Supporting your research with our capabilities

BD Accuri™ C6 Plus Personal Flow Cytometer
BD FACSCelesta™ Cell Analyzer
BD LSRFortessa™ X-20 Cell Analyzer
BD FACSMelody™ Cell Sorter
One of the largest portfolios of reagents

Learn more >
Alcohol consumption and prostate cancer incidence and progression: A Mendelian randomisation study

Clair Brunner1,2, Neil M. Davies1,2, Richard M. Martin1,2,3, Rosalind Eeles4,5, Doug Easton6, Zsofia Kote-Jarai6, Ali Amin Al Olama6, Sara Benlloch7, Kenneth Muir7, Graham Giles8,9, Fredrik Wiklund10, Henrik Gronberg10, Christopher A. Haiman11, Johanna Schleutker11,12,13, Børge G. Nordestgaard13,14, Ruth C. Travis15, David Neal16,17, Jenny Donovan1, Freddie C. Hamdy18, Nora Pashayan19,20, Kay-Tee Khaw21, Janet L. Stanford22,23, William J. Blot24, Stephen Thibodeau25, Christiane Maier26,27, Adam S. Kibel28,29, Cezary Cybulski30, Lisa Cannon-Albright31, Hermann Brenner32,33,34, Jong Park35, Radka Kaneva36, Jyotsna Batra37, Manuel R. Teixeira38, Hardev Pandha39, the PRACTICAL Consortium,40,41 and Luisa Zuccolo1,2

1 School of Social and Community Medicine, University of Bristol, Bristol, United Kingdom
2 MRC/University of Bristol Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom
3 The NIH Bristol Nutrition Biomedical Research Unit, University Hospitals Bristol NHS Foundation Trust and the University of Bristol, Bristol, United Kingdom
4 The Institute of Cancer Research, London, SM2 5NG, United Kingdom
5 Royal Marsden NHS Foundation Trust, London, SW3 6JJ, United Kingdom

Key words: alcohol, prostate cancer, alcohol metabolising genes, Mendelian randomisation

Abbreviations: ADH: alcohol dehydrogenase; ALDH: aldehyde dehydrogenase; CI: confidence interval; HR: hazard ratio; LD: linkage disequilibrium; OR: odds ratio; PRACTICAL: PRostate assoCiation group to Investigate Cancer-associated AlLerations in the genome; SNP: single nucleotide polymorphism

DOI: 10.1002/ijc.30436

History: Received 14 Apr 2016; Accepted 29 July 2016; Online 19 Sep 2016

Correspondence to: Luisa Zuccolo, MRC/University of Bristol Integrative Epidemiology Unit, University of Bristol, Bristol, United Kingdom, Tel: +44 [117] 3310066, E-mail: l.zuccolo@bristol.ac.uk

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Additional Supporting Information may be found in the online version of this article.

This article was published online on 19 September 2016. Supporting Information file was added. This notice is included in the online and print versions to indicate that both have been corrected 21 April 2017.

Grant sponsor: UK MRC Special Training Fellowship; Grant number: G0501864/76656; Grant sponsor: UK MRC Population Health Scientist fellowship; Grant number: G0902144; Grant sponsor: UK MRC; Grant number: G0600705; Grant sponsor: University of Bristol; Grant number: MC_UU_12013/1; Grant sponsor: The European Commission’s Seventh Framework Programme; Grant number: HEALTH-F2-2009-223175; Grant sponsor: Cancer Research UK; Grant number(s): C5047/A7357, C1287/A10118, C5047/A3354, C5047/A10692, C1693/A6135, C12292/A11174, C1281/A12014, C5047/A8384, C5047/A15007, C18281/A19169; Grant sponsor: National Institutes of Health; Grant number: CA128978; Grant sponsor: National Institute of Health (NIH) Cancer Post-Cancer GWAS; Grant number(s): U19 CA148537, U19 CA148065, U19 CA148112; Grant sponsor: Canadian Department of Defence; Grant number: W81XWH-10-1-0341; Grant sponsor: UK Department of Health; Grant number: HTA Programme of the NIHR Research; Grant number(s): HTA 96/20/99, ISRCTN20141297; Grant sponsor: National Cancer Research Institute (Department of Health, the Medical Research Council and Cancer Research UK); Grant number: G0500966/75466.

The PRACTICAL Consortium (in addition to those named in the author list)

Information on the consortium can be found at http://practical.cge.medschl.cam.ac.uk/

Additional members from the consortium are Margaret Cook,1 Angela Morgan,2 Artitaya Lophatananon,3,4 Cyril Fisher,2 Daniel Leongamornlert,5 Edward J. Saunders,6 Emma J. Sawyer,7 Koveela Govindasami,2 Malgorzata Tymrakiewicz,2 Michelle Guy,2 Naomi Livni,2 Rosemary Wilkinson,2 Sara Jugurnauth-Little,2 Steve Hazel,2 Tokhir Dadave,7 Melissa C. Southey,9 Liesel M. Fitzgerald,9 John Pedersen,9 John Hopper,9 Carin Cavin-Bjoerkman,9 Jan-Erik Johansson,9 Jan Adolfsen,9 Markus Aly,9,10 Michael Broms,9 Paer Stattin,9 Brian E. Henderson,9 Fredrick Schumacher,11 Anssi Auvinen,11 Kimmo Taari,13 Lisa Maeeataen,14 Paula Kujala,15 Teuvo I.J. Tammela,16,17 Teuvo I.J. Tammela,16,17 Tessa R. Gillen,16,17 Tiina Wallholf,18 Martin Andreas Roder,19 Peter Iversen,19 Peter Klawars,9 Sune F. Nielsen,21,22 Tim J. Key,23 Hans Wallander,24 Paul Brown,25 son,24 Jenny L. Donovan,25 Freddie Hamdy,26 Angela Cox,27 Anne George,28 Athene Lane,28 Gemma Marsden,26 Michael Davis,25 Shaun Paul Pharaoh,29 Lisa B. Signorello,30,31 Wei Zheng,32 Shannon K. McDonnell,33 Daniel J. Schaid,33 Liang Wang,33 Lori Tillmans,33,34 Wojciech Wojciech Risa,33 Thomas Schnoeller,34 Kathleen Herkommer,15 Manuel Luedeke,34 Walther Vogel,34 Dominika Wokozorczyk,37 Jan Lubinski,40 Julio Kluzniak,40 Katja Butterbach,40 Christa Stegmaier,37 Bernd Holleczek,39 Babu Zachariah,41 Hui-Yi Lim,41 Hyun Park,42 James Haley,43 Pow-Sang,44 Maria Rincon,44 Selina Radlein,44 Thomas Sellers,44 Chavdar Slavov,44 Aleksandra Vlahova,44 Atanaska Mitkova,44 Darina Kachyova,44 Elenko Popov,44 Svetla Christova,44 Tihomir Dokov,44 Vanio Mitev,44 Allison Eckert,44 Amanda Spurdle,45 Angus Collins,45 Wood,45 Greg Malone,45 Judith A. Clements,45 Kris Kerr,45 Megan Turner,45 Pamela Saunders,45 Peter Heathcote,45 Srilakshmi Srinivasan,47 Rui Leire Moya,45 Trina Yeaton,45 Australian Prostate Cancer BioResource,47 Joana Santos,47 Carmen Jerónimo,47 Paula Paula,47 Pedro Pinto,47 Henrique,47 Sofia Maia,47 Agnieszka Michal,48 Andrej Kierzek,48 Huihai Wu48 and Robert J. MacInnis,48

Int. J. Cancer: 140, 75–85 (2017) © 2016 The Authors International Journal of Cancer published by John Wiley & Sons Ltd on behalf of UICC
Conflict of interest

Rosalind Eeles reports an honoraria to attend the Genitourinary Cancers Symposium at the American Society of Clinical Oncology Meeting January 2016.
Prostate cancer is the most common cancer in men in developed countries, and is a target for risk reduction strategies. The effects of alcohol consumption on prostate cancer incidence and survival remain unclear, potentially due to methodological limitations of observational studies. In this study, we investigated the associations of genetic variants in alcohol-metabolising genes with prostate cancer incidence and survival. We analysed data from 23,868 men with prostate cancer and 23,051 controls from 25 studies within the international PRACTICAL Consortium. Study-specific associations of 68 single nucleotide polymorphisms (SNPs) in 8 alcohol-metabolising genes (Alcohol Dehydrogenases (ADHs) and Aldehyde Dehydrogenases (ALDHs)) with prostate cancer diagnosis and prostate cancer-specific mortality, by grade, were assessed using logistic and Cox regression models, respectively. The data across the 25 studies were meta-analysed using fixed-effect and random-effects models. We found little evidence that variants in alcohol metabolising genes were associated with prostate cancer diagnosis. Four variants in two genes exceeded the multiple testing threshold for associations with prostate cancer mortality in fixed-effect meta-analyses. SNPs within ALDH1A2 associated with prostate cancer mortality were rs1441817 (fixed effects hazard ratio, $HR_{\text{fixed}} = 0.78$; 95% confidence interval (95%CI):0.66,0.91; $p$ values = 0.002); rs12910509, $HR_{\text{fixed}} = 0.76$; 95%CI:0.64,0.91; $p$ values = 0.003); and rs8041922 ($HR_{\text{fixed}} = 0.76$; 95%CI:0.64,0.91; $p$ values = 0.002). These SNPs were in linkage disequilibrium with each other. In ALDH1B1, rs10973794 ($HR_{\text{fixed}} = 1.43$; 95%CI:1.14,1.79; $p$ values = 0.002) was associated with prostate cancer mortality in men with low-grade prostate cancer. These results suggest that alcohol consumption is unlikely to affect prostate cancer incidence, but it may influence disease progression.

What’s new?
Alcohol may spur prostate cancer progression, though it does not appear to affect incidence, according to new analysis. Variation in genes involved in alcohol metabolism affect how much the body is exposed to carcinogenic metabolites. These authors examined 68 genetic variants in alcohol metabolising enzymes (ADHs) and the enzyme dehydrogenase (ALDHs) to understand the role of genetic variation in prostate cancer risk. While there was no evidence that these variants alter prostate cancer incidence, previous studies showed that SNPs in the ALDH1A2 gene affect prostate cancer mortality. From a public health standpoint, these results suggest reducing alcohol consumption could slow prostate cancer disease progression.

Introduction
Prostate cancer is the most common cancer in men in developed countries, with 758,700 new cases diagnosed and 142,000 deaths in 2012. With increasing uptake of prostate-specific antigen (PSA) testing and the ageing population, prostate cancer incidence is increasing. The factors influencing prostate cancer incidence and survival after diagnosis are poorly understood, therefore more evidence is needed.

Alcohol is a carcinogen associated with oropharyngeal, liver, breast, colorectal and oesophageal cancers. Functional variation in the genes involved in alcohol metabolism result in altered exposure to the carcinogenic metabolites of ethanol, suggesting a mechanism for genetic sensitivity to alcohol to influence the pathogenesis of cancers. For example, populations with an increased prevalence of common genetic variation in the alcohol dehydrogenase gene, that results in
reduced enzyme activity, have an increased risk of oesophageal cancer compared with populations with the fully active enzyme.\(^6\)

At present the role of alcohol use on prostate cancer remains uncertain. The World Cancer Research Fund’s extensive report based on systematic reviews described the evidence as limited and inconclusive,\(^6\) and the International Association for Research on Cancer did not list this cancer site amongst others more apparently caused by alcohol in their Monograph on alcohol’s carcinogenicity.\(^1\) There have been conflicting reports of possible associations of alcohol with various stages or histological grades of prostate cancer,\(^7,12\) and meta-analyses have highlighted the inconsistencies, emphasising the need for further research in this area.\(^13,14\)

The majority of evidence about the effects of alcohol on prostate cancer is from observational studies. One potential limitation of traditional observational research is that the findings can potentially be explained by common causes of both exposure and outcome (confounding factors). Other potential sources of bias are reverse causation and recall bias, where having prostate cancer affects drinking behaviour or its reporting, rather than alcohol consumption increasing the risk of prostate cancer. A prospective study design could mitigate both of these problems, but could still be affected by bias in the form of the “sick quitter” effect, where former heavy drinkers reduce their alcohol intake in middle-age because of comorbidities that may be alcohol-related.

Mendelian randomisation is an approach that uses genetic variants robustly associated with exposures of interest, or their metabolic effects, as instrumental variables to test the un-confounded and unbiased causal effects of those exposures and their metabolic effects with cancer.\(^15\) Mendelian randomisation analyses rely on two approximate laws of Mendelian genetics,\(^15,16\) that at meiosis alleles segregate without any influence of environmental factors and that the inheritance of one trait is independent of the inheritance of others. This allows genetic variation to be used in epidemiological studies as an un-confounded proxy for an environmental exposure,\(^15–17\) in this case alcohol consumption, to estimate the influence of cumulative life-time risk of exposure, to reduce recall bias and the “sick-quitter” effect and to negate reverse causation. All these features are limitations of previous conventional observational studies.\(^7,8,13\) Mendelian randomisation has already been used successfully in both cardiovascular\(^18,19\) and cancer epidemiology\(^20–24\) to clarify the causal effects of alcohol on disease.

In this study, we undertook Mendelian randomisation analyses in which we used variants in alcohol metabolising genes influencing metabolism and intake, to test the causal effect of alcohol exposure on prostate cancer risk and progression. The motivation is that if alcohol intake causally increases prostate cancer risk or progression, then genetic variants associated with metabolic effects of alcohol or increased intake will be differentially represented in cases and controls. We stratified the analysis by histological prostate cancer grade, based on Gleason score, as low- and high-grade prostate cancers have differing natural histories which could be influenced by different risk factors.

**Material and Methods**

**Study populations**

We used phenotypic and genotypic data from 46,919 men (23,868 cases) in the international Prostate cancer association group to investigate cancer-associated alterations in the genome (PRACTICAL) consortium. Data were provided by 25 studies within the consortium, based in USA, Australia and European countries. This study population was limited to those of European ethnicity. The studies used a number of methods of recruitment, including screen and clinically detected cases and participants selected due to a family history of prostate cancer. The background characteristics of the participants of each study are shown in Table 1. Gleason scores were used to categorise cancers as low grade (Gleason score ≤6) or high grade (Gleason score ≥7). Further details are available from the consortium website (practical.cge.medschl.cam.ac.uk). All studies adhered both to national ethical guidelines and to the principles of the Declaration of Helsinki.

**Genotyping data**

The participants were genotyped using a custom Illumina Infinium genotyping array (iCOGS), which was specifically designed for the Collaborative Oncological Gene-environmental Study (COGS) and recorded 211,155 SNPs (details available from: http://ec.europa.eu/research/health/medical-research/cancer/fp7-projects/cogs_en.html).\(^25,26\) The iCOGS array was designed to investigate SNPs in regions thought to be associated with breast, ovarian and prostate cancer; 68,638 of the SNPs on the array were chosen because of their potential role in prostate cancer aetiology. The other 125,877 SNPs were selected on the basis of potential importance for other cancers and common SNPs known to be associated with any other traits. Individuals with fewer than 95% of genotypes called, or high or low heterozygosity ($p < 1 \times 10^{-5}$) were dropped from our analysis. In total 201,598 SNPs passed quality control. The genotypic data were used to impute SNPs which were not directly genotyped, but were in linkage disequilibrium (LD) with genotyped SNPs. We used the HapMap 2 CEU reference panel and IMPUTE2 software.\(^27\)

In this study, we searched the iCOGS array database for all alcohol-metabolising genetic variants (within Alcohol Dehydrogenases (ADHs) or Aldehyde Dehydrogenases (ALDHs) genes), and identified 68 common variants in 5 distinct genomic regions: the ADH cluster on chromosome 4, comprising ADH1A, ADH1B, ADH1C and ADH7; ALDH1A1 and ALDH1B1 on chromosome 9; ALDH1A2 and ALDH1A3 on chromosome 15. Of these SNPs, 67 were directly genotyped, and one was imputed. An overview of the genes’ role in alcohol metabolism and behaviour is shown in Supplementary material.
Table S1. The characteristics of the SNPs included in this study are shown in Supplementary material Table S2.

Statistical analysis
We converted the genotypic data for each SNP into a count of the number of minor alleles at each locus. We used logistic regression to estimate the associations of the SNPs with prostate cancer risk (cases vs. controls), for all cases and stratified by high- vs. low-grade disease. In a case-only analysis, we used Cox proportional hazards regression to estimate associations of each SNP with prostate cancer-specific mortality, stratified by grade of prostate cancer. All regression analyses were adjusted for the first 8 principal components of population stratification, since these genomic regions show marked variation across different populations and so do prostate cancer incidence and survival.

When checking the proportional hazards assumption, we found little evidence of violation. These regressions were performed for each study and then meta-analysed using both fixed- and random-effects models. Studies were excluded from the survival meta-analysis if there were <5 deaths during the follow up period or <90% completion of follow up data. We investigated between-study heterogeneity using the Stata metan command to estimate the $I^2$ statistic assuming a fixed-effect model; we also report random-effect models for completeness as such models may be relevant where $I^2$ values are high (e.g., >75%).

Using meta-regression, we investigated whether heterogeneity could be explained by the following a priori defined study-specific characteristics: mean age at diagnosis, mean PSA at diagnosis, country of study (USA vs. elsewhere), and the percentage of participants with a family history of prostate cancer. Manhattan plots of the associations in the five chromosomal regions were constructed to identify SNPs exceeding the Nyholt corrected $p$ values threshold for association—a multiple testing correction which accounts for LD between the SNPs.

Sensitivity analyses were conducted by reclassifying low- and high-grade disease as <8 and 8–10 Gleason grade, respectively.

The power of our study was also assessed using reverse power calculations to demonstrate the effect size we would...
expect to detect given our sample size and \( \alpha = 0.05 \) with SNPs of a range of minor allele frequencies. The analysis was carried out using Stata v.13.1. The statistical code used to produce these results can be accessed here (https://github.com/nmdavies/practical-alcohol/).

**Results**

The background characteristics of the participants are summarised in Table 1, by study. Variation between studies reflects their individual recruitment methods (e.g., some studies selected for those men with a positive family history of prostate cancer).

The Manhattan plots with results from both fixed and random effects meta-analyses testing associations between SNPs in the five genomic regions and prostate cancer risk are presented in Figure 1 (detailed results available in Supplementary material Tables S4–S9, and sensitivity analyses with alternative definitions of low- and high-grade presented in Supplementary material Tables S16–S19). The figure and Supplementary material tables show that no SNP exceeded...
the Nyholt corrected $p$ values threshold for association with prostate cancer risk. In case-only analyses, four SNPs exceeded the Nyholt corrected $p$ values threshold for association with prostate cancer-specific mortality in the fixed-effect meta-analysis (summary Manhattan plots presented in Figure 2, and individual SNP results presented in Supplementary material Tables S10–S15, with results of sensitivity analyses with alternative definitions of low- and high-grade presented in Supplementary material Tables S20–S23). Three SNPs within ALDH1A2 were associated with prostate cancer mortality following diagnosis with any prostate cancer: rs1441817 (fixed effects hazard ratio, HR\textsubscript{fixed} = 0.78; 95% confidence interval (95%CI):0.66,0.91, $p$ values = 0.002, $I^2$ = 19.4);
There is evidence that genetic variations in ADH and ALDH genes affecting ethanol metabolism are associated with altered alcohol intake and risk of alcohol dependence. The most extensively studied SNP in the context of alcohol intake is rs1229984 in ADH1B. It has been shown to be associated with increased adverse effects from alcohol intake and reduced consumption. On average minor allele carriers drink 17.2% fewer units/week (95% CI: 15.6%, 18.9%), are less likely to be in the top third of alcohol drinking volume (odds ratio, OR = 0.7; 95% CI: 0.68, 0.73) and are less likely to binge drink (OR = 0.78; 95% CI: 0.73, 0.84). It has also been reported to affect cancer risk at various sites. In our study, the associations of this SNP with prostate cancer diagnosis and survival were OR fixed = 1.00 (95% CI: 0.96, 1.03, p values = 0.87), and HR fixed = 1.11 (95% CI: 0.95, 1.30, p values = 0.17), respectively. Combining the effects of the ADH1B SNP on alcohol intake with the upper confidence intervals from our results implies that a 17% reduction in alcohol consumption could be associated with a reduced risk of prostate cancer.

**Table 2. Results of univariate meta-regressions to test if the association of the two SNPs (representing the two signals observed) is affected by selected study characteristics**

| Single nucleotide polymorphism | Study characteristic | Ratio of odds-ratios | Confidence intervals | p Values |
|-------------------------------|----------------------|----------------------|----------------------|---------|
| rs1441817                     | PSA                  | 1.48                 | 0.75                 | 2.93    | 0.19 |
| (ALDH1A2)                     |                      | 0.63                 | 0.35                 | 1.14    | 0.10 |
| USA                           |                      | 0.69                 | 0.30                 | 1.57    | 0.30 |
| Age                           |                      | 1.58                 | 1.00                 | 2.51    | 0.05 |
| rs10973794                    | PSA                  | 0.65                 | 0.23                 | 1.80    | 0.33 |
| (ALDH1B1)                     |                      | 0.65                 | 0.25                 | 1.68    | 0.27 |
| USA                           |                      | 1.30                 | 0.29                 | 5.72    | 0.65 |
| Age                           |                      | 1.30                 | 0.62                 | 2.72    | 0.40 |

PSA, mean PSA at diagnosis. FHX, percentage family history. USA, study location in USA vs. rest of world. Age, mean age at diagnosis.
alcohol consumption is unlikely to reduce prostate cancer risk by >3% and prostate cancer mortality by >5%.

Alcohol is metabolised to acetaldehyde, a known carcinogen, and there is evidence to support the theory that genetic variants in alcohol metabolising genes, which control the production and breakdown of acetaldehyde, contribute to carcinogenesis.4,5,20,24 There is also evidence of a tissue-specific interaction in the prostate between ethanol and retinoic acid, through modulations of ALDH1A1, ALDH1A2 and ALDH1A3 levels.38 To our knowledge, this is the first comprehensive investigation of the association between ADH and ALDH variants, as genetic proxies for alcohol, and prostate cancer to date. Genetic predisposition to prostate cancer has been examined by GWASs, which shows common genetic variants can explain 33% heritability of prostate cancer but no genome-wide significant hits are in ADHs or ALDHs39,40 (however, this lack of evidence from GWASs could be a type 2 error). Similarly, we did not find any evidence of genetic association between ADH/ALDH variants and prostate cancer incidence in this study. Possible reasons for this include type 2 error, especially if the underlying effects of alcohol on prostate cancer incidence are small and limited to the very heavy drinking behaviours and/or to the more aggressive forms of disease, as possibly suggested by the recent literature.7,12

We have shown that SNPs in ALDH1A2 are associated with altered prostate cancer-specific mortality in a case-only analysis. None of these SNPs appear to have regulatory features (www.ensemble.org), so they are unlikely to be causal variants themselves but rather they could be in LD with the causal variants. Recently, ALDH isoforms have been suggested as possible mechanistic mediators of metastasis in prostate cancer in particular41 and other solid tumours in general.42 One study found lack of compelling evidence linking variation in ALDH1 (including ALDH1A1, ALDH1A2, ALDH1A3 and ALDH1B1) with prostate cancer progression,41 but another had reported preliminary evidence for a potential role of ALDH1A2 as a tumour suppressor gene in prostate cancer cell lines43 and decreased expression of ALDH1A2 has been associated with shorter recurrence free survival in patients with prostate cancer.43 In our study, three intronic SNPs in ALDH1A2 were associated with longer survival, none of which were directly or indirectly (through LD) associated with alcohol-related phenotypes (http://www.ebi.ac.uk/gwas/). One potential explanation for our results may be that these SNPs, or others in LD with them, lead to increased activity in ALDH1A2. We speculate that the observed prostate cancer survival effect could be the result of a net increase in the synthesis of retinoic acid (by ALDH1A2), which is particularly beneficial when the rate of conversion is affected by slower ADH activity in the presence of alcohol consumption (retinol and ethanol both being ADH substrates,44 and ethanol modulating retinoic acid synthesis in the rat prostate45).

Another intronic SNP in ALDH1B1 (also close to IGFBP1) was found to be associated with increased mortality following a diagnosis of low-grade prostate cancer. ALDH1B1 is the second most abundant mitochondrial ALDH, after ALDH2, with documented involvement in alcohol metabolism and dependence.45 However, this specific SNP is not known to be in LD with any of the variants associated with alcohol phenotypes to date, therefore we cannot speculate on its specific role in relation to alcohol. Evidence has been previously found linking levels of ALDH1B1 to survival following gastric cancer46 and non-small-cell lung cancer,47 but not prostate cancer,41 however the latter was a study in vitro Conversely, ALDH1A1 expression in the prostate has been reported to be a good candidate prognostic biomarker, based on all cause mortality and to a lesser extent prostate cancer-specific mortality,48 and ALDH1A3 expression is thought to be involved with initiation and progression of several cancers,49 however we did not observe an association with common germ-line mutations in either of these genes, or did we observe associations with variation in ADH genes. This could be due to different functional effects of variants on metabolic levels (e.g., alcohol and acetaldehyde peak levels and cumulative concentrations), affecting prostate cancer proliferation or survival differently both in terms of effect sizes and pathways.

Differences by tumour grade

In this study, we have found that genetic variants in genes involved in alcohol metabolism were associated with disease-specific mortality in men with prostate cancer, most of whom had been diagnosed with low-grade disease. We found a signal specific to low-grade prostate cancer survival, but none for high-grade disease. Possible reasons why stronger associations were not seen with high-grade cancers include: limited power, as there were smaller numbers of high compared with low-grade cases (this was investigated using a reverse power calculation, to assess the power of this study to detect small associations (Supplementary material Table S3)); patients behaviour may change following a diagnosis, e.g. patients with high-grade prostate cancer may be too ill to drink and the effect of the SNPs cannot be seen in the absence of drinking; or the findings could reflect a true clinical difference in the way alcohol affects survival for the different grades of prostate cancer.

Strengths and limitations

The strengths of this study include the large sample size and availability of data on both risk and mortality stratified by grade, which is an important predictor of prognosis. Importantly, we used a Mendelian randomisation approach, which minimises the potential for bias due to confounding, information bias (recall bias and sick-quitter effect) and reverse causation, major limitations of previous studies in this area.7,8,13 We were also able to control for confounding by population stratification by adjusting for basic population
characteristics. A potential limitation of our study is its power to detect small effects of alcohol on high-grade disease, with fewer of these cases having been diagnosed and followed-up.

Data for this study were contributed to the PRACTICAL Consortium from many studies with varying recruitment and inclusion/exclusion criteria, as different screening practices could complicate the interpretation of our results. For example, CAPS participants were all diagnosed clinically, whereas ProtecT participants were all screen detected. While the consortium provides a large sample size for investigation, there is inevitably some heterogeneity in the contributing studies. The effect of this was investigated using random effects meta-analysis and meta-regression. No one study-level characteristic had a strong influence on the results, and we were unable to clearly determine the reason for the modest levels of heterogeneity observed, and the consequent variation between the fixed-effect and random-effect analyses. Potential explanations include: systematic differences in smaller vs. larger studies, and the former being assigned larger weights in random-effect models; true variation in the effects of alcohol in the different study populations; effects of study designs that we were not able to investigate, e.g. the different ways cases were ascertained/recruited.

Another possible limitation to consider is the potential influence of pleiotropy. There may be other direct pathways through which the SNPs influence prostate cancer mortality independently of alcohol metabolism and intake. In particular, SNPs in ALDH1A2 could have a role in retinoic acid independently of alcohol metabolism and intake. For example, ProtecT participants were all screen detected. While the consortium provides a large sample size for investigation, there is inevitably some heterogeneity in the contributing studies. The effect of this was investigated using random effects meta-analysis and meta-regression. No one study-level characteristic had a strong influence on the results, and we were unable to clearly determine the reason for the modest levels of heterogeneity observed, and the consequent variation between the fixed-effect and random-effect analyses. Potential explanations include: systematic differences in smaller vs. larger studies, and the former being assigned larger weights in random-effect models; true variation in the effects of alcohol in the different study populations; effects of study designs that we were not able to investigate, e.g. the different ways cases were ascertained/recruited.

Future directions

There are a number of ways in which this work could be taken forward. These include further analysis in larger consortia with longer follow up data availability, or repeating within individual subsets with certain study design characteristics to increase similarity of studies included in analysis (direct replication). Analysis of further genetic variants with known effects on alcohol metabolism or behaviour would also allow further development of this work (indirect replication), as would establishing the magnitude and direction of effect of genetic variants in alcohol metabolising genes on alcohol intake. It would also be interesting to investigate patient behaviour following diagnosis to establish if the varied effect seen between high- and low-grade disease could be due to differences in behaviour.

Conclusion

If confirmed in independent studies or through direct or indirect replication, these findings suggest a role for alcohol in the progression of prostate cancer, whilst also confirming that alcohol is unlikely to have a large impact on prostate cancer carcinogenesis. This has potential public health implications and alcohol intake could be targeted to improve survival from prostate cancer as part of holistic care.

Acknowledgements

The Collaborative Oncological Gene-environment Study (COGS), within which the PRACTICAL Consortium was assembled, would not have been possible without the contributions of the following: Per Hall (COGS); Douglas F. Easton, Paul Pharoah, Kyriaki Michailidou, Manjeet K. Bolla, Qin Wang (BCAC), Andrew Berchuck (OCAC), Rosalind A. Eeles, Douglas F. Easton, Ali Amin Al Olama, Zsofia Kote-Jarai, Sara Benlloch (PRACTICAL), Georgia Chenevix-Trench, Antonis Antoniou, Lesley Mcguinness, Ferrus Cousin and Ken Offit (CIMBA), Joe Dennis, Alison M. Dunning, Andrew Lee, and Ed Dicks, Craig Luccarini and the staff of the Centre for Genetic Epidemiology Laboratory, Javier Benitez, Anna Gonzalez-Neira and the staff of the CNIO genotyping unit, Jacques Simard and Daniel C. Tessier, Francois Bacot, Daniel Vincent, Sylvie LaBoissiere and Frederic Robidoux and the staff of the McGill University and Genomique Quebec Innovon Centre, Stig E. Bojesen, Sune F. Nielsen, Borge G. Nordestgaard, and the staff of the Copenagen DNA laboratory, and Julie M. Cunningham, Sharon A. Windebank, Christopher A. Hilker, Jeffrey Meyer and the staff of Mayo Clinic Genotyping Core Facility.

References

1. Torre LA, Bray F, Siegel RL, et al. Global cancer statistics, 2012. Cancer J Clin 2013;63:8–29.
2. Crawford ED. Understanding the epidemiology, natural history, and key pathways involved in prostate cancer. Urolology 2009;73:3–4–10.
3. Andriole GL. Overview of pivotal studies for prostate cancer risk reduction, past and present. Urology 2009;73:36–40.
4. Baan R, Straif K, Grosse Y, et al. Carcinogenicity of alcoholic beverages. Lancet Oncol 2007;8:292–3.
5. Druenne-Pecollo N, Tehard B, Mallet Y, et al. Alcohol and genetic polymorphisms: effect on risk of alcohol-related cancer. Lancet Oncol 2009;10:173–80.
6. Research WCRFaAIC. Alcoholic beverages. In: Research WCRFaAIC, ed. Systematic literature review on diet and prostate cancer. Food, nutrition, physical activity, and the prevention of cancer: a global perspective. Second Expert Report ed. Washington DC: AICR, 2007.
7. Zuccolo L, Lewis SJ, Donovan JL, et al. Alcohol consumption and PSA-detected prostate cancer risk—a case-control nested in the ProtecT study. Int J Cancer 2013;132:2176–85.
8. Sawada N, Inoue M, Iwasaki M, et al. Alcohol and smoking and subsequent risk of prostate cancer in Japanese men: the Japan Public Health Center-based prospective study. Int J Cancer 2014;134:971–8.
9. Watters JL, Park Y, Holmbeck A, et al. Alcoholic beverages and prostate cancer in a prospective US cohort study. Am J Epidemiol 2010;172:773–80.
10. Chao C, Haque R, Van Den Eeden SK, et al. Red wine consumption and risk of prostate cancer: the California men’s health study. Int J Cancer 2010;126:171–9.
11. Breslow RA, Chen CM, Graubard BI, et al. Prospective study of alcohol consumption quantity and frequency and cancer-specific mortality in the US population. Am J Epidemiol 2011;174:1044–53.
12. Gong ZH, Kristal AR, Schenk JM, et al. Alcohol consumption, finasteride, and prostate cancer risk results from the prostate. Cancer 2009;115:3661–9.

13. Middleton FK, Chikritzhs T, Stockwell T, et al. Alcohol use and prostate cancer: a meta-analysis. Mol Nutr Food Res 2009;53:240–55.

14. Rota M, Scotti I, Turati F, et al. Alcohol consumption and prostate cancer risk: a meta-analysis of the dose-risk relation. Eur J Cancer Prev 2012;21:350–9.

15. Davey Smith G, Ebrahim S. ‘Mendelian randomization’: can genetic epidemiology contribute to understanding environmental determinants of disease? Int J Epidemiol 2003;32:1–22.

16. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23: R89–R98.

17. Davey Smith G, Lawlor DA, Harbord R, et al. Clustered environments and randomized genes: a fundamental distinction between conventional and genetic epidemiology. PLoS Med 2007;4:e352.

18. Holmes MV, Dale CE, Zuccolo L, et al. Association between alcohol and cardiovascular disease: Mendelian randomisation analysis based on individual participant data. BMJ 2014;349:g4164.

19. Han HG, Wang HS, Yin ZT, et al. Association of genetic polymorphisms in ADH and ALDH2 with risk of coronary artery disease and myocardial infarction: a meta-analysis. Gene 2013;526: 134–41.

20. Boccia S, Hashibe M, Galli P, et al. Aldehyde dehydrogenase 2 and head and neck cancer: a meta-analysis implementing a Mendelian randomization approach. Cancer Epidemiol Biomarkers Prev 2009;18:248–54.

21. Hashibe M, McKay JD, Curado MP, et al. Multiple ADH genes are associated with upper aerodigestive cancers. Nat Genet 2008;40:707–9.

22. Guo XF, Wang J, Yu SJ, et al. Meta-analysis of the ADH1B and ALDH2 polymorphisms and the risk of colorectal cancer in East Asians. Intern Med 2013;52:2693–9.

23. Tsai ST, Wong TY, Ou CY, et al. The interplay between alcohol consumption, oral hygiene, ALDH2 and ADH1B in the risk of head and neck cancer. Int J Cancer 2014;135:2424–36.

24. Hidaka A, Sasazuki S, Matsuo K, et al. Genetic polymorphisms of ADH1B, ADH1C and ALDH2, alcohol consumption, and the risk of gastric cancer: the Japan Public Health Center-based prospective study. Carcinogenesis 2015;36:223–31.

25. Kote-Jarai Z, Easton DF, Stanford JL, et al. Multiple novel prostate cancer predisposition loci confirmed by the PRACTICAL Consortium. Cancer Epidemiol Biomarkers Prev 2008;17:2052–61.

26. Eeles RA, Al Olama AA, Benlloch S, et al. Identification of 23 new prostate cancer susceptibility loci using the iCOGS genotyping array. Nat Genet 2013;45:385–91.

27. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet 2009;5:e1000529.

28. Higgins JPT, Thompson SG, Deeks JJ, et al. Measuring inconsistency in meta-analyses. Br Med J 2003;327:557–60.

29. Nyholt DR. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. Am J Hum Genet 2004;74:765–9.

30. Purcell S. Genetic Power Calculator, 2008. Available from: http://pngu.mgh.harvard.edu/~purcell/gpc/cci.html.

31. Frank J, Cichon S, Treutlein J, et al. Genome-wide significant association between alcohol dependence and a variant in the ADH1 gene cluster. Addict Biol 2012;17:171–80.

32. Gyamfi MA, Kocsis MG, He L, et al. The role of retinoic X receptor alpha in regulating alcohol metabolism. J Pharmacol Exp Ther 2006;319:360–8.

33. Birley AJ, James MR, Dickson PA, et al. ADH1 single nucleotide polymorphism associations with alcohol metabolism in vivo. Hum Mol Genet 2009;18:1533–42.

34. Edenberg HJ. The genetics of alcohol metabolism—role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 2003;27:170–4.

35. Linneberg A, Gonzalez-Quintela A, Vidal C, et al. Genome-wide association study of alcohol consumption and health in the Danish population. PLoS Genet 2009;5:e1000529.

36. Macgregor S, Lind PA, Bucsholz KK, et al. Association of ADH and ALDH2 gene variation with self reported alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet 2009;18:880–93.

37. Lind PA, Eriksson CJ, Wilhelmsen KC. The role of aldehyde dehydrogenase-1 (ALDH1A1) polymorphisms in harmful alcohol consumption in a Finnish population. Hum Genomics 2008;3:24–35.

38. Fioruci-Fontanelli BA, Chaffa LF, Mendes LO, et al. Ethanol modulates the synthesis and catabolism of retinoic acid in the rat prostate. Reprod Toxicol 2015;53:1–9.

39. Al Olama AA, Kote-Jarai Z, Berndt SI, et al. A meta-analysis of 87,040 individuals identifies 23 new susceptibility loci for prostate cancer. Nat Genet 2014;46:1103–9.

40. Eeles R, Goh C, Castro E, et al. The genetic epidemiology of prostate cancer and its clinical implications. Nat Rev Urol 2014;11:18–31.

41. van den Hoogen C, van der Horst G, Cheung H, et al. High aldehyde dehydrogenase activity identifies tumor-initiating and metastasis-initiating cells in human prostate cancer. Cancer Res 2010;70:5163–73.

42. Rodriguez-Torres M, Allan AL. Aldehyde dehydrogenase as a marker and functional mediator of metastasis in solid tumors. Clin Exp Metastasis 2016;33:97–113.

43. Kim H, Lapointe J, Rawaygus G, et al. The retinoic acid synthesis gene ALDH1a2 is a candidate tumor suppressor in prostate cancer. Cancer Res 2005;65:818–24.

44. Chou CF, Lai CL, Chang YC, et al. Kinetic mechanism of human class IV alcohol dehydrogenase functioning as retinol dehydrogenase. J Biol Chem 2002;277:25209–16.

45. Jackson BC, Holmes RS, Backos DS, et al. Comparative genomics, molecular evolution and computational modeling of ALDH1B1 and ALDH2. Chem Biol Interact 2013;202:11–21.

46. Shen JX, Liu J, Li GW, Huang YT, Wu HT. Mining distinct aldehyde dehydrogenase 1 (ALDH1) isoforms in gastric cancer. Oncotarget 2013;7: 25340–9.

47. You Q, Guo H, Xu D. Distinct prognostic values and potential drug targets of ALDH1 isoforms in non-small-cell lung cancer. Drug Des Dev Ther 2015;9:5087–97.

48. Zhao I, Yu N, Guo T, et al. Tissue biomarkers for prognosis of prostate cancer: a systematic review and meta-analysis. Cancer Epidemiol Biomarkers Prev 2014;23:1047–54.

49. Duan JJ, Cai J, Guo YF, Bian XW, Yu SC. ALDH1A3, a metabolic target for cancer diagnosis and therapy. Int J Cancer 2016;139:965–75.

50. Garcia-Chonas M, de Gonzalez AB. Invited commentary: screening and the elusive etiology of prostate cancer. Am J Epidemiol 2015;182:390–3.

51. Cai Z, Chen HT, Boyle B, et al. Identification of a novel insulin-like growth factor binding protein gene homologue with tumor suppressor like properties. Biochem Biophys Res Commun 2005;331:261–6.