Correlates of anti-EBV EBNA1 IgA positivity among unaffected relatives from nasopharyngeal carcinoma multiplex families

CM Chang*,1, KJ Yu1, WL Hsu2,3, JM Major4, JY Chen4, PJ Lou5, MY Liu6, SR Diehl7, AM Goldstein1, CJ Chen2,3 and A Hildesheim1

1Division of Cancer Epidemiology and Genetics, National Cancer Institute, NIH, DHHS, 6120 Executive Blvd., EPS 7073, Rockville, MD, USA; 2Graduate Institute of Epidemiology, College of Public Health, National Taiwan University, Taipei, Taiwan; 3Genomics Research Center, Academia Sinica, Taipei, Taiwan; 4National Institute of Cancer Research, National Health Research Institutes, 35, Keyan Road, Zhunan Town, Miaoli County 350, Taiwan; 5Department of Otolaryngology, National Taiwan University Hospital and National Taiwan University College of Medicine, Taipei, Taiwan; 6Center of General Education, National Taipei University of Nursing and Health Sciences, Taipei, Taiwan; 7Center for Pharmacogenomics and Complex Disease Research, New Jersey Dental School, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA

BACKGROUND: To determine whether non-viral nasopharyngeal carcinoma (NPC) risk factors might be associated with (and mediated through) Epstein–Barr virus (EBV) serological responses linked to NPC risk, we evaluated predictors of risk of anti-EBV EBNA1 IgA seropositivity and other markers among unaffected relatives from a large NPC family study in Taiwan.

METHODS: Multivariate logistic regression conditioned on family was used to examine the associations between sociodemographic, dietary, lifestyle, and occupational variables and risk of anti-EBV EBNA1 IgA positivity, anti-VCA IgA, and anti-DNase positivity.

RESULTS: Among 2393 unaffected relatives from 319 multiplex families, 1180 (49.3%) were anti-EBV EBNA1 IgA seropositive. None of the associations with anti-EBNA1 IgA were statistically significant, except for being 31–50 years of age (ORs 0.51–0.57). For one or more EBV serological markers, there were suggestive associations for older age, GuangDong firm salted fish, betel use, current alcohol use, and male gender.

CONCLUSION: Overall, we found little evidence to suggest that non-viral NPC risk factors significantly alter EBV serological patterns, suggesting that non-viral NPC risk factors act through pathways independent of EBV serological responses.

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Nasopharyngeal carcinoma (NPC) is rare, albeit relatively common in southern China, southeast Asia, the Arctic, and North Africa (Chang and Adami, 2006). NPC risk factors include male gender, increased age, southern Chinese ethnicity, less education, consumption of salted fish and other preserved foods containing elevated levels of nitrate/nitrosamines, reduced fruit and vegetable consumption, cigarette smoking, betel nut chewing, alcohol consumption, and occupational exposures (Chang and Adami, 2006). Epstein–Barr virus (EBV), a ubiquitous herpesvirus that infects over 90% of adults worldwide, is believed to be necessary, but not sufficient for developing NPC (Chang and Adami, 2006). Longitudinal studies in China and Taiwan have shown that positivity for anti-EBNA1 IgA, anti-VCA IgA, and anti-DNase antibodies were associated with a significantly elevated NPC risk (Zeng et al., 1983, 1985; Chien et al., 2001; Ji et al., 2007; Hsu et al., 2009; Ng et al., 2010; Yu et al., 2011).

Whether non-viral risk factors act independently of anti-EBV serological responses or mediate EBV effects on NPC development is not known. To address this question, we evaluated the association between non-viral NPC risk factors and EBV serological responses in a group of healthy individuals from high-risk NPC multiplex families in Taiwan.

METHODS

Participants in the present study were part of the NPC multiplex family study in Taiwan, previously described (Pickard et al., 2004; Yang et al., 2005; Yu et al., 2009). Over 300 NPC multiplex families were identified and recruited, including 659 NPC cases and 2557 unaffected parents, siblings, spouses, and children.

Risk factors were assessed by questionnaire for all individuals by a trained nurse. Sociodemographic characteristics were age, gender, ethnicity, and education. Lifestyle factors included smoking, betel use, and alcohol use. Occupational exposures evaluated included duration of formaldehyde exposure and wood exposure.

Dietary intake during ages 10–30 (representing consumption during adolescence and adulthood) was assessed by food frequency questionnaire, such as: consumption of salted fish, other preserved foods (salted meat, smoked foods, preserved eggs, fried/fermented bean curd, fermented rice, and fermented flour sauce), and fruits and vegetables. Other preserved foods were combined into a single variable by summing across the variables, and dividing into categories corresponding to the questionnaire diet frequency categories.
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RESULTS

There were a total of 2393 unaffected family members in this study. The mean age was 46 years (47 in women and 44 in men), and 53% were women.

Overall, there were 1180 (49.3%) individuals seropositive for anti-EBNA1 IgA (OD405 > 0.1). In the full model, being 31–40 years old and 41–50 years old (vs 18–30 years) were inversely associated with anti-EBNA1 IgA positivity (adjusted OR

Table 1 Adjusted odds ratios (aORs) for the associations between NPC risk factors and risk of EBV antibody seropositivity for anti-EBNA1 IgA (OD405 > 0.1), anti-VCA IgA (OD405 > 0.2), and anti-DNase (≥160)

| Factor                       | Anti-EBNA1 IgA-positive (≥0.1) | Anti-EBNA1 IgA-positive (≥0.2) | Anti-VCA IgA-positive (≥1:10) | Anti-DNase-positive (≥160) |
|------------------------------|---------------------------------|---------------------------------|-------------------------------|----------------------------|
| Age                          |                                 |                                 |                               |                            |
| 18–30                        | 429                             | 216                             | 50.4                          | 1.0 (Reference)            |
| 31–40                        | 558                             | 250                             | 44.8                          | 0.51 (0.32, 0.83)          |
| 41–50                        | 546                             | 268                             | 49.1                          | 0.37 (0.35, 0.91)          |
| >60                          | 488                             | 255                             | 52.3                          | 0.77 (0.45, 1.3)           |
| P-value trend                | 0.74                            | 0.18                            |                               |                            |
| Gender                       |                                 |                                 |                               |                            |
| Female                       | 1269                            | 588                             | 46.3                          | 1.0 (Reference)            |
| Male                         | 1124                            | 592                             | 52.7                          | 1.1 (0.73, 1.5)            |
| Smoking                      |                                 |                                 |                               |                            |
| Never                        | 1616                            | 762                             | 47.2                          | 1.0 (Reference)            |
| Former                       | 186                             | 105                             | 56.5                          | 1.7 (0.91, 3.0)            |
| Current                      | 585                             | 310                             | 53.0                          | 0.95 (0.64, 1.4)           |
| Betel                        |                                 |                                 |                               |                            |
| Never                        | 2143                            | 1033                            | 48.2                          | 1.0 (Reference)            |
| Former                       | 84                              | 52                              | 61.9                          | 1.3 (0.66, 2.8)            |
| Current                      | 159                             | 91                              | 57.2                          | 1.3 (0.72, 2.4)            |
| Alcohol                      |                                 |                                 |                               |                            |
| Never                        | 1818                            | 873                             | 48.0                          | 1.0 (Reference)            |
| Former                       | 100                             | 56                              | 56.0                          | 0.8 (0.36, 1.6)            |
| Current                      | 469                             | 248                             | 52.9                          | 1.1 (0.77, 1.7)            |
| Duration of formaldehyde     |                                 |                                 |                               |                            |
| None                         | 1042                            | 484                             | 46.5                          | 1.0 (Reference)            |
| <10 years                    | 155                             | 71                              | 45.8                          | 1.3 (0.84, 2.0)            |
| >10 years                    | 216                             | 122                             | 56.5                          | 1.3 (0.84, 2.0)            |
| P-value trend                | 0.74                            | 0.87                            | 0.78                          |                            |
| Fruits and vegetables        |                                 |                                 |                               |                            |
| Less than once a day         | 257                             | 142                             | 55.3                          | 1.0 (Reference)            |
| 1–2 times a day              | 1324                            | 645                             | 48.7                          | 0.88 (0.54, 1.4)           |
| 2 or more times a day        | 784                             | 377                             | 48.1                          | 0.86 (0.51, 1.5)           |
| P-value trend                | 0.62                            | 0.46                            |                               |                            |
| Guangdong salted fish        |                                 |                                 |                               |                            |
| Never                        | 1941                            | 960                             | 49.5                          | 1.0 (Reference)            |
| Mouldy fragrant fish only    | 45                              | 23                              | 51.1                          | 1.4 (0.58, 3.3)            |
| Firm fish only               | 40                              | 30                              | 60.0                          | 1.8 (0.89, 3.8)            |
| Both mouldy and firm fish    | 98                              | 40                              | 40.8                          | 0.98 (0.51, 1.79)          |

aAdjusted odds ratios are from full models containing the following variables: age, gender, smoking, betel use, alcohol use, duration of formaldehyde exposure, fruit and vegetable intake, and type of salted fish intake.
(aOR) = 0.51, 95% CI = 0.32–0.83 and aOR = 0.57, 95% CI = 0.35–0.91, respectively), but associations with the older age groups were not significant. There was a suggestive, nonsignificant association between GuangDong firm salted fish (vs never; aOR = 1.8) and anti-EBNA1 positivity (Table 1).

A total of 384 (16.1%) individuals were seropositive for anti-EBNA1 IgA (Odds ratio (aOR) = 1.8–2.5, 95% CI = 1.0–2.8; Table 1). Former betel use (vs never use) was associated with anti-EBNA1 IgA positivity (aOR = 1.7, 95% CI = 1.0–2.8). Female sex was associated with anti-DNase positivity (aOR = 1.8–2.9). Males were at lower risk of anti-DNase seropositivity compared with females with anti-DNase seropositivity (aORs 2.2–2.9). Males were at significant risk of anti-DNase seropositivity (aORs 2.2–2.9). Mouldy and firm salted fish (vs never; aOR = 2.1–2.9) and GuangDong firm salted fish, and both mouldy and firm salted fish (vs never; aOR = 3.1–2.9) were nonsignificantly associated with anti-EBNA1 IgA positivity. There were nonsignificantly inverse associations with anti-EBNA1 IgA positivity for the three middle age groups (31–40, 41–50, and 51–60 years; aORs 0.63–0.86), but no association with the oldest age group (Table 1).

A total of 491 (26.3%) individuals were seropositive for anti-VCA IgA. Compared with the youngest age group (less than 30), the oldest age group (greater than 60; aOR = 1.9, 95% CI = 1.1–3.5) was associated with anti-VCA IgA positivity (P-trend = 0.012). Not reaching statistical significance, GuangDong firm salted fish (vs never; aOR = 2.0, 95% CI = 0.88–4.5) was associated with anti-VCA IgA positivity (Table 1).

A total of 767 (32.1%) individuals were seropositive for anti-DNase. Former and current betel use (vs never use) and GuangDong firm salted fish (vs never) were significantly associated with anti-DNase seropositivity (aORs 2.2–2.9). Males were at lower risk of anti-DNase seropositivity compared with females (aOR = 0.64, 95% CI = 0.43–0.94). Age greater than 30 (vs less than 30) was associated with anti-DNase seropositivity (aORs 1.8–2.5, P-trend = 0.81).

**DISCUSSION**

With a couple of notable exceptions discussed below, we saw little evidence to indicate that non-viral NPC risk factors influence anti-EBV seroreactivity. This suggests that non-viral NPC risk factors are unlikely to influence NPC risk by altering anti-EBV serological profiles.

We observed a U-shaped curve for age, with anti-EBNA1 IgA positivity being higher among the youngest and oldest, and lower among the middle age categories. The higher antibody positivity rate observed among older individuals, also seen for anti-VCA IgA, could reflect immunosenescence in older age, leading to more frequent viral lytic reactivation (Agarwal and Busse, 2010). The fact that anti-EBNA1 IgA positivity, but not other markers, was also higher among the youngsters, is not clearly understood.

There was a suggestive elevated risk for all EBV markers (aORs 1.6–2.7) associated with GuangDong firm salted fish, but not mouldy fragrant fish. In an *in vitro* study, aqueous extracts of Cantonese salted fish activated EBV lytic replication in Raji cells in a dose-dependent manner by causing cells to express EBV early antigen (Shao et al, 1988). However, it is unclear why one type of GuangDong salted fish would activate EBV, but not another type.

A significant association was observed between betel use and anti-DNase positivity, and was suggestive for anti-EBNA1. Although betel nut use is classified as a group 1 carcinogen in humans, there is no data on whether or not betel use can lead to EBV reactivation (IARC, 2004). Betel nut ingredients have induced inflammation *in vitro*, supporting the biological plausibility of this association (Jeng et al, 2000, 2003; Chang et al, 2005).

There may be recall bias of diet between the ages of 10–30, such that young subjects may be prone to recall their diet in their adulthood. EBV serology was measured at only one point in time and may not capture all episodes of EBV lytic replication. We would not have detected associations with other aspects of EBV exposure and/or host response to EBV. Our results may have been affected by the reproducibility for anti-VCA IgA testing, which was modest (agreement ~68%, k = 0.29–0.38; Pickard et al, 2004). Our findings for a high-risk population may not represent that of the general population, and associations may be attenuated due to similarity of exposures within high-risk families (Yang et al, 2005). However, by studying unaffected relatives from NPC multiplex families, the population is enriched in terms of EBV IgA antibody positivity, which occurs at much lower frequencies in the general population (Pickard et al, 2004). Additional strengths of the study include the large sample size, recruitment through cases identified from the national cancer registry, availability of detailed risk factor information, as well as multiple EBV serological markers.

In summary, the majority of NPC risk factors were not found to be significantly associated with anti-EBNA1 IgA positivity, the strongest predictor of NPC risk in our study population. In conclusion, our data suggest that non-viral NPC risk factors affect NPC risk via mechanisms other than through effects on EBV reactivation or host antibody responses to such infections.

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