An International Case-Control Study of *Interleukin-4Rα*, *Interleukin-13*, and *Cyclooxygenase-2* Polymorphisms and Glioblastoma Risk

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Abstract

Previous studies found that allergies are inversely related to risk of glioma. In an earlier publication, using data from a Swedish case-control study, Schwartzbaum et al. report an inverse relation between risk of glioblastoma and four single nucleotide polymorphisms (SNP) on two genes [*interleukin (IL)-4Rα, IL-13*] that are associated with allergies. In addition, recent studies suggest that IL-4 and IL-13 induce cyclooxygenase-2 (COX-2) to resolve brain inflammation. To see whether previous Swedish results (110 cases, 430 controls) would be replicated, we estimated the association between glioblastoma and two *IL-4Rα* (*rs1805015, rs1801275*) and two *IL-13* (*rs20541, rs1800925*) SNPs and their haplotypes and one *COX-2* SNP (*−765GC*) using additional English, Danish, and Finnish data (217 cases, 1,171 controls). Among general population controls, we evaluated associations between these haplotypes, the *COX-2* SNP, and self-reported allergies. Our data did not support our original observations relating individual *IL-4Rα, IL-13*, or *COX-2* SNPs to glioblastoma risk. However, the *T-G IL-4Rα* haplotype was associated with glioblastoma risk (odds ratio, 2.26; 95% confidence interval, 1.13-4.52) and there was a suggestion of an inverse relation between this haplotype and hayfever prevalence among controls (odds ratio, 0.38; 95% confidence interval, 0.14-1.03). The lack of support for a link between four *IL-4Rα* and *IL-13* SNPs and glioblastoma may reflect the absence of associations or may result from uncontrolled confounding by haplotypes related both to those that we examined and glioblastoma. Nonetheless, the association between the *T-G IL-4Rα* haplotype and glioblastoma risk may indicate a role of immune factors in glioblastoma development. (Cancer Epidemiol Biomarkers Prev 2007;16(11):2448–54)

Glioblastoma, an aggressive primary malignant brain tumor, is the most common type of adult glioma and has a poor prognosis with only ~3% of glioblastoma patients surviving 5 years after diagnosis (0.3% for people older than 65 years at diagnosis; ref. 1). Inherited syndromes account for only a small proportion of glioblastoma but familial aggregation of this tumor has been observed (2). The strongest known environmental risk factor for glioma is exposure to therapeutic doses of ionizing radiation (3); however, this risk factor accounts for only a small proportion of cases. In addition, evidence from one cohort and nine case-control studies (e.g., refs. 4-6) is consistent for an inverse association between self-reported asthma and allergic conditions and risk of glioma. Wiemels et al. (7) found that glioma patients have lower serum IgE levels than controls; however, the possibility that immunosuppression by the tumor itself or by its standard treatment with immunosuppressive drugs may lower IgE levels or eliminate allergies cannot be excluded.

IL-4 and *IL-13* are genes that share a common *IL-4Rα* chain on their receptors and code cytokines (immunoregulatory proteins) that share functions. These

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cytokines play a central role in allergy by stimulating IgE synthesis (8), show strong antitumor activity in mice, and inhibit proliferation of astrocytoma and low-grade glioma in human cell lines (9, 10). The anti-inflammatory properties of IL-4 and IL-13 are probably based on their ability to induce regulatory T-cell expression (11) or, in the brain, to enhance cyclooxygenase (COX)-2 expression, thereby causing cell death of activated microglia (major inflammatory cells of the central nervous system; refs. 12-14). In addition to its potential role in inhibiting brain inflammation, COX-2 expression is associated with glioma angiogenesis (15). Consistent with this evidence, Sivak-Sears et al. (16) found an inverse association between nonsteroidal anti-inflammatory drug use and glioblastoma risk.

Studies of germ line polymorphisms of the *IL-4Rα* and *IL-13* genes provide relatively strong support for a role of these genes in IgE production or allergy. For example, in a study of 4,570 DNA samples from a British cohort, Maier et al. (17) found ''robust confirmation'' for an association between allelic variation of the *IL-13* gene and variation in total serum IgE levels. They further state that the strength and consistency of their findings show that this association is valid and not a false-positive result. These authors also report a statistically significant association ($P = 0.04$) between an *IL-4Rα* germ line variant (*rs1801275*) and IgE levels. In addition, a recent meta-analysis of seven studies of the association between this same *IL-4Rα* variant and allergic asthma (18) found evidence for an association [OR, 1.54; 95% confidence interval (95% CI), 1.14-2.08]. In the absence of functional studies, it is not possible to identify precisely which single nucleotide polymorphisms (SNP) are important in determining the association between the *IL-4Rα* and *IL-13* genes and IgE or allergy (due in part to the correlation among SNPs on the same gene); however, these two genes seem to play a central role in IgE production and allergic disease.

In a previous analysis of data from a Swedish population-based case-control study, Schwartzbaum et al. (19) used four germ line polymorphisms on the *IL-4Rα* and *IL-13* genes as indicators of allergy risk. The rationale for using SNPs related to allergies rather than self-report of allergy or measurement of IgE after glioma diagnosis is that, unlike these two estimates of the probability that an individual has allergic disease, SNPs provide an indicator that is not subject to differential case-control error. Schwartzbaum et al. found that genetic variants that increase the risk of IgE or allergies also decrease glioblastoma risk. Their finding for the *IL-13, rs1800925* SNP was confirmed for glioma by Wiemels et al. (20) in a large case-control study of glioma (456 cases, 541 controls). Furthermore, Wiemels et al. report that the same *IL-13* SNP was inversely associated with IgE levels among controls ($P = 0.04$) as was the *IL-13 rs20541* SNP ($P < 0.001$). However, Wiemels et al. did not observe the relationships between the *IL-13 rs20541* SNP and glioma nor did they find the same associations between the *IL-4Rα* SNPs and glioma previously seen for glioblastoma by Schwartzbaum et al. Differences between the two studies may be attributed to chance or variations in the distribution of SNPs among populations. Therefore, it is important that the association between these allergy-related SNPs and glioma be evaluated in diverse populations.

The purpose of the present study was to determine whether the same associations between *IL-4Rα* and *IL-13* SNPs and glioblastoma that had previously been observed by Schwartzbaum et al. (19) in the relatively small Swedish data set (110 cases, 430 controls), would be found in a data set that included three additional population-based case-control studies from Southeast England, Denmark, and Finland. In addition to examining individual SNPs, as had Schwartzbaum et al., we also considered whether *IL-4Rα* or *IL-13* haplotypes might be related to glioblastoma. A haplotype consists of sets of alleles on the same chromosome that are inherited together and may have more functional importance than individual SNPs. Therefore, evaluating haplotypes rather than SNPs may increase statistical power. Furthermore, because our selection of *IL-4Rα* and *IL-13* SNPs was based on the central role of these genes in IgE-mediated allergies, we estimated associations between their haplotypes and self-reported allergies among general population controls.

The previous Schwartzbaum et al. study also evaluated the *−765GC COX-2* polymorphism for evidence of an association with glioblastoma. Although they observed no statistically significant association in this study, the number of cases was relatively small and because there is evidence for a role of the *COX-2* gene in brain inflammation and glioma angiogenesis, it seemed worthwhile to repeat the initial analysis in a larger study.

Materials and Methods

Study Design and Recruitment. Four population-based case-control studies of primary brain tumors were carried out in Sweden, Denmark, Finland, and Southeast England. These studies were conducted in the Stockholm, Gothenburg, Lund, and Umeå regions of Sweden; throughout Denmark; all regions except Northern Lapland and Åland in Finland; and the Thames regions of Southeast England. All four studies followed the core protocol of the INTERPHONE Study, coordinated by the IARC (21), but with extensions to the study design such as a wider age range of patients, an extended questionnaire and collection of blood samples. Results from the Swedish study were reported previously (19); however, since the previous study was published, the sample of participants whose blood we had collected has changed slightly. One misclassified glioblastoma case was removed from the sample and 14 controls that had been inadvertently excluded were added. Nonetheless, because of the similarity of the published Swedish data set and the Swedish data set that we now analyze, inferences based on results of analyses of both samples are the same.

With the aim of reducing phenotypic heterogeneity, we restricted the present study to glioblastoma patients whose histologic diagnoses were coded 9440, 9441, or 9442 using criteria in the International Classification of Diseases for Oncology, third edition, 2000. These patients were identified through neurosurgery, neuropathology, oncology, and neurology centers. Lists of case patients were also obtained from the appropriate population-based cancer registries to ensure completeness of ascertainment. Eligible case patients were individuals diagnosed with primary glioblastoma between September

1, 2000, to February 29, 2004 (the dates of case ascertainment within this period vary among centers), at ages 20 to 69 years in the Nordic countries and 18 to 59 years in England, and resident in the study region at the time of diagnosis. We restricted the present analyses to cases diagnosed with glioblastoma, the most common type of adult glioma, to reduce possible etiologic heterogeneity among different categories of glioma.

Control participants in the Nordic centers were randomly selected from the population register for each study area and frequency matched to all adult brain tumor cases (including all glioma and meningioma cases) on age, sex, and region. In England, frequency-matched controls were randomly selected from general practitioners' practice lists. Control participants were subject to the same age and residence criteria as case patients and the criterion that they had not been previously diagnosed with a brain tumor.

Eligible cases were approached either by mail, or personally at the clinics with written information about the study, and asked to participate in the study, whereas the controls were first approached by mail. If the subjects contacted by mail did not respond, another letter was sent or the subject was approached by telephone. Before asking for participation, study subjects received both an invitation letter and written information about the study. Informed consent was obtained from all study participants. Each study was approved by local ethics committees and informed consent was obtained from all study participants before the start of interview.

Data and Blood Collection. Computer-assisted personal interviews were conducted in participants' homes or other convenient locations (e.g., hospital rooms, offices, etc.) by trained interviewers. Information collected on allergic conditions included questions concerning previous diagnoses of asthma, hayfever, or eczema and whether these conditions were still present at the time of interview. In Sweden, Southeast England, and Denmark, a blood sample was drawn from cases and controls who participated in the interview and agreed to give blood. Finnish investigators did not attempt to collect blood from all interviewed participants; rather, they drew blood from a convenience sample, of predetermined size, from patients in three hospitals or controls living in the area served by these hospitals.

Genotyping. Dynamic allele-specific hybridization (22-26) was conducted to identify the *IL-4Rα* (*rs1805015, rs1801275*) and *IL-13* (*rs20541, rs1800925*) genotypes. For this, two PCR primers and one dynamic allele-specific hybridization probe per target SNP were designed by means of custom software (27) supplied by DynaMetrix Ltd. To verify successful PCR amplification, several randomly chosen samples were examined on a 3.0% low-melt agarose gel. Dynamic allele-specific hybridization analysis of the PCR product was then conduced on membrane macroarrays, using the dynamic allele-specific hybridization-2 protocol (25). A random sample of 15% of all DNA samples was reassayed and the genotype assignment was confirmed. An error rate of <1% was observed.

Statistical Analysis. We assessed country-specific odds ratios (OR) using unconditional logistic regression adjusted for the variables sex and age at diagnosis for cases (or for the controls, their age at their interview date with adjustment for the interval between the diagnostic and interview date of the cases and the difference between the mean interview dates of cases and controls). We also examined whether region within each country, on which controls were also matched (except in Denmark), should be included in the country-specific models. To do so, we conducted within-country analyses adjusted for age, sex, and region and found that results were almost identical to within-country analyses adjusted for age and sex only. We therefore did not adjust for region within country of residence as a potentially confounding variable.

To find out whether we could legitimately estimate the combined effects of polymorphisms across countries of residence, we conducted global tests for heterogeneity among countries. We used log-likelihood ratio tests to compare models with glioblastoma as the dependent variable, including main SNP and country effects and SNP-country interaction terms to models with only SNP and country main effects. Results suggested heterogeneity among countries for both *IL-4Rα* SNPs ($P < 0.10$) but none for the *IL-13* SNPs or the COX-2 SNP. ORs for two *IL-4Rα* haplotypes and one *IL-13* haplotype also showed evidence of heterogeneity among countries. We therefore present country-specific *IL-4Rα* SNP results as well as total and, for consistency, also show country-specific as well as total findings for the *IL-13* SNPs and all seven *IL-4Rα* and *IL-13* haplotypes. When we restricted the analysis to controls and used all allergies combined as the dependent variable, we found no evidence for heterogeneity. We therefore do not present country-specific findings for the haplotype analyses of allergies among glioblastoma general population controls.

To pool the SNP and haplotype data over countries, we used two-stage random effects meta-analysis (28) where country-specific ORs were first adjusted for sex and age and then summarized using random-effects meta-analysis. For our data, ORs using this meta-analytic method were similar to those obtained by excluding the Finnish data (Finnish results for the *IL-4Rα* SNP seemed to differ from those of the other three countries) and pooling data from the remaining three countries. However, because CIs for the ORs produced by the meta-analytic method were slightly wider than those produced by pooling data from three of four countries, we report only ORs from the meta-analysis. Our rationale for this choice is based on the high prevalence of false-positive genotyping findings in the literature. However, because our meta-analytic method rests on country-specific results, we did not have adequate numbers of observations to evaluate genotype trends.

Haplotype probabilities for the two *IL-4Rα* (*rs1805015, rs1801275*) and two *IL-13* (*rs20541, rs1800925*) sequence variants were estimated using Proc Haplotype's (SAS Genetics) implementation of the expectation-maximization algorithm. Each individual in the sample is assigned a probability of having each haplotype or combination of SNPs (e.g., for *IL-13* haplotypes, *G-C, G-T, A-C*, and *A-T*). The probability assigned by the program is based on individual genotypes together with the prevalence of their SNPs in the population. These probabilities are then used as weights to estimate haplotype frequencies. We excluded the *IL-4Rα C-A* haplotype from the analysis because it was found in less

than 5% of the total population and therefore was more likely to produce an unstable result. We next estimated country-specific sex and age-adjusted ORs for each haplotype using unconditional logistic regression and summarized them using random-effects meta-analysis (28). Each haplotype is included in a separate model with the reference category consisting of individuals who did not have that specific haplotype.

To find out whether the *IL-4Rα* or *IL-13* haplotypes were associated with self-reported allergies among controls in our data set, we divided controls into inclusive categories representing controls reporting any allergy, asthma, eczema, or hayfever and a comparison group representing controls reporting no allergies. The dependent variable for each of four unconditional logistic regression models consisted of one allergic condition category and the control group consisted of people who reported no allergies. Independent variables were three *IL-4Rα* and four *IL-13* haplotypes, country, sex, and age.

Results

In Table 1, the percentage males, median age at diagnosis, and associations between any allergy and glioblastoma are comparable for case and control participants in each country who did and did not have their blood drawn. Age and sex differences among cases and controls reflect our use of participants who were initially matched to the age and sex distribution of all glioma and meningioma case patients (see Materials and Methods).

In Table 2, summary ORs for both *IL-4Rα* polymorphisms provide no evidence for an association with glioblastoma (rs1805015 OR, 1.08; 95% CI, 0.70-1.66; rs1801275 OR, 1.00; 95% CI, 0.79-1.69) although ORs from the Southeast English data set (rs1805015 OR, 1.36; 95% CI, 0.87-2.12; rs1801275 OR, 1.45; 95% CI, 0.94-2.24) are consistent with previously reported Swedish findings (ref. 19; rs1805015 OR, 1.57; 95% CI, 1.01-2.45; rs1801275 OR, 1.56; 95% CI, 1.02-2.39).

Table 3 shows associations between haplotypes and glioblastoma risk. Only the T-G *IL-4Rα* haplotype is clearly related to glioblastoma risk (summary OR, 2.26; 95% CI, 1.13-4.52). Swedish, Southeast English, and Danish findings are consistent with this result but the Finnish findings are not, possibly because they are based on smaller numbers of observations.

As can be seen by comparing ORs in Table 4 with those in the last column of Table 3, 26 of 28 allergy-haplotype ORs are on the opposite side of the null from the corresponding glioblastoma-haplotype ORs. The *T-G IL-4Rα* haplotype ORs in Table 3 that is associated with glioblastoma (OR, 2.26; 95% CI, 1.13-4.52) is also related to a decreased risk of hayfever in Table 4 (OR, 0.38; 95% CI, 0.14-1.06).

Discussion

We did not confirm associations between two *IL-4Rα* and two *IL-13* polymorphisms and glioblastoma risk that were originally observed in the Swedish data set (19). We did, however, find an increased risk of glioblastoma among people with the *T-G IL-4Rα* haplotype. Whether this effect is merely a false-positive finding is not known, but it is noteworthy that a similar raised risk was apparent in several, although not all, countries. The inverse OR for hayfever, which would be expected if the *T-G IL-4Rα* haplotype accounts, in part, for the reduced risk of glioblastoma among people reporting hayfever, provides some additional plausibility for this finding.

In their large case-control study of glioma, Wiemels et al. (20) also found suggestive evidence for an association of borderline statistical significance between an *IL-4Rα* haplotype and glioma (OR, 1.49; 95% CI, 0.99-2.25). However, their haplotype includes four SNPs in addition to the two that we evaluated. When they restricted their haplotype analysis to the same *IL-4Rα* SNPs that we examined, they found no evidence for an association with glioma (OR, 1.13; 95% CI, 0.83-1.53). Although they do not report specific

Table 1. Country-specific and total sex and age distribution of glioblastoma case patients and population controls; sex-, age-, and country-specific; and country-adjusted associations between any self-reported allergy and glioblastoma among study participants by blood collection status

	Sweden		Southeast England		Denmark		Finland		Total	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Total number of cases and controls interviewed										
Number	176	632	146	630	128	819	115	870	565	2,951
%Male	59.7	48.1	69.2	46.7	56.3	48.4	58.3	42.0	61.1	46.1
Median age (y)	56.2	53.8	50.2	47.9	55.5	53.2	57.4	52.4	54.6	51.7
No allergies (OR)	1.00		1.00		1.00		1.00		1.00	
Any allergy* [OR (95% CI)]	0.73 (0.50-1.05)		0.53 (0.37-0.78)		0.69 (0.44-1.10)		0.71 (0.47-1.09)		0.66 (0.54-0.80)	
Cases and controls interviewed and blood collected and analyzed										
Number	110	436	107	459	66	602	44	110	327	1,607
%Male	59.1	45.4	71.0	45.4	60.6	46.7	61.4	36.4	63.6	45.3
Median age (y)	55.7	54.3	49.4	48.8	55.0	53.0	58.2	53.5	53.4	52.0
No allergies (OR)	1.00		1.00		1.00		1.00		1.00	
Any allergy* [OR (95% CI)]	0.67 (0.42-1.06)		0.45 (0.29-0.70)		0.73 (0.42-1.28)		0.75 (0.32-1.77)		0.59 (0.46-0.77)	

NOTE: Data are results from population-based case-control studies conducted in Sweden, Southeast England, Denmark, and Finland (2000-2004).
*Includes self-reported asthma, hayfever, eczema, food allergy, and all other allergic conditions.

Table 2. Sex- and age-adjusted country-specific and total country-adjusted associations between *IL-4Rα*, *IL-13*, and *COX-2* polymorphisms and glioblastoma

Gene (SNP) genotype	Sweden		Southeast England		Denmark		Finland		Total
	Cases/controls* (*n* = 110/436)	OR (95% CI)	Cases/controls* (*n* = 107/459)	OR (95% CI)	Cases/controls* (*n* = 66/602)	OR (95% CI)	Cases/controls* (*n* = 44/110)	OR (95% CI)	OR† (95% CI)
IL-4Rα (*rs1805015*)									
TT	65/298	1.00	63/308	1.00	46/406	1.00	36/75	1.00	1.00
TC, CC	43/127	1.57 (1.01-2.45)	43/149	1.36 (0.87-2.12)	19/191	0.88 (0.50-1.55)	8/35	0.46 (0.19-1.12)	1.08 (0.70-1.66)
IL-4Rα (*rs1801275*)									
AA	54/253	1.00	54/275	1.00	40/365	1.00	32/64	1.00	1.00
AG, GG	55/165	1.56 (1.02-2.39)	52/180	1.45 (0.94. 2.24)	26/236	1.00 (0.59-1.68)	12/45	0.54 (0.25-1.19)	1.16 (0.79-1.69)
IL-13 (*rs20541*)									
GG	75/266	1.00	70/308	1.00	42/387	1.00	20/42	1.00	1.00
AG, AA	35/168	0.74 (0.47-1.16)	37/151	1.10 (0.70-1.72)	24/212	1.10 (0.62-1.79)	24/67	0.66 (0.31-1.37)	0.92 (0.71-1.12)
IL-13 (*rs1800925*)									
CC	84/286	1.00	68/294	1.00	44/350	1.00	28/72	1.00	1.00
CT, TT	21/131	0.56 (0.33-0.95)	36/146	0.99 (0.62-1.57)	22/249	0.70 (0.41-1.21)	16/38	1.04 (0.49-2.22)	0.78 (0.59-1.04)
COX-2 −765GC									
GG	79/298	1.00	80/329	1.00	57/443	1.00	35/86	1.00	1.00
GC, CC	29/114	0.95 (0.59-1.53)	27/128	0.85 (0.52-1.38)	8/155	0.40 (0.19-0.87)	8/23	0.96 (0.38-2.42)	0.81 (0.60-1.08)

NOTE: Data are results from population-based case-control studies conducted in Sweden, Southeast England, Denmark, and Finland (2000-2004).
*Number of cases and controls varies slightly depending on success of each SNP assay.
†ORs estimated using two-stage random-effects meta-analysis (28).

results of this analysis for glioblastoma cases (which would have allowed direct comparison with our findings), overall, they state that they observed no difference in outcomes when their analyses were limited to glioblastoma cases.

An additional finding of Wiemels et al. that may support results of the present study is the association that they observed between the *IL-13 rs1800925* SNP and glioma (OR, 0.76; 95% CI, 0.57-1.00), which is similar to the result that we observed in the present study between

Table 3. Sex- and age-adjusted country-specific and country-adjusted associations between *IL-4Rα* and *IL-13* haplotypes and glioblastoma

Haplotype	Sweden (102/390)*		Southeast England (102/435)*		Denmark (65/592)*		Finland (44/108)*		Total (313/1,525)*	
	Haplotype frequencies (cases/controls)	OR† (95% CI)	Haplotype frequencies (cases/controls)	OR† (95% CI)	Haplotype frequencies (cases/controls)†	OR† (95% CI)	Haplotype frequencies (cases/controls)†	OR† (95% CI)	Haplotype frequencies (cases/controls)†	OR‡ (95% CI)
IL-4Rα										
H1, T-G	0.07/0.05	2.12 (0.66-6.80)	0.07/0.05	2.86 (0.81-10.11)	0.08/0.05	2.46 (0.61-9.91)	0.06/0.06	1.15 (0.13-10.17)	0.07/0.05	2.26 (1.13-4.52)
H2, C-G	0.22/0.17	1.83 (0.86-3.89)	0.22/0.17	1.81 (0.81-4.07)	0.15/0.17	0.72 (0.26-1.95)	0.10/0.16	0.29 (0.05-1.54)	0.19/0.17	1.14 (0.58-2.27)
H3, T-A	0.71/0.78	0.48 (0.25-0.95)	0.71/0.78	0.45 (0.22-0.93)	0.77/0.78	0.96 (0.41-2.33)	0.84/0.78	2.23 (0.57-8.87)	0.74/0.78	0.69 (0.38-1.24)
IL-13										
J1, G-C	0.79/0.71	2.16 (1.02-4.57)	0.73/0.75	0.95 (0.46-1.95)	0.73/0.71	1.20 (0.53-2.71)	0.62/0.56	1.79 (0.62-5.21)	0.74/0.71	1.40 (0.94-2.10)
J2, A-C	0.09/0.11	0.88 (0.47-1.64)	0.07/0.08	0.90 (0.28-2.91)	0.09/0.06	2.66 (0.69-10.31)	0.18/0.24	0.45 (0.11-1.78)	0.10/0.09	0.94 (0.55-1.61)
J3, G-T	0.04/0.08	0.24 (0.05-1.22)	0.07/0.08	0.83 (0.23-2.30)	0.07/0.09	0.53 (0.12-2.29)	0.04/0.07	0.53 (0.04-7.48)	0.06/0.08	0.57 (0.28-1.16)
J4, A-T	0.08/0.10	0.55 (0.18-1.72)	0.12/0.10	1.36 (0.50-3.74)	0.11/0.14	0.58 (0.17-1.92)	0.16/0.13	1.06 (0.25-4.48)	0.11/0.12	0.84 (0.47-1.51)

NOTE: Data are results from population-based case-control studies conducted in Sweden, Southeast England, Denmark, and Finland (2000-2004).
*Numbers differ from those in Table 2 because either SNP in haplotype can have missing values.
†Each haplotype is included in a separate model with the reference category consisting of individuals who did not have that specific haplotype.
‡ORs estimated using two-stage random-effects meta-analysis (28).

Table 4. Sex-, age-, and country-adjusted associations between *IL-4Rα* and *IL-13* haplotypes and allergies among glioblastoma controls

Haplotype	All allergies,* (n = 744/741) †,‡	All allergies*	Asthma cases (n = 152)	Asthma	Eczema cases (n = 307)	Eczema	Hayfever cases (n = 342)	Hayfever
	Haplotype frequencies	OR§ (95% CI)	Haplotype frequencies	OR§ (95% CI)	Haplotype frequencies	OR§ (95% CI)	Haplotype frequencies	OR§ (95% CI)
IL-4Rα								
H1, T-G	0.04/0.05	0.59 (0.30-1.17)	0.04	0.59 (0.16-2.15)	0.05	0.75 (0.31-1.81)	0.03	0.38 (0.14-1.03)
H2, C-G	0.17/0.18	0.86 (0.58-1.26)	0.18	1.08 (0.56-2.11)	0.17	0.94 (0.57-1.55)	0.18	0.94 (0.57-1.54)
H3, T-A	0.80/0.77	1.31 (0.92-1.86)	0.78	1.06 (0.58-1.95)	0.78	1.14 (0.72-1.79)	0.79	1.31 (0.83-2.06)
IL-13								
J1, G-C	0.70/0.72	0.71 (0.51-0.98)	0.69	0.70 (0.40-1.23)	0.67	0.58 (0.38-0.89)	0.71	0.77 (0.50-1.19)
J2, A-C	0.09/0.09	1.08 (0.62-1.87)	0.09	0.81 (0.32-2.05)	0.10	1.53 (0.77-3.04)	0.09	1.13 (0.56-2.27)
J3, G-T	0.09/0.08	1.42 (0.80-2.51)	0.11	2.46 (0.97-6.23)	0.09	1.67 (0.80-3.46)	0.08	1.09 (0.52-2.31)
J4, A-T	0.13/0.11	1.51 (0.94-2.41)	0.12	1.30 (0.57-2.93)	0.13	1.52 (0.84-2.76)	0.12	1.44 (0.78-2.66)

NOTE: Data are results from population-based case-control studies conducted in Sweden, Southeast England, Denmark, and Finland (2000-2004).
*Includes self-reported asthma, hayfever, eczema, food allergy, and all other allergic conditions.
†Number of controls (n = 1,485) differs from those in Table 3 (n = 1,525) because people with missing values for allergies are excluded from Table 4 analyses.
‡Controls are people reporting no allergies.
§Each haplotype is included in a separate model with the reference category consisting of individuals who did not have that specific haplotype.

the same SNP and glioblastoma (OR, 0.78; 95% CI, 0.59-1.04). However, they found no evidence for a relation between the two *IL4-Rα* SNPs that we studied and glioma.

The majority of haplotype-allergy ORs are on the opposite side of the null from the haplotype-glioblastoma ORs. Whether this pattern is attributable to sampling variation is not known; however, if it is not, then it suggests that the *IL-4Rα* and *IL-13* haplotypes play a role in both conditions. Wiemels et al. observed an inverse pattern for both *IL-13* SNPs and IgE levels among the controls; however, they did not find associations between the *IL-4Rα* SNPs and IgE levels. Furthermore, this inverse association observed by Wiemels et al. is consistent both with prior knowledge about the link between the *IL-4Rα* and *IL-13* SNPs and allergies (29, 30) and the reported inverse relation between allergies and glioblastoma (4-6).

Although our sample is one of the largest reported glioblastoma case-control data sets, it is still possible that this sample was not large enough to identify case-control differences in the distribution of *IL-4Rα* or *IL-13* SNPs. The pooled OR for the total sample was close to the null value and the previously reported *IL-4Rα* SNP Swedish findings (19) are replicated only in the Southeast English data set, which has approximately the same number of cases (n = 107) as the Swedish sample (n = 110). The Danish (n = 66) and Finnish (n = 44) samples are smaller and provide no evidence for these associations. Possibly more important in accounting for our failure to duplicate the *IL-4Rα* SNP Swedish findings than sample size was the evidence for heterogeneity of the *IL4Rα* SNP ORs among countries. This heterogeneity may reflect the role of unknown and therefore uncontrolled environmental factors in obscuring the effects of the allergy SNPs. Our failure to replicate our findings may be attributed to the differing prevalence among countries of genotypes on the *IL-4/IL-13* pathway with which the *IL-4Rα* or *IL-13* SNPs may interact. It is becoming clear that SNPs or even individual genes cannot be examined for their effect on disease in isolation but rather whole genetic pathways must be simultaneously investigated as well as pathways that perform similar functions.

Of central importance in determining the validity of our findings is the presence of prior knowledge (31). There are several lines of evidence that suggest a role for IL-4 and IL-13 cytokines in glioma etiology. In addition to their central role in allergic conditions, these cytokines resolve brain inflammation by inducing *COX-2* and killing microglia. Both IL-4 and IL-13 suppress cell proliferation in the normal astrocytic and low-grade astrocytoma cell lines (9, 10), possibly by blocking angiogenesis (32). Additional evidence for an association between IL-13 and glioblastoma includes the fact that glioblastoma tissue is characterized by the presence of *IL-13Rα2* decoy receptors that limit the biological availability of IL-13 (33).

Yet, in spite of a relatively strong biological rationale, we were unable to provide evidence for associations between the *IL-4Rα* and *IL-13* SNPs and glioblastoma originally observed in the Swedish data set. We did, however, find an association between the *T-G IL-4Rα* haplotype and glioblastoma risk and also a suggestion of an inverse relation between *IL-4Rα* and *IL-13* haplotypes and allergies. Potential reasons for our lack of confirmation of previous results include the absence of a relationship between the cytokines coded by these genes and glioblastoma risk or our failure to simultaneously consider other genes on the *IL-4/IL-13* pathway or to identify the specific SNPs on the *IL-4Rα* and *IL-13* genes involved in glioblastoma development. Cousin et al. (34) reported that at least six SNPs are required to characterize the variability of the *IL-13* gene, whereas (35) four are needed to represent that of *IL-4Rα*. However, we evaluated only two *IL-4Rα* and two *IL-13* SNPs in the present article. Although our findings weakly indicate an association between the *IL-4Rα* gene and glioblastoma risk, the relative strength of the previous biological evidence suggests that further research is needed to evaluate the possible role of the entire *IL-4/IL-13* pathway in glioblastoma development.

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