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Low vitamin D exposure and risk of nasopharyngeal carcinoma: Observational and genetic evidence from a multicenter case–control study

Zhi-Ming Mai [a, *], Roger Kai-Cheong Ngan [b, c], Wai-Tong Ng [b, d], Jia-Huang Lin [a], Dora Lai-Wan Kwong [b, e], Kam-Tong Yuen [b, f], Cheuk Kwong Lee [g], Jennifer Ngar-Sze Leung [g], Dennis Kai-Ming Ip [a], Yap-Hang Chan [a, e], Anne Wing-Mui Lee [b, d, h], Maria Li Lung [b], Tai-Hing Lam [a, **], Sai-Yin Ho [a]

[a] School of Public Health, The University of Hong Kong, Hong Kong SAR, China
[b] Department of Clinical Oncology, The University of Hong Kong, Hong Kong SAR, China
[c] Department of Clinical Oncology, Queen Elizabeth Hospital, Hong Kong SAR, China
[d] Department of Clinical Oncology, Pamela Youde Nethersole Eastern Hospital, Hong Kong SAR, China
[e] Department of Medicine, Queen Mary Hospital, The University of Hong Kong, Hong Kong SAR, China
[f] Department of Oncology, Princess Margaret Hospital, Hong Kong SAR, China
[g] Hong Kong Red Cross Blood Transfusion Service, Hong Kong SAR, China
[h] Clinical Oncology Center, The University of Hong Kong-Shenzhen Hospital, Shenzhen, China

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SUMMARY

Background & aims: Little is known about the risk of nasopharyngeal carcinoma (NPC) in relation to vitamin D exposure. The aim of this study was to examine the associations of NPC risk with serum level of 25-hydroxyvitamin D (25OHD) and genetic predicted 25OHD, and potential effect modification by several putative risk factors of NPC.

Methods: Our multicenter case–control study in Hong Kong recruited 815 NPC cases and 1502 frequency-matched (by sex and age) hospital controls from five major regional hospitals, and recruited 299 healthy subjects from blood donation centers (2014–2017). Circulating level of 25-hydroxyvitamin D (25OHD) and genetic predicted 25OHD (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) were measured by validated enzyme immunoassay and the iPLEX assay on the MassARRAY System, respectively. Data were also collected on demographics, lifestyle factors, ultraviolet radiation exposure, and potential confounders using a computer-assisted, self-administered questionnaire with satisfactory test-retest reliability. Unconditional logistic regression models were used to estimate ORs and 95% CIs.

Results: Despite no significant association of NPC risk with circulating 25OHD and genetic predicted 25OHD, there was evidence for an inverse association in participants with normal body mass index (between 18.5 and 27.5) across categories of 25OHD ($P_{trend} = 0.003$), and a positive association in those with low socioeconomic status across categories based on the genetic score ($P_{trend} = 0.005$). In addition, risk of NPC diagnosed at an early stage was higher for genetically lower 25OHD level (adjusted OR = 3.09, 95% CI = 1.04–9.21, $P_{trend} = 0.022$).

Conclusions: Findings of this first comprehensive study to investigate the positive association of NPC risk with vitamin D deficiency need to be confirmed and be best interpreted with results of further similar studies. Our findings may inform possible etiological mechanisms of the associations with several putative risk/protective factors of NPC.

* Corresponding author. School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, China.
** Corresponding author. School of Public Health, Li Ka Shing Faculty of Medicine, The University of Hong Kong, G/F, Patrick Manson Building (North Wing), 7 Sassoon Road, Pok Fu Lam, Hong Kong SAR., China. Fax: +852 2855 9528.
E-mail addresses: maizm@connect.hku.hk (Z.-M. Mai), hrmrlth@hku.hk (T.-H. Lam).

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1. Introduction

Nasopharyngeal carcinoma (NPC) shows marked differences in racial and geographical distribution and is particularly prevalent in East and Southeast Asia [1]. While NPC age-standardized incidence rates (ASIRs) in both sexes were only 0.4 per 100,000 person-years in western populations that are mainly white, it reached 3.0 in China with over 60,000 new cases per year (>47% of all 129,000 NPCs worldwide in 2018) [2]. The ASIRs were particularly high in South China (28.9 and 11.3 for men and women in Sihui [3], 26.9 and 10.1 in Zhongshan [4], 13.4 and 5.2 in Guangzhou [5], and 13.0 and 4.0 in Hong Kong [6]). Decreasing trends in NPC incidence have been consistently reported across the world, with average annual percent changes of −0.9% to −5.4% in men and −1.1% to −4.1% in women during 1970–2007 [2]. The decline in ASIRs of NPC in endemic regions coincided with socioeconomic development [7] probably because of related changes in exposure to putative NPC risk and protective factors, including older age of first Epstein–Barr virus (EBV) infection [8], less preserved food [9] and more fresh food consumption [10], and lower smoking prevalence [11,12]. However, in Hong Kong, where NPC is endemic and a decline was first reported in the 1980s, the trend has become stagnant in recent years (ASIRs of about 7–8 per 100,000 person-years in 2010–2018). In addition, NPC incidence peaks in relatively young patients (from 45 years in Hong Kong) typically in their prime of life. As NPC is usually diagnosed at advanced stages, the adverse impacts on quality of life and socioeconomic conditions are enormous. Further elucidating the etiology of NPC with discovery of potential new environmental factors will help to further reduce the disease burden.

Vitamin D is synthesized when the skin is exposed to ultraviolet radiation (UVR) or obtained from nutritional sources [13]. Vitamin D deficiency is hypothesized to increase the risk of developing cancer [14–18]. While evidence is lacking on the association between vitamin D exposure and NPC risk, vitamin D deficiency has been linked to several risk factors of NPC, including low SES [19], obesity [20], smoking [21,22] and EBV [23–26]. European studies in patients with multiple sclerosis reported inverse associations between vitamin D exposure and EBV serostatus [23–25]. Our recent study [26] showed personal UVR was associated with EBV viral capsid antigen (VCA-IgA) seropositivity, but not with observational or genetic 25-hydroxyvitamin D (25OHD) levels in Hong Kong, where UVR levels are high due to its latitude (22.3° N) yet vitamin D deficiency is common [27]. Given the close relation between EBV and NPC, we hypothesized vitamin D deficiency could have a role in the etiology of NPC. An ecological study has found satellite based UVR exposure inversely correlated with NPC mortality and incidence in China [28], but the study did not have data on circulating or genetic predicted 25OHD [29,30]. In a case–control study with 171 NPC patients and 176 non-cancer controls in the West China Hospital, Sichuan, a low-risk region of NPC [31], NPC was not associated with two variants (rs10735810 and rs1544410) of vitamin D receptor. These two variants have not been found to be associated with vitamin D exposure [32–34], and the study did not adjust for potential confounders [31].

We examined the independent associations of NPC risk with vitamin D exposure, and potential effect modification by several putative risk factors of NPC using data from the Hong Kong Area of Excellence NPC case–control (HKAoENPC) study. As we found no similar papers in our search of PubMed, Web of Science, CNKI and Wanfang, our study is the first to include a comprehensive list of potential confounders to examine such associations using both circulating 25OHD and a refined measure of vitamin D exposure (genetically instrumented based on single-nucleotide polymorphism that relates to vitamin D synthesis and/or catabolism) [35].

2. Material &methods

2.1. Study population

The HKAoENPC study is a multicenter case–control study conducted from March 2014 to September 2017 in five major regional hospitals (Queen Mary Hospital, Pamela Youde Nethersole Eastern Hospital, Queen Elizabeth Hospital, Princess Margaret Hospital and Tuen Mun Hospital) that treated up to 75% of all NPC new cases in Hong Kong. Detailed descriptions of the study design have been reported [36,37].

Briefly, the cases were 815 histologically and/or radiologically confirmed incident NPC patients. Of 1039 eligible NPC cases approached by trained research staff, 815 completed the computer-based questionnaire (response rate: 78.4%). We included two types of controls. The first type of controls were 1502 frequency-matched (by sex and 5-year age group) new patients or referrals with a new health complaint in the past 12 months in specialist outpatient clinics, or new inpatients admitted in the past 3 months in the same hospitals. Those with a history of NPC, dementia, or suspected NPC symptoms such as recent unilateral facial nerve palsy, tinnitus, unilateral hearing loss and epistaxis were excluded. Following the AsiaLymph guideline of the U.S. National Cancer Institute [23], we also specified that no more than 15% of controls would have the same specific disease type. A limited number of specific diagnoses were further excluded, based on a known or suspected relation with vitamin D exposure and immunological, infectious, and/or inflammatory etiology. Of 1765 eligible hospital controls approached, 1502 completed the questionnaire (response rate: 85.1%).

The second type of controls were 299 healthy subjects in three of the largest blood donation centers of the Hong Kong Red Cross Blood Transfusion Service (Causeway Bay, Tsuen Wan and Kwun Tong Donor Centers) to minimize potential biases related to hospitalization status. As the healthy subjects were found to differ from NPC patients in all factors except for composite allele score based on five variants, they were treated as controls in the genetic analysis only.

Ten milliliters of peripheral blood were collected on the recruitment date (centrifuged at 3000 rpm at 4 °C for 10 min). All samples were then stored at −80 °C before measurements of EBV VCA-IgA serostatus, circulating 25OHD concentration, and DNA extraction for genotyping. Genomic DNA for genetic analysis was extracted from the buffy coat using the ReliaPrep Blood gDNA Miniprep System (Promega, Madison, WI, USA) extraction kits according to the manufacturer's instructions.

The present study was restricted to the subset of participants who had completed the questionnaire and provided blood for 25OHD measurement (516 cases, 1034 hospital controls and 194 healthy subjects). No significant difference between included and excluded subjects in sex, age, household income, smoking status and family history of cancer was observed (Supplementary Table 1).

2.2. Vitamin D exposure

Detailed descriptions of the measurements have been reported [26]. Briefly, we measured both serum level of 25OHD and genetic predicted 25OHD using validated enzyme immunoassay (Abbott ARCHITECT i2000SR) and the iPLEX assay on the MassARRAY System (Sequenom, San Diego, CA, USA), respectively. Circulating 25OHD was classified into three categories based on cutoff points in previous studies for the main analysis: <37.5, 37.5-<75, and ≥75 nmol/L, or classified into quartiles (Q4: 59.5–128, Q3: 48-<59.5, Q2: 37.7-<48, Q1: 7-<37.7) in the sensitivity analysis. Models categorizing 25OHD levels into tertiles or quintiles generated

similar results (data not shown). We used 5 genetic instruments (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) in all four 25OHD-related genes to calculate a composite genetic score (linear continuous: 0–9) based on the summation method [38]. A higher score represented a proxy to greater lifelong status of vitamin D deficiency (circulating 25OHD was 1.78, 1.85 and 1.88 nmol/L lower per score increase in NPC patients, non-NPC hospital controls, and healthy subjects, respectively).

2.3. Covariables

Information on demographics, lifestyle factors and personal UVR exposure 10 years before recruitment was collected using a computer-assisted, self-administered questionnaire with satisfactory test-retest reliability (24), including sex, age, SES score (ranged from −1 [lowest] to 13 [highest], calculated by the subject's, and their father's and mother's education, housing type at age 10, personal income, and household income), smoking and drinking status, body mass index (BMI), family history of cancer, exposure to any occupational hazards, the season when blood was taken, and salted fish consumption at aged 13–18, dietary vitamin D intake and total energy intake 10 years before recruitment, and duration of sunlight exposure, use of sunscreens and hand skin tone 10 years before recruitment. Antibodies of EBV VCA-IgA were measured using a commercial kit (EUROIMMUN AG, Lübeck, Germany), and results were evaluated semi-quantitatively by calculating the ratio of the optical density (OD) value of the sample to the optical density value of the calibrator, expressed as relative OD. According to the manufacturer's instruction, the serostatus of VCA-IgA was classified as seronegative (relative OD value: <1.2) or seropositive (relative OD value: ≥1.2).

2.4. Statistical analysis

To assess the association between vitamin D exposure and NPC risk, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression models. The following variables were considered potential confounders because they were significantly associated with both vitamin D exposure and NPC but not believed to be on the causal pathway: sex, age, SES, months of blood drawing, BMI and hand skin tone at recruitment, duration of sunlight exposure and use of sunscreen 10 years before recruitment, exposure to any occupational hazards, family history of cancer, smoking status and EBV VCA IgA serostatus (Supplementary Table 2). Models mutually adjusting for circulating 25OHD and genetic predicted 25OHD did not change the coefficients. Missing values were coded as separate categories and included as indicator variables in the models. Consumption of salted fish at age 13–18 was included *a priori* in all models because it is a strong risk factor for NPC [9,39].

Final models were adjusted for sex and 5-year age group (crude model because of frequency-matching in subject recruitment), and SES (coded continuously from −1 to 13), consumption of salted fish at aged 13–18 (no, yes), season when blood was taken (winter, summer), BMI (<18.5, 18.5-<23, 23-<27.5, 27.5+) and hand skin tone (coded continuously 1–4) at recruitment, duration of sunlight exposure (<2, 2–4, 5–7, 8–10 h/day) and use of sunscreen (no, yes) 10 years before recruitment, exposure to any occupational hazards (never, ever), family history of cancer (none; yes, but not NPC; yes, NPC), smoking status (never, ever) and EBV VCA IgA serostatus (seronegative, seropositivity). To assess dose–response relationship, a test for trend was examined in models with circulating 25OHD and genetic predicted 25OHD included as an ordinal variable. To determine whether associations between vitamin D exposure and NPC risk were modified by SES (first half [low] versus second half [high]), BMI (18.5-<27.5 [normal] versus ≤18.5 or ≥27.5 [abnormal]), smoking (ever versus never), and EBV (seropositivity versus seronegative), statistical interactions were assessed based on the likelihood ratio test that compared nested models with and without interaction terms.

We conducted several additional analyses as follows. We examined the associations of NPC with quartiles of circulating 25OHD, and genetic predicted 25OHD with NPC only using 2 strong genetic instruments (rs12794714 and rs4588; F-statistic>10). We also investigated whether NPC cases diagnosed at early (American Joint Committee on Cancer [AJCC] staging system stage I or II) or late stage (III or IV) impacted our findings (Fig. 1). All statistical analyses were done with Stata version 15.0 (Tx: StataCorp LLC), and all tests were two-sided with $P < 0.05$ indicating statistical significance.

3. Results

The great majority of NPC cases were non-keratinizing undifferentiated carcinoma (WHO type III; 94%), and diagnosed at a later stage (AJCC stage III or IV; 84%). Compared with 1034 hospital controls, the 516 NPC cases had a lower SES and BMI, but higher proportions of family history of NPC, ever-smoking, EBV seropositivity, and exposure to any occupational hazards (all *P*-values < 0.05). No difference was observed between NPC cases and hospital controls for other factors, and between NPC cases and both control groups for genetically lower 25OHD (Table 1).

We found no significant association of NPC risk (versus hospital controls) with circulating 25OHD levels (Table 2). While the risk of NPC was non-significantly lower in lower circulating 25OHD levels in the crude model (sex- and age-adjusted; <37.5 versus ≥75.0 nmol/L, crude OR = 0.85, 95% CI = 0.55–1.32, *P* for trend = 0.59), it was non-significantly higher in the adjusted model (adjusted OR = 1.39, 0.69–2.82, 0.27). The associations were not modified by SES, smoking status and EBV VCA IgA serostatus (*P*-values for interaction>0.05). An exception was that BMI marginally modified such association (*P* for interaction = 0.06). In subjects with BMI of 18.5-<27.5, we found a significantly increased NPC risk among subjects who were vitamin D deficient (25OHD<37.5 versus ≥75.0 nmol/L, adjusted OR = 3.13, 95% CI = 1.29–7.62, *P* for trend = 0.003). However, in subjects with BMI≤18.5 or ≥27.5, lower circulating 25OHD was not associated with NPC (25OHD<37.5 versus ≥75.0 nmol/L, adjusted OR = 1.16, 95% CI = 0.19–7.24, *P* for trend = 0.59).

Table 3 shows no significant association of NPC risk (versus non-NPC controls) with genetic predicted 25OHD based on five variants (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) inversely associated with 25OHD. However, the risk of NPC was marginally (*P* for trend = 0.07) higher in genetically lower 25OHD level (composite allele score 6–9 versus 0–2, adjusted OR = 1.51, 95% CI = 0.83–2.73). Circulating 25OHD level (quartile) and genetic predicted 25OHD level based on two variants (rs12794714 and rs4588) also were not associated with NPC risk (Supplementary Table 3). While the associations of NPC risk (versus non-NPC controls) with genetic predicted 25OHD level were not modified by SES, BMI, smoking status and EBV VCA IgA serostatus (*P*-values for interaction≥0.25), the positive association between genetically lower 25OHD level and NPC risk appeared to be stronger and became significant in lower SES (6–9 versus 0–2, adjusted OR = 2.58, 95% CI = 1.14–5.88, *P* for trend = 0.005) (Table 3).

Genetically lower 25OHD level based on five variants was significantly associated with higher risk of NPC diagnosed at an early stage (AJCC stage I or II) (composite allele score 6–9 versus 0–2, adjusted OR = 3.09, 95% CI = 1.04–9.21, *P* for trend = 0.022). However, no significant association for circulating 25OHD level by NPC AJCC stage was found (Fig. 1).

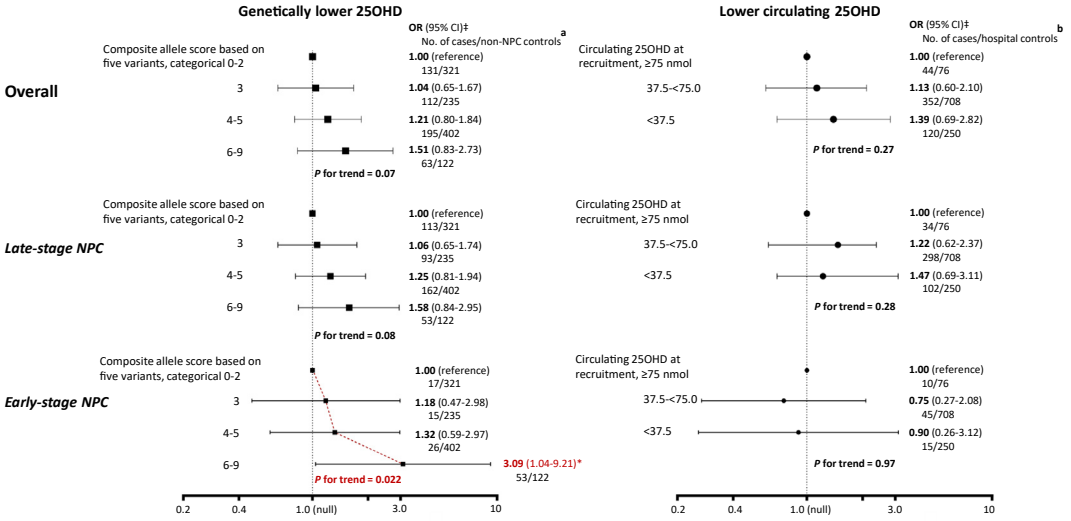

Fig. 1. Associations of NPC for genetically lower 25OHD and lower circulating 25OHD by AJCC staging system (late-, early-stage) in Hong Kong, China, 2014–2017. Abbreviations: NPC, nasopharyngeal carcinoma; 25OHD, 25-hydroxyvitamin D; OR, odds ratio; CI, confidence interval; EBV VCA-IgA, IgA against Epstein–Barr virus viral capsid antigen. [a] Includes 516 NPC cases and 1228 non-NPC controls (1034 hospital controls and 194 healthy subjects combined), but numbers may be inconsistent because of missing values. [b] Includes 516 NPC cases and 1034 hospital controls, but numbers may be inconsistent because of missing values. ‡ Adjusted for sex, 5-year age group, SES (coded continuously from −1 to 13), consumption of salted fish at aged 13–18 (no, yes), season when blood was taken (winter, summer), BMI (<18.5, 18.5-<23, 23-<27.5, 27.5+) and hand skin tone (coded continuously 1–4) at recruitment, duration of sunlight exposure (<2, 2–4, 5–7, 8–10) and use of sunscreen (no, yes) 10 years before recruitment, exposure to any occupational hazards (no, yes), family history of cancer (no, yes but not NPC, yes NPC), smoking status (no, yes) and EBV VCA IgA serostatus (seronegative, seropositivity). Trend tests were conducted by modeling ordinal categories as continuous. * $P < 0.05$.

4. Discussion

In this large case–control study in Hong Kong, while the association between circulating 25OHD and NPC risk was non-significantly inverse in the crude model, the association became non-significantly positive after adjusting for potential confounders, which suggested negative confounding. In the present study, we used an allele score that predicted lower 25OHD level as a surrogate [40] for lifelong differences in vitamin D exposure to test whether vitamin D deficiency is associated with NPC, because the effect of genetics on disease is generally unaffected by confounding or reverse causality. We did observe a marginal positive association between genetically lower 25OHD level and a higher risk of NPC. This positive association became statistically significant, and appeared to be stronger in subjects with lower SES, and for early-stage NPC. However, these stratified estimates should be interpreted with caution because these stratified factors were also associated with the primary outcomes assessed (e.g. more NPC cases with low SES, and the use of chemotherapy [induction or concurrent] in patients with advanced-stage NPC may alter their level of circulating and genetic predicted 25OHD).

Our study is the first to report an association of NPC risk with genetically lower 25OHD level, which is considered the best marker of vitamin D status [29,30]. Four previous studies have investigated the associations between indicators of vitamin D exposure and NPC but they did not specifically study circulating and genetic predicted 25OHD levels [28,31,37,41]. Our recent case–control study showed an inverse association of NPC with consumption of milk [37], which is usually fortified with vitamin D. An ecological study of 263 counties in China reported that NPC incidence rates increased with exposure to UVR [28], which is generally associated with higher vitamin D exposure. However, while the authors adjusted for sex, age, and location, further controlling for risk factors of NPC, including EBV, smoking and salted fish consumption at the individual level was impossible because of lack of data. A case–control study of 171 NPC patients and 176 non-cancer controls in Sichuan (West China) showed no association between two variants (rs10735810 and rs1544410) of vitamin D receptor and NPC [31]. These two variants were not associated with vitamin D exposure [32–34]. The other case–control study of 48 NPC patients and 48 healthy controls in a low-risk region (Yangzhou) showed Fok-I polymorphism was significantly associated with a higher risk of NPC, but potential confounders were not controlled for [41].

Although studies have found that vitamin D could reduce the risk of multiple kinds of cancer [42–44], potential biological mechanisms to explain the lower risk might not apply to NPC, and the exact biological mechanisms between vitamin D exposure and NPC development remain unclear. Several studies have shown that vitamin D decreased cancer risk through regulation of cellular proliferation and differentiation, induction of apoptosis, and inhibition of angiogenesis [15,45]. Vitamin D deficiency has been associated with tumor necrosis factor-alpha (TNF-α) [46], which in turn has been associated with EBV [47]. We also hypothesized vitamin D exposure was associated with EBV reactivation. Indeed, our recent study found higher sunlight exposure was associated with EBV VCA-IgA seropositivity in our hospital controls, but circulating 25OHD and genetic predicted 25OHD levels were not associated with EBV VCA-IgA serostatus [26]. The association between UVR and NPC may be positive via EBV reactivation due to UVR-induced immunosuppression [48]. However, the association between vitamin D exposure and NPC was inverse in our study. These conflicting observations made conclusive interpretation of the current evidence in the literature more difficult. Research on other factors, such as nitric oxide that has been associated with EBV reactivation [49] in relation to NPC is warranted. In addition, the role of vitamin D in NPC may involve several risk factors for NPC, including SES [19], BMI [20], smoking [21,22] and EBV [23–26] in previous studies, and smoking status, family history of NPC and exposure to any occupational hazards in the present analysis. However, even when we considered that these factors might be in

Table 1
Characteristics of nasopharyngeal carcinoma (NPC) cases and controls in Hong Kong, China, 2014–2017.

	NPC patients (N = 516)			Hospital controls (N = 1034)				Healthy subjects (N = 194)			
	n	%		n	%		P^b	n	%		P^b
NPC histology, % (case only)											
WHO type I	12	2.3									
II	13	2.5									
III	486	94.2									
Unknown	5	1.0									
NPC AJCC stage, % (case only)											
Early (stage I or II)	70	13.6									
Late (stage III or IV)	434	84.1									
Unknown	12	2.3									
Age at recruitment							0.55				<0.001
Mean, SD	516	51.8	11.1	1034	51.4	12.7		194	40.5	11.5	
Sex							0.32				<0.001
Male	379	73.5		735	71.1			103	50.1		
Low socioeconomic status[a]	319	61.8		505	48.8		<0.001	47	24.2		<0.001
Family history of cancer							<0.001				<0.001
None	176	34.1		508	49.1			86	44.3		
Yes, but not NPC	167	32.4		398	38.5			97	50.0		
Yes, NPC	82	15.9		52	5.0			10	5.2		
Unknown	91	17.6		76	7.4			1	0.5		
Exposure to any occupational hazards							<0.001				<0.001
Never	180	34.9		528	51.1			147	75.8		
Ever	241	46.7		402	38.9			30	15.5		
Unknown	95	18.4		104	10.1			17	8.8		
Smoking status							<0.001				<0.001
Never	264	51.2		644	62.3			142	62.3		
Ever	248	48.1		387	37.4			52	37.4		
Unknown	4	0.78		3	0.3			0	–		
EBV VCA-IgA serostatus							<0.001				<0.001
Seronegativity	55	10.7		895	86.6			174	89.7		
Seroposivity	450	87.2		121	11.7			20	10.3		
Missing	11	2.1		18	1.7			0	–		
Body mass index at recruitment, kg/m^2							0.008				<0.001
<18.5	35	6.8		58	5.6			7	3.6		
18.5-<23	180	34.9		360	34.8			76	39.2		
23-<27.5	90	17.4		172	16.6			83	42.8		
≥27.5	112	21.7		333	32.2			28	14.4		
Unknown	99	19.2		111	11			0	–		
Months of blood draw							0.05				<0.001
December–February	137	26.6		240	23.2			77	39.7		
March–May	110	21.3		242	23.4			79	40.7		
June–August	136	26.4		328	31.7			4	2.1		
September–November	133	25.8		224	21.7			34	17.5		
Composite allele score based on five variants (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) negatively associated with 25OHD, categorical							0.56				0.44
0–2	131	25.4		263	25.4			58	25.4		
3	112	21.7		202	19.5			33	19.5		
4–5	195	37.8		328	31.7			74	31.7		
6–9	63	12.2		100	9.7			22	9.7		
Missing	15	2.9		141	13.6			0	–		
Serum 25OHD, nmol/L											
<37.5	44	8.5		76	7.4		0.69	9	4.6		0.06
37.5-<75.0	352	68.2		708	68.5			147	65.5		
≥75.0	120	23.3		250	24.2			58	29.9		

Abbreviations: NPC, nasopharyngeal carcinoma; 25OHD, 25-hydroxyvitamin D; EBV VCA-IgA, IgA against Epstein–Barr virus viral capsid antigen; SD, standard deviation; WHO, World Health Organization; AJCC, American Joint Committee on Cancer staging system.

[a] Low (from −1 to 3) socioeconomic position score was calculated by the subject's, and their father's and mother's education, housing type at age 10, and personal and household income.

[b] t-test and Chi-square test were used to compare the mean of continuous factors, and proportions of categorical factors between NPC cases and hospital controls, or between NPC cases and healthy subjects.

the causal pathway, the effects through them were modest, indicating that vitamin D has independent roles in NPC development.

A major strength of the present study was the inclusion of a comprehensive list of indicators of vitamin D exposure in an NPC endemic region where vitamin D deficiency is common. Furthermore, as we measured vitamin D-related single nucleotide polymorphisms, a strong surrogate of lifelong exposure to vitamin D [29,30], our study could limit potential selection bias and reverse causality that would be introduced if only circulating 25OHD level or indicators of UVR exposure were analyzed.

Several limitations need to be acknowledged. First, as our sample was of Chinese ethnicity aged 18–90 recruited in five major hospitals in Hong Kong, our results may not be generalized to other populations or ethnic groups. Second, we assumed a linear inverse

Table 2
Odds ratio and 95% confidence interval of NPC for circulating 25OHD at recruitment in 516 NPC cases and 1034 hospital controls in Hong Kong, China, 2014–2017.

	All		No. of cases and hospital controls		Crude OR[a]	95% CI		*P for trend*	Adjusted OR[b]	95% CI		*P for trend*
Serum 25-hydroxyvitamin D at recruitment, nmol/L												
≥75.0			44	76	1.00 (ref)				1.00 (ref)			
37.5-<75.0			352	708	0.87	0.58	1.28		1.13	0.60	2.10	
<37.5			120	250	0.85	0.55	1.32	0.59	1.39	0.69	2.82	0.27
Low or high socioeconomic status	*P* for interaction	0.11										
	Serum 25-hydroxyvitamin D at recruitment, nmol/L											
Low	≥75.0		24	43	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		236	352	1.16	0.69	1.97		1.74	0.76	3.98	
	<37.5		59	110	0.92	0.50	1.67	0.45	1.57	0.61	4.05	0.57
High	≥75.0		20	33	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		116	356	0.54	0.30	1.00		0.68	0.25	1.90	
	<37.5		61	140	0.75	0.39	1.44	0.79	1.47	0.47	4.66	0.14
Underweight/obese or normal or overweight	*P* for interaction	0.06										
BMI≤18.5 or ≥27.5	≥75.0		4	15	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		60	135	1.69	0.53	5.35		2.04	0.38	11.0	
	<37.5		22	79	0.30	0.31	3.56	0.34	1.16	0.19	7.24	0.59
BMI≥18.5&<27.5	≥75.0		27	60	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		225	503	1.03	0.63	1.66		1.39	0.65	2.96	
	<37.5		79	131	1.45	0.84	2.50	0.06	3.13	1.29	7.62	0.003
Ever or never smoking	*P* for interaction	0.77										
Ever	≥75.0		23	33	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		171	268	0.92	0.52	1.63		1.38	0.52	3.68	
	<37.5		54	86	0.90	0.47	1.71	0.76	1.70	0.55	5.32	0.37
Never	≥75.0		20	43	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		178	439	0.86	0.49	1.51		1.37	0.52	3.60	
	<37.5		66	162	0.85	0.46	1.56	0.68	2.64	0.88	7.96	0.032
EBV VCA-IgA serostatus	*P* for interaction	0.80										
Seropositivity	≥75.0		39	12	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		309	85	1.13	0.56	2.25		1.37	0.60	3.11	
	<37.5		102	24	1.31	0.59	2.93	0.47	2.16	0.81	5.72	0.10
Seronegative	≥75.0		5	62	1.00 (ref)				1.00 (ref)			
	37.5-<75.0		33	613	0.71	0.27	1.88		1.06	0.33	3.35	
	<37.5		17	220	1.16	0.41	3.32	0.33	1.97	0.55	7.09	0.14

Abbreviations: NPC, nasopharyngeal carcinoma; 25OHD, 25-hydroxyvitamin D; OR, odds ratio; CI, confidence interval; EBV VCA-IgA, IgA against Epstein–Barr virus viral capsid antigen.
Includes 516 NPC cases and 1034 hospital controls, but numbers may be inconsistent because of missing values.
Trend tests were conducted by modeling ordinal categories as continuous.
Interaction tests were assessed based on the likelihood ratio test that compared nested models with and without interaction terms.
[a] Adjusted for sex and 5-year age group (frequency-matching in subject recruitment).
[b] Adjusted for sex, 5-year age group, SES (coded continuously from −1 to 13), consumption of salted fish at aged 13–18 (no, yes), season when blood was taken (winter, summer), BMI (<18.5, 18.5-<23, 23-<27.5, 27.5+) and hand skin tone (coded continuously 1–4) at recruitment, duration of sunlight exposure (<2, 2–4, 5–7, 8–10) and use of sunscreen (no, yes) 10 years before recruitment, exposure to any occupational hazards (no, yes), family history of cancer (none; yes, but not NPC; yes, NPC), smoking status (ever, never) and EBV VCA IgA serostatus (seronegative, seropositivity).

association of serum 25OHD level with NPC risk. However, the association was somewhat curvilinear than linear, as suggested by some of our stratified analyses. Although high levels of 25OHD (≥250 nmol/L) is toxic [50], vitamin D intoxication is extremely rare [51–54]. As the highest concentration found in our samples (127.3 nmol/L) was substantially lower than the toxic level, our study could not provide information on the optimal level of 25OHD for NPC prevention. Furthermore, we arbitrarily categorized serum 25OHD level into three groups (<37.5, 37.5 < 75, and ≥75 nmol/L) because population-based studies defining the range of 25OHD in normal Chinese population is lacking in the literature. The number of participants with NPC was relatively small in the present study, which had detailed information on confounders of NPC, and a comprehensive list of indicators of vitamin D exposure.

In summary, this multicenter case–control study has shown that vitamin D deficiency may be associated with higher risk of NPC. Our findings may inform possible etiological mechanisms of the associations with several putative risk/protective factors of NPC. Findings of this first comprehensive study to investigate the association of NPC risk with vitamin D exposure need to be confirmed and be best interpreted with results of further similar studies.

Publication history

The contents of this manuscript have not been presented or published in any form.

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Availability of data and material

The data of the Hong Kong Area of Excellence NPC case–control study underlying this article will be shared on reasonable request to the corresponding authors.

Table 3
Odds ratio and 95% confidence interval of NPC for genetic predicted 25OHD at recruitment in 516 NPC cases and 1228 non-NPC controls in Hong Kong, China, 2014–2017.

	All		No. of cases and non-NPC controls		Crude OR[a]	95% CI			*P for trend*	Adjusted OR[b]	95% CI			*P for trend*
Composite allele score based on five variants (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) negatively associated with 25OHD, categorical														
0–2			131	321	1.00 (ref)					1.00 (ref)				
3			112	235	1.16	0.86	1.58			1.04	0.65	1.67		
4–5			195	402	1.20	0.92	1.57			1.21	0.80	1.84		
6–9			63	122	1.25	0.87	1.81		0.19	1.51	0.83	2.73		0.07
Low or high socioeconomic status	*P* for interaction	0.25												
	Composite allele score based on five variants (rs12785878, rs11234027, rs12794714, rs4588 and rs6013897) negatively associated with 25OHD, categorical													
Low	0–2		77	154	1.00 (ref)					1.00 (ref)				
	3		72	107	1.33	0.89	2.00			1.52	0.79	2.93		
	4–5		122	168	1.47	1.02	2.10	*		1.79	1.01	3.17	*	
	6–9		41	49	1.67	1.01	2.75	*	0.039	2.58	1.14	5.88	*	0.005
High	0–2		54	167	1.00 (ref)					1.00 (ref)				
	3		40	128	0.97	0.60	1.55			0.68	0.32	1.45		
	4–5		73	234	0.97	0.65	1.45			0.81	0.42	1.55		
	6–9		22	73	0.93	0.53	1.64		0.81	0.85	0.34	2.12		0.70
Underweight/obese or normal or overweight	*P* for interaction	0.74												
BMI≤18.5 or ≥27.5	0–2		48	89	1.00 (ref)					1.00 (ref)				
	3		47	76	1.15	0.69	1.90			0.81	0.37	1.75		
	4–5		65	124	0.98	0.61	1.55			0.93	0.44	1.99		
	6–9		18	41	0.81	0.42	1.56		0.64	0.79	0.27	2.36		0.83
BMI≥18.5&<27.5	0–2		83	232	1.00 (ref)					1.00 (ref)				
	3		65	159	1.13	0.77	1.66			1.20	0.65	2.21		
	4–5		130	278	1.33	0.96	1.85			1.40	0.83	2.36		
	6–9		45	81	1.54	0.99	2.41		0.06	1.85	0.89	3.85		0.06
Ever or never smoking	*P* for interaction	0.84												
Ever	0–2		65	114	1.00 (ref)					1.00 (ref)				
	3		59	90	1.12	0.71	1.76			1.09	0.54	2.21		
	4–5		85	132	1.13	0.74	1.70			1.01	0.53	1.93		
	6–9		33	44	1.33	0.77	2.30		0.34	1.55	0.63	3.80		0.22
Never	0–2		64	206	1.00 (ref)					1.00 (ref)				
	3		52	145	1.16	0.76	1.78			1.17	0.60	2.29		
	4–5		109	270	1.30	0.91	1.87			1.43	0.80	2.55		
	6–9		30	78	1.21	0.73	2.01		0.28	1.70	0.73	3.96		0.11
EBV VCA-IgA serostatus	*P* for interaction	0.93												
Seropositivity	0–2		115	39	1.00 (ref)					1.00 (ref)				
	3		100	30	1.13	0.65	1.96			1.10	0.60	2.00		
	4–5		168	48	1.22	0.75	1.98			1.15	0.67	1.95		
	6–9		56	12	1.56	0.75	3.21		0.11	1.69	0.76	3.71		0.13
Seronegative	0–2		16	277	1.00 (ref)					1.00 (ref)				
	3		11	201	0.92	0.42	2.03			0.74	0.32	1.72		
	4–5		19	351	0.96	0.48	1.91			0.97	0.47	2.00		
	6–9		7	110	1.09	0.43	2.73		0.77	1.14	0.43	2.99		0.61

Abbreviations: NPC, nasopharyngeal carcinoma; 25OHD, 25-hydroxyvitamin D; OR, odds ratio; CI, confidence interval; EBV VCA-IgA, IgA against Epstein–Barr virus viral capsid antigen.
Includes 516 NPC cases and 1228 non-NPC controls (1034 hospital controls and 194 healthy subjects combined), but numbers may be inconsistent because of missing values.
Trend tests were conducted by modeling ordinal categories as continuous.
Interaction tests were assessed based on the likelihood ratio test that compared nested models with and without interaction terms.
[a] Adjusted for sex and 5-year age group (frequency-matching in subject recruitment).
[b] Adjusted for sex, 5-year age group, SES (coded continuously from −1 to 13), consumption of salted fish at aged 13–18 (no, yes), season when blood was taken (winter, summer), BMI (<18.5, 18.5-<23, 23-<27.5, 27.5+) and hand skin tone (coded continuously 1–4) at recruitment, duration of sunlight exposure (<2, 2–4, 5–7, 8–10) and use of sunscreen (no, yes) 10 years before recruitment, exposure to any occupational hazards (no, yes), family history of cancer (none; yes, but not NPC; yes, NPC), smoking status (ever, never) and EBV VCA IgA serostatus (seronegative, seropositivity).

Code availability

The code underlying this article will be shared on reasonable request to the corresponding authors.

Authors' contributions

ZMM, JHL, YHC, and THL designed the study; ZMM performed the statistical analysis and drafted the manuscript; ZMM, JHL., RKCN, DLWK, WTN, KTY, CKL, JNSL, AWML and MLL collected data. All authors critically reviewed data for important intellectual content and contributed to final approval of the paper.

Ethics approval

The Institutional Review Board of the HKU/Hospital Authority HK West Cluster (UW 11–192), the HK East Cluster Research Ethics Committee (HKEC-2012-043), the Research Ethics Committee of the Hospital Authority Kowloon Central/Kowloon East (KC/KE-13-0115/ER-2), the Research Ethics Committee of the Kowloon West Cluster [KW/EX-13-073 (63-11)], and the NTW Cluster Clinical & Research Ethics Committee (NTWC/CREC/1239-13) approved the study.

Consent to participate

Informed consent was obtained from all subjects included in the study.

Conflicts of interest

The authors declare no conflict of interest.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2021.07.034.

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