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A prospective study of plasma 25-hydroxyvitamin D concentration and prostate cancer risk

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Abstract

Mechanistic hypotheses suggest that vitamin D and the closely related parathyroid hormone (PTH) may be involved in prostate carcinogenesis. However, epidemiological evidence is lacking for PTH and inconsistent for vitamin D. Our objectives were to prospectively investigate the association between vitamin D status, vitamin D-related gene polymorphisms, PTH and prostate cancer risk. A total of 129 cases diagnosed within the Supplémentation en Vitamines et Minéraux Antioxydants cohort were included in a nested case–control study and matched to 167 controls (13 years of follow-up). 25-Hydroxyvitamin D (25(OH)D) and PTH concentrations were assessed from baseline plasma samples. Conditional logistic regression models were computed. Higher 25(OH)D concentration was associated with decreased risk of prostate cancer ($OR_{Q4\ v.\ Q1}$ 0·30; 95 % CI 0·12, 0·77; $P_{trend}=0·007$). PTH concentration was not associated with prostate cancer risk ($P_{trend}=0·4$) neither did the studied vitamin D-related gene polymorphisms. In this prospective study, prostate cancer risk was inversely associated with 25(OH)D concentration but not with PTH concentration. These results bring a new contribution to the understanding of the relationship between vitamin D and prostate cancer, which deserves further investigation.

Key words: 25-Hydroxyvitamin D: Parathyroid hormone: Prostate cancer risk: SNP: Nested case–control studies

Vitamin D is a prohormone synthesised in the skin from UVB exposure and absorbed from scarce dietary sources. It is first converted to 25-hydroxyvitamin D (25(OH)D) – its main circulating form – and then to 1,25-dihydroxyvitamin D ($1,25(OH)_2D$) – its biologically active form. As 25(OH)D-to-$1,25(OH)_2D$ conversion and $1,25(OH)_2D$ signalling can take place directly in prostate tissues[1], vitamin D is thought to play a role in the prevention of prostate cancer through pro-differentiation, pro-apoptosis, anti-proliferative and growth control activities, as suggested by experimental studies[2–4]. However, so far, epidemiological evidence regarding the relationship between 25(OH)D concentration and prostate cancer risk has been inconsistent. On the basis of a dose–response meta-analysis that involved fifteen prospective studies, the World Cancer Research Fund/American Institute for Cancer Research (WCRF/AICR)[5], as part of the Continuous Update Project 2014 on prostate cancer, stated that the level of proof for the association between 25(OH)D concentration and prostate cancer risk was still 'limited-no conclusion'. Most of the studies included in this meta-analysis observed null results.

Besides, vitamin D is primarily involved in Ca homoeostasis: $1,25(OH)_2D$ increases Ca concentration through enhanced intestinal Ca absorption, reabsorption of Ca from kidneys and bone resorption. Renal 25(OH)D-to-$1,25(OH)_2D$ conversion is induced by parathyroid hormone (PTH) secretion in response to low Ca concentration. $1,25(OH)_2D$ exerts in turn a negative feedback on PTH secretion[6–8]. Vitamin D and PTH are thus closely related. To our knowledge, only one prospective study

Abbreviations: 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-dihydroxyvitamin D; *CaSR*, Ca-sensing receptor; *CYP24A1*, 1,25-dihydroxyvitamin D_3 24-hydroxylase; *GC*, vitamin D-binding globulin, gc-globulin or group-specific component; MAF, minor allele frequency; PTH, parathyroid hormone. *RXR*, retinoid X receptor; SU.VI.MAX, Supplémentation en Vitamines et Minéraux Antioxydants; *VDR*, vitamin D receptor.

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has investigated the association between PTH concentration and prostate cancer risk, with null result[9].

Several genes involved in vitamin D metabolism, in particular signalling (vitamin D receptor (*VDR*) and retinoid X receptor (*RXR*)), transportation (vitamin D-binding protein, also known as gc-globulin or group-specific component (*GC*)) and degradation (1,25-dihydroxyvitamin D3 24-hydroxylase (*CYP24A1*)), or in Ca homoeostasis (Ca-sensing receptor (*CaSR*)) could also play a role in prostate cancer aetiology[2]. Recent meta-analyses found null associations between *VDR* BsmI, FokI and Cdx2 polymorphisms and prostate cancer risk[10–12]. The epidemiological literature dealing with polymorphisms of other genes (*GC*, *CYP24A1*, *RXR* and *CaSR*) in relation to prostate cancer risk is scarce[13–16].

Thus, our objective was to prospectively investigate the associations between prostate cancer risk and vitamin D status (25(OH)D concentration), plasma PTH concentration and polymorphisms of genes involved in vitamin D metabolism.

Methods

Subjects

The Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) study was initially designed as a double-blind placebo-controlled trial (Trial Registration clinicaltrials.gov Identifier: NCT00272428) with purpose to assess the influence of a daily supplementation with nutritional doses of antioxidants (single capsule of a combination of ascorbic acid (120 mg), vitamin E (30 mg), β-carotene (6 mg), Se (100 μg) and Zn (20 mg) or placebo) on the incidence of CVD and cancers[17]. A total of 13 017 participants were enrolled in 1994–1995 for an 8-year-intervention trial and followed up for health events until September 2007. Participants were advised against taking any self-prescribed supplements (vitamin D and others) during the trial.

Ethical approvals

This study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (CCPPRB no. 706/no. 2364) and the 'Commission Nationale de l'Informatique et des Libertés' (CNIL no. 334641/no. 907094). Written informed consent was obtained from all participants.

Case ascertainment

Health events were self-reported by the participants. Then, all relevant medical information and pathological reports were gathered through participants, physicians and/or hospitals and reviewed by an independent physician expert committee. Histologically validated cases were classified according to the *International Chronic Diseases Classification, 10th Revision, Clinical Modification*[18]. All first-incident primary prostate cancers were considered as cases in this study.

Nested case–control study

All prostate cancer cases diagnosed during follow-up (1994–2007 i.e. 13-year of follow-up) were included in a nested case–control study: one or two controls per case were randomly selected among subjects without prostate cancer by the end of follow-up and matched according to the following baseline criteria: age (<40/40–44/45–49/50–54/55–65 years), intervention group of the initial SU.VI.MAX trial (placebo/antioxidants), season of blood draw (*a priori* defined periods: June–October/November–May) and BMI (<25/≥25 kg/m^2).

Baseline data collection

Information on socio-demographics, smoking habits, alcohol consumption, physical activity, medication use and health status was collected at baseline through self-administered questionnaires. Participants underwent a clinical examination by the study nurses and physicians with anthropometric measurements (in particular height and weight) and a blood draw occurring in the early morning after an overnight fasting period of 12 h. A volume of 35 ml venous blood samples was collected in vacutainer tubes (Becton Dickinson) and immediately centrifuged to get plasma aliquots (preserved in Na heparin), and buffy-coat fractions, allowing future DNA extraction. Both were stored frozen in liquid N_2. Participants were asked to provide repeated 24 h-dietary records every 2 months, completed through a French telephone-based terminal equivalent to an internet prototype (Minitel). Portion sizes were assessed by referring to a validated picture booklet[19]. The amounts consumed from composite dishes were estimated using French recipes validated by food and nutrition professionals. Mean daily energy and nutrient intakes were estimated from all available 24 h-dietary records completed during the first 2 years of follow-up, using a published French Food Composition Table[20].

Laboratory assay of plasma 25-hydroxyvitamin D and parathyroid hormone concentrations

25(OH)D and PTH plasma concentrations were determined on baseline samples, as previously described in detail[21,22]. Plasma 25(OH)D concentration was measured using the Roche Cobas® electrochemiluminescence total 25(OH)D assay (Roche Diagnostics), based on the principle of competitive binding[23]. Inter-assay CV was <10 % (eight samples of various 25(OH)D concentrations tested in forty-two separate runs), whereas intra-assay CV was <6·6 % (the same eight samples tested twenty-one times in the same run). Plasma PTH concentration was assessed with the Roche Cobas® electrochemiluminescence immunometric assay (Roche Diagnostics), a second-generation PTH assay that uses two anti-PTH antibodies – one directed towards the 26–32 portion of the PTH molecule and another directed towards the 53–84 portion[24]. Inter-assay CV was <2·9 % (three samples of various PTH concentrations tested in forty-two separate runs), and intra-assay CV was <1·4 % (the same three samples tested twenty-one times in the same run).

Genotyping

One to three SNP were selected for each gene of interest (*VDR*, *CYP24A1*, *GC*, *RXR* and *CaSR*) on the basis of their relatively high frequency in Caucasian populations

(http://www.ncbi.nlm.nih.gov/guide/howto/viewgen-freq/): *VDR* rs1544410 (BsmI, minor allele frequency (MAF): T = 0·2959), rs2228570/10735810 (FokI, MAF: A = 0·3285) and rs11568820 (Cdx2, MAF: T = 0·4569); *CYP24A1* rs4809958 (MAF: G = 0·1907); *GC* rs4588 (MAF: T = 0·2079) and rs7041 (MAF: C = 0·3816); *RXR* rs7861779 (MAF: T = 0·2804) and rs12004589 (T = 0·1304); *CaSR* rs1801725 (MAF: T = 0·0942) and rs4678174 (MAF: C = 0·4619), and of their predicted functional effect (Pupasuite database, http://snpeffect.vib.be and http://pupasuite.bioinfo.cipf.es/). Genomic DNA was extracted from each patient's mononuclear cells in peripheral blood using a MagNA Pure Compact Instrument with a magnetic-bead technology for the isolation process (Roche Diagnostics). Genetic polymorphisms were assessed by allelic discrimination using fluorogenic probes and the 5' nuclease (TaqMan) assay (Applied Biosystems). Quality control of genotyping was carried out for each SNP by investigating any departure from Hardy–Weinberg's equilibrium and comparing observed distributions with those of European reference populations: CSHL-HapMap-CEU and 1000GENOMES-phase_1_EUR (http://www.ensembl.org/) by χ^2 tests.

Statistical analyses

Baseline characteristics were compared between prostate cancer cases and controls using χ^2 tests for categorical variables and Fisher's tests (from ANOVA models) for continuous variables. Associations between prostate cancer risk and 25(OH)D plasma concentration, PTH plasma concentration, dietary Ca intake and SNP were characterised by OR and 95 % CI derived from multivariate conditional logistic regression models. Models were adjusted for several potential confounders that were as follows: (1) factors constitutive to the study design (intervention group of the initial SU.VI.MAX trial (placebo/antioxidants) and month of blood draw (2-month periods in order to take into account the seasonal variation of 25(OH)D concentration)) and (2) cancer risk factors: major socio-demographic variables (age at baseline (continuous) and educational level (primary/secondary/superior)), lifestyle factors (physical activity (irregular/<1 h/d walking equivalent/ ≥1 h/d walking equivalent), alcohol intake (g/d, continuous) and smoking status (never/former/current smoker)), anthropometric variables (height (cm, continuous) and BMI (kg/m^2, continuous)), factors indicating higher susceptibility to prostate cancer (family history of prostate cancer (yes/no) and baseline serum prostate-specific antigen concentration (<3/>3 μg/l)). SNP models were also adjusted for 25(OH)D concentration (ng/ml, continuous) in order to investigate the effect of SNP, at equal levels of 25(OH)D. Further adjustments were tested: energy intake (without alcohol, kJ/d (kcal/d), continuous), dietary variables for which a possible association with prostate cancer has been reported[5], such as dietary intakes of Ca (mg/d, continuous) and dairy products (g/d, continuous), plasma Se (μmol/l, continuous) and α-tocopherol (μmol/l, continuous) concentrations and a mutual adjustment for 25(OH)D and PTH concentrations. Although dietary vitamin D intake was not associated with vitamin D status in SU.VI.MAX, as published previously[21], associations between prostate cancer risk and dietary intakes of vitamin D and Ca were also investigated using conditional logistic regression models (energy-adjusted variables and residual method[25]).

For all models involving dietary intake data, only subjects who provided at least three valid 24 h-dietary records during the first 2 years of follow-up (ninety-six cases and 123 matched controls) were included.

Plasma 25(OH)D concentration was coded as a continuous variable, as quartiles and as insufficiency (<20 ng/ml) according to the US Institute of Medicine's[26] recommendations for the general population. Plasma PTH concentration was coded as a continuous variable and as quartiles. As there is no established hypothesis on the dominant, codominant or recessive character of the studied SNP, the three following codings were tested: codominant (heterozygous type (HT) *v.* wild type (WT) and homozygous mutant type (MT) *v.* WT), dominant (HT + MT *v.* WT) and recessive (MT *v.* WT + HT). Besides, considering the relationships existing between 25(OH)D concentration, polymorphisms of vitamin D-related genes, PTH concentration and Ca[6–8], two-way interactions between 25(OH)D concentration, PTH concentration and dietary Ca intake, and between the ten SNP and 25(OH)D concentration were tested by introducing the product of the two variables into the main model. For all covariates, missing data represented <5 % and were replaced by the mode.

All statistical tests were two-sided and $P < 0{\cdot}05$ was considered significant. Analyses were performed using SAS software version 9.3 (SAS Institute).

Results

A total of 129 prostate cancer cases diagnosed within the SU.VI.MAX cohort were included in this study. Mean age at diagnosis was 63·0 years and mean baseline-to-diagnosis time was 8·3 years. Of the cases, 49·2 % had a Gleason's score ≥7. A total of 167 controls were randomly selected and matched to the cases. Table 1 summarises the characteristics of prostate cancer cases and controls. Compared with controls, prostate cancer cases were more likely to have a lower vitamin D status at baseline and to be better educated. Severe vitamin D deficiency (<10 ng/ml) was observed for 14·0 % of cases and 13·8 % of controls, and vitamin D insufficiency (<20 ng/ml) was observed for 62·8 % of cases and 54·5 % of controls, with no statistically significant difference between cases and controls. A seasonal fluctuation of vitamin D status was observed in controls with decreasing vitamin D status from October to March (shortening days) and increasing vitamin D status in April–May (extending days). All studied SNP respected the Hardy–Weinberg's equilibrium ($P > 0{\cdot}05$). The repartition of subjects across the different genotypes was in accordance with that observed in European reference populations (CSHL-HapMap-CEU and 1000GENOMES-phase_1_EUR) for all SNP ($P > 0{\cdot}05$).

25(OH)D concentration was inversely associated with prostate cancer risk ($OR_{per\ 1\ ng/ml}$ 0·96; 95 % CI 0·93, 1·00; $P_{trend} = 0{\cdot}04$; $OR_{Q4\ v.\ Q1}$ 0·30; 95 % CI 0·12, 0·77; $P_{trend} = 0{\cdot}007$; $OR_{<20\ v.\ \geq 20\ ng/ml}$ 0·44; 95 % CI 0·23, 0·85; $P = 0{\cdot}01$, Table 2; $OR_{per\ 30\ nmol/l}$ 0·64; 95 % CI 0·42, 0·97; $P_{trend} = 0{\cdot}04$, data not tabulated). Using the quartile coding this inverse association was observed in particular for cases with a Gleason's score <7 (sixty-nine cases/ninety controls, $OR_{Q4\ v.\ Q1}$ 0·03; 95 % CI 0·003, 0·40; $P_{trend} = 0{\cdot}02$; data not tabulated), whereas it was not significant for cases with a Gleason's score ≥7 (sixty cases/seventy-seven controls, $OR_{Q4\ v.\ Q1}$ 0·96; 95 % CI 0·23, 4·05; $P_{trend} = 0{\cdot}5$; data not tabulated). However, using

Table 1. Baseline characteristics of prostate cancer cases and controls, Supplémentation en Vitamines et Minéraux Antioxydants cohort, France (1994–2007)
(Numbers and percentages; mean values and standard deviations)

	Prostate cancer cases (*n* 129)				Controls (*n* 167)				
	n	%	Mean	SD	*n*	%	Mean	SD	*P**
Age (years)			54·7	4·7			54·6	4·5	0·9
BMI (kg/m²)			25·8	3·2			25·6	3·1	0·6
									0·9
<25	55	42·6			70	41·9			
≥25	74	57·4			97	58·1			
Height (cm)			173·5	6·6			172·8	6·7	0·4
Intervention group									0·9
Antioxidants	55	42·6			72	43·1			
Placebo	74	57·4			95	56·9			
Smoking status									0·8
Never	54	41·9			65	38·9			
Former	59	45·7			78	46·7			
Current	16	12·4			24	14·4			
Physical activity									0·7
Irregular	28	21·7			36	21·6			
<1 h/d walking equivalent	37	28·7			41	24·6			
≥1 h/d walking equivalent	64	49·6			90	53·9			
Educational level									0·03
Primary	26	20·2			56	33·5			
Secondary	44	34·1			54	32·3			
Superior	59	45·7			57	34·1			
Prostate-specific antigen (μg/l)			3·4	3·6			1·3	1·3	<0·0001
≥3	42	32·6			14	8·4			<0·0001
Family history of prostate cancer (yes)†	14	10·9			9	5·4			0·1
Gleason's score ≥7‡	60	49·2							
Alcohol intake (g/d)			28·5	21·4			28·5	23·6	0·98
Energy intake (without alcohol, kJ/d)			9151·2	2545·5			9597·7	2008·3	
Energy intake (without alcohol, kcal/d)§			2187·2	608·4			2293·9	480·0	0·1
Dietary Ca intake (mg/d)§			1002·7	394·0			1052·8	331·2	0·3
Dietary vitamin D intake (μg/d)§			2·9	2·0			3·2	2·1	0·3
Month of blood draw									0·2
October–November	10	7·8			26	15·6			
December–January	38	29·5			47	28·1			
February–March	62	48·1			69	41·3			
April–May	19	14·7			25	15·0			
Plasma 25-hydroxyvitamin D (ng/ml)			18·7	8·9			20·5	9·7	0·1
October–November			22·5	10·8			24·2	8·1	
December–January			20·2	10·4			23·5	11·3	
February–March			17·9	7·9			17·4	8·4	
April–May			16·5	6·7			19·5	8·6	
Plasma parathyroid hormone (pg/ml)			26·4	7·4			27·3	9·6	0·4
VDR BsmI rs1544410‖									0·6
C/C (WT)	45	36·3			53	33·8			
C/T (HT)	62	50·0			75	47·8			
T/T (MT)	17	13·7			29	18·5			
VDR FokI rs2228570‖									0·1
G/G (WT)	45	35·4			75	45·7			
A/G (HT)	64	50·4			62	37·8			
A/A (MT)	18	14·2			27	16·5			

Table 1. *Continued*

	Prostate cancer cases (*n* 129)				Controls (*n* 167)				
	n	%	Mean	SD	*n*	%	Mean	SD	*P**
VDR Cdx2 rs11568820‖									0·3
C/C (WT)	75	60·5			86	54·1			
C/T (HT)	41	33·1			66	41·5			
T/T (MT)	8	6·5			7	4·4			
CYP24A1 rs4809958‖									0·7
G/G (WT)	84	67·7			110	67·9			
G/T (HT)	39	31·5			49	30·3			
T/T (MT)	1	0·8			3	1·9			
GC rs4588‖									0·3
G/G (WT)	71	57·3			82	49·7			
G/T (HT)	43	34·7			63	38·2			
T/T (MT)	10	8·1			20	12·1			
GC rs7041‖									0·2
A/A (WT)	19	15·0			39	23·6			
A/C (HT)	63	49·6			76	46·1			
C/C (MT)	45	35·4			50	30·3			
RXR rs7861779‖									0·7
C/C (WT)	93	73·8			116	73·9			
C/T (HT)	31	24·6			40	25·5			
T/T (MT)	2	1·6			1	0·6			
RXR rs12004589‖									0·5
G/G (WT)	104	81·9			129	78·7			
G/T (HT)	21	16·5			34	20·7			
T/T (MT)	2	1·6			1	0·6			
CaSR rs1801725‖									0·4
G/G (WT)	91	72·8			114	70·4			
G/T (HT)	29	23·2			45	27·8			
T/T (MT)	5	4·0			3	1·9			
CaSR rs4678174‖									0·8
T/T (WT)	62	50·8			76	47·2			
C/T (HT)	52	42·6			72	44·7			
C/C (MT)	8	6·6			13	8·1			

VDR, vitamin D receptor; WT, wild type; HT, heterozygous type; MT, homozygous mutant type; *CYP24A1*, 1,25-dihydroxyvitamin D_3 24-hydroxylase; *GC*, vitamin D-binding protein, also known as gc-globulin or group-specific component; *RXR*, retinoid X receptor; *CaSR*, Ca-sensing receptor.

* *P* value for the comparison between cases and controls using χ^2 tests or Fisher's tests as appropriate.

† Among first-degree relatives.

‡ Data available for 122 cases.

§ Mean dietary intakes from 24 h-dietary records during the first 2 years of follow-up, data available for 167 controls and 122 cases.

‖ Missing data were as follows: 15 (rs1544410), 5 (rs2228570), 13 (rs11568820), 10 (rs4809958), 7 (rs4588), 4 (rs7041), 13 (rs7861779), 5 (rs12004589), 9 (rs1801725), 13 (rs4678174).

Table 2. Associations between 25-hydroxyvitamin D (25(OH)D) and parathyroid hormone (PTH) plasma concentrations, and prostate cancer risk, from conditional logistic regression, Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) cohort, France (1994–2007)
(Odds ratios and 95 % confidence intervals)

				Quartiles*								Insufficiency			
	Per 1 unit increment			Q1	Q2		Q3		Q4				<20 ng/ml	≥20 ng/ml	
	OR	95 % CI	P_{trend}	OR	OR	95 % CI	OR	95 % CI	OR	95 % CI	P_{trend}	OR	OR	95 % CI	*P*
25(OH)D (ng/ml)															
Cases/controls	129/167			42/32	31/43		25/49		31/43			81/91	48/76		
Model 1†	0·96	0·93, 1·00	0·04	1·00	0·44	0·19, 1·04	0·18	0·07, 0·49	0·30	0·12, 0·77	0·007	1·00	0·44	0·23, 0·85	0·01
Cases/controls	96/123			27/20	23/35		20/35		26/33			57/66	39/57		
Model 2†	0·95	0·91, 1·00	0·06	1·00	0·35	0·12, 1·07	0·13	0·04, 0·49	0·25	0·08, 0·81	0·02	1·00	0·43	0·19, 1·00	0·05
Cases/controls	96/123			27/20	23/35		20/35		26/33			57/66	39/57		
Model 3†	0·96	0·91, 1·01	0·08	1·00	0·33	0·11, 1·03	0·12	0·03, 0·46	0·28	0·08, 0·95	0·03	1·00	0·43	0·18, 1·01	0·05
PTH (pg/ml)															
Cases/controls	129/167			31/43	34/40		34/40		30/44						
Model 1†	0·97	0·94, 1·01	0·1	1·00	0·90	0·40, 2·05	0·95	0·40, 2·27	0·66	0·28, 1·55	0·4				
Cases/controls	96/123			20/35	30/26		27/30		19/32						
Model 2†	0·96	0·91, 1·01	0·09	1·00	1·72	0·60, 4·90	1·95	0·62, 6·18	0·77	0·25, 2·36	0·6				
Cases/controls	96/123			20/35	30/26		27/30		19/32						
Model 3†	0·96	0·91, 1·01	0·1	1·00	1·63	0·56, 4·77	2·25	0·67, 7·59	0·81	0·25, 2·62	0·8				

Q, quartiles.

* Model 1: cut-offs for quartiles of 25(OH)D plasma concentration (ng/ml) and PTH plasma concentration (pg/ml) were, respectively, 12·9/18·2/24·7 and 20·9/26·0/30·6. Models 2 and 3 are restricted to men who provided at least three valid 24 h-dietary records (ninety-six cases/123 controls): cut-offs for quartiles of 25(OH)D plasma concentration (ng/ml) and PTH plasma concentration (pg/ml) were, respectively, 13·7/18·5/25·2 and 20·9/25·9/30·2.

† Model 1 was adjusted for age at baseline (continuous, matching factor), intervention group of the initial SU.VI.MAX trial (antioxidants/placebo, matching factor), month of blood draw (October–November/December–January/February–March/April–May), educational level (primary/secondary/superior), physical activity (irregular/<1 h/d walking equivalent/≥1 h/d walking equivalent), alcohol intake (g/d, continuous), smoking status (never/former/current), height (cm, continuous), BMI (kg/m², continuous, matching factor), family history of prostate cancer (yes/no) and baseline serum prostate-specific antigen concentration (<3/≥3 ng/l). Model 2 corresponds to model 1 further adjusted for energy intake (without alcohol) (continuous, kJ/d (kcal/d)), dietary intakes of Ca intake (continuous, mg/d) and dairy products (continuous, g/d), plasma Se (continuous, μmol/l) and *α*-tocopherol (continuous, μmol/l) concentrations. Model 3 corresponds to model 2 with further mutual adjustment for 25(OH)D and PTH plasma concentrations (continuous).

Table 3. Associations between SNP of genes involved in vitamin D metabolism and prostate cancer risk, from conditional logistic regression, Supplémentation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) cohort, France (1994–2007)
(Odds ratios and 95 % confidence intervals)

	WT	HT		MT		
	OR	OR	95 % CI	OR	95 % CI	P_{trend}
VDR BsmI rs1544410						
Cases/controls*	42/52	60/73		16/27		
Model†	1·00	1·13	0·57, 2·22	0·81	0·34, 1·92	0·8
VDR FokI rs2228570						
Cases/controls*	43/74	64/61		18/26		
Model†	1·00	1·86	0·98, 3·52	1·06	0·46, 2·48	0·5
VDR Cdx2 rs11568820						
Cases/controls*	72/83	38/62		8/7		
Model†	1·00	0·52	0·27, 1·01	0·61	0·17, 2·20	0·1
CYP24A1 rs4809958						
Cases/controls*	82/106	39/46		1/3		
Model†	1·00	0·79	0·40, 1·57	0·25	0·02, 3·56	0·3
GC rs4588						
Cases/controls*	70/79	42/59		10/20		
Model†	1·00	0·90	0·47, 1·70	0·69	0·24, 1·96	0·5
GC rs7041						
Cases/controls*	19/38	61/75		45/49		
Model†	1·00	1·34	0·54, 3·33	1·39	0·60, 3·26	0·5
RXR rs7861779						
Cases/controls*	87/114	30/38		2/1		
Model†	1·00	1·19	0·59, 2·41	4·49	0·21, 95·0	0·5
RXR rs12004589						
Cases/controls*	102/128	21/32		2/1		
Model†	1·00	0·71	0·34, 1·52	2·76	0·14, 52·9	0·6
CaSR rs1801725						
Cases/controls*	86/111	29/43		5/3		
Model†	1·00	0·72	0·35, 1·46	2·47	0·36, 16·8	0·9
CaSR rs4678174						
Cases/controls*	60/72	49/67		7/12		
Model†	1·00	0·74	0·40, 1·39	0·37	0·09, 1·43	0·1

WT, wild type; HT, heterozygous type; MT, homozygous mutant type; *VDR*, vitamin D receptor; *CYP24A1*, 1,25-dihydroxyvitamin D_3 24-hydroxylase; *GC*, vitamin D-binding protein, also known as gc-globulin or group-specific component; *RXR*, retinoid X receptor; *CaSR*, Ca-sensing receptor.

* Missing data were as follows: 15 (rs1544410), 5 (rs2228570), 13 (rs11568820), 10 (rs4809958), 7 (rs4588), 4 (rs7041), 13 (rs7861779), 5 (rs12004589), 9 (rs1801725), 13 (rs4678174). Because of the conditional logistic regression model (matched analyses), cases with no control and controls with no case were deleted from the analysis.

† Adjusted for 25-hydroxyvitamin D concentration (continuous, ng/ml), age at baseline (continuous, matching factor), intervention group of the initial SU.VI.MAX trial (antioxidants/placebo, matching factor), educational level (primary/secondary/superior), physical activity (irregular/<1 h/d walking equivalent/≥1 h/d walking equivalent), alcohol intake (g/d, continuous), smoking status (never/former/current), height (cm, continuous), BMI (kg/m², continuous, matching factor), family history of prostate cancer (yes/no) and baseline serum prostate-specific antigen concentration (<3/≥3 ng/l).

the continuous 25(OH)D variable or the 20 ng/ml cut-off, these associations were not significant in both Gleason's subgroups. Exclusion of cases diagnosed during the first 5 years of follow-up provided similar results (109 cases/140 controls, $OR_{per\ 1\ ng/ml}$ 0·96; 95 % CI 0·93, 1·00; $P_{trend} = 0{\cdot}04$; $OR_{Q4\ v.\ Q1}$ 0·33; 95 % CI 0·12, 0·86; $P_{trend} = 0{\cdot}01$; $OR_{<20\ v.\ \geq 20\ ng/ml}$ 0·45; 95 % CI 0·23, 0·89; $P = 0{\cdot}02$; data not tabulated). No interaction was observed between 25(OH)D concentration and the intervention group of the SU.VI.MAX trial ($P_{interaction} > 0{\cdot}1$ for all codings).

Plasma PTH concentration was not associated with prostate cancer risk ($OR_{Q4\ v.\ Q1}$ 0·66; 95 % CI 0·28, 1·55; $P_{trend} = 0{\cdot}4$) (Table 2). This result was similar (124 cases/157 controls) after removing participants with possibly abnormal PTH values that may suggest potential hyperparathyroidism (i.e. PTH ≥ 50·8 pg/ml if 25(OH)D <20 ng/ml, PTH ≥ 45·5 pg/ml if 20 ng/ml ≤ 25(OH)D < 30 ng/ml and PTH ≥ 45·3 pg/ml if 25(OH)D ≥ 30 ng/ml, as previously recommended[22]).

Dietary Ca intake was not associated with prostate cancer risk (ninety-six cases/123 controls, $OR_{Q4\ v.\ Q1}$ 0·83; 95 % CI 0·20, 3·43; $P_{trend} = 0{\cdot}5$, data not tabulated), nor did dietary intake of vitamin D (ninety-six cases/123 controls, $OR_{Q4\ v.\ Q1}$ 1·05; 95 % CI 0·40, 2·81; $P_{trend} = 0{\cdot}7$, data not tabulated).

All results were similar when models were further adjusted for dietary variables (although some of the results were only borderline significant due to loss of statistical power: ninety-six cases/123 controls), dietary Ca and mutual adjustments for 25(OH)D and PTH. Two-way interactions between 25(OH)D, PTH and dietary Ca intake were not statistically significant (all $P > 0{\cdot}1$, data not shown).

No association was observed between the ten studied vitamin D-related SNP and prostate cancer risk in the codominant (Table 3), dominant and recessive models (data not tabulated). No interaction was observed between the SNP and 25(OH)D concentration (all $P > 0{\cdot}1$, data not shown). As no association was detected between the ten SNP and prostate cancer with a P value

threshold of 0·05, no association was detected after adjustment for multiple testing (Bonferroni correction) (data not shown).

Discussion

In this prospective study, plasma 25(OH)D concentration was inversely associated with prostate cancer risk. No association was detected for plasma PTH concentration or the studied SNP.

We observed an inverse association between 25(OH)D concentration and prostate cancer risk. Recently, a high *v.* low meta-analysis by Xu *et al.*[27] (summary $OR_{high\ v.\ low}$ 1·17; 95 % CI 1·05, 1·30) and a dose–response meta-analysis by the WCRF[5] (summary $RR_{per\ 30\ nmol/l}$ 1·04; 95 % CI 1·00, 1·07) suggested an increased risk. However, in a previous study by Tuohimaa *et al.*[28], both high and low 25(OH)D concentrations were associated with increased prostate cancer risk: increased risk was observed for 25(OH)D concentration ≥32 or <15·6 ng/ml compared with 16–23·6 ng/ml. This U-shaped association is supported by the evidence of non-linearity observed in the WCRF dose–response meta-analysis[5]. In our study, the range of 25(OH)D concentrations observed (95th percentile = 36·3 ng/ml) may be positioned in the left part of this U-shaped curve, which may explain why a decreased prostate cancer risk was observed for 25(OH)D ≥ 20 ng/ml (insufficiency) or ≥18·2 ng/ml (median) compared with 25(OH)D < 20 or <12·9 ng/ml (quartile 1 (Q1)), respectively. Consistently, a recent study by Kristal *et al.*[29] observed a decreased prostate cancer risk associated with 25(OH)D concentrations between 23·3 and 29·2 ng/ml (3rd quintile) compared with 25(OH)D < 17·7 ng/ml (1st quintile). In contrast, some studies observing an increased risk may involve 25(OH)D concentrations situated in the right part of the U-shaped curve. For example, Brandstedt *et al.*[9] observed an increased risk for 25(OH)D concentrations ≥34 ng/ml compared with 25(OH)D concentrations ≤27·2 ng/ml, and Meyer *et al.*[30] observed an increased risk for 25(OH)D concentrations ≥28 ng/ml compared with 25(OH)D concentrations between 20 and 28 ng/ml. Studies observing non-significant results may involve middle-range concentrations (such as the study by Skaaby *et al.*[31]). However, this point remains unclear as some studies that involved high 25(OH)D concentrations observed non-significant results[32,33], and some other studies observed a significant direct association between prostate cancer risk and 25(OH)D concentrations, even at relatively low levels[34]. Thus, further studies are needed that take into account the distribution of 25(OH)D concentrations in the studied population and its position in the potential U-shaped curve. In addition, it has been suggested that large seasonal fluctuations of vitamin D status may also contribute to explain the positive association between 25(OH)D concentration and prostate cancer risk in some studies[35], adding to the complexity of this relationship. In the SU.VI.MAX cohort (Touvier *et al.*[21] and Table 1), seasonal fluctuation of vitamin D status was moderate with the lowest 25(OH)D concentrations observed in late winter/early spring (shorter days), consistently with the existing literature in France[36] and in other countries such as the USA[37–39].

The potentially protective role of vitamin D in prostate carcinogenesis observed in our study is supported by mechanistic hypotheses. Indeed, prostate cells can express the 25(OH)D-to-1,25$(OH)_2$D conversion enzyme and the vitamin D receptor[1] and vitamin D is thought to be involved in several cell regulation pathways: pro-differentiation, pro-apoptosis, anti-proliferation and cell growth[2–4].

In our study, when 25(OH)D was coded into quartiles, a decreased prostate cancer risk was observed for Gleason's score <7 but not for Gleason's score ≥7. However, when using the other codings (continuous and 20 ng/ml cut-off), the association was non-significant in both cancer subgroups. As statistical power was limited in stratified analyses, these results should be considered with caution and further explored in large prospective studies. Thus far, the results regarding potential differences according to prostate cancer stage/grade are unclear, as shown in the WCRF meta-analysis[5], where no difference was observed between advanced/high-grade or non-advanced/low-grade prostate cancers (non-significant results in both groups), or in a recent study by Kristal *et al.*[29], where a decreased prostate cancer risk was observed whatever the Gleason's score.

The lack of association between the ten studied SNP and prostate cancer risk in our study does not seem to support the protective role of vitamin D in prostate carcinogenesis suggested by our results on plasma 25(OH)D concentration. However, in this study, statistical power was limited in the analyses of SNP, especially for the homozygote mutant genotypes. This could explain the null associations observed. Consistent with our findings, several meta-analyses[10–12] and one recent prospective study[13] found null associations between *VDR* BsmI, FokI and Cdx2 polymorphisms and prostate cancer risk. Another study (not included in these meta-analyses) observed an increased prostate cancer associated with *VDR* BsmI GG genotype among men in the first tertile of plasma 25(OH)D concentration. The epidemiological literature dealing with the other studied polymorphisms is scarce. One study[13] observed an increased prostate cancer risk associated with *GC* rs4588 T allele or *GC* rs7041 A allele. In SU.VI.MAX[21], these alleles were associated with a lower vitamin D status. Another study[15] observed a decreased lethal prostate cancer risk associated with *CaSR* rs1801725 among men with low plasma 25(OH)D concentration. To our knowledge, no study has investigated the other selected SNP (*CYP24A1* rs4809958, *RXR* rs7861779 and rs12004589 and *CaSR* rs4678174) in relation to prostate cancer risk. Besides, other vitamin D-related SNP than the ones included in the present study may also be associated with prostate cancer risk, as observed by Mondul *et al.*[14], and deserve further investigation.

Plasma PTH concentration was not associated with the risk of prostate cancer. To our knowledge, our study was only the second to investigate this relationship, the first one having observed null results[9]. In a previous study performed in the SU.VI.MAX cohort[22], we observed an inverse correlation between 25(OH)D and PTH concentrations, with a threshold value for PTH when 25(OH)D was approximately 30 ng/ml. Thus, it could be expected that PTH concentration would decrease as 25(OH)D concentration increases. Mechanistic data are unclear regarding a potential involvement of PTH in prostate carcinogenesis. Although some data have suggested a potential pro-carcinogenic role of PTH[40–42] (potential mitogenic activity in preneoplastic lesions), others support a potential protective role. Indeed, high PTH concentration may decrease growth hormone secretion, thereby decreasing

circulating insulin-like growth factor-1 (IGF-1) concentration[43,44]; IGF-1 being considered as a potential risk factor for prostate cancer[45,46]. Thus, further investigation is needed on the association between PTH concentration and prostate cancer risk.

Strengths of our study pertained to its prospective design, long follow-up, simultaneous assessment of 25(OH)D and PTH plasma concentrations, vitamin D-related gene polymorphisms and dietary intakes, and the consideration of numerous confounding factors. However, limitations should be acknowledged. First, blood Ca concentration was not available in our study. Ca concentration would have provided more information regarding the association between 25(OH)D, PTH, Ca and prostate cancer risk. Dietary Ca intake was available, but intakes within normal range are poorly correlated with blood Ca concentration[47], which is under homoeostatic control. Second, only one plasma 25(OH)D and PTH measurement was available at baseline. Repeated measures could have been of interest to study their evolution across time. Third, although the number of cases was appropriate for the analyses described here, it has limited our ability to perform separate analyses in specific subgroups, in particular regarding genetic polymorphisms or prostate cancer grade. Finally, the observed inverse association between vitamin D status and prostate cancer could be partly explained by reverse causality, considering the long lasting development of this cancer. However, results were similar when excluding cases diagnosed within the first 5 years of follow-up, thus arguing against reverse causality.

In this prospective study, the association between vitamin D and prostate cancer risk was addressed through 25(OH)D concentration, polymorphisms of vitamin D-related genes and PTH concentration. Prostate cancer risk was inversely associated with 25(OH)D concentration but not with PTH concentration. These results, supported by mechanistic data, bring a new contribution to the understanding of the relationship between vitamin D and prostate cancer risk and deserve further exploration.

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The author's responsibilities were as follows – M. D. and M. T. designed the research; S. H., P. G., M. T., A. S. and N. C. conducted the research; M. D. performed statistical analysis; M. D. and M. T. wrote the paper; J.-C. S., P. L.-M., A. S., N. C., N. D.-P., P. G., S. H., S. L. C., E. K.-G. and K. E. contributed to the data interpretation and revised each draft for important intellectual content; and M. D. and M. T. had the primary responsibility for final content. All authors read and approved the final manuscript.

The authors declare that they have no conflicts of interest.

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