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Nasopharyngeal carcinoma (NPC) is caused by Epstein-Barr virus (EBV) and is more likely to occur in susceptible families. Whether genetic susceptibility operates through altered EBV control is incompletely understood. We used a NPC risk prediction model based on 14 EBV markers to compare risk score distribution in unaffected members from multiplex families with that in population-based controls. Despite the absence of NPC at the time of antibody measurement, we observed an upward shift in risk score among multiplex family members compared to the general population, consistent with the possibility that genetic factors affect NPC risk through alterations in EBV control.

**Keyword.** virus control; immune; genetic susceptibility; host-virus interactions; multiplex antibody array.

Undifferentiated nasopharyngeal carcinoma (NPC) is caused by Epstein-Barr virus (EBV) [1]. Studies have shown that anti-EBV antibodies are associated with NPC risk [1]. As a result, EBV-based screening has been proposed for the early detection and treatment of NPC in endemic areas and within high-risk groups [2–4]. We recently developed a NPC risk prediction model that incorporates 14 anti-EBV antibody measures into a single risk score [5]. The EBV markers included in this model were defined using a case-control study of NPC in Taiwan and then independently validated in 2 prospective cohorts from that country.

Other than EBV, one of the strongest and most consistently reported risk factors for NPC is family history of the disease. Individuals with a family history of NPC have been reported to be at a 4 to 10-fold increased risk of the disease [1]. Furthermore, NPC multiplex family members (families with 2 or more NPC cases) have been shown to have a 10-fold higher NPC risk compared to the general populations from which they derive [6]. Linkage studies conducted within NPC multiplex families have discovered rare gene variants that might be linked to NPC within these families [7, 8]. Importantly, many of the genes reported (ITGB6, BCL2L12, and NEDD4L) are known or suspected to be involved in EBV infection acquisition and control, suggesting that the increased susceptibility to NPC in some of these families might be mediated primarily through alterations in host EBV control [8].

Data in support of this idea derive from studies that have reported elevated rates of positivity to specific EBV antibodies known to be associated with NPC, including viral capsid antigen (VCA) IgA and EBV nuclear antigen 1 (EBNA1) IgA, among unaffected (NPC-free) individuals from multiplex families [5, 9–12]. However, whether unaffected individuals from these multiplex families have increased risk prediction scores using the newly identified 14-marker model has not been evaluated.

**METHODS**

We evaluated this question using data from 2 studies in Taiwan nested within a multiplex family study cohort of 2557 individuals and a general population cohort of 23,943 individuals. Both cohorts have been described in detail previously and are expected to be broadly representative of Taiwanese NPC multiplex families and the general population, respectively [9, 13]. In brief, the TFS cohort recruited individuals from 358 NPC multiplex families with 2 or more first- or second-degree family members affected by NPC. The other cohort recruited subjects from the general population living in 7 townships. All participants at study entry were free of NPC and followed for incidence of NPC by computerized data linkage to the National Cancer Registration Profiles system. Sample collection and storage and follow-up for cancer incidence were performed in a comparable manner in both cohorts. The study nested within the multiplex family cohort included 26 incident NPC cases and 77 unaffected individuals. The study nested within the general population cohort included 37 incident NPC cases and 114 controls. In both studies, controls were frequency matched to cases on age and sex. Anti-EBV IgG and IgA antibody data measured using a comprehensive EBV proteome array was available from these participants [5], including antibody data against the 14 markers included in the recently developed NPC
risk score. These 14 markers were measured in a single, multiplex array format as previously described [5]. Samples from both studies were tested together and by the same technician using a single batch of microarray slides to avoid batch effects. Furthermore, the technician was masked to study and sample status at the time of testing.

**Statistical Analysis**

Levels of each of the 14 EBV antibody markers among controls in the 2 cohorts were compared by Wilcoxon rank-sum tests. Logistic regression was used to develop a risk prediction model that included the 14 EBV antibody markers. To avoid using a risk prediction model biased toward either of the 2 cohorts, we combined

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**Figure 1.** A, Distribution of predicted nasopharyngeal carcinoma (NPC) probabilities among unaffected controls in the NPC multiplex families (blue) and the general population (red). B, Comparison of individual EBV antibody marker reactivity among unaffected controls in the NPC multiplex families (blue) and the general population (red).
Table 1. Epstein-Barr Virus-Based Nasopharyngeal Carcinoma (NPC) Risk Predicted Score Among Unaffected Controls in NPC Multiplex Family Members and the General Population

<table>
<thead>
<tr>
<th>NPC predicted Probability</th>
<th>Members of NPC Multiplex Families (n = 77)</th>
<th>General Population (n = 114)</th>
<th>PValue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± SD</td>
<td>0.16 ± 0.21</td>
<td>0.10 ± 0.12</td>
<td>.023/ .013</td>
</tr>
<tr>
<td>Minimum/maximum</td>
<td>0.007/0.90</td>
<td>0.005/0.72</td>
<td></td>
</tr>
<tr>
<td>Predicted probability &lt; 0.30, No. (%)</td>
<td>64 (83.1)</td>
<td>107 (93.9)</td>
<td>.03</td>
</tr>
<tr>
<td>Predicted probability ≥ 0.30, No. (%)</td>
<td>13 (16.9)</td>
<td>7 (6.1)</td>
<td></td>
</tr>
</tbody>
</table>

The groups were compared by the parametric 2 sample t test.
The groups were compared by the nonparametric Wilcoxon 2-sample test.
The groups were compared by the Fisher exact test.

data from the 2 studies and defined regression coefficients for each of the 14 markers in this combined dataset using logistic regression with the 14 EBV antibodies parameterized as continuous variables (Supplementary Table 1). We then applied this risk prediction model to NPC-free controls from our 2 studies and calculated a risk score for each individual. The NPC risk scores among unaffected individuals within multiplex families and population-based controls were compared by 2-sample t tests. To account for the nonnormal distribution observed, we also compared the ranks using the nonparametric Wilcoxon 2-sample tests. In addition, we set up cutoff values of risk scores and compared the distributions of the scores among the unaffected members in multiplex families and general population by Fisher exact tests. P values < .05 were considered as a statistical significance. All of the data analyses were performed by SAS (version 9.4; SAS Institute).

RESULTS

The distribution of NPC risk scores among members of multiplex families and general population controls are presented in Figure 1A. Individuals from multiplex families had a mean score of 0.16 (SD 0.21) compared to 0.10 (SD 0.12) among individuals from the general population (P value = .02 by t test). We also compared ranks and again observed suggestive evidence of higher NPC risk scores among members of the multiplex families (P value = .10). Furthermore, using a risk score of 0.30 as the cutoff, 16.9% of individuals from multiplex families had elevated scores compared to 6.1% of individuals from the general population (P value = .03; Table 1). Use of alternative cutoffs led to similar patterns (Supplementary Table 2); unaffected members in multiplex families tended to have higher predicted risk scores regardless of the cutoff used. We also evaluated each of the 14 EBV markers individually and observed a general pattern of increased antibody reactivity among multiplex family members compared to general population controls, with significant differences noted for BRLF1 (Rta) IgA and suggestive differences noted for VCAp18 IgA (P value = .06), thymidine kinase IgA (P value = .07), and thymidine kinase IgG (P value = .10; Figure 1B).

DISCUSSION

EBV is one of the most common viruses in humans and is highly prevalent throughout the world. More than 95% of adults have been infected by EBV by the age of 30 years and these infections persist lifelong. In the present study, we show that individuals from families who are genetically susceptible to NPC have an elevated EBV antibody-based NPC risk score. This finding is consistent with and bolsters the hypothesis that underlying genetic factors that predispose to NPC within multiplex families affect disease risk through alterations in host EBV control. While previous published studies have indicated that members of NPC multiplex families have elevations in individual anti-EBV antibodies that are associated with NPC [9–11], this is the first report to examine a risk score-based approach using a comprehensive set of EBV antibodies that have been shown to predict NPC with high accuracy [5].

Previous published work in these NPC multiplex families has identified several candidate susceptibility genes that predispose to NPC within multiplex families [8]. Many of them, including NOTCH1, DLL3, NIPAL1, ITGB6, BCL2L12, and NEDD4L, are known to have biological functions that could be important in the modulation and control of EBV infections. Further supporting the idea that NPC genetic susceptibility factors act biologically by affecting/modulating host responses to EBV come from reports that have demonstrated that polymorphism in HLA genes (genes whose proteins are involved in the presentation of exogenous antigens to the immune system) are strongly associated with NPC risk [7, 14] and, more recently, that HLA genes are associated with EBV antibody responses [15].

In conclusion, our study found healthy members of NPC multiplex families have elevated EBV antibody responses compared to general population levels, suggesting that susceptibility in these families might be partly driven by their lack of ability to adequately control EBV infection. Given these findings, additional efforts to better understand the natural history of EBV infection among individuals from families that are genetically susceptible to NPC are warranted.

Supplementary Data

Supplementary materials are available at The Journal of 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.
Notes

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