Research Article

Cancer Epidemiology, Biomarkers & Prevention

Reassessing the Association between Circulating Vitamin D and IGFBP-3: Observational and Mendelian Randomization Estimates from Independent Sources

Vanessa Y. Tan[1,2], Kalina M. Biernacka[3,4], Tom Dudding[1,2], Carolina Bonilla[1,2], Rebecca Gilbert[2], Robert C. Kaplan[5], Qi Qibin[5], Alexander Teumer[6], Richard M. Martin[1,2,4], Claire M. Perks[3]; the PRACTICAL consortium; Nicholas J. Timpson[1,2], and Jeff M.P. Holly[3]

Abstract

Background: Circulating insulin-like growth factor binding protein 3 (IGFBP-3) has been associated with prostate cancer. Preclinical studies found that vitamin D regulates IGFBP-3 expression, although evidence from epidemiologic studies is conflicting.

Methods: Mendelian randomization analyses (MR) were conducted to reassess associations between IGFBP-3 and prostate cancer risk and advanced prostate cancer using summary statistics from the PRACTICAL consortium (44,825 cases; 27,904 controls). Observational and MR analyses were conducted to assess the relationship between inactive vitamin D [25(OH)D] and IGFBP-3 using data from the ProtecT study (1,366 cases;1,071 controls) and summary statistics from the CHARGE consortium ($n = 18{,}995$).

Results: The OR for prostate cancer per SD unit increase in circulating IGFBP-3 was 1.14 [95% confidence interval (CI), 1.02–1.28]. The OR for advanced prostate cancer per SD unit increase in IGFBP-3 was 1.22 (95% CI, 1.07–1.40). Observationally, a SD increase in 25(OH)D was associated with a 0.1SD (95% CI, 0.05–0.14) increase in IGFBP-3. MR analyses found little evidence for a causal relationship between circulating 25(OH)D and IGFBP-3 in the circulation.

Conclusions: This study provided confirmatory evidence that IGFBP-3 is a risk factor for prostate cancer risk and progression. Observationally, there was evidence that 25(OH)D is associated with IGFBP-3, but MR analyses suggested that these findings were unlikely to be causal. Findings may be limited by the nature of instrumentation of 25(OH)D and IGFBP-3 and the utility of circulating measures. 25(OH)D appears unlikely to be causally related to IGFBP-3 in the circulation, however, our findings do not preclude causal associations at the tissue level.

Impact: IGFBP-3 is a prostate cancer risk factor but 25(OH)D are unlikely to be causally related to IGFBP-3 in the circulation. *Cancer Epidemiol Biomarkers Prev; 27(12); 1462–71. ©2018 AACR.*

Introduction

Insulin-like growth factor binding protein 3 (IGFBP-3) is the most abundant circulating IGFBP and modulates the bioactivity of IGFs (1). Independent of IGFs, IGFBP-3 regulates cell proliferation, leading to increased interest in its role in carcinogenesis (2). A meta-analysis found that circulating IGFBP-3 was associated with increased odds of prostate cancer [OR for highest versus lowest quintile: 1.25; 95% confidence interval (CI),1.12–1.40; ref. 3]. This is in line with results from a nested case–control study within the Prostate Testing for Cancer and Treatment (ProtecT) trial, which found evidence that circulating IGFBP-3 was associated with increased prostate cancer odds (OR 1.28 per SD increase in IGFBP-3; 95% CI, 1.21–1.36; ref. 4). There is inconsistent evidence regarding the association between circulating IGFBP-3 and aggressive prostate cancer. However, a Mendelian randomization (MR) study concluded that the IGFs may be associated with more aggressive prostate cancer, in particular IGFBP-3 (5).

Active vitamin D [1,25-hydroxyvitamin D;1,25$(OH)_2$D] upregulates IGFBP-3 expression by binding to the vitamin D receptor

[1]Medical Research Council (MRC) Integrative Epidemiology Unit, Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom. [2]Population Health Sciences, Bristol Medical School, University of Bristol, Bristol, United Kingdom. [3]IGFs & Metabolic Endocrinology Group, School of Translational Health Sciences, Learning & Research Building, Southmead Hospital, Bristol, United Kingdom. [4]National Institute for Health Research (NIHR) Bristol Biomedical Research Centre, University Hospitals Bristol NHS Foundation Trust and the University of Bristol, Bristol, United Kingdom. [5]Department of Epidemiology and Population Health, Albert Einstein College of Medicine, Bronx, New York. [6]Institute for Community Medicine, University Medicine Greifswald, Greifswald, Germany.

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

Members from the PRACTICAL Consortium (http://PRACTICAL.icr.ac.uk) are provided in the Supplementary Materials.

Corresponding Author: Nicholas J. Timpson, University of Bristol, MRC IEU, Oakfield House, Oakfield Grove, Bristol, BS8 2BN, UK. Phone: 44 117 3310131; E-mail: N.J.Timpson@bristol.ac.uk

doi: 10.1158/1055-9965.EPI-18-0113

which in turn binds to the vitamin D response element in the *IGFBP-3* promoter (6, 7). In the context of this relationship, there is motivation to assess the relationship between IGFBP-3 and the inactive form of vitamin D [25-hydroxyvitamin D;25(OH)D], as it acts as a proxy for $1,25(OH)_2D$ and is the most likely form of supplementation (8, 9). Evidence from observational studies examining the direct association between circulating 25(OH)D and $1,25(OH)_2D$ with prostate cancer incidence and progression been inconclusive (10). However, there is evidence that SNPs in vitamin D pathway are associated with higher prostate cancer grade (11). Despite this, a recent MR study using SNPs [representing lower 25(OH)D] failed to provide evidence of a causal association between 25(OH)D and risk of seven types of cancers including prostate cancer (12). Currently, it is unlikely that 25(OH)D exerts large linear effects on prostate cancer, but small or nonlinear effects cannot be ruled out (13). Indeed, preclinical studies have suggested that a possible mechanism for a vitamin D effect could be through the regulation of IGFBP-3 (14–16).

Evidence from observational studies investigating the association between circulating 25(OH)D and IGFBP-3 has been conflicting. To compound this, observational studies can suffer from the effects of confounding, bias and reverse causation (17–20). Specifically, reverse causation could exist as IGFBP-3 can affect 25(OH)D through its effect on IGF-I and residual confounding might be present due to inadequate control for confounders such as BMI. Further examination of the relationship between 25(OH)D and IGFBP-3 is needed to clarify if vitamin D affects cancer via IGFBP-3.

MR, which uses genetic variants as proxies of an exposure, is a causal analysis method used to investigate causality between exposures and health outcomes. Associations between SNPs and outcomes can provide evidence of causation because they are not subjected to bias (i.e., reverse causation and confounding) found in observational studies (21). Recently, two meta-analyses of genome-wide association studies (GWASs) identified SNPs associated with circulating IGFBP-3 (22) and 25(OH)D (23), respectively. The SNPs associated with circulating IGFBP-3 and 25(OH)D provide a framework to assess the consequence of lifelong 25(OH)D on IGFBP-3 independent of other factors.

In this study, we aimed to re-assess the causal relationship between IGFBP-3 and prostate cancer. We also aimed to undertake both observational and one-sample MR analyses within ProtecT to assess the observational and causal relationship between 25(OH)D and IGFBP-3 and to compare these in order to assess the impact of potential bias, confounding or reverse causality. We also aimed to investigate whether there is a causal effect of 25(OH)D on IGFBP-3 using two-sample MR in independent data from the IGF working group of the CHARGE consortium.

Materials and Methods

Study design

This investigation had three components (Fig. 1): (i) two-sample MR analyses were conducted to reassess the causal relationship between IGFBP-3 and prostate cancer risk and advanced prostate cancer (Gleason $\geq$ 8, death from prostate cancer, PSA > 100 or metastasis) using summary statistics from the PRACTICAL consortium; (ii) linear regression analyses were conducted to investigate the observational relationship between circulating 25(OH)D and IGFBP-3 using individual level data from ProtecT; and (iii) one-sample MR analyses using individual level data from ProtecT and two-sample MR analyses (24, 25) using summary statistics from the IGFBP-3 GWAS were conducted to investigate the causal relationship between 25(OH)D and IGFBP-3.

Study populations and data sources for observational and MR analyses

Individual level analyses (observational and one-sample MR) were examined in a nested case–control study within the ProtecT

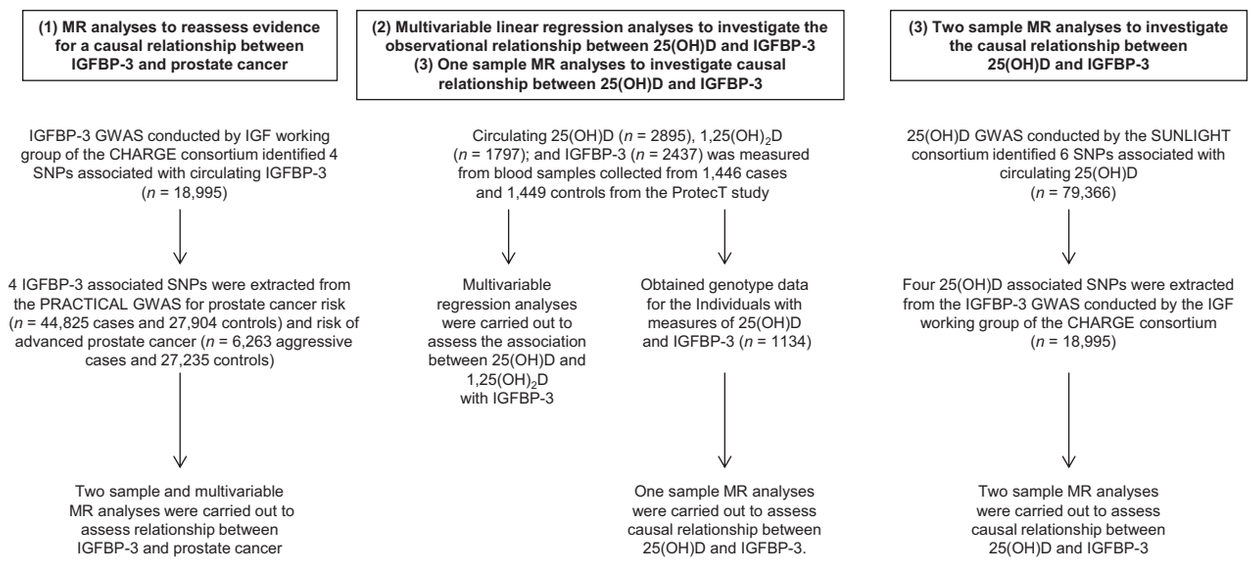

Figure 1.
Flow diagram of study design. Flow diagram showing how the study population or data were selected for observational and MR analyses.

trial (details of the cohort are described in the Supplementary Materials and Methods; refs. 26, 27). For the observational analyses, 1,446 cases and 1,449 controls with 25(OH)D and IGFBP-3 measured at diagnosis and before treatment were included. Circulating 25(OH)D in blood plasma was measured using tandem mass spectrometry, as described previously (28). Circulating $1,25(OH)_2D$ were measured in blood plasma using immunoassay, as previously described (29). Circulating IGFBP-3 in blood serum was measured using in-house radioimmunoassay, as previously described (Supplementary Materials and Methods; ref. 4). Seasonality in 25(OH)D and $1,25(OH)_2D$ levels were adjusted using the cosinor method as previously described (30). This method was chosen instead of adjusting models for season of blood draw, as it is a more accurate way to assess an individual's vitamin D status (31). Details of how potential confounders (BMI, exercise, smoking, alcohol consumption, family history of prostate cancer, history of benign prostatic hyperplasia, diabetes, social class, and ethnicity) were measured are provided in the Supplementary Materials and Methods. Genome-wide genotyping of participants was carried out using the Illumina Human660W array (Supplementary Materials and Methods). Altogether, 674 cases and 410 controls with genotype data and IGFBP-3 measured were included in the one-sample MR analyses.

A previous MR study (5) had investigated the association between the IGF axis with risk of prostate cancer by identifying SNPs associated with the IGFs from a GWAS for IGFBP-3 (n = 10,018; ref. 32) and candidate gene studies and using summary statistics from a prostate cancer risk GWAS (n = 22,898 cases and 23,054 controls) genotyped using the iCOGS array. A new GWAS for risk of prostate cancer (33) (n = 44,825 cases and 27,904 controls) genotyped using Oncoarray and an updated GWAS for IGFBP-3 (n = 18,995; ref. 22) were recently published. We updated the previous MR analysis for IGFBP-3 and risk of prostate cancer using the latest prostate cancer GWAS meta-analysis (33) and the latest IGFBP-3 GWAS meta-analysis (Supplementary Materials and Methods; ref. 22). For our MR analyses investigating the causal relationship between 25(OH)D and IGFBP-3, we used the following summary statistics: (i) IGFBP-3 GWAS (22) conducted by the IGF working group of the CHARGE consortium (n = 18,995); and (ii) 25(OH)D GWAS (n = 79,366; ref. 34) conducted by the SUNLIGHT consortium (Supplementary Materials and Methods).

Identification of genetic instruments for MR analyses

Instruments for IGFBP-3. The previous MR study (5) investigating the causal relationship between IGFs and prostate cancer used individual SNPs identified either by the discovery IGF GWAS (32) or by candidate gene studies. For our MR analyses, we constructed a new instrument for IGFBP-3 by selecting four independent IGFBP-3 associated SNPs (rs11977526, rs700753, rs1065656, rs4234798) identified by the updated IGFBP-3 GWAS (22). rs700753 was not present in the 1000 genomes imputation reference used in the prostate cancer GWAS. We identified a proxy SNP, rs700752, which was in high linkage disequilibrium ($r^2 > 0.8$) using SNAP (https://www.broadinstitute.org/mpg/snap/).

Instruments for 25(OH)D. To-date, GWAS studies (23, 35, 36) have focused on identifying SNPs associated with 25(OH)D as it is more stable than $1,25(OH)_2D$ in the circulation. The latest 25(OH)D GWAS meta-analysis (34) conducted in Europeans identified two novel loci [*SEC23A* (rs8018720) and *AMDHD1* (rs10745742)] with a genome-wide significant association with 25(OH)D and confirmed four previously identified loci (CYP2R1(rs10741657); DHCR7(rs12785878); GC(rs3755967); and CYP24A1(rs17216707); ref. 23) located in or near genes involved in 25(OH)D synthesis and metabolism. We constructed an instrument for 25(OH)D (allele score) by using the six 25(OH)D-associated SNPs. As sensitivity analyses, we constructed a synthesis (rs12785878 and rs10741657) and metabolism (rs3755967 and rs17216707) score using SNPs in genes involved in 25(OH)D synthesis and metabolism.

Statistical analyses

Causal association between IGFBP-3 with overall cancer risk and advanced prostate cancer. SNP-exposure (IGFBP-3) and SNP-outcome (prostate cancer) estimates for the four IGFBP-3–associated SNPs were combined using inverse-variance weighted (IVW) and maximum likelihood method to provide a weighted average of the causal estimates (37). The previous MR study found that SNPs associated with IGFBP-3 have pleiotropic effects on other biomarkers of the IGF pathway (5). The latest IGF GWAS found that two IGFBP-3–associated SNPs (rs700753 and rs1065656) were also associated with IGF-I at genome-wide significant levels (22). To account for the effect of the IGFBP-3–associated SNPs on IGF-I, we used multivariable MR, a new MR method that estimates causal effects using multiple SNPs associated with multiple exposures simultaneously (38, 39). Multivariable MR was conducted by regression of the SNP-prostate cancer estimates on SNP-IGFBP-3 and SNP-IGF-I estimates in a multivariable weighted regression model. As the IGF GWAS did not analyze other IGFs, the multivariable MR method could not take into account the association between SNPs and other biomarkers of the IGF pathway.

Observational relationship between circulating 25(OH)D and IGFBP-3 in ProtecT. Observational associations between 25(OH)D and IGFBP-3 were assessed using linear regression for cases and controls combined into one cohort or stratified by case–control status. Additional analyses were adjusted for potential confounders (age, center, BMI, smoking, and diabetes status). Associations of 25(OH)D and IGFBP-3 with potential confounders (listed above) were estimated using linear regression. Potential nonlinear effects of 25(OH)D and IGFBP-3 were tested by carrying out linear regression with quadratic terms. Details of the analysis investigating the observational relationship between circulating $1,25(OH)_2D$ and IGFBP-3 in ProtecT are provided in the Supplementary Materials and Methods.

Causal associations between 25(OH)D and IGFBP-3: one-sample MR in ProtecT. A weighted genetic risk score (GRS) for 25(OH)D was generated by taking the sum of genotypes (coded 0,1,2) multiplied by the strength of the effect of each SNP on 25(OH)D recorded by the 25(OH)D GWAS (23). Two-stage least squares analyses using the weighted GRS was used to obtain estimates of the associations between season-adjusted 25(OH)D and IGFBP-3 (40, 41). The properties of the 25(OH)D-associated SNPs as instruments were assessed in ProtecT by examination of: (i) first-stage F-statistic (measure of the strength of the association between the instrument and exposure) and (ii) associations

of the SNPs with potential confounders. The first-stage *F*-statistic was obtained from the regression of 25(OH)D on the 25(OH)D genetic instrument (first stage of the two-stage least squares MR analysis). Sensitivity analyses were also performed by combining the estimates from SNPs involved in the synthesis and metabolism of vitamin D separately. We compared the instrumental variable estimates with those from observational analyses using the Durbin form of the Durbin–Wu–Hausman statistic (42).

Causal associations between 25(OH)D and IGFBP-3: two-sample MR. The SNP-exposure [25(OH)D] and SNP-outcome (IGFBP-3) estimates for the six 25(OH)D-associated SNPs were combined using the IVW method (37). The IVW method (similar method to that used in the two-sample MR analysis of IGFBP-3 and prostate cancer) is equivalent to the two-stage least square analyses using data from ProtecT (43). Sensitivity analyses were performed by combining the estimates from SNPs involved in the synthesis and metabolism pathways separately. As only a small number of SNPs were included in our MR analyses, methods to test for pleiotropy such as MR-Egger regression (43), weighted median (44), and mode method (45) were not conducted as these lack power with small number of SNPs. A leave-one-out analysis, which repeats the MR analysis by leaving out each SNP in turn, was applied to assess whether any single SNP was driving the causal estimate. In the reverse direction, we investigated the causal effect of IGFBP-3 on 25(OH)D using the two-sample MR analyses (see Supplementary Materials and Methods).

Power

Power for one-sample MR analyses to detect the causal effect of 25(OH)D on IGFBP-3 was estimated using the online tool (http://glimmer.rstudio.com/kn3in/mRnd/; ref. 46), specifying $\alpha = 0.05$, $R^2 = 0.05$ (35) and using the observational estimates from the ProtecT sample set. Given a sample size of 1,134, in one-sample MR, a causal effect of 0.39 SD units per SD unit change in 25(OH)D was required to yield 80% power. For two-sample MR analysis of the same relationship, power to detect the causal effect of 25(OH)D on IGFBP-3 was estimated using the "pwr.r.test" function (implemented in the pwr package in R) specifying $\alpha = 0.05$. The correlation between 25(OH)D and IGFBP-3 was obtained by taking the square-root of the variance in IGFBP-3 explained by the 25(OH)D associated SNPs. Given a sample size of 18,995 in the two-sample MR analysis, a causal effect needed to yield 80% power is 0.09 SD units. This method is not dissimilar to that suggested elsewhere noting that power in a two-sample procedure is limited by the direct SNP–outcome association (47). All analyses were performed in R (version 3.0.1), Stata version 14 (Stata Corp) and using MR-Base (www.mrbase.org).

Results

Association between IGFBP-3 with overall cancer risk and advanced prostate cancer

In agreement with the previous MR of IGFBP-3 and prostate cancer (5), two-sample MR analyses using the latest prostate cancer GWAS found that the OR for prostate cancer per SD unit increase in circulating IGFBP-3 was 1.12 (95% CI, 0.91–1.36; $P = 0.18$) from IVW analyses. The multivariable estimate, which took into account the effect of the SNPs on IGF-I, was of similar magnitude to the IVW analyses and gave stronger evidence with an OR of 1.14 (95% CI, 1.02–1.28; $P = 0.02$; Supplementary Table S1). The OR for advanced prostate cancer per SD unit increase in IGFBP-3 was 1.16 (95% CI, 0.87–1.55; $P = 0.19$) from IVW analyses. The multivariable estimate was of similar magnitude to the IVW analyses, but gave stronger evidence with an OR of 1.22 (95% CI, 1.07–1.40; $P = 0.004$) (Supplementary Table S1).

Observational association between circulating 25(OH)D and IGFBP-3 in ProtecT

Mean circulating IGFBP-3 levels were higher in the cases compared with the controls in ProtecT (4634.77 ng/mL *vs.* 4502.19 ng/mL, *P* for difference = 0.002). Mean season-adjusted circulating 25(OH)D levels did not differ between cases and controls (22.75 ng/mL vs. 22.71 ng/mL, respectively, *P* for difference = 0.87). Mean season-adjusted $1,25(OH)_2D$ levels did not differ between cases and controls (40.71 pg/mL vs. 42.06 pg/mL, respectively, *P* for difference = 0.12; Table 1). For cases and controls combined, circulating 25(OH)D and season-adjusted 25(OH)D were positively correlated with $1,25(OH)_2D$ levels ($r = 0.24$; $P < 0.001$ and $r = 0.22$; $P < 0.001$, respectively; Supplementary Table S2).

For cases and controls combined, an SD unit increase (SD = 7.8 ng/mL) in circulating 25(OH)D was associated with a 0.09 SD (95% CI, 0.05–0.13; $P < 0.001$) increase in circulating IGFBP-3 levels. A 0.09 SD unit increase in IGFBP-3 is equivalent to 93.3 ng/mL. There was no strong evidence for difference between cases and controls (Table 2). We found evidence that circulating 25(OH)D and IGFBP-3 are associated with potential confounding factors including age, BMI, and diabetes status (Supplementary Table S3). After adjustment for confounders, the effect ($\beta = 0.09$; 95% CI, 0.04–0.14; $P < 0.001$) was similar in magnitude to the unadjusted analysis. When stratified by case–control status, the precision of the estimates was reduced due to smaller sample sizes (Table 2). We found little evidence for a nonlinear effect of vitamin D on IGFBP-3 ($P = 0.43$).

For cases and controls combined, an SD unit increase in circulating $1,25(OH)_2D$ was associated with a 0.09 SD (95% CI, 0.04–0.14; $P = 0.001$) increase in IGFBP-3 (Table 2). We found evidence for association between $1,25(OH)_2D$ and potential confounders including BMI, family history of prostate cancer, smoking, and diabetes status (Supplementary Table S3). After adjustment for potential confounders, the effect ($\beta = 0.09$; 95% CI, 0.03–0.15; $P = 0.003$) was similar in magnitude to the unadjusted analysis. When stratified by case–control status, the precision of the estimates was reduced due to smaller sample sizes (Table 2).

Validation of the instruments for circulating 25(OH)D in ProtecT

An MR assumption is that there is a reliable association between the genetic instrument and the exposure (48). We validated this using data from ProtecT in which 1,416 individuals had both genotype data and 25(OH)D measured. In cases and controls combined, per unit increase in the allele score was associated with a 0.11 SD unit increase in 25(OH)D. There was no strong evidence for difference between cases and controls. For an individual with the maximum number of 25(OH)D increasing alleles (12), this corresponds to a 1.32 SD

Table 1. Baseline characteristics of participants in the ProtecT study

	Controls ($N = 1,449$)		Cases ($N = 1,446$)	
Continuous variables	***N***	**Mean (SD or IQR)**	***N***	**Mean (SD or IQR)**
IGFBP-3 (ng/mL)	1071	4502.19 (1026.06)	1366	4634.77 (1041.58)
25(OH)D (ng/mL)	1449	22.93 (8.36)	1446	23.05 (8.71)
Season-adjusted 25(OH)D (ng/mL)	1449	22.73 (7.86)	1446	22.75 (7.97)
$1,25(OH)_2D$ (pg/mL)	872	42.03 (18.05)	925	40.85 (18.42)
Season-adjusted $1,25(OH)_2D$ (pg/mL)	872	42.06 (18.02)	925	40.71 (18.24)
Age (years)	1449	62.38 (5.02)	1446	62.59 (5.00)
BMI (kg/m^2)	1067	27.35 (3.94)	1177	27.17 (3.62)
PSA (ng/mL)	1449	1.39 (1.27)	1446	9.39 (26.46)
IGF-I (ng/mL)	1082	165.46 (54.49)	1405	157.69 (52.61)
IGF-II (ng/mL)	1072	759.93 (270.14)	1366	851.38 (314.96)
IGFBP-2 (ng/mL)	1069	694.08 (410.05)	1404	716.26 (407.67)
Categorical variables	***N***	**%**	***N***	**%**
[a]Social class				
Managerial and professional	608	41.96	594	41.08
Intermediate	235	16.22	219	15.15
Working	586	40.44	587	40.59
Missing	20	1.38	46	3.18
[b]Family history of BPH				
No	62	4.28	54	3.73
Yes	1272	87.78	1297	89.70
Possible	84	5.80	63	4.36
Not known/Not given	31	2.14	32	2.21
[c]Family history of prostate cancer				
No	1234	85.16	1182	81.74
Yes	75	5.18	107	7.40
Missing	140	9.66	157	10.86
[d]Smoking				
Never	300	20.70	438	30.29
Ever	705	48.65	768	53.11
Missing	364	25.12	240	16.60
Ethnicity				
White	1428	98.55	1414	97.79
Others	15	1.04	17	1.18
Missing	6	0.41	15	1.04
[e]Diabetes				
Yes	936	64.60	1045	72.27
No	86	5.94	66	4.56
Missing	427	29.47	335	23.17
Study center				
Sheffield	216	14.91	213	14.73
Newcastle	193	13.32	193	13.35
Bristol	132	9.11	130	8.99
Cardiff	148	10.21	147	10.17
Edinburgh	129	8.90	130	8.99
Birmingham	32	2.21	33	2.28
Leicester	265	18.29	264	18.26
Cambridge	163	11.25	162	11.20
Leeds	171	11.80	174	12.03
[f]Physical activity (per week)				
None	481	33.20	550	38.04
1–2 times	355	24.50	367	25.38
3–4 times	159	10.97	184	12.72
5+ times	64	4.42	78	5.39
Missing	390	26.92	267	18.46

NOTE: Normally distributed variables are presented as the means ± standard deviation, non-normally distributed variables are presented as medians (interquartile range) (*).

All men included in the table have both (25(OH)D and IGFBP-3 measured.

Abbreviations: BPH, benign prostatic hyperplasia; BMI, body mass index; IGFBP-2, insulin-like growth factor binding protein-2; IGF-1, insulin-like growth factor 1; IGF-2, insulin-like growth factor 2; *N*, sample size.

[a]Three-class social categorization from Rose and O'Reilly (1998).

[b]Family history of benign prostatic hyperplasia is categorized as "yes," "no," "possible," and "unknown."

[c]Family history of prostate cancer is coded yes if father or brother were diagnosed with prostate cancer.

[d]Smoking status was dichotomized and defined as `ever' (ex-smoker or current smoker) versus "never smokers."

[e]Diabetes status as diagnosed by a doctor and was categorized as "yes" or "no."

[f]Weekly exercise calculated from the frequency of participation in exercise of mild, moderate and strenuous intensity. Weekly exercise was categorized as "None," "1–2 times," "3–4 times," ">5 times."

Table 2. Association between circulating season-adjusted 25(OH)D (ng/mL) and 1,25$(OH)_2$D (ng/mL) with IGFBP-3 for cases and controls in the ProtecT study

	25(OH)D						1,25$(OH)_2$D					
		[a]SD unit change in IGFBP-3 (ng/mL) per SD increase in 25(OH)D			[b]SD unit change in IGFBP-3 (ng/mL) per SD increase in 25(OH)D			[a]SD unit change in IGFBP-3 (ng/mL) per SD increase in 1,25$(OH)_2$D			[b]SD unit change in IGFBP-3 (ng/mL) per SD increase in 1,25$(OH)_2$D	
	N	(95% CI)	*P* value	*N*	(95% CI)	*P* value	*N*	(95% CI)	*P* value	*N*	(95% CI)	*P* value
Cases and controls	2437	0.09 (0.05, 0.13)	<0.001	1559	0.09 (0.04, 0.14)	<0.001	1428	0.09 (0.04, 0.14)	0.001	955	0.09 (0.03, 0.15)	0.003
Controls only	1071	0.07 (0.01, 0.14)	0.02	666	0.07 (−0.001, 0.15)	0.05	562	0.10 (0.03, 0.20)	0.01	354	0.12 (0.03, 0.22)	0.01
Cases only	1366	0.11 (0.05, 0.16)	<0.001	893	0.10 (0.04, 0.16)	0.002	866	0.08 (0.01, 0.15)	0.02	601	0.07 (−0.01, 0.15)	0.10

Abbreviation: *N*, sample size.
[a]Adjusted for case–control status.
[b]Adjusted for age, center, case–control status, PSA levels, smoking status, social class, BMI, ethnicity, family history of prostate cancer, history of benign hyperplasia, physical activity, and diabetes status.

unit difference (on average) in 25(OH)D when compared with an individual with the minimum (0) number of 25(OH)D increasing alleles. This is higher in magnitude and is comparable to that of vitamin D supplementation which increases 25(OH)D by 0.45 SD units (35). In cases and controls combined, the metabolism score was associated with increased 25(OH)D ($P < 0.001$). For the metabolism score, there was no strong evidence for difference between cases and controls. In cases and controls combined, the synthesis score was found to be associated with increased 25(OH)D; however, the precision of the estimates was reduced when stratified by case–control status due to reduced sample size (Supplementary Table S4).

Using the allele score, each additional 25(OH)D-related allele was associated with a 0.05 pg/mL increase in 1,25$(OH)_2$D in cases and controls combined. There was no strong evidence for difference between cases and controls. In cases and controls combined, the metabolism score was also found to be associated with increased 1,25$(OH)_2$D ($P = 0.005$); however, the precision of the estimates was reduced when stratified by case–control status due to reduced sample size (Supplementary Table S4).

Another MR assumption is that the genetic instrument should not be associated with confounders (measured or unmeasured) of the exposure–outcome relationship (48). There was little evidence that the allele, metabolism, or synthesis score were associated with potential confounders (Supplementary Table S5).

Causal associations between 25(OH)D and IGFBP-3: one-sample MR in ProtecT

The causal effect of 25(OH)D on IGFBP-3 was estimated using one-sample MR (Fig. 2). Using the allele score as an instrument for 25(OH)D, for cases and controls combined, the instrumental variable (IV) estimate of the causal relationship between a one SD increase in 25(OH)D and IGFBP-3 for cases and controls combined was −0.32 SD units (95% CI, −0.64 to −0.01; $P = 0.04$). When stratified by case–control status, the IV estimate for the controls was −0.10 (95% CI, −0.62–0.41; $P = 0.69$) and the IV estimate for the cases was −0.30 (95% CI, −0.79 to −0.02; $P = 0.04$). Sensitivity analyses based on the use of individual SNPs, synthesis, and metabolism score showed little evidence that 25(OH)D was causally associated with IGFBP-3 (Table 3; Fig. 3). The first-stage *F*-statistic using the allele score and metabolism score was 46.76 and 41.12, respectively. There was evidence of a departure of instrumental variable-derived estimates from those derived from observational analyses (Durbin–Wu–Hausman test $P = 0.007$; Table 3).

Causal associations between 25(OH)D and IGFBP-3: two-sample MR

Using the six 25(OH)D-associated SNPs for the two-sample MR analysis, the IVW estimate between a one log-unit increase in 25(OH)D and IGFBP-3 was 0.11 SD units (95% CI, −0.10–0.31; $P = 0.32$; Fig. 3). In leave-one-out analyses, sequentially omitting each of the six 25(OH)D-associated SNPs provided similar causal estimates to IVW analysis (Supplementary Table S6).

Sensitivity analyses using SNPs involved in 25(OH)D synthesis found that the IVW estimate between a one log-unit increase in 25(OH)D and IGFBP-3 was 0.15 SD units (95% CI, −0.30–0.61; $P = 0.51$). Using SNPs involved in 25(OH)D metabolism, the IVW estimate between a one log-unit increase in 25(OH)D and IGFBP-3 was 0.07 SD units (95% CI, −0.17–0.31; $P = 0.59$; Fig. 3).

In the reverse direction, we investigated the causal effect of IGFBP-3 on 25(OH)D using the two-sample MR. The IVW estimate between a one SD increase in IGFBP-3 and 25(OH)D was 0.01 log-units (95% CI, −0.003–0.03; $P = 0.10$; Supplementary Table S7).

Discussion

This study supported results from previous studies that the IGF pathway is associated with increased odds of prostate cancer and of advanced prostate cancer—in particular, IGFBP-3 (4, 5).

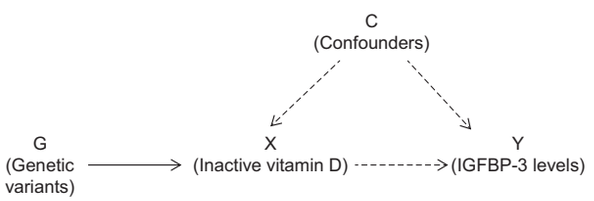

Figure 2.
Mendelian randomization to infer causal nature of relationship between vitamin D and IGFBP-3. Genetic variants associated with circulating 25(OH)D levels are used as proxies for circulating vitamin D levels to assess the causal association between vitamin D and IGFBP-3. Dashed arrows show the potential influence of confounding factors on the original observational association which can also be affected by reverse causation. In Mendelian Randomization analyses, bias due to confounding and reverse causation is greatly reduced as the genetic variants are assigned at conception and remain unchanged throughout a person's lifetime and are not associated with confounders.

Table 3. Beta estimates of SD unit change in IGFBP-3 per SD unit increase in 25(OH)D and 1,25$(OH)_2$D based on one-sample Mendelian randomization analysis in the ProtecT study

	25(OH)D					1,25$(OH)_2$D				
Instruments	***N***	[a]β **(95% CI)**	***P* value**	[b]***F*-statistic**	[c]***P* value (DWH)**	***N***	[a]β **(95% CI)**	***P* value**	[b]***F*-statistic**	[c]***P* value (DWH)**
rs3755967 (overall)	1207	−0.30 (−0.63–0.04)	0.08	39.04	0.02	1,195	−5.30 (−1.24–0.18)	0.14	11.06	0.03
rs3755967 (controls)	487	−0.23 (−0.77–0.31)	0.40	14.67	0.32	485	−0.35 (−1.24–0.53)	0.44	6.35	0.23
rs3755967 (cases)	720	−0.31 (−0.72–0.11)	0.15	24.85	0.04	710	−0.59 (−1.63–0.44)	0.26	5.30	0.10
rs12785878 (overall)	1134	0.30 (−1.47–2.07)	0.74	1.27	0.80	1,124	0.44 (−1.32–2.20)	0.62	1.39	0.71
rs12785878 (controls)	460	1.26 (−9.32–11.83)	0.82	0.08	0.72	458	0.32 (−0.95–1.60)	0.62	2.52	0.76
rs12785878 (cases)	674	0.23 (−1.33–1.79)	0.77	1.58	0.86	666	1.64 (−11.47–14.74)	0.81	0.07	0.68
rs10741657 (overall)	1207	0.03 (−0.67–0.73)	0.93	7.59	0.91	1,195	0.15 (−1.24–1.55)	0.83	1.99	0.97
rs10741657 (controls)	487	1.12 (−0.42–2.65)	0.15	3.57	0.04	485	2.14 (−2.35–6.63)	0.35	0.97	0.05
rs10741657 (cases)	720	−0.76 (−2.01–0.49)	0.23	4.10	0.08	710	−1.36 (−4.75–2.04)	0.43	1.05	0.13
rs17216707 (overall)	1207	−0.36 (−1.69–0.98)	0.60	2.48	0.50	1,195	−1.69 (−11.40–8.01)	0.73	0.18	0.45
rs17216707 (controls)	487	−0.42 (−1.47–0.63)	0.43	4.22	0.35	485	27.35 (−1533.39–1588.10)	0.97	0.001	0.36
rs17216707 (cases)	720	−0.40 (−7.84–7.03)	0.92	0.08	0.88	710	−0.45 (−4.48–3.58)	0.83	0.31	0.75
rs10745742 (overall)	1207	−2.53 (−17.64–12.59)	0.74	0.13	0.35	1195	−6.92 (−103.79–89.94)	0.89	0.02	0.31
rs10745742 (controls)	487	0.04 (−4.43–4.50)	1.00	0.19	1.00	485	−0.10 (−3.80–3.59)	0.95	0.31	0.90
rs10745742 (cases)	720	−7.13 (−105.48–91.21)	0.89	0.02	0.28	710	−1.73 (−7.81–4.35)	0.58	0.45	0.21
rs8018720 (overall)	1207	−1.35 (−3.91–1.21)	0.30	1.76	0.06	1,195	4.48 (−16.65–25.62)	0.68	0.17	0.07
rs8018720 (controls)	487	0.77 (−0.64–2.17)	0.29	3.01	0.21	485	2.46 (−6.73–11.66)	0.60	0.29	0.22
rs8018720 (cases)	720	−0.43 (−1.14–0.29)	0.24	9.24	0.11					
[d]Allele score (overall)	1134	−0.32 (−0.64–−0.01)	0.04	46.76	0.007	1124	−0.61 (−1.33–0.11)	0.10	11.72	0.01
[d]Allele score (controls)	460	−0.10 (−0.62–0.41)	0.69	15.01	0.59	458	−0.15 (−0.99–0.68)	0.72	6.20	0.49
[d]Allele score (cases)	674	−0.40 (−0.79–−0.02)	0.04	32.71	0.004	666	−0.87 (−2.01–0.26)	0.13	5.96	0.02
[e]Synthesis score (overall)	1134	0.03 (−0.69–0.76)	0.93	7.29	0.92	1,124	0.15 (−1.06–1.37)	0.81	2.66	0.96
[e]Synthesis score (controls)	460	0.97 (−0.74–2.69)	0.27	2.44	0.14	458	0.97 (−0.59–2.52)	0.22	2.76	2.77
[e]Synthesis score (cases)	674	−0.42 (−1.40–0.56)	0.40	4.99	0.24	666	−0.98 (4.59–2.64)	0.60	0.65	0.38
[f]Metabolism score (overall)	1207	−0.30 (−0.63–0.03)	0.07	41.12	0.02	1,195	−0.56 (−1.29–0.16)	0.13	10.94	0.02
[f]Metabolism score (controls)	487	−0.25 (−0.74–0.23)	0.31	18.14	0.23	485	−0.45 (−1.40–0.50)	0.35	5.94	0.17
[f]Metabolism score (cases)	720	−0.31 (−0.74–0.12)	0.16	23.50	0.05	710	−0.58 (−1.60–0.43)	0.26	5.46	0.10

Abbreviaions: β, regression coefficient; DWH, Durbin–Wu–Hausman; *N*, sample size.

[a]β refers to the SD unit change in IGFBP-3 levels per 1 SD unit increase in 25(OH)D or 1,25$(OH)_2$D.

[b]F statistic indicates how much of the variability in 25(OH)D is explained by each SNP or allele score (i.e., the strength of each SNP as an instrument for vitamin D levels. $F > 10$ indicates a strong instrument.

[c]*P* value(DWH) is the *P* value for a test (the Durbin form of the Durbin–Wu–Hausman test) for the difference between the estimates from linear regression (without additional adjustment) and instrumental variable analysis.

[d]Allele score: rs8018720, rs10745742, rs10741657, rs12785878, rs3755967, and rs17216707.

[e]Synthesis score: rs12785878 and rs10741657.

[f]Metabolism score: rs3755967 and rs17216707.

Existing preclinical research has implicated 1,25$(OH)_2$D in the regulation of IGFBP-3 expression and in this context we set out to assess the causal relationship between the closest modifiable proxy for this (25(OH)D) and IGFBP-3. Using data from ProtecT, this study found observational evidence that 25(OH)D and 1,25$(OH)_2$D are associated with IGFBP-3 in the circulation. However, MR analyses using data from ProtecT were not suggestive of a causal effect for 25(OH)D. Using summary statistics from the new IGFBP-3 GWAS, two-sample MR analyses found little evidence of a causal relationship between circulating 25(OH)D and IGFBP-3.

The relationship of vitamin D and IGFBP-3 is complex due to the coexisting endocrine and autocrine/paracrine interactions (6). At the autocrine/paracrine level, several preclinical studies have demonstrated that 1,25$(OH)_2$D regulates IGFBP-3 expression (6, 7) and that the growth inhibitory effects of 1,25$(OH)_2$D on prostate cancer cells is altered by IGFBP-3 (14–16). At the endocrine level, evidence from observational studies examining whether 25(OH)D or 1,25$(OH)_2$D influence IGFBP-3 in the circulation have been conflicting, but inferences of causality are limited with observational studies due to effects of confounding and reverse causation (17–20). Our MR results suggesting that 25(OH)D does not have a causal effect on IGFBP-3 levels in the circulation are in agreement with the results from a randomized phase II trial of men with localized prostate cancer and high-grade prostatic intraepithelial neoplasia which found that supplementation with the vitamin D analog,1-alpha-hydroxyvitamin D2, did not have an effect on circulating IGFBP-3 levels (49). Another vitamin D supplementation trial conducted among women with high risk of breast cancer also found little evidence that circulating IGFBP-3 levels changed after supplementation with 25(OH)D (50). This does not preclude that increasing the availability of 25(OH)D with supplementation may lead to increased production of 1,25$(OH)_2$D within tissues that could then stimulate the local production of IGFBP-3 that is not detected in the circulation.

Evidence from this study corroborates that the IGF pathway is a risk factor for prostate cancer risk and progression (3–5), but challenges assumptions about the possible route from supplement-derived precursor vitamin D, through IGFBP-3 to cancer. This work sits in the broader context of a prospective nested case–control study which found evidence for interactions between plasma 25(OH)D and plasma IGF-I/IGFBP-3 ratio with regard to colorectal cancer risk (20). Another study of premenopausal women also reported that vitamin D supplementation was negatively associated with mammographic breast density, and that the association was stronger in those with high IGF-I and IGFBP-3 levels (18).

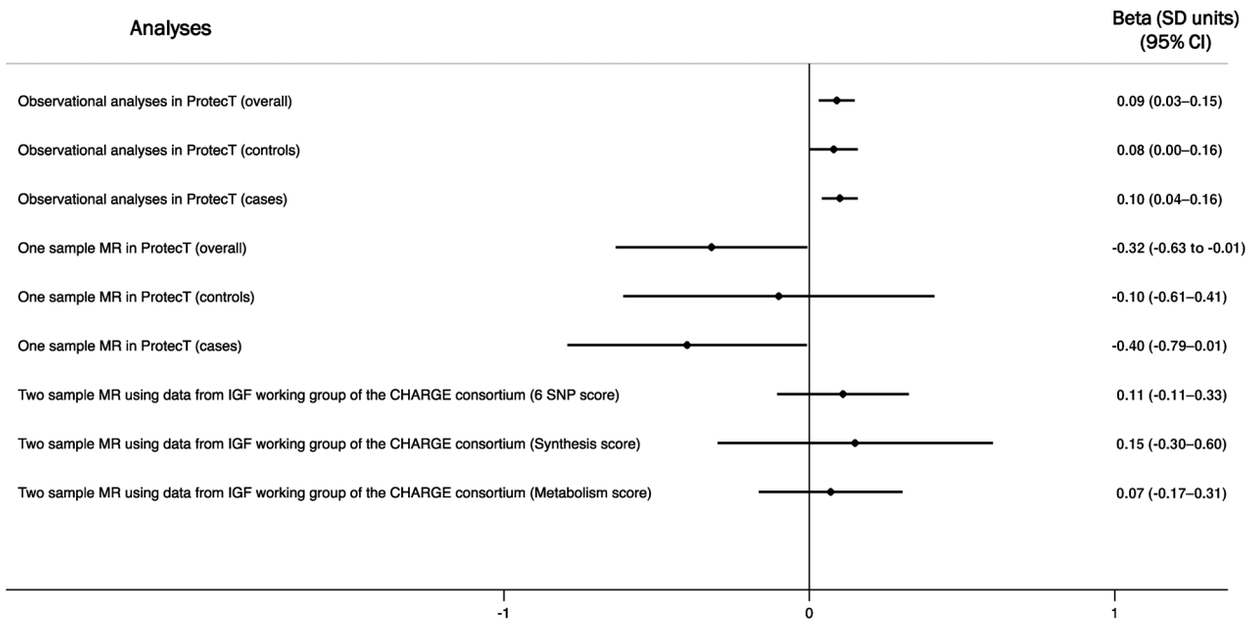

Figure 3.

Forest plots of observational and instrumental variable estimates of the relationship between 25(OH)D and IGFBP-3. The forest plot shows the estimate of the effect of 25(OH)D on IGFBP-3 from observational and MR analyses using individual level data from ProtecT or summary statistics from IGF GWAS conducted by the IGF working group of the CHARGE consortium. Diamonds represent individual study point estimates. Horizontal lines represent the 95% confidence intervals.

There could be several reasons why our MR findings differ from results from the observational studies. First, the point estimates are close to the null for our two sample MR analyses. It is possible that the true effect of 25(OH)D on IGFBP-3 could be small and undetectable using a causal analysis framework. Second, the genetic instruments for 25(OH)D may not be acting as a true proxy for $1,25(OH)_2D$. Our analyses using the allele and metabolism score found that each additional 25(OH)D-related allele was associated with increased $1,25(OH)_2D$ levels for controls and cases combined (Supplementary Table S4). However, the *F*-statistic for instrumental analyses using allele, metabolism, and synthesis scores was lower for $1,25(OH)_2D$ compared with 25(OH)D (Table 3), indicating that they are weaker instruments for $1,25(OH)_2D$. Our observational analyses in ProtecT found that $1,25(OH)_2D$ is associated with IGFBP-3 in the circulation; however, it is still unclear if a causal relationship exists between circulating $1,25(OH)_2D$ and IGFBP-3. Thirdly, given that vitamin D activating enzymes are present in many tissues and could influence $1,25(OH)_2D$ concentrations within tissues, including prostate tissues (51, 52), circulating 25(OH)D and $1,25(OH)_2D$ may not reflect the true bioavailability of 25(OH)D and $1,25(OH)_2D$ within tissues. Therefore, the endocrine relationship between 25(OH)D and IGFBP-3 may not be reflective of the autocrine/paracrine relationship.

Our study has several limitations. The SNPs associated with IGFBP-3 have been shown to be associated with more than one part of the IGF pathway. Although the multivariable MR method enables the determination of the causal effect of IGFBP-3 on prostate cancer independent of IGF-I, we cannot rule out associations with other members of the IGF pathway (5). As only a small number of SNPs were included in our MR analyses, methods to test for pleiotropy such as the MR-Egger (43), weighted median (44), and mode (45) method were not conducted as these lack power with small number of SNPs. As the SNPs associated with 25(OH)D only explained a small proportion of the variability in circulating 25(OH)D, large sample sizes are required to detect expected influences on IGFBP-3 levels and to be able to provide precise estimates for this effect. Although our point estimates are close to the null for the two-sample MR analyses, we cannot rule out the possibility that 25(OH)D may have small effects on IGFBP-3. Finally, our study population was limited to Europeans. Although population homogeneity eliminates population admixture as a potential confounder in our analyses, as 25(OH)D levels vary with sun exposure and skin color, the findings drawn from this study might not be applicable to other ethnic groups or individuals in different geographical locations.

Conclusions

This study confirmed results from previous studies that members of the IGF pathway, in particular IGFBP-3, has a causal effect on prostate cancer and advanced prostate cancer. Observationally, there is evidence that circulating 25(OH)D is positively associated with circulating IGFBP-3, but MR analyses indicated these findings were unlikely to be causal. Findings here are limited by the nature of instrumentation of both 25(OH)D and IGFBP-3 and the utility of circulating measures, but are important as they suggest that circulating 25(OH)D are unlikely to be causally related to circulating IGFBP-3, although vitamin D supplementation could affect local tissue levels.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: V.Y. Tan, K.M. Biernacka, R.M. Martin, C.M. Perks, N.J. Timpson, J.M.P. Holly
Development of methodology: N.J. Timpson
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.M. Biernacka, R. Gilbert, R.C. Kaplan, Q. Qibin, R.M. Martin, the PRACTICAL consortium
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): V.Y. Tan, K.M. Biernacka, T. Dudding, C. Bonilla, Q. Qibin, A. Teumer, C.M. Perks, N.J. Timpson, J.M.P. Holly
Writing, review, and/or revision of the manuscript: V.Y. Tan, K.M. Biernacka, T. Dudding, C. Bonilla, R. Gilbert, R.C. Kaplan, Q. Qibin, A. Teumer, R.M. Martin, C.M. Perks, the PRACTICAL consortium, N.J. Timpson, J.M.P. Holly
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): V.Y. Tan, R. Gilbert, Q. Qibin, the PRACTICAL consortium
Study supervision: R.M. Martin, C.M. Perks, N.J. Timpson, J.M.P. Holly

Acknowledgments

This work was supported by CRUK (The Integrative Cancer Epidemiology Programme, C18281/A19169). V.Y. Tan, T. Dudding, C. Bonilla, N.J. Timpson and R.M. Martin work in the Medical Research Council Integrative Epidemiology Unit at the University of Bristol which is supported by the Medical Research Council and the University of Bristol (grant number MC_UU_12013/3). N.J. Timpson is a Wellcome Trust Investigator (202802/Z/16/Z) and works within the University of Bristol NIHR Biomedical Research Centre (BRC). T. Dudding is funded by The Wellcome Trust (grant number 201237/Z/16/Z), K.M. Biernacka is funded by Cancer Research UK (C11046/A10052) and by the National Institute for Health Research (NIHR) Bristol Biomedical Research Centre based at University Hospitals Bristol NHS Foundation Trust and the University of Bristol. Q. Qibin is supported by a Scientist Development Award (K01HL129892) from the NHLBI. The views expressed are those of the authors and not necessarily those of the NHS, the NIHR, or the Department of Health.

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 23, 2018; revised June 18, 2018; accepted June 19, 2018; published first August 2, 2018.

References

1. Johnson MA, Firth SM. IGFBP-3: a cell fate pivot in cancer and disease. Growth Horm IGF Res 2014;24:164–73.
2. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824–54.
3. Travis RC, Appleby PN, Martin RM, Holly JM, Albanes D, Black A, et al. A meta-analysis of individual participant data reveals an association between circulating levels of IGF-I and prostate cancer risk. Cancer Res 2016;76:2288–300.
4. Rowlands MA, Holly JM, Gunnell D, Donovan J, Lane JA, Hamdy F, et al. Circulating insulin-like growth factors and IGF-binding proteins in PSA-detected prostate cancer: the large case-control study ProtecT. Cancer Res 2012;72:503–15.
5. Bonilla C, Lewis SJ, Rowlands MA, Gaunt TR, Davey Smith G, Gunnell D, et al. Assessing the role of insulin-like growth factors and binding proteins in prostate cancer using Mendelian randomization: genetic variants as instruments for circulating levels. Int J Cancer 2016;139:1520–33.
6. Ameri P, Giusti A, Boschetti M, Murialdo G, Minuto F, Ferone D. Interactions between vitamin D and IGF-I: from physiology to clinical practice. Clin Endocrinol (Oxf) 2013;79:457–63.
7. Peng L, Malloy PJ, Feldman D. Identification of a functional vitamin D response element in the human insulin-like growth factor binding protein-3 promoter. Mol Endocrinol 2004;18:1109–19.
8. Dubbelman R, Jonxis JH, Muskiet FA, Saleh AE. Age-dependent vitamin D status and vertebral condition of white women living in Curacao (the Netherlands Antilles) as compared with their counterparts in the Netherlands. Am J Clin Nutr 1993;58:106–9.
9. Lips P, Wiersinga A, van Ginkel FC, Jongen MJ, Netelenbos JC, Hackeng WH, et al. The effect of vitamin D supplementation on vitamin D status and parathyroid function in elderly subjects. J Clin Endocrinol Metab 1988; 67:644–50.
10. Jacobs ET, Kohler LN, Kunihiro AG, Jurutka PW. Vitamin D and colorectal, breast, and prostate cancers: a review of the epidemiological evidence. J Cancer 2016;7:232–40.
11. Gilbert R, Bonilla C, Metcalfe C, Lewis S, Evans DM, Fraser WD, et al. Associations of vitamin D pathway genes with circulating 25-hydroxyvitamin-D, 1,25-dihydroxyvitamin-D, and prostate cancer: a nested case-control study. Cancer Causes Control 2015;26:205–18.
12. Dimitrakopoulou VI, Tsilidis KK, Haycock PC, Dimou NL, Al-Dabhani K, Martin RM, et al. Circulating vitamin D concentration and risk of seven cancers: Mendelian randomisation study. BMJ 2017;359:j4761.
13. Manousaki D, Richards JB. Low vitamin D levels as a risk factor for cancer. BMJ 2017;359:j4952.
14. Krishnan AV, Shinghal R, Raghavachari N, Brooks JD, Peehl DM, Feldman D. Analysis of vitamin D-regulated gene expression in LNCaP human prostate cancer cells using cDNA microarrays. Prostate 2004;59:243–51.
15. Boyle BJ, Zhao XY, Cohen P, Feldman D. Insulin-like growth factor binding protein-3 mediates 1 alpha,25-dihydroxyvitamin d(3) growth inhibition in the LNCaP prostate cancer cell line through p21/WAF1. J Urol 2001; 165:1319–24.
16. Stewart LV, Weigel NL. Role of insulin-like growth factor binding proteins in 1alpha,25-dihydroxyvitamin D(3)-induced growth inhibition of human prostate cancer cells. Prostate 2005;64:9–19.
17. DeLellis K, Rinaldi S, Kaaks RJ, Kolonel LN, Henderson B, Le Marchand L. Dietary and lifestyle correlates of plasma insulin-like growth factor-I (IGF-I) and IGF binding protein-3 (IGFBP-3): the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 2004;13:1444–51.
18. Diorio C, Berube S, Byrne C, Masse B, Hebert-Croteau N, Yaffe M, et al. Influence of insulin-like growth factors on the strength of the relation of vitamin D and calcium intakes to mammographic breast density. Cancer Res 2006;66:588–97.
19. Holmes MD, Pollak MN, Willett WC, Hankinson SE. Dietary correlates of plasma insulin-like growth factor I and insulin-like growth factor binding protein 3 concentrations. Cancer Epidemiol Biomarkers Prev 2002;11:852–61.
20. Wu K, Feskanich D, Fuchs CS, Chan AT, Willett WC, Hollis BW, et al. Interactions between plasma levels of 25-hydroxyvitamin D, insulin-like growth factor (IGF)-1 and C-peptide with risk of colorectal cancer. PLoS One 2011;6:e28520.
21. Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. Hum Mol Genet 2014;23: R89–98.
22. Teumer A, Qi Q, Nethander M, Aschard H, Bandinelli S, Beekman M, et al. Genomewide meta-analysis identifies loci associated with IGF-I and IGFBP-3 levels with impact on age-related traits. Aging Cell 2016;15: 811–24.
23. Manousaki D, Dudding T, Haworth S, Hsu Y, Liu C, Medina-Gómez C, et al. Low frequency coding variation in CYP2R1 has large effects on vitamin D level and risk of multiple sclerosis. Am J Hum Genet 2017; 101:227–38.
24. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. Stat Med 2008;27:1133–63.
25. Burgess S, Scott RA, Timpson NJ, Davey Smith G, Thompson SG, EPIC-InterAct Consortium. Using published data in Mendelian randomization: a blueprint for efficient identification of causal risk factors. Eur J Epidemiol 2015;30:543–52.
26. Lane A, Metcalfe C, Young GJ, Peters TJ, Blazeby J, Avery KN, et al. Patient-reported outcomes in the ProtecT randomized trial of clinically localized prostate cancer treatments: study design, and baseline urinary, bowel and sexual function and quality of life. BJU Int 2016;118:869–79.

27. 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.
28. Gilbert R, Metcalfe C, Fraser WD, Donovan J, Hamdy F, Neal DE, et al. Associations of circulating 25-hydroxyvitamin D with prostate cancer diagnosis, stage and grade. Int J Cancer 2012;131:1187–96.
29. Gilbert R, Metcalfe C, Fraser WD, Donovan J, Hamdy F, Neal DE, et al. Associations of circulating retinol, vitamin E, and 1,25-dihydroxyvitamin D with prostate cancer diagnosis, stage, and grade. Cancer Causes Control 2012;23:1865–73.
30. Lawlor DA, Wills AK, Fraser A, Sayers A, Fraser WD, Tobias JH. Association of maternal vitamin D status during pregnancy with bone-mineral content in offspring: a prospective cohort study. Lancet 2013;381:2176–83.
31. Degerud E, Hoff R, Nygard O, Strand E, Nilsen DW, Nordrehaug JE, et al. Cosinor modelling of seasonal variation in 25-hydroxyvitamin D concentrations in cardiovascular patients in Norway. Eur J Clin Nutr 2016;70: 517–22.
32. Kaplan RC, Petersen AK, Chen MH, Teumer A, Glazer NL, Doring A, et al. A genome-wide association study identifies novel loci associated with circulating IGF-I and IGFBP-3. Hum Mol Genet 2011;20:1241–51.
33. Schumacher FR, Al Olama AA, Berndt SI, Benlloch S, Ahmed M, Saunders EJ, et al. Prostate cancer meta-analysis of more than 140,000 men identifies 63 novel prostate cancer susceptibility loci. Nat Genet 2018;50:928–36.
34. Jiang X, O'Reilly PF, Aschard H, Hsu YH, Richards JB, Dupuis J, et al. Genome-wide association study in 79,366 European-ancestry individuals informs the genetic architecture of 25-hydroxyvitamin D levels. Nat Commun 2018;9:260.
35. Wang TJ, Zhang F, Richards JB, Kestenbaum B, van Meurs JB, Berry D, et al. Common genetic determinants of vitamin D insufficiency: a genome-wide association study. Lancet 2010;376:180–8.
36. Ahn J, Yu K, Stolzenberg-Solomon R, Simon KC, McCullough ML, Gallicchio L, et al. Genome-wide association study of circulating vitamin D levels. Hum Mol Genet 2010;19:2739–45.
37. Burgess S, Butterworth A, Thompson SG. Mendelian randomization analysis with multiple genetic variants using summarized data. Genet Epidemiol 2013;37:658–65.
38. Burgess S, Dudbridge F, Thompson SG. Re: "Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects". Am J Epidemiol 2015;181:290–1.
39. Burgess S, Thompson SG. Multivariable Mendelian randomization: the use of pleiotropic genetic variants to estimate causal effects. Am J Epidemiol 2015;181:251–60.
40. Greenland S. An introduction to instrumental variables for epidemiologists. Int J Epidemiol 2000;29:722–9.
41. Timpson NJ, Lawlor DA, Harbord RM, Gaunt TR, Day IN, Palmer LJ, et al. C-reactive protein and its role in metabolic syndrome: mendelian randomisation study. Lancet 2005;366:1954–9.
42. Baum CF, Schaffer ME, Stillman S. IVENDOG: Stata module to calculate Durbin-Wu-Hausman endogeneity test after ivreg. Statistical Software Components, S494401, Boston College Department of Economics; revised 29 May 2007.
43. Bowden J, Davey Smith G, Burgess S. Mendelian randomization with invalid instruments: effect estimation and bias detection through Egger regression. Int J Epidemiol 2015;44:512–25.
44. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. Genet Epidemiol 2016;40:304–14.
45. Hartwig FP, Davey Smith G, Bowden J. Robust inference in summary data Mendelian randomization via the zero modal pleiotropy assumption. Int J Epidemiol 2017;46:1985–98.
46. Brion MJ, Shakhbazov K, Visscher PM. Calculating statistical power in Mendelian randomization studies. Int J Epidemiol 2013;42:1497–501.
47. Pierce BL, Burgess S. Efficient design for Mendelian randomization studies: subsample and 2-sample instrumental variable estimators. Am J Epidemiol 2013;178:1177–84.
48. Haycock PC, Burgess S, Wade KH, Bowden J, Relton C, Davey Smith G. Best (but oft-forgotten) practices: the design, analysis, and interpretation of Mendelian randomization studies. Am J Clin Nutr 2016;103:965–78.
49. Gee J, Bailey H, Kim K, Kolesar J, Havighurst T, Tutsch KD, et al. Phase II open label, multi-center clinical trial of modulation of intermediate endpoint biomarkers by 1alpha-hydroxyvitamin D2 in patients with clinically localized prostate cancer and high grade pin. Prostate 2013; 73:970–8.
50. Crew KD, Xiao T, Thomas PS, Terry MB, Maurer M, Kalinsky K, et al. Safety, feasibility, and biomarker effects of high-dose vitamin D supplementation among women at high risk for breast cancer. Int J Food Sci Nutr Diet 2015;2015(Suppl 1):1–16.
51. Chen TC, Sakaki T, Yamamoto K, Kittaka A. The roles of cytochrome P450 enzymes in prostate cancer development and treatment. Anticancer Res 2012;32:291–8.
52. Blomberg Jensen M, Nielsen JE, Jorgensen A, Rajpert-De Meyts E, Kristensen DM, Jorgensen N, et al. Vitamin D receptor and vitamin D metabolizing enzymes are expressed in the human male reproductive tract. Hum Reprod 2010;25:1303–11.