

Circulating Metabolic Biomarkers of Screen-Detected Prostate Cancer in the ProtecT Study



Charleen D. Adams^{1,2}, Rebecca Richmond^{1,2}, Diana L. Santos Ferreira^{1,2}, Wes Spiller^{1,2}, Vanessa Tan^{1,2}, Jie Zheng^{1,2}, Peter Würtz⁴, Jenny Donovan², Freddie Hamdy⁵, David Neal⁵, J. Athene Lane^{2,3}, George Davey Smith^{1,2,3}, Caroline Relton^{1,2,3}, Rosalind A. Eeles^{6,7}, Christopher A. Haiman⁸, ZSofia Kote-Jarai⁶, Fredrick R. Schumacher^{9,10}, Ali Amin Al Olama^{11,12}, Sara Benlloch^{6,11}, Kenneth Muir^{13,14}, Sonja I. Berndt¹⁵, David V. Conti⁸, Fredrik Wiklund¹⁶, Stephen J. Chanock¹⁵, Susan Gapstur¹⁷, Victoria L. Stevens¹⁷, Catherine M. Tangen¹⁸, Jyotsna Batra^{19,20}, Judith A. Clements^{19,20}, Henrik Gronberg¹⁶, Nora Pashayan^{21,22}, Johanna Schleutker^{23,24}, Demetrius Albanes¹⁵, Alicja Wolk²⁵, Catharine M.L. West²⁶, Lorelei A. Mucci²⁷, Géraldine Cancel-Tassin^{28,29}, Stella Koutros¹⁵, Karina Dalsgaard Sorensen^{30,31}, Lovise Maehle³², Ruth C. Travis³³, Robert J. Hamilton³⁴, Sue Ann Ingles⁸, Barry S. Rosenstein^{35,36}, Yong-Jie Lu³⁷, Graham G. Giles^{38,39}, Adam S. Kibel⁴⁰, Ana Vega⁴¹, Manolis Kogevinas^{42,43,44,45}, Kathryn L. Penney⁴⁶, Jong Y. Park⁴⁷, Janet L. Stanford^{48,49}, Cezary Cybulski⁵⁰, Børge G. Nordestgaard^{51,52}, Hermann Brenner^{53,54,55}, Christiane Maier⁵⁶, Jeri Kim⁵⁷, Esther M. John^{58,59}, Manuel R. Teixeira^{60,61}, Susan L. Neuhausen⁶², Kim De Ruyck⁶³, Azad Razack⁶⁴, Lisa F. Newcomb^{48,65}, Davor Lessel⁶⁶, Radka P. Kaneva⁶⁷, Nawaid Usmani^{68,69}, Frank Claessens⁷⁰, Paul A. Townsend⁷¹, Manuela Gago Dominguez^{72,73}, Monique J. Roobol⁷⁴, Florence Menegaux⁷⁵, Kay-Tee Khaw⁷⁶, Lisa A. Cannon-Albright^{77,78}, Hardev Pandha⁷⁹, and Stephen N. Thibodeau⁸⁰, on behalf of the PRACTICAL consortium; and Richard M. Martin^{1,2,31}

¹MRC Integrative Epidemiology Unit at the University of Bristol, Bristol, United Kingdom. ²University of Bristol, Bristol, United Kingdom. ³Bristol National Institute of Health Research Biomedical Research Centre, Bristol, United Kingdom. ⁴Research Programs Unit, Diabetes and Obesity, University of Helsinki and Nightingale Health Ltd., Helsinki, Finland. ⁵Nuffield Department of Surgical Sciences, University of Oxford and Faculty of Medical Science, John Radcliffe Hospital, University of Oxford, Oxford, United Kingdom. ⁶The Institute of Cancer Research, London, United Kingdom. ⁷Royal Marsden NHS Foundation Trust, London, United Kingdom. ⁸Department of Preventive Medicine, Keck School of Medicine, University of Southern California/Norris Comprehensive Cancer Center, Los Angeles, California. ⁹Department of Epidemiology and Biostatistics, Case Western Reserve University, Cleveland, Ohio. ¹⁰Seidman Cancer Center, University Hospitals, Cleveland, Ohio. ¹¹Centre for Cancer Genetic Epidemiology, Department of Public Health and Primary Care, University of Cambridge, Strangeways Research Laboratory, Cambridge, United Kingdom. ¹²University of Cambridge, Department of Clinical Neurosciences, Cambridge, United Kingdom. ¹³Division of Population Health, Health Services Research and Primary Care, University of Manchester, Manchester, United Kingdom. ¹⁴Warwick Medical School, University of Warwick, Coventry, United Kingdom. ¹⁵Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, Bethesda, Maryland. ¹⁶Department of Medical Epidemiology and Biostatistics, Karolinska Institute, Stockholm, Sweden. ¹⁷Epidemiology Research Program, American Cancer Society, Atlanta, Georgia. ¹⁸SWOG Statistical Center, Fred Hutchinson Cancer Research Center, Seattle, Washington. ¹⁹Australian Prostate Cancer Research Centre-Qld, Institute of Health and Biomedical Innovation and School of Biomedical Science, Queensland University of Technology, Brisbane, Queensland, Australia. ²⁰Translational Research Institute, Brisbane, Queensland, Australia. ²¹University College London, Department of Applied Health Research, London, United Kingdom. ²²Centre for Cancer Genetic Epidemiology, Department of Oncology, University of Cambridge, Strangeways Laboratory, Cambridge, United Kingdom. ²³Department of Medical Biochemistry and Genetics, Institute of Biomedicine, University of Turku, Turku, Finland. ²⁴Tyks Microbiology and Genetics, Department of Medical Genetics, Turku University Hospital, Turku, Finland. ²⁵Division of Nutritional Epidemiology, Institute of Environmental Medicine, Karolinska Institutet, Stockholm, Sweden. ²⁶Division of Cancer Sciences, University of Manchester, Manchester Academic Health Science Centre, Radiotherapy Related Research, Manchester NIHR Biomedical Research Centre, The Christie Hospital NHS Foundation Trust, Manchester, United King-

dom. ²⁷Department of Epidemiology, Harvard T.H. Chan School of Public Health, Boston, Massachusetts. ²⁸CeRePP, Tenon Hospital, Paris, France. ²⁹UPMC Sorbonne Universités, GRC N°5 ONCOTYPE-URO, Tenon Hospital, Paris, France. ³⁰Department of Molecular Medicine, Aarhus University Hospital, Aarhus, Denmark. ³¹Department of Clinical Medicine, Aarhus University, Aarhus, Denmark. ³²Department of Medical Genetics, Oslo University Hospital, Oslo, Norway. ³³Cancer Epidemiology Unit, Nuffield Department of Population Health University of Oxford, Oxford, United Kingdom. ³⁴Department of Surgical Oncology, Princess Margaret Cancer Centre, Toronto, Canada. ³⁵Department of Radiation Oncology, Icahn School of Medicine at Mount Sinai, New York, New York. ³⁶Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, New York. ³⁷Centre for Molecular Oncology, Barts Cancer Institute, Queen Mary University of London, John Vane Science Centre, London, United Kingdom. ³⁸Cancer Epidemiology & Intelligence Division, The Cancer Council Victoria, Melbourne, Victoria, Australia. ³⁹Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Australia. ⁴⁰Division of Urologic Surgery, Brigham and Women's Hospital, Boston, Massachusetts. ⁴¹Fundación Pública Galega de Medicina Xenómica-SERGAS, Grupo de Medicina Xenómica, CIBERER, IDIS, Santiago de Compostela, Spain. ⁴²Centre for Research in Environmental Epidemiology (CREAL), Barcelona Institute for Global Health (ISGlobal), Barcelona, Spain. ⁴³CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain. ⁴⁴MIM (Hospital del Mar Research Institute), Barcelona, Spain. ⁴⁵Universitat Pompeu Fabra (UPF), Barcelona, Spain. ⁴⁶Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital/Harvard Medical School, Boston, Massachusetts. ⁴⁷Department of Cancer Epidemiology, Moffitt Cancer Center, Tampa, Florida. ⁴⁸Division of Public Health Sciences, Fred Hutchinson Cancer Research Center, Seattle, Washington. ⁴⁹Department of Epidemiology, School of Public Health, University of Washington, Seattle, Washington. ⁵⁰International Hereditary Cancer Center, Department of Genetics and Pathology, Pomeranian Medical University, Szczecin, Poland. ⁵¹Faculty of Health and Medical Sciences, University of Copenhagen, Copenhagen, Denmark. ⁵²Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Herlev, Denmark. ⁵³Division of Clinical Epidemiology and Aging Research, German Cancer Research Center (DKFZ), Heidelberg, Germany. ⁵⁴German Cancer Consortium (DKTK), German Cancer Research Center (DKFZ), Heidelberg, Germany. ⁵⁵Division of Preventive Oncology, German Cancer Research Center (DKFZ) and National Center for

Abstract

Background: Whether associations between circulating metabolites and prostate cancer are causal is unknown. We report on the largest study of metabolites and prostate cancer (2,291 cases and 2,661 controls) and appraise causality for a subset of the prostate cancer–metabolite associations using two-sample Mendelian randomization (MR).

Methods: The case-control portion of the study was conducted in nine UK centers with men ages 50–69 years who underwent prostate-specific antigen screening for prostate cancer within the Prostate Testing for Cancer and Treatment (ProtecT) trial. Two data sources were used to appraise causality: a genome-wide association study (GWAS) of metabolites in 24,925 participants and a GWAS of prostate cancer in 44,825 cases and 27,904 controls within the Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium.

Results: Thirty-five metabolites were strongly associated with prostate cancer ($P < 0.0014$, multiple-testing threshold). These fell into four classes: (i) lipids and lipoprotein subclass characteristics (total cholesterol and ratios, cholesterol esters and ratios, free cholesterol and ratios, phospholipids and ratios, and triglyceride ratios); (ii) fatty acids and ratios; (iii) amino acids; (iv) and fluid balance. Fourteen top metabolites were proxied by genetic variables, but MR indicated these were not causal.

Conclusions: We identified 35 circulating metabolites associated with prostate cancer presence, but found no evidence of causality for those 14 testable with MR. Thus, the 14 MR-tested metabolites are unlikely to be mechanistically important in prostate cancer risk.

Impact: The metabolome provides a promising set of biomarkers that may aid prostate cancer classification.

Introduction

Prostate cancer is the most frequently diagnosed malignancy among men worldwide (1). Despite huge geographical variation in incidence and mortality (suggestive of environmental causes), the only established risk factors are age, race, and family history (2), while adiposity is associated with advanced prostate cancer (3). Metabolic dysregulation is a hallmark of carcinogenesis (4), and various circulating metabolites have been associated with both prostate cancer presence and aggressiveness (2, 5–15). However, there are limitations with the existing evidence-base. First, the metabolites detected as being associated with prostate cancer have varied between studies, with no clear pattern of associations emerging. Second, most previous studies have been small, having fewer than 100 cases (Supplementary Table S1 in the Supplementary Data; ref. 5) and hence liable to the play of

chance, yielding both false-positive and false-negative findings. Third, the nature of any observed associations must be interpreted cautiously, because epidemiologic studies are highly susceptible to various biases that preclude causal inference (16, 17). For instance, metabolites may be associated with other factors that affect prostate cancer development (confounding), or the presence of prostate cancer may alter metabolites (reverse causation). Mendelian randomization (MR) is a method for appraising causality that uses genetic instrumental variables to proxy for exposures that can be otherwise confounded or subject to reverse causation. Such an approach may be used to distinguish causally relevant intervention targets from biomarkers that are noncausally associated with prostate cancer. The latter may nevertheless be of potential value in risk prediction (e.g., if the biomarker acts as a strong proxy for other factors leading to disease) or disease

Tumor Diseases (NCT), Heidelberg, Germany. ⁵⁶Institute for Human Genetics, University Hospital Ulm, Ulm, Germany. ⁵⁷Department of Genitourinary Medical Oncology, The University of Texas MD Anderson Cancer Center, Houston, Texas. ⁵⁸Cancer Prevention Institute of California, Fremont, California. ⁵⁹Department of Health Research & Policy (Epidemiology) and Stanford Cancer Institute, Stanford University School of Medicine, Stanford, California. ⁶⁰Department of Genetics, Portuguese Oncology Institute of Porto, Porto, Portugal. ⁶¹Biomedical Sciences Institute (ICBAS), University of Porto, Porto, Portugal. ⁶²Department of Population Sciences, Beckman Research Institute of the City of Hope, Duarte, California. ⁶³Ghent University, Faculty of Medicine and Health Sciences, Basic Medical Sciences, Ghent, Belgium. ⁶⁴Department of Surgery, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia. ⁶⁵Department of Urology, University of Washington, Seattle, Washington. ⁶⁶Institute of Human Genetics, University Medical Center Hamburg-Eppendorf, Hamburg, Germany. ⁶⁷Molecular Medicine Center, Department of Medical Chemistry and Biochemistry, Medical University, Sofia, Bulgaria. ⁶⁸Department of Oncology, Cross Cancer Institute, University of Alberta, Edmonton, Alberta, Canada. ⁶⁹Division of Radiation Oncology, Cross Cancer Institute, Edmonton, Alberta, Canada. ⁷⁰Molecular Endocrinology Laboratory, Department of Cellular and Molecular Medicine, KU Leuven, Leuven, Belgium. ⁷¹Institute of Cancer Sciences, Manchester Cancer Research Centre, University of Manchester, Manchester Academic Health Science Centre, St. Mary's Hospital, Manchester, United Kingdom. ⁷²Genomic Medicine Group, Galician Foundation of Genomic Medicine, Instituto de Investigación Sanitaria de Santiago de Compostela (IDIS), Complejo Hospitalario Universitario de Santiago, Servicio Galego de Saúde, SERGAS, Santiago De

Compostela, Spain. ⁷³University of California San Diego, Moores Cancer Center, La Jolla, California. ⁷⁴Department of Urology, Erasmus University Medical Center, Rotterdam, the Netherlands. ⁷⁵Cancer & Environment Group, Center for Research in Epidemiology and Population Health (CESP), INSERM, University Paris-Sud, University Paris-Saclay, Villejuif, France. ⁷⁶Clinical Gerontology Unit, University of Cambridge, Cambridge, United Kingdom. ⁷⁷Division of Genetic Epidemiology, Department of Medicine, University of Utah School of Medicine, Salt Lake City, Utah. ⁷⁸George E. Wahlen Department of Veterans Affairs Medical Center, Salt Lake City, Utah. ⁷⁹The University of Surrey, Guildford, Surrey, United Kingdom. ⁸⁰Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, Minnesota.

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

Information for the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium can be found at <http://practical.icr.ac.uk/>.

Corresponding Author: Charleen D. Adams, Department of Population Sciences, Beckman Research Institute of City of Hope, Duarte, CA 91010. Phone: 626-841-3937; E-mail: chaadams@coh.org

doi: 10.1158/1055-9965.EPI-18-0079

©2018 American Association for Cancer Research.

detection (e.g., if early disease leads to alterations in the circulating metabolome).

Materials and Methods

Observational study

We undertook a case-control study within the Prostate Testing for Cancer and Treatment (ProtecT) trial (ISRCTN20141297; refs. 18–20). During recruitment to ProtecT, 228,966 men ages 50–69 years at 337 general practices in nine UK centers (Birmingham, Bristol, Cambridge, Cardiff, Edinburgh, Leeds, Leicester, Newcastle, Sheffield) were invited between 2001 and 2009 to attend a clinic for consideration of a prostate-specific antigen (PSA) test. Overall, 100,444 men attended the clinic where a PSA test was offered to 82,429 men deemed eligible to enroll into the ProtecT treatment trial, after a fully informed, 30-minute discussion with a nurse. Men with PSA levels ≥ 3 ng/mL were offered a 10-core diagnostic biopsy. Tumors were histologically confirmed, assigned a Gleason score by urologists, and clinically staged using the TNM classification (21). Men with a PSA < 3 ng/mL or a raised PSA (≥ 3 ng/mL) but a negative biopsy without development of prostate cancer during the follow-up protocol were eligible as controls. Controls were randomly selected from the same five-year age-band (age at PSA test) and GP/family practice, as cases (22).

In this analysis, in concert with the guidelines of the National Institute for Health and Care Excellence (NICE; ref. 23), men with stage T3 or T4 tumors ("localized advanced"), Gleason score ≥ 8 , or with a PSA level at diagnosis > 20 ng/mL, were classified as "high-risk." Men with stage T1 or T2 tumors, Gleason score < 8 , or with a PSA level at diagnosis ≤ 20 ng/mL were classified as "low-risk." Participants in the present analysis consisted of those with self-reported (white) European ancestry.

Ethics

All men provided written informed consent prior to inclusion into ProtecT. The Trent Multicentre Research Ethics Committee (MREC) approved ProtecT (MREC/01/4/025) and the linked ProMPT study, which collected biological material (MREC/01/4/061), including serum used for the current study of metabolites.

Laboratory analyses

Two hundred and twenty-seven quantified metabolic traits (henceforth "metabolites") were obtained per sample of serum using a proton nuclear magnetic resonance (NMR) spectroscopy-based metabolomics platform (Nightingale Health, Helsinki, Finland). Details of the methodology have been described elsewhere (24). Briefly, 100- μ L serum was mixed with sodium phosphate buffer and transferred to NMR tubes using an eight-channel, Varispan Janus liquid handling robot (PerkinElmer). Two 1D NMR spectra were acquired using a 500 MHz Bruker Avance III HD spectrometer and analyzed bioinformatically for absolute quantification of lipoprotein subclasses, their particle concentrations and composition, lipoprotein particle size, apolipoprotein A-I and B, multiple cholesterol and triglyceride measures, albumin, various fatty acids, as well as numerous low-molecular-weight metabolites covering amino acids (including branched-chained and aromatic), glycolysis-related measures, and ketone bodies. The method has been widely used in epidemiologic research and recently reviewed in refs. 24, 25.

Statistical analysis

All analyses were performed using R (version 3.4.1). A total of 2,291 men with screen-detected prostate cancer (348 high-risk; 1,939 low-risk) and 2,661 controls had NMR metabolites measured in ProtecT. The distribution of baseline characteristics in cases versus controls was compared using Wilcoxon rank-sum tests for continuous variables and a χ^2 statistic for categorical variables. Multiple imputation using the "mice" R package, and based on a subset of 78 metabolites chosen at random (given imputation constraints), was used to impute family history of prostate cancer, unknown for 11% of participants. Family history and age were selected as covariates in multivariable models of prostate cancer risk, as those factors are strongly associated with prostate cancer and are potential confounders of the exposure-outcome relationship. We also adjusted for the primary care center where patients were registered. Metabolite trait concentrations/ratios were log-transformed and then scaled to SD scores to allow direct comparison of the magnitude of the effect of traits with different units on prostate cancer. A dictionary of metabolic traits with units before standardization is available in Supplementary Table S2.

Multivariable logistic regression was performed to compare the odds of total prostate cancer (versus controls) per log-transformed, then SD-scaled metabolite concentration, such that each metabolite has a standard deviation of one. As a sensitivity analysis, we also examined the odds of prostate cancer by high- and low-risk case status and performed tests of the differences between ORs [took the absolute difference between the ORs (δ); calculated the standard error (SE) for δ using the SEs from each comparison set, such that SE_1^2 and SE_2^2 refer, respectively, to the SEs of the first comparison and second comparison sets, $\sqrt{SE_1^2 + SE_2^2}$; calculated z scores, $\frac{\delta}{SE(\delta)}$; and calculated p-values for the z scores] for the following comparisons: differences in odds ratios for high-risk results and total results, the low-risk results and total results, and high-risk and low-risk results. In addition (also as a sensitivity analysis) we examined the correlation between the metabolites and PSA, given that our population of participants was screen- (PSA) detected.

To account for multiple testing and the correlation between the metabolic measures, principal component analysis was carried out on z-scored metabolic trait data (26). We calculated that the first 37 principal components explained $> 99\%$ of the variance in the data and set our statistical threshold to $P < 0.05/37$ ($= 0.0014$), equivalent to $P < 0.05$ after adjusting for multiple testing.

Causal analysis

To assess causality, we used MR, a causal analysis method which exploits the random assortment of alleles in an instrumental variable (IV) framework to address confounding and reverse causation that preclude causal inference in observational studies (27, 28). Germline genetic variants associated with each metabolite of interest can serve as proxies (IVs) for those metabolites in models examining the causal effects of metabolic traits on prostate cancer, if a number of assumptions are met: (i) the IVs (genetic variants) are robustly associated with metabolites; (ii) the IVs are independent of confounders of the metabolites and prostate cancer; and (iii) the IVs are not pleiotropically associated with the prostate cancer; i.e. they are associated with prostate cancer only through the metabolites they are instrumenting and not associated with prostate cancer through other exposures (29).

From the literature, we know there are strong associations between single-nucleotide polymorphisms (SNPs) and metabolite levels (30–33); therefore, these SNPs can serve as instruments in Mendelian randomization analyses (34–36). For instance, the median proportion of variance explained for metabolite associations in Kettunen and colleagues (2016) was 5% and ranged from 0.2% for acetoacetate to 12.5% for glycine (33). To implement MR, we identified independent (those not in linkage disequilibrium; $r^2 < 0.01$) SNPs that were robustly associated at genome-wide significance (i.e. $P < 5 \times 10^{-8}$) with metabolites in the Kettunen and colleagues (2016) genome-wide association study (GWAS) of 123 circulating metabolites in 24,925 participants from 14 European cohorts (33). These SNPs were chosen as IVs for our metabolites. We could not instrument 113 of the 227 NMR-quantified metabolic traits; 65 of these traits were ratio measures not included in the GWAS and 48 were other types of traits that had no genetic proxy.

To leverage power from large samples, we performed two-sample MR (27, 37, 38), whereby we obtained summary data on the effects of the SNPs that acted as genetic instruments for each metabolite on prostate cancer from a separate data source, the Prostate Cancer Association Group to Investigate Cancer Associated Alterations in the Genome (PRACTICAL) consortium. This consortium involved 52 prostate cancer case-control studies on which genome-wide association studies (GWAS) had been conducted (39–44). The current MR analysis was based on 44,825 prostate cancer cases and 27,904 controls from within 48 of the 52 PRACTICAL cohorts of men with European ancestry.

To implement MR, we undertook the following process for each of the SNPs identified as a proxy for each metabolite: we extracted the effect and noneffect allele, and the log OR and standard error per copy of the effect allele from the PRACTICAL GWAS. We used these data to construct our genetic instruments for our circulating metabolic traits and calculated the log OR for prostate cancer per SD increase in metabolic measure levels using the two-sample MR Maximum likelihood estimator (ref. 45; Supplementary Table S3 contains the characteristics of the genetic variants associated with metabolites that were selected as instruments.) In computing the effect estimates based on MR, the effect estimates for each SNP-prostate cancer association were meta-analyzed.

Two-sample MR analyses were performed in R using the TwoSampleMR package (46).

Results

Characteristics of men in the ProtecT study

Table 1 compares the distribution of selected characteristics in cases versus controls. The median age (63 years) and body mass index (BMI, 27 kg/m²) were the same, but more cases than controls had a family history of prostate cancer (8% vs. 5%; $P < 0.001$).

Observational associations of metabolic traits on prostate cancer (ProtecT)

Thirty-five metabolites were associated with odds of prostate cancer at $P < 0.0014$ (Table 2; Fig. 1; Supplementary Table S4). The following increased the odds of prostate cancer: (i) lipids and lipoprotein particle concentration, specifically, total lipids (TL) in small high-density lipoprotein (HDL) and concentration of small HDL particles; (ii) total cholesterol (TC) and TC compositional ratios, namely, TC in HDL3, TC in medium low-density lipopro-

Table 1. Baseline characteristics (medians and interquartile ranges, or percents) for ProtecT cases and controls

Characteristic	Case (n = 2291)	Control (n = 2661)	P ^a
Age	63 (59–67)	63 (59–66)	0.709
Family history of prostate cancer (%) ^b	170 (8)	128 (5)	<0.001
BMI (kg/m ²) ^c	27 (24–29)	27 (24–29)	0.872

ProtecT = Prostate Testing for Cancer and Treatment trial.

^aP value based on χ^2 tests (for categorical variables) and Wilcoxon rank-sum tests (for continuous variables).

^bFamily history data available on only 90% of these subjects.

^cBMI data available on only 64% of these subjects.

tein (LDL), small HDL, and small LDL; and TC:TL ratios in medium LDL, small HDL, small LDL, and small very low-density lipoprotein (VLDL); (iii) cholesterol esters (CE) and CE compositional ratios, specifically: CE in medium LDL, small HDL, and small LDL; CE-to-TL ratios in medium LDL, CE:TL ratios in small HDL and small LDL; (iv) free cholesterol (FC) and a FC compositional ratios, namely, FC in IDL, large LDL, and medium HDL, and FC-to-TL ratio in medium HDL; (v) phospholipids (PL) and PL compositional ratios, including: PL in intermediate-density lipoprotein (IDL) and very small VLDL, and PL:TL ratios in medium VLDL and very small VLDL; (vi) and the protein albumin; (vii) the ratio of omega-6 fatty acids (FA) to total FA.

The following decreased the odds of total prostate cancer: VLDL particle size, PL-to-TL ratios in medium LDL; triglycerides (TG)-to-total lipid ratios in small and medium VLDL; amino acids (AA), specifically, the branched chain AA, isoleucine, leucine, and valine, and the aromatic AA tyrosine; and saturated FA-to-total FA.

In the sensitivity analysis of the effect of metabolic traits on high-risk prostate cancer versus controls, albumin was associated with high-risk case status [OR 1.12; 95% confidence interval (CI) 1.08–1.36; $P < 0.0014$]; 138 (61%) had ORs reversed from those in the combined (total, case-control) analysis; and, although 53 ORs were statistically different from those in the total analysis (P value threshold < 0.05), none of the differences survived multiple comparisons ($P < 0.05/227$; 0.0002; Supplementary Tables S5 and S6). Consistent with these results, in the comparison of high-versus low-risk ORs, 78 metabolites had ORs that were statistically different at the < 0.05 threshold and two at the multiple-testing threshold ($P < 0.0002$: TC:TL in small HDL and PL:TL in small HDL); 63% of metabolites had directionally reversed ORs. Notably, among the 35 top metabolites in the total analysis, eight were included in the set of those with statistically different ORs in the high- versus low-risk comparison (P value for multiple testing set to $0.05/35 = 0.0014$; Supplementary Tables S7 and S8). Conversely, the sensitivity analysis for the effect of metabolites on low-risk prostate cancer (vs. controls) revealed patterns of association that mirrored the magnitude and direction of effects observed for the total analysis versus controls; only four (0.02%) metabolites in the low-risk analysis had ORs directionally reversed from those in the total analysis; and none of the ORs were statistically different from those in the total analysis ($P < 0.05$) (Supplementary Tables S9 and S10).

None of the metabolite-PSA correlations exceeded $|0.06|$ (Supplementary Table S11).

Mendelian randomization causal analysis (PRACTICAL)

Fourteen of the top 35 metabolites observationally associated with total prostate cancer were analyzable using MR. Of the 14

Table 2. Among ProtecT cases and controls, odds of prostate cancer for top metabolites^a

Name	OR (95% CI)	P ^b
Lipids and lipoprotein subclass characteristics		
Small HDL (particle concentration)	1.102 (1.042–1.167)	0.00070
VLDL (mean particle diameter)	0.906 (0.856–0.958)	0.00056
Cholesterol esters (CE)		
CE in medium LDL	1.105 (1.044–1.170)	0.00058
CE to total lipids ratio in medium LDL	1.108 (1.044–1.180)	0.00062
CE in small HDL	1.135 (1.071–1.205)	0.00002
CE to total lipids ratio in small HDL	1.100 (1.038–1.167)	0.00111
CE in small LDL	1.099 (1.039–1.165)	0.00107
CE to total lipids ratio in small LDL	1.100 (1.037–1.169)	0.00139
Free (FC) and total cholesterol (TC)		
FC in IDL	1.105 (1.044–1.170)	0.00057
FC in large LDL	1.101 (1.041–1.166)	0.00080
FC in medium HDL	1.109 (1.045–1.179)	0.00060
FC to total lipids ratio in medium HDL	1.109 (1.045–1.179)	0.00055
TC in HDL3	1.098 (1.038–1.162)	0.00109
TC in medium LDL	1.100 (1.039–1.164)	0.00095
TC to total lipids ratio in medium LDL	1.100 (1.039–1.167)	0.00105
TC in small HDL	1.144 (1.080–1.213)	<0.00001
TC to total lipids ratio in small HDL	1.099 (1.039–1.165)	0.00106
TC in small LDL	1.097 (1.037–1.161)	0.00132
TC to total lipids ratio in small LDL	1.100 (1.039–1.166)	0.00107
TC to total lipids ratio in small VLDL	1.099 (1.038–1.163)	0.00105
Phospholipids (PL) and total lipids (TL)		
PL in IDL	1.100 (1.040–1.164)	0.00092
PL to total lipids ratio in medium LDL	0.904 (0.853–0.957)	0.00046
PL to total lipids ratio in medium VLDL	1.145 (1.082–1.211)	<0.00001
PL in very small VLDL	1.099 (1.039–1.163)	0.00103
PL to total lipids ratio in very small VLDL	1.120 (1.056–1.190)	0.00013
TL in small HDL	1.108 (1.048–1.173)	0.00035
Triglycerides (TG)		
TG to total lipids ratio in medium VLDL	0.907 (0.857–0.959)	0.00064
TG to total lipids ratio in small VLDL	0.906 (0.856–0.958)	0.00055
Fatty acids (FA)		
Ratio of omega-6 FA to total FA	1.102 (1.041–1.166)	0.00080
Ratio of saturated FA to total FA	0.890 (0.841–0.942)	0.00006
Amino acids		
Isoleucine	0.893 (0.844–0.944)	0.00008
Leucine	0.901 (0.851–0.953)	0.00027
Tyrosine	0.886 (0.837–0.937)	0.00003
Valine	0.913 (0.863–0.965)	0.00139
Fluid balance		
Albumin	1.104 (1.043–1.168)	0.00065

Abbreviations: HDL, high-density lipoprotein; IDL, intermediate-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

^aModels adjusted for age, center, and imputed family history of prostate cancer (imputed because family history was only available for 90% of subjects).

^bP value threshold corrected for multiple testing ($P < 0.05/37 = 0.0014$).

metabolites that were instrumental, none appear causal for prostate cancer risk (Fig. 2; Supplementary Tables S12 and S13).

Discussion

Main findings

We identified 35 potential biomarkers for prostate cancer. The majority of these were cholesterol, followed by glycerides and phospholipids. Steroid hormones, including androgens that drive prostate cancer, are derived from cholesterol (47), and high levels of cholesterol are required by rapidly proliferating cells (48). Hence, it is possible that our findings point to the underlying relationship between prostate cancer and androgens. Moreover, the observed effects appear to be driven solely by the low-risk cases, which were more abundant in our screen-detected cohort. The weak correlations between the metabolites and PSA suggest that our findings are not a byproduct of screening.

Fifteen of the top noninstrumented metabolites were ratios, which means that we were able to test the causal effects for the majority (70%) of our top metabolites that were not ratios (14/20).

Comparison with previous literature

A few recent studies have explored the relationship between serum metabolites and prostate cancer using metabolites detected from chromatography–mass spectrometry (2, 14, 15). In a pilot study, Mondul and colleagues (2014) compared 420 metabolic compounds in fasting serum collected prospectively from 74 clinically detected prostate cancer cases and 74 matched controls within the Alpha-Tocopherol, Beta-Carotene Cancer Prevention Study (ATBC) cohort. In their study, circulating 1-stearoylglycerol (1-SG) was inversely associated with prostate cancer (OR 0.34; 95% CI, 0.20–0.58; ref. 15). In this study, we did not quantify 1-SG. In their replication study, also within the ATBC cohort,

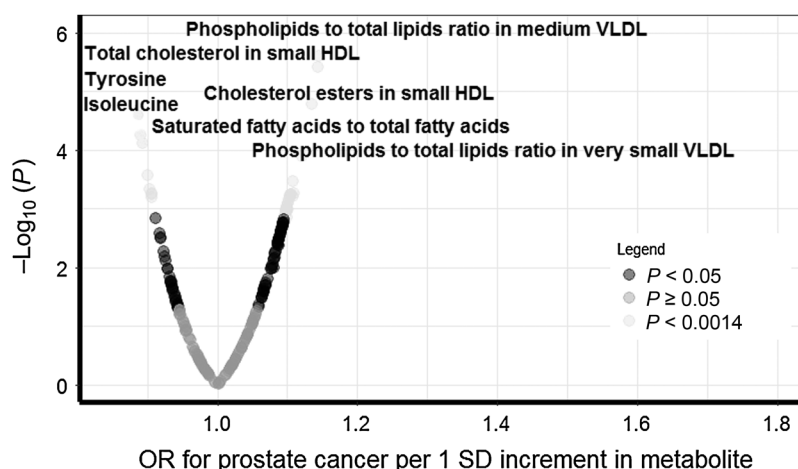


Figure 1. Volcano plot of the odds of prostate cancer in ProtecT. Labeled metabolites are Bonferroni significant ($<0.05/227$). Light gray dots indicate $P < 0.0014$; dark gray dots indicate $P < 0.05$; and medium gray dots indicate $P \geq 0.05$.

Mondul and colleagues (2015) analyzed fasting serum collected prospectively for 626 metabolic compounds in 200 clinically detected cases and 200 matched controls (14). Notably, there was no overlap between the findings of this study and those of Mondul and colleagues (2015; 14).

Similarly, Huang and colleagues (2016) undertook an investigation of prostate cancer within the Prostate, Lung, Colorectal, and Ovarian Cancer Screening Trial (PLCO), for which they prospectively examined 695 known serum

metabolites in 380 screen-detected cases and 380 matched controls. Their set of top metabolites differed from both from ours and the set observed in the ATBC (clinically detected) studies (2).

This study differed from both the two ATBC and the PLCO metabolome studies—studies perhaps the most comparable with ours—in that, instead of using chromatography–mass spectrometry to detect agnostically any measurable serum metabolites, we used a quantitative high-throughput NMR metabolomics

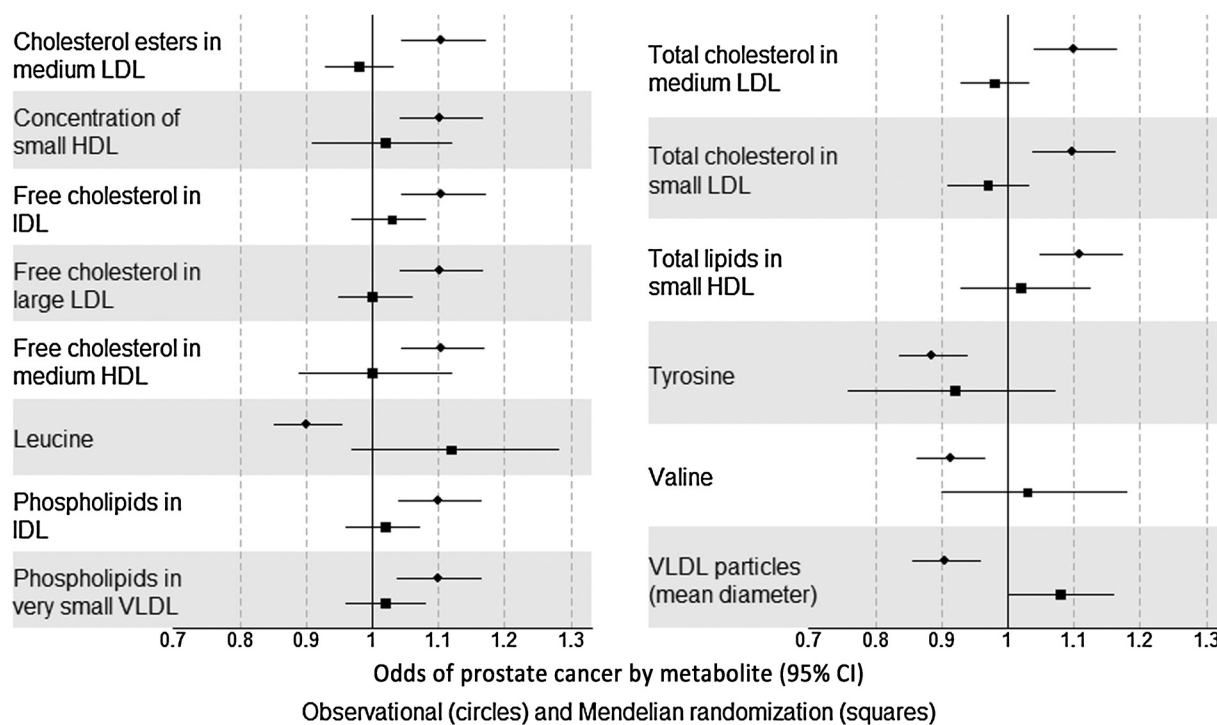


Figure 2. Forest plot showing odds of prostate cancer by metabolite for top observational findings in ProtecT along with causal estimates from Mendelian randomization. Figure 2 is a forest plot of the odds of prostate cancer by metabolite for top observational findings in the ProtecT trial with models adjusted for age, center, and imputed family history of prostate cancer. Summary data for the effects of metabolite loci on prostate cancer for the Mendelian randomization analysis was obtained from the PRACTICAL consortium. The squares and lines indicate odds ratios and 95% CIs for top findings in ProtecT. The circle dots and lines indicate the causal estimates for the effects of the metabolites on prostate cancer in PRACTICAL.

platform with a prechosen set of metabolites that cover metabolic pathways for lipoprotein lipids and subclasses, fatty acids, amino acids, and glycolysis precursors. As such, some of the deviation between our findings and theirs are explained by this—we examined different sets of metabolites. Another difference is that the ATBC and PLCO studies were prospective and the observational portion of this study is cross-sectional.

We observed a family history of prostate cancer in 8% of cases, likely reflecting that they were identified in a screening versus a clinical setting.

Strengths

Our analysis uses MR to interrogate whether some of our top findings (those with genetic instruments) appear causal. It is the first study of circulating metabolic traits and prostate cancer to do so. Moreover, it is the largest (more than 4× larger than the largest previous study; ref. 2) examination of the role of circulating metabolites in prostate cancer, and it yielded novel and promising associations with metabolic traits that may be useful clinically as biomarkers to better distinguish presence of disease and disease severity.

Limitations

Our study has a few limitations. As the blood samples were collected at diagnosis for cases, we were unable to determine the direction of causality in our observational analyses. Likewise, there is potential, due to the way we selected our controls [men with PSA <3 ng/mL or a raised PSA (≥3 ng/mL) and negative biopsy] for there to be some misclassification of case status. Use of MR, at least for the instrumentable metabolites, allowed us, nonetheless, to appraise causality for a subset of our top findings, and we had at least 80% power to detect effect estimates within the range of those observed in our observational analysis for most metabolites. Another limitation is that there was a lack of specificity for many of the available genetic instruments, potentially biasing our causal analysis toward the null. Given this, while our MR found no evidence for causality, future MR analyses containing a larger number of specific genetic instruments for the metabolites are needed to strengthen causal assessment of the role of the metabolites we have detected as marking the presence of prostate cancer.

Conclusion

We identified 35 circulating metabolites associated with prostate cancer presence, but found no evidence of causality for those 14 testable with MR. Thus, the 14 metabolites tested with MR are unlikely to be mechanistically important in prostate cancer risk. We cannot speculate about the causality for those not tested with MR.

Disclosure of Potential Conflicts of Interest

P. Wurtz is an employee of and has ownership interest in Nightingale Health Ltd. R.A. Eeles reports receiving speakers bureau honoraria from Janssen. N. Usmani reports receiving commercial research funding from Best Medical and is a consultant/advisory board member for Bayer, Amgen, and Astellas. P. Townsend reports receiving speakers bureau honoraria from MedLabs; is named as an inventor in patents unrelated to this manuscript; is a consultant/advisory board member for DeepMed, NED, and is a scientific advisor for Aptamer Group; and receives shares/license fees from Karus Therapeutics Ltd., Pentagon Biotechnology Ltd, and PRE-Cignature Ltd. No potential conflicts of interest were disclosed by the other authors.

Authors' Contributions

Conception and design: C. Adams, G.D. Smith, C.L. Relton, B.E. Henderson, F.R. Schumacher, A. Wolk, A.S. Kibel, M. Kogevinas, M.G. Dominguez, R.M. Martin

Development of methodology: C. Adams, D.L. Santos Ferreira, A. Razack, R.M. Martin

Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): P. Wurtz, J.L. Donovan, F.C. Hamdy, D.E. Neal, R.A. Eeles, C.A. Haiman, Z. Kote-Jarai, F.R. Schumacher, K. Muir, S.I. Berndt, F. Wiklund, S.J. Chanock, S.M. Gapstur, C.M. Tangen, J. Batra, J.A. Clements, N. Pashayan, J. Schleutker, D. Albanes, A. Wolk, C.M.L. West, L.A. Mucci, G. Cancel-Tassin, S. Koutros, K.D. Sørensen, L. Maehle, R. Hamilton, S.A. Ingles, B.S. Rosenstein, Y.-J. Lu, A. Vega, M. Kogevinas, J.Y. Park, J.L. Stanford, C. Cybulski, B.G. Nordestgaard, H. Brenner, E.M. John, M.R. Teixeira, S.L. Neuhausen, K. DeRuyck, A. Razack, L.F. Newcomb, D. Lessel, R.P. Kaneva, N. Usmani, F. Claessens, P. Townsend, M.G. Dominguez, M.J. Roobol, F. Menegaux, K.-T. Khaw, L.A. Cannon-Albright, H. Pandha, S.N. Thibodeau, R.M. Martin

Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): C. Adams, R.C. Richmond, W. Spiller, V.Y. Tan, J. Zheng, P. Wurtz, A.A. Al Olama, D.V. Conti, S.J. Chanock, B.S. Rosenstein, A.S. Kibel

Writing, review, and/or revision of the manuscript: C. Adams, R.C. Richmond, D.L. Santos Ferreira, V.Y. Tan, J.L. Donovan, D.E. Neal, J.A. Lane, G.D. Smith, C.L. Relton, R.A. Eeles, Z. Kote-Jarai, F.R. Schumacher, A.A. Al Olama, S. Benlloch, K. Muir, S.I. Berndt, F. Wiklund, S.M. Gapstur, V.L. Stevens, J. Batra, J.A. Clements, N. Pashayan, J. Schleutker, D. Albanes, A. Wolk, L.A. Mucci, G. Cancel-Tassin, S. Koutros, K.D. Sørensen, L. Maehle, R.C. Travis, R. Hamilton, S.A. Ingles, B.S. Rosenstein, Y.-J. Lu, G.G. Giles, A.S. Kibel, A. Vega, M. Kogevinas, J.Y. Park, B.G. Nordestgaard, H. Brenner, C. Maier, J. Kim, E.M. John, M.R. Teixeira, S.L. Neuhausen, A. Razack, R.P. Kaneva, N. Usmani, P. Townsend, M.J. Roobol, K.-T. Khaw, H. Pandha, R.M. Martin

Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): R.A. Eeles, A. Wolk, K.D. Sørensen, Y.-J. Lu, M.R. Teixeira, R.P. Kaneva, M.G. Dominguez, K.-T. Khaw, R.M. Martin

Study supervision: H. Gronberg, Y.-J. Lu, A.S. Kibel, M.G. Dominguez, R.M. Martin

Other (coding): D.L. Santos Ferreira

Other (PRACTICAL consortium member; contributed samples): K.L. Penney

Other (provided primary sample material for analysis): P. Townsend

Other (implementation of PRAGGA study in Galicia, Spain): M.G. Dominguez

Acknowledgments

This work was supported by Cancer Research UK (CRUK; C18281/A19169) and the Medical Research Council Integrative Epidemiology Unit at the University of Bristol, which is supported by the Medical Research Council (MRC) and the University of Bristol (MC_UU_12013/2; author C. Adams). R.C. Richmond is supported by CRUK (C18281/A19169) and MRC (MC_UU_12013/2). W. Spiller is supported by MRC (MC_UU_12013/1) and a Wellcome Trust studentship (108902/15/Z). V.Y. Tan is supported by CRUK (C18281/A19169). P. Wurtz is funded by the Academy of Finland (grant nos: 312476 and 312477). D.E. Neal is funded by (C11043/A4286, C18281/A8145, and C18281/A11326). J.A. Lane is supported by CRUK (C18281/A19169). C.L. Relton is supported by MRC (MC_UU_12013/2) and CRUK (C18281/A19169). R.A. Eeles is supported by CRUK (C5047/A17528). Z. Kote-Jarai is supported by CRUK (C5047/A17528). K.-T. Khaw with EPIC Norfolk is supported by grants from the MRC (MR/N003284/1; G1000143) and CRUK (14136). R.C. Travis is supported by CRUK (C8221/A19170), CRUK (14136 for EPIC-Norfolk and C570/A16491 for EPIC-Oxford), and the MRC (1000143 for EPIC-Norfolk and MR/M012190/1 for EPIC-Oxford). R.M. Martin is supported by CRUK (C18281/A19169). The National Institute for Health Research Biomedical Research Centre supported R.M. Martin, C.L. Relton, G.D. Smith. Genotyping of the OncoArray was funded by the US NIH [U19 CA 148537 for ELucidating Loci Involved in Prostate cancer Susceptibility (ELLIPSE) project and X01HG007492 to the Center for Inherited Disease Research (CIDR) under contract number HHSN268201200008]. Additional analytic support was provided by NIH NCI U01 CA188392 (principal investigator: F.R. Schumacher). The PRACTICAL consortium was supported by Cancer Research UK Grants C5047/A7357, C1287/A10118, C1287/A16563, C5047/A3354, C5047/A10692, C16913/A6135, European Commission's Seventh Framework Programme grant agreement no. 223175 (HEALTH-F2-2009-223175), and The

NIH Cancer Post-Cancer GWAS initiative grant: no. 1 U19 CA 148537-01 (the GAME-ON initiative). We would also like to thank the following for funding support: The Institute of Cancer Research and The Everyman Campaign, The Prostate Cancer Research Foundation, Prostate Research Campaign UK (now Prostate Action), The Orchid Cancer Appeal, The National Cancer Research Network UK, The National Cancer Research Institute (NCRI) UK. We are grateful for support of NIHR funding to the NIHR Biomedical Research Centre at The Institute of Cancer Research and The Royal Marsden NHS Foundation Trust.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received January 26, 2018; revised March 25, 2018; accepted October 15, 2018; published first October 23, 2018.

References

- Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global Cancer Statistics, 2012. *CA Cancer J Clin* 2015;65:87–108.
- Huang J, Mondul AM, Weinstein SJ, Koutros S, Derkach A, Karoly E, et al. Serum metabolomic profiling of prostate cancer risk in the prostate, lung, colorectal, and ovarian cancer screening trial. *Br J Cancer* 2016;115:1087–95.
- Markozannes G, Tzoulaki I, Karli D, Evangelou E, Ntzani E, Gunter MJ, et al. Diet, body size, physical activity and risk of prostate cancer: an umbrella review of the evidence. *Eur J Cancer* 2016;69:61–9.
- Pavlova NN, Thompson CB. The emerging hallmarks of cancer metabolism. *Cell Metab* 2016;23:27–47.
- Kelly RS, Heiden MG, Giovannucci E, Mucci LA. Metabolomic biomarkers of prostate cancer: Prediction, diagnosis, progression, prognosis, and recurrence. *Cancer Epidemiol Biomarkers Prev* 2016;25:887–906.
- Osl M, Dreiseitl S, Pfeifer B, Weinberger K, Klocker H, Bartsch G, et al. A new rule-based algorithm for identifying metabolic markers in prostate cancer using tandem mass spectrometry. *Bioinformatics* 2008;24:2908–14.
- Lokhov PG, Dashtiev MI, Moshkovskii SA, Archakov AI. Metabolite profiling of blood plasma of patients with prostate cancer. *Metabolomics* 2010;6:156–63.
- Fan Y, Murphy TB, Byrne JC, Brennan L, Fitzpatrick JM, Watson RWG. Applying random forests to identify biomarker panels in serum 2D-DIGE data for the detection and staging of prostate cancer. *J Proteome Res* 2011;10:1361–73.
- Miyagi Y, Higashiyama M, Gochi A, Akaike M, Ishikawa T, Miura T, et al. Plasma free amino acid profiling of five types of cancer patients and its application for early detection. *PLoS One* 2011;6:1–12.
- Saylor P. Prospective study of changes in the metabolomic profiles of men during their first three months of androgen deprivation therapy for prostate cancer. *Clin Cancer Res* 2012;18:3677–85.
- Zhou X, Mao J, Ai J, Deng Y, Roth MR, Pound C, et al. Identification of plasma lipid biomarkers for prostate cancer by lipidomics and bioinformatics. *PLoS One* 2012;7:1–11.
- Huang G, Liu X, Jiao L, Xu C, Zhang Z, Wang L, et al. Metabolomic evaluation of the response to endocrine therapy in patients with prostate cancer. *Eur J Pharmacol* 2014;729:132–7.
- Zang X, Jones CM, Long TQ, Monge ME, Zhou M, Walker LD, et al. Feasibility of detecting prostate cancer by ultra performance liquid chromatography-mass spectrometry serum metabolomics. *J Proteome Res* 2014;3:3444–54.
- Mondul AM, Moore SC, Weinstein SJ, Karoly ED, Sampson JN, Albanes D. Metabolomic analysis of prostate cancer risk in a prospective cohort: the alpha-tocopherol, beta-carotene cancer prevention (ATBC) study. *Int J Cancer* 2015;137:2124–32.
- Mondul AM, Moore SC, Weinstein SJ, Männistö S, Sampson JN, Albanes D. 1-Stearoylglycerol is associated with risk of prostate cancer: results from a serum metabolomic profiling analysis. *Metabolomics* 2014;1–6.
- Fewell Z, Davey Smith G, Sterne JAC. The impact of residual and unmeasured confounding in epidemiologic studies: a simulation study. *Am J Epidemiol* 2007;166:646–55.
- Davey Smith G, Ebrahim S. Data dredging, bias, or confounding. *BMJ* 2002;325:1437–8.
- Lane JA, Hamdy FC, Martin RM, Turner EL, Neal DE, Donovan JL. Latest results from the UK trials evaluating prostate cancer screening and treatment: the CAP and ProtecT studies. *Eur J Cancer* 2010;46:3095–101.
- Hamdy FC. The prostate testing for cancer and treatment (ProtecT) study: what have we learnt? *BJU Int* 2016;118:843.
- Hamdy FC, Donovan JL, Lane JA, Mason M, Metcalfe C, Holding P, et al. 10-year outcomes after monitoring, surgery, or radiotherapy for localized prostate cancer. *N Engl J Med* 2016;375:1415–24.
- Lane JA, Donovan JL, Davis M, Walsh E, Dedman D, Down L, et al. Active monitoring, radical prostatectomy, or radiotherapy for localised prostate cancer: study design and diagnostic and baseline results of the ProtecT randomised phase 3 trial. *Lancet Oncol* 2014;15:1109–18.
- Bonilla C, Lewis SJ, Martin RM, Donovan JL, Hamdy FC, Neal DE, et al. Pubertal development and prostate cancer risk: mendelian randomization study in a population-based cohort. *BMC Med* 2016;14:66.
- National Institute For Health and Care Excellence. Prostate cancer: diagnosis and management. Available from: <https://www.nice.org.uk/guidance/cg175>.
- Würtz P, Kangas AJ, Soyninen P, Lawlor DA, Davey Smith G, Ala-Korpela M. Quantitative serum NMR metabolomics in large-scale epidemiology: a primer on -omic technology. *Am J Epidemiol* 2017;186:1084–96.
- Würtz P, Havulinna AS, Soyninen P, Tynkynen T, Prieto-Merino D, Tillin T, et al. Metabolite profiling and cardiovascular event risk: a prospective study of 3 population-based cohorts. *Circulation* 2015;131:774–85.
- Gao X, Starmer J, Martin ER. A multiple testing correction method for genetic association studies using correlated single nucleotide polymorphisms. *Genet Epidemiol* 2008;32:361–9.
- Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet* 2014;23:R89–98.
- Davey Smith G, Ebrahim S. "Mendelian randomization": can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol* 2003;32:1–22.
- Didelez V, Sheehan N. Mendelian randomization as an instrumental variable approach to causal inference. *Stat Methods Med Res* 2007;16:309–30.
- Joshi PK, Esko T, Mattsson H, Eklund N, Gandin I, Nutile T, et al. Directional dominance on stature and cognition in diverse human populations. *Nature* 2015;523:459–62.
- Petersen AK, Zeilinger S, Kastenmuller G, Römisch-Margl W, Brügger M, Peters A, et al. Epigenetics meets metabolomics: an epigenome-wide association study with blood serum metabolic traits. *Hum Mol Genet* 2014;23:534–45.
- Gieger C, Geistlinger L, Altmaier E, Hrabé de Angelis M, Kronenberg F, Meitinger T, et al. Genetics meets metabolomics: a genome-wide association study of metabolite profiles in human serum. *PLoS Genet* 2008;4:e1000282.
- Kettunen J, Demirkan A, Würtz P, Draisma HH, Haller T, Rawal R, et al. Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun* 2016;7:1–9.
- Wurtz P, Wang Q, Kangas AJ, Richmond RC, Skarp J, Tiainen M, et al. Metabolic signatures of adiposity in young adults: mendelian randomization analysis and effects of weight change. *PLoS Med* 2014;11:e1001765.
- Lotta LA, Scott RA, Sharp SJ, Burgess S, Luan J, Tillin T, et al. Genetic predisposition to an impaired metabolism of the branched-chain amino acids and risk of type 2 diabetes: a Mendelian randomisation analysis. *PLoS Med* 2016;13:1–22.
- Varbo A, Benn M, Tybjaerg-Hansen A, Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG. Remnant cholesterol as a causal risk factor for ischemic heart disease. *J Am Coll Cardiol* 2013;61:427–36.
- Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. *Eur J Epidemiol* 2015;30:543–52.

38. Pierce BL, Burgess S. Efficient design for mendelian randomization studies: subsample and 2-sample instrumental variable estimators. *Am J Epidemiol* 2013;178:1177–84.
39. Kote-jarai Z, Easton DF, Stanford JL, Ostrander EA, Schleutker J, Ingles SA, et al. Multiple novel prostate cancer predisposition loci confirmed by an international study: the PRACTICAL consortium. *Cancer Epidemiol Biomarkers Prev* 2008;17:2052–61.
40. Eeles RA, Kote-jarai Z, Amin A, Giles GG, Guy M, Severi G, et al. Identification of seven new prostate cancer susceptibility loci through a genome-wide association study. *Nat Genet* 2010;41:1116–21.
41. Kote-Jarai Z, Olama AAA, Giles GG, Severi G, Schleutker J, Weischer M, et al. Seven prostate cancer susceptibility loci identified by a multi-stage genome-wide association study. *Nat Genet* 2011;43:785–91.
42. Al Olama AA, Kote-Jarai Z, Schumacher FR, Wiklund F, Berndt SI, Benlloch S, et al. A meta-analysis of genome-wide association studies to identify prostate cancer susceptibility loci associated with aggressive and non-aggressive disease. *Hum Mol Genet* 2013;22:408–15.
43. Eeles Rosalind A, Benlloch S, Saunders EJ, Leongamornlert DA, Tymrakiewicz M, et al. Identification of 23 new prostate cancer susceptibility loci using the iCOGS custom genotyping array. *Nat Genet* 2013;45:1–22.
44. Liao F, Xu H, Torrey N, Road P, Jolla L. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. *Nat Genet* 2014;46:1103–9.
45. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. *Genet Epidemiol* 2013;37:658–65.
46. Hemani G, Zheng J, Wade KH, Laurin C, Elsworth B, Burgess S, et al. MR-Base: a platform for systematic causal inference across the phenome using billions of genetic associations. *BioRxiv* 2016;1–31.
47. Berg JM, Tymoczko JL, Stryer L. Section 26.4, important derivatives of cholesterol include bile salts and steroid hormones. In: *Biochemistry*. 5th edition. New York, NY: W H Freeman; 2002. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK22339/>.
48. Harshman LC, Wang X, Nakabayashi M, Xie W, Valenca L, Werner L, et al. Statin use at the time of initiation of androgen deprivation therapy and time to progression in patients with hormone-sensitive prostate cancer. *JAMA Oncol* 2015;1:495.