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Original Article

A polymorphism in the glucokinase gene that raises plasma fasting glucose, rs1799884, is associated with diabetes mellitus and prostate cancer: findings from a population-based, case-control study (the ProtecT study)

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Abstract: Epidemiological studies have identified a positive association between prostate cancer and recent onset type 2 diabetes mellitus but an increasingly inverse association with greater duration of type 2 diabetes. The mechanisms underlying these paradoxical associations are not clear. A single nucleotide polymorphism in the glucokinase gene, rs1799884, is associated with higher circulating plasma fasting glucose and with an increased risk of type 2 diabetes. We report a case-control study nested within the population-based Prostate testing for cancer and Treatment (*ProtecT*) study ISRCTN20141297. Men aged 50-69 years based around 9 UK cities were invited for a prostate specific antigen (PSA) test between June 2002 and November 2006. 1,551 cases and 2,993 controls were genotyped. We observed suggestive evidence for a positive association between the AA variant rs1799884 and PSA-detected prostate cancer ($OR_{AA\ v\ GG}$= 1.40, 95% CI= 0.95 to 2.07). There was little evidence that this effect was greater for more advanced stage / grade cancers ($OR_{AA\ v\ GG}$= 1.78, 95% CI= 0.99 to 3.21) versus less advanced cancers ($OR_{AA\ v\ GG}$= 1.23, 95% CI= 0.77 to 1.94) (p for interaction = 0.33). The rs1799884 genotype was not associated with PSA concentration, suggesting that any effect on prostate cancer risk is not attributable to PSA detection bias. Our results provide suggestive evidence for a link between a genotype associated with type 2 diabetes mellitus and PSA-detected prostate cancer. We hypothesize that hyperglycaemia may be important in mediating this relationship.

Keywords: Single nucleotide polymorphisms, glucokinase, GCK, rs1799884, prostate cancer, diabetes, insulin, case-control study, prostate specific antigen

Introduction

Epidemiological studies consistently report an inverse association between type 2 diabetes mellitus (T2DM) and prostate cancer, but the mechanism by which these two diseases are related remains unclear. Several studies reported that risk of prostate cancer decreases with increasing time since diagnosis of T2DM [1-3] and there is some evidence that prostate cancer risk is increased in the years immediately following diagnosis [1-5].

These findings have led some investigators to hypothesize that the effects of T2DM on prostate cancer risk are mediated through changes in insulin concentration. The early stages of T2DM are characterized by hyperinsulinaemia, as the pancreas augments insulin secretion to counter increasing insulin resistance [6]. This is followed by worsening hypoinsulinaemia secondary to progressive pancreatic β-cell failure [6]. Insulin has a mitogenic effect on rat prostate adenocarcinoma cells *in vitro* [7] and is associ-

ated with a higher incidence of incident [8], recurrent [9], advanced [10] and lethal [11] prostate cancer in humans. A transient increase in insulin concentration could therefore explain an increased risk of prostate cancer early in T2DM, whilst a subsequent reduction in insulin concentration might explain the protective effect of established diabetes. Other hypotheses to explain the inverse relationship between T2DM and prostate cancer include an effect of testosterone [2, 12], which is reduced in T2DM [13, 14], the possibility of unmeasured biases or residual confounding [15], for example by diabetic medication [16], and that the two diseases share a common underlying aetiology [17].

Studies incorporating germline genetic variation offer a new way to study the association between T2DM and prostate cancer. Genetic studies are less susceptible to confounding than observational epidemiology [18, 19] and when combined with knowledge of the functional effects of genetic variation can provide clues as to how associations between exposures, intermediate phenotypes and disease outcomes arise.

rs1799884 is a polymorphism located in the β-cell specific promoter region of the Glucokinase (*GCK*) gene. This region demonstrates strong homology amongst humans, mice and rats, suggesting that it plays an important role in the transcriptional regulation of *GCK* [20]. The AA variant of rs1799884 has been associated with an elevated fasting glucose concentration [20-23]. It has also been linked with an increased risk of T2DM, both in a meta-analysis (OR= 1.08, no CI provided, p=0.004)[24] and a large, prospective study (OR= 2.39, 95% CI= 1.30 to 4.42 under a recessive model) [25]. However, most, but not all [26], studies have found no evidence that rs1799884 is associated with impaired insulin secretion [20, 27, 28]. Whilst impairment of insulin secretion is a feature of established T2DM, current T2DM diagnostic criteria are solely dependent upon demonstration of hyperglycaemia [29], which might explain how a SNP could predispose to T2DM without impairing insulin secretion. Thus, by raising glucose concentration, thereby increasing *in vivo* insulin levels, the AA genotype of rs1799884 may predispose to prostate cancer.

We analysed data from a large, population-based case-control study nested within the Prostate testing for cancer and Treatment (ProtecT) trial to test the hypothesis that the AA genotype of rs1799884 predisposes to prostate cancer (Online Mendelian Inheritance in Man number: #176807).

Materials and Methods

Nested case-control study within ProtecT

ProtecT is an ongoing, multi-centre, randomized controlled trial that will compare the efficacy, cost-effectiveness and acceptability of treatments for localized prostate cancer [30]. Between 2001 and 2008 more than 100,000 men aged 50 to 69 years were invited to prostate check clinics, where histologically confirmed prostate cancer cases were identified through a combination of PSA testing, digital rectal examination (DRE) and, for men with a raised PSA or abnormal DRE, 10-core transrectal ultrasound-guided biopsy. In this study, our case population consisted of all men with prostate cancer identified at prostate check clinics conducted before the end of November 2006 who gave consent for genotyping. We defined non-aggressive prostate cancer cases as those of TNM Stage < 3 and Gleason Score < 7. We defined aggressive prostate cancer cases as those of TNM Stage ≥ 3 and/or Gleason Score ≥ 7. This definition is equivalent to that used in other studies [31]. All participants with no evidence of prostate cancer after PSA testing, DRE and/or biopsy were eligible to be controls. Two non-overlapping groups of controls were selected. One group included only participants without a diagnosis of prostate cancer and with a PSA concentration < 0.5ng/ml ('low PSA controls'). The other group included only participants without a diagnosis of prostate cancer and placed no restriction on PSA concentration ('unrestricted controls'). Controls were stratum matched to cases by age (5-year bands) and the primary care centre (general practice) from which men were recruited. We restricted cases and controls to self-reported 'white' males (98.9% of participants). Additional data were collected on participants' exact age, family history of prostate cancer (in a father or brother) and self-reported diabetes (using the question "have you ever been told by a doctor that you have diabetes?"). Detailed descriptions of ProtecT and the protocol for nested case-control selection are published elsewhere [30, 32, 33].

Genotyping

Consent for genotyping and blood samples were taken from all patients enrolled in ProtecT under the auspices of the Prostate Mechanisms of Progression and Treatment (ProMPT) collaborative and approved by Trent Multicentre Research and Ethics Committee.

DNA extraction was performed by Tepnel (http://www.tepnel.com) and the Institute for Cancer Studies, University of Sheffield. Genotyping for single nucleotide polymorphism (SNP) analysis was performed by KBioscience (www.kbioscience.co.uk), whose personnel were blinded to patient status, using their proprietary KASPar PCR technique and Taqman™. Genotype calling was performed using an automated system, the results of which were checked manually by study personnel using SNPviewer software. Investigators reviewing genotyping data were blinded to patient status. To further ensure adequate quality control, we included 412 duplicate samples in our genotype request. Concordance for these 412 unblinded replicates was 100%.

Statistical methods

A Pearson χ^2-test was performed amongst controls to ensure that genotype distribution did not deviate from Hardy-Weinberg equilibrium. Odds ratios (OR) and 95% confidence intervals (95% CI) for the association of rs1799884 genotype with overall prostate cancer, non-aggressive prostate cancer and aggressive prostate cancer were calculated using conditional logistic regression, to account for the stratum matching of cases to controls, further adjusted for exact age and a family history of prostate cancer.

We also used multinomial logistic regression to determine whether there was evidence of a heterogeneous effect across non-aggressive and aggressive prostate cancer cases [34]. This required comparing a referent group (controls) with non-aggressive cancers and aggressive cancers using simultaneous logistic regression [35], whilst controlling for exact age and family history of prostate cancer. A p-value for heterogeneity in odds ratios comparing non-aggressive cancers and aggressive cancers was calculated by applying a post-estimation Wald χ^2 test to the results [34].

To assess the potential for PSA detection bias [36], associations of rs1799884 with mean serum PSA levels by genotype were investigated in 'unrestricted controls' using multivariable linear regression adjusted for exact age and a family history of prostate cancer.

All analysis was performed using Stata 10 software (StataCorp, TX, USA).

Results

We submitted blood samples from 1,565 cases and 3,009 controls for genotyping. The 1,565 cases comprised 1,053 non-aggressive cases, 507 aggressive cases and 5 indeterminate cases. The 3,009 controls comprised 1,181 'low PSA controls' and 1,828 'unrestricted controls'. We received completed genotypes for 1,551 (99.1%) cases and 2,993 (99.5%) controls. There was no evidence of departure from Hardy-Weinberg equilibrium amongst controls (p=0.74). Cases were 7 months older than controls, more likely to have a family history of prostate cancer and less likely to have diabetes (**Table 1**).

There was some evidence that the AA genotype of rs1799884 was associated with a 40% increased prostate cancer risk (**Table 2**), but the result was not statistically significant at the conventional 5% level. Restricting analysis to men without diabetes increased the magnitude of this association ($OR_{AA\ v\ GG}$= 1.62, 95% CI= 0.92 to 2.87, p= 0.10). ORs were higher for aggressive ($OR_{AA\ v\ GG}$= 1.78, 95% CI= 0.99 to 3.21, p= 0.05) versus non-aggressive cancers ($OR_{AA\ v\ GG}$= 1.23, 95% CI= 0.77 to 1.94, p= 0.39) but without statistical evidence of a difference between these odds ratios ($p_{heterogeneity}$= 0.33). Similar results were obtained when 'low PSA controls' and 'unrestricted controls' were compared with cases separately (data on request).

The AA genotype of rs1799884 was associated with a 2.7-fold increased risk of diabetes amongst cases and controls (**Table 3**). We found no evidence that rs1799884 was associated with PSA concentration amongst 'unrestricted controls' (p> 0.25 for all genotypes compared against GG as the reference group (data on request)).

Discussion

In this nested, case-control study we have found suggestive evidence that the AA genotype of

Table 1. Characteristics of the ProtecT study population

	Cases (n= 1,565) *n* (%)		Controls (n= 3,009) *n* (%)	
Age at selection (years)				
Mean age	62.55		61.96	
SD	5.12		5.25	
Family history of prostate cancer (in father and/or brother)				
Yes	1,450	(92.6)	2,870	(95.4)
No	115	(7.4)	139	(4.6)
Diabetes (self reported)				
Yes	71	(4.5)	175	(5.8)
No	890	(56.9)	1,704	(56.6)
Unspecified	604	(38.6)	1,130	(37.6)
PSA (ng/ml)				
≤0.5	0	(0.0)	1745	(58.0)
0.5- 4.0	465	(29.7)	1197	(39.8)
4.0- 9.9	787	(50.3)	60	(2.0)
10- 49.9	281	(18.0)	7	(0.2)
^{3}50	32	(2.0)	0	(0)
Tumour stage (TNM)				
T1	953	(60.9)	-	-
T2	181	(11.6)	-	-
T3	120	(7.7)	-	-
T4	5	(0.3)	-	-
Unspecified	306	(19.6)	-	-
Gleason score				
2-4	2	(0.1)	-	-
5-6	1,106	(70.7)	-	-
7-10	452	(28.9)	-	-
Unspecified	5	(0.3)	-	-

SD, standard deviation; PSA, prostate specific antigen.

rs1799884 was associated with an increased risk of prostate cancer. We also replicated the association between rs1799884 and increased T2DM risk. The rs1799884 genotype was not associated with PSA concentration, suggesting that any effect on prostate cancer risk is not attributable to PSA detection bias.

Several other groups have demonstrated an association between the AA genotype of rs1799884 and T2DM [24, 25]. This association is plausible because rs1799884 increases plasma fasting glucose concentration [20-23], upon which diagnosis of T2DM relies [29]. To the best of our knowledge, no other group has found evidence in support of an association between rs1799884 and prostate cancer.

Our results provide suggestive evidence to support the hypothesis that the AA genotype of rs1799884 predisposes to prostate cancer. An effect of this polymorphism on overall prostate cancer risk is biologically plausible because of the believed carcinogenic effects of insulin [7]. There was no strong evidence that this effect was greater for more advanced PSA-detected prostate cancers, despite emerging evidence that phenotypically heterogeneous prostate cancers may have differing aetiology [37-39] and several studies that have suggested aggressive prostate cancers are more likely to have a genetic basis than their indolent counterparts [38, 40, 41]. It is likely that our sample size was too small to detect heterogeneity in odds ratios for associations of genotype on prostate cancer stratified by aggressive versus non-aggressive subtypes, although restricting analysis to two disease phenotypes that are extremely distinct, aggressive prostate cancer cases versus low PSA controls, may reduce the misclassification of cases as controls and controls as cases [42, 43]. We cannot be sure how many ProtecT

Table 2. Association of rs1799884 genotype with overall prostate cancer risk

Genotype	Number (%) of subjects				OR (95% CI)[a]	*p* value[a]
	Cases (n=1551)		All controls (n = 2993)			
GG	1,035	(66.7)	2,044	(68.3)	Referent	-
GA	463	(29.9)	862	(28.8)	1.11 (0.96 to 1.28)	0.18
AA	53	(3.4)	87	(2.9)	1.40 (0.95 to 2.07)	0.09
GA/AA	516	(33.3)	949	(31.7)	1.13 (0.98 to 1.30)	0.09
Per allele	-	-	-	-	1.12 (0.99 to 1.27)	0.06

[a]Conditional logistic regression to account for stratum matching of cases and controls on 5-year age bands and primary care recruiting centre, further adjusted for exact age and family history of prostate cancer.
OR, odds ratio; CI, confidence interval.

Table 3. Association of rs1799884 genotype with self-reported type 2 diabetes mellitus

Genotype	*n* (%)				OR[a] 95% CI	*p* value[a]
	Diabetes N=243		No diabetes N=2581			
GG	163	(67.1)	1,748	(67.7)	Referent	-
GA	64	(26.3)	761	(29.5)	0.96 (0.68 to 1.35)	0.82
AA	16	(6.6)	72	(2.8)	2.70 (1.25 to 5.85)	0.01
GA/AA	80	(32.9)	833	(32.3)	1.09 (0.79 to 1.50)	0.59
Per allele	-	-	-	-	1.20 (0.91 to 1.57)	0.20

[a]Logistic regression, adjusted for exact age and recruitment centre. OR, odds ratio; CI, confidence interval.

cases and controls were misclassified but there is evidence that DRE and the use of PSA thresholds fail to identify a substantial minority of men with indolent prostate cancer [44, 45]. It is therefore possible that our control group contains a number of men with unrecognized prostate cancer. This may have had the effect of diluting an association between rs1799884 genotype and prostate cancer risk.

One group has identified a SNP in *HNF1B* (rs4430796) that protects against T2DM and also increases risk of prostate cancer [17]. This association has been replicated [46-49] but it is not known whether rs4430796 exerts a functional effect [47]. Our study adds to that of Gudmundsson et al. by providing further evidence for a possible genetic link between T2DM and prostate cancer and suggestive evidence for a biological mechanism linking the two diseases. Whilst rs1799884 increases plasma glucose concentration it does not appear to impair insulin secretion [20, 27, 28]. Most carriers of the AA genotype of rs1799884 will therefore be exposed to high insulin concentrations throughout their lifetime. Insulin has a mitogenic effect on prostatic adenocarcinoma cells [7] and this could explain the increased risk of prostate cancer seen amongst this group in our study.

Our finding that the risk of prostate cancer increased when participants self-reporting diabetes were excluded from analysis may support the hypothesis that insulin concentrations are

important in determining prostate cancer risk in patients with T2DM. In established T2DM, pancreatic β-cells cannot synthesize insulin, resulting in hypoinsulinaemia even in the presence of hyperglycaemia. Therefore, if insulin concentrations are important in determining prostate cancer risk amongst patients with T2DM, we would expect the AA genotype of rs1799884 to exert a greater effect on prostate cancer risk when patients with established T2DM are excluded from analysis. Similarly, we would expect prostate cancer risk to gradually return to, and ultimately fall below, normal levels in patients with the AA genotype of rs1799884 who develop β-cell failure.

Unfortunately, our data do not allow us to distinguish between patients self-reporting T2DM who have pancreatic β-cell failure and those who do not. This is of particular relevance here because the AA genotype of rs1799884 increases fasting plasma glucose concentration without impairing insulin secretion. This means that many of the carriers of the rs1799884 AA genotype who self-report diabetes may not suffer from pancreatic β-cell failure. This, together with the fact that we do not know the temporal relationship between a fall in insulin concentration and a consequent fall in risk of prostate cancer associated with chronic T2DM, makes subgroup analysis within the diabetic population difficult and is a limitation of our study. Future studies of the effect of rs1799884 on prostate cancer risk could include measurements of pancreatic β-cell function in order to further analyse the effects of this SNP.

Our study had other limitations. The population based PSA testing design meant that our study contained a relative paucity of aggressive prostate cancers. This may have limited our ability to detect a difference between the effects of rs1799884 on aggressive prostate cancer risk and non-aggressive prostate cancer risk. Our study cannot inform on the effect of rs1799884 genotype by different ethnic groups. We did not test for T2DM but relied on participants' self-report of diabetes status. Whilst self-reporting of T2DM has been shown to be both sensitive and specific [50-52], we cannot exclude the possibility that some T2DM cases were missed or that non-cases were erroneously included. Furthermore we cannot differentiate between type 1 and type 2 diabetes mellitus with our data, although the relative prevalence of these two diseases means that we would expect the great majority of diabetes cases within ProtecT to be T2DM.

Other SNPs exist in the promoter region of the glucokinase gene (http://hapmap.ncbi.nlm.nih.gov/). We used rs1799884 because this SNP has repeatedly been shown to be associated with fasting glucose levels and we wanted to use this consistent association to make inferences about glucose levels and prostate cancer risk. Weedon et al [21] genotyped 22 tagging SNPs in the glucokinase gene and found this SNP to have the strongest association with glucose levels. Therefore, whilst we could have genotyped other SNPs and performed haplotype analysis, we feel it is unlikely that this would have affected our conclusions.

The results for both diabetes and prostate cancer are consistent with either a co-dominant or a recessive model, if the focus is on the confidence intervals rather than the point estimates. The number of men with diabetes is small and therefore it is difficult to determine a genetic model from these data. For this reason we have presented results for each genotype, plus a per allele analysis.

In conclusion, our case-control study provides suggestive evidence that the AA genotype of rs1799884 may be associated with an increased risk of prostate cancer. We hypothesize that the possible effect on prostate cancer is mediated through alterations in insulin concentration consequent on hyperglycaemia. We have also replicated the association between rs1799884 and increased T2DM risk. Further investigations into this SNP, using cohorts with a larger number of aggressive prostate cancer cases, and the roles of other diabetes-associated SNPs in determining prostate cancer risk are indicated.

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