T3 or T4), metastatic or fatal disease (**Figure 2**, **Table S15**).

Of the 255 PCa risk variants that are polymorphic (MAF≥1%) in African populations, 17 variants were nominally associated (P<0.05) with risk of aggressive versus non-aggressive disease (**Table S16**). The PCa risk allele of 14 variants was associated with a higher risk of aggressive disease while the novel variant rs73991216 and two known variants (rs2659051 and rs76765083) at the *KLK3*/PSA locus were inversely associated with disease aggressiveness

(**Table 3**). Of the 14 variants positively associated with aggressive PCa, the removal of rs72725854 at 8q24 from the PRS led to the largest decrease in the PRS association with aggressive (21.6% decrease in OR, P-heterogeneity=1.6×10^{-3}) and non-aggressive disease (16.2% decrease in OR, P-heterogeneity=6.1×10^{-4}), and a null association with aggressive disease in the case-case analysis (P=0.09; **Table S17)**. Removing each of the other variants had less impact on the PRS association with aggressive and non-aggressive disease, and the positive association with aggressive disease remained nominally significant in the case-case analysis (P<0.03; **Table S17**).

DISCUSSION

In the largest genetic study of PCa in African ancestry men, we identified nine novel risk variants, seven of which were at substantially higher frequencies and/or only polymorphic in populations of African ancestry. A PRS comprised of the known and novel risk variants was effective in stratifying PCa risk, with replication of the PRS association demonstrated in an independent sample. For men in the top PRS decile, we observed a significantly greater risk of aggressive PCa than non-aggressive disease.

This study highlights the importance of including African ancestry samples in genetic analysis to reveal susceptibility loci that cannot be discovered without sampling a more ancestrally diverse and heterogeneous populations. A notable example is rs60985508 at the anoctamin 7 (*ANO7*) risk region on 2q37.3, which creates a premature termination codon (S860X) within the penultimate exon of the *ANO7* long isoform. *ANO7* is a prostate-specific gene shown to be an independent predictor of PCa prognosis, lymph node metastasis, and early biochemical recurrence[24,25]. Previous studies in European populations have identified three *ANO7* variants (rs77559646/R158H, rs77482050/E226*, and rs76832527/A759T), of which two

are rare in African ancestry populations (MAF < 1%)[1,2]. Together with I448S in *CHEK2*[6] and X285K in *HOXB13*[12], S860X in *ANO7* represents another example of risk-associated protein-altering variation that is unique to African ancestry men.

Six other novel risk variants were discovered in known susceptibility regions. Chromosome 5p15.33/*TERT* (telomerase reverse transcriptase) is a well-established cancer susceptibility locus where several PCa risk variants have been identified (rs2242652, rs71595003, rs2736098, rs7725218, and rs10069690). The novel intronic variant rs13172201 represents the strongest independent association with PCa risk in this region for African ancestry men. At 2p21, the African ancestry-specific variant rs73923570 is in intron 30 of *THADA* (thyroid adenoma-associated) and in proximity (86-487 kb) to three independent PCa risk signals in the region (rs6738169, rs7591218, and rs28514770). Germline *THADA* variants have been associated with several traits that were linked with PCa risk, such as waist-hip ratio[26], testosterone levels[27], and type 2 diabetes[28,29], with several variants in moderate to high correlation with known PCa risk variants.

Novel risk variant rs114053368 at 14q23.2 is in intron 79 of *SYNE2* (spectrin repeat containing nuclear envelope protein 2), and ~90 kb from a known East Asian PCa risk variant rs58262369 in the 3'UTR of the *ESR2* (estrogen receptor 2) gene[30]. We also identified a novel intronic variant rs150947563 in *ZBTB46* and *ZBTB46-AS1* at 20q13.33, ~67 kb from a known PCa risk variant (rs1058319). In several studies, overexpression of *ZBTB46* induced by androgen deprivation promoted castration-resistant PCa and neuroendocrine differentiation of PCa[31–33]; however, whether these variants alter the expression or function of *ZBTB46* has not been investigated. The novel variant rs9895704 at 17p13.1 is in intron 11 of the *CHD3* (chromodomain helicase DNA binding protein 3) gene, ~2 kb from a known risk variant

(rs28441558). *CHD3* encodes an ATPase subunit of the nucleosome remodeling deacetylase complex that represses the activity of early growth response 1 (EGR1)[34,35], a transcription factor shown to promote PCa metastasis[36,37]. At 17q11.2, the novel lead variant rs73991216 is intergenic, ~29 kb downstream of the gene *RAB11FIP4* and ~200 kb from known risk variant rs4795646. However, the mechanisms and genes involved are unclear and warrant further investigation.

Two novel PCa risk variants define new susceptibility regions for PCa. The lead variant rs72960383 at 3q13.31 is in intron 1 of the transcription factor gene *ZBTB20* (zinc finger and BTB domain containing 20). *ZBTB20* was included in a nine-gene expression profile identified in prostate tumors that acquired treatment resistance, which was found to be associated with time to biochemical relapse and PCa metastasis[38]. *ZBTB20* was also a *PTEN*-cooperating tumor suppressor gene, co-downregulated with *PTEN* in both primary and metastatic prostate tumor samples, with lower expression associated with a shorter time to recurrence[39,40]. The lead variant rs144842076 at 4q21.1 is an intergenic variant between the *SHROOM3* (~88 kb) and *SEPT11* (~78 kb) genes in a region not previously implicated in PCa.

We constructed the PRS using external weights from a previous trans-ancestry GWAS to mitigate the potential inflation in PRS associations due to the overlapped samples in PRS development and testing. While adding the nine novel risk variants to the previous 269-variant PRS did not lead to a marked improvement in PRS performance[1], the replication of PRS associations in an independent sample of African ancestry men, and the similar risk associations observed in studies from African and non-African countries, demonstrated the robustness of the multi-ancestry PRS in risk stratification across African populations with varying degrees of admixture. Consistent with previous findings in European and African populations[1,18], the

association of the top PRS decile was greater for younger compared with older men, which highlights the contribution of genetics in earlier- versus late-onset disease.

Despite greater statistical power in studies of European ancestry (21,919 aggressive and 39,426 non-aggressive cases), the 269-variant PRS was equally associated with aggressive and non-aggressive PCa[1]. Here we provide the first evidence that a PRS can differentiate risk of aggressive and non-aggressive PCa for African ancestry men in the top PRS decile. A significantly higher risk of high-grade, advanced, metastatic, or fatal disease was also observed for men in the top PRS decile. This association was not driven by the greater effect in younger versus older men since age at diagnosis was similar in aggressive and non-aggressive cases across studies. The African-specific variant rs72725854 at 8q24, which accounts for the largest fraction of PCa risk of all variants known to date, made the greatest contribution to the PRS-aggressive disease association. Men of European ancestry do not harbor this risk variant, which could explain the difficulty in associating the PRS with disease aggressiveness in European populations.

This study underscores the importance of large-scale genetic analysis in African ancestry men for a better understanding of PCa susceptibility in this high-risk population. In addition to the discovery of nine novel risk variants, PRS was validated as an effective tool for PCa risk stratification in African ancestry men. Importantly, we found that PRS could distinguish an African ancestry men's risk of developing aggressive versus non-aggressive disease. As the first evidence of this association, future studies are warranted to further validate and characterize this relationship. Risk-stratified screening studies in African ancestry populations are needed to determine the benefits of an earlier and more frequent PSA screening strategy for those at high genetic risk.

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DATA AVAILABILITY

The summary statistics, genotype data and/or relevant covariate information used in this study are deposited in dbGaP (https://www.ncbi.nlm.nih.gov/gap/) under accession codes phs001120.v2.p2, phs001391.v1.p1, phs001120.v2.p2, and phs000838.v1.p1. The MVP individual level data is

available to approved VA researchers through standard mechanisms. Full MVP GWAS summary statistics can be found in dbGaP under the MVP accession (phs001672).

All analyses were performed using R statistical packages freely available at https://cran.r-project.org/mirrors.html. The R code for the PRS association analysis was modified from the code available at https://github.com/USCmec/Polfus_Darst_HGGA_2021/.

FIGURE LEGENDS

Figure 1 Genome-wide associations with prostate cancer risk. The association for each variant was estimated in each study/consortium and meta-analyzed across studies using a fixed-effect inverse-variance-weighted method. The nine novel association signals were highlighted in orange. The known risk associations were not shown in this plot. The dash line represents the genome-wide significance at $P < 5\times 10^{-8}$.

Figure 2 Association of the multi-ancestry PRS with aggressive and non-aggressive forms of prostate cancer. Association was assessed comparing prostate cancer cases by Gleason score, tumor stage, metastatic or fatal prostate cancer to controls. Results were obtained from each individual study and then meta-analyzed across studies. The x-axis indicates the PRS category. The y-axis indicates the ORs with error bars representing the 95% CIs for each PRS category compared to the 40%-60% PRS category. The dotted horizontal line corresponds to an OR of 1. ORs and 95% CIs for each PRS decile and/or strata are provided in **Table S13** and **Table S15**.

TABLES

Table 1. Nine novel risk regions/variants associated with prostate cancer in men of African ancestry

rsID[a]	Chromosomal Position	Alleles[b]	Nearest Gene (consequence)	RAF[c]	RAF in 1KG (AFR, EUR, EAS)[d]	OR	95% CIs	P value[e]
rs73923570[f]	2:43551893 (2p21)	G/A	*THADA* (intron)	0.12	0.13, 0, 0	1.12	1.08-1.17	1.46×10^{-8}
rs60985508[f]	2:242163365 (2q37.3)	T/TCA	*ANO7* (stop-gained)	0.31	0.34, < 0.01, 0	1.11	1.08-1.15	1.48×10^{-13}
rs72960383	3:114732510 (3q13.31)	A/T	*ZBTB20* (intron)	0.33	0.40, 0.02, < 0.01	1.09	1.06-1.12	5.46×10^{-9}
rs144842076	4:77792911 (4q21.1)	C/T	-- (intergenic)	0.97	0.98, 0.95, 1.00	1.25	1.16-1.35	1.12×10^{-8}
rs13172201[f]	5:1271661 (5p15.33)	C/T	*TERT* (intron)	0.40	0.45, 0.24, 0.86	1.10	1.07-1.13	2.36×10^{-11}
rs114053368[f]	14:64606132 (14q23.2)	T/A	*SYNE2* (intron)	0.20	0.24, 0.06, < 0.01	1.12	1.08-1.16	7.07×10^{-12}
rs9895704[f]	17:7801082 (17p13.1)	T/C	*CHD3* (intron)	0.89	0.88, 1.00, 1.00	1.13	1.08-1.18	9.80×10^{-9}
rs73991216[f]	17:29893888 (17q11.2)	G/A	- (intergenic)	0.89	0.86, 1.00, 1.00	1.19	1.14-1.24	5.34×10^{-14}
rs150947563[f]	20:62441171 (20q13.33)	C/T	*ZBTB46* (intron)	0.98	0.98, 1.00, 1.00	1.47	1.31-1.66	3.24×10^{-10}

[a] Only the most significant variant defining each association signal was reported.

[b] Prostate cancer risk allele/other allele

[c] Weighted mean of risk allele frequency (RAF) estimated in controls across individual African ancestry studies in the meta-analysis.

[d] Risk allele frequency in 1000 Genomes Project (1KG) African (AFR), European (EUR) and East Asian (EAS) populations.

[e] P value from the fixed-effect inverse-variance-weighted meta-analysis.

[f] Variant within ± 800 kb of a known risk variant reported in Conti, Darst et al., *Nature Genetics*, 2021.

Table 2 Association of PRS with prostate cancer risk in men of African ancestry.

	Discovery Samples[a] 18,018 cases, 64,034 controls				Replication Samples[b] 405 cases, 396 controls			
PRS Category[c]	**Controls**	**Cases**	**OR (95% CI)**	**P**	**Controls**	**Cases**	**OR (95% CI)**	**P**
[0%-10%]	6407	493	0.33 (0.29-0.37)	7.49×10^{-93}	40	15	0.53 (0.26-1.06)	0.07
(10%-20%]	6402	780	0.51 (0.47-0.56)	4.83×10^{-45}	40	22	0.71 (0.37-1.33)	0.3
(20%-30%]	6403	916	0.62 (0.56-0.67)	3.26×10^{-27}	39	18	0.57 (0.30-1.12)	0.10
(30%-40%]	6402	1024	0.68 (0.63-0.74)	1.53×10^{-18}	40	38	1.19 (0.67-2.10)	0.6
(40%-60%]	12806	2960	1.00 (Reference)		79	62	1.00 (Reference)	
(60%-70%]	6402	1901	1.28 (1.19-1.38)	3.12×10^{-11}	39	40	1.36 (0.77-2.40)	0.3
(70%-80%]	6403	2271	1.52 (1.41-1.63)	9.24×10^{-31}	40	46	1.53 (0.88-2.66)	0.14
(80%-90%]	6402	2867	1.94 (1.81-2.07)	3.84×10^{-81}	39	63	2.13 (1.25-3.64)	5.52×10^{-3}
(90%-100%]	6407	4806	3.19 (3.00-3.40)	1.22×10^{-281}	40	101	3.52 (2.12-5.84)	1.12×10^{-6}
(99%-100%][d]	643	870	5.75 (5.06-6.53)	4.30×10^{-160}	4	21	7.55 (2.42-23.6)	5.02×10^{-4}

[a] Discovery samples included men of African ancestry from the AAPC Consortium, the ELLPSE OncoArray Consortium, the California and Uganda Prostate Cancer Study, the Ghana Prostate Study, the NCI-Maryland Prostate Cancer Case-Control Study, and the Million Veteran Program. Odds ratios (ORs) and 95% confidence intervals (CIs) were estimated in logistic regression analysis adjusting for age, sub-study (if applicable) and up to ten principal components in each study/consortium, and meta-analyzed across the studies using a fixed-effects inverse-variance-weighted method.

[b] Replication samples were from the Men of African Descent and Carcinoma of the Prostate (MADCaP) Network, which was not part of any previous prostate cancer GWAS.

[c] PRS was constructed from the 269 known prostate cancer risk variants and the 9 novel variants, weighted by the multi-ancestry effects from the previous trans-ancestry prostate cancer GWAS. PRS percentile categories were based on observed distribution in controls.

[d] A separate analysis was performed to evaluate the PRS association with prostate cancer risk in men with extremely high genetic risk (99% - 100%).

Table 3 The prostate cancer risk variants associated with disease aggressiveness in case-case analysis (P < 0.05)

rsID (Effect /Other Allele[a])	Nearest Gene	EAF[b] (AFR, EUR)	Aggressive vs. Non-aggressive[c]	Gleason ≥ 8 vs. Gleason = 6	Stage T3/T4 vs. Stage T1/T2	Metastatic vs. Non-aggressive	Fatal vs. Non-aggressive
			OR (95% CI), P value[d]				
rs708723 (C/T)	*RAB29*	0.83, 0.47	1.09 (1.02-1.17)*	1.09 (1.00-1.18) *	1.10 (0.98-1.23)	1.11 (0.93-1.32)	1.05 (0.85-1.29)
rs11691517 (T/G)	*BCL2L11*	0.79, 0.75	1.08 (1.00-1.16) *	0.97 (0.89-1.06)	1.04 (0.92-1.17)	0.99 (0.83-1.19)	1.04 (0.84-1.30)
rs2293607 (T/C)	*TERC*	0.96, 0.76	1.16 (1.02-1.32) *	1.02 (0.88-1.19)	1.16 (0.93-1.45)	1.15 (0.83-1.60)	1.00 (0.70-1.41)
rs13142786 (T/A)	*RASSF6*	0.59, 0.50	1.08 (1.01-1.14) *	1.07 (1.00-1.15) *	1.07 (0.97-1.18)	1.05 (0.90-1.21)	1.15 (0.96-1.38)
rs339351 (C/A)	*RFX6*	0.74, 0.69	1.14 (1.07-1.23) **	1.16 (1.07-1.25) **	1.13 (1.01-1.27) *	1.13 (0.95-1.34)	1.03 (0.84-1.27)
rs4513875 (T/C)	*MAD1L1*	0.08, 0.40	1.10 (1.00-1.20) *	0.99 (0.89-1.11)	1.07 (0.92-1.24)	0.99 (0.77-1.26)	1.02 (0.79-1.33)
rs834608 (A/T)	*TNS3*	0.62, 0.60	1.07 (1.00-1.13) *	1.05 (0.98-1.13)	1.07 (0.97-1.18)	1.01 (0.87-1.16)	1.04 (0.87-1.25)
rs72725854 (T/A)	-- (8q24)	0.08, 0.00	1.14 (1.05-1.25) *	1.25 (1.13-1.39) **	1.09 (0.95-1.26)	1.31 (1.06-1.62) *	1.35 (1.04-1.75) *
rs72725879 (T/C)	-- (8q24)	0.37, 0.20	1.07 (1.00-1.13) *	1.09 (1.02-1.17) *	1.06 (0.96-1.16)	1.24 (1.07-1.43) *	1.01 (0.85-1.21)
rs68010938 (T/TA)	*SLC39A13*	0.01, 0.29	1.16 (1.02-1.33) *	1.17 (1.00-1.36) *	1.04 (0.83-1.31)	1.28 (0.92-1.80)	1.20 (0.83-1.72)
rs12785905 (C/G)	*KDM2A*	0.001, 0.05	1.54 (1.14-2.08) *	1.46 (1.03-2.05) *	1.84 (1.10-3.06) *	0.94 (0.38-2.31)	2.88 (1.44-5.76) *
rs11228580 (C/T)	*MYEOV*	0.18, 0.18	1.12 (1.04-1.20) *	1.11 (1.02-1.21) *	1.14 (1.02-1.29) *	1.37 (1.16-1.63) **	1.16 (0.94-1.43)
rs75823044 (T/C)	*IRS2*	0.04, 0.00	1.23 (1.05-1.45) *	1.28 (1.05-1.57) *	1.60 (1.27-2.02) **	1.64 (1.09-2.46) *	1.52 (0.97-2.37)
rs17565772 (G/A)	*COX16*	0.16, 0.47	1.08 (1.01-1.16) *	1.02 (0.94-1.11)	1.09 (0.98-1.23)	1.07 (0.90-1.27)	1.26 (1.03-1.54) *
rs73991216 (G/A)	-- (17q11.2)	0.86,1.00	0.89 (0.80-0.98) *	0.90 (0.80-1.01)	0.90 (0.76-1.05)	0.78 (0.62-0.99) *	1.00 (0.73-1.37)
rs2659051 (G/C)	*KLK15/KLK3*	0.85, 0.79	0.89 (0.82-0.97) *	0.86 (0.78-0.94) *	0.90 (0.79-1.03)	0.97 (0.79-1.18)	0.89 (0.70-1.13)
rs76765083 (T/G)	*KLK3*	1.00, 0.93	0.69 (0.53-0.90) *	0.57 (0.41-0.78) **	0.75 (0.46-1.22)	0.42 (0.22-0.81) *	0.90 (0.43-1.85)

[a] Effect allele was set to be the prostate cancer risk-increasing allele.

[b] Effect allele frequency (EAF) in 1000 Genomes Project (1KG) African (AFR) and European (EUR) populations.

[c] Cases were considered as aggressive if one of the following criteria was met: tumor stage T3/T4, reginal lymph node involvement, metastatic disease, Gleason score ≥ 8, PSA ≥ 20 ng/mL or prostate cancer as the underlying cause of death. Cases without any aggressive features and met one or more of the following criteria were considered nonaggressive: Gleason score ≤ 7, PSA < 20 ng/mL and stage ≤ T2.

[d] ORs and 95% CIs were estimated in logistic regression analysis adjusting for age, sub-study (if applicable) and up to ten principal components in each study/consortium, and meta-analyzed across the studies using a fixed-effects inverse-variance-weighted method.

* P value < 0.05; ** P value < 0.001

FIGURES

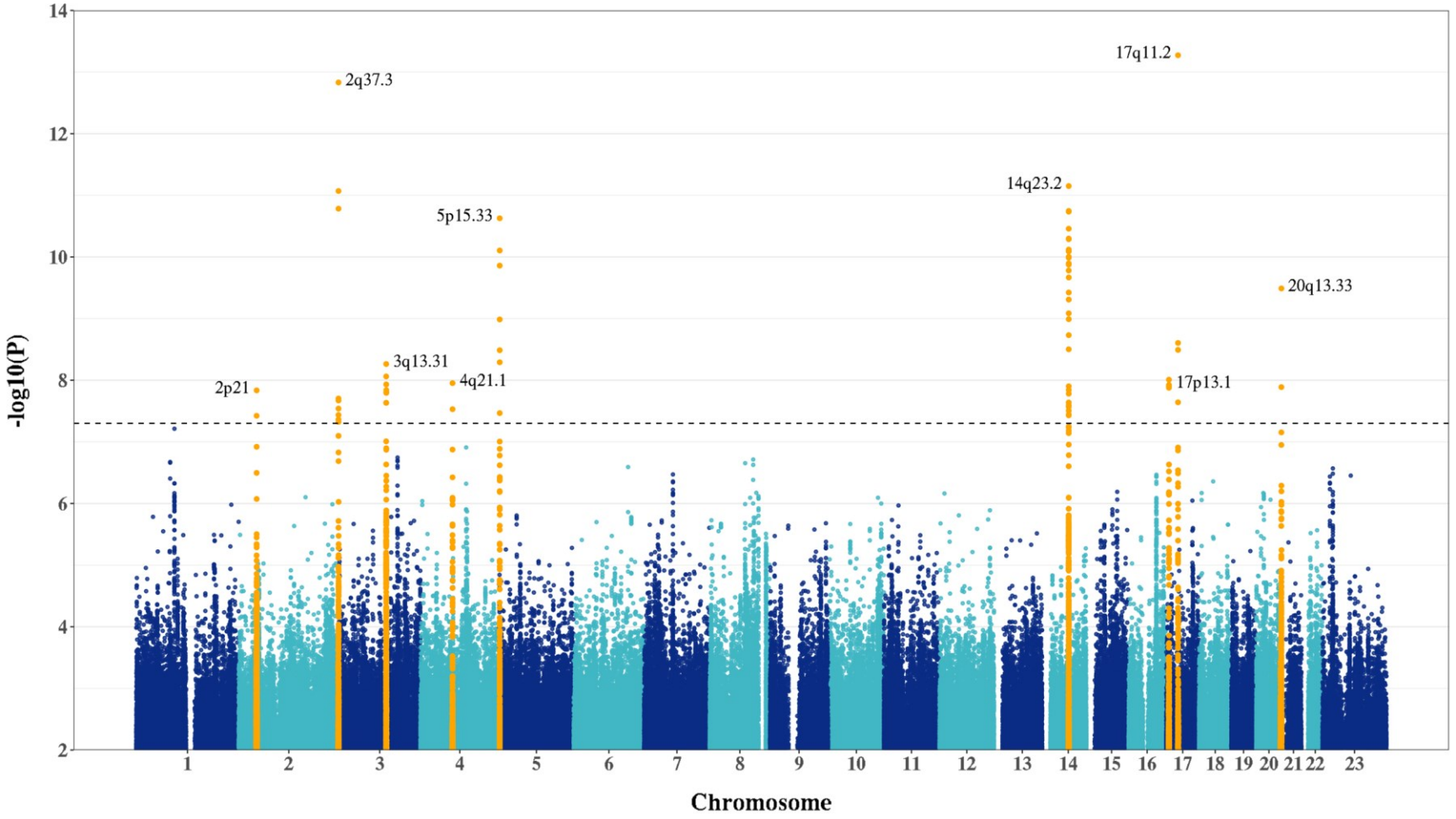

Figure 2 Genome-wide associations with prostate cancer risk.

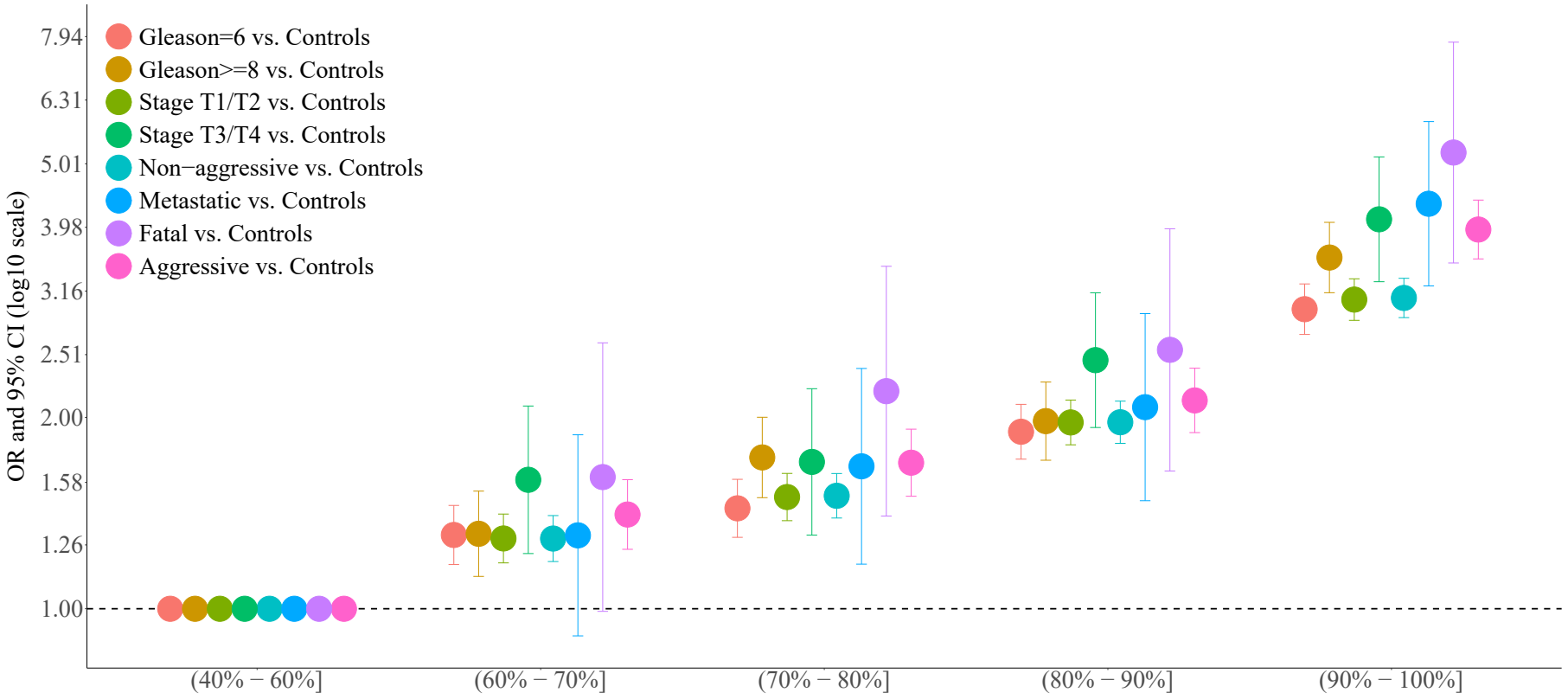

Figure 2 Association of the multi-ancestry PRS with aggressive and non-aggressive forms of prostate cancer.

TAKE HOME MESSAGE

Nine novel susceptibility loci for prostate cancer were identified in men of African ancestry. A multi-ancestry PRS was validated as an effective tool for PCa risk stratification and shown to differentiate the aggressive and non-aggressive prostate cancer in men of African ancestry.