Solar ultraviolet radiation and vitamin D deficiency on Epstein-Barr virus reactivation: observational and genetic evidence from a nasopharyngeal carcinoma endemic population

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UV radiation, vitamin D and EBV

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

Acknowledgment of support: This study was supported by the Hong Kong RGC Area of Excellence Scheme (Grant Number AoE/M-06/08), and the World Cancer Research Fund UK (WCRF UK) and Wereld Kanker Onderzoek Fonds (WCRF NL), as part of the WCRF International Grant Programme (Grant 2011/460). The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or report writing.

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Conflict of Interest: The authors declare no potential conflicts of interest.

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Abstract
Background
We investigated the relationship of Epstein-Barr virus viral capsid antigen (EBV VCA-IgA) serostatus with ambient and personal ultraviolet radiation (UVR) and vitamin D exposure.

Methods
Using data from a multicenter case-control study, we included 1026 controls subjects in 2014-2017 in Hong Kong, China. Odds ratios (ORs) and 95% confidence intervals (CIs) of the association between UVR exposure and EBV VCA-IgA (seropositivity vs seronegativity) were calculated using unconditional logistic regression models adjusted for potential confounders.

Results
We observed a large increase in seropositivity of EBV VCA-IgA in association with duration of sunlight exposures at both 10 years before recruitment and age 19-30 years (adjusted OR = 3.59, 95% CI = 1.46-8.77; and 2.44, 1.04-5.73 for ≥8 vs <2 hours/day; P for trend = 0.005 and 0.048, respectively). However, no association of EBV VCA-IgA serostatus with other indicators of UVR exposure was found. In addition, both circulating 25-hydroxyvitamin D (25OHD) and genetic predicted 25OHD were not associated with EBV VCA-IgA serostatus.

Conclusions
Our results suggest that personal UVR exposure may be associated with higher risk of EBV reactivation, but we did not find clear evidence of vitamin D exposure (observational or genetic), a molecular mediator of UVR exposure. Further prospective studies in other populations are needed to confirm this finding, and to explore the underlying biological mechanisms. Information on photosensitizing agents, and serological markers of EBV, and biomarkers related to systemic immunity and inflammation should be collected and are also highly relevant in future studies.

Keywords: ultraviolet radiation; vitamin D; Epstein-Barr virus; reactivation; genetic epidemiology; nasopharyngeal carcinoma
Introduction

Epstein-Barr virus (EBV) is the most common human virus, infecting and persisting latently in more than 90% of the adult population worldwide, but EBV only accounts for over 200,000 new cancer cases each year. Although most infected individuals establish a life-long immunity to the virus and do not develop the associated illness, EBV can be reactivated and then cause clinical disease when the cellular immune response is compromised. Immunosuppression is thought to contribute to EBV reactivation, and elevated risks in EBV reactivation have been observed among organ-transplantation recipients and human immunodeficiency virus patients, which may subsequently be associated with higher risks in EBV-related malignancies. EBV reactivation also can be induced by DNA-damaging agents.

Solar ultraviolet radiation (UVR), an omnipresent non-ionizing radiation, can damage DNA and induce immunosuppression, but the underlying mechanisms between UVR and EBV-related malignancies remain unclear. Higher risks in EBV-related diseases such as nasopharyngeal carcinoma (NPC) were found in individuals with higher exposure to solar UVR, while UVR was associated with lower risks of multiple sclerosis, and lymphomas, suggesting that UVR-induced EBV reactivation may play a dual etiological role in human health. However, the precise role of UVR exposure in EBV reactivation is unknown, particularly for an ultimate biomarker of UVR with potential immunomodulatory and anti-inflammatory effects – vitamin D. Vitamin D, a surrogate of sunlight exposure, has traditionally been viewed as a contributor for the UVR-induced immunomodulation and anti-inflammation. Few studies have assessed the association between vitamin D and EBV reactivation. A cross-sectional study in 71 plasma samples of EBV-seropositive young adults in the United Kingdom showed no correlation of circulating 25-hydroxivitamin D3 (25OHD) for EBV load or anti-EBNA-1 titers. In a vitamin D supplementation study on 37 healthy Antarcticans, participants with higher serum 25OHD were more likely to have less Epstein-Barr virus in saliva. A randomized controlled trial in 53 patients with relapsing-remitting multiple sclerosis showed high-dose vitamin D3 supplementation (14,000 IU/day; n=30) reduced anti-EBNA-1 antibody levels. These studies did not include other
races/ethnicities (especially Chinese) and areas with higher UV levels due to latitude, both being strong modifying factors of vitamin D exposure. Moreover, these studies did not control for potential confounders, including smoking, occupation, socioeconomic status, dietary vitamin D intake, and ambient or personal UVR exposure.

We examined the associations of EBV viral capsid antigen (VCA-IgA) serostatus with ambient and personal UVR, and vitamin D exposure using data from 1026 hospital-based non-NPC patients recruited in a multicenter NPC case-control study in 2014-2017 in Hong Kong, China where UV levels are high due to latitude while vitamin D deficiency is common, and NPC is endemic. This is the first report including a comprehensive list of UVR exposure indicators (integrating both personal behavior and ambient UVR), and is the largest study to examine the associations between vitamin D exposure and EBV VCA-IgA serostatus using both serum vitamin D and a refined measure of vitamin D exposure (genetically instrumented based on single-nucleotide polymorphism that relates to vitamin D synthesis and/or catabolism).

**Materials and Methods**

**Study subjects**

Subject recruitment of the multicenter NPC case-control study was 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 treat up to 90% of all NPC new cases in Hong Kong.

Only non-NPC patients were included in the present analysis, while NPC cases were excluded. The non-NPC patients were selected from patients who attended the clinics or admitted to the hospitals with a wide range of medical diseases unrelated to NPC. These non-NPC patients were frequency-matched by age (5-year age groups) and sex to the NPC cases, and were new patients or referrals of a new health complaint in the past 12 months in specialist outpatient clinics, or new inpatients admitted in the past three months in the same hospitals. We excluded those who had any possibly NPC related symptoms such as hearing
problems, epistaxis or cranial nerve palsy. Following the AsiaLymph guideline of the US National Cancer Institute, we also specified that no more than 15% of controls had the same specific type of disease. 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. In addition, because of the potential associations of EBV reactivation with sleep disturbances, fatigue and fever, 13 non-NPC patients with these conditions were excluded in the present analysis. The disease list is shown in Supplementary Part I. Ten milliliters of peripheral blood were collected at the same date of recruitment (centrifuged at 3000 rpm at 4 degrees Celsius for 10 minutes), and then all samples were stored at −80 degrees Celsius before measurements of EBV VCA-IgA serostatus, circulating 25OHD concentration, and DNA extraction for genotyping.

**Exposure**

**Ambient and personal UVR**

Exposure to ambient UVR was derived by linking the date of blood taken reported by 1026 subjects to the Hong Kong Observatory (HKO) by using the daily mean UV index. The HKO used a Yankee Environmental Systems broadband UVB-1 pyranometer for measuring the UV index. The daily mean UV index for a given day was defined as the averages of all the 15-minute mean UV Index values between 7 a.m. and 6 p.m. in the day. Information on personal UVR exposure over four life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment) was collected by a computer-assisted, self-administered questionnaire with satisfactory test-retest reliability, including duration of sunlight exposure (reliability coefficients ranged from 0.3 to 0.9), use of sunscreens (0.3-0.5) and hand skin tone (0.4-0.6).

**Vitamin D**

**Circulating 25OHD**

Serum level of 25OHD was measured using validated enzyme immunoassay (Abbott ARCHITECT i2000SR). The sensitivity was 4.75 nmol/L and the range was 0-400 nmol/L, and no sample had a concentration below or about these limits. The intra-assay coefficient of variation was 4.3-8.1% by repeating measurements of 50 samples, and the reliability
coefficient was acceptable (<10%). Circulating 25OHD was classified into three a priori
categories based on clinically relevant cut-off points for the main analysis: <37.5 (deficient),
37.5<75 (insufficient), and ≥75 (sufficient) nmol/L.

*Genetic predicted 25OHD*

Genomic DNA for genetic analysis was extracted from buffy coat using the ReliaPrep
Blood gDNA Miniprep System (Promega, Madison, WI, USA) extraction kits according to the
manufacturer’s instructions. Common genetic variants have been identified in the recent
genome-wide association studies of circulating 25OHD level, and eight variants that passed a
geno analytic association threshold (√5×10−8) and had been replicated were selected.27-29.
These genetic instruments locate in or near four 25OHD related genes: 7-dehydrocholesterol
reductase (DHCR7), cytochrome P450 family 2, subfamily R, polypeptide 1 (CYP2R1), group-
specific component (GC), and cytochrome P450, family 24, polypeptide 1 (CYP24A1). The
metabolic pathways of vitamin D have been shown (Supplementary Figure 1), including
rs7977926, rs12785878, rs3829251 and rs11234027 (DHCR7), rs12794714 (CYP2R1), rs4588
and rs1155563 (GC), and rs6013897 (CYP24A1). All SNPs chosen had a minor allele frequency
of ≥5%. Genotyping of these eight SNPs was performed at the Centre for PanorOmics
Sciences, The University of Hong Kong using the iPLEX assay on the MassARRAY System
(Sequenom, San Diego, CA, USA). The rs7944926 was excluded due to the deviation from
Hardy-Weinberg equilibrium (√0.05) (Supplementary Table 1). As the pairs of rs3829251 &
rs11234027 (DHCR7) and rs4588 & rs1155563 (GC) were in linkage disequilibrium (D’>0.80,
the information from one can represent the other), only one of them (rs11234027 and
rs4588) was selected as the candidate SNPs. Furthermore, rs12785878, rs11234027 and
rs6013897 were excluded due to the weak instrument bias (F-statistic<10). Finally, two
variants (rs12794714 and rs4588) were used in the present analysis to calculate a composite
genetic score (linear continuous: 0–4) based on the summation method.22 A higher score
represented a proxy to greater lifelong status of vitamin D-deficiency.
Outcome assessment (EBV VCA-IgA serostatus)

Antibody of EBV VCA-IgA was measured using a commercial kit (EUROIMMUN AG, Lübeck, Germany) based on the standard method of ELISA in subjects who had agreed to provide blood. To minimize bias, the laboratory personnel was blinded to the disease status of the samples. A calibrator for calculation, and a negative control and positive control for internal quality assessment were included on each plate. Results were evaluated semi-quantitatively by calculating the ratio of the optical density (OD) value of the sample over 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).

Covariables

Information on demographic and lifestyle factors was collected by the questionnaire, including sex, age, socioeconomic status (ranged from 1 [lowest] to 13 [highest], calculated by the subject’s, and his/her father’s and mother’s education, housing type at age 10, personal income, and household income), smoking status, body mass index, family history of cancer, exposure to any occupational hazards, season when blood was taken, and salted fish consumption, dietary vitamin D intake and total energy intake over four periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment).

Statistical analysis

We examined the associations of EBV VCA-IgA (seropositivity vs seronegativity) with UVR exposure (daily mean UV index, and duration of sunlight exposure, use of sunscreens and hand skin tone) and vitamin D exposure (categorical serum 25OHD and composite genetic score) by calculating odds ratios (ORs) and 95% confidence intervals (CIs) using unconditional logistic regression models adjusted for sex and 5-year age group, socioeconomic position score, smoking status (never and ever), consumption of salted fish (never and ever), exposure to any occupational hazards (never and ever), season of blood taking (winter and summer), body mass index (≥18.5–23.0, <18.5, ≥23.0–25.0 and ≥25.0), tertiles of dietary vitamin D intake (<12.4, ≥12.4–22.5, ≥22.5–40.7 and ≥40.7–<637 IU/day) and total energy intake over four life periods. To assess dose-response effect, a test for
trend was examined for a model that included UV index, duration of sunlight exposure, hand skin tone, and serum 25OHD and composite genetic score as an ordinal variable. All statistical analyses were done with Stata version 15.0 (TX: StataCorp LLC), and all tests were two-sided (P < 0.05 indicating statistical significance).

**Results**

**UVR exposure and EBV VCA-IgA**

Duration of sunlight exposures at both 10 years before recruitment and age 19-30 years were associated with higher seropositivity of EBV VCA-IgA with dose-response relationships (adjusted OR = 3.59, 95% CI = 1.46-8.77; and 2.44, 1.04-5.73 for ≥8 vs <2 hours/day; P for trend = 0.005 and 0.048, respectively) (Table 1). No association of EBV VCA-IgA serostatus with duration of sunlight exposure at other periods (age 13-18 and 6-12 years), and with UV index, use of sunscreens and hand skin tone over different periods was found.

**Vitamin D exposure and EBV VCA-IgA**

Both circulating 25OHD and genetic predicted 25OHD were not associated with EBV VCA-IgA serostatus (Table 2). A positive association (without dose-response relationship) between higher serum levels of 25OHD and EBV VCA-IgA seropositivity was found in the age- and sex-adjusted model (adjusted OR = 1.80, 95% CI = 1.04-3.14; P for trend = 0.06 for 75-<127.3 vs <37.5 nmo/L of 25OHD), but this association appeared to be null after adjusting for potential confounders in Models 1 and 2.



discussion

uvr and ebv

This is the first report showing personal UVR exposure could be a potential inducer of EBV reactivation in an NPC endemic region. We found strong evidence that longer duration of sunlight exposure was associated with EBV VCA-IgA seropositivity. These results remained robust with adjustment for multiple and relevant confounders. Although no study has examined such association, to some extent, our findings are consistent with previous studies of the positive association between UVR exposure and herpes simplex virus (HSV) reactivation. Exposure to solar UVR was associated with higher risk of HSV reactivation. Evidence from a randomization controlled trial of the effect of sunscreen on UV-induced herpes labialis has suggested that UV light is a potent stimulus for inducing reactivation of herpes. Solar UVR may be related to virus reactivation through immunosuppression. Indeed, higher risks in EBV-related diseases have been consistently observed in patients with immunosuppressive diseases. Furthermore, exposure to solar UV has recently been associated with higher circulating levels of cutaneous T cell-attracting chemokine (CTACK) in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial (data not published) in which CTACK has been linked to EBV reactivation.

UVR, vitamin D and EBV

Although the mechanism of UV-induced EBV reactivation is unknown, vitamin D, a circulating mediator reflective of recent UVR exposure is thought to contribute through its immunomodulatory effect. However, in the present analysis, we did not find any association between vitamin D exposure (either circulating 25OHD or genetic predicted 25OHD) and EBV VCA-IgA serostatus. The null association of our results is consistent with those of observational studies in patients with multiple sclerosis that examined circulating concentration of vitamin D and anti-EBV nuclear antigen (EBNA) complex IgG and EBNA-2, EBV load or anti-EBNA-1 IgG. However, other studies showed inverse correlations between serum 25OHD and anti-EBNA-1, and that vitamin D supplementation reduced anti-EBNA-1 titers. Our study has provided additional robust evidence of the association between vitamin D and EBV with several strengths. First, given additionally measuring SNPs as proxy
to lifelong status of vitamin D-deficiency, our study can limit potential selection bias and reverse causality that can be introduced if only circulating levels of vitamin D were analyzed.\textsuperscript{41} Second, we adjusted for a comprehensive list of vitamin D-related factors and other potential confounders.

**Strengths and limitations**

The present study had two additional strengths. First, we collected inclusive indicators of both ambient and personal UVR exposure over four life periods (age 6–12, 13–18, and 19–30, and 10 years before recruitment), which showed satisfactory test-retest reliability,\textsuperscript{26} thus limiting recall errors (random and systematic). Second, the large sample size of the present analysis had 83.3\% statistical power to detect a crude difference of 0.5 nmol/L or greater in 25OHD level between subjects with EBV VCA-IgA seropositivity and seronegativity.\textsuperscript{42}

However, this study had several limitations. First, we only used EBV VCA-IgA status as a proxy for EBV activation. Although there is no gold standard to evaluate EBV activation, using other serological markers to explore inducers of EBV activation is needed, including IgA antibody against latent membrane protein 1, antibodies against EBNA-1, Zta and EA. Second, the status of EBV reactivation and vitamin D may vary from time to time. In our study, VCA-IgA and 25OHD were only captured at one time point because we collected blood samples once per subject. The fluctuations of these markers, if any, cannot be documented and studied. Potential associations of EBV VCA-IgA serostatus with vitamin D exposure warrant further investigation in large prospective studies. Third, reverse causality of UVR exposure could be a concern, though we examined the associations over four life periods and similar results were observed. Fourth, although we had adjusted for the most relevant and potential confounders, residual confounding is still a possibility.
Conclusions

This is the first report with comprehensive examination of EBV reactivation with ambient and personal UVR, and vitamin D exposure, showing that longer duration of sunlight exposure per day was significantly associated with increased risk of EBV VCA-IgA seropositivity. However, vitamin D exposure (observational or genetic), a molecular mediator of UVR exposure, was not associated with EBV VCA-IgA. Further prospective studies in other populations are needed to confirm this finding, and to explore the underlying biological mechanisms. Information on photosensitizing agents, and serological markers of EBV reactivation, and biomarkers related to systemic immunity and inflammation should be collected and are also highly relevant in future studies.
Acknowledgments

The chief acknowledgment is to the subjects who provided information for this study, and the research staff and Dr Kam-tong Yuen for data collection.

Funding

This study was supported by the Hong Kong RGC Area of Excellence Scheme (Grant Number AoE/M-06/08), and the World Cancer Research Fund UK (WCRF UK) and Wereld Kanker Onderzoek Fonds (WCRF NL), as part of the WCRF International Grant Programme (Grant 2011/460). The sponsors of the study had no role in study design, data collection, data analysis, data interpretation, or report writing.

Declaration of Competing Interest

The authors declare no conflict of actual or potential competing financial interests.

Patient Consent Statement

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

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.

Contributors

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

**Table 1.** Odds ratio (OR) and 95% confidence interval (CI) of EBV VCA-IgA (seropositivity versus seronegativity) with ambient and personal UVR exposure in Hong Kong, China 2014-2017

**Table 2.** Odds ratio (OR) and 95% confidence interval (CI) of EBV VCA-IgA serostatus (seropositivity vs seronegativity) with serum 25-hydroxyvitamin D concentration and genetic predicted 25-hydroxyvitamin D concentration in Hong Kong, China 2014-2017
Table 1. OR and 95% CI of EBV VCA-IgA (seropositivity versus seronegativity) with personal UVR exposure in Hong Kong, China 2014-2017

| Variable                                                                 | Number of EBV VCA-IgA status | Age- and sex-adjusted model | Multivariable adjusted model |
|--------------------------------------------------------------------------|------------------------------|-----------------------------|-----------------------------|
|                                                                           | Positive | Negative | 95% CI    | OR | 95% CI    | OR | 95% CI    |
| Daily mean UV index at the date of blood taken, 0-6                       |          |          |           |    |            |    |            |
| Low (0-2)                                                                | 139      | 334      | 1 (ref.)  | 0.71 (ref.) | 1 | 0.69 (ref.) |
| Moderate (3-5)                                                           | 118      | 301      | 0.95 (1.28) | 0.69 (1.28) | 0.99 | 1.44 (1.83) |
| High (≥6)                                                                | 23       | 46       | 1.19 (2.04) | 0.8 (2.04) | 1.61 | 3.13 (3.13) |
| P for trend                                                              | 0.8      | 2        |            | 0.96 |            |    |            |
| 10 years before recruitment                                              |          |          |           |    |            |    |            |
| Duration of sunlight exposure, hours/day                                 |          |          |           |    |            |    |            |
| <2                                                                       | 112      | 350      | 1 (ref.)  | 0.73 (ref.) | 1 | 0.77 (ref.) |
| ≥2-<5                                                                    | 88       | 277      | 1.00 (1.38) | 1.24 (1.38) | 1.13 | 1.65 (1.65) |
| ≥5-<8                                                                    | 32       | 51       | 2.03 (3.33) | 1.39 (3.33) | 1.74 | 3.18 (3.18) |
| ≥8                                                                       | 16       | 17       | 2.87 (5.91) | 0.0 (5.91) | 3.59 | 8.77 (8.77) |
| P for trend                                                              | 0.0      | 01       |            | 0.00 |            |    |            |
| Use of sunscreens                                                        |          |          |           |    |            |    |            |
| Never                                                                    | 121      | 295      | 1 (ref.)  | 0.58 (ref.) | 1 | 0.60 (ref.) |
| Ever                                                                     | 126      | 402      | 0.78 (1.05) | 0.53 (1.05) | 0.86 | 1.22 (1.22) |
| Hand skin tone, 1-3                                                      |          |          |           |    |            |    |            |
| 1 (Light)                                                                | 62       | 201      | 1 (ref.)  | 0.93 (ref.) | 1 | 0.94 (ref.) |
| 2                                                                        | 150      | 364      | 1.31 (1.84) | 1.14 (1.84) | 1.41 | 2.13 (2.13) |
| 3 (Dark)                                                                 | 36       | 133      | 0.85 (1.36) | 0.67 (1.36) | 0.86 | 1.20 (1.20) |
| P for trend                                                              | 0.7      | 06       |            | 0.39 |            |    |            |
| Age 19-30 years                                                          |          |          |           |    |            |    |            |
| Duration of sunlight exposure, hours/day                                 |          |          |           |    |            |    |            |
| <2                                                                       | 100      | 317      | 1 (ref.)  | 0.78 (ref.) | 1 | 0.79 (ref.) |
| ≥2-<5                                                                    | 98       | 292      | 1.08 (1.50) | 1.05 (1.50) | 1.16 | 1.69 (1.69) |
| ≥5-<8                                                                    | 32       | 61       | 1.71 (2.79) | 1.14 (2.79) | 1.35 | 2.45 (2.45) |
| ≥8                                                                       | 16       | 23       | 2.27 (4.51) | 0.0 (4.51) | 2.44 | 5.73 (5.73) |
| P for trend                                                              | 0.0      | 07       |            | 0.04 |            |    |            |
| Use of sunscreens                                                        |          |          |           |    |            |    |            |
| Never                                                                    | 117      | 312      | 1 (ref.)  | 0.69 (ref.) | 1 | 0.74 (ref.) |
| Ever                                                                     | 129      | 383      | 0.92 (1.24) | 0.67 (1.24) | 1.06 | 1.50 (1.50) |
| Hand skin tone, 1-3                                                      |          |          |           |    |            |    |            |
| 1 (Light)                                                                | 81       | 248      | 1 (ref.)  | 0.85 (ref.) | 1 | 0.87 (ref.) |
| 2                                                                        | 123      | 320      | 1.17 (1.63) | 0.67 (1.63) | 1.27 | 1.87 (1.87) |
| 3 (Dark)                                                                 | 44       | 129      | 1.03 (1.57) | 0.96 (1.57) | 0.96 | 1.61 (1.61) |
### Age 13-18 years

**Duration of sunlight exposure, hours/day**

| Exposure | <2 | ≥2-<5 | ≥5-<8 | ≥8 |
|----------|----|-------|-------|----|
|          | 83 | 125   | 25    | 15 |
| Reference| 224| 371   | 79    | 20 |
| Odds Ratio | 0.67-0.71 | 1.29-1.54 | 0.84-1.42 | 0.99-4.15 |
| Confidence Interval | 0.50-0.61 | 1.11-2.01 | 0.99-1.29 | 1.05-4.44 |

**P for trend**

|        | 0.7 | 4   | 0.85 |

### Use of sunscreens

|        | Never | Ever |
|--------|-------|------|
| Odds Ratio | 0.92-0.93 | 1.24-1.66 |
| Confidence Interval | 0.67-1.86 | 1.32-1.86 |

### Hand skin tone, 1-3

| Skin tone | 1 (Light) | 2 | 3 (Dark) |
|-----------|-----------|---|---------|
| Odds Ratio | 0.83-0.80 | 1.17-1.71 | 0.85-0.83 |
| Confidence Interval | 0.57-0.60 | 0.50-0.71 | 0.57-0.71 |

**P for trend**

|        | 0 | 9 | 0.11 |

### Age 6-12 years

**Duration of sunlight exposure, hours/day**

| Exposure | <2 | ≥2-<5 | ≥5-<8 | ≥8 |
|----------|----|-------|-------|----|
|          | 82 | 129   | 32    | 5  |
| Reference| 272| 333   | 72    | 17 |
| Odds Ratio | 0.95-0.99 | 1.31-2.09 | 1.45-2.64 | 0.94-2.63 |
| Confidence Interval | 0.89-0.94 | 1.44-2.64 | 1.49-2.64 | 0.87-2.25 |

**P for trend**

|        | 0.1 | 0 | 0.09 |

### Use of sunscreens

|        | Never | Ever |
|--------|-------|------|
| Odds Ratio | 0.80-0.81 | 1.09-1.66 |
| Confidence Interval | 0.67-1.66 | 1.16-1.66 |

### Hand skin tone, 1-3

| Skin tone | 1 (Light) | 2 | 3 (Dark) |
|-----------|-----------|---|---------|
| Odds Ratio | 0.71-0.70 | 1.37-1.48 | 0.77-1.15 |
| Confidence Interval | 0.52-0.38 | 1.02-1.48 | 0.62-1.01 |

**P for trend**

|        | 0.2 | 5 | 0.09 |

Abbreviations: OR, odds ratio; CI, confidence interval; EBV VCA-IgA, IgA against Epstein-Barr virus viral capsid antigen; UVR, ultraviolet radiation.

Multivariable adjusted model included all variables above, and sex, 5-year age group (frequency-matching in subject recruitment), and socioeconomic position score (ranged from -1 [lowest] to 13 [highest], and calculated by the subject’s, and his/her father’s and mother’s education, housing type at age 10, personal income, and household income), smoking status (ever/never), consumption of salted fish (ever/never), exposure to any occupational hazards (ever/never), season of blood draw (summer/winter), body mass index (<18.5/18.5-23.0/18.5-23.0-25.0/18.5-25.0), dietary vitamin D intake (<12.4/12.4-22.5/12.4-22.5-40.7/12.4>40.7) IU/day) and total energy intake over four life periods (age 6-12, 13-18, and 19-30, and 10 years before recruitment) as appropriate.
Table 2. OR and 95% CI of EBV VCA-IgA serostatus (seropositivity vs seronegativity) with serum 25-hydroxyvitamin D concentration and genetic predicted 25-hydroxyvitamin D concentration in Hong Kong, China 2014-2017

| Variables (Number of EBV seropositivity vs seronegativity) | Age- and sex-adjusted | Model 1 | Model 2 |
|-------------------------------------------------------------|------------------------|---------|---------|
| Serum 25OHD, nmol/L |
| <37.5 (61 vs 186) | 1 (ref.) | 1 (ref.) | 1 (ref.) |
| 37.5-75 (192 vs 500) | 1.16 0.83-1.63 | 1.10 0.80-1.62 | 1.02 0.67-1.55 |
| 75-<127.3 (29 vs 47) | 1.80 1.04-3.14 | 1.48 2.76 | 1.31 0.67-2.59 |
| P for trend | 0.06 | 0.27 | 0.54 |

Composite genetic score based on two genetic variants (rs1279471 and rs4588) associated with higher 25OHD (about -3.4 nmol/L per one score/allele decreased; ranged from 4-0)

| Variables (Number of EBV seropositivity vs seronegativity) | Age- and sex-adjusted | Model 1 | Model 2 |
|-------------------------------------------------------------|------------------------|---------|---------|
| 3-4 (31 vs 63) | 1 (ref.) | 1 (ref.) | 1 (ref.) |
| 1-2 (180 vs 412) | 0.75 0.44-1.27 | 0.85 0.54 | 0.82 0.43-1.54 |
| 0 (60 vs 160) | 0.88 0.55-1.40 | 0.93 0.54 | 0.96 0.55-1.70 |
| P for trend | 0.25 | 0.57 | 0.44 |

Abbreviations: OR, odds ratio; CI, confidence interval; EBV VCA-IgA, IgA against Epstein-Barr virus viral capsid antigen; 25OHD, 25-hydroxivitamin D; CYP2R1, Cytochrome P450 Family 2 Subfamily R Member 1; GC, Group-specific Component; rs, RefSNPs.

Adjusted for sex and 5-year age group (frequency-matching in subject recruitment).

Model 1: adjusted additionally for putative NPC risk factors (consumption of salted fish [ever/never], family history of cancer [No/Yes, non-NPC/Yes, NPC], exposure to any occupational hazards [ever/never], socioeconomic position score [ranged from -1 (lowest) to 13 (highest), and calculated by the subject’s, and his/her father’s and mother’s education, housing type at age 10, personal income, and household income], and smoking status [ever/never]).

Model 2: Model 1 additionally adjusted for factors of vitamin D exposure (season of blood draw [summer/winter], daily mean UV index at the date of blood draw, and 10 years before recruitment duration of sun exposure [<2/>=2, <<5/>=5, <8/>=8 hours/day], use of sunscreen [ever/never], and hand skin tone [1: light-3: dark], body mass index [<18.5/>=18.5-23.0/>=23.0-25.0/>=25.0], dietary vitamin D intake [<12.4/>=12.4-22.5/>=22.5-40.7/>=40.7-<637 IU/day] and total energy intake.