

Allergy-Related Polymorphisms Influence Glioma Status and Serum IgE Levels

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Abstract

Previous studies have shown that glioma patients report allergies less frequently than controls, harbor lower atopy-associated IgE levels, and harbor different frequencies of polymorphisms in the *IL13* and *IL4* pathways than controls. We sought to confirm this latter result and extend the analysis to IgE levels. Glioma patients ($n = 456$) and controls ($n = 541$) were genotyped for genetic variants in *IL4*, *IL4R*, and *IL13* and tested for total IgE levels ($n = 248$ controls and 289 cases). Among Whites, *IL4* and *IL4R* polymorphisms and haplotypes were neither significantly associated with IgE levels in controls nor associated with glioma status. *IL13* *R110G* and *C-1112T* were associated with increased IgE levels in controls ($P < 0.001$ and $P = 0.04$, respectively), and *IL13* *C-1112T* was inversely associated with case-control status ($P = 0.05$, test for

trend in dose model). An *IL4R* haplotype was borderline associated with increased risk in case-control analysis [odds ratio (OR), 1.5; 95% confidence interval (95% CI), 1.0-2.3]. In addition, a rare haplotype for *IL4* was associated with decreased risk (OR, 0.23; 95% CI, 0.07-0.83), and a common haplotype in *IL13* was associated with decreased risk (OR, 0.73; 95% CI, 0.53-1.00). Our data provide evidence for a role of *IL13* polymorphisms on IgE levels and a role for *IL4*, *IL4R*, and *IL13* haplotypes on case-control status. We did not find any evidence that the interleukin (IL) polymorphisms exerted their effect on glioma risk via their effects on IgE levels. Further exploration of immune susceptibility factors, including genetics, in glioma etiology is advisable. (Cancer Epidemiol Biomarkers Prev 2007;16(6):1229-35)

Introduction

Glioma is a rare and rapidly fatal disease. Research efforts have made little headway in discovering the causes and reducing morbidity of glioma; however, in both of these areas, there are recent indications that immune factors may play a strong role. A long-standing observation that allergies are reported less frequently among glioma cases than healthy controls has been bolstered in recent case-control and cohort studies (1-5). This observation is reinforced by the presence of higher IgE levels among controls than glioma patients (6), a strong relationship between IgE levels and survival among glioma patients (7), and the finding that allergy-associated genetic variants in *IL13* and *IL4R* are inversely related to glioma (8). These cytokine networks are also a subject of fervent research with regards to glioma treatment; for example, the presence of high levels of interleukin 13 (IL13) receptors on glioma cells has provided a basis for chemotherapy delivery via an IL13 tether (9); in animal models, i.v. IL4 treatment helps to clear transplanted glioma tumors by inducing a strong eosinophilia (10).

A notable theme of the studies mentioned above is the prominence of molecules that feature attributes of the T-cell helper-2 (Th2) branch of immunity, which is critical for clearance of certain human parasites and features strongly in conditions such as allergy and successful pregnancy. Successful immune reactions against cancer are thought to consist of largely a Th1 phenomenon, with the notable presence of

cytotoxic T cells, natural killer cells, and activated macrophages (11). This branch of the immune system is effective at eradicating viral infections and other conditions that involve the creation of new intracellular antigens, such as cancer. Brain tumors (as well as normal brain tissue), however, strongly restrain the activity of Th1 immune reactions by means of suppressive cytokines; a physical architecture constrained to inhibit inflammation; a restricted repertoire of supportive immune cells, such as professional antigen-presenting cells; and low expression of stimulating cell surface major histocompatibility proteins (12-14). The available epidemiologic and clinical data noted above suggest that features associated with Th2-type immunity, such as allergies and high IgE levels, may help to improve anti-glioma immunity.

We recently showed that glioma cases had lower IgE levels, mirroring their lower reported allergy levels, than population-based frequency-matched controls (6). The decreased rate of allergies and lower IgE levels in glioma patients may have an underlying genetic basis. We now report on the role of common, well-characterized single nucleotide polymorphisms (SNP) and haplotypes in our study population in cytokine genes critical for allergy and essential for IgE production (*IL4*, *IL4R*, and *IL13*). These genes are among the most intensely studied SNPs for allergy and IgE production (15), providing an adequate initial survey into the genetics of immunoregulation and glioma. The receptors for *IL13* are not appreciably polymorphic at the planning of this study and were not assessed here.

Materials and Methods

Study Participants. Study subjects were recruited by population-based methods in the six San Francisco Bay Area counties in two separate ascertainment periods (1991-1994 and 1997-1999). Cases were identified via rapid case ascertainment methods using the Northern California Cancer Registry as previously described (5). Controls were selected through

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random digit dialing and frequency matched to cases by age, race, and gender. Subjects or their proxies completed a detailed in-person interview including history of allergies (5). Cases included all individuals diagnosed with *International Classification of Diseases* codes 9380 to 9481, but the individuals used in the current genotyping analysis were confined to astrocytic cases. These were 60% glioblastoma, 17% astrocytoma and anaplastic astrocytoma, 17% oligodendroglioma and oligoastrocytoma, and 6% others. All study methods were approved by the University of California, San Francisco Committee on Human Research, and all participants (or their proxies if deceased) signed detailed consent forms, in accordance with the Helsinki Doctrine. Diagnostic pathology slides and reports were obtained from hospitals and reviewed and classified by a single neuropathologist. Blood and sera were collected either at the time of interview or at a later time and stored. DNA was isolated using conventional methods before genotyping. Buccal cells were collected on cytobrushes from individuals who refused blood collection. For controls ($N = 541$), 452 (83.5%) were blood, and 89 (16.5%) were buccal; and for cases ($N = 461$), 410 (88%) were blood, and 56 (12%) were buccal. IgE levels were assessed as described previously (6), and we only report on IgE levels determined in our study population during the ascertainment period 1997 to 1999, from which sera were available.

Genotyping. DNA was isolated from heparinized whole blood using Qiagen column purification. For subjects who provided buccal specimens, buccal swabs were inserted into a 1.5-mL tube with 300 to 600 μ L of 50 mmol/L NaOH and vortexed. The brush was then removed from the tube, making sure all liquid was reserved. The tube was boiled in a water bath at 95°C for 5 min. The tube was centrifuged at 14,000 rpm for 1 min; the liquid was transferred into a new vial; and the solution was neutralized by adding a 1:10 volume (10% final concentration) of 1 mol/L Tris-EDTA (pH 8.0). DNA concentration was measured using Hoescht-33258 fluorimetry. Up to 10 μ L was used in a 50 μ L PCR reaction. All PCR reactions

were at 94°C for 30 s at the various annealing temperatures specific for each gene sequence for another 30 s then at 72°C for a final 30 s. A portion was run on 3% to 4% agarose gel, and another portion of the product digested overnight or for 4 h at 37°C with a restriction enzyme that distinguished two alleles (variable depending on the sequence). A second electrophoresis run of the digested products indicated the genotype. Quality control measures include blinded analyses and the routine running of replicates of 10% of samples, DNA samples of known genotypes, and negative controls (no DNA). The dbSNP reference numbers are as follows: for *IL4R*, the numbers are rs1805010, rs1805011, rs1805012, rs1805015, rs1801275, rs1805016 for I75V, E400A, R431C, P503S, Q576R, and A752S, respectively. For *IL4*, the numbers are rs2243250 and rs2070874 for C-589T and C-34T, respectively. Finally, for *IL13*, the numbers are rs1800925 and rs20541 for C-1112T and Arg110Gln, respectively. Primers for genotyping *IL4R* and *IL4* C-34T have been published (7). Additional primers include *IL4* C-589T, ACCCAAAGTAGGCCTCACCT and ACAGGTG-GCATCTTGGAAAC; *IL13* C-1112T, GGAATCCAGCATG-CCTTGTGAGG and GTCGCCTTTTCCTGCTCTCCCCG; and *IL13* Arg110Gln, GAAACTTTTCGCGAGGGGC and GAAA-CTTTTCGCGAGGGGC.

Data Analysis. When considered singly, genotypes were considered both in a "dose-model" (heterozygotes having intermediate function between the two homozygote genotypes) and also in a "dominant model" (both heterozygote and homozygote variants are functionally equivalent). Because haplotypes are more rare, they were considered in the dominant model only. IgE levels were normalized by a transformation step: taking the least-squared means. Haplotypes at each locus were predicted using a Bayesian method implemented in PHASE 2.0 (16) and also using an expectation maximization algorithm in SAS/GENETICS (SAS Institute). Because of fairly strong linkage disequilibrium, results from both methods of haplotype prediction were virtually identical. Global likelihood ratio tests were done over all haplotypes

Table 1. Characteristics of participants with and without genotyping data: age, gender, ethnicity, education, income, and smoking

	Participants								Non-participants*	
	Cases				Controls				Cases [†]	Controls [‡]
	All ($n = 873$)	With genotyping ($n = 466$)	No genotyping ($n = 407$)	$P^{\S}$	All ($n = 864$)	With genotyping ($n = 541$)	No genotyping ($n = 323$)	P^*	($n = 108$)	($n = 240$)
Mean age $\pm$ SE	55.4 $\pm$ 0.5	50.8 $\pm$ 0.7	60.6 $\pm$ 0.8	<0.0001	54.9 $\pm$ 0.6	55.3 $\pm$ 0.7	54.3 $\pm$ 1.0	0.3742	60.1 $\pm$ 1.6	55.6 $\pm$ 1.1
% White	82.8	83.1	82.6	0.8476	83.8	87.3	77.5	0.0002	13.0	54.2
% Male	56.4	59.2	53.1	0.0673	54.4	53.9	55.3	0.7460	63.0	58.8
% GBM	59.5	49.1	71.3	<0.0001	—	—	—	—	70.4	—
% College graduate	40.1	46.8	32.4	<0.0001	44.6	48.0	38.8	0.0074	8.7	37.4
Mean education (y)	14.3 $\pm$ 0.1	14.9 $\pm$ 0.2	13.6 $\pm$ 0.2	<0.0001	14.9 $\pm$ 0.1	15.2 $\pm$ 0.1	14.4 $\pm$ 0.2	0.0004	NC	NC
Household income (US\$/y)										
≤\$29,999 (%)	26.0	16.9	36.6	<0.0001	25.4	21.7	31.7	<0.0001	NC	NC
\$30-49,999 (%)	23.7	23.4	24.0		21.2	20.4	22.6			
\$50-69,999 (%)	15.8	17.8	13.4		19.6	19.5	19.9			
\$70-99,999 (%)	15.9	19.4	11.8		17.0	19.5	12.8			
≥\$100,000 (%)	18.7	22.5	14.2		16.8	18.9	13.1			
Smoking										
% Never	44.1	47.6	40.2	0.0852	42.7	42.1	43.8	0.9430	34.8	43.4
% Past	41.0	38.4	44.1		41.0	41.5	40.0		47.8	38.0
% Current	14.8	14.0	15.8		16.3	16.4	16.3		17.4	18.7

NOTE: The San Francisco Bay Area Adult Glioma Study 1991-1999.

Abbreviations: NC, data not collected; GBM, glioblastoma multiforme.

*Non-participants are eligible cases identified through RCA (series 2 only) and controls identified by random digit dialing (series 1 and 2) who did not complete a full questionnaire.

[†]For cases, ethnicity, age, gender, and histology are based on data from Surveillance, Epidemiology, and End Results, and education and smoking are from the abbreviated questionnaire ($n = 23$).

[‡]For controls, all variables listed are from the abbreviated questionnaire.

[§] P values adjusted for all variables listed comparing with versus without genotyping data.

Table 2. Log(IgE) by IL genotypes for series 2 (cases from 1997-2000), White controls only

Gene	SNP name		White controls mean log(IgE) by genotype			ANOVA <i>P</i> *
<i>IL4</i>	C-589T	Genotype	CC	TC	TT	0.59
		<i>n</i>	172	69	6	
		Adjusted LS mean [†]	3.3	3.5	3.4	
	C-34T	Genotype	WW	VV	VV	0.37
		<i>n</i>	180	63	5	
		Adjusted LS mean	3.3	3.6	3.2	
<i>IL4R</i>	I75F	Genotype	AA	AG	GG	0.40
		<i>n</i>	87	116	44	
		Adjusted LS mean	3.3	3.5	3.1	
	E400A	Genotype	AA	AC	CC	0.82
		<i>n</i>	195	47	5	
		Adjusted LS mean	3.4	3.2	3.3	
	R431C	Genotype	TT	TC	CC	0.86
		<i>n</i>	197	45	4	
		Adjusted LS mean	3.4	3.3	3.2	
	P503S	Genotype	TT	TC	CC	0.81
		<i>n</i>	174	63	10	
		Adjusted LS mean	3.4	3.4	3.1	
	Q576R	Genotype	AA	AG	GG	0.86
		<i>n</i>	155	78	14	
		Adjusted LS mean	3.3	3.4	3.4	
<i>IL13</i>	A752S	Genotype	TT	TG	GG	0.63
		<i>n</i>	219	26	1	
		Adjusted LS mean	3.4	3.5	2.0	
	Arg110Gln	Genotype	GG	GA	AA	<0.001
		<i>n</i>	150	90	7	
		Adjusted LS mean	3.1	3.6	5.4	
	C-1112T	Genotype	CC	CT	TT	0.04
		<i>n</i>	156	78	12	
		Adjusted LS mean	3.2	3.7	3.9	

NOTE: The San Francisco Bay Area Adult Glioma Study 1991-1999.

Abbreviation: LS, least square.

*All *P* values for LS means are type 3 from Proc GLM.

† All least squared means adjusted for age, gender, ethnicity, smoking, and education level (6).

and estimates of exact *P* values were computed using Monte Carlo methods with 10,000 permutations. Multiple logistic regression was used to model the log odds of disease as a function of the individual's haplotype probabilities, adjusting for age, gender, and ethnicity. In addition, the diplotype, or combination of two haplotypes, was predicted for each subject in the study, and each diplotype category was compared with regard to case-control status and IgE levels. IgE levels were adjusted for age, gender, smoking, ethnicity, and education level as described (6), as these factors were associated with IgE levels in many population studies. For case-control comparisons not involving IgE, adjustments for age and gender (only) were made. IgE levels were considered on a categorical scale for some analyses ("undetectable," "borderline," and "elevated"), as these categories have clinical significance (6); for other analyses, the least square means of IgE levels was used as a continuous variable as noted above. This transformed value of IgE approximates a Gaussian distribution.

Results

Participant Population. Participants were enrolled over two time periods (series 1 and 2) from the same geographic region. A detailed description of total numbers of cases and controls contacted, participant counts from each series, refusal rates, and reasons for refusal were presented previously (17) and will not be repeated here except for a few salient points. Case subjects with available DNA were 10 years younger on average than those with no available DNA, due to lack of available sample from older patients who generally have more rapidly fatal outcomes. There were also significant differences among participants with DNA from those without for years of education and household income for both cases and controls, histology among cases, and ethnicity among controls (Table 1).

Genotypes, IgE, and Allergies. All (100%) blinded repeat samples were concordant with each other. No individual SNPs were divergent from Hardy-Weinberg equilibrium, by χ^2 analysis. Individuals carrying the heterozygote or homozygote rare-variant of polymorphisms in *IL4* and *IL4R* did not differ from those carrying the homozygote common-variant in IgE levels among the controls (Table 2). *IL13* polymorphisms on the other hand were significantly associated with IgE levels: the coding variant *Arg110Gln* (*P* < 0.001) and the promoter variant *C-1112T* (*P* = 0.04). Among the controls, there were no associations of any SNPs tested with numbers of allergies or IGE levels (data not shown). When control carriers of specific "risk" alleles (see below) were compared with the rest of controls, some trends emerged. Control carriers of *IL4R* 111110 haplotype were more likely to exhibit "elevated" serum IgE (4 of 15 controls or 27%) than controls that did not carry the haplotype (16 of 171 or 9.3%; *P* = 0.06, Fisher's exact test), but the least-squared mean of log(IgE) levels was not higher among carriers (*P* = 0.82). Control carriers of the 11 haplotype of *IL13* had higher IgE levels than controls without this haplotype (least-squared mean IgE, 3.97 versus 3.25; *P* = 0.001). Finally, we did ANOVA tests to determine whether SNPs or diplotypes of *IL4R*, *IL4*, and *IL13* were linked to having allergies or numbers of allergies and found no associations between these variables (data not shown).

Genotypes, Haplotypes, and Case-Control Status. Because of potential bias introduced by ethnic population substructure, only Whites were included for case-control analyses, as these represent the largest subgroup in the study. With the exception of *IL13*, no significant associations were noted between individual genotypes and case/control status (Table 3). The *IL13* promoter polymorphism (*C-1112T*) was associated with a reduced risk of brain cancer when considered as a codominant trait (dose model, Table 3; *P* = 0.05). When the disease category was confined to the largest homogeneous subtype, stage IV

glioblastoma, this relationship was not found to be stronger (data not shown).

The overall distribution of haplotypes among all chromosomes were compared among cases and controls showing some differences in frequencies of minor haplotypes (i.e., *IL4* 01 and *IL13* 11; Table 4). These differences among frequencies do not take into account the haplotypes carried by individual study participants or genetic mode of expression.

A single *IL4R* haplotype (111110) was carried more among the cases compared with controls [odds ratio (OR), 1.5; 95% confidence interval (95% CI) 1.0-2.3; Table 5]. Interestingly, this same haplotype, which is associated with increased risk of glioma, was also associated with longer survival time in the same study group (7). Because of this association, we did a case-control comparison using only those cases who had been diagnosed less than 3 months before blood draw, thereby lessening the potential bias from survival. For this group ($n = 431$ subjects), the OR was very similar (arguing against the

bias) and not significant (OR, 1.52; 95% CI, 0.72-3.21). In contrast, a rare *IL4* haplotype 01 was significantly underrepresented among the cases (OR, 0.23; 95% CI, 0.07-0.83; Table 5). A common *IL13* haplotype, which consists of the more rare variant at both positions (11), was underrepresented among the cases compared with controls (OR, 0.73; 95% CI, 0.53-1.00; Table 5). To better compare our results with Schwartzbaum et al. (8), we did *IL4R* haplotype analysis with a reduced data set, including only the *S503P* and *Q576R* SNPs. Haplotypes were constructed with the two SNPs, and both haplotype frequencies and diplotypes individuals were compared between cases and controls. This reduced data set did not yield any significant associations, although the risk ratios were in the same direction as Schwartzbaum reported (OR, 1.13; 95% CI, 0.83-1.53 for carriers versus non-carriers; ref. 8).

Further analyses were done to examine gender-specific effects and interactions between IgE, allergies, and *IL* SNPs and haplotypes. We did not find consistent differences

Table 3. Individual genotype case-control ORs, Whites only

Genotype	Cases		Controls		OR* (reference = wt/wt only)	P	OR* (dose response model)
	Total N	n (%)	Total N	n (%)			
<i>IL4</i> /C-589T							
CC	384	278 (72)	468	313 (67)	1.00		1.00
TC		95 (25)		144 (31)	0.77 (0.57-1.0)	0.093	0.74 (0.55-1.0)
TT		11 (3)		11 (2)			1.2 (0.49-2.7)
$P_{\text{trend}} = 0.188$							
<i>IL4</i> /C-34T							
CC	386	281 (73)	471	328 (70)	1.0		1.00
CT		93 (24)		134 (28)	0.87 (0.64-1.2)	0.366	0.82 (0.60 - 1.1)
TT		12 (3)		9 (2)			1.6 (0.64 - 3.8)
$P_{\text{trend}} = 0.645$							
<i>IL4R</i> /175F							
AA	387	119 (31)	471	148	1.0		1.00
AG		196 (51)		232 (49)	1.0 (0.77-1.4)	0.830	1.0 (0.77-1.4)
GG		72 (19)		91 (19)			1.0 (0.67-1.5)
$P_{\text{trend}} = 0.970$							
<i>IL4R</i> /E400A							
AA	387	301 (78)	471	386	1.0		1.00
AC		78 (20)		79 (17)	1.3 (0.95-1.9)	0.101	1.3 (0.92-1.9)
CC		8 (2)		6 (1)			1.6 (0.54-4.7)
$P_{\text{trend}} = 0.095$							
<i>IL4R</i> /R431C							
TT	386	310 (80)	469	388	1.0		1.00
TC		69 (18)		76 (16)	1.2 (0.83-1.7)	0.351	1.2 (0.80-1.7)
CC		7 (2)		5 (1)			1.6 (0.50-5.2)
$P_{\text{trend}} = 0.298$							
<i>IL4R</i> /P503S							
TT	386	274 (71)	470	341 (73)	1.0		1.00
TC		99 (26)		113 (24)	1.1 (0.81-1.5)	0.547	1.1 (0.81-1.5)
CC		13 (3)		16 (3)			0.98 (0.46-2.1)
$P_{\text{trend}} = 0.643$							
<i>IL4R</i> /Q576R							
AA	384	243 (63)	469	303	1.0		1.00
AG		126 (33)		144 (31)	1.1 (0.82-1.4)	0.576	1.1 (0.83-1.5)
GG		15 (4)		22 (5)			0.86 (0.43 -1.7)
$P_{\text{trend}} = 0.782$							
<i>IL4R</i> /A752S							
TT	382	343 (90)	462	405	1.0		1.00
TG		39 (10)		55 (12)	0.84 (0.55-1.3)	0.449	0.88 (0.57-1.4)
GG		0		2 (0)			—
$P_{\text{trend}} = \text{not valid}$							
<i>IL13</i> /Ara110Gln							
GG	385	247 (64)	468	281 (60)	1.0		1.00
GA		123 (32)		169 (36)			0.85 (0.64 - 1.1)
AA		15 (4)		18 (4)	0.86 (0.65-1.1)	0.298	0.92 (0.45 -1.9)
$P_{\text{trend}} = 0.359$							
<i>IL13C</i> -1112T							
CC	379	256 (68)	459	281 (61)	1.0		1.00
CT		112 (30)		156 (34)	0.76 (0.57-1.0)	0.068	0.78 (0.58-1.1)
TT		11 (3)		22 (5)			0.60 (0.28-1.3)
$P_{\text{trend}} = 0.053$							

NOTE: The San Francisco Bay Area Adult Glioma Study 1991-1999.

*OR and 95% CI were calculated using multiple logistic regression adjusting for age and gender.

Table 4. Haplotype frequencies among white adult glioma cases and controls

Gene/haplotype	Cases	Controls	χ^2 *	P	OR (95% CI) [†]
<i>IL4R</i>[‡]					
000000	48.2	47.4	0.62	0.73	1.03 (0.85-1.25)
011110	3.2	3.4	0.03	0.85	0.92 (0.54-1.58)
100000	31.1	31.7	0.06	0.81	0.97 (0.79-1.19)
100111	4.3	5.2	0.80	0.37	0.85 (0.54-1.34)
111110	7.5	5.5	2.80	0.09	1.44 (0.97-2.13)
Pooled [§]	5.7	6.9	1.03	0.31	0.82 (0.55-1.22)
Gene copies	774	944			
Global test : $\chi^2 = 33.43$ ($P = 0.129$)					
<i>IL4</i>*					
00	84.5	82.3	1.41	0.24	1.16 (0.89-1.50)
11	14.9	16.0	0.39	0.53	0.94 (0.72-1.23)
01	0.4	1.6	5.90	0.02	0.22 (0.06-0.78)
10	0.3	0.1	0.57	0.45	1.74 (0.15-19.57)
Gene copies	772	944			
Global test : $\chi^2 = 7.69$ ($P = 0.069$)					
<i>IL13</i>*					
00	73.8	70.9	1.81	0.18	1.14 (0.92-1.41)
01	6.4	7.0	0.29	0.59	0.90 (0.61-1.33)
10	8.3	7.0	0.93	0.998	1.19 (0.83-1.70)
11	11.5	15.1	4.57	0.03	0.76 (0.57-1.01)
Gene copies	772	942			
Global test : $\chi^2 = 5.60$ ($P = 0.143$)					

NOTE: San Francisco Bay Area Glioma Study 1991-1999.

*Pearson χ^2 test was used to compare an individual haplotype versus all others combined in a 2 × 2 table.[†]Haplotype OR and 95% CI were calculated using multiple logistic regression modeling haplotype probabilities and adjusting for age and gender (each haplotype is compared with all other haplotypes).[‡]The order of the *IL4R* SNP loci in the haplotypes is *I75F*, *E400A*, *R431C*, *P503S*, *Q576R*, and *A752S*. The order of the *IL4* SNP loci in the haplotypes is *C-34T* and *C-589T*. The order of the *IL13* SNP loci in the haplotypes is *Arg110Gln* and *C-1112T*. For each loci, the more frequent allele is indicated with a 0, and the less frequent allele with a 1. The nucleotide identity of the alleles is shown in Table 3.[§]Pooled rare haplotypes include 000001, 000010, 000011, 000100, 000110, 000111, 001110, 010000, 010010, 011100, 011111, 100001, 100010, 100110, 101110, 110000, 110001, 110011, 110110, and 111100. Other potential haplotypes (of 64 possible) not observed.^{||}Global likelihood ratio test done over all haplotypes. Estimates of exact *P* values were computed using Monte Carlo methods with 10,000 permutations.

between males and females. When *IL* SNPs and IgE levels or allergies were included in multivariable models with an interaction term, no statistical interaction between these factors was evident, nor did *IL* SNP or IgE OR change in direction or magnitude (data not shown).

Discussion

We assessed immune gene polymorphisms in glioma etiology for two reasons. First, the consistently observed association of allergies and asthma to glioma risk begs the question whether similar genetic factors lie behind both allergy and glioma onset (5). Second, Schwartzbaum et al. discovered associations between these genes and glioma risk in a smaller population from Sweden, requiring independent confirmation, and extension to IgE levels (8). Common SNPs in *IL4*, *IL4R*, and *IL13* were not individually strongly associated with glioma in our population; however, two *IL13* polymorphisms affected IgE levels significantly among non-diseased controls (Table 2). These polymorphisms also were linked to IgE in the same direction in several other studies (reviewed in ref. 18). One of these polymorphisms, a promoter SNP at the -1112 position, was borderline significantly associated with glioma case-control status (Table 3), but refinement of the disease category to those with grade 4 glioblastoma did not enhance this or other associations (data not shown). Diplotype analysis helped to reveal potential associations between specific haplotypes and case-control status; for two of these genes (*IL4R* and *IL13*), these associations were predicted from prior results on the

genetics of allergy and brain tumors as well as our studies on brain tumor survival, as explained below.

The associations detected here between *IL13* C-1112T polymorphism and case-control status is consistent with the data of Schwartzbaum et al., who observed a case-control OR of 0.56 (95% CI, 0.33-0.96) for this SNP in the codominant model with 105 cases and 403 controls (8), but other polymorphisms were null in the current study when assessed singly. The 95% CIs in the two studies overlap for all SNPs studied in common, indicating that the data are in effect

Table 5. Case-control comparisons of haplotypes of *IL4R*, *IL4*, and *IL13* polymorphisms, using a dominance genetic model

<i>IL4R</i> diplotypes*	Cases (n = 387)	Controls (n = 472)	OR (95% CI) [†]
000000			
Other/other	97 (25.1)	121 (25.9)	1.00 (reference)
≥1 copy	290 (74.9)	350 (74.1)	1.03 (0.75-1.40)
011110			
Other/other	363 (93.8)	443 (93.4)	1.00 (reference)
≥1 copy	24 (6.2)	31 (6.6)	0.91 (0.52-1.59)
100000			
Other/other	188 (48.6)	223 (47.3)	1.00 (reference)
≥1 copy	199 (51.4)	249 (52.8)	0.94 (0.72-1.23)
100111			
Other/other	354 (91.5)	424 (89.8)	1.00 (reference)
≥1 copy	33 (8.5)	48 (10.2)	0.86 (0.54-1.38)
111110			
Other/other	331 (85.5)	422 (89.6)	1.00 (reference)
≥1 copy	56 (14.5)	49 (10.4)	1.51 (0.996-2.29)
Pool [‡]			
Other/other	346 (89.4)	411 (87.1)	1.00 (reference)
≥1 copy	41 (10.6)	61 (12.9)	0.81 (0.53-1.23)
<i>IL4</i> diplotypes*			
00			
Other/other	13 (3.4)	11 (2.3)	1.00 (reference)
≥1 copy	373 (96.6)	461 (97.7)	0.69 (0.30-1.57)
01			
Other/other	383 (99.2)	458 (97.0)	1.00 (reference)
≥1 copy	3 (0.8)	14 (3.0)	0.23 (0.07-0.83)
10			
Other/other	384 (99.5)	471 (99.8)	1.00 (reference)
≥1 copy	2 (0.5)	1 (0.2)	1.74 (0.15-19.75)
11			
Other/other	281 (72.8)	330 (69.9)	1.00 (reference)
≥1 copy	105 (27.2)	142 (30.1)	0.89 (0.66-1.21)
<i>IL13</i> diplotypes*			
00			
Other/other	22 (5.7)	33 (7.0)	1.00 (reference)
≥1 copy	364 (94.3)	438 (93.0)	1.21 (0.69-2.12)
01			
Other/other	340 (88.1)	408 (86.6)	1.00 (reference)
≥1 copy	46 (11.9)	63 (13.4)	0.88 (0.58-1.32)
10			
Other/other	326 (84.5)	408 (86.6)	1.00 (reference)
≥1 copy	60 (15.5)	63 (13.4)	1.18 (0.80-1.74)
11			
Other/other	301 (78.0)	336 (71.3)	1.00 (reference)
≥1 copy	85 (22.0)	135 (28.7)	0.73 (0.53-1.00)

NOTE: The San Francisco Bay Area Glioma Study 1992-1999.

*The order of the *IL4R* SNP loci in the haplotypes is *I75F*, *E400A*, *R431C*, *P503S*, *Q576R*, and *A752S*. The order of the *IL4* SNP loci in the haplotypes is *C-34T* and *C-589T*. The order of the *IL13* SNP loci in the haplotypes is *Arg110Gln* and *C-1112T*.[†]OR and 95% CI were calculated using multiple logistic regression adjusting for age and gender. The reference (other/other, or reference) category includes individuals who do not carry the indicated haplotype on either chromosome. The ≥1 copy category includes individuals who carry at least one copy of the indicated haplotype.[‡]Pooled rare haplotypes include 000001, 000010, 000011, 000100, 000110, 000111, 001110, 010000, 010010, 011100, 011111, 100001, 100010, 100110, 101110, 110000, 110001, 110001, 110011, 110110, and 111100. Other potential haplotypes (of 64 possible) not observed.

consistent (Table 3; ref. 8). These include two SNPs for *IL4R* (*P503S* and *Q576R*), which, when combined into haplotypes with four other SNPs *111110* (Table 5), yielded increased risk of brain cancer in case-control analysis, consistent in direction with Schwartzbaum, who observed a case control OR of 1.64 (95% CI, 1.05-2.55) for *P503S* and 1.61 (95% CI, 1.05-2.47) for *Q576R* (8). SNPs *S503P* and *Q576R* considered as haplotypes yielded a null result in our data (see Results), indicating the importance of including additional SNPs in the haplotype assessment and also suggesting that the *S503P* and *Q576R* are not the "causal" SNPs within the *IL4R* haplotype.

It should be emphasized that the *IL4R 111110* haplotype was borderline associated with "risk" of brain tumor in this analysis (OR, 1.49; 95% CI, 0.99-2.25) in the opposite direction to our previous analysis in which this haplotype was highly associated with "protection" measured by longer survival of glioma patients in a case-only analysis (HR, 0.64; 95% CI, 0.47-0.87; $P = 0.004$). Our patient population for the survival study (7) essentially overlaps with this case-control study; therefore, there are not any obvious differences in population structure that could explain the discrepancy. Versions of this haplotype (using some but not all the same SNPs) were previously linked to increased atopic asthma, decreased type I diabetes, and increased sensitivity of the *IL4R* receptor or decreased *IL4R* activity (19-22). However, the minor alleles *503P* and *576R* have been associated with decreased IgE levels and atopy in other studies (23, 24). These minor alleles comprise the fourth and fifth position of the haplotype *111110*. In our population, we were not able to detect an effect of *111110* or other *IL4R* haplotypes on allergies or IgE levels to help explain the difference in association between case-control and survival analysis. One possible explanation might be a survival bias: those individuals carrying *111110* may simply live long enough for ascertainment into our study (because it is associated with better survival), leading to a higher frequency of this haplotype among the cases, hence leading to the appearance of "risk" in the case-control analysis. From our series 2 patients (collected from 1997 to 1999), 73% of participating cases and 94% of controls provided blood for genotyping. (From series 1, only 39% of cases had blood drawn, but this was primarily due to our lack of resources for biological sample collection early in the study.) Most cases not providing blood (in series 2) were not alive at the time of interview/blood collection. Three months is the average time from diagnosis to ascertainment in our glioma study. We repeated the *111110* case-control analysis with patients and controls who had blood drawn within 3 months of diagnosis (patients) or identification (controls) and found that our OR did not change (OR, 1.50 for whole data set and OR, 1.52 for <3-month blood draw), arguing that the SNP may have an etiologic significance in addition to its previously shown survival effect. Survival bias (and etiologic bias in the case of survival studies!) should be carefully considered in future studies on this population.

In contrast to the *IL4* haplotype, the *IL13* haplotype was associated with protection in this analysis (Table 5). *IL13* had no effect on survival in our case only analysis (OR, 0.93; 95% CI, 0.69-1.2), also in contrast to *IL4R*. Because the *IL13* haplotype had no effect on survival of the genotyped cases, a potential survival bias is less likely in the case-control analysis. This result is consistent with an earlier report (25) and also consistent with the hypothesis that a polymorphism that is a risk factor for allergy should be protective for glioma. We believe that this result, not sullied by a potential survival bias as in the case of *IL4R*, is the most consistent result presented in this study, especially as *IL13* polymorphisms are also associated with IgE levels in the expected direction (Table 2). This result is less likely to be due to chance or bias. Individuals who are genetically capable of producing higher levels or a more active *IL13* cytokine may be protected from glioma. Such protection may

become moot when full-blown cancer is present, in which *IL4/IL13* pathways may be corrupted by the expression of aberrant levels of the *IL13RA2* decoy receptor (26).

Reasons for lack of concordance of the OR estimates of the singleton genotypes between Schwartzbaum et al. (8) and our study may include several factors. First, environmental differences between Sweden and California may differentially affect allergy onset and the mechanism by which these immune factors affect glioma. Second, there may be genetic linkage differences between Swedes and U.S. residents, or unmeasured population substructure differences between cases and controls that is likely to be more complex in the San Francisco Bay Area than Sweden, thus biasing our results towards the null. Third, the smaller numbers in the Swedish study may be unstable, and the point estimates in the current study may be more accurate. When examined as a haplotype, our results are quite consistent with the Swedish study (8) indicating that there are likely to be genetic factor(s) in linkage disequilibrium with particular haplotypes in *IL4R* and *IL13* that affects the risk of brain cancer and likely via an immune mechanism. A new study using a U.K./Danish cohort has also found haplotypes associated with glioma and will help further refine the associations observed here (25).

Because functional variants by themselves (except *IL13 C-1112T*) were not significantly related to case-control status, and specific haplotypes were, there remains the possibility that the associated haplotypes are in linkage disequilibrium with neighboring genes. Interestingly, the *IL4R* gene is located in chromosome 16 next to *IL21R*, a receptor critical to natural killer cell function, an important component of anti-immunity. *IL4* and *IL13* genes are both located next to each other within a cluster of cytokine-related genes in chromosome 5q31, further supporting this supposition. Future studies should assess genes neighboring to ones considered here.

This study has several strengths and limitations. Strengths include the large size (for a glioma study), relative ethnic homogeneity (for the bulk of the presented analyses), and limited number of measurements that are based on solid hypotheses. Limitations of the study include the role of chance that might lead to false-positive or false-negative findings, uncertain functional relevance of some of the SNPs measured, and limited statistical power particularly in relation to interaction tests.

The genetics of the immune system may be related to glioma under two scenarios. A genetic factor may underlie allergy onset, which may then affect the capacity of an individual's immune system to recognize and delete nascent brain tumors. Alternatively, a genetic factor may be related to allergy onset and glioma risk independently via other mechanisms on separate causal pathways. The SNPs that we assessed are good candidates for affecting IgE levels (in particular, *IL13* SNPs) and allergies but only account for a small percentage of factors that affect IgE levels. For instance, *IL13* haplotypes were predicted to account for only 0.59% of total IgE levels in a large study despite highly significant associations (reviewed in ref. 18). Other factors determining a point measurement of total IgE would include seasonality, proximity to allergen challenge, diet, and circadian rhythms. We put IgE and our allergy SNPs into the same model, including interaction terms, and found little or no effect on point estimates of these two factors and providing no evidence for interaction. It is highly unlikely given the evidence above that the SNPs measured here are the basis for the IgE-glioma or allergy-glioma connection previously discovered in this patient population (5, 6). Given that significant results were found for both IgE levels and SNPs, different, although not necessarily unrelated, pathways may be suggested by these two measurements.

In sum, this assessment of SNPs in immune-related genes in glioma etiology, when viewed as haplotypes, provides some confirmatory results to Schwartzbaum et al. (8) in a larger

more diverse population and extends the analysis to IgE and allergies. This study invites a comprehensive assessment of polymorphisms in other related immune-related genes.

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