Journal of Nutritional Science

cambridge.org/jns

The vitamin D receptor TaqI TT genotype is associated with type 1 diabetes in the Black South African population

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Research Article

Cite this article: Bhola S, Cave EM, Prigge KL, Bhana S, Crowther NJ, and Padoa CJ (2024). The vitamin D receptor TaqI TT genotype is associated with type 1 diabetes in the Black South African population. *Journal of Nutritional Science* **13**: e73, 1–8. doi: 10.1017/jns.2024.77

Received: 14 March 2024
Revised: 20 August 2024
Accepted: 10 October 2024

Keywords:
Polymorphisms; South African population; Type 1 diabetes; Vitamin D receptor

Abbreviations:
VDR, Vitamin D receptor; T1D, Type 1 diabetes; RXR, Retinoid X receptor; UTR, Untranslated region; HbA1c, Glycated haemoglobin

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Abstract

Polymorphisms in the vitamin D receptor (VDR) gene (BsmI (rs1544410), FokI (rs2228570), ApaI (rs7975232), TaqI (rs731236)) and low vitamin D concentrations have previously been associated with type 1 diabetes (T1D). Vitamin D is thought to mediate the switch from a pro-inflammatory Th1 response to an anti-inflammatory Th2 response which is protective against the development of T1D. These associations are inconsistent across studies and population groups. These associations have not been investigated in the South African black population. Thus, this observational, case-control study aims to address this knowledge gap. South African black participants with T1D (cases; n = 182) and healthy controls (n = 151) were genotyped for the four VDR polymorphisms using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Vitamin D levels were measured using high performance liquid chromatography (HPLC). Vitamin D levels were not significantly different between cases and controls (62.8 ± 20.7 vs. 59.5 ± 17.0 nmol/l, respectively; P = 0.122). Higher vitamin D levels were associated with the TaqI TT (P = 0.045) and FokI TT/TC (P = 0.014) genotypes in multivariate analyses. Furthermore, the TaqI TT genotype was associated with T1D status in multivariate analysis (P = 0.040). The FokI CC genotype increases the transcription of *CYP24A1*, resulting in vitamin D catabolism and thus decreased vitamin D concentration through the action of 24-hydroxlase. The TaqI TT genotype results in increased vitamin D potentially through calcium metabolism feedback pathways. In addition, the TaqI TT genotype is associated with T1D through a vitamin D-independent mechanism and may be in linkage disequilibrium with a true causative variant.

Introduction

Type 1 diabetes (T1D) is a multifactorial disease characterised by insulin deficiency as a result of the autoimmune destruction of pancreatic beta cells.[1] An imbalance between the Th1/Th2 pathways i.e. a shift toward the pro-inflammatory Th1 pathway is thought to result in beta cell destruction.[2] Several studies have shown that vitamin D is protective against T1D.[3–6] In addition, studies have shown a negative correlation between vitamin D and HbA1c levels in participants with T1D. Furthermore, following vitamin D supplementation, these participants showed improved glycaemic control.[7,8]

Vitamin D exerts its effects through the vitamin D receptor (VDR). Upon vitamin D binding there is a conformational change and subsequent heterodimerisation of VDR with the retinoid X receptor (RXR). This complex mediates the downregulation of pro-inflammatory cytokines (e.g. IL-2, IL-12 and IFNγ) through direct binding to transcription factors, vitamin D response elements or promotor regions of target genes.[9] The decreased pro-inflammatory cytokine production inhibits the proliferation of Th1 cells thus preventing beta cell death.[10]

The VDR belongs to the steroid receptor superfamily of transcription factors.[11] The *VDR* gene consists of a 5' promoter region (containing the untranslated exons 1a–1f), exons 2–9 (which encode the N-terminal dual zinc finger DNA-binding domain, C-terminal ligand-binding domain and an unstructured region that links the two functional domains) and a 3′ untranslated region (UTR).[12] Four polymorphisms in the *VDR* gene, namely, BsmI (rs1544410), FokI (rs2228570), ApaI (rs7975232) and TaqI (rs731236) have previously been associated with the development of T1D.[13,14]

The FokI polymorphism, in exon 2, results in a C>T substitution which creates a start codon three amino acids upstream of the original start codon, resulting in a VDR protein that is three amino acids longer than the wildtype protein.[15] The shorter variant is more active in transactivation of vitamin D signalling than the longer variant.[16]

The BsmI (A>G) and ApaI (C>A) polymorphisms (found in intron 8) and the synonymous TaqI (T>C) polymorphism (located in exon 9) are in linkage disequilibrium with a poly(A) microsatellite repeat in the 3' UTR of the VDR gene.[17] This repeat influences *VDR* mRNA stability and regulates gene expression.[18]

Numerous studies have shown an association between T1D and the VDR polymorphisms. However, results are inconsistent based on the population group and the polymorphism studied.[13,19–29] To the best of our knowledge, there is no data on these polymorphisms in black South African participants with T1D, an under-studied population for which there is very little information on the molecular aetiology of T1D. We therefore aimed to determine the association of vitamin D levels and *VDR* polymorphisms with T1D in black South African individuals.

Methods

Study participants

Adult South African black participants (≥ 18 years of age;) for this observational (case and control) study were recruited between 16 October 2014 and 20 September 2015 through convenience sampling. Participants with T1D (cases; n = 182) were recruited from diabetic clinics at tertiary hospitals in the greater Johannesburg area, namely, Chris Hani Baragwanath Academic Hospital and Charlotte Maxeke Johannesburg Academic Hospital. These are state clinics and therefore anyone can attend who has been referred through the public health care system. Criteria for T1D diagnosis were the initiation of insulin therapy within one year of diagnosis in individuals <30 years of age or initiation of insulin treatment within the first-year post diagnosis in non-obese participants >30 years of age at diagnosis. Patient glycated haemoglobin (HbA1c) levels measured on the DCA Vantage point of care analyser (Siemens, Munich, Germany), within three months of date of recruitment, were obtained from patient files. Patient glucose levels were measured on an Accu-chek active glucometer (Roche, Basel, Switzerland) by the clinic on the day of recruitment.

Non-diabetic black participants (controls; n = 156) were recruited from South African National Blood Service blood drives in the greater Johannesburg area. Control participants were excluded from the study if they had a random blood glucose measurement > 11.1 mmol/l (n = 3), a family history of T1D (n = 0), type 2 diabetes (n = 1), chronic pancreatitis (n = 0), were pregnant (n = 0) or had two or more beta cell autoantibodies (n = 1).

This study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human subjects/patients were approved by the University of the Witwatersrand human research ethics committee; (M180334 and M150885) and by the South African National Blood Service Research Ethics Committee (clearance certificate number 2014/19). Written informed consent was obtained from all subjects/patients. All participants completed a questionnaire self-reporting age at diagnosis.

Sample size

The number of participants used in this study was calculated based on the ability to determine differences in allele frequencies between cases and controls for the VDR polymorphisms with the highest (62% for the A allele; rs7975232) and lowest (24% for the A allele; rs1544410) recorded disease-associated allele frequencies in African populations.[30] Thus, with an N of 150 participants in each group we will be able to detect an 11% difference in the A allele for rs7975232 between cases and controls and a 10% difference in the A allele for rs1544410 at $P < 0.05$.

Genotyping participants for the BsmI, FokI, ApaI and TaqI polymorphisms

DNA was extracted from whole blood using the Invisorb Spin Blood Mini DNA Extraction Kit (Stratatec Biomedical AG, Birkenfield, Germany) according to the manufacturer's instructions. Participants were genotyped for the four polymorphisms using PCR-RFLP. Primer sequences were obtained from the literature.[13,31] Supertherm Gold Taq polymerase (JMR Holdings, London, United Kingdom) was used to amplify the regions flanking the ApaI, FokI and TaqI polymorphisms while OneTaq 2x mastermix (New England Biolabs Inc., Massachusetts, USA) was used to amplify the region flanking the BsmI polymorphism. The resultant PCR products were digested with ApaI (10U), FokI (5U), TaqI (10U) and BsmI (10U), restriction endonucleases (New England Biolabs Inc., Massachusetts, USA) and participants genotyped based on the resultant fragment sizes.

Measurement of vitamin D

Vitamin D levels were measured using the ClinRep High Performance Liquid Chromatography complete kit 25-OH-Vitamin D_2/D_3 (RECIPE, Munich, Germany) according to the manufacturer's instructions. Vitamin D status was based on the National Academy of Medicine guidelines for the classification of Vitamin D status (> 50 nmol/l = sufficient; 30–50 nmol/l = insufficient; < 30.0 nmol/l = deficient).[32]

Measurement of control plasma glucose concentration

The enzymatic hexokinase method was used to measure random plasma glucose concentrations in control participants on the ADVIA Chemistry System (Siemens Health Care Diagnostics Inc., New York, USA) in the Chemical Pathology National Health Laboratory Service diagnostic laboratory.

Measurement of autoantibodies

GAD65 and IA-2 autoantibody positivity was measured by ELISA (Kronus, Star, ID, USA) according to the manufacturer's instructions. Each ELISA plate was run in the same laboratory using serum samples from both cases and controls. Participants were considered GAD65 and IA-2 autoantibody positive when the concentration was ≥5 IU/ml and ≥15 IU/ml, respectively, based on the manufacturer's recommendations.

Sun exposure

All participants completed a questionnaire in which they reported their daily time spent in the sun (<5, 5–30 and >30 min) and degree of skin exposure (four categories ranging from face and hands only to "bathing suit") for one week.[33] The weekly sun exposure score (0–58) was calculated from the sum of the daily sun exposure scores (the product of time spent outdoors and skin exposure; 0–8).

Anthropometric measurements

The weight of participants was measured in kilograms on an electronic calibrated weighing scale (Seca, Hamburg, Germany)

after removing shoes and any heavy clothing. Height was measured in centimetres without shoes.

Statistical analysis

Statistical analysis was performed using Statistica software v14.0.0.15 (StatSoft, Oklahoma, United States of America). Results with a P value < 0.05 were considered to be statistically significant. Data that were not normally distributed (disease duration and glucose) were log-transformed to normality. Data with a normal distribution were represented as mean ± standard deviation and non-parametric data as median (lower quartile; upper quartile). Categorical data was represented as per cent (%) or frequency. The control population was tested for Hardy–Weinberg equilibrium. Continuous variables were compared between cases and controls using the two-tailed Student's non-paired *t*-test. Categorical variables were compared using a chi-squared (χ^2) test. Significant levels are two-sided. Due to the low frequency of participants with the BsmI (AA), TaqI (CC) and FokI (TT) genotypes they were combined with the heterozygous genotype for statistical analysis. A two-tailed Student's non-paired *t*-test was used to compare continuous variables between the BsmI, TaqI and FokI genotypes and an ANOVA was used to compare continuous variables across the three ApaI genotypes.

Backward stepwise multivariable linear regression analysis was performed with vitamin D concentration as the dependent variable. Independent variables (season of sampling, sex, sun exposure and VDR genotypes) were selected based on scientific plausibility and subjected to univariate analysis with the dependent variable. When the resultant P-value was < 0.2, the variable was included in the multivariable regression model. The same process was used for the backwards stepwise multivariable logistic regression analysis of T1D status. In this model, the independent variables included in the univariate analyses were sex, family history of diabetes, vitamin D levels and VDR genotypes.

Results

Clinical and phenotypic characteristics of participants with T1D and control participants

The clinical and phenotypic characteristics of cases and controls are summarised in Table 1. The mean age at diagnosis of T1D was 20.7 ± 8.4 years and the median disease duration was 7.0 (2.0; 11.0) years. Glucose levels were significantly higher in the patient group than in the control group (9.2 (5.7; 13.2) vs. 5.6 (4.7; 7.6); P < 0.001). The mean HbA1c levels in cases was 10.6 ± 3.4 indicative of poor glycaemic control. Participants with T1D had a significantly lower body mass index (BMI) than control participants (25.0 ± 5.9 vs. 28.0 ± 5.9; P < 0.001). No other variable differed between groups. Vitamin D concentrations ranged from 21.3 to 159.6 nmol/l. The majority (70.1%) of participants had sufficient levels (> 50 nmol/l) of vitamin D, 27.2% had insufficient levels (30–50 nmol/l) and 2.7% of the cohort were deficient in vitamin D (< 30 nmol/l). The percentage of participants who were deficient, insufficient and sufficient for vitamin D did not differ between patients and controls (2.7, 26.9, 70.3 vs. 2.7, 27.5, 69.8 %, respectively; P = 0.992).

Genotypic and allelic frequencies of the VDR gene polymorphisms in cases and controls

All four *VDR* gene polymorphisms were found to be in Hardy–Weinberg equilibrium (P > 0.05). There was no significant difference in allelic and genotypic frequencies between participants with T1D and control participants for any of the *VDR* gene polymorphisms (Table 2).

Table 1. Clinical and phenotypic characteristics of type 1 diabetes patients and control participants

Variables	Cases n = 182	Controls n = 151	P value
Age (years)	28.9 (9.3)[a]	29.2 (9.7)[b]	0.804
Age at diagnosis (years)	20.7 (8.4)[a]	–	–
Duration of disease (years)	7.0 (2.0; 11.0)[c]	–	–
Gender (% male, n)	54.4 (99)	43.7 (66)	0.052
Glucose (mmol/l)	9.2 (5.7; 13.2)[d]	5.6 (4.7; 7.6)	<0.001
HbA1c (%)	10.6 (3.4)[d]	–	–
BMI (kg/m^2)	25.0 (5.9)[e]	28.0 (5.9)[e]	<0.001
Waist circumference (cm)	77.9 (13.0)[g]	80.8 (13.3)[a]	0.053
Vitamin D (nmol/l)	62.8 (20.7)	59.5 (17.0)[a]	0.122

Data displayed as mean (SD) or median (lower quartile; upper quartile); Missing data: [a]2, [b]10, [c]1, [d]14, [e]3, [f] 8, [g]7.

Associations of the VDR gene polymorphisms with vitamin D levels in black South African participants

The associations between *VDR* genotypes and vitamin D levels in the total population are shown in Table 3. Participants with the FokI CC genotype had significantly lower vitamin D levels compared to participants with the TC/ TT genotypes (59.3 ± 18.2 vs. 65.1 ± 20.7 nmol/l; P = 0.009). Similarly, participants with the TaqI CC/TC combined genotypes had significantly lower vitamin D levels than participants with the TT genotype (58.4 ± 18.5 vs. 62.8 ± 19.5 nmol/l; P = 0.047). The remaining *VDR* polymorphisms did not show an association with vitamin D levels.

VDR genotypes and clinicopathological variables in participants with T1D

The *VDR* gene polymorphisms and their association with different clinicopathological variables are summarised in Tables 4–7. The FokI CC genotype was associated with significantly lower vitamin D levels compared to the CT/TT genotypes (60.3 ± 19.2 vs. 68.2 ± 23.0; P = 0.017). The remaining *VDR* polymorphisms showed no statistically significant difference with any of the clinicopathological variables investigated.

Determinants of vitamin D levels in the total cohort

Upon multiple regression analysis, sampling in spring/winter, sex, FokI and TaqI genotype were associated with vitamin D concentrations in the total cohort when controlling for sun exposure (Table 8). Vitamin D levels were 9.6 nmol/l lower in individuals recruited during winter/spring than participants recruited in summer/autumn (P < 0.001). In addition, vitamin D levels were 5.8 nmol/l higher in males compared to females (P = 0.005). Individuals with the FokI CT/TT genotypes had vitamin D levels 5.4 nmol/l higher than those with the CC genotype (P = 0.014).

Table 2. Genotypic and allelic frequencies of vitamin D receptor gene polymorphisms in cases and controls

SNP	Cases Frequency (n)	Controls Frequency (n)	P value
BsmI			
Genotype model			
AA	0.05 (9)	0.05 (7)	0.448
AG	0.28 (49)	0.34 (47)	
GG	0.67 (120)	0.61 (84)	
Allele model			
A	0.19 (67)	0.22 (61)	0.309
G	0.81 (289)	0.78 (215)	
FokI			
Genotype model			
CC	0.69 (125)	0.63 (95)	0.256
CT	0.29 (53)	0.31 (47)	
TT	0.02 (4)	0.05 (8)	
Allele model			
C	0.83 (303)	0.79 (237)	0.163
T	0.17 (61)	0.21 (63)	
ApaI			
Genotype model			
CC	0.13 (24)	0.09 (13)	0.389
CA	0.43 (78)	0.43 (64)	
AA	0.44 (79)	0.48 (72)	
Allele model			
C	0.35 (126)	0.30 (90)	0.210
A	0.65 (236)	0.69 (208)	
TaqI			
Genotype model			
TT	0.69 (123)	0.60 (89)	0.189
TC	0.27 (48)	0.36 (54)	
CC	0.04 (7)	0.04 (6)	
Allele model			
T	0.83 (294)	0.78 (232)	0.129
C	0.17 (62)	0.22 (66)	

Participants with the TaqI TT had vitamin D levels 4.3 nmol/l higher than participants with the TC/CC genotypes (P = 0.045).

Logistic regression analysis for the determinants of T1D

The main determinants of T1D were a family history of T1D and TaqI TT genotype when controlling for vitamin D levels, sex, and FokI genotypes (Table 9). A positive family history of T1D was associated with a 5 times higher risk of T1D (P < 0.001). In addition, participants with the Taq CT/CC genotypes had a 41% lower risk of T1D compared to participants with the TT genotype (P = 0.040).

Table 3. Associations of the vitamin D receptor polymorphisms with vitamin D levels in black South African participants

VDR polymorphism	Genotype (n)	Vitamin D (nmol/l)	P value
BsmI	GG (204)	61.9 (19.2)	0.56
	AA + AG (110)[a]	60.6 (19.5)	
FokI	CC (218)[a]	59.3 (18.2)	0.009
	TC + TT (112)	65.1 (20.7)	
ApaI	CC (37)	63.2 (17.0)	0.743
	CA (141)[b]	61.6 (18.6)	
	AA (150)[b]	60.6 (20.3)	
TaqI	TT (211)[b]	62.8 (19.5)	0.047
	TC + CC (113)[a]	58.4 (18.5)	

Data displayed as mean (SD); Missing data: [a]2, [b]1.

Table 4. Association of the BsmI vitamin D receptor gene polymorphism with clinicopathological variables in participants with type 1 diabetes

Variable	BsmI genotype GG (n = 120)	AA+ AG (n = 58)	P value
Age at diagnosis (years)	21.3 (7.6)[a]	19.1 (9.2)	0.096
Male (%, n)	53.3 (64)	58.6 (34)	0.506
Glucose (mmol/l)	8.3 (5.4; 13.0)[b]	10.2 (6.5; 13.3)[c]	0.240
HbA1c (%)	10.6 (3.5)[d]	10.4 (3.2)[d]	0.772
BMI (kg/m^2)	25.1 (6.1)[a]	24.9 (5.7)[e]	0.831
Waist (cm)	78.2 (14.1)[c]	77.7 (11.0)[e]	0.818
Vitamin D (nmol/l)	63.6 (20.8)	61.6 (21.0)	0.556

Data displayed as mean (SD) or median (lower quartile; upper quartile); Missing data: [a]2, [b]8, [c]6, [d]7, [e]1.

Table 5. Association of the FokI vitamin D receptor gene polymorphism with clinicopathological variables in participants with type 1 diabetes

Variable	FokI genotype CC (n = 125)	CT + TT (n = 57)	P value
Age at diagnosis (years)	20.1 (8.3)[a]	21.9 (8.4)[a]	0.200
Male (%, n)	56.0 (70)	50.9 (29)	0.520
Glucose (mmol/l)	9.0 (5.8; 14.1)[b]	9.3 (5.5; 13.0)[c]	0.588
HbA1c (%)	10.4 (3.3)[d]	11.0 (3.7)[a]	0.275
BMI (kg/m^2)	25.3 (6.2)[a]	24.4 (5.3)[e]	0.350
Waist (cm)	78.8 (13.4)[f]	76.1 (12.1)[e]	0.205
Vitamin D (nmol/l)	60.3 (19.2)	68.2 (23.0)	0.017

Data displayed as mean (SD) or median (lower quartile; upper quartile); Missing data: [a]1, [b]11, [c]3, [d]13, [e]2.

Discussion

The associations of VDR polymorphisms with vitamin D levels and T1D in the black South African population have not previously been investigated. The FokI polymorphism, TaqI polymorphism,

Table 6. Association of the ApaI vitamin D receptor gene polymorphism with clinicopathological variables in participants with type 1 diabetes

	ApaI genotype			
	CC (n = 24)	CA (n = 78)	AA (n = 79)	
Variable	Mean/median	Mean/median	Mean/median	P value
Age at diagnosis (years)	21.1 (10.0)	20.4 (8.05)[a]	20.8 (8.3)	0.914
Male (%, n)	45.8 (11)	56.4 (44)	54.4 (43)	0.660
Glucose (mmol/l)	10.4 (5.7; 16.8)[b]	8.1 (5.3; 13.0)[c]	9.8 (6.1; 13.3)[d]	0.215
HbA1c (%)	10.8 (3.5)[e]	10.7 (3.7)[e]	10.6 (3.1)[f]	0.962
BMI (kg/m^2)	24.9 (6.5)[b]	24.7 (5.8)	20.2 (5.9)[a]	0.882
Waist (cm)	75.6 (12.7)	77.7 (13.0)[g]	78.7 (13.2)[e]	0.592
Vitamin D (nmol/l)	61.8 (19.4)	65.1 (20.6)	61.0 (21.4)	0.454

Data displayed as mean (SD) or median (lower quartile; upper quartile); Missing data: [a]2, [b]1, [c]6, [d]7, [e]3, [f]8, [g]4.

Table 7. Association of the TaqI vitamin D receptor gene polymorphism with clinicopathological variables in participants with type 1 diabetes

	TaqI genotype		
	TT (n = 123)	TC + CC (n = 55)	
Variable	Mean/median	Mean/median	P value
Age at diagnosis (years)	20.9 (8.2)[a]	20.2 (8.8)	0.636
Male (%, n)	52.8 (65)	56.4 (31)	0.664
Glucose (mmol/l)	9.9 (5.7; 15.0)[b]	8.2 (5.7; 12.9)[c]	0.248
HbA1c (%)	10.4 (3.3)[d]	11.3 (3.6)[e]	0.128
BMI (kg/m^2)	24.7 (5.4)[f]	25.4 (7.0)[a]	0.527
Waist (cm)	77.4 (12.9)[e]	78.7 (13.4)[a]	0.561
Vitamin D (nmol/l)	63.5 (21.4)	60.7 (19.7)	0.396

Data displayed as mean (SD) or median (lower quartile; upper quartile); Missing data: [a]1, [b]9, [c]5, [d]8, [e]6, [f]2.

Table 8. Multivariable linear regression model to determine the main factors influencing vitamin D concentration

Dependent variables	Independent variables	b value	P value
Vitamin D (n = 324)	Season[a]	−9.60	<0.001
	Sex[b]	5.76	0.005
	FokI genotype[c]	5.35	0.014
	TaqI genotype[d]	4.30	0.045

For full model $R^2 = 0.111$ and $P < 0.001$.
[a]Season code: Autumn/summer = 0, winter/spring = 1.
[b]Sex: female = 0, male = 1.
[c]FokI genotype: CC = 0, CT/TT = 1.
[d]TaqI genotype: TC/CC = 0, TT = 1.

Table 9. Logistic regression model for the determinants of type 1 diabetes (T1D) status

Dependent variable	Independent variable	Odds ratio (95% CI)	P value
T1D status[a]	Family history[b]	5.00 (3.08–8.11)	<0.001
	TaqI genotype[c]	0.59 (0.36–0.98)	0.040

For full model $P < 0.001$ (n = 324).
[a]case = 1, control = 0.
[b]positive family history = 1, negative family history = 0.
[c]TC + CC genotype = 1; TT genotype = 0.

season of sampling and sex were associated with vitamin D levels. Furthermore, the VDR TaqI TT genotype and a family history of T1D were associated with T1D in the present study.

Reduced vitamin D levels have been associated with T1D in Egyptian, Australian, Saudi Arabian, Indian and Scandinavian populations.[34–39] This is in contrast to our study where vitamin D levels were not associated with T1D status. Similarly, studies in Chilean, American and Danish populations found no difference in vitamin D levels between participants with T1D and healthy controls.[20,40,41] The conflicting results in these studies may be due to differences in participant ethnicity, age, BMI, sun exposure, and the method used for vitamin D measurements. In addition, the studies showing an association between vitamin D levels and T1D tended to have a smaller sample size (36–100 participants with T1D) compared to those studies which did not show an association (156–907 participants with T1D) and which includes the current study. It is therefore possible that the associations observed in the smaller studies were due to chance i.e. a type 1 error.

The current study found that participants with the FokI CC genotype had significantly lower levels of serum vitamin D compared to participants with the FokI TC/TT genotype. Similarly, in Egyptian children with COVID-19 (n = 180), Jordanian men with prostate cancer (n = 124) and healthy Jordanian men (n = 100) the FokI CC genotype was associated with lower vitamin D levels.[42,43] In addition, in Brazilian participants with T1D (n = 65), the FokI CT/CC genotypes were associated with lower levels of vitamin D compared to the TT genotype.[44] In contrast, the FokI CC genotype was associated with higher vitamin D levels in a healthy Turkish Cypriot population (n = 320), Egyptian children with T1D (n = 132), Americans with actinic keratoses (n = 137) and a Han Chinese population with high levels of low density lipoprotein (n = 192) compared to participants with the FokI TT genotype.[45–48] Furthermore, no association between FokI genotype and vitamin D levels was seen in a healthy adolescent Brazilian population.[49] Similarly, no association was found between FokI genotype and vitamin D levels in a heterogenous South African population (white (n = 73), black (n = 175) and Indian (n = 21)) with chronic kidney disease[50] and in a South African mixed-ancestry population (n = 968) with and

without hyperglycaemia/diabetes.[51] The reasons for the differences in the outcomes of these studies, in addition to the factors mentioned above for the discrepancies in the T1D-vitamin D investigations, may include the disease status of the participants. The lack of agreement of our study with the two South African studies may be that these studies looked at different ethnic groups and did not report the results for the black participants separately and the use of a mixed-ancestry population with no adjustment for ethnic admixture.

The VDR-RXR-vitamin D complex activates the transcription of *CYP24A1*, which encodes 24-hydroxlase, the enzyme responsible for the catabolism of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3 to their less active metabolites lowering the concentrations of 25-hydroxyvitamin D_3 and 1,25-dihydroxyvitamin D_3.[52] In South African black participants, the VDR FokI CC genotype has been found to increase the transcription of *CYP24A1* mRNA.[53] Thus, with increased degradation of vitamin D, the serum vitamin D levels are expected to be lower. Alternatively, the shorter VDR protein encoded by the C allele is known to have increased transactivational activity due to an increased binding affinity with the VDR co-activator, transcription factor II B.[54,55] It is therefore possible that the increased transactivation results in increased internalisation of vitamin D-bound VDR and thus lower serum vitamin D concentrations.

In addition, the TaqI TT genotype was associated with higher vitamin D concentrations compared to the TC/CC genotypes. This is similar to results from Polish, Egyptian and Greek populations.[56–58] Similarly, in the Chinese population, participants with the TT and TC genotypes had higher vitamin D levels than participants with the CC genotype.[59] However, studies in the Iranian and Chinese populations found no association between TaqI genotype and vitamin D levels.[60,61]

Bhanushali and colleagues hypothesised that the mechanism through which TaqI influences vitamin D levels is through VDR-mediated calcium metabolism.[62] The TaqI polymorphism is in linkage disequilibrium with a poly(A) microsatellite repeat (long and short forms) in the 3' untranslated region and regulates gene expression through modulation of mRNA stability.[63] A study by Durrin and colleagues found no association between the poly(A) microsatellite repeat length and mRNA stability.[17] However, the poly(A) microsatellite long form and the TaqI T allele have been associated in cell culture with increased VDR mRNA transcription resulting in higher levels of VDR.[54,64] Thus, the presence of the T allele may result in higher concentrations of VDR which when activated by binding to 1,25-dihydroxyvitamin D and RXR regulates the expression of genes involved in increased calcium absorption from the gut, together with calcium reabsorption in the kidney and bone resulting in high serum calcium concentrations.[65] High serum calcium inhibits the conversion of 25 hydroxyvitamin D to 1,25-dihydroxyvitamin D in the kidney through decreased parathyroid hormone production.[66] The levels of 25 hydroxyvitamin D are therefore higher in the presence of the T allele.

Previous studies looking at the association of VDR polymorphisms with T1D have shown contradictory results. In our study, we found that only the TaqI TT genotype was associated with T1D in multivariate analysis. This is similar to studies in the Dalmation and Greek populations which found the TT genotype associated with T1D.[28,67,68] However, in the Kuwaiti, Egyptian and Iranian populations, the C allele was associated with T1D .[22,24,69] Furthermore, no association between the TaqI polymorphism and T1D was found in the Pakistani, Chilean, Han Chinese, Kurdish and Egyptian populations.[13,20,23,29,70] The discrepancy in results may be due to environmental factors, different populations studied, epigenetic factors or linkage disequilibrium.

Higher vitamin D levels have been shown to be protective against the development of T1D.[4–6] As the TaqI TT genotype is associated with increased vitamin D levels in the South African black population, it is unlikely that TaqI TT increases the risk of T1D through vitamin D-mediated VDR signalling pathways. Therefore, we hypothesise that the VDR TaqI polymorphism is in linkage disequilibrium with a gene region which is the causative factor for T1D.

The strength of this study is that, to the best of our knowledge, it is the first study examining the associations of VDR polymorphisms and vitamin D levels with T1D in the under-represented South African black population. The limitations of this study were a small sample size, participants with T1D were not newly diagnosed, lack of haplotype analysis and the cross-sectional design of the study. All participants were diagnosed by a clinician and met all inclusion criteria; however, we cannot completely rule out that some participants with maturity onset diabetes of the young or other types of diabetes may have been misdiagnosed. In addition, the cases and controls were not matched for season of recruitment and although season of recruitment was adjusted for in our models, it is possible that this was only partially effective at controlling for this confounder. We also cannot rule out other sources of residual confounding. In addition, all participants were recruited from a particular geographic area of South Africa (greater Johannesburg) and therefore our findings may be subject to some level of selection bias.

In conclusion, the TaqI TT genotype is associated with T1D in the black South African population through a mechanism which is independent of vitamin D levels. It is possible that TaqI is not a functional polymorphism but is in linkage disequilibrium with a true causative polymorphism that is responsible for an increased risk of T1D. Future work would include functional studies to determine the true risk alleles for T1D. The FokI CC and TaqI CT/CC genotypes were associated with lower vitamin D levels and thus individuals with these genotypes may need increased vitamin D supplementation compared to participants with the FokI T allele.

Acknowledgements. We would like to acknowledge Prof Raal for allowing us access to the diabetic clinic, Sr Angeline Naidoo for blood draws and the participants for agreeing to partake in this study.

Authors' contributions. CJP designed the study. SBhola recruited participants, collected the data and helped prepare the first draft of the manuscript. EMC and CJP performed statistical analysis, writing and critical review of the manuscript. NJC was involved in critical review of the manuscript. KLP performed and interpreted vitamin D assays. SB provided access to participant samples.

Funding. This work was supported by a National Health Laboratory Service Research Trust Development Grant (C.P. Grant number 94398) and a University of the Witwatersrand Faculty of Health Sciences Faculty Research Committee Grant (S.B). The authors received no funding from commercial or not-for-profit sectors for this research.

Competing interests. The authors declare no conflict of interest.

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