

Genetic variants in inflammation pathway genes and asthma in glioma susceptibility

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Single nucleotide polymorphisms (SNPs) in inflammation-related genes have previously been shown to alter risks of developing various cancers. However, the effects of such SNPs on glioma risk remain unclear. We used a multistrategic approach to elucidate the relationship between glioma risk, asthma/allergies, and 23 literature-based functional SNPs in 11 inflammation genes. Genotyping was conducted on 373 histologically confirmed adult glioma patients and 365 cancer-free controls from the Harris County Brain Tumor Study. Deviations from the Hardy–Weinberg equilibrium were assessed using the χ^2 -test, and Akaike's information criterion was used to determine the best genetic model for each SNP. Odds ratios (ORs) were calculated both for each SNP individually and for grouped analyses, examining the effects of the numbers of adverse alleles on glioma risk in participants with and without asthma. In the single-locus analysis of the 23 examined SNPs, 1 pro-inflammatory and 2 anti-inflammatory gene SNPs were significantly associated with glioma risk (*COX2/PTGS2*, rs20417 [OR = 1.41]; *IL10*, rs1800896 [OR = 1.57]; and *IL13*, rs20541 [OR = 0.39], respectively). When we examined the joint effects of the risk-conferring alleles of these 3 SNPs, we found a significant trend indicating that the risk increases as the number of adverse alleles increase ($P = .005$). Stratifying by asthma status, we found that this dose–response-like trend of increasing risk is only present among those without asthma/allergies ($P < .0001$). Our study indicates that polymorphisms in inflammation genes are associated with glioma

susceptibility, especially when a history of asthma/allergies is absent.

Keywords: allergy, asthma, glioma, inflammation.

The role of the immune system in cancer etiology has long been in question. Single nucleotide polymorphisms (SNPs) in inflammation-related genes have been shown in previously published literature to alter the risks of various cancers, including lung, prostate, and bladder cancer.^{1–3} However, the effects of such SNPs on glioma risk have not yet been clarified. Although cytokine and other inflammation gene polymorphisms have been examined in relation to glioma risk,⁴ a pathway-based, multistrategic approach has not yet been utilized to explore the impact of such SNPs and their potential higher-order interactions on gliomagenesis.

Although the impact of inflammation pathway SNPs on glioma risk still needs clarification, some relatively consistent findings in the literature lend credibility to the potential role of these polymorphisms in gliomagenesis. In particular, some SNPs that confer asthma risk have been found to be inversely associated with glioma risk.^{5–9} Similarly, a history of asthma/allergies, as well as higher immunoglobulin E (IgE) levels, appear to be associated with a protective effect against glioma development and prognosis.^{10,11} By using the SNPs related to asthma risk as biomarkers, many of these studies have illustrated that the inverse relationship between asthma, IgE, and glioma is not likely attributable to poor recall or a change in the patient's immunology due to the cancer or the subsequent treatment. Rather, the relationship is likely reflective of an intricate immunological pathway that provides protection against gliomagenesis through its many facets.

Although previous research has examined the influence of various aspects of the immune system on glioma development, it appears that studies utilizing a pathway-based, exploratory approach to this topic are

Received February 2, 2009; accepted August 13, 2009.

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uncommon in the literature. The purpose of our study was to utilize such an approach to elucidate the relationship between glioma risk, asthma, and 23 functional SNPs in 11 inflammation-related genes that likely act in concert by potentially impacting either the amount or the functional efficiency of the produced cytokines.

Materials and Methods

Study Population

The study population and subject characteristics are described in detail elsewhere.¹² Briefly, this study included 373 glioma patients and 365 cancer-free controls. All the subjects were Caucasian adults (18 years or older) and were from the 15 counties (Austin, Brazoria, Chambers, Colorado, Fort Bend, Galveston, Harris, Jefferson, Liberty, Montgomery, Orange, San Jacinto, Walker, Waller, and Wharton counties) surrounding the metropolitan Houston area in Texas. Patients newly diagnosed with histopathologically confirmed malignant gliomas (ICD-9 codes 9380–9481) were consecutively recruited between January 2001 and January 2006, without the restrictions of age, sex, and histology. Cancer-free controls were recruited during the same period through random-digit dialing using standard methods. All the control subjects were frequency-matched to the cases by age (± 5 years), gender, and ethnicity and were English-speaking residents of the same counties in Houston. The participation rates for cases and controls were 77% and 83%, respectively.

Each participant completed a detailed in-person or telephone interview after written informed consent was obtained. The structured questionnaire was administered by interviewers to collect information on demographic factors, personal and family medical history, and environmental exposure history. Information about history of asthma or allergies was ascertained through questions on whether the individuals had ever been diagnosed with asthma, eczema, hay fever, or any other allergy and about the age at onset of each condition. A family history of brain tumor was defined as any self-reported brain tumor in a first-degree relative (parents, siblings, or children). Proxy interviews, often with the spouse, were conducted for approximately 4% of the study population, when the subject was very ill or had died. Approximately 20 mL of venous blood was collected from each participant. The research protocol was approved by the University of Texas M. D. Anderson Cancer Center Institutional Review Board.

Selection of Candidate SNPs and Genotyping Assays

The 11 inflammation-related genes included interleukin 4 receptor (*IL4R*), interleukin 10 (*IL10*), interleukin 13 (*IL13*), interleukin 1 beta (*IL1B*), interleukin 2 (*IL2*), interleukin 6 (*IL6*), interleukin 18 (*IL18*), cyclooxygenase 2 (*COX2/PTGS2*), nuclear factor-kappa B

(*NFKB1*), and the IgE receptor (*MS4A2*).^{6,13–21} The interleukin gene products are anti-inflammatory cytokines (*IL-4*, *IL-4R*, *IL-10*, and *IL-13*) and pro-inflammatory cytokines (*IL-1B*, *IL-2*, *IL-6*, and *IL-18*). The other included genes encode factors related to inflammatory or atopic reactions (*NFKB1*, *COX2/PTGS2*, and *MS4A2*). *NFKB1* is a well-known transcription factor gene, responsive to various cytokines, that is involved in immune response and regulation, apoptosis, and the cell cycle. *COX2* (also known as *PTGS2*), which is downregulated by *IL-13*, is involved in prostaglandin generation and is also related to C-reactive protein levels postsurgery.^{14,15} IgE is the major antibody involved in allergic responses and asthma.¹⁰

This study focused on polymorphisms located in the promoter, 5'-untranslated, and coding regions of the genes and on the basis of commonality (minor allele frequencies > 0.05). Overall, a total of 23 potential functional polymorphisms in 11 key genes were chosen for this study, of which 12 SNPs are involved in the anti-inflammatory pathway and 11 SNPs belong to the proinflammatory pathway (Table 1). The basis of the selection of these SNPs is given in Table 1. The genes and their corresponding SNPs were chosen based on previous epidemiologic associations reported in the literature (especially associations with inflammatory diseases or cancers) and the biological plausibility of the involvement of the genes in gliomagenesis.

Genomic DNA was extracted from peripheral blood lymphocytes using the Qiagen Blood Kit (Qiagen Inc., Valencia, California). Genotyping was performed using the Sequenom MassARRAY iPLEXTM platform (<http://www.sequenom.com/seq.genotyping.html>). MassARRAY Workstation version 3.3 software was used to process and analyze iPLEX SpectroCHIP bioarrays. The quality-control analyses included the genotyping of internal positive-control samples, the use of no template controls, and replicates for 10% of the samples.

Statistical Analysis

For each polymorphism, deviation of the genotype frequencies from those expected under the Hardy–Weinberg equilibrium (HWE) was assessed in the controls by the χ^2 -test. We compared genotype frequencies in cases and controls using the χ^2 -test, and used Akaike's information criterion (AIC) to determine the best genetic model for each SNP.²² Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by unconditional logistic regression analysis with adjustment for age and gender. Analyses were stratified by glioma histologic types because of differences in the genetics and biology of glioblastoma (WHO Grade IV) vs medium-grade (WHO Grade III) and low-grade (WHO Grade II) tumors. We performed permutation testing (10 000 times) to reduce the potential for spurious findings due to multiple testing. Joint effects by the number of adverse genotypes identified from the SNP analyses were examined using the multivariate logistic regression models. All statistical tests were 2-sided.

Table 1. Information about 23 genotyped SNPs in 11 inflammation pathway genes

Chromosome Location	Genes	SNP ID	SNP region (alias)	Base change	P value ^a	Basis of selection						
						Reported functions	References	Epidemiologic association	References			
Anti-inflammatory												
5q31.1	<i>IL4</i>	rs2243250	5' UTR, −524 (−589)	C>T	0.966	Increased transcriptional activity	16	Asthma, atopy, rheumatoid arthritis	16,17,28–31			
		rs2070874	5' UTR, Ex1–168	C>T	0.781						Asthma	16
16p11.2–12.1	<i>IL4R</i>	rs1805011	Exon 11, A400E	A>C	0.478						Asthma, atopy	5,17,28,30
		rs1805012	Exon 11, R431C	T>C	0.412							
		rs1805015	Exon 11, P503S	T>C	0.574							
		rs1801275	Exon 11, R576Q	A>G	0.987							
		rs1805016	Exon 11, A752S	T>G	0.993							
1q31–q32	<i>IL10</i>	rs1800896	5' UTR, −1082 (−1116)	G>A	0.087	Decreased expression; interaction	19–21	Asthma, lymphoma, melanoma, systemic lupus erythematosus, multiple sclerosis, Alzheimer's disease	32–39			
		rs1800871	5' UTR, −819 (−853)	C>T	0.221	Decreased production						
		rs1800872	5' UTR, −592 (−626)	C>A	0.175	Decreased production, serum levels						
5q31	<i>IL13</i>	rs20541	Exon 4, R144Q	C>T	0.135	Increased expression	25	Asthma, atopy	40–43			
		rs1800925	5' UTR, −1069	C>T	0.818							
Proinflammatory												
2q14	<i>IL1B</i>	rs16944	5' UTR,	A>G	0.633	Increased expression, protein secretion	27	Hepatitis B, rheumatoid arthritis, gastric cancer	44–51			
		rs1143627	5' UTR, −31	C>T	0.559							
		rs1143634	Exon 4, F105F	C>T	0.340	Influence production						
4q26–q27	<i>IL2</i>	rs2069762	5' UTR, −384	G>T	0.746			Infection susceptibility	52			
7p21	<i>IL6</i>	rs1800795	5' UTR, −174 (−236)	C>G	0.722	Increased expression	8	Stroke, rheumatoid arthritis	53,54			
		rs1800796	5' UTR, −572	G>C	0.678							
		rs1800797	5' UTR, −596 (−597)	G>A	0.775					Alters transcription	28	
11q22.2–q22.3	<i>IL18</i>	rs187238	5' UTR, −9211	G>C	0.776							
1q25.2–q25.3	<i>Cox2 /PTGS2</i>	rs20417	5' UTR, −765	C>G	0.052	Reduced expression, affect binding of TF	14,15					
4q23–q24	<i>NFKB1</i>	rs1020759	Intron 11	C>T	0.967							
11q13	<i>MS4A2</i>	rs569108	Exon 7, G237E	T>C	0.164							

^a10 000 permutation P value for difference in allele distributions between cases and controls.

The pairwise linkage disequilibrium (LD) among the SNPs was examined using Lewontin's standardized coefficient D' and LD coefficient r^2 .²³ Haplotype analyses were conducted using Haplo.stats (<http://www.mayo.edu/stagen>), which is a score test based on the generalized linear model framework that allows for the adjustment of possible confounding variables and provides both global and haplotype-specific tests.²⁴

Results

Characteristics of Study Subjects

The distributions of selected characteristics between glioma cases and cancer-free controls are presented in Table 2. Briefly, in our analysis, cases were less likely to report a history of allergies or asthma than controls. Of the 373 cases, 214 (57.4%) were glioblastoma (WHO Grade IV), 77 (20.6%) were anaplastic astrocytoma (WHO Grade III), and 82 (22.0%) were other low-grade glioma (WHO Grade II).

Individual SNP Association Analysis

Information about the 23 genotyped SNPs is given in Table 1. The genotype distributions of all 23 selected SNPs in the controls were consistent with those expected

from the HWE. Genotype distribution of these SNPs in the cases and controls are summarized in Table 3. The logistic regression analyses revealed that in the dominant-effect model as assessed by the AIC, a significant increased risk effect was associated with the G allele of COX2 rs20417 (among the overall study population, adjusted OR: 1.41, 95% CI: 1.01–1.96 for CG/GG compared with the CC genotype; among Grade IV glioma, adjusted OR: 1.32, 95% CI: 0.93–1.92). Meanwhile, in the recessive-effect model, compared with the wild-type homozygote's and heterozygous carriers (AA/GA), a significantly increased risk was associated with variant homozygote's (GG) of IL10 rs1800896 (overall adjusted OR: 1.57, 95% CI: 1.11–2.23 for the GG genotype compared with the AA/GA genotypes; adjusted OR: 1.61, 95% CI: 1.07–2.44 for Grade IV glioma), whereas a significant protective effect was associated with the variant homozygotes of IL13 rs20541 (overall adjusted OR: 0.39, 95% CI: 0.16–0.93 for the TT genotype compared with the CC/CT genotypes; adjusted OR: 0.30, 95% CI: 0.11–1.02 for Grade IV glioma).

The allele frequencies of COX2 rs20417 and IL10 rs1800896 were marginally different between the cases and the controls, after 10 000 permutations ($P = .052$ and $.087$, respectively). No differences in allele frequency distribution was observed for IL13 rs20541 after 10 000 permutations ($P = .135$).

Joint Effect of Multiple SNP Association

To test our hypothesis that multiple SNPs in the inflammation pathways act in concert to modulate glioma risk, we estimated the joint effect of the three adverse SNPs that were significantly associated with glioma risk in the single-locus analysis. First, we conducted the joint effect analysis for the 3 adverse SNP variant genotypes. In this analysis, we treated the minor allele of the 2 risk SNPs (COX2 rs20417 and IL10 rs1800896) and the common allele of the protective SNP (IL13 rs20541) as the adverse allele and regarded individuals with 1 or fewer adverse alleles as the referent group. Unfortunately, we were unable to use individuals with no adverse alleles as the referent group in the joint effect analyses due to small numbers. As shown in Table 4, the risk of glioma increased progressively as the number of adverse genotypes increased ($P_{\text{trend}} = .005$). In other words, individuals with 2 risk-referring alleles appear to have a higher glioma risk than those with 1 or no adverse alleles. That is, the groups with 2 and 3 adverse genotypes all exhibited a significantly increased glioma risk, with adjusted ORs of 1.41 (95% CI: 1.03–1.95) and 2.33 (95% CI: 1.26–4.35), respectively.

Stratification by asthma and allergy history revealed a significant dose–response effect on glioma risk only among those with no history of asthma/allergies ($P_{\text{trend}} < .0001$). Specifically, among those with no asthma/allergy history, individuals with 2 or 3 adverse alleles showed a significantly elevated glioma risk

Table 2. Frequency distribution of selected characteristics of study subjects by case–control status

Variable	Cases ($n = 373$) (No. [%]) ^a	Controls ($n = 365$) (No. [%]) ^a
Age (y)		
≤ 45	142 (38.1)	143 (39.2)
> 45	231 (61.9)	222 (60.8)
Gender		
Male	212 (56.8)	159 (43.6)
Female	161 (43.2)	206 (56.4)
Asthma		
No	314 (84.2)	251 (68.8)
Yes	59 (15.8)	114 (31.2)
Smoking status		
Never	222 (59.7)	205 (56.2)
Ever	150 (40.3)	160 (43.8)
Family history of cancer		
No	161 (56.8)	182 (50.0)
Yes	212 (43.2)	182 (50.0)
Family history of brain tumor		
No	339 (96.0)	326 (97.3)
Yes	14 (4.0)	9 (2.7)
Histologic type		
WHO Grade IV	214 (57.4)	
WHO Grade III	77 (20.6)	
WHO Grade II	82 (22.0)	

^aNumbers do not add up to the column totals due to missing values.

Table 3. Case–control distribution of genotypes for various inflammation gene polymorphisms

Pathway	Gene	SNP ID	Model	Genotype	Overall study population (n = 373 cases; 365 controls)		Among WHO Grade IV cases ^c	
					OR (95% CI) ^a	P value ^b	OR (95% CI) ^a	P value ^b
Anti-inflammatory	<i>IL10</i>	rs1800896	Codominant	AA	1.00	.021		
				GA	0.82 (0.59–1.18)			
				GG	1.39 (0.94–2.09)			
			Dominant Recessive	GA/GG	0.98 (0.72–1.36)	.892		
				AA/GA	1.00		1.00	
				GG	1.57 (1.11–2.23)	.012	1.61 (1.07–2.44)	.016
	<i>IL13</i>	rs20541	Codominant	CC	1.00	.081		
				CT	0.99 (0.73–1.38)			
				TT	0.39 (0.17–0.94)			
			Dominant Recessive	CT/TT	0.91 (0.66–1.24)	.526		
				CC/CT	1.00		1.00	
				TT	0.39 (0.16–0.93)	.023	0.30 (0.11–1.02)	.058
Pro-inflammatory	<i>Cox2</i>	rs20417	Codominant	CC	1.00	.124	1.00	
				CG	1.43 (1.02–1.98)			
				GG	1.20 (0.43–3.24)			
			Dominant Recessive	CG/GG	1.41 (1.01–1.96)	.041	1.32 (0.93–1.92)	.063
				CC/CG	1.00			
				GG	1.10 (0.35–3.12)	.865		

The AIC was used to determine the best genetic model for each SNP. OR for analyses conducted among WHO Grade IV (glioblastoma) cases and cancer-free controls are only provided for the best genetic models.

^aAdjusted for age and gender.

^bP values calculated for the difference in genotype distribution between cases and controls using χ^2 -tests.

^cTotal number of WHO Grade IV cases = 214.

Bold values indicate the odds ratios that are statistically significant.

compared with individuals with 1 or fewer adverse alleles [adjusted OR: 1.70 (1.19–2.48) and 2.52 (1.23–5.24), respectively]. Whereas individuals with a

positive asthma/allergy history who had zero to one adverse alleles had a dramatically decreased glioma risk and individuals with a positive asthma/allergy history who had 2 to 3 adverse alleles had an even further reduced glioma risk [adjusted OR: 0.58 (0.35–0.94) and 0.44 (0.26–0.79), respectively] (Table 4).

Table 4. Gene dose and asthma in glioma cases and controls

Number of adverse genotypes	Number of cases/controls	OR (95% CI) ^a	P _{trend}
Three risk-conferring SNPs			.005
0–1 ^b	187/222	1.00	
2	143/122	1.41 (1.03–1.95)	
3	37/19	2.33 (1.26–4.35)	
Asthma and the three risk-conferring SNPs			<.0001
No asthma and 0–1 ^c	151/157	1.00	
No asthma and 2	128/79	1.70 (1.19–2.48)	
No asthma and 3	31/13	2.52 (1.23–5.24)	
Have asthma and 0–1	36/65	0.58 (0.35–0.94)	
Have asthma and 2–3	21/49	0.44 (0.26–0.79)	

^aAdjusted for age and gender.

^bWe treated the minor allele of the 2 risk-effect SNPs and the common allele of the protective-effect SNPs as the adverse allele, and set individuals with one or no adverse allele as the reference group. Adverse genotypes: for the protective SNP, *IL13* rs20541 (recessive, WW + WV); for the 2 risk SNPs, *COX2* rs20417 (dominant, WV + VV) and *IL10* rs1800896 (recessive, VV).

^cThe reference group consists of those with no asthma and one or no adverse allele.

Haplotype Analysis

Strong LD was observed between each pair of the SNPs in the *IL4*, *IL4R*, *IL10*, and *IL6* genes, but not *IL13* and *IL1B* genes (data not shown). The associations between the frequency distributions of the *IL1*, *IL4*, *IL4R*, *IL6*, and *IL10* haplotypes and the risk of glioma were examined; however, none of the haplotypes of these genes were found to be significantly associated with glioma risk or protection (Table 5). Only a marginal effect was observed for the *IL10* "ATA" haplotype (adjusted OR: 0.81, 95% CI: 0.61–1.05) compared with the common "GCC" haplotype. The global score test also showed no statistically significant differences in the haplotype profiles for these genes between cases and controls.

Discussion

In this study, we utilized a multianalytic strategy to explore the potential associations between a panel of

Table 5. Haplotype analyses of glioma risk

Gene	Haplotype	Frequency			OR (95% CI) ^a	Global score test P value ^b
		Total	Case	Control		
<i>IL1</i>	C T	0.464	0.467	0.461	1.00	.67
	C C	0.307	0.293	0.320	1.10 (0.86–1.42)	
	T T	0.199	0.203	0.195	1.01 (0.75–1.36)	
	T C	0.031	0.038	0.025	0.67 (0.30–1.47)	
<i>IL4</i>	C C	0.734	0.747	0.721	1.00	.60
	C T	0.125	0.113	0.137	1.24 (0.88–1.74)	
	T C	0.073	0.075	0.071	0.99 (0.64–1.52)	
	T T	0.068	0.065	0.071	1.10 (0.70–1.73)	
<i>IL4R</i>	A T T T A	0.773	0.773	0.774	1.00	.65
	C C C T G	0.104	0.110	0.099	0.86 (0.61–1.21)	
	A T C G G	0.62	0.062	0.063	1.03 (0.66–1.60)	
	A T T T G	0.050	0.045	0.056	1.29 (0.80–2.08)	
<i>IL6</i>	G G G	0.546	0.545	0.548	1.00	.93
	C G A	0.385	0.388	0.381	0.97 (0.78–1.21)	
	G C G	0.060	0.056	0.063	1.04 (0.66–1.63)	
<i>IL10</i>	G C C	0.475	0.455	0.475	1.00	.32
	A C C	0.288	0.281	0.295	1.12 (0.88–1.43)	
	A T A	0.237	0.225	0.251	1.20 (0.93–1.56)	

^aAdjusted for age and gender.

^b*IL10*: 1082 G>A, –819 C>T, –592 C>A; generated by permutation test (10 000 times).

inflammation gene SNPs and glioma risk. In the single-locus analysis, 1 pro-inflammatory gene SNP (*COX2* rs20417, -765) and 2 anti-inflammatory gene SNPs (*IL10* rs1800896, -1082 and *IL13* rs20541, R144Q) were significantly associated with glioma risk. When we examined the joint effects of the risk-referring alleles of these 3 SNPs, we found a significant trend indicating that the risk increases as the number of adverse alleles increases. Stratification by asthma status showed that this dose-response-like trend is only present among individuals without a history of allergies or asthma.

Previous studies have assessed SNPs in cytokine genes for their associations with glioma risk with mixed results. It has been reported that the *IL6* rs1800795 SNP may be associated with risk of glioma,⁸ but this could not be replicated in our study. Furthermore, Schwartzbaum et al.⁵ found that *IL4R* rs1805015 and rs1801275 were associated with increased glioblastoma risk and that the CT/TT genotypes of *IL13* C1112T (rs1800925) were significantly protective against glioblastoma. However, a subsequent study failed to confirm these findings.⁶ Similarly, our study failed to find significant associations of these variants with the risk of glioma, but found evidence of an association between *IL13* rs20541 and glioma risk. The failure to replicate the published results of some single-locus analyses might be due to the complex genetic architecture, in which locus-locus and gene-gene interactions are the expected phenomenon rather than the exception, especially in intricate pathways such as inflammation. Unlike our study, these previous studies did not explore potential higher-order interactions between genes, and thus, may have only had the ability to reveal pieces of the complex network of interactions at play in the pathway of interest.

Although the functional relevance of these SNPs is not completely understood, several lines of evidence suggest that the associations found in our study appear to be biologically plausible. Studies have suggested that the variant (T) allele of *IL13* rs20541 can increase IL-13 production,²⁵ a pivotal inflammatory cytokine. Not surprisingly, IL-13 has been positively associated with having higher IgE levels and with the development of allergic asthma.^{26,27} We found this same *IL13* rs20541 genotype (TT) to have a significant protective effect against gliomagenesis. *COX2* (PTGS2), which is downregulated by IL-13, is involved in the generation of prostaglandins, which mediate inflammation in bronchial asthma and negatively impact the secretion of glucose-stimulated insulin.^{14,15} We found an increase in glioma risk associated with the variant allele (G) of *COX2* rs20417; however, to date, the functional effect of this SNP on gene expression remains unknown.

IL-10 is an anti-inflammatory cytokine and can generally be considered an immunosuppressive factor.¹⁹⁻²¹ Of the three SNPs found to be associated with the development of glioma, *IL10* rs1800896 seemed to be consistently important throughout different types of analyses (ie, single-locus, haplotype, and joint effect analyses).

The protective effect of the *IL10* ATA haplotype, compared with GCC (rs1800896, rs1800871, and rs1800872), is consistent with the reported function of this variant haplotype, which appears to result in lower IL-10 expression.²¹ The fact that the protective effect of this haplotype was only of marginal statistical significance may be due to inadequate statistical power. Regardless, the results of this study imply that alterations in these inflammation pathway genes may have a major influence on glioma risk through both known and yet undiscovered pathways. Thus, further investigation of their roles in gliomagenesis in other larger study populations is warranted.

Perhaps, the most interesting finding in our study was that an increasing number of adverse alleles (of *IL13* rs20541, *COX2/PTGS2* rs20417, and *IL10* rs1800896) were associated with increasing glioma risk among those without a history of asthma/allergies. The discrepancy in this trend between those with and without asthma/allergies indicates that asthma status strongly modifies the effect of the risk-conferring SNPs. The protection conferred by having asthma/allergies may be stronger than the risk conferred by the adverse alleles. Thus, the trend of increasing risk with increasing number of adverse alleles that is present among the individuals without asthma may be neutralized or counterbalanced in those who do have a history of asthma/allergies. In fact, there may possibly be the opposite trend among those with asthma/allergies, in that an increase in the number of alleles appears to confer a decrease in risk. One implication of our findings could be true biological importance; however, these findings may also be due to a statistical fluctuation in the data (ie, from low precision, etc.).

As with any epidemiologic study, our study has some limitations. The main limitation of the current study is that the history of asthma/allergies was established through self-report. Little is known about the accuracy of self-reported lifetime asthma/allergy history. However, as asthma tends to be a relatively severe atopic condition, it is unlikely that the quality of the data would suffer from poor recall. Several previous studies also use the self-reported history of allergic conditions as an indicator of allergic status. In fact, relatively few questionnaires that attempt to measure a history of asthma and/or allergies have been validated. Validating asthma questionnaires is particularly difficult because of the absence of a universally accepted definition of asthma. Regardless, if there were differential recall bias present, cases would be more likely to report a history of asthma/allergies than controls, leading to a bias toward the null. In the present study, we did not ask about specific types of allergies, antihistamines, or asthma severity, but we plan to gather additional data on these topics in future studies to allow for more detailed analyses. Another limitation of our study is the sample size. Although we had a priori hypotheses to examine interactions, this study was meant to be mainly exploratory, and as such, has provided several hypotheses to be confirmed in a larger

study. Some of the inconsistencies between our study and those previously published may be related to the statistical fluctuation present when there is a lack of an adequate sample size—a common problem in studies of outcomes as rare as glioma. Larger studies with greater statistical power will be necessary to confirm the associations reported both here and in previous literature.

In summary, this case–control study indicates that polymorphisms in inflammation pathway genes are associated with glioma susceptibility. In addition, we identified a joint effect between allergy/asthma history and polymorphisms in these genes. These findings suggest that there might be significant gene–gene and gene–environment interactions that comodulate adult glioma susceptibility. Confirmation of these findings in

a larger epidemiologic study and further research on the functional significance of these SNPs and the biological mechanisms underlying such association are warranted.

Conflict of interest statement. None declared.

Funding

This study was supported by grants from the NIH (R01CA070917, PI: M.L.B.). Fellowship support for E.A. was provided through the Cancer Prevention Research Training Program (CA056452; PI: R.M. Chamberlain).

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