Allergy and glioma risk: test of association by genotype

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ABSTRACT

While epidemiological studies have suggested an association between atopy and glioma risk these observations have been based on self-reporting of allergic conditions raising the possibility that associations may be non-causal and arise as a consequence of bias, reverse causation or other artefacts. Genetic information provides an alternative approach to investigate the relationship avoiding such biases. We analysed 1,878 glioma cases and 3,670 controls for variants at 2q12, 5q12.1, 11q13, and 17q21 that are associated with asthma or eczema risk at $P < 5.0 \times 10^{-7}$. The SNP rs7216389, which tags the 3' flanking region of ORMDL3 at 17q21 and has been associated with childhood asthma, was correlated with increased glioma risk (OR = 1.10; 95% CI: 1.01-1.19). These data provide evidence for a correlation between asthma susceptibility and glioma risk and illustrate the value of using genetics as an investigative tool for developing etiological hypotheses.
INTRODUCTION

Gliomas account for ~80% of malignant primary brain tumors (PBT)\(^1\). In the United States ~21,000 individuals are diagnosed with glioma annually and for most the prognosis is dismal\(^1\). To date few lifestyle exposures have consistently been linked to glioma risk except ionizing radiation\(^1\), which accounts for only a few cases.

An inverse association between self-reported allergic conditions and glioma has been reported in several cohort and case-control studies\(^2\). Such data have been interpreted as reflecting a relationship between heightened immune function, seen in atopy, and protection against tumour development. Alternatively the associations observed may be non-causal, arising as a consequence of methodological biases inherent in the study design\(^2\). These biases include possible selection bias in controls, recall bias from self-reported allergy assessment, and reverse causation or confounding from unmeasured effects. Furthermore, a high frequency of exposure ascertainment by proxy may lead to bias, especially if proxies systematically underreport allergic diseases.

Genetic information provides an alternative approach to assessing the relationship between susceptibility to atopy and tumour risk avoiding these biases. Twin studies have long shown considerable heritability for asthma and atopy phenotypes and furthermore they are strongly correlated with each other\(^4\). Recent genome-wide association (GWA) studies have identified polymorphisms influencing eosinophil counts\(^5\) and risk of both eczema\(^6\) and asthma\(^5,7,8\). The substantial evidence supporting these findings, including sizeable power and replication in large samples, indicates that the associations are highly robust.

To determine whether asthma and allergic condition polymorphisms are inversely related to glioma, we interrogated data from the two GWA studies of glioma that we have recently conducted. Since we have used germline polymorphisms as biomarkers of susceptibility to asthma and
allergic conditions, our results mitigate against recall bias or effects of glioma on the immune system.
MATERIALS AND METHODS

We extracted data from two GWA studies of glioma\(^9\). The first was a UK GWA study of 636 cases ascertained through the INTERPHONE Study\(^10\) with individuals from the 1958 Birth Cohort serving as controls\(^11\). The second was a US GWA study of 1,247 cases ascertained through M.D. Anderson Cancer Center, Texas with individuals from CGEMS serving as controls\(^12, 13\). Collection of blood samples and clinical information from subjects was undertaken with informed consent and relevant ethical review board approval in accordance with the tenets of the Declaration of Helsinki.

As previously described\(^9\), a genome-wide scan of tagging SNPs was conducted using Illumina Hap550K and Human610-Quad BeadChips according to the manufacturer's protocols (Illumina, San Diego, USA). We subjected cases and controls to rigorous quality control in terms of excluding samples with cryptic relatedness and non-CEU ancestry\(^9\).

To identify polymorphisms robustly associated with atopic risk we searched PubMed for published articles (January 2000-August 2009) using “atopy, allergy, asthma, eczema, hayfever” in conjunction with “genome-wide association” as reference terms. To guard against analyzing variants that may be type 1 errors we took forward only (1) variants that had been replicated in more than one series or had been validated in multi-phase studies involving independent case-control series and (2) that had shown evidence for an association at \(P < 5.0 \times 10^{-7}\) in combined analyses, the significance level advocated for GWA studies. From the literature we identified four loci robustly associated with risk of developing atopy under these criterion. Specifically, rs7216389 mapping to 17q21 (ORMDL3)\(^8, 14-17\) and rs1588265 mapping to 5q12.1 (PDE4D)\(^7\), associated with childhood asthma; rs7927894 mapping to 11q13 (C11orf30) associated with atopic dermatitis\(^6, 18\) and rs1420101 mapping to 2q12 (IL1RL1) associated with eosinophil count and risk of asthma\(^5, 19\).
All SNPs, except for rs7927894, had been genotyped in both GWA studies of glioma. To examine the association between variation at this locus and glioma risk we made use of rs7130588 which is in strong linkage disequilibrium with rs7927894 ($r^2=0.97$).

The risk of glioma associated with SNPs was estimated by odds ratios (ORs) using unconditional logistic regression and associated 95% confidence intervals (CIs) were calculated. All analyses were undertaken using R software and STATA (Version 8.2, Stata Corporation, College Station, TX, USA). A $P$-value of $<0.05$ was considered statistically significant in all analyses. While our study design precludes adjustment for age effects or putative behavioral and environmental risk factors, these adjustments are unlikely to materially change our conclusions as SNPs are unlikely to have profound age specific effects or strong interactions.
RESULTS AND DISCUSSION

No significant association with glioma risk was seen between rs7130588 or rs1588265 in either case-control series (Table 1). An increased risk of glioma was observed between the atopic risk allele of rs1420101 and glioma risk in the US series, but no support was provided by the UK series (combined $P = 0.25$). A significant association between rs7216389 and glioma risk was shown in the combined dataset (combined $P = 0.022$); whereby an increased glioma risk was associated with the risk allele for asthma. While rs7216389 was reported to provide the strongest evidence at 17q21 for an association with asthma, other SNPs mapping to the region were also supportive of a relationship. In view of this we interrogated the region more fully, annotating all SNPs showing significant LD with rs7216389 in order to examine whether a stronger association might emerge (Figure 1). Four SNPs mapping to the region ($r^2 > 0.8$ with rs7216389) also provide evidence for a relationship between variation at 17q21 and glioma risk (rs2290400, rs8067378, rs11557467, rs9303277) although none provided superior evidence for a relationship (respective $P$-values 0.024, 0.028, 0.021, 0.030).

Although rs7216389 is located within intron 1 of GSDML/GSDMB, rs7216389 genotype strongly influences the expression of the nearby gene ORMDL3 (orm1 like protein 3; MIM 610075) through chromatin remodelling. ORMDL3 is a ubiquitously expressed transmembrane protein participating in signalling and facilitation of ER-mediated inflammatory responses. Since a relationship between rs7216389 and childhood asthma was first reported, the association has been replicated by several independent studies and the variant underscores ~17% of asthma in European populations.

While requiring replication our observation provides evidence of a positive association between asthma and glioma. This is counter to the consensus among epidemiological studies which in general report an inverse relationship between atopy and glioma risk. Although it is possible that other genetic risk factors for asthma may have opposite effects on glioma
risk, methodological issues, such as recall bias from self reported allergy assessment, may well have systematically biased previous epidemiological studies. Indeed, the odds ratios among previous allergy/brain tumour studies have been found to be inversely related to the proportion of proxy respondents.

This positive association between asthma and glioma is in keeping with the recent observation of higher serum IgE levels in recently diagnosed glioma cases compared with controls irrespective of Temozolomide treatment. However, inference from such a complex phenotype is not necessarily straightforward and such observations could be a consequence of reverse causality. In view of this observation, we extracted data from our glioma case-control series for two genetic variants (rs2251746, rs2040704) found in a GWA study to influence levels of IgE. Neither were found to be significant (rs2251746 $P=0.78$; rs6871536, $r^2=1$ with rs2040704, $P=0.30$).

A major strength of our study is that it has been based on a large series of patients and our analysis is unlikely to be confounded by population stratification as we have excluded non-Western European ancestral cases from the analyses. Furthermore, we have only evaluated genetic variants that have previously been shown to be robustly associated with risk of allergy which have been identified by GWA studies of atopy and which were annotated by SNPs typed in our GWA studies of glioma. While this strategy avoids type 1 errors, it has precluded analysis of other potential disease-causing variants. It is also acknowledged that our level of statistical support for an association between $ORMDL3$ variation and glioma risk does not preclude type 1 error on correction for multiple testing and, as with all association studies, our findings require independent replication.

The overall evidence concerning the nature of the relationship between cancer and allergy is complex and often conflicting with respect to specific tumour types. The immune surveillance hypothesis, which proposes that allergic conditions may lead to decreased risk of malignancy by
enhancing the ability of the immune system to detect and eliminate malignant cells\textsuperscript{27}, has enjoyed popular support resulting from the multitude of studies showing an inverse relationship between cancer and allergy. However there is still no clear consensus, with many epidemiological studies providing varied evidence for an association in addition to the problems of potential methodological biases. The alternative hypothesis, whereby inflammation associated with allergy acts to promote cancer, has supporting evidence from studies of haematological malignancies\textsuperscript{26,28,29} and liver cancer\textsuperscript{30}. A possible basis for this relationship between allergy and cancer is provided by the enhanced cell survival associated with pro-inflammatory states\textsuperscript{31}.

This wealth of evidence suggests a complex relationship between cancer and allergy, and highlights the requirement for studies in this area to be clearly defined and free from potential bias. In this study we aim to eliminate bias by using genetic information only. This approach has the advantage that these germ line polymorphisms cannot be influenced by the presence of the cancer, unlike self-reporting of allergy or measurement of IgE levels.

This study serves to illustrate the value of using genetics as a tool to develop aetiological hypotheses for cancer risk thus avoiding potential bias in case-control analyses and providing robust evidence in order to determine the true relationship between allergy and glioma.

**COMPETING INTERESTS STATEMENT**
The authors declare no competing financial interests

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TABLE AND FIGURE LEGENDS

TABLE 1: Glioma risk associated with rs7216389, rs7130588, rs1588265, rs1420101

Shown, for the four SNPs of interest, are the numbers of cases and controls with each genotype in the UK, US and combined GWA studies. Odds ratios (OR) and associated 95% confidence intervals are given along with the Cochran-Armitage trend test \( P \) values. Genes shown are within 20kb of the SNP. Locations are given according to build NCBI 36.3. The observed control genotype frequencies of SNPs were in accordance with Hardy-Weinberg equilibrium, providing no evidence of population stratification within either dataset (i.e. \( P>0.05 \)).

FIGURE 1: Regional plot of the 17q21 association with glioma risk

Armitage trend test \( P \) values (as \(-\log_{10} \) values; left y axis) are shown for SNPs from the combined analysis of the glioma GWA studies. Each square represents a SNP found in this locus with the asthma-risk SNP, rs7216389, marked by a red diamond. The color intensity of each square reflects the extent of LD with rs7216389 – red \( (r^2=1) \) through to white \( (r^2=0) \). Recombination rates in HapMap CEU across the region are shown in blue (right y axis). Also shown are the relative positions of genes mapping to the region. Chromosomal positions and genes are based on NCBI build 36 coordinates.
REFERENCES


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