Common Variation in Genes Related to Innate Immunity and Risk of Adult Glioma

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Abstract

Current evidence suggests that immune system alterations contribute to the etiology of adult glioma, the most common adult brain tumor. Although previous studies have focused on variation in candidate genes in the adaptive immune system, the innate immune system has emerged as a critical avenue for research given its known link with carcinogenesis. To identify genetic markers in pathways critical to innate immunity, we conducted an association study of 551 glioma cases and 865 matched controls of European ancestry to investigate "tag" single nucleotide polymorphisms (SNP) in 148 genetic regions. Two independent U.S. case-control studies included were as follows: a hospital-based study conducted by the National Cancer Institute (263 cases, 330 controls) and a community-based study conducted by the National Institute for Occupational Safety and Health (288 cases, 535 controls). Tag SNPs (1,397) chosen on the basis of an r^2 of >0.8 and minor allele frequency of >5% in Caucasians in HapMap1 were genotyped. Glioma risk was estimated by odds ratios. Nine SNPs distributed across eight genetic regions (*ALOX5, IRAK3, ITGB2, NCF2, NFKB1, SELP, SOD1*, and *STAT1*) were associated with risk of glioma with *P* value of <0.01. Although these associations were no longer statistically significant after controlling for multiple comparisons, the associations were notably consistent in both studies. Region-based tests were statistically significant ($P < 0.05$) for *SELP, SOD*, and *ALOX5*. Analyses restricted to glioblastoma ($n = 254$) yielded significant associations for the *SELP, DEFB126/127, SERPINI1*, and *LY96* genetic regions. We have identified a promising set of innate immunity-related genetic regions for further investigation. (Cancer Epidemiol Biomarkers Prev 2009;18(5):1651–8)

Introduction

Epidemiologic studies have consistently observed an inverse association between risk of adult glioma and personal history of allergy (1-8) as well as, to a lesser extent, autoimmune disease (3, 4, 9). Consequently, it has been hypothesized that germline and somatic alterations in the immune system may contribute to the pathogenesis of adult glioma. It is now possible to comprehensively study common genetic germline variants in immune genes and estimate the effect of low penetrance alleles on risk of glioma. Already, a series of individual studies have reported that common single nucleotide polymorphisms (SNP) in cytokine genes (*IL4, IL4RA, IL13*, and *IL6*) seem to be associated with risk of glioma development (7, 10-12) and/or survival (13). It is notable that the same variants seem to be associated with risk of allergic and autoimmune disorders, but further work is needed to mechanistically link the observations.

The adaptive immune response within the brain could be limited by unique features of the brain, namely the presence of the blood brain barrier which restricts the access of immune mediators and cells from the blood, and a well-established limited capacity to process antigens (14, 15). Resident microglia and astrocytes, which share many characteristics of classic innate immune cells have emerged as important effector cells (16, 17) in the central nervous system. The activation of these cells has been implicated in the pathogenesis of central nervous system infections, brain injury, cerebral ischemia, autoimmune disorders, and neurodegenerative diseases (16, 17). The innate immune system, which is phylogenetically ancient, works closely with adaptive immunity in an integrated process to ensure effective responses to a wide range of antigenic challenges, including tumors (18).

In view of the epidemiologic and biological evidence implicating alterations in the immune system and central

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Table 1. Descriptive characteristics of the participants in a hospital-based case-control study of adult glioma conducted by the NCI and in a population-based case-control study of adult glioma conducted by the NIOSH

Characteristic	NCI						NIOSH					
	Cases (n = 489)			Controls (n = 799)			Cases (n = 798)			Controls (n = 1,175)		
	All* (n = 444)	With blood (n = 391)	Genotyped (n = 263)	All (n = 715)	With blood (n = 549)	Genotyped (n = 330)	All* (n = 783)	With blood (n = 320)	Genotyped (n = 288)	All (n = 1152)	With blood (n = 578)	Genotyped (n = 535)
Male, %	57.7	56.2	55.5	46.7	47.0	46.4	57.2	58.1	59.4	55.0	55.5	55.7
Mean age, years	52.1	51.7	51.1	50.4	49.6	49.4	52.0	44.5	44.8	54.8	55.6	55.4
Education, %												
Less than high school	10.6	9.4	7.2	10.8	10.9	10.9	17.8	10.0	9.7	17.5	16.8	16.6
High school or general equivalency diploma or 3 y of college	53.6	53.7	52.1	61.0	60.5	59.1	65.5	70.9	71.5	65.5	65.2	65.6
Complete college or GRA or professional school	33.3	34.6	37.6	25.9	26.6	28.5	16.6	19.1	18.8	16.8	18.0	17.8
Unknown	2.5	2.3	3.0	2.4	2.0	1.5	0.1	0	0	0.1	0	0

*Limited to individuals of European background.

nervous system disorders, including adult glioma, we conducted an association study of adult glioma, pooling data from two independent studies to investigate tag SNPs in 148 innate immune genes and their surrounding regions.

Materials and Methods

Study Population and Setting. Details of the studies conducted by the National Cancer Institute (NCI) and the National Institute for Occupational Safety and Health (NIOSH) have been described previously (19, 20) and are summarized in Table 1.

Briefly, the NCI study was conducted between June 1994 and August 1998 at Brigham and Women's Hospital in Boston, MA; St Joseph's Hospital and Medical Center in Phoenix, AZ; and Western Pennsylvania Hospital in Pittsburgh, PA. Eligible cases were patients with newly diagnosed, histologically confirmed intracranial glioma or neuroepitheliomatous tumors (ICDO-O-2 codes 9380-9473 and 9490-9506). Cases had to be at least age 18 y, English or Spanish-speaking, and residing within 50 miles of the hospital (or within Arizona for the Phoenix hospital) at the time of diagnosis. Ninety percent (n = 489) of potentially eligible cases agreed to participate in the study. Controls were patients admitted to the same hospitals for a variety of nonmalignant conditions, with the most common reasons being injuries and poisoning (n = 197; ICD-9 codes 800-999, V01-V82, and E800-E999) and diseases of the circulatory (n = 179; ICD-9 390-459), musculoskeletal (n = 172; ICD-9 710-739), digestive (n = 92; ICD-9 520-579), and nervous systems (n = 58; ICD-9 320-389). Controls were frequency matched (1:1) to a total case series (glioma, meningioma, and acoustic neuroma) by hospital, age (in 10-y strata), sex, race/ethnicity, and distance of residence from hospital. Of the potentially eligible controls contacted, 86% (n = 799) participated.

Eligible cases from NIOSH were patients age 18 to 80 y, newly diagnosed between January 1995 and January 1997 with histologically confirmed intracranial gliomas (ICD-O-2 codes 9380-9473) in participating medical facilities and neurosurgical offices in 4 upper Midwestern states (Iowa, Michigan, Minnesota, and Wisconsin), in counties where the largest population center had <250,000 residents. Of the cases invited to participate in the study, 92% (n = 798) agreed. Population controls were glioma-free individuals randomly selected from 10-y age-sex–specific strata of the state driver's license or nondriver identification records (for those between ages 18 and 64 y) or from the Health Care Financing Administration Medicare records (for those between ages 65 and 80 y). Controls were frequency matched to cases (1.5:1) by state of residence, sex, and age. Of the eligible controls contacted, 70% (n = 1,175) participated.

The analysis was conducted for 551 glioma cases and 865 controls of European ancestry from both studies with an adequate amount of DNA extracted from blood samples (described below). Both the NCI and NIOSH studies were reviewed and approved by the respective institutional review boards, and all participants signed an informed consent upon enrollment.

Laboratory Methods

Blood Sample Collection and DNA Extraction. 391 Subjects with glioma (88%) and 549 controls (77%) of European ancestry provided blood samples in the NCI study (Table 1). In the NIOSH study (Table 1), blood samples were collected from 320 cases (41% of participating cases; 73% of 440 cases alive at the time of interview) and 578 controls (72% of 805 approached) of European ancestry. The low participation in blood sample draw for NIOSH cases was due to the fact that many patients with glioma were not alive at the time of sample collection.

Two different extraction methods were used on peripheral WBC: the NCI samples were extracted using a phenol-chloroform method (21), whereas a sodium perchlorate-chloroform method was done on the NIOSH samples (22).

Genotyping. A total of 551 cases of glioma (263 from NCI; 288 from NIOSH) and 865 matched controls (330 from NCI and 535 from NIOSH) were genotyped at the NCI Core Genotyping Facility (Advanced Technology Corporation) using an Illumina GoldenGate OPA panel designed to tag 148 candidate innate immunity genes and their surrounding regions [20 kb 5′ of the start of transcription (exon 1) and 10 kb 3′ of the end of the last exon (N) of each candidate gene]. Genes for the innate immunity panel were selected from known innate immune pathways (oxidative response, pattern recognition molecules and antimicrobials, integrins and adhesion molecules, complement, chemokines with their receptors and signaling molecules, and response genes and tissue factors), and a small number of SNPs were forced into the choice of tag SNPs based on prior evidence from association studies. Less than 5% of SNPs were forced into the panel, creating a negligible effect on the tagging algorithm. Tag SNPs were chosen from the SNPs that were genotyped as part of the International HapMap (23) using the TagZilla algorithm[5] with the following parameters: minor allele frequency of >5% in HapMap Caucasian (CEU) samples, r^2 of >0.8, and greater weighting for SNPs with a design score of 1.1 (SNPs with a design score of <0.4 were designated as ''obligate excludes'').

Quality control specimens included replicate samples from seven nonstudy participants (3 from NCI, 4 from NIOSH) and blinded duplicate samples from 58 participants (21 from NCI and 37 from NIOSH) interspersed among cases and controls. Out of 1,536 SNPs originally chosen, 72 assays failed in manufacturing or provided only monoallelic calls. For the study analyses, any SNPs that did not satisfy Hardy-Weinberg Equilibrium at P values of <0.001 in both sets of control subjects were excluded (n = 24). Additional genotype assays were excluded for low completion rate of <90% (n = 27), or poor concordance rates of <95% (n = 16). Results for SNPs with concordance rates of 95% to 97% were flagged (n = 9, after Hardy-Weinberg Equilibrium exclusion). Percent agreement between the 7 nonstudy replicates for the remaining 1,397 SNPs was 100% for all SNPs. Concordance for study duplicates ranged from 97% to 100%, and the genotyping success rate ranged from 96% to 100%.

To test for possible differences regarding ethnicity-related substructure between the base population of cases and controls, we conducted a principal component analysis (24, 25). Analyses of all loci and of loci with pairwise r^2 value of <0.01 indicated that population stratification was negligible in this data set. A complete list of genes, chromosomal location and position, and genotypic change for the 1,397 SNPs included in the final analysis is provided in Supplementary Table S1.

Statistical Analysis. The two studies were first analyzed separately. Unconditional multivariate logistic regression models (PROC LOGISTIC, SAS 8.2) were used to estimate odds ratios (OR) and calculate 95% confidence intervals (CI) for main effect for each individual SNP. We used the homozygous wild-type genotype as the reference category, and estimated separate ORs for heterozygotes and rare homozygous allele groups, adjusting for study-specific matching factors (hospital, age, sex, and residential distance from hospital for NCI; sex, age, and state of residence for NIOSH). Data from both studies were then pooled to increase power. Adjusted pooled ORs were estimated using fixed effects models (26), and a likelihood ratio test of linear trend was conducted for each SNP using a 3-level ordinal variable corresponding to 0, 1, or 2 minor alleles for that SNP. All analyses were repeated, restricted to the subset of 254 cases of glioblastoma, the most common and aggressive subtype of glioma. To evaluate possible bias introduced by using disease controls, regression models were repeated for each SNP excluding one major subset of hospital controls at a time. We also evaluated possible survival bias in the NIOSH study by conducting separate analyses for cases with interval between time of diagnosis and blood draw of 155 d or less, and cases with interval of >155 d.

For the subset of SNPs with a P value of a linear trend of <0.01 in the pooled analysis, we conducted further genetic region-based analyses. Linkage disequilibrium for SNPs within each region was estimated in controls using the Haploview package (27). A haplotype sliding window approach was used to evaluate potential disease loci in small genetic regions that may have been overlooked with a single locus analysis, with windows composed of three and five SNPs (28). Haplotype frequencies for 3- and 5-SNP mini-blocks, as well for conventional blocks, were estimated using the expectation-maximization algorithm (29), and overall differences in haplotype frequencies between cases and controls were assessed using a global score test (30). Haplotype ORs and 95% CIs were adjusted for study (NCI versus NIOSH), and for study-specific matching factors.

To adjust SNP and region-based findings for multiple testing while accounting for correlations among SNPs induced by linkage disequilibrium, we used the rank truncated product (31). The rank truncated product is an appropriate test in scenarios with a small set of true effects among a large number of null effects. This test statistic is based on the product of the most significant P values overall, and within a gene. Permutation based P values for the rank truncated product statistic were computed based on 20,000 permutations of case-control status under the null hypotheses of no association with genotype.

[5] http://tagzilla.nci.nih.gov/ last accessed 22 December 2008.

Gene-region based tests were conducted for all regions. We further conducted haplotype and sliding window analyses for those gene regions that included SNPs with the lowest P values ($P < 0.01$), which was in fact consistent with significant gene regions of $P < 0.01$ (3 for glioma and 4 for glioblastoma).

Results

Five hundred fifty-one cases of glioma (263 from NCI; 288 from NIOSH) and 865 controls (330 from NCI and 535 from NIOSH) were successfully genotyped for 1,397 SNPs in 148 genetic regions. The observed distribution of the P values of trend for all 1,397 SNPs did not differ significantly from the expected (uniform) null distribution, making the possibility of systematic bias in the study unlikely (Fig. 1).

Eighty-seven SNPs in 42 genetic regions were significantly associated with risk of adult glioma at a P value of <0.05 (Supplementary Table S2), and 9 SNPs in 8 genetic regions were associated at a P value of <0.01 (Table 2). Associations for the top nine SNPs (i.e., the SNPs with the smallest P values) did not vary significantly by sex. However, *ITGB2* rs235325 and *ALOX5* rs2291427 were more strongly associated with risk of glioma in younger individuals compared with older ones ($P_{interaction} = 0.003$ and 0.019, respectively). After correcting for multiple testing based on the threshold truncated product, none of the SNPs from the single SNP analysis remained statistically significant. However, we note the striking consistency of risk estimates for the two studies (NCI versus NIOSH), with both studies showing borderline significant or statistically significant associations for *SELP* rs2236868, *ITGB2* rs235325, *ALOX5* rs2291427, and *NCF2* rs11579965. Region-based tests identified 3 regions as statistically significant: *SOD1* ($P = 0.02$), *SELP* ($P = 0.04$), and *ALOX5* ($P = 0.04$). An analysis of haplotypes using both the 3- and 5-SNP sliding windows confirmed the position of signal but did not show any stronger signals. The 2 SNPs significant at a P value of <0.01 in *SELP* (rs3917727, $P_{trend} = 0.001$; and rs2236868, $P_{trend} = 0.007$) were in strong linkage disequilibrium ($D' = 0.99$ and $r^2 = 0.59$) in controls. Analyses excluding one set of disease control at a time indicated no effect of control selection on either the NCI study-specific or the pooled estimates (data not shown). Analyses stratified by median interval between time of diagnosis and blood draw (<155 days) showed that the main glioma findings, while demonstrating some variation, did not vary significantly according to time from diagnosis to blood draw. In fact, ORs in cases with a shorter time interval exhibited trends similar to the overall pooled results reported in Table 2 (data not shown).

In further analyses, we restricted testing to 254 glioblastoma cases while using all controls (results for all SNPs provided in Supplementary Table S3). Although glioblastoma accounts for only 46% of the glioma cases, the associations for glioblastoma with the nine SNPs with the smallest P values were similar in magnitude to the estimated effects for all glioma. Of particular note were the significant associations observed for the two SNPs in the *SELP* genetic region (rs2236868 and rs3917727) and the SNP in the *STAT1/STAT4* region (rs2066804). Overall, 16 SNPs in nine genetic regions were significant at a P value of <0.01 (Table 3). Again, individual SNP associations for glioblastoma results were not significant after correction for multiple hypotheses testing using the threshold truncated product method. Associations for glioblastoma were significant for the genetic regions *SELP* ($P = 0.02$), *DEFB126/127* ($P = 0.002$), *SERPINI1* ($P = 0.02$), and *LY96* ($P = 0.03$).

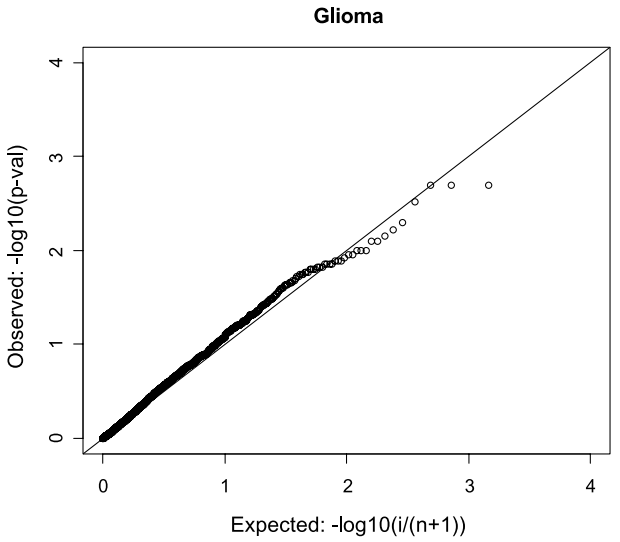

Figure 1. Observed and expected distributions for the results of test of trend with tag-SNPs in 148 innate immunity gene regions in hospital-based case-control study of adult glioma conducted by the NCI and population-based case-control study of adult glioma conducted by the NIOSH.

Discussion

Based on the accumulation of evidence that alterations in the immune system may contribute to the etiology of brain tumors (8, 32), we have conducted a detailed association study of adult glioma and common tagging SNPs in 148 innate immune genes and their surrounding regions. In our pooled analysis of two independent case-control studies, we identified three genetic regions of particular interest within two innate immune pathways: *SELP* and *ALOX5* (integrins/cell surface receptors), and *SOD1* (oxidative response). In an analysis restricted to glioblastoma, the most common and aggressive tumor subtype, *SELP* was again implicated, as were three additional genetic regions: *DEFB126/DEFB127*, *LY96* (pattern recognition and antimicrobials), and *SERPINI1* (response genes and tissue factors).

The *SELP* gene exhibited the most consistent association with risk of overall glioma and glioblastoma in our analyses. In fact, the only SNP that was significantly associated with risk of glioblastoma in both NCI and NIOSH studies was an intronic SNP (rs2236868) in the *SELP* gene. We are not aware of published epidemiologic studies that have evaluated *SELP* polymorphisms in this gene in relation to risk of any cancer, including glioma, but there are several reports in which polymorphisms

Table 2. Tag-SNPs associated with risk of glioma at a P_{trend} of <0.01 in pooled hospital-based case-control study of adult glioma conducted by the NCI and population-based case-control study of adult glioma conducted by the NIOSH

Region Gene	SNP ID	Genotype	NCI			NIOSH			Pooled
			Control *n* (%)	Case *n* (%)	OR*,† (95% CI)‡	Control *n* (%)	Case *n* (%)	OR§ (95% CI)	OR‖ (95% CI)
SELP									
SELP	rs3917727¶	AA	132 (40.1)	129 (49.2)	1.00	213 (39.8)	137 (48.1)	1.00	1.00
		AG	155 (47.1)	102 (38.9)	0.67 (0.47-0.96)	246 (46.0)	122 (42.8)	0.72 (0.52-1.00)	0.70 (0.55-0.89)
		GG	42 (12.8)	31 (11.8)	0.79 (0.46-1.35)	76 (14.2)	26 (9.1)	0.51 (0.30-0.87)	0.63 (0.43-0.91)
		P_{trend}			0.103			0.005	0.001
	rs2236868¶	GG	86 (26.2)	91 (34.6)	1.00	140 (26.3)	94 (32.8)	1.00	1.00
		AG	159 (48.5)	113 (43.0)	0.66 (0.44-0.97)	259 (48.7)	131 (45.6)	0.75 (0.53-1.08)	0.71 (0.54-0.92)
		AA	83 (25.3)	59 (22.4)	0.67 (0.43-1.06)	133 (25.0)	62 (21.6)	0.67 (0.44-1.03)	0.67 (0.49-0.91)
		P_{trend}			0.068			0.059	0.007
STAT1/STAT4									
STAT1	rs2066804	GG	166 (50.3)	164 (62.4)	1.00	296 (55.4)	172 (60.1)	1.00	1.00
		AG	142 (43.0)	85 (32.3)	0.57 (0.40-0.81)	210 (39.3)	103 (36.0)	0.85 (0.61-1.17)	0.71 (0.56-0.90)
		AA	22 (6.7)	14 (5.3)	0.62 (0.30-1.27)	28 (5.2)	11 (3.9)	0.57 (0.27-1.23)	0.61 (0.36-1.02)
		P_{trend}			0.004			0.113	0.002
ITGB2									
ITGB2	rs235325	GG	80 (24.2)	91 (34.7)	1.00	177 (33.3)	110 (38.2)	1.00	1.00
		AG	180 (54.6)	127 (48.5)	0.61 (0.42-0.89)	262 (49.3)	137 (47.6)	0.84 (0.60-1.17)	0.73 (0.56-0.94)
		AA	70 (21.2)	44 (16.8)	0.54 (0.33-0.88)	93 (17.5)	41 (14.2)	0.67 (0.42-1.07)	0.61 (0.43-0.85)
		P_{trend}			0.007			0.086	0.002
ALOX5									
ALOX5	rs2291427	GG	170 (51.7)	110 (41.8)	1.00	240 (44.9)	125 (43.4)	1.00	1.00
		AG	137 (41.6)	128 (48.7)	1.47 (1.04-2.09)	244 (45.7)	120 (41.7)	1.01 (0.72-1.40)	1.21 (0.95-1.53)
		AA	22 (6.7)	25 (9.5)	1.76 (0.93-3.33)	50 (9.4)	43 (14.9)	1.85 (1.12-3.04)	1.85 (1.25-2.74)
		P_{trend}			0.014			0.065	0.003
NFKB1									
NFKB1	rs4647992	CC	315 (95.7)	234 (89.0)	1.00	485 (90.7)	257 (89.2)	1.00	1.00
		CT	14 (4.3)	28 (10.7)	2.85 (1.45-5.64)	48 (9.0)	31 (10.8)	1.43 (0.86-2.39)	1.84 (1.23-2.73)
		TT	0 (0.0)	1 (0.4)	∞	2 (0.4)	0 (0.0)	0.00	1.01 (0.08-12.36)
		P_{trend}			0.001			0.340	0.005
IRAK3									
IRAK3	rs2701652	GG	167 (50.8)	152 (57.8)	1.00	243 (45.8)	149 (51.9)	1.00	1.00
		CG	128 (38.9)	85 (32.3)	0.71 (0.50-1.02)	230 (43.3)	122 (42.5)	0.86 (0.62-1.19)	0.80 (0.63-1.02)
		CC	34 (10.3)	26 (9.9)	0.87 (0.49-1.53)	58 (10.9)	16 (5.6)	0.40 (0.22-0.76)	0.60 (0.40-0.91)
		P_{trend}			0.191			0.011	0.006
SOD1									
SOD1	rs202445	TT	210 (64.0)	173 (65.8)	1.00	334 (63.6)	207 (72.4)	1.00	1.00
		CT	103 (31.4)	84 (31.9)	0.95 (0.66-1.35)	169 (32.2)	74 (25.9)	0.74 (0.52-1.05)	0.83 (0.65-1.07)
		CC	15 (4.6)	6 (2.3)	0.46 (0.17-1.25)	22 (4.2)	5 (1.8)	0.30 (0.11-0.84)	0.37 (0.18-0.74)
		P_{trend}			0.271			0.007	0.006
NCF2									
NCF2	rs11579965	CC	293 (88.8)	221 (84.0)	1.00	471 (88.2)	239 (83.0)	1.00	1.00
		CG	35 (10.6)	38 (14.5)	1.48 (0.90-2.45)	62 (11.6)	48 (16.7)	1.47 (0.95-2.27)	1.47 (1.06-2.04)
		GG	2 (0.6)	4 (1.5)	2.61 (0.46-14.72)	1 (0.2)	1 (0.4)	4.24 (0.26-69.36)	2.99 (0.67-13.40)
		P_{trend}			0.068			0.059	0.007

*OR.
†ORs adjusted for sex, age, study hospital, and distance of residence from hospital.
‡95% CIs.
§ORs adjusted for sex, age, and state of residence.
‖ORs adjusted for study, sex, residence, and age within each study.
¶D′ = 0.99 and r^2 = 0.59 for pooled controls of European background.

in this gene have been associated with increased risk of premature coronary heart disease (33), myocardial infarction (34), childhood-onset systemic lupus (35), and decreased risk of cognitive deficit after cardiac surgery (36), potentially underscoring the importance of *SELP* polymorphisms in chronic inflammatory conditions. *SELP* codes for the membrane glycoprotein P-selectin, an endothelial cell adhesion molecule that plays an important role in inflammatory responses in normal tissues (including the brain) by facilitating the recruitment, transendothelial migration, and proliferation of inflammatory cells in the extravascular compartment. In addition to *SELP*, the *ALOX5* gene from the pathway of integrins, adhesion and related molecules was associated with risk of overall glioma. The involvement of this pathway in glioma pathogenesis is plausible biologically in the view of the high migratory and invasive potential of glioma cells (37).

Our data also suggest a role for genetic variation in oxidative pathway (*SOD1*). Mutations in the *SOD1*gene have been commonly reported in individuals with amyotrophic lateral sclerosis, a severe neurodegenerative disease (38, 39). In addition, the *SOD1* gene was found to be overexpressed in glial cell lines and contribute to their

Table 3. Tag-SNPs associated with risk of glioblastoma at P_{trend} of <0.01 in pooled hospital-based case-control study of adult glioma conducted by the NCI and population-based case-control study of adult glioma conducted by the NIOSH

Region Gene	SNP ID	Genotype	NCI			NIOSH			Pooled
			Control *n* (%)	Case *n* (%)	OR*,† (95% CI)‡	Control *n* (%)	Case *n* (%)	OR§ (95% CI)	OR‖ (95% CI)
STAT1/STAT4									
STAT1	rs2066804¶	GG	166 (50.3)	83 (66.4)	1.00	296 (55.4)	78 (61.4)	1.00	1.00
		AG	142 (43.0)	40 (32.0)	0.48 (0.30-0.78)	210 (39.3)	45 (35.4)	0.77 (0.51-1.16)	0.63 (0.46-0.86)
		AA	22 (6.7)	2 (1.6)	0.14 (0.03-0.66)	28 (5.2)	4 (3.2)	0.50 (0.17-1.50)	0.30 (0.12-0.72)
		P_{trend}			0.00008			0.102	0.0001
GLS	rs13035504¶	CC	245 (74.5)	79 (63.2)	1.00	395 (74.4)	84 (65.1)	1.00	1.00
		CG	81 (24.6)	43 (34.4)	1.63 (1.00-2.66)	123 (23.2)	39 (30.2)	1.50 (0.96-2.34)	1.55 (1.12-2.15)
		GG	3 (0.9)	3 (2.4)	1.58 (0.30-8.52)	13 (2.5)	6 (4.7)	2.28 (0.82-6.34)	2.05 (0.85-4.97)
		P_{trend}			0.055			0.030	0.004
DEFB127/126									
DEFB127	rs1434789**	TT	143 (43.3)	46 (36.8)	1.00	215 (40.3)	37 (28.9)	1.00	1.00
		GT	147 (44.6)	62 (49.6)	1.26 (0.78-2.04)	270 (50.6)	64 (50.0)	1.40 (0.89-2.19)	1.34 (0.97-1.86)
		GG	40 (12.1)	17 (13.6)	1.44 (0.71-2.94)	49 (9.2)	27 (21.1)	3.91 (1.81-5.98)	2.39 (1.51-3.78)
		P_{trend}			0.24			0.0002	0.0004
DEFB126	rs6054706**	TT	105 (31.9)	32 (25.6)	1.00	174 (32.7)	31 (24.2)	1.00	1.00
		CT	165 (50.2)	68 (54.4)	1.45 (0.86-2.45)	269 (50.6)	65 (50.8)	1.41 (0.88-2.27)	1.43 (1.00-2.04)
		CC	59 (17.9)	25 (20.0)	1.49 (0.77-2.88)	89 (16.7)	32 (25.0)	2.01 (1.14-3.55)	1.79 (1.16-2.76)
		P_{trend}			0.191			0.013	0.006
	rs13036802**	TT	131 (39.8)	41 (32.8)	1.00	222 (41.8)	43 (33.3)	1.00	1.00
		CT	161 (48.9)	66 (52.8)	1.40 (0.85-2.28)	247 (46.5)	65 (50.4)	1.40 (0.91-2.16)	1.41 (1.02-1.95)
		CC	37 (11.3)	18 (14.4)	1.66 (0.81-3.40)	62 (11.7)	21 (16.3)	1.79 (0.96-3.24)	1.74 (1.09-2.77)
		P_{trend}			0.112			0.040	0.009
SERPINI1									
SERPINI1	rs10513634††	TT	232 (70.5)	104 (83.2)	1.00	408 (76.7)	108 (84.4)	1.00	1.00
		AT	87 (26.4)	20 (16.0)	0.45 (0.26-0.80)	120 (22.6)	19 (14.8)	0.61 (0.36-1.05)	0.53 (0.36-0.79)
		AA	10 (3.0)	1 (0.8)	0.22 (0.03-1.92)	4 (0.8)	1 (0.8)	0.84 (0.09-7.54)	0.38 (0.08-1.78)
		P_{trend}			0.002			0.079	0.001
	rs1552746††	TT	204 (61.8)	91 (72.8)	1.00	365 (68.6)	98 (76.6)	1.00	1.00
		CT	109 (33.0)	33 (26.4)	0.71 (0.43-1.16)	152 (28.6)	28 (21.9)	0.68 (0.43-1.09)	0.69 (0.49-0.98)
		CC	17 (5.2)	1 (0.8)	0.12 (0.02-0.95)	15 (2.8)	2 (1.6)	0.56 (0.12-2.46)	0.26 (0.08-0.90)
		P_{trend}			0.014			0.079	0.003
LY96									
LY96	rs16938755	TT	261 (79.1)	87 (69.6)	1.00	404 (75.8)	91 (70.5)	1.00	1.00
		CT	63 (19.1)	33 (26.4)	1.51 (0.89-2.56)	123 (23.1)	34 (26.4)	1.35 (0.86-2.12)	1.42 (1.00-2.00)
		CC	6 (1.8)	5 (4.0)	4.98 (1.32-18.79)	6 (1.1)	4 (3.1)	3.03 (0.81-11.34)	3.77 (1.48-9.59)
		P_{trend}			0.014			0.069	0.003
SELP									
SELP	rs2236868‡‡	GG	86 (26.2)	42 (33.6)	1.00	140 (26.3)	47 (36.7)	1.00	1.00
		AG	159 (48.5)	60 (48.0)	0.77 (0.46-1.29)	259 (48.7)	54 (42.2)	0.63 (0.40-0.99)	0.69 (0.49-0.97)
		AA	83 (25.3)	23 (18.4)	0.49 (0.26-0.92)	133 (25.0)	27 (21.1)	0.59 (0.34-1.01)	0.54 (0.36-0.82)
		P_{trend}			0.027			0.040	0.003
	rs3917727‡‡	AA	132 (40.1)	65 (52.0)	1.00	213 (39.8)	65 (51.2)	1.00	1.00
		AG	155 (47.1)	48 (38.4)	0.68 (0.42-1.08)	246 (46.0)	49 (38.6)	0.63 (0.41-0.96)	0.65 (0.48-0.89)
		GG	42 (12.8)	12 (9.6)	0.62 (0.29-1.32)	76 (14.2)	13 (10.2)	0.58 (0.30-1.12)	0.60 (0.36-0.98)
		P_{trend}			0.084			0.028	0.005
	rs17523783‡‡	GG	167 (50.8)	74 (59.2)	1.00	259 (48.5)	76 (58.9)	1.00	1.00
		GT	136 (41.3)	45 (36.0)	0.81 (0.51-1.29)	223 (41.8)	47 (36.4)	0.69 (0.45-1.04)	0.74 (0.54-1.01)
		TT	26 (7.9)	6 (4.8)	0.53 (0.20-1.41)	52 (9.7)	6 (4.7)	0.43 (0.18-1.05)	0.47 (0.24-0.91)
		P_{trend}			0.152			0.028	0.005
C2									
C2	rs7746553	CC	232 (70.3)	82 (65.6)	1.00	377 (70.6)	75 (58.6)	1.00	1.00
		CG	87 (26.4)	37 (29.6)	1.19 (0.72-1.95)	144 (27.0)	48 (37.5)	1.74 (1.14-2.64)	1.48 (1.08-2.04)
		GG	11 (3.3)	6 (4.8)	1.72 (0.56-5.29)	13 (2.4)	5 (3.9)	2.15 (0.72-6.43)	1.99 (0.90-4.37)
		P_{trend}			0.292			0.006	0.006

(*Continued on the following page*)

Table 3. Tag-SNPs associated with risk of glioblastoma at P_{trend} of <0.01 in pooled hospital-based case-control study of adult glioma conducted by the NCI and population-based case-control study of adult glioma conducted by the NIOSH (Cont'd)

Region Gene	SNP ID	Genotype	NCI			NIOSH			Pooled
			Control *n* (%)	Case *n* (%)	OR*, † (95% CI) ‡	Control *n* (%)	Case *n* (%)	OR§ (95% CI)	OR‖ (95% CI)
CCL2/CCL7/CCL11/CCL8/CCL13/CCL1									
CCL2	rs2857653	CC	205 (63.1)	71 (56.8)	1.00	357 (67.1)	77 (59.7)	1.00	1.00
		CT	105 (32.3)	50 (40.0)	1.81 (1.11-2.94)	166 (31.2)	43 (33.3)	1.23 (0.80-0.96)	1.46 (1.06-2.00)
		TT	15 (4.6)	4 (3.2)	0.72 (0.22-2.38)	9 (1.7)	9 (7.0)	4.90 (1.83-13.13)	1.99 (0.95-0.98)
		P_{trend}			0.169			0.015	0.006
JAK3									
JAK3	rs3212741§§	GG	192 (58.2)	81 (64.8)	1.00	319 (59.6)	94 (72.9)	1.00	1.00
		AG	119 (36.1)	40 (32.0)	0.86 (0.53-1.39)	196 (36.6)	31 (24.0)	0.53 (0.34-0.83)	0.66 (0.48-0.92)
		AA	19 (5.8)	4 (3.2)	0.51 (0.16-1.66)	20 (3.7)	4 (3.1)	0.67 (0.22-2.05)	0.56 (0.25-1.27)
		P_{trend}			0.251			0.009	0.007
	rs2072496§§	GG	270 (81.8)	92 (73.6)	1.00	438 (82.0)	100 (77.5)	1.00	1.00
		AG	59 (17.9)	31 (24.8)	1.49 (0.88-2.55)	95 (17.8)	26 (20.2)	1.22 (0.75-2.01)	1.34 (0.93-1.92)
		AA	1 (0.3)	2 (1.6)	8.22 (0.44-151.78)	1 (0.2)	3 (2.3)	17.6 (1.76-175.30)	14.2 (2.38-84.78)
		P_{trend}			0.061			0.080	0.010
MIF									
MIF	rs738807	CC	244 (73.9)	103 (82.4)	1.00	392 (73.3)	104 (80.6)	1.00	1.00
		CT	80 (24.2)	22 (17.6)	0.65 (0.37-1.13)	137 (25.6)	23 (17.8)	0.58 (0.35-0.97)	0.61 (0.42-0.89)
		TT	6 (1.8)	0 (0.0)	0.00	6 (1.1)	2 (1.6)	1.52 (0.29-8.09)	0.53 (0.11-2.57)
		P_{trend}			0.028			0.093	0.007

*OR.
†ORs adjusted for sex, age, study hospital, and distance of residence from hospital.
‡95% CIs.
§ORs adjusted for sex, age, and state of residence.
‖ORs adjusted for study, sex, residence, and age within each study.
¶D′ = 1.00 and r^2 = 0.06.
** Pairwise $0.74 \le D' \le 1.00$ and $0.52 \le r^2 \le 0.72$.
††D′ = 0.99 and r^2 = 0.67.
‡‡Pairwise D′ = 1.00 and $0.42 \le r^2 \le 0.72$.
§§D′ = 0.65 and r^2 = 0.01. All D′ and r^2 are reported for pooled controls of European background.

radioresistance (40), suggesting a potential role in the biology of glioma.

Analyses restricted to glioblastoma revealed several additional genetic regions of interest in two pathways: *DEFB126/DEFB127, LY96* (pattern recognition and antimicrobials), and *SERPINI1* (response genes and tissue factors). Although this might be due, to some extent, to chance variation with a smaller sample size, it is also possible that restricting to a more homogeneous set of tumors yielded information specific to the etiology of these aggressive tumors. Interestingly, the *SERPINI1* gene that encodes neuroserpin is predominantly expressed in the brain and inhibits tissue type plasminogen-activator (41); also, mutations in *SERPINI1* have been associated with early familial encephalopathy (42). Taken together, this may point to a yet unknown role of *SERPINI1* in the development of glioblastoma. At present, there seems to be no published evidence on whether the SNPs in *DEFB126/DEFB127* and *LY96* genes are associated with risk of other cancers, inflammatory conditions or central nervous system disorders.

As with all studies, our results are subject to some caveats. Given that the SNPs were chosen as tagging markers for the genetic region and not based on known function, the observed associations could be due to linkage disequilibrium with the true unobserved causal SNPs. Because the median coverage for the genetic regions was 59% (range 7-100%), it is also possible that additional loci could be associated with risk for glioma. Although our top associations were consistent in magnitude and direction in two independent studies, these did not withstand adjustment for multiple comparisons, and replication of these results is required to rule out the possibility of chance findings (43). We have assembled a large pooled study of adult glioma that had ~80% power to detect strong effects (OR, ≥2) for common alleles (minor allele frequency, ~20%) after taking multiple comparisons into account. We had limited statistical power to detect modest associations (OR of ≤1.5), associations with less common alleles, and risks for specific glioma subtypes. We attempted to quantify possible survival bias in the SNP results due to differences in participation rates for blood sampling (41% of all NIOSH cases compared with 88% of NCI cases) by conducting stratified analyses for individuals based on time interval between diagnosis and blood draw. We found that results for the key findings, when restricted to those with a shorter interval between diagnosis and blood draw, were similar to the pooled findings. This indicates that our main findings are unlikely to represent false positives due to survival bias. However, we may have a higher proportion of false negatives if inclusion of cases with a longer time to blood draw attenuated associations.

From a set of tag SNPs in 148 genes critical to innate immunity, our pooled study of adult glioma identified 6 genetic regions of major interest (*SELP, SOD1, ALOX5* for glioma; *SELP, DEFB126/127, SERPINI1*, and *LY96* for glioblastoma). We note the consistent association observed for *SELP* rs2236868 for glioma and glioblastoma,

in the NCI and NIOSH studies. This is the first report of these associations to our knowledge. Replication of these findings in large studies, such as consortial efforts of brain tumors with increased coverage of the identified genes of interest, will be essential.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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