Environmental Factors for Epstein-Barr Virus Reactivation in a High-Risk Area of Nasopharyngeal Carcinoma: A Population-Based Study

Yufeng Chen,1 Ellen T. Chang,2,3 Qing Liu,4,5 Yonglin Cai,5,6 Zhe Zhang,7,8 Guomin Chen,9 Qi-Hong Huang,10 Shang-Hang Xie,11 Su-Mei Cao,12 Wei-Hua Jia,4,13 Yuming Zheng,14 Yancheng Li,15 Longde Lin,16 Ingemar Ernberg,17,14 Guangwu Huang,14 Yi-Xin Zeng,15 Hans-Olov Adami,18,14 and Weimin Ye15

1Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden, 2Exponent, Inc., Center for Health Sciences, Menlo Park, California, USA, 3Department of Cancer Prevention Center, Sun Yat-sen University Cancer Center, Guangzhou, China, 4State Key Laboratory of Oncology in South China & Collaborative Innovation Center for Cancer Medicine & Guangdong Key Laboratory of Nasopharyngeal Carcinoma Diagnosis and Therapy, Guangzhou, China, 5Department of Clinical Laboratory, Wuzhou Red Cross Hospital, Wuzhou, China, 6Department of Otolaryngology-Head & Neck Surgery, First Affiliated Hospital of Guangxi Medical University, Nanning, China, 7Key Laboratory of High-Intensity-Tumor Prevention & Treatment (Guangxi Medical University), Ministry of Education, Nanning, China, 8State Key Laboratory for Infectious Diseases Prevention and Control, Institute for Viral Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Beijing, China, 9Shi Cancer Institute, Shihui, China, 10Cangwu Institute for Nasopharyngeal Carcinoma Control and Prevention, Wuzhou, China, 11Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Stockholm, Sweden, 12Beijing Hospital, Beijing, China, 13Clinical Effectiveness Group, Institute of Health and Society, University of Oslo, Oslo, Norway, 14Department of Epidemiology and Health Statistics & Key Laboratory of Ministry of Education for Gastrointestinal Cancer, Fujian Medical University, Fuzhou, China.

Background. Epstein-Barr virus (EBV) reactivation from latent to lytic infection has been considered as a key step in nasopharyngeal carcinoma oncogenesis. However, epidemiological evidence regarding environmental risk factors for EBV reactivation on a population level remains largely lacking.

Methods. We enrolled 1916 randomly selected adults from the general population of Guangdong and Guangxi, China, from 2010 to 2014. Information on environmental factors was collected via a structured interview. Serum immunoglobulin A antibodies against EBV viral capsid antigen and nuclear antigen 1 were measured by enzyme-linked immunosorbent assay to evaluate EBV reactivation status. We used logistic regression to calculate odds ratios (ORs) with 95% confidence intervals (CIs) for the associations of EBV reactivation with various environmental factors.

Results. No associations were observed between EBV reactivation and extensive environmental factors, including alcohol or tea drinking, a history of chronic ear/nose/throat diseases, use of medications or herbs, consumption of salted fish or preserved foods, oral hygiene, sidship structure, and various residential and occupational exposures. Only cigarette smoking was associated with EBV reactivation (current smokers vs never smokers; OR = 1.37; 95% CI = 1.02–1.83), with positive exposure-response trends with increasing intensity, duration, and pack-years of smoking.

Conclusions. Consistent with previous studies, we found an association between cigarette smoking and EBV reactivation. Other examined exposures were not associated with EBV reactivation. These null results could suggest either more complex interactions between exposures and EBV reactivation or a predominant role of host and/or viral genetic variation.

Keywords. EBV reactivation; environmental factors; Epstein-Barr virus; nasopharyngeal carcinoma; risk factor; serology.

Nasopharyngeal carcinoma (NPC) has a distinct geographic and racial distribution across the world, with an especially high incidence in the Cantonese-speaking population of southern China [1, 2]. Epstein-Barr virus (EBV) plays a necessary etiologic role in the development of NPC in endemic areas [3, 4].

Epstein-Barr virus, a ubiquitous B-lymphotropic herpesvirus, is the first human tumor virus that was found to contribute to the development of a wide range of lymphoid and epithelial malignancies, including NPC [5, 6]. Among human tumors, EBV infection is most strongly associated with undifferentiated NPC, the predominant histological type in endemic regions [7, 8]. The life cycle of EBV includes latent and lytic phases. In general, after primary infection, EBV establishes asymptomatic, life-long latent infection in the resting B lymphocytes of typical adults [9]. Approximately 95% of the world’s population sustains asymptomatic, life-long infection with EBV [7]. However, EBV can be periodically reactivated under endogenous and environmental stress, in which the virus enters into a lytic replication phase [7, 10]. Upon reactivation, a series of EBV lytic genes are expressed, large amounts of viral particles are produced and released, and host levels of serum immunoglobulin A (IgA)
antibodies against multiple EBV antigens, including early antigen (EA), viral capsid antigen (VCA), and nuclear antigen 1 (EBNA1), are substantially elevated [11, 12]. Elevated EBV lytic antibody levels are associated with significantly higher risk of NPC several years later [13–15], indicating that EBV reactivation is involved in the pathogenesis of NPC. Although latent EBV infection is thought to be largely responsible for viral oncogenesis [16], increasing evidence from molecular research shows that the EBV lytic phase contributes to oncogenesis primarily through 2 ways: (1) the production of infectious particles to infect more cells; and (2) the regulation of cellular oncogenic pathways by both cell-autonomous and noncell-autonomous signaling mechanisms [17].

Given the importance of EBV reactivation in NPC oncogenesis, identifying environmental factors that can induce EBV reactivation may facilitate primary prevention of NPC and also enlighten us as to whether a causal effect is mediated by or independent of EBV. However, although evidence from experimental studies shows that several chemicals, such as phorbol esters, sodium butyrate, nitrosamines, fatty acids, and extracts from Chinese herbs or cigarette smoke, can induce the EBV lytic phase [18–21], epidemiological evidence on environmental risk factors for EBV reactivation remains sparse [11, 18, 22].

Due to these knowledge gaps with potential public-health importance, we conducted a post hoc analysis with 1916 population-based individuals derived from the control group of a large epidemiological case-control study of NPC in southern China, where the highest incidence rate of NPC occurs in the world, to unveil the links between environmental exposures and EBV reactivation.

MATERIALS AND METHODS

Patient Consent Statement
Written or oral informed consent was obtained from all study participants. The study was approved by the institutional review boards of Harvard T.H. Chan School of Public Health, Sun Yat-sen University Cancer Center, the Institute for Viral Disease Control and Prevention of the Chinese Center for Disease Control and Prevention, First Affiliated Hospital of Guangxi Medical University, and the Regional Ethical Review Board in Stockholm, Sweden.

Study Population
The present study is based on the control population from a multicenter collaborative population-based case-control study of NPC entitled “NPC Genes, Environment, and EBV”. Details of the study were described previously [23]. In brief, the study base was defined as individuals officially living in 13 cities/counties in Guangdong Province and Guangxi Autonomous Region in southern China between 2010 and 2014. The 13 study cities/counties have an approximate population of 8 million. Eligible participants were aged 20–74 years, residing in the study area, and without a history of malignant disease or congenital or acquired immunodeficiency. In total, 3047 eligible histopathologically confirmed, incident NPC cases were identified and contacted between March 2010 and December 2013; 2554 (84%) agreed to participate. The number of ascertained NPC cases was similar to the total number expected in the study area. Controls without NPC were randomly selected every 6–12 months from the total population registries covering the study areas between 2010 and 2014, with frequency matching to the expected distribution of NPC cases based on age (within 5 years), sex, and residential area. We anticipated the participation rate of controls to be approximately 10% lower than that of cases; therefore, we increased the number of controls accordingly during control sampling. Of the 3202 selected controls, 2648 (83%) consented to participate. To increase the controls’ participation rate, we enlisted help from local village doctors and community leaders. Interviews were conducted at the subject’s home or a nearby hospital. In addition, we attempted to contact potential controls just before the Chinese Spring Festival, when many people return to their hometown for the holiday. Furthermore, for a small set of control subjects, we performed the interview by telephone after several failed attempts for a face-to-face interview.

Because EBV is reactivated in virtually all NPC patients, we assessed the association of environmental factors with EBV reactivation only among the population-based controls, excluding the NPC cases. During data cleaning, 51 subjects were excluded due to missing questionnaire data or being outside the eligible age range. We further excluded 681 subjects without blood samples, leaving 1916 subjects for inclusion in the final analysis. No differences in age ($\chi^2$ test, $P = .98$), sex ($P = .95$), or education ($P = .55$) were found between the final dataset ($N = 1916$) and the full dataset ($N = 2597$) of controls.

Data Collection
Each participant completed a face-to-face or telephone interview administered by a trained interviewer using a structured electronic questionnaire. The study questionnaire was designed to assess long-term (3 months or longer) environmental exposures but not transient or short-term exposures; therefore, we use the term “stable” for the environmental exposures examined in this study. The questionnaire covered demographics, body size, residential and occupational history, history of chronic ear/nose/throat (ENT) diseases family history of NPC and other cancers, cigarette smoking, alcohol consumption, tea consumption, dietary habits, and use of Chinese herbal medicine. Extensive efforts were made to minimize information bias and ensure the quality of questionnaire data, as described previously [23]. For instance, interviewers were trained with a manual that described standard survey techniques to be implemented for all participants, logic checks were built into the
electronic questionnaire, and interviews were audiotaped for quality control.

**Epstein-Barr Virus Serological Tests**

Blood samples were collected at the time of interview. Serum was separated and temporarily stored at local laboratories using standard operating procedures and then transported to the central laboratories at the Sun Yat-sen University Cancer Center (for samples collected at Guangdong sites) and Guangxi Medical University (for samples collected at Guangxi sites) through cold-chain for storage at −80°C before testing. Antibody levels of VCA/IgA (EUROIMMUNAG, Lübeck, Germany) and EBNA1/IgA (Zhongshan Bio-Tech Company, Zhongshan, China) were measured by commercial enzyme-linked immunosorbent assay kits following the manufacturers’ instructions. Serum antibody levels of VCA/IgA and EBNA1/IgA were presented as relative optical density values, calculated as the ratio of the sample optical density to a reference control (calibrator). Across-batch coefficients of variation for a control serological sample were 9.1% for VCA/IgA and 9.2% for EBNA1/IgA. Kappa coefficients for test-retest values for approximately 10% of samples that were randomly retested were 0.88 (P < .001) for VCA/IgA and 0.85 (P < .001) for EBNA1/IgA.

In this study population of adults in southern China, where virtually all individuals undergo primary EBV infection in early childhood, elevated VCA/IgA and EBNA1/IgA were implicitly assumed to indicate EBV reactivation, as opposed to primary infection. Study subjects were classified as exhibiting serological evidence of EBV reactivation (Score ≥ 0.65) or not (Score < 0.65) using an EBV-based risk score [24, 25]: Score = [e\(^{-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA}}\)]/[1 + e\(^{-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA}}\)]. We also classified study subjects as “low-risk” (Score < 0.65), “medium-risk” (0.65 ≤ Score < 0.98), or “high-risk” (Score ≥ 0.98), using standard cutoffs in the context of NPC screening [25, 26]. The EBV-based risk score based on the combination of VCA/IgA and EBNA1/IgA was previously established under screening scenarios to identify high-risk individuals (ie, EBV seropositive) for NPC in endemic regions [24, 27]. The EBV-based score had a high discriminatory performance (ie, the area under the receiver-operator-characteristic curve was 0.95; 95% confidence interval [CI] = 0.93–0.97]), which was validated in an ongoing NPC screening trial in southern China with 51 235 adult participants [25].

**Statistical Analysis**

Differences in demographic characteristics between subjects with and without EBV reactivation were compared using \(\chi^2\) tests. We used multivariate logistic regression to calculate odds ratios (ORs) and corresponding CIs for associations between EBV reactivation and environmental factors, adjusting for age (continuous variable), sex, geographic area (Zhaoqing, Wuzhou, or Guiping/Pingnan), and educational level (≤6, 7–9, ≥10 years). Linear trend tests for associations between environmental factors and EBV reactivation were conducted by using the median value within each category or by treating the categorical variable as an ordinal variable, where applicable. Multinomial logistic regression was used to evaluate the relationship of EBV reactivation status, which were categorized into 3 levels (low-, medium-, or high-risk) with environmental factors. We used SAS version 9.4 (SAS Institute, Inc., Cary, NC) for all analyses, and 2-sided \(P < .05\) were considered statistically significant.

**RESULTS**

**Study Population Characteristics**

Table 1 presents the distribution of EBV reactivation status across the baseline characteristics of the 1916 participants. The overall prevalence of EBV reactivation was 22.5% (431 of 1916), with higher levels among older individuals and those who lived in the Zhaoqing area. The EBV reactivation status did not differ by educational level, first-degree family history of NPC, or body mass index 10 years before the interview, and it was only marginally higher in men than in women.

**Associations Between Environmental Factors and Epstein-Barr Virus Reactivation**

**Lifestyle Factors**

Table 2 shows the associations between lifestyle factors and EBV reactivation. Current smoking was associated with a high prevalence of EBV reactivation (OR = 1.37; 95% CI = 1.02–1.83), whereas former smoking was not (OR = 1.16; 95% CI = 0.71–1.92). Other lifestyle factors, including alcohol or tea drinking, a history of chronic ENT diseases, use of aspirin or nasal drops/balm/oil, use of herbal medicine, consumption of herbal tea/soup, consumption of salted fish or other preserved foods, oral hygiene conditions, and sibship structure, were not significantly associated with EBV reactivation.

We used more refined measures of smoking exposure to examine potential exposure-response relationships with risk of EBV reactivation (Table 3). We found that among ever smokers, earlier age at smoking initiation, longer duration of smoking, more cumulative pack-years of smoking, consumption of unfiltered cigarettes, and having ever engaged in deep inhalation when smoking all exhibited significant positive exposure-response trends with risk of EBV reactivation. Similar results were obtained when current smokers were compared with never smokers, excluding former smokers (see Supplementary Table 1). However, no significant exposure-response trends by smoking intensity, duration, pack-years, or other characteristics were observed in analyses restricted only to current smokers that used the lowest category of current smokers as the reference group (results not shown).
Table 4. The Characteristics of 1916 Population-Based Individuals Stratified by EBV Reactivation Status, Southern China, 2010–2014

| Characteristics | EBV Reactivation Status| n (%) | n (%) | P Value |
|----------------|------------------------|-------|-------|---------|
|                |                         | Negative (N = 1485) | Positive (N = 431) |       |
| Area           |                         | 585 (74.2) | 203 (25.8) | .01     |
| Zhaoqing       |                         | 455 (78.9) | 122 (21.1) |       |
| Wuzhou         |                         | 445 (80.8) | 106 (19.2) |       |
| Sex            |                         | 400 (79.1) | 106 (20.9) | .33     |
| Female         |                         | 1085 (77.0) | 325 (23.0) |       |
| Male           |                         | 1085 (77.0) | 325 (23.0) |       |
| Age, Years     |                         | 44 (78.6) | 12 (21.4) | <.001   |
| 20–29          |                         | 222 (82.5) | 47 (175) |       |
| 30–39          |                         | 527 (80.8) | 125 (19.2) |       |
| 40–49          |                         | 424 (78.6) | 128 (23.2) |       |
| 50–59          |                         | 268 (69.3) | 119 (30.7) |       |
| ≥60–74         |                         | 166 (24.9) | 157 (19.9) |       |
| Educational Level, Years | | 352 (76.5) | 108 (23.5) | .06     |
| ≥10            |                         | 1417 (77.5) | 412 (22.5) |       |
| 7–9            |                         | 43 (79.6) | 11 (20.4) |       |
| <6             |                         | 25 (75.8) | 8 (24.2) |       |
| First-Degree Family History of NPC | | 1417 (77.5) | 412 (22.5) | .91     |
| No             |                         | 43 (79.6) | 11 (20.4) |       |
| Yes            |                         | 25 (75.8) | 8 (24.2) |       |
| Unknown        |                         | 149 (73.8) | 53 (26.2) |       |
| BMI 10 years ago (kg/m²) | | 958 (79.0) | 255 (21.0) | .11     |
| <18.5          |                         | 334 (78.3) | 104 (23.7) |       |
| 18.5–22.9      |                         | 42 (68.9) | 19 (31.1) |       |
| 23.0–27.4      |                         | 149 (73.8) | 53 (26.2) |       |
| ≥27.5          |                         | 958 (79.0) | 255 (21.0) |       |

Abbreviations: BMI, body mass index; EBNA1/IgA, IgA antibodies against EBV nuclear antigen 1; EBV, Epstein-Barr virus; IgA, immunoglobulin A; NPC, nasopharyngeal carcinoma; VCA/IgA, IgA antibodies against EBV capsid antigens.

aTwo EBV serological markers (VCA/IgA, EBNA1/IgA) were used to determine the status of EBV reactivation. An EBV score was calculated using a formula: Score = [e^{-(3.934 + 2.203 × VCA/IgA + 4.797 × EBNA1/IgA)]/[1 + e^{-(3.934 + 2.203 × VCA/IgA + 4.797 × EBNA1/IgA)}]. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

bP-values were determined using the χ² test.

Residential Characteristics and Occupational Exposures

Table 4 shows the associations of EBV reactivation with residential and occupational exposures. None of the residential-related factors, including type of residential structure, type of cooking fuel, source of drinking water, and ventilation in the home, were associated with EBV reactivation. Exposure to occupational dust showed an inverse association with EBV reactivation (OR = 0.78; 95% CI = .62–0.98), whereas no associations were observed with exposure to occupational chemical vapors, smokes/exhausts, or acids/alkalis, or with current job category.

Associations With Epstein-Barr Virus Reactivation Risk Categories

The odds ratios were essentially unchanged even when we assessed the association of the exposures with the risk categories (low-, medium-, and high-risk) derived from the EBV-based risk score, and the differences observed with respect to cigarette smoking and occupational dust exposure were attenuated (see Supplementary Tables 2 and 3).

DISCUSSION

In this post hoc analysis with 1916 randomly selected controls from a previous large case-control study of NPC in an endemic area, we present a rich data resource to investigate potential environmental influences on EBV reactivation/lytic status. In general, we found that a wide range of environmental factors were not associated with EBV reactivation, except for a positive association with cigarette smoking. Our findings support previous studies suggesting a link between smoking and EBV reactivation and deliver new insight into the relationship between many other environmental factors and EBV reactivation. In particular, our predominantly null findings suggest that nonenvironmental factors, including host genetic susceptibility and viral genetic variation, may be the primary determinants of EBV reactivation in this population. Alternatively (or in addition), short-term environmental exposures not captured by our questionnaire, such as transient sources of endogenous or environmental stress, may influence EBV reactivation.
| Variables                                             | EBV Reactivation Status<sup>a</sup> | OR (95% CI)<sup>b</sup> | PValue<sup>b</sup> |
|------------------------------------------------------|--------------------------------------|-------------------------|-------------------|
| Smoking Status                                       |                                      |                         |                   |
| Never smoker                                         | 719                                  | 176                     | 1.00 (ref.)       |
| Former smoker                                        | 86                                   | 28                      | 1.16 (0.71–1.92)  |
| Current smoker                                       | 679                                  | 227                     | 1.37 (1.02–1.83)  |
| Alcohol Drinking                                     |                                      |                         |                   |
| Never                                                | 1042                                 | 283                     | 1.00 (ref.)       |
| Former                                               | 43                                   | 17                      | 1.25 (0.70–2.26)  |
| Current                                              | 393                                  | 127                     | 1.17 (0.90–1.51)  |
| Tea Drinking                                         |                                      |                         |                   |
| No                                                    | 898                                  | 256                     | 1.00 (ref.)       |
| Yes                                                   | 586                                  | 175                     | 0.93 (0.73–1.18)  |
| History of Chronic ENT Diseases                      |                                      |                         |                   |
| No                                                    | 1319                                 | 382                     | 1.00 (ref.)       |
| Yes                                                   | 166                                  | 49                      | 1.03 (0.73–1.45)  |
| Use of Aspirin                                       |                                      |                         |                   |
| No                                                    | 1414                                 | 409                     | 1.00 (ref.)       |
| Yes                                                   | 71                                   | 22                      | 1.01 (0.61–1.67)  |
| Use of Nasal Drops/Nasal Balm/Flower Oil             |                                      |                         |                   |
| No                                                    | 1390                                 | 409                     | 1.00 (ref.)       |
| Yes                                                   | 95                                   | 22                      | 0.72 (0.44–1.17)  |
| Use of Herbal Medicine                               |                                      |                         |                   |
| No                                                    | 1388                                 | 404                     | 1.00 (ref.)       |
| Yes                                                   | 77                                   | 20                      | 0.88 (0.52–1.46)  |
| Herbal Tea Consumption                                |                                      |                         |                   |
| Yearly or less                                       | 831                                  | 241                     | 1.00 (ref.)       |
| Monthly                                              | 511                                  | 143                     | 0.97 (0.76–1.24)  |
| Weekly or more                                       | 121                                  | 38                      | 1.09 (0.72–1.63)  |
| Herbal Soup Consumption                               |                                      |                         |                   |
| Yearly or less                                       | 356                                  | 104                     | 1.00 (ref.)       |
| Monthly                                              | 668                                  | 188                     | 0.95 (0.71–1.26)  |
| Weekly or more                                       | 440                                  | 132                     | 0.97 (0.7–1.34)   |
| Salted Fish Consumption in Adulthood                 |                                      |                         |                   |
| Yearly or less                                       | 1113                                 | 313                     | 1.00 (ref.)       |
| Monthly                                              | 289                                  | 89                      | 1.01 (0.77–1.34)  |
| Weekly or more                                       | 80                                   | 29                      | 1.11 (0.71–1.75)  |
| Preserved Vegetables Consumption in Adulthood        |                                      |                         |                   |
| No                                                    | 119                                  | 40                      | 1.00 (ref.)       |
| Yes                                                   | 1343                                 | 386                     | 0.86 (0.59–1.27)  |
| Salted Fish Consumption in Adolescence               |                                      |                         |                   |
| Yearly or less                                       | 1141                                 | 318                     | 1.00 (ref.)       |
| Monthly                                              | 220                                  | 78                      | 1.09 (0.81–1.47)  |
| Weekly or more                                       | 121                                  | 35                      | 0.78 (0.52–1.18)  |
| Teeth Lost After Age 20 Years                        |                                      |                         |                   |
| No                                                    | 748                                  | 193                     | 1.00 (ref.)       |
| Yes                                                   | 736                                  | 238                     | 0.99 (0.78–1.25)  |
| Number of Filled Teeth                               |                                      |                         |                   |
| None                                                  | 1263                                 | 366                     | 1.00 (ref.)       |
| 1–3                                                   | 172                                  | 52                      | 1.09 (0.78–1.53)  |
| ≥ 4                                                   | 49                                   | 13                      | 0.96 (0.51–1.80)  |
| Daily Tooth Brushing, Times                          |                                      |                         |                   |
| ≤1                                                    | 859                                  | 250                     | 1.00 (ref.)       |
| ≥2                                                    | 621                                  | 180                     | 1.12 (0.89–1.41)  |
| Birth Order                                          |                                      |                         |                   |
| 1                                                     | 382                                  | 134                     | 1.00 (ref.)       |
| 2–3                                                   | 646                                  | 183                     | 0.83 (0.62–1.08)  |
| ≥4                                                    | 457                                  | 114                     | 0.76 (0.57–1.02)  |
To date, only a few epidemiological studies have investigated environmental inducers of EBV reactivation [11, 18, 22, 28]. Two hospital-based studies reported that smoking was linked to seropositivity for EBV VCA/IgA, EBNA1/IgA, and Zta/IgA in healthy males from endemic and nonendemic areas. No association was detected with 7 other suspected risk factors for NPC, including family history of NPC and consumption of alcohol, tea, Chinese herbal tea, Cantonese slow-cooked soup, salted fish, or preserved vegetables [11, 18]. Likewise, a screening-based cohort study conducted in southern China showed that smoking was associated with EBV seropositivity for VCA/IgA and EBNA1/IgA among NPC-free individuals at baseline and at 3–5 years of follow-up, whereas no association was observed with salted food consumption or family history of NPC [22]. In a case-control study conducted in Taiwan, Hsu et al [29] also reported a higher VCA/IgA seropositivity rate in current smokers than never smokers among the controls. However, in a study with 313 male subjects by Chen et al [30], no association was found between smoking and VCA/IgA seropositivity. The latter findings may be different because the study subjects were previously seropositive and the sample size was relatively small. A more recent study in Hong Kong suggests a possible association between seropositivity of VCA/IgA and sunlight exposures, but no association with vitamin D level, a molecular mediator of sunlight exposure [28]. Besides confirming the positive association of smoking with serological evidence of EBV reactivation, we also found that a history of ENT diseases, use of ENT-related medications or herbal medicine, oral hygiene conditions, sibling structure, residential exposures, and occupational exposures (except, possibly, for dust) are not associated with EBV reactivation.

Xu et al [18] showed, using in vitro assays, that cigarette smoke extract promoted EBV replication and enhanced the expression levels of lytic-phase genes. Combined with our findings and those of prior epidemiological studies [11, 18, 22] as well as the direct exposure of the nasopharyngeal epithelium to tobacco smoke, these observations suggest that cigarette smoking might contribute to NPC oncogenesis not only by a direct carcinogenic effect of tobacco smoke, but also indirectly by induction of EBV reactivation.

Other environmental factors, such as household indoor air pollution, early-life salted-fish consumption, and residential and occupational exposures, have also been linked to the development of NPC [3, 31–33]. Our population-based study plus prior hospital-based studies [11, 18], however, found no relationship between a broad range of environmental factors and EBV reactivation. In addition, the prevalence of positive EBV reactivation status differs so minimally between women and men in our study as well as in previous research [22] notwithstanding the substantial gender disparity (a ratio of male vs female = 2–3:1) in NPC incidence. Together, these null findings suggest that stable environmental factors are unlikely to be important inducers of the switch from EBV latent infection to lytic infection. These observations also imply that the oncogenic mechanisms of environmental risk factors for NPC may be independent of EBV reactivation.

By contrast, recent genomic analyses showed that host and viral genetic variation may affect EBV lytic reactivation. A study that analyzed paired EB viral and human genomic data from 268 human immunodeficiency virus-infected individuals reported significant associations between 25 human single-nucleotide polymorphisms and viral variants mapping to 3 EBV regions including BALF5, BBRF1, and BRLF1 [34]. These genes are involved in controlling EBV reactivation from latency and regulation of viral deoxyribonucleic acid (DNA) replication. The study in southern China by Xu et al [35] identified 2 nonsynonymous EBV variants within the BALF2 gene, a core component of lytic viral DNA replication machinery, that were associated with a 6.1- to 8.7-fold increased risk of NPC. In addition, Xue et al [36] conducted a comprehensive genetic analysis of 22 critical viral genes that are involved in the EBV replication, and they identified new high-risk EBV.

Table 2. Continued

| Variables         | EBV Reactivation Statusa   | OR (95% CI)b | PValueb |
|-------------------|---------------------------|-------------|---------|
| Number of Siblings|                           |             |         |
| 0–1               | 143                       | 46          | 1.00 (ref.) |
| 2–3               | 559                       | 162         | 0.93 (0.63–1.36) | .70 |
| ≥4                | 783                       | 223         | 0.93 (0.54–1.35) | .70 |
| Number of Younger Siblings|                 |             |         |
| 0                 | 380                       | 110         | 1.00 (ref.) |
| 1–2               | 668                       | 161         | 0.82 (0.62–1.08) | .15 |
| ≥3                | 437                       | 160         | 1.24 (0.93–1.66) | .14 |

Abbreviations: CI, confidence interval; EBNA1/IgA, IgA antibodies against EBV nuclear antigen 1; EBV, Epstein-Barr virus; ENT, ear, nose, and throat; IgA, immunoglobulin A; OR, odds ratio; ref., reference; VCA/IgA, IgA antibodies against EBV capsid antigens.

Two EBV serological markers (VCA/IgA, EBNA1/IgA) were used to determine the status of EBV reactivation. An EBV score was calculated using a formula: Score = [e^(-3.934 + 2.203 × VCA/IgA + 1.370 × EBNA1/IgA) + e^1 + 2.00 × VCA/IgA + 0.50 × EBNA1/IgA]. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

OR estimates and P-values were calculated using logistic regression, adjusted for age (continuous variable), sex, geographic area, and educational level.
subtypes including 4 Chinese-specific NPC-associated amino acid substitutions (BALF2 V317M, BNRFI G696R, BNRFI V1222I, and RPMS1 D51E). The EBV subtypes defined by the 4 substitutions conferred a profoundly higher risk of NPC in China (ORs = 4.8, 20.0, 18.2, and 32.0 for 1, 2, 3, and 4 substitutions, respectively). These findings suggest that human and viral genetic diversity, particularly variation in viral genes, may have an important role in disease development via regulation of the EBV lytic cycle. Hence, a vaccine against high-risk EBV strains in the future may be an effective public health approach to disease prevention for EBV-associated diseases including NPC.

In the present study, serological evidence of EBV reactivation was assessed based on a combination of 2 markers, VCA/IgA and EBNA1/IgA, the 2 most commonly used indicators of EBV reactivation. However, further studies should examine whether other serological markers such as EA/IgA, Zta/IgA, Rta/IgA, and plasma EBV load, and other noninvasive markers based on saliva/mouthwash and nasopharyngeal swab/brushings, could serve as better markers of EBV reactivation.

Table 3. Associations Between Cigarette Smoking and EBV Reactivation in Population-Based Individuals, Southern China, 2010–2014

| Variables                              | EBV Reactivation Statusa | OR (95% CI)b | PValueb |
|----------------------------------------|--------------------------|--------------|---------|
|                                        | Negative                 | Positive     |         |
| Cigarette Smoking                      |                          |              |         |
| Never smokerc                          | 719                      | 176          | 1.00 (ref.) |
| Former smoker                          | 86                       | 28           | 1.16 (0.71–1.92) | .55 |
| Current smoker                         | 679                      | 227          | 1.37 (1.02–1.83) | .03 |
| P_trend d                              |                          |              | 0.03    |
| Age at Smoking Initiation, Years       |                          |              |         |
| ≥30                                   | 113                      | 30           | 0.99 (0.62–1.60) | .98 |
| 20 to <30                             | 352                      | 115          | 1.31 (0.95–1.82) | .10 |
| <20                                   | 300                      | 109          | 1.51 (1.08–2.11) | .02 |
| P_trend d                              |                          |              | 0.01    |
| Cigarettes Smoked Per Day              |                          |              |         |
| <10                                   | 204                      | 73           | 1.35 (0.93–1.95) | .11 |
| 10 to <20                             | 213                      | 74           | 1.44 (1.00–2.06) | .05 |
| ≥20                                   | 276                      | 80           | 1.21 (0.85–1.72) | .298 |
| P_trend d                              |                          |              |         |
| Duration of Smoking, Years             |                          |              |         |
| <10                                   | 60                       | 12           | 0.99 (0.50–1.94) | .97 |
| 10 to <20                             | 157                      | 40           | 1.22 (0.79–1.87) | .37 |
| ≥30                                   | 224                      | 66           | 1.27 (0.88–1.84) | .20 |
| P_trend d                              |                          |              |         |
| Pack-Years of Smoking                  |                          |              |         |
| <10                                   | 234                      | 67           | 1.21 (0.84–1.75) | .31 |
| 10 to <20                             | 164                      | 49           | 1.30 (0.87–1.95) | .20 |
| ≥30                                   | 213                      | 86           | 1.49 (1.03–2.14) | .03 |
| P_trend d                              |                          |              |         |
| Type of Cigarette                      |                          |              |         |
| Filtered                               | 541                      | 155          | 1.22 (0.90–1.65) | .21 |
| Unfiltered                             | 224                      | 100          | 1.67 (1.17–2.38) | .01 |
| P_trend d                              |                          |              |         |
| Type of Smoking, Inhaled or Not        |                          |              |         |
| Not deeply inhaled                     | 418                      | 130          | 1.25 (0.91–1.72) | .17 |
| Deeply inhaled                         | 347                      | 125          | 1.45 (1.05–2.00) | .02 |
| P_trend d                              |                          |              | 0.02    |

Abbreviations: CI, confidence interval; IgA, immunoglobulin A; EBNA1/IgA, IgA antibodies against EBV nuclear antigen 1; EBV, Epstein-Barr virus; OR, odds ratio; ref, reference; VCA/IgA, IgA antibodies against EBV capsid antigens.

aTwo EBV serological markers (VCA/IgA, EBNA1/IgA) were used to determine the status of EBV reactivation. An EBV score was calculated using a formula: Score = [e^(-3.934 + 2.203 × VCA/IgA + 4.797 × EBNA1/IgA)] / [1 + e^(-3.934 + 2.203 × VCA/IgA + 4.797 × EBNA1/IgA)]. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.
bOR estimates and P values were calculated using logistic regression, adjusted for age (continuous variable), sex, geographic area, and educational level.
cNever smokers were the reference group for all comparisons.
dLinear trend tests were conducted by using the median value within each category or by treating the categorical variable as an ordinal variable.
| Variables                                      | EBV Reactivation Status\( ^a \) | OR (95% CI)\( ^b \) | PValue\( ^b \) |
|-----------------------------------------------|----------------------------------|----------------------|---------------|
| **House Category**                            |                                  |                      |               |
| Building                                      | 1173                             | 330                  | 1.00 (ref.)   |
| Cottage/boat                                  | 312                              | 101                  | 1.05 (0.81–1.37) | .72 |
| **Cooking Fuel**                              |                                  |                      |               |
| Gas/electricity                               | 525                              | 157                  | 1.00 (ref.)   |
| Wood                                          | 936                              | 268                  | 0.93 (0.74–1.18) | .55 |
| Coal/Aerosene                                 | 24                               | 6                    | 0.83 (0.33–2.10) | .70 |
| **Source of Drinking Water**                  |                                  |                      |               |
| Tap water                                     | 818                              | 259                  | 1.00 (ref.)   |
| Wells                                         | 427                              | 108                  | 0.86 (0.66–1.13) | .29 |
| Rivers                                        | 35                               | 9                    | 0.80 (0.38–1.70) | .56 |
| Pond/stream                                   | 205                              | 55                   | 0.83 (0.59–1.16) | .27 |
| **Cooking smoke**                             |                                  |                      |               |
| No smoke/a little smoke                       | 712                              | 228                  | 1.00 (ref.)   |
| Some smoke                                    | 383                              | 100                  | 0.83 (0.63–1.09) | .18 |
| A lot of smoke                                | 135                              | 33                   | 0.75 (0.49–1.13) | .17 |
| **Burning Incense**                           |                                  |                      |               |
| Never/occasionally                            | 913                              | 265                  | 1.00 (ref.)   |
| Twice per month\( ^c \)                       | 478                              | 138                  | 0.92 (0.73–1.18) | .52 |
| Every day                                     | 94                               | 28                   | 0.98 (0.62–1.54) | .93 |
| **Burning Mosquito Coils in Summer**          |                                  |                      |               |
| No                                            | 432                              | 128                  | 1.00 (ref.)   |
| Yes                                           | 1053                             | 303                  | 0.97 (0.76–1.24) | .83 |
| **Proximity to a source of pollution, meters\( ^d \)** | | | |
| >1000                                         | 569                              | 179                  | 1.00 (ref.)   |
| 300–1000                                      | 212                              | 62                   | 0.98 (0.70–1.37) | .91 |
| <300                                          | 700                              | 188                  | 0.91 (0.72–1.16) | .46 |
| **Bedroom Windows**                           |                                  |                      |               |
| Large                                         | 556                              | 159                  | 1.00 (ref.)   |
| Medium                                        | 434                              | 127                  | 0.96 (0.72–1.28) | .80 |
| Small                                         | 488                              | 144                  | 0.93 (0.68–1.25) | .61 |
| **Hall Windows**                              |                                  |                      |               |
| Large                                         | 474                              | 135                  | 1.00 (ref.)   |
| Medium                                        | 509                              | 160                  | 1.03 (0.78–1.35) | .84 |
| Small                                         | 475                              | 130                  | 1.00 (0.70–1.41) | .98 |
| **Kitchen Windows**                           |                                  |                      |               |
| Large                                         | 453                              | 148                  | 1.00 (ref.)   |
| Medium                                        | 525                              | 142                  | 0.80 (0.61–1.05) | .11 |
| Small                                         | 487                              | 131                  | 0.76 (0.57–1.02) | .07 |
| **Current Job Category**                      |                                  |                      |               |
| White collar                                  | 217                              | 70                   | 1.00 (ref.)   |
| Farmer                                        | 586                              | 176                  | 0.80 (0.56–1.14) | .22 |
| Blue collar                                   | 523                              | 129                  | 0.75 (0.52–1.07) | .11 |
| Other                                         | 155                              | 56                   | 0.91 (0.59–1.42) | .68 |
| **Exposed to Occupational Dust**              |                                  |                      |               |
| No                                            | 586                              | 201                  | 1.00 (ref.)   |
| Yes                                           | 892                              | 230                  | 0.78 (0.62–0.98) | .04 |
| **Exposed to Occupational Chemical Vapor**    |                                  |                      |               |
| No                                            | 857                              | 248                  | 1.00 (ref.)   |
| Yes                                           | 617                              | 181                  | 0.96 (0.77–1.21) | .70 |
| **Exposed to Occupational Smoke**             |                                  |                      |               |
| No                                            | 1067                             | 338                  | 1.00 (ref.)   |
| Yes                                           | 404                              | 93                   | 0.80 (0.61–1.05) | .10 |
| **Exposed to Occupational Acid/Alkali**       |                                  |                      |               |
To our knowledge, the present study is the only large, population-based study in a NPC-endemic region to evaluate potential environmental risk factors for EBV reactivation. Our study is strengthened by its random sampling from total population registries, a high participation rate, use of a standardized questionnaire assessing dozens of environmental exposures, and reliable measurement of EBV antibodies. Our study is limited by its self-reported evaluation of environmental exposures, although subjects were unaware of their EBV infection status, making systematic recall bias highly improbable. Nondifferential misclassification, however, might partly explain the largely null findings in our study. We cannot rule out uncontrolled or residual confounding, for example, by socioeconomic conditions. Finally, given the lack of biological plausibility, the observed inverse association with occupational dust exposure may be due to chance.

CONCLUSIONS

In conclusion, we found that exposure to an extensive variety of stable environmental factors, with the exception of cigarette smoking, is not likely associated with EBV reactivation, suggesting that stable environmental factors are not likely to be primary determinants of EBV reactivation. Thus, environmental risk factors for NPC may contribute to nasopharyngeal oncogenesis through other mechanisms that merit further investigation. To elucidate the determinants of EBV reactivation, future studies may be better focused on viral and host genetic variants.

Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Acknowledgments

We sincerely thank our deceased colleague Professor Yi Zeng who played a pivotal role during the planning and conduct of this study. We thank the members of the External Advisory Board, including Drs. Curtis Harris and Allan Hildesheim (National Cancer Institute, USA), Dr. Youlin Qiao (Chinese Academy of Medical Sciences, China), Dr. Xihong Liu (Harvard T.H. Chan School of Public Health, USA), and Dr. Weicheng You (Peking University Health Science Center, China).

Author contributions. Y. Ce. contributed to investigations, formal analysis, writing the original draft, and writing, review, and editing. E. T. C. contributed to conceptualization, methodology, and writing, review, and editing. Z. Z. contributed to investigation and funding acquisition. G. C. contributed to investigation and methodology; Q. L., Y. Ca., Q.-H. H., S.-H. X., S.-M. C., W.-J. Y., Y. Z., Y. L., and L. L. contributed to investigation. I. E. contributed to conceptualization and methodology. G. H. contributed to conceptualization, methodology, and project administration. Y.-X. Z. contributed to conceptualization, methodology, funding acquisition, and supervision. H.-O. A. and W. Y. contributed to conceptualization, project administration, funding acquisition, and supervision. All authors read and approved the final manuscript.

Disclaimer. The content is solely the responsibility of the authors and does not necessarily represent the official views of the funding agencies.

Financial support. This work was funded by the National Cancer Institute at the US National Institutes of Health (Grant Number R01CA115873; to E. T. C., H.-O. A., and Y.-X. Z.), the Swedish Research Council (Grant Numbers 2015-02625, 2015-06268, and 2017-05814; to W. Y.), and the Swedish Foundation for International Cooperation in Research and Higher Education (Grant Number CH2015-6223; to W. Y.). Work in the Guiping/Pingnan was funded by the Program for New Century Excellent Talents in University (Grant Number NCET-12-0654; to Z. Z.); National Basic Research Program of China (Grant Number 2011CB504300; to Y.-X. Z.); and Guangxi Natural Science Foundation (Grant Number 2013GXNSFGA019002; to Z. Z.). This work also was funded by a Karolinska Institutet Distinguished Professor Award (Grant Number 20160016007; to Y. Ce.).

Potential conflicts of interest. E. T. C. is an employee of Exponent, an international science and engineering consulting company that provides consulting support to clients in industry, government, academia, and the private sector on some of the environmental exposures addressed in this study. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Tao Q, Chan AT. Nasopharyngeal carcinoma: molecular pathogenesis and therapeutic developments. Expert Rev Mol Med 2007; 9:1–24.
2. Jia WH, Qin HD. Non-viral environmental risk factors for nasopharyngeal carcinoma: a systematic review. Semin Cancer Biol 2012; 22:117–26.
3. Chang ET, Adamo HO. The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomarkers Prev 2006; 15:765–77.
4. Raab-Traub N. Epstein–Barr virus in the pathogenesis of NPC. Semin Cancer Biol 2002; 12:431–41.
5. Hsu JL, Glaser SL. Epstein–Barr virus-associated malignancies: epidemiologic patterns and etiologic implications. Crit Rev Oncol Hematol 2000; 34:27–53.
6. Ko YH, EBV and human cancer. Exp Mol Med 2015; 47:e130.

7. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer 2016; 16:789–802.

8. Chen Y-P, Chan ATC, Le Q-T, et al. Nasopharyngeal carcinoma. The Lancet 2019; 394:64–80.

9. Laichalk LL, Hochberg D, Babcock GJ, et al. The dispersal of mucosal memory B cells: evidence from persistent EBV infection. Immunity 2002; 16: 745–54.

10. Shannon-Lowe CD, Neuhiel B, Baldwin G, et al. Resting B cells as a transfer vehicle for Epstein-Barr virus infection of epithelial cells. Proc Natl Acad Sci U S A 2006; 103:7605–7.

11. He YQ, Xue WQ, Xu FH, et al. The relationship between environmental factors and the profile of Epstein-Barr virus antibodies in the lytic and latent infection periods in healthy populations from endemic and non-endemic nasopharyngeal carcinoma areas in China. EBioMedicine 2018; 30:184–91.

12. Middeldorp JM. Epstein-Barr Virus-Specific Humoral Immune Responses in Health and Disease. In: Münz C, editor. Epstein Barr Virus Volume 2. One Herpes Virus: Many Diseases. Heidelberg, New York, Dordrecht, London: Springer International Publishing Switzerland; 2015; 39: pp 289–323.

13. Ji MF, Wang DK, Yu YL, et al. Sustained elevation of Epstein-Barr virus antibody levels preceding clinical onset of nasopharyngeal carcinoma. Br J Cancer 2007; 96:623–30.

14. Cao SM, Liu Z, Jia WH, et al. Fluctuations of Epstein-Barr virus serological antibody levels preceding clinical onset of nasopharyngeal carcinoma. EBioMedicine 2021; 60:216–25.

15. Chien YC, Chen JY, Liu MY, et al. Serologic markers of Epstein-Barr virus in the 20-year follow-up. PLoS One 2011; 6:e19100.

16. Tsao SW, Tsang CM, Lo KW. Epstein-Barr virus infection and nasopharyngeal carcinoma. Philos Trans R Soc Lond B Biol Sci 2017; 372:20160270.

17. Chien YC, Chen JY, Liu YY, et al. Genome sequencing analysis identifies Epstein-Barr virus capsid antigen: a mediation analysis. Cancer Med 2020; 9:1867–76.

18. Chen Y, Xu Y, Zhao W, et al. Lack of association between cigarette smoking and Epstein-Barr virus reactivation in the nasopharynx in people with elevated EBV IgA antibody titres. BMC Cancer 2018; 18:190.

19. Chen Y, Chang ET, Liu Z, et al. Residence characteristics and risk of nasopharyngeal carcinoma in southern China: a population-based case-control study. Environ Int 2021; 151:106455.

20. Chen Y, Chang ET, Liu Q, et al. Occupational exposures and risk of nasopharyngeal carcinoma in a high-risk area: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 2021; 30:1035–47.

21. Chen Y, Chang ET, Liu Z, et al. Smoking can increase nasopharyngeal carcinoma risk by repeatedly reactivating Epstein-Barr virus: an analysis of a prospective study in southern China. Cancer Med 2019; 8:2561–71.

22. Ye W, Chang ET, Liu Z, et al. Development of a population-based cancer case-control study in southern China. Oncotarget 2017; 8:87073–85.

23. Young LS, Yap LF, Murray PG. Epstein-Barr virus: more than 50 years old and still providing surprises. Nat Rev Cancer 2016; 16:789–802.

24. Chen Y-P, Chan ATC, Le Q-T, et al. Nasopharyngeal carcinoma. The Lancet 2019; 394:64–80.

25. Chen Y, Chang ET, Liu Z, et al. Development of a population-based cancer case-control study in southern China. Oncotarget 2017; 8:87073–85.

26. Chen Y, Chang ET, Liu Z, et al. Two Epstein-Barr virus-related serologic antibody tests in nasopharyngeal carcinoma screening: results from the initial phase of a cluster randomized controlled trial in Southern China. Am J Epidemiol 2013; 177:242–50.