Prospective associations between vitamin D status, vitamin D–related gene polymorphisms, and risk of tobacco-related cancers$^{1,2}$

Mélanie Deschasaux,$^{3,*}$ Jean-Claude Souberbielle,$^{4}$ Paule Latino-Martel,$^{3}$ Angela Sutton,$^{5}$ Nathalie Charneau,$^{5}$ Nathalie Drauene-Pecollo,$^{5}$ Pilar Galan,$^{5}$ Serge Hercberg,$^{3,6}$ Sigrid Le Clerc,$^{7}$ Emmanuelle Kesse-Guyot,$^{3}$ Khaled Ezzedine,$^{3,8}$ and Mathilde Touvier$^{5}$

$^{3}$Paris 13 University, Nutritional Epidemiology Research Team, French National Institute of Health and Medical Research (Inserm) U1153, French National Institute for Agricultural Research (Inra) U1125, French National Conservatory of Arts and Crafts (CNAM), Sorbonne Paris Cité University, Bobigny, France; $^{4}$Physiology Department, Necker Hospital, Inserm U845, Paris, France; $^{5}$Biochemistry Department, Jean Verdier Hospital, Inserm U698, Paris 13 University, Bondy, France; $^{6}$Public Health Department, Avicenne Hospital, Bobigny, France; $^{7}$CNAM, Genomics, Bioinformatics and Applications Team (EA4627), Paris, France; and $^{8}$Dermatology Department, Saint André Hospital, Bordeaux, France

ABSTRACT

Background: Experimental evidence has suggested that vitamin D may be protective against tobacco-related cancers through the inhibition of the formation of tumors induced by tobacco carcinogens. To our knowledge, only one previous epidemiologic study investigated the association between vitamin D status and tobacco-related cancer risk, and no study has focused on vitamin D–related gene polymorphisms.

Objective: Our objective was to prospectively study the association between plasma 25-hydroxyvitamin D [25(OH)D] concentrations, vitamin D–related gene polymorphisms, and risk of tobacco-related cancers.

Design: A total of 209 tobacco-related cancers were diagnosed within the SU.VI.MAX (Supplémentation en vitamines et minéraux antioxydants) cohort (1994–2007) and were matched with 418 controls as part of a nested case-control study. Tobacco-related cancers (i.e., cancers for which tobacco is one of the risk factors) included several sites in the respiratory, digestive, reproductive, and urinary systems. Total plasma 25(OH)D was assessed with the use of an electrochemiluminescent assay. Polymorphisms were determined with the use of a TaqMan assay. Conditional logistic regression models were computed.

Results: A 25(OH)D concentration $\geq 30$ ng/mL was associated with reduced risk of tobacco-related cancers (OR for $\geq 30$ vs. $< 30$ ng/mL: 0.59; 95% CI 0.35, 0.99; $P = 0.046$). This association was observed in former and current smokers (OR for $\geq 30$ vs. $< 30$ ng/mL: 0.43; 95% CI: 0.23, 0.84; $P = 0.01$) but not in never smokers ($P = 0.8$). The vitamin D receptor (VDR) FokI AA genotype and retinoid X receptor (RXR) rs7861779 TT genotype were associated with increased risk of tobacco-related cancers [OR for homozygous mutant type (MT) vs. wild type (WT): 1.87; 95% CI: 1.08, 3.23; $P$-trend = 0.02; OR for heterozygous type (HT) plus MT vs. WT: 1.60; 95% CI: 1.07, 2.38; $P = 0.02$].

Conclusions: In this prospective study, high vitamin D status [25(OH)D concentration $\geq 30$ ng/mL] was associated with decreased risk of tobacco-related cancers, especially in smokers. These results, which are supported by mechanistic plausibility, suggest that vitamin D may contribute to the prevention of tobacco-induced cancers in smokers and deserve additional investigation. The SU.VI.MAX trial was registered at clinicaltrials.gov as NCT00272428.

INTRODUCTION

Tobacco-smoking is a major risk factor for several cancers (in particular, respiratory, digestive, and urinary cancers) because of many carcinogens released during cigarette combustion (1). Experimental studies have suggested that vitamin D could be beneficial in cancer prevention through several cell regulation properties (e.g., antiproliferation, pro-apoptosis, and growth control) (2) and, especially in tobacco-related cancers, through its ability to inhibit the formation of chemically induced tumors that result from exposure to tobacco carcinogens (3–6).

Because tobacco-related cancers [i.e., cancers for which tobacco is one of the risk factors (7)] are supposed to share a common cause linked to tobacco smoking, and because vitamin D may be protective against smoking carcinogens, it is relevant to consider tobacco-related cancers as a single outcome when studying their association with vitamin D (8). To our knowledge, only one prospective study has previously investigated the association between vitamin D status (25-hydroxyvitamin D [25(OH)D]$^9$ plasma concentration) and risk of tobacco-related cancers; the study observed increased risk associated with a low

Keywords: nested case-control study, single nucleotide polymorphisms, smoking status, tobacco-related cancers, 25-hydroxyvitamin D

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$^{*}$To whom correspondence should be addressed. E-mail: m.deschasaux@eren.smbb.univ-paris13.fr.

$^{9}$Abbreviations used: CaSR, calcium-sensing receptor; CYP24A1, 1,25-dihydroxyvitamin D3 24-hydroxylase; GC, vitamin D binding protein; HT, heterozygous type; MAF, minor allele frequency; MT, homozygous mutant type; RXR, retinoid X receptor; SNP, single nucleotide polymorphism; SU.VI.MAX, Supplémentation en vitamines et minéraux antioxydants; VDR, vitamin D receptor; WT, wild type; 25(OH)D, 25-hydroxyvitamin D.
25(OH)D concentration (8). When tobacco-related cancer sites have been considered separately, current epidemiologic evidence has supported a protective role of vitamin D in colorectal carcinogenesis as expressed in 2008 by the International Agency for Research on Cancer in its report on vitamin D and cancer (9) and as observed in recent meta-analyses (10, 11). Literature that has dealt with other tobacco-related cancer sites individually (such as of the lung, bladder, esophagus, stomach, pharynx and larynx, liver, kidneys, and pancreas) has been scarce and inconsistent (12–20). However, for several of these cancer sites, previous studies have suggested a more-pronounced protective effect of vitamin D in smokers (8, 15, 21, 22).

Polymorphisms of genes involved in vitamin D metabolism [in particular, those for signaling (vitamin D receptor (VDR) and retinoid X receptor (RXR)), transportation vitamin D binding protein (GC), degradation 1,25-dihydroxyvitamin D3 24-hydroxylase (CYP24A1), and calcium metabolism calcium-sensing receptor (CaSR)] may also be involved in carcinogenesis through a potential influence on vitamin D activity. To our knowledge, no previous study investigated the association between polymorphisms of these genes and tobacco-related cancers as the overall outcome. Studies have been performed on specific sites with inconsistent results that mainly focused on colorectal cancer risk and VDR polymorphisms (11, 23), suggesting inverse associations with VDR BsmI (B allele vs. b) and VDR Cdx2 (c allele vs. C). Evidence for other tobacco-related cancer sites or other gene polymorphisms has been sparse (21, 24–29).

Thus, our objective was to study the association between plasma 25(OH)D concentrations, 10 single nucleotide polymorphism (SNPs) of genes involved in vitamin D metabolism (VDR BsmI, FokI, and Cdx2, CYP24A1 rs4809958, GC rs4588 and rs7041, RXR rs7861779 and rs12004589, and CaSR rs1801725 and rs4678174), and risk of tobacco-related cancers.

METHODS

Subjects

The SU.VI.MAX (Supplémentation en vitamines et minéraux antioxydants) study (clinicaltrials.gov; NCT00272428) was initially designed as a double-blind placebo-controlled trial with the purpose to assess the influence of a daily supplementation with nutritional doses of antioxidants (a daily capsule of a combination of 120 mg ascorbic acid, 30 mg vitamin E, 6 mg β carotene, 100 µg Se, and 20 mg Zn) on the incidence of cardiovascular diseases and cancers (30, 31). A total of 13,017 participants were enrolled in 1994–1995 for an 8-y intervention trial and were followed up for health events until September 2007. All subjects gave their written informed consent to participate in the study. Vitamin D was not part of the trial supplementation, and participants were advised against taking any self-prescribed supplements (vitamin D or others) during the trial. The study was conducted according to the Declaration of Helsinki guidelines and was approved by the Ethics Committee for Studies with Human Subjects of Paris-Cochin Hospital (CCPPRB 706/2364) and the ‘Commission Nationale de l’Informatique et des Libertés’ (334641/907094).

Case ascertainment

Health events were self-reported by the participants. All relevant medical information and pathologic reports were gathered through participants, physicians, and hospitals and reviewed for validation by an independent physician expert committee. Validated cancer cases were classified according to the International Chronic Diseases Classification, 10th Revision, Clinical Modification (32). All first-incident primary tobacco-related cancers were considered as cases in this study. Tobacco-related cancers included all of the following cancer sites for which an association with tobacco smoking has been established with sufficient evidence according to the International Agency for Research on Cancer (7): the lung, oral cavity, pharynx, larynx, esophagus, pancreas, urinary bladder, ureter, kidney, nasal sinuses, stomach, liver, uterine cervix, ovary, myeloid leukemia, colon-rectum, and anus.

Nested case-control study

All participants who were diagnosed with a tobacco-related cancer during follow-up (1994–2007; i.e., 13 y of follow-up) were included in a nested case-control study whereby 2 control subjects per tobacco-related cancer case were randomly selected in cancer-free subjects and were matched according to the following baseline criteria: sex (female or male), age (<40, 40–44, 45–49, 50–54, or 55–65 y), intervention group of the initial SU. VI.MAX trial (placebo or supplemented), season of blood draw (June through October or November through May), and, for women, menopausal status (premenopause or postmenopause) and use of hormonal treatment of menopause (yes or no).

Baseline data collection

Self-administered questionnaires were used at baseline to get information on sociodemographics, physical activity, medication use, health status, and smoking habits.

Participants underwent a clinical examination by the study nurses and physicians with anthropometric measurements and a blood draw that occurred in the early morning after an overnight (12-h) fasting period. Thirty-five–milliliter venous blood samples were collected in evacuated tubes and immediately centrifuged to get plasma aliquots (preserved in sodium heparin) and buffy-coat fractions, thereby allowing for future DNA extraction. Both aliquots and fractions were stored frozen in liquid nitrogen. Dietary intakes were collected with the use of repeated 24-h dietary records that were completed through the Minitel Telematic Network, which is a French telephone-based terminal that is equivalent to an Internet prototype. Portion sizes were assessed with the use of a validated picture booklet (33), and the amounts consumed from composite dishes were estimated with the use of French recipes that were validated by food and nutrition professionals. Mean daily energy, alcohol, and nutrient intakes were estimated with the use of a published French food-composition table (34).

Laboratory assay of plasma 25(OH)D concentration

The 25(OH)D plasma concentration was determined on baseline samples as previously described in detail (35, 36). The plasma 25(OH)D concentration was measured with the use of the
Roche Cobas electrochemoluminescent total 25(OH)D assay (Roche Diagnostics), which is based on the principle of competitive binding (37). The interassay CV was <10% [8 samples of various 25(OH)D concentrations tested in 42 separate runs], whereas the intra-assay CV was <6.6% (the same 8 samples tested 21 times in the same run).

**Genotyping**

One to 3 SNPs were selected for each gene of interest (VDR, CYP24A1, GC, RXR, and CASR) on the basis of two criteria: 1) relatively high frequency in Caucasian populations (http://www.ncbi.nlm.nih.gov/guide/howto/viewgen-freq/) and 2) predicted functional effect according to the PUPA database (http://snpeffect.vib.be and http://pupasuite.bioinfo.cipf.es/). The selected SNPs were as follows: 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 (MAF: T, 0.1304), and CaSR rs1801725 (MAF: T, 0.0942) and rs4678174 (MAF: C, 0.4619). Genomic DNA was extracted from each patient's mononuclear cells in peripheral blood with the use of a MagNA Pure Compact Instrument with magnetic-bead technology for the isolation process (Roche Diagnostics). Genetic polymorphisms were assessed by allelic discrimination with the use of 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 the Hardy-Weinberg equilibrium and comparing observed distributions to those of European reference populations [CSHL-HapMap-CEU and 1000GENOMES-phase_1-EUR (http://www.ensembl.org/)] with the use of chi-square tests.

**Statistical analyses**

Baseline characteristics were compared between tobacco-related cancer cases and controls with the use of chi-square tests for categorical variables or Fisher tests (from ANOVA models) for continuous variables. Associations between the plasma 25(OH)D concentration, dietary vitamin D intake, studied SNPs, and tobacco-related cancer risk were characterized by ORs and 95% CIs that were derived from multivariate logistic regressions. All models were conditional except for stratified analyses and SNPs analyses. Participants were matched for sex, age at baseline, intervention group of the initial SU.VI.MAX trial, season of blood draw, menopausal status (women), and use of hormonal treatment of menopause (women), and models were further adjusted for educational level, physical activity, alcohol intake, smoking status, height, BMI, and family history of cancer. SNPs models were also adjusted for 25(OH)D concentrations. Sensitivity analyses were carried out by further adjusting for energy intake (without alcohol) or for professional categories. For all models that involved dietary intake data, only subjects who provided ≥3 valid 24-h dietary records were included. Dietary vitamin D intake was treated as an energy-adjusted variable with the use of the residual method (38).

The plasma 25(OH)D concentration was coded as ≥30 and <30 ng/mL. These cutoffs correspond to the threshold values that are used to define vitamin D insufficiency and sufficiency, respectively, according to the official recommendations of the US Endocrine Society (39). Therefore, individuals with a 25(OH)D concentration ≥30 ng/mL may be considered as having “optimal vitamin D status.” Subjects were classified into 5 categories according to their baseline smoking status as follows: never smokers, former smokers with past cigarette consumption ≤10 or >10 cigarettes/d, and current smokers with cigarette consumption ≤10 or >10 cigarettes/d. A combined variable of vitamin D status (<30 and ≥30 ng/mL) and smoking status (never smokers and ever smokers) was also computed. A test for a linear trend was performed across the 4 categories with the use of the ordinal value of this combined variable (1) 25(OH)D concentration <30 ng/mL and ever smokers; 2) 25(OH)D concentration ≥30 ng/mL and ever smokers, 3) 25(OH)D concentration <30 ng/mL and never smokers; and 4) 25(OH)D concentration ≥30 ng/mL and never smokers). For each SNP, the following codings were tested: codominant [heterozygous type (HT) vs. wild type (WT) and homozygous mutant type (MT) vs. WT), dominant (HT and MT vs. WT), and recessive (MT vs. WT and HT).

Two-way interactions were tested between the 25(OH)D concentration, the 10 SNPs, and smoking status, and stratified analyses were performed when appropriate. All statistical tests were 2 sided, and P < 0.05 was considered significant. A power analysis was performed with the use of PS Power and Sample Size calculator (version 3.0) (40). Analyses were performed with the use of SAS software (version 9.3; SAS Institute).

**RESULTS**

During the 13 y of follow-up (1994-2007), the 209 tobacco-related cancer cases of the following sites were diagnosed within the SU.VI.MAX cohort: lung (n = 32), oral cavity (n = 9), pharynx (n = 2), larynx (n = 6), esophagus (n = 9), pancreas (n = 14), urinary bladder (n = 12), ureter (n = 1), kidney (n = 15), nasal sinuses (n = 2), stomach (n = 4), liver (n = 4), uterine cervix (n = 24), ovary (n = 15), myeloid leukemia (n = 4), colon/rectum (n = 52), and anus (n = 4). A total of 418 controls were randomly selected and matched with the cases. The mean age at diagnosis was 57.7 y, and the mean baseline-to-diagnosis time was 6.3 y. With 209 cancer cases and 2 matched controls per case, a type I error probability of 0.05, and a power of 0.8, we were able to detect ORs ≤0.6 or ≥1.4.

**Table 1** summarizes the baseline characteristics of tobacco-related cancer cases and controls. Compared with controls, cases were more likely to smoke, be obese, have lower intake of dietary vitamin D, and possess the T allele (CT and TT genotypes) of the RXR rs7861779 polymorphism. All studied SNPs respected the Hardy-Weinberg equilibrium (P > 0.05). The re-partition 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 SNPs (P > 0.05).

A 25(OH)D concentration ≥30 ng/mL was associated with reduced risk of tobacco-related cancers (OR for ≥30 vs. <30 ng/mL: 0.59; 95% CI: 0.35, 0.99; P = 0.046) (**Table 2**). This association was observed for subjects who have ever smoked (former or
TABLE 1
Baseline characteristics of tobacco-related cancers and controls (SU.VI.MAX cohort, France; 1994–2007) 1

<table>
<thead>
<tr>
<th></th>
<th>Tobacco-related cancer cases</th>
<th>Controls</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>(n = 209)</td>
<td>(n = 418)</td>
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</tr>
<tr>
<td>Age, y</td>
<td>—</td>
<td>—</td>
<td>0.6</td>
</tr>
<tr>
<td>Sex</td>
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<tr>
<td>Men</td>
<td>106 (50.7)</td>
<td>212 (50.7)</td>
<td></td>
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<tr>
<td>Women</td>
<td>103 (49.3)</td>
<td>206 (49.3)</td>
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</tr>
<tr>
<td>BMI, kg/m²</td>
<td>—</td>
<td>—</td>
<td>0.09</td>
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<tr>
<td>&lt;18.5 (underweight)</td>
<td>8 (3.8)</td>
<td>8 (1.9)</td>
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<tr>
<td>≥18.5 to &lt;25 (normal weight)</td>
<td>113 (54.1)</td>
<td>257 (61.5)</td>
<td></td>
</tr>
<tr>
<td>≥25 to &lt;30 (overweight)</td>
<td>64 (30.6)</td>
<td>130 (31.1)</td>
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<tr>
<td>≥30 (obese)</td>
<td>24 (11.5)</td>
<td>23 (5.5)</td>
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<tr>
<td>Height, cm</td>
<td>—</td>
<td>167.9 ± 8.0</td>
<td>0.5</td>
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<td>Intervention group</td>
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<td>—</td>
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<tr>
<td>Antioxidants</td>
<td>105 (50.2)</td>
<td>210 (50.2)</td>
<td></td>
</tr>
<tr>
<td>Placebo</td>
<td>104 (49.8)</td>
<td>208 (49.8)</td>
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<td>—</td>
<td>&lt;0.0001</td>
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<tr>
<td>Never</td>
<td>73 (34.9)</td>
<td>193 (46.2)</td>
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<tr>
<td>Former, ≤10</td>
<td>29 (13.9)</td>
<td>61 (14.6)</td>
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<td>Former, &gt;10</td>
<td>54 (25.8)</td>
<td>114 (27.3)</td>
<td></td>
</tr>
<tr>
<td>Current, ≤10</td>
<td>15 (7.2)</td>
<td>31 (7.4)</td>
<td></td>
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<tr>
<td>Current, &gt;10</td>
<td>38 (18.2)</td>
<td>19 (4.5)</td>
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<td>Physical activity</td>
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<td>Irregular</td>
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<td>≥1-h/d walking equivalent</td>
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<td>189 (45.2)</td>
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<td>—</td>
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<tr>
<td>Primary</td>
<td>39 (18.7)</td>
<td>91 (21.8)</td>
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<tr>
<td>Secondary</td>
<td>90 (43.1)</td>
<td>152 (36.3)</td>
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<tr>
<td>Superior</td>
<td>80 (38.3)</td>
<td>175 (41.9)</td>
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<td>155 (37.1)</td>
<td>0.8</td>
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<td>Alcohol intake, g/d</td>
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<td>22.6 ± 25.0</td>
<td>0.09</td>
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<td>Energy intake without alcohol, kcal/d</td>
<td>—</td>
<td>1919.5 ± 553.3</td>
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<tr>
<td>Dietary vitamin D intake, μg/d</td>
<td>—</td>
<td>2.6 ± 1.7</td>
<td>0.03</td>
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<tr>
<td>Plasma 25-hydroxyvitamin D, ng/mL</td>
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<td>20.4 ± 10.5</td>
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<td>40 (19.1)</td>
<td>79 (18.9)</td>
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<tr>
<td>December through January</td>
<td>59 (28.2)</td>
<td>134 (32.1)</td>
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<td>February through March</td>
<td>84 (40.2)</td>
<td>156 (37.3)</td>
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<tr>
<td>April through May</td>
<td>26 (12.5)</td>
<td>49 (11.7)</td>
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<tr>
<td>VDR BsmI rs1544410</td>
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<tr>
<td>C/C (WT)</td>
<td>69 (36.3)</td>
<td>150 (38.2)</td>
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<tr>
<td>C/T (HT)</td>
<td>96 (50.5)</td>
<td>171 (43.5)</td>
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<td>25 (13.2)</td>
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<td>69 (32.5)</td>
<td>168 (40.9)</td>
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<tr>
<td>A/G (HT)</td>
<td>98 (50.5)</td>
<td>193 (46.9)</td>
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<tr>
<td>A/A (MT)</td>
<td>33 (17.0)</td>
<td>50 (12.2)</td>
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<td>VDR Cdx2 rs11568820</td>
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<td>221 (54.8)</td>
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<td>C/T (HT)</td>
<td>83 (42.3)</td>
<td>159 (39.5)</td>
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<td>5 (2.6)</td>
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<td>132 (69.8)</td>
<td>285 (70.7)</td>
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<td>0.5</td>
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<td>22 (11.1)</td>
<td>35 (8.7)</td>
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(Continued)
current smokers) (OR for $\geq 30$ vs. $< 30$ ng/mL: $0.43; 95\%$ CI: $0.23, 0.84; P = 0.01$) but not in never smokers (OR for $\geq 30$ vs. $< 30$ ng/mL: $1.01; 95\%$ CI: $0.50, 2.42; P = 0.8$). However, the P-interaction between 25(OH)D and smoking was NS ($P$-interaction = 0.2).

Similar results were obtained when we excluded colorectal cancer cases ($n = 52$), which represented $24.9\%$ of all tobacco-related cancer cases in the study, although the $P$ value did not reach significance because of a loss of statistical power [ORs for $\geq 30$ vs. $< 30$ ng/mL: overall, $0.56 (95\%$ CI: $0.30, 1.05; P = 0.07$); for ever smokers, $0.48 (95\%$ CI: $0.23, 1.00; P = 0.05$); and for never smokers, $P = 0.6$ (data not tabulated)]. Results were also similar when we adjusted for energy intake or for professional categories (data not shown).

Stratified analyses on the average baseline-to-diagnosis time ($< 6$ and $\geq 6$ y) were performed. Similar trends were observed for cancer cases diagnosed $< 6$ and $\geq 6$ y after baseline although the trends were NS or were borderline significant because of restricted statistical power (ORs for $\geq 30$ vs. $< 30$ ng/mL—baseline-to-diagnosis time $< 6$ y; overall (102 cases and 204 controls), $0.58 (95\%$ CI: $0.27, 1.22; P = 0.1$) and in ever smokers (70 cases and 113 controls), $0.49 (95\%$ CI: $0.19, 1.25; P = 0.1$); baseline-to-diagnosis time $\geq 6$ y; overall (107 cases and 214 controls), $0.67 (95\%$ CI: $0.31, 1.45; P = 0.3$) and in ever smokers (66 cases and 112 controls), $0.35 (95\%$ CI: 0.13, 0.94; $P = 0.04$).

With the use of a combined variable of vitamin D status and smoking status, we observed that, compared with smokers with 25(OH)D concentrations $< 30$ng/mL (reference), lowest risk of tobacco-related cancer was observed for never smokers with 25(OH)D concentrations $\geq 30$ ng/mL (OR: $0.13; 95\%$ CI: $0.05, 0.35$), with an overall $P$-trend = 0.046 across the 4 categories (data not tabulated). This result was similar after the exclusion of colorectal cancers (OR: $0.09; 95\%$ CI: $0.03, 0.29$; $P$-trend = 0.07).

No association was observed between dietary vitamin D intake and risk of tobacco-related cancers [143 cases and 286 controls; OR for Quartile 4 vs. Quartile 1: $0.84; 95\%$ CI: $0.46, 1.51$; $P$-trend = 0.8 (data not tabulated)].

Associations between the 10 studied SNPs and risk of tobacco-related cancers are presented in Table 3. The genotype AA of the VDR FokI polymorphism was associated with increased risk of tobacco-related cancers (OR for MT vs. WT = 1.87; 95% CI: 1.08, 3.23; $P$-trend = 0.02) as was the genotype TT of RXR rs7861779 (OR for HT plus MT vs. WT: 1.60; 95% CI: 1.07, 2.38; $P = 0.02$). No association was observed for the other studied SNPs. Similar results were obtained when colorectal cancer cases were excluded [VDR FokI: OR for MT vs. WT, 1.99 (95% CI: 1.04, 3.83; $P$-trend = 0.02); RXR rs7861779: OR for HT plus MT vs. WT, 1.64 (95% CI: 1.02, 2.64; $P = 0.04$)]. No 2-way interaction was detected between the SNPs and the 25(OH)D concentration or smoking status.
TABLE 2

Associations between 25(OH)D concentrations and risk of tobacco-related cancers from logistic regression models overall and according to smoking status (SU.VIMAX cohort, France; 1994–2007)\(^1\)

<table>
<thead>
<tr>
<th>25(OH)D, ng/mL</th>
<th>&lt;30</th>
<th>≥30</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>All</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls, n</td>
<td>182/335</td>
<td>27/83</td>
<td>—</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>0.59 (0.35, 0.99)</td>
<td>0.046</td>
</tr>
<tr>
<td>Never smoker(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls, n</td>
<td>62/163</td>
<td>11/30</td>
<td>—</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>1.10 (0.50, 2.42)</td>
<td>0.8</td>
</tr>
<tr>
<td>Ever smoker (former and current)(^2)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cases/controls, n</td>
<td>120/172</td>
<td>16/53</td>
<td>—</td>
</tr>
<tr>
<td>OR (95% CI)</td>
<td>1.00</td>
<td>0.43 (0.23, 0.84)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

\(^1\)Tobacco-related cancer sites were as follows: lung, oral cavity, pharynx, larynx, esophagus, pancreas, urinary bladder, ureter, kidney, nasal sinuses, stomach, liver, uterine cervix, ovary, myeloid leukemia, colon-rectum, and anus. Participants were matched for sex, age at baseline, intervention group of the initial SU.VIMAX trial, season of blood draw, menopausal status, and use of hormonal treatment of menopause, and models were further adjusted for educational level (primary, secondary, and superior), physical activity (irregular, <1-h/d walking equivalent, and ≥1-h/d walking equivalent), alcohol intake (continuous; in g/d), smoking status (never; former, ≤10 cigarettes/d; former, >10 cigarettes/d; current, ≤10 cigarettes/d; and current, >10 cigarettes/d), height (continuous; in cm), BMI (continuous; in kg/m\(^2\)), and family history of overall cancer (yes or no). HT, heterozygous; mer, equivalent; alcohol intake (continuous; in g/d), smoking status (never; former, ≤10 cigarettes/d; former, >10 cigarettes/d; current, ≤10 cigarettes/d; and current, >10 cigarettes/d), height (continuous; in cm), BMI (continuous; in kg/m\(^2\)), and family history of overall cancer (yes or no). HT, heterozygous type; MT, homozygous mutant type; UT, wild type; 25(OH)D, 25-hydroxyvitamin D.

\(^2\)P-interaction = 0.2 between 25(OH)D plasma concentration and smoking status (never or ever).

DISCUSSION

In this nested case-control study, higher vitamin D status [25(OH)D concentration ≥30 ng/mL] was inversely associated with risk of tobacco-related cancers, particularly in smokers. Two polymorphisms of genes involved in vitamin D metabolism (VDR FokI allele and RXR Rs7861779 T allele) were positively associated with tobacco-related cancer risk.

To our knowledge, only one recent prospective study investigated a possible association between vitamin D status and tobacco-related cancer risk (8). Its results were in line with ours (i.e., increased risk associated with low vitamin D status, especially in smokers). Our results were also consistent with several studies on tobacco-related cancer sites considered separately that showed an inverse association with vitamin D status that was modulated by smoking status (15, 22). Zheng et al. (22) observed reduced risk of colorectal adenoma with vitamin D status that was only significant for active smokers. Consistently, Amaral et al. (15) observed increased bladder cancer risk associated with low vitamin D status in smokers only.

These results suggest that vitamin D could contribute to the prevention of tobacco-related cancers, which is supported by experimental data. Smoking is a known risk factor for many cancers because of released carcinogens (1). Several experimental studies have shown that vitamin D may be involved in the prevention of chemically induced tumors and tumors that are induced by tobacco carcinogens in particular (3–6). Indeed, vitamin D is supposed to enhance apoptosis, thereby suppressing cells that are damaged because of carcinogens and limiting tumor progression (2, 41), to be a factor of detoxification (42), and to reduce the susceptibility to carcinogens (41).

From a public health standpoint, our results on the combined 25(OH)D concentration–smoking status variable indicated that tobacco-related cancer risk was maximal in smokers, especially if their vitamin D status was <30 ng/mL, and was minimal in nonsmokers with vitamin D status ≥30 ng/mL. To the best of our knowledge, our study is the first one to investigate the relation between several SNPs of genes involved in vitamin D metabolism and tobacco-related cancer risk.

Epidemiologic evidence regarding the association between VDR FokI polymorphisms and separate cancer sites has been inconsistent. In our study, we observed increased risk of tobacco-related cancers with the A (f) allele [compared with the G (F) allele], which was consistent with some studies that observed a direct association between the f allele and increased risk of colon (43) or liver cancer in patients with hepatitis B (25), whereas other studies showed decreased risk of head and neck cancer (44) or lung cancer (45) with this same allele or no association with colorectal cancer (11, 23), renal cell carcinoma (24), or bladder cancer (29). Experimental studies have provided support for our results because they suggested that the f allele produces a longer form of the VDR protein, which leads to a less-effective interaction of this receptor with transcription factors and results in a less-effective activity of vitamin D (46, 47). This less-effective activity of vitamin D is consistent with increased risk of tobacco-related cancers when the potentially protective effect of vitamin D that has been suggested for these cancers is considered.

Very limited information exists regarding the SNP RXR Rs7861779 for which a positive association was observed between the T allele and tobacco-related cancer risk in our analyses. One previous epidemiologic study observed increased risk of proximal colon cancer that was associated with this same allele (27). RXR forms a heterodimer with VDR that enables the latter to interact with target genes (2). It may be hypothesized that the T allele of RXR Rs7861779 results in a less-effective RXR-VDR dimer, which hinders a proper vitamin D action in cancer prevention.

There was no association observed with the other studied SNPs. The literature regarding these SNPs and separate tobacco-related cancer sites has been uneven and inconsistent; most studies dealt with VDR BsmI or Cdx2, especially as they relate to risk of colorectal cancer for which an inverse association has been observed with the B allele and a direct association with the C allele, respectively (11, 23). Few studies have investigated these SNPs of VDR with other cancer such as head and neck cancer (null result) (26), lung cancer (decreased risk with the A allele) (21), or renal cancer (null result) (24), and even fewer studies have investigated other gene polymorphisms such as GC rs4588, GC rs7041, CaSR rs4678174, and CaSR rs1801725, and, again, most of these studies investigated these polymorphisms in association with colorectal cancer risk and observed null results (27, 28, 48, 49).

Strengths of our study pertained to its prospective design and a well-characterized population with available plasma 25(OH)D concentrations and genotypes for 10 vitamin D–related SNPs. However, some limitations should be acknowledged. First, although the number of cases was sufficient to perform analyses...
on tobacco-related cancers overall, the number of cases for each site was limited so that statistical power was not sufficient to allow separate analyses by cancer sites. Furthermore, colorectal cancer was the most-represented site in our sample, and because of the established inverse association between vitamin D status and colorectal cancer risk, it could be hypothesized that our results were driven by this site. However, because all results were similar when colorectal cancer cases were excluded but became borderline significant, the results suggest that, although colorectal cancer did contribute to the findings, the observed associations were not entirely explained by this cancer site. Statistical power was also limited in the stratified and in genetic analyses. Although this limitation may have restricted our ability to detect some of the associations, this drawback was unlikely to explain the observed relations that were significant despite this potential power limitation. Additional levels of stratifications (e.g., by BMI or alcohol intake) were also not possible in this study because of the limited sample size. Finally, smoking status was only assessed at baseline, and no detailed information was available on the duration of smoking or the time since former smokers quit smoking.

In conclusion, this prospective study observed decreased risk of tobacco-related cancers for 25(OH)D concentrations ≥30 ng/mL, particularly in smokers. Consistently, 2 genetic polymorphisms were shown to be associated with increased risk of tobacco-related cancers (i.e., the A allele of VDR FokI and the T allele of RXR rs7861779), which could reflect the less-effective activity of vitamin D induced by these polymorphisms. Our results on vitamin D status were consistent with the only existing prospective study that has dealt with tobacco-related cancers, and to our knowledge, our study is the first to investigate the link between vitamin D–related gene polymorphisms and risk of tobacco-related cancers overall, the number of cases for each site was limited so that statistical power was not sufficient to allow separate analyses by cancer sites. Furthermore, colorectal cancer was the most-represented site in our sample, and because of the established inverse association between vitamin D status and colorectal cancer risk, it could be hypothesized that our results were driven by this site. However, because all results were similar when colorectal cancer cases were excluded but became borderline significant, the results suggest that, although colorectal cancer did contribute to the findings, the observed associations were not entirely explained by this cancer site. Statistical power was also limited in the stratified and in genetic analyses. Although this limitation may have restricted our ability to detect some of the associations, this drawback was unlikely to explain the observed relations that were significant despite this potential power limitation. Additional levels of stratifications (e.g., by BMI or alcohol intake) were also not possible in this study because of the limited sample size. Finally, smoking status was only assessed at baseline, and no detailed information was available on the duration of smoking or the time since former smokers quit smoking.

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tobacco-related cancers. These results, which are supported by mechanistic data from experimental studies, provide insight into the role of vitamin D in the prevention of tobacco-related cancers and deserve additional exploration in future large prospective studies.

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The authors’ responsibilities were as follows—MD: performed the statistical analysis; MD and MT: designed the research, wrote the manuscript, and had primary responsibility for the final content of the manuscript; J-CS, PL-M, AS, NC, ND-D-P, PG, SH, SLC, EK-G, and KE: contributed to the data interpretation and revised each draft of the manuscript for important intellectual content; SH, PG, MT, AS, and NC: conducted the research; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

REFERENCES