Open Forum Infectious Diseases
MAJOR ARTICLE

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

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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, sibship 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].

Received 8 December 2021; editorial decision 7 March 2022; accepted 14 March 2022; published online 15 March 2022.

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Open Forum Infectious Diseases®2022

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https://doi.org/10.1093/ofid/ofac128

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 (χ^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 manufactures' 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]: $\text{Score} = [e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]/[1 + e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]$. 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]= .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 χ^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 = .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 1. The Characteristics of 1916 Population-Based Individuals Stratified by EBV Reactivation Status, Southern China, 2010–2014

	EBV Reactivation Status[a]		
	Negative (N = 1485)	Positive (N = 431)	
Characteristics	n (%)	n (%)	*P* Value[a]
Area			.01
Zhaoqing	585 (74.2)	203 (25.8)	
Wuzhou	455 (78.9)	122 (21.1)	
Guiping and Pingnan	445 (80.8)	106 (19.2)	
Sex			.33
Female	400 (79.1)	106 (20.9)	
Male	1085 (77.0)	325 (23.0)	
Age, Years			<.001
20–29	44 (78.6)	12 (21.4)	
30–39	222 (82.5)	47 (17.5)	
40–49	527 (80.8)	125 (19.2)	
50–59	424 (76.8)	128 (23.2)	
60–74	268 (69.3)	119 (30.7)	
Educational Level, Years			.06
≥10	501 (75.1)	166 (24.9)	
7–9	632 (80.1)	157 (19.9)	
≤6	352 (76.5)	108 (23.5)	
First-Degree Family History of NPC			.91
No	1417 (77.5)	412 (22.5)	
Yes	43 (79.6)	11 (20.4)	
Unknown	25 (75.8)	8 (24.2)	
BMI 10 years ago (kg/m^2)			.11
<18.5	149 (73.8)	53 (26.2)	
18.5–22.9	958 (79.0)	255 (21.0)	
23.0–27.4	334 (76.3)	104 (23.7)	
≥27.5	42 (68.9)	19 (31.1)	

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.

[a]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 = $[e^{(-3.934 + 2.203 \times VCA/IgA + 4.797 \times EBNA1/IgA)}]/[1 + e^{(-3.934 + 2.203 \times VCA/IgA + 4.797 \times EBNA1/IgA)}]$. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

[b]*P* values were determined using the χ^2 test.

Residential Characteristics and Occupational Exposures

Table 4 shows the associations of EBV reactivation with residential and occupational exposures. None of the residence-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.

Table 2. Associations Between Lifestyle Factors and EBV Reactivation in Population-Based Individuals, Southern China, 2010–2014

Variables	EBV Reactivation Status[a]		OR (95% CI)[b]	P Value[b]
	Negative	Positive		
Smoking Status				
Never smoker	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
Alcohol Drinking				
Never	1042	283	1.00 (ref.)	
Former	43	17	1.25 (0.70–2.26)	.45
Current	393	127	1.17 (0.90–1.51)	.23
Tea Drinking				
No	898	256	1.00 (ref.)	
Yes	586	175	0.93 (0.73–1.18)	.54
History of Chronic ENT Diseases				
No	1319	382	1.00 (ref.)	
Yes	166	49	1.03 (0.73–1.45)	.88
Use of Aspirin				
No	1414	409	1.00 (ref.)	
Yes	71	22	1.01 (0.61–1.67)	.97
Use of Nasal Drops/Nasal Balm/Flower Oil				
No	1390	409	1.00 (ref.)	
Yes	95	22	0.72 (0.44–1.17)	.18
Use of Herbal Medicine				
No	1388	404	1.00 (ref.)	
Yes	77	20	0.88 (0.52–1.46)	.61
Herbal Tea Consumption				
Yearly or less	831	241	1.00 (ref.)	
Monthly	511	143	0.97 (0.76–1.24)	.81
Weekly or more	121	38	1.09 (0.72–1.63)	.69
Herbal Soup Consumption				
Yearly or less	356	104	1.00 (ref.)	
Monthly	668	188	0.95 (0.71–1.26)	.71
Weekly or more	440	132	0.97 (0.7–1.34)	.84
Salted Fish Consumption in Adulthood				
Yearly or less	1113	313	1.00 (ref.)	
Monthly	289	89	1.01 (0.77–1.34)	.92
Weekly or more	80	29	1.11 (0.71–1.75)	.65
Preserved Vegetables Consumption in Adulthood				
No	119	40	1.00 (ref.)	
Yes	1343	386	0.86 (0.59–1.27)	.45
Salted Fish Consumption in Adolescence				
Yearly or less	1141	318	1.00 (ref.)	
Monthly	220	78	1.09 (0.81–1.47)	.56
Weekly or more	121	35	0.78 (0.52–1.18)	.24
Teeth Lost After Age 20 Years				
No	748	193	1.00 (ref.)	
Yes	736	238	0.99 (0.78–1.25)	.91
Number of Filled Teeth				
None	1263	366	1.00 (ref.)	
1–3	172	52	1.09 (0.78–1.53)	.61
≥ 4	49	13	0.96 (0.51–1.80)	.90
Daily Tooth Brushing, Times				
≤1	859	250	1.00 (ref.)	
≥2	621	180	1.12 (0.89–1.41)	.34
Birth Order				
1	382	134	1.00 (ref.)	
2–3	646	183	0.83 (0.62–1.08)	.16
≥4	457	114	0.76 (0.57–1.02)	.06

Table 2. Continued

Variables	EBV Reactivation Status[a]		OR (95% CI)[b]	*P* Value[b]
	Negative	Positive		
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.

[a]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: $\text{Score} = [e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]/[1 + e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]$. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

[b]OR estimates and *P* values were calculated using logistic regression, adjusted for age (continuous variable), sex, geographic area, and educational level.

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

	EBV Reactivation Status[a]			
Variables	Negative	Positive	OR (95% CI)[b]	*P* Value[b]
Cigarette Smoking				
Never smoker[c]	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 (1.00–2.08)	.05
20 to <30	276	80	1.21 (0.85–1.72)	.298
≥30	72	27	1.49 (0.90–2.49)	.13
P_{trend}[d]			0.16	
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
20 to <30	224	66	1.27 (0.88–1.84)	.20
≥30	324	136	1.52 (1.08–2.13)	.02
P_{trend}[d]			0.02	
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
20 to <30	154	52	1.39 (0.93–2.08)	.11
≥30	213	86	1.49 (1.03–2.14)	.03
P_{trend}[d]			0.03	
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]			0.01	
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.

[a]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: $Score = [e^{(-3.934 + 2.203 \times VCA/IgA + 4.797 \times EBNA1/IgA)}]/[1 + e^{(-3.934 + 2.203 \times VCA/IgA + 4.797 \times EBNA1/IgA)}]$. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

[b]OR estimates and *P* values were calculated using logistic regression, adjusted for age (continuous variable), sex, geographic area, and educational level.

[c]Never smokers were the reference group for all comparisons.

[d]Linear trend tests were conducted by using the median value within each category or by treating the categorical variable as an ordinal variable.

subtypes including 4 Chinese-specific NPC-associated amino acid substitutions (*BALF2* V317M, *BNRF1* G696R, *BNRF1* 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 4. **Associations Between Residential Characteristics, Occupational Exposures and EBV Reactivation in Population-Based Individuals, Southern China, 2010–2014**

Variables	EBV Reactivation Status[a]		OR (95% CI)[b]	*P* Value[b]
	Negative	Positive		
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/kerosene	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				

Table 4. Continued

Variables	EBV Reactivation Status[a]		OR (95% CI)[b]	*P* Value[b]
	Negative	Positive		
No	1402	416	1.00 (ref.)	
Yes	70	14	0.70 (0.39–1.26)	.24

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

[a]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: $\text{Score} = [e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]/[1 + e^{(-3.934 + 2.203 \times \text{VCA/IgA} + 4.797 \times \text{EBNA1/IgA})}]$. Score < 0.65 was defined as negative, whereas Score ≥ 0.65 was defined as positive.

[b]OR estimates and *P* values were calculated using logistic regression, adjusted for age (continuous variable), sex, geographic area, and educational level.

[c]Twice per month represents the 1st and 15th of every lunar month.

[d]Sources of pollution include main road, factory, and mining areas.

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 Lin (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.-H. J., 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 2013GXNSFGA 019002; to Z. Z.). This work also was funded by a Karolinska Institutet Distinguished Professor Award (Grant Number 2368/10-221; to H.-O. A.) and a scholarship from China Scholarship Council (Grant Number 201600160071; 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.

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