Single nucleotide polymorphisms in *IL-4Ra*,*IL-13* and *STAT6* genes occurs in brain glioma

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1. ABSTRACT

Gliomas are aggressive brain tumor. Association studies were consistent for an inverse association between asthma and allergic conditions (IgE levels) and risk of glioma. Studies reported that the *IL-4Ra*, *IL-13* and *STAT6* genes played a relatively strong role in IgE production or allergy. This population-based case-control study aimed to find potential association between single nucleotide polymorphisms *IL-13*rs20541, *IL-4Ra*rs1801275 and glioma susceptibility in population, as well as *STAT6* rs1059513 and *STAT6* rs324015. Among non-smokers, homozygote GG of *STAT6* 4610A/G showed an increased association with risk of glioma compared with AA (adjusted OR=1.691, 95%CI=1.152-2.481, p=0.007, corrected p=0.028), and the haplotype with A allele at rs1059513 and G allele at rs324015 was revealed to increase glioma risk significantly (OR=1.321,95%CI= 1.081-1.614, p=0.007,corrected p=0.028). GG genotype of *STAT6* 4610A/G was a significant risk factor compared with AA in glioblastoma (adjusted OR=1.856, 95%CI=1.153-2.987, p=0.011, corrected p=0.044). GG of *STAT6* 4610A/G was significantly related to increased WHO IV risk compared with AA (adjusted OR=1.591,95%CI=1.030-2.459, p=0.036, corrected p=0.144). Interaction between *IL-13* Arg130Gln and *IL-4Ra* Gln576Arg was observed in decreasing glioma risk (p=0.045).

2. INTRODUCTION

Gliomas are aggressive lethal solid brain tumor arising from support cells in the central nervous system, which can be divided into astrocytic tumors, oligodendrogliomas and oligoastrocytomas. According to the WHO classification, gliomas can be graded into four hitological degrees of malignancy (1). Astrocytomas, amounting to 80-85% of all gliomas, are tumors composed of neoplastic astrocytes predominantly and graded from low (grade I) to high (grade IV) according to hallmarks of the tumor histological aberrations (2). Grade IV astrocytomas are known as glioblastoma multiforme (GBM), the most common type of adult glioma with a poor prognosis. Inherited syndromes account for only a small proportion of glioblastoma but familial aggregation of this tumor has been observed (3) Oligodendrogliomas and oligoastrocytomas are tiered into grade II, and anaplastic is grade III lesions. Despite the development of therapy technology, the death rate of glioblastoma patients decreases a little (4). The strongest known environmental risk factor for glioma is exposure to therapeutic doses of ionizing radiation (5) and genetic factors such as single nucleotide polymorphisms (SNPs) might also associate with glioma risk (6). Evidence from cohort and case-control studies was consistent for an inverse association between self-reported asthma and allergic conditions and risk of glioma (7-9). Wiemels *et al.* examined whether

allergic disease reduces brain tumor risk by comparing serum IgE levels (10). Although lower serum IgE levels were found in glioma cases than controls, the possibility that the immunosuppression by the tumor itself or by its standard treatment related to lower IgE levels or eliminate allergies can not be excluded.

Genes *IL-4* and *IL-13* share a common receptor component, *IL-4Ra* chain, and code immunoregulatory cytokines that share functions. Many of the actions of IL-13 closely resemble those of IL-4. Both cytokines play a central role in allergy by stimulating IgE synthesis in B lymphocytes (11) and reduce production of pro-inflammatory cytokines by macrophages (12). Previous studies showed they have strong antitumor activity in mice and inhibit proliferation of astrocytoma and low-grade glioma in human cell lines (13, 14). The pleiotropic effects of IL-13 and IL-4 are mediated through the IL-4R, which is composed of the common IL4-Ra subunit and either a gamma subunit or IL-13 receptor (IL-13R) alpha subunit (low-affinity IL-13Ra1 (15)or a high-affinity IL-13Ra2 subunit (16)). IL-13Ra2 acts as an inhibitor of IL-4-dependent signal transduction and STAT6-responsive gene expression. The inhibition is likely mediated through physical interaction between cytoplasmic domain of the IL-4Ra chain and short intracellular domain of IL-13Ra2 protein (17). Human gene signal transducer and activator of transcription 6 (*STAT6*) spans 19 kb of genomic DNA containing 23 exons in region 12q12.3-14.1 (18). The phosphorylation of the intracellular molecule STAT6 through the activated Janus tyrosine kinases leaded to a homodimerisation of STAT6, which migrated to the nucleus and bound to a specific region found within promoters of IL-4–inducible and IL-13–inducible genes (19).

Studies of germ line polymorphisms of the *IL-4Ra*, *IL-13* and *STAT6* genes provide relatively strong support for a role of these genes in IgE production or allergy (20-22). Previous analysis of data from a population-based case-control study in Sweden indicated genetic variants that increase the risk of IgE or allergies also decrease glioblastoma risk. Polymorphism in *IL-4Ra* Gln576Arg increased GBM risk (p=0.02) (23), but this difference was not confirmed by analysis in larger population in European (24) or United States (25). Although the combination of IL-13 and IL-4Ra relate to asthma and plasma IgE concentration (26, 27), no previous studies have indicated the roles of their interaction in glioma susceptibility. For SNPs in *STAT6*, association studies were conducted for asthma and IgE level (28, 29), but no association study in *STAT6* had been reported in glioma. Here we reported population-based case-control study aimed to address potential association of SNPs *IL-13* rs20541, *IL-4Ra* rs1801275 and their interaction in glioma susceptibility, as well as *STAT6* SNPs rs324015 and rs1059513.

3. METHODS

3.1. Study population

The population in this case-control study was similar to Liu *et al.* (30). All subjects were genetically unrelated ethnic Han from Shanghai and the surrounding provinces (Zhejiang, Jiangsu and Anhui) in east China. Patients diagnosed with histopathologically confirmed glioma were consecutively recruited between October 2004 and May 2006 in the Department of Neurosurgery at Huashan Hospital of Fudan University (Shanghai, China) with no restrictions of age, gender and histology. Cancer-free controls were recruited during the same period including trauma outpatients (20%) from the Emergency Medical Centre and hospital visitors (80%) who came to the health examination clinic for an annual check-up at the same hospital (Huashan Hospital). The exclusion criteria for healthy subjects included central nervous system-related disease, self-reported history of any cancer and previous radiotherapy and chemotherapy for unknown disease conditions. All the control subjects were frequency matched to the cases on age (+/-5 years), gender and residence area (urban or rural). The research protocol was reviewed and approved by the Fudan University Ethics Committee for Human Subject Research.

3.2. Questionnaire

Each eligible subject was interviewed by trained personnel who were not aware of the case and control with a structured questionnaire to obtain detailed information on demographic factors, family history of cancer (fmc), smoking status, and health characteristics. Fmc was defined as any self-reported cancer in the first-degree relatives (parents, siblings, or children). Never-smokers were defined as those who had smoked less than one cigarette per day and less than 1 year in their lifetime. Smokers were classified into ever-smokers and current-smokers. After interview, 3-5ml venous blood specimen was collected from each subject after the informed consent was obtained.

3.3. Laboratory genotyping

EDTA-containing tubes were used to collect blood samples and then Qiagen Blood Kit (Qiagen, Chatsworth, CA, USA) was applied to extract genomic DNA. Polymerase chain reaction-ligation detection reaction (PCR-LDR) method was used to perform the genotyping.

Specific primers were summarized in Table 1 (Table 1). PCR was conducted on the ABI 9600 (Applied Biosystems, Foster City, CA, USA) in a system with total volume of 15 ul containing 1 ul genomic DNA, 1.5 ul 10×PCR Buffer, 0.13 uM each primer, 0.2 mM dNTP, 0.25 ul Taq DNA polymerase (Qlagen Gmbh, Hllden, Germany) and 7.5 ul H_2O. The cycling parameters were: 94 °C for 1 min; 35 cycles at 94 °C for 10 s, 56 °C for 20 s, 72 °C for 40 s; and a final extension step at 72 °C for 3 min. For each PCR product, the ligation reaction was performed in a final volume of 10 ul including 2 ul of PCR product, 1 ul 10×Taq DNA ligase buffer, 0.02 uM of probe mixture, 5 U Taq DNA ligase (New England Biolabs, Beverly, Mass, USA) and 6 ul H_2O. The LDR parameters were as follows: 25 cycles at 94 °C for 30 s and 55 °C for 4 min. The LDR reaction products were analyzed on ABI 377 DNA Sequencer (Applied Biosystems). To confirm the accuracy of PCR-LDR genotyping method, direct DNA

Table 1. Primers for PCR and probes for LDR detection

SNPs	PCR primers	Probes for LDR
Rs20541	F:TTCCCGCCTACCCAAGACATT R:GAGACAGTCCCTGGAAAGCCC	C-specific: tttttATGCTTTCGAAGTTTCAGTTCAACC T-specific: ttttttttttATGCTTTCGAAGTTTCAGTTCAACT R: P-GTCCCTCGCGAAAAAGTTTCTTTAAtt-FAM
Rs1801275	F:AGGAAGTAGAACCCGAGATGC R:GTCCAGTCCAAAGGTGAACAA	G-specific: CTCGGCCCCCACCAGTGGCAATCG A-specific: tttttCTCGGCCCCCACCAGTGGCAATCA R: -P-GGAGTTTGTACATGCGGTGGAG-FAM-
Rs324015	F: GCTCTTCTACTACCCCCACAG R:CGTAGGCAAAAGCAGATAGAC	A-specific: ttttttttttAGGGAAGTTCAGGCTCTGAGTCACA G-specific: ttttttttttGTGTATGAGACTATGCAAAACTACG R:-P-CCCCAACATGCCTGCACCTGCAGCGtt-FAM-
Rs1059513	F: GCTCTTCTACTACCCCCACAG R:CGTAGGCAAAAGCAGATAGAC	G-specific: ttttttttttGTGTATGAGACTATGCAAAACTACG A-specific:ttttttttttttttttGTGTATGAGACTATGCAAAACTACA R:-P-AGGGCTGAGATTCTTCGTGTATAGCtttttt-FAM-

F: forward primer, R:reverse primer, FAM: 6-carboxyXuorescein

sequencing of randomly selected PCR products was performed. The proportion of the sequencing samples was about 5%.

3.4. Statistical analysis

To examine Hardy-Weinberg Equilibrium, a Chi-Square test for goodness of fit was performed using a web-based program (http://ihg2.helmholtz-muenchen.de/cgi-bin/hw/hwa1.pl). The Fisher's exact Chi-Square test was conducted to compare the frequency distribution of age, gender, smoking status and fmc between cases and controls. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated by univariate logistic regression analyses and multivariate logistic regression analyses adjusted for age and sex, or age, fmc and smoking status under dominant genetic model, recessive genetic model and additive model.

The patients were stratified into three subgroups according to histology: glioblastoma, astrocytomas (including diffuse astrocytomas, anaplastic astrocytomas or other astrocytomas except for glioblastoma) and other gliomas (including oligodendroglimas, enpendymomas or mixed glioma). Patients were also grouped with WHO grade to glioma: WHOI, WHOII, WHOIII and WHOIV. Subgroup analyses according to smoking status, histology and WHO grade were performed to estimate the specific ORs and CIs. Haplotype frequencies for *STAT6* were estimated from genotype data using the expectation-maximization algorithm in Haploview 4.1 (Broad Institute, Cambridge, MA). Gene-Gene interaction analysis between *IL13* and *IL4Ra* was performed by introducing an interaction term into the univariate and multivariate logistic regression which was adjusted by age, sex, fmc, smoking status and genotype.

All the regression analyses were performed using SPSS 16.0 (SPSS Inc., Chicago, IL, USA). QUANTO (version 1.2.4) software was used to calculate the statistic power**.** All p-values were two-sided, and considered significant if a p-value is less than 0.05. We adjusted multiple test using Bonferroni corrections.

4.RESULTS

4.1. Characteristics of study populations

806 glioma cases and 910 control subjects were recruited without the restrictions of age, sex and glioma histology. Among all participants, DNA samples and questionnaires were available from 677 cases and 698 cancer-free control subjects representing an 84.0% and 76.7% of all eligible case and control subjects, respectively.

Table 2 showed the summarized characteristics of case patients and control subjects included in our study (Table 2). The mean ages were 41.6 years (+/-16.3 years, ranging from 2-79 yeas) for the cases and 39.6 years (+/-18.3 years, ranging from 1-86 years) for the controls. No significant differences on age, sex and smoking status between cases and controls (p=0.355 for age stratification, 0.141 for sex and 0.093 for smoking status) were observed. Among 677 case patients, 256 (37.8%) had astrocytomas, 220 (32.5%) had glioblastoma and 201 (29.7%) had other gliomas.

4.2. Association study without stratification

The genotype distributions of controls were in Hardy-Weinberg equilibrium (data not shown). No significant association between *IL-13* Arg130Gln (CT and TT genotypes) and glioma risk comparing to CC was observed in the univariate and multivariate logistic regression analysis The similar result was also observed in *IL-4Ra* Gln576Arg (rs1801275) when AG and GG genotypes comparing to AA. *STAT6* 4219A/G (rs324015) and *STAT6* 4610A/G (rs1059513) showed no significant association to glima risk either (Table 3). Dominant genetic model, recessive genetic model and additive genetic model were used to estimate specific ORs and no significant association was found. For each SNP, statistical powers of dominant model, recessive model and additive model were evaluated, and additive model was found with highest statistical power in the study (data not shown). Thus, additive model was used for all the following association analysis.

4.3. Association analysis after stratification

Stratification analyses were performed by smoking status, histology subtypes and WHO grade. Subjects were grouped as never-, ever- and current-smokers as described above. Among never smokers, homozygote GG of *STAT6* 4610A/G showed an increased association with risk of glioma compared with AA (adjusted OR=1.691, 95% CI=1.152-2.481, p=0.007, corrected p=0.028) (Table 4). No significant association was observed among ever- and current-smokers. Table 5 summarized logistic regression analysis stratified by histology subtypes (Table 5). GG genotype of *STAT6*

Table 2. Characteristics of selected patients with glioma and controls

Variable	Patients (n=677) No.(%)	Controls(n=698)No. (%)	P value*
Gender			0.141
Male	400(59.1)	384(55.0)	
Female	277(40.9)	314(45.0)	
Age(years)			0.355
Children(≤18)	69(10.2)	60(8.6)	
Adults(>18)	608(89.8)	638(91.4)	
Smoking status[1]			0.093
Never	386(65.8)	454(65.3)	
Ever	72(12.3)	111(16.0)	
Current	129(22.0)	130(18.7)	
Family history of cancer			0.056
No	566(83.6)	609(87.2)	
Yes	111(16.4)	89(12.8)	
Histology			
Astrocytomas[2]	256(37.8)		
Glioblastoma	220(32.5)		
Other gliomas[3]	201(29.7)		
WHO			
WHOI	61(9.0)		
WHOII	236(34.9)		
WHOIII	118(17.4)		
WHOIV	262(38.7)		

*Two-sides χ^2 test, [1] Smoking status information was absent for 90 patients and 3 controls, [2] Astrocytomas including diffuse astrocytomas, anaplastic astrocytomas, and other astrocytomas except for glioblastoma, [3] Other gliomas including oligodendrogliomas, ependymomas, medulloblastoma, gliomatosis cerebri or mixed gliomas

Table 3. Analysis of association between the polymorphisms and risk of glioma with multivariate logistic regression analyze

	Case(%) N=672	Contro(%) N=693	Crude p	Crude OR (95%CI)	Adjusted P*	Adjusted OR*(95%CI)	Bonferroni-corrected p**
IL-13							
rs20541							
CC	316(47.0)	300(43.3)		1.000		1.000	
CT	293(43.6)	319(46.0)	0.230	0.872(0.697-1.091)	0.740	0.961(0.760-1.215)	1.000
TT	63(9.4)	74(10.7)	0.261	0.808(0.558-1.172)	0.639	0.912(0.621-1.339)	1.000
CT+TT/CC	356/316	393/300	0.166	0.860(0.695-1.065)	0.665	0.952(0.761-1.190)	1.000
CC+CT/TT	609/63	619/74	0.423	0.865(0.607-1.233)	0.700	0.931(0.646-1.341)	1.000
additive			0.155	0.889(0.756-1.046)	0.612	0.957(0.808-1.134)	1.000
IL-4Ra							
rs1801275							
AA	462(68.8)	466(67.0)		1.000		1.000	
GA	196(29.2)	205(29.5)	0.762	0.964(0.763-1.219)	0.823	0.972(0.761-1.242)	1.000
GG	14(2.1)	25(3.6)	0.093	0.565(0.290-1.100)	0.275	0.685(0.348-1.350)	1.000
GA+GG/AA	210/462	230/466	0.477	0.921(0.734-1.156)	0.625	0.943(0.744-1.195)	1.000
AA+GA/GG	658/14	671/25	0.098	0.571(0.294-1.108)	0.283	0.691(0.353-1.356)	1.000
additive			0.252	0.890(0.729-1.086)	0.443	0.922(0.749-1.134)	1.000
STAT6							
rs324015							
AA	160(23.8)	187(26.9)		1.000		1.000	
GA	328(48.9)	340(48.9)	0.366	1.127(0.869-1.462)	0.418	1.119(0.852-1.469)	1.000
GG	183(27.3)	169(24.3)	0.120	1.266(0.940-1.703)	0.068	1.334(0.978-1.818)	0.272
GA+GG/AA	511/160	509/187	0.199	1.173(0.919-1.498)	0.182	1.190(0.922-1.536)	0.728
AA+GA/GG	488/183	527/169	0.206	1.169(0.917-1.491)	0.097	1.239(0.962-1.595)	0.388
additive			0.120	1.125(0.970-1.305)	0.068	1.155(0.989-1.349)	0.272
rs1059513							
AA	581(86.5)	605(86.9)		1.000		1.000	
GA	90(13.4)	90(12.9)	0.800	1.041(0.761-1.425)	0.972	1.006(0.724-1.398)	1.000
GG	1(0.1)	1(0.1)	0.977	1.041(0.065-16.687)	0.889	1.220(0.076-19.658)	1.000
GA+GG/AA	91/581	91/605	0.799	1.041(0.762-1.423)	0.961	1.008(0.727-1.399)	1.000
AA+GA/GG	671/1	695/1	0.980	1.036(0.065-16.593)	0.889	1.219(0.076-19.634)	1.000
additive			0.801	1.040(0.765-1.414)	0.949	1.011(0.733-1.394)	1.000

*:adjusted for age, sex, family history of cancer (fmc) and smoking status, **:adjusted multiple test using Bonferroni corrections

4610A/G was a significant risk factor compared with AA in glioblastoma (adjusted OR=1.856, 95%CI= 1.153-2.987, p=0.011, corrected p=0.044). No significant association was found in astrocytomas or other gliomas including oligodendroglimas, enpendymomas and mixed glioma. In stratification analysis by WHO grade, GG genotype of *STAT6* 4610A/G was significantly related to increased WHO IV risk compared with AA (adjusted OR=1.591,95%CI=1.030-2.459, p=0.036, corrected p=0.144). Additive model showed similar results (adjusted OR=1.265, 95%CI=1.017-1.575, p=0.035, corrected p=0.140) (Table 6).

Table 4. Stratified analysis of association by Smoking status between the genotypes and risk of glioma

Smoking status	SNPs	Case/control	Crude p	Crude OR (95%CI)	Adjusted P*	Adjusted OR*(95%CI)	Bonferroni-corrected p**
Never	rs20541						
	CC	167/193		1.000		1.00	
	CT	169/207	0.695	0.944(0.706-1.261)	0.795	0.962(0.719-1.288)	1.000
	TT	45/54	0.869	0.963(0.616-1.505)	0.864	0.961(0.614-1.506)	1.000
	additive		0.764	0.969(0.792-1.187)	0.806	0.975(0.795-1.195)	1.000
	rs1801275						
	AA	257/302		1.000		1.00	
	GA	114/133	0.963	1.007(0.746-1.360)	0.931	1.013(0.750-1.370)	1.000
	GG	10/18	0.291	0.653(0.296-1.439)	0.295	0.653(0.295-1.448)	1.000
	additive		0.574	0.931(0.725-1.195)	0.600	0.935(0.727-1.202)	1.000
	Rs324015						
	AA	84/126		1.000		1.000	
	GA	179/223	0.283	1.204(0.858-1.690)	0.296	1.199(0.853-1.685)	1.000
	GG	117/104	0.007	1.687(1.152-2.473)	0.007	1.691(1.152-2.481)	0.028
	additive		0.007	1.301(1.075-1.576)	0.007	1.303(1.075-1.578)	0.028
	Rs1059513						
	AA	326/393		1.000		1.000	
	GA	54/59	0.627	1.103(0.742-1.641)	0.624	1.105(0.741-1.646)	1.000
	GG	1/1	0.895	1.206(0.075-19.348)	0.907	1.180(0.073-19.033)	1.000
	additive		0.617	1.103(0.751-1.619)	0.617	1.103(0.751-1.622)	1.000
Ever	rs20541						
	CC	33/56		1.000		1.000	
	CT	33/42	0.368	1.333(0.712-2.495)	0.637	1.171(0.608-2.255)	1.000
	TT	6/10	0.974	1.018(0.339-3.058)	0.860	0.904(0.293-2.786)	1.000
	additive		0.605	1.129(0.713-1.787)	0.900	1.031(0.640-1.662)	1.000
	rs1801275						
	AA	47/75		1.000		1.000	
	GA	24/34	0.714	1.126(0.596-2.130)	0.471	1.275(0.658-2.472)	1.000
	GG	1/2	0.855	0.798(0.070-9.045)	0.693	0.608(0.052-7.143)	1.000
	additive		0.808	1.075(0.602-1.918)	0.655	1.145(0.631-2.078)	1.000
	Rs324015						
	AA	17/32		1.000		1.000	
	GA	42/49	0.192	1.613(0.787-3.309)	0.115	1.823(0.864-3.845)	0.460
	GG	13/30	0.649	0.816(0.339-1.961)	0.732	0.853(0.342-2.124)	1.000
	additive		0.726	0.928(0.610-1.411)	0.835	0.955(0.618-1.475)	1.000
	rs1059513						
	AA	63/93		1.000		1.000	
	GA	9/18	0.490	0.738(0.312-1.747)	0.431	0.697(0.285-1.709)	1.000
	GG	0/0	N/A	N/A	N/A	N/A	N/A
	additive		0.490	0.738(0.312-1.747)	0.431	0.697(0.285-1.709)	1.000
Still	rs20541						
	CC	62/50		1.000		1.000	
	CT	60/68	0.190	0.712(0.428-1.184)	0.327	0.770(0.457-1.299)	1.000
	TT	7/10	0.279	0.565(0.200-1.590)	0.324	0.584(0.201-1.700)	1.000
	additive		0.132	0.731(0.486-1.100)	0.219	0.767(0.503-1.170)	0.876
	rs1801275						
	AA	95/87		1.000		1.000	
	GA	31/38	0.304	0.747(0.428-1.303)	0.516	0.828(0.468-1.464)	1.000
	GG	3/4	0.629	0.687(0.149-3.156)	0.852	0.863(0.184-4.043)	1.000
	additive		0.284	0.773(0.482-1.239)	0.542	0.860(0.530-1.396)	1.000
	Rs324015						
	AA	38/29		1.000		1.000	
	GA	59/66	0.210	0.682(0.375-1.240)	0.167	0.650(0.353-1.198)	0.668
	GG	32/34	0.342	0.718(0.363-1.422)	0.407	0.744(0.370-1.497)	1.000
	additive		0.341	0.847(0.602-1.192)	0.405	0.862(0.608-1.222)	1.000
	rs1059513						
	AA	116/116		1.000		1.00	
	GA	13/13	1.000	1.000(0.445-2.249)	0.991	0.995(0.436-2.270)	1.000
	GG	0/0	N/A	N/A	N/A	N/A	N/A
	additive		1.000	1.000(0.445-2.249)	0.991	0.995(0.436-2.270)	1.000

N/A: not available, *:adjusted for age, sex, family history of cancer (fmc) and smoking status, **:adjusted multiple test using Bonferroni corrections

4.4. Haplotype analysis for STAT6

The two tag SNPs in *STAT6* were considered in the same linkage disequilibrium block, three haplotypes composed of them were constructed and identified using the Haploview software (Table 7a). Haplotype frequencies were 0.498 for AA, 0.435 for AG and 0.067 for GG. Logistic regression analysis between haplotypes and glioma was summarized in Table 7 (Table 7), using most frequent haplotype Hap1 as reference. No significant association was observed between the haplotypes and glioma risk in all subjects.

We further performed stratification analysis according to smoking status, WHO grade, and histology.

Table 5. Stratified analysis of association by histology between the genotypes and risk of glioma

Histology subtypes	SNPs	case/control	Crude p	Crude OR (95%CI)	Adjusted P*	Adjusted OR*(95%CI)	Bonferroni-corrected p**
Astrocytoma[1]	rs20541						
	CC	121/300		1.000		1.000	
	CT	110/319	0.310	0.855(0.632-1.157)	0.914	0.982(0.712-1.356)	1.000
	TT	24/74	0.399	0.804(0.485-1.334)	0.849	0.951(0.563-1.604)	1.000
	additive		0.260	0.881(0.706-1.098)	0.848	0.978(0.775-1.232)	1.000
	rs1801275						
	AA	174/466		1.000		1.000	
	GA	74/205	0.835	0.967(0.704-1.328)	0.903	0.979(0.699-1.372)	1.000
	GG	8/25	0.711	0.857(0.379-1.936)	0.948	1.028(0.450-2.346)	1.000
	additive		0.712	0.952(0.731-1.238)	0.957	0.992(0.752-1.309)	1.000
	Rs324015						
	AA	61/187		1.000		1.000	
	GA	125/340	0.508	1.127(0.791-1.606)	0.810	1.047(0.720-1.522)	1.000
	GG	69/169	0.274	1.252(0.837-1.872)	0.317	1.240(0.814-1.888)	1.000
	additive		0.274	1.119(0.915-1.368)	0.317	1.114(0.902-1.377)	1.000
	Rs1059513						
	AA	226/605		1.000		1.000	
	GA	29/90	0.516	0.863(0.552-1.347)	0.122	0.668(0.400-1.114)	0.488
	GG	1/1	0.487	2.677(0.167-42.979)	0.385	3.459(0.211-56.742)	1.000
	additive		0.657	0.908(0.592-1.392)	0.209	0.732(0.450-1.191)	0.836
Glioblastoma	rs20541						
	CC	99/300		1.000		1.000	
	CT	101/319	0.799	0.959(0.697-1.320)	0.902	1.022(0.718-1.455)	1.000
	TT	20/74	0.472	0.819(0.476-1.411)	0.735	0.902(0.495-1.642)	1.000
	additive		0.515	0.925(0.733-1.169)	0.856	0.976(0.755-1.263)	1.000
	rs1801275						
	AA	152/466		1.000		1.000	
	GA	64/205	0.798	0.957(0.685-1.338)	0.645	0.916(0.631-1.330)	1.000
	GG	3/25	0.106	0.368(0.110-1.236)	0.195	0.436(0.124-1.530)	0.780
	additive		0.263	0.847(0.634-1.133)	0.279	0.837(0.605-1.156)	1.000
	Rs324015						
	AA	49/187		1.000		1.000	
	GA	104/340	0.429	1.167(0.795-1.713)	0.342	1.232(0.801-1.897)	1.000
	GG	66/169	0.065	1.490(0.975-2.278)	0.011	1.856(1.153-2.987)	0.044
	additive		0.063	1.224(0.989-1.514)	0.010	1.371(1.079-1.743)	0.040
	Rs1059513						
	AA	181/605		1.000		1.000	
	GA	38/90	0.103	1.411(0.933-2.135)	0.080	1.498(0.953-2.354)	0.320
	GG	0/1	1.000	0.000	1.000	0.000	1.000
	additive		0.131	1.370(0.910-2.062)	0.098	1.458(0.933-2.277)	0.392
Other gliomas[2]	rs20541						
	CC	96/300		1.000		1.000	
	CT	82/319	0.199	0.803(0.575-1.122)	0.418	0.863(0.605-1.232)	1.000
	TT	19/74	0.436	0.802(0.461-1.396)	0.726	0.902(0.508-1.603)	1.000
	additive		0.224	0.859(0.673-1.097)	0.512	0.918(0.710-1.186)	1.000
	rs1801275						
	AA	136/466		1.000		1.000	
	GA	58/205	0.861	0.969(0.684-1.374)	0.873	1.031(0.713-1.490)	1.000
	GG	3/25	0.151	0.411(0.122-1.383)	0.203	0.451(0.133-1.534)	0.812
	additive		0.342	0.864(0.640-1.168)	0.545	0.907(0.662-1.243)	1.000
	Rs324015						
	AA	50/187		1.000		1.000	
	GA	99/340	0.663	1.089(0.742-1.599)	0.652	1.098(0.731-1.650)	1.000
	GG	48/169	0.791	1.062(0.679-1.662)	0.756	1.078(0.671-1.732)	1.000
	additive		0.785	1.031(0.826-1.288)	0.748	1.039(0.822-1.315)	1.000
	Rs1059513						
	AA	174/605		1.000		1.000	
	GA	23/90	0.635	0.889(0.545-1.448)	0.813	0.941(0.569-1.556)	1.000
	GG	0/1	1.000	0.000	1.000	0.000	1.000
	additive		0.572	0.870(0.537-1.410)	0.754	0.924(0.562-1.519)	1.000

[1]Astrocytomas including diffuse astrocytomas, anaplastic astrocytomas, and other astrocytomas except for glioblastoma, [2]Other gliomas including oligodendrogliomas, ependymomas, medulloblastoma, gliomatosis cerebri or mixed gliomas, *:adjusted for age, sex, family history of cancer (fmc) and smoking status, **:adjusted multiple test using Bonferroni corrections

Among non-smokers, Hap 2 (A allele at SNP Rs1059513 and G allele at SNP Rs324015) was revealed to increase glioma risk significantly (OR=1.321, 95%CI= 1.081-1.614, p=0.007, corrected p=0.021). We did not observe significant association in WHO stratification analysis.

4.5. Gene-Gene interaction between IL-13 and IL-4Ra for susceptibility to glioma

The gene-gene interaction between *IL-13* and *IL-4Ra* was analyzed between glioma and control subjects. Multivariate logistic regression analysis indicated the

Table 6. Stratified analysis of association by WHO between the genotypes and risk of glioma

WHO	SNPs	case/control	Crude p	Crude OR (95%CI)	Adjusted P*	Adjusted OR*(95%CI)	Bonferroni-corrected p**
I	rs20541						
	CC	28/300		1.000		1.000	
	CT	29/319	0.924	0.974(0.566-1.676)	0.795	1.083(0.593-1.976)	1.000
	TT	3/74	0.179	0.434(0.129-1.468)	0.278	0.503(0.145-1.743)	1.000
	additive		0.304	0.803(0.529-1.219)	0.507	0.860(0.550-1.345)	1.000
	rs1801275						
	AA	42/466		1.000		1.000	
	GA	17205	0.781	0.920(0.512-1.655)	0.683	1.140(0.608-2.137)	1.000
	GG	1/25	0.431	0.444(0.059-3.358)	0.489	0.485(0.063-3.762)	1.000
	additive		0.500	0.840(0.506-1.394)	0.911	0.971(0.574-1.642)	1.000
	Rs324015						
	AA	15/187		1.000		1.000	
	GA	32/340	0.624	1.173(0.619-2.222)	0.574	`1.229(0.599-2.524)	1.000
	GG	13/169	0.915	0.959(0.443-2.074)	0.583	1.259(0.553-2.865)	1.000
	additive		0.938	0.985(0.680-1.427)	0.580	1.121(0.749-1.678)	1.000
	rs1059513						
	AA	55/605		1.000		1.000	
	GA	5/90	0.305	0.611(0.238-1.567)	0.444	0.687(0.263-1.796)	1.000
	GG	0/1	1.000	0.000	1.000	0.000	1.000
	additive		0.288	0.602(0.237-1.534)	0.425	0.678(0.261-1.761)	1.000
II	rs20541						
	CC	120/300		1.000		1.000	
	CT	89/319	0.026	0.697(0.508-0.957)	0.155	0.783(0.558-1.097)	0.620
	TT	25/74	0.508	0.845(0.512-1.393)	0.903	0.968(0.577-1.626)	1.000
	additive		0.112	0.831(0.662-1.044)	0.448	0.912(0.718-1.158)	1.000
	rs1801275						
	AA	157/466		1.000		1.000	
	GA	72/205	0.801	1.042(0.754-1.441)	0.798	1.046(0.740-1.480)	1.000
	GG	6/25	0.465	0.712(0.287-1.768)	0.702	0.836(0.333-2.098)	1.000
	additive		0.828	0.970(0.739-1.274)	0.983	0.997(0.747-1.330)	1.000
	Rs324015						
	AA	55/187		1.000		1.000	
	GA	120/340	0.328	1.200(0.832-1.730)	0.560	1.122(0.762-1.650)	1.000
	GG	59/169	0.426	1.187(0.778-1.810)	0.602	1.126(0.721-1.758)	1.000
	additive		0.424	1.089(0.884-1.341)	0.598	1.061(0.851-1.324)	1.000
	Rs1059513						
	AA	204/605		1.000		1.000	
	GA	31/90	0.924	1.022(0.659-1.583)	0.534	0.856(0.525-1.397)	1.000
	GG	0/1	1.000	0.000	1.000	0.000	1.000
	additive		0.992	0.998(0.647-1.538)	0.473	0.838(0.516-1.360)	1.000
III	rs20541						
	CC	47/300		1.000		1.000	
	CT	58/319	0.483	1.161(0.766-1.759)	0.222	1.321(0.845-2.064)	0.888
	TT	11/74	0.884	0.949(0.469-1.918)	0.653	1.180(0.575-2.421)	1.000
	additive		0.810	1.037(0.769-1.399)	0.366	1.156(0.845-1.580)	1.000
	rs1801275						
	AA	80/466		1.000		1.000	
	GA	32/205	0.673	0.909(0.585-1.414)	0.476	0.842(0.524-1.353)	1.000
	GG	4/25	0.898	0.932(0.316-2.749)	0.848	1.113(0.373-3.321)	1.000
	additive		0.696	0.930(0.647-1.338)	0.670	0.919(0.624-1.354)	1.000
	Rs324015						
	AA	28/187		1.000		1.000	
	GA	53/340	0.872	1.041(0.637-1.702)	0.976	1.008(0.600-1.695)	1.000
	GG	35/169	0.238	1.383(0.807-2.370)	0.289	1.359(0.770-2.399)	1.000
	additive		0.232	1.182(0.898-1.556)	0.282	1.173(0.877-1.569)	1.000
	rs1059513						
	AA	98/605		1.000		1.000	
	GA	17/90	0.591	1.166(0.666-2.043)	0.421	1.269(0.710-2.269)	1.000
	GG	1/1	0.199	6.173(0.383-99.511)	0.185	6.605(0.404-108.031)	0.740
	additive		0.370	1.272(0.752-2.152)	0.239	1.385(0.805-2.381)	0.956
IV	rs20541						
	CC	121/300		1.000		1.000	
	CT	117/319	0.533	0.909(0.674-1.226)	0.889	0.977(0.706-1.353)	1.000
	TT	24/74	0.399	0.804(0.485-1.334)	0.575	0.853(0.489-1.488)	1.000
	additive		0.353	0.902(0.724-1.122)	0.634	0.944(0.744-1.198)	1.000
	rs1801275						
	AA	183/466		1.000		1.000	
	GA	75/205	0.659	0.932(0.680-1.277)	0.774	0.951(0.675-1.340)	1.000
	GG	3/25	0.055	0.306(0.091-1.024)	0.105	0.359(0.104-1.239)	0.420
	additive		0.150	0.817(0.621-1.076)	0.259	0.842(0.625-1.135)	1.000
	Rs324015						

	AA	62/187		1.000		1.000	
	GA	123/340	0.629	1.091(0.766-1.554)	0.416	1.176(0.796-1.738)	1.000
	GG	76/169	0.130	1.356(0.914-2.013)	0.036	1.591(1.030-2.459)	0.144
	additive		0.128	1.167(0.957-1.423)	0.035	1.265(1.017-1.575)	0.140
	Rs1059513						
	AA	224/605		1.000		1.000	
	GA	37/90	0.619	1.110(0.735-1.677)	0.692	1.095(0.699-1.715)	1.000
	GG	0/1	1.000	0.000	1.000	0.000	1.000
	additive		0.702	1.083(0.720-1.627)	0.752	1.074(0.689-1.674)	1.000

*:adjusted for age,sex,family histoy of cancer and smoking status when stratifed by histology and WHO, for age, sex, family history of cancer by smoking status, **:adjusted multiple test using Bonferroni corrections

Table 7. Haplotype analysis of *STAT6*

A. Haplotype construction by haploview software and regression analysis							
Haplotypes	**SNPs**	**Case n (%)**	**Control N (%)**	**OR(95%CI)**	**p-value**	**Bonferroni-corrected p****	
	Rs1059513	Rs324015					
	Rs1059513	Rs324015					
Hap1	A	A	649(48.3)	712(51.2)	1.000		
Hap2	A	G	603(44.9)	587(42.4)	1.127(0.965-1.317)	0.132	0.396
Hap3	G	G	92(6.8)	91(6.5)	1.109(0.815-1.510)	0.511	1.000

B. Haplotype ananlysis after stratification					
	Haplotypes	**Haplotype frequencies (case/control)**	**OR(95%CI)**	**p-value**	**Bonferroni-corrected p****
Smoking status					
Never	Hap1 AA	0.457/0.524	1.000		
	Hap2AG	0.470/0.408	1.321(1.081-1.614)	0.007	0.021
	Hap3GG	0.073/0.067	1.253(0.850-1.847)	0.255	0.765
ever	Hap1 AA	0.528/0.509	1.000		
	Hap2AG	0.410/0.410	0.964(0.622-1.494)	0.870	1.000
	Hap3GG	0.062/0.081	0.743(0.317-1.742)	0.495	1.000
current	Hap1 AA	0.522/0.475	1.000		
	Hap2AG	0.428/0.474	0.823(0.577-1.174)	0.282	0.846
	Hap3GG	0.049/0.045	0.982(0.426-2.265)	0.966	1.000
Histology					
Astrocytoma[1]	Hap1 AA	0.485/0.512	1.000		
	Hap2AG	0.455/0.422	1.140(0.924-1.405)	0.222	0.666
	Hap3GG	0.061/0.065	0.978(0.635-1.507)	0.920	1.000
Glioblastoma	Hap1 AA	0.461/0.512	1.000		
	Hap2AG	0.453/0.422	1.187(0.948-1.485)	0.134	0.402
	Hap3GG	0.086/0.065	1.488(0.988-2.243)	0.057	0.171
Other gliomas[2]	Hap1 AA	0.505/0.512	1.000		
	Hap2AG	0.437/0.422	1.047(0.830-1.319)	0.700	1.000
	Hap3GG	0.058/0.065	0.914(0.563-1.484)	0.717	1.000
WHO grade					
I	Hap1 AA	0.516/0.512	1.000		
	Hap2AG	0.442/0.422	1.035(0.706-1.518)	0.860	1.000
	Hap3GG	0.041/0.065	0.638(0.250-1.629)	0.347	1.000
II	Hap1 AA	0.492/0.512	1.000		
	Hap2AG	0.442/0.422	1.092(0.879-1.357)	0.426	1.000
	Hap3GG	0.066/0.065	1.050(0.680-1.620)	0.826	1.000
III	Hap1AA	0.469/0.512	1.000		
	Hap2AG	0.449/0.422	1.155(0.864-1.544)	0.329	0.987
	Hap3GG	0.081/0.065	1.379(0.808-2.353)	0.238	0.714
IV	Hap1 AA	0.473/0.512	1.000		
	Hap2AG	0.456/0.422	1.169(0.948-1.440)	0.143	0.429
	Hap3GG	0.071/0.065	1.172(0.779-1.764)	0.446	1.000

*: adjusted for age,sex,family histoy of cancer and smoking status when stratifed by histology and WHO, for age, sex, family history of cancer by smoking status, **:adjusted multiple test using Bonferroni corrections,[1] Astrocytomas including diffuse astrocytomas, anaplastic astrocytomas, and other astrocytomas except for glioblastoma, [2] Other gliomas including oligodendrogliomas, ependymomas, medulloblastoma,gliomatosis cerebri or mixed gliomas

interaction effect of *IL-13* Arg130Gln and *IL-4Ra* Gln576Arg might be protective factor for glioma risk (crude OR=0.730, 95%CI=0.537-0.993, p=0.045), and the effect was more significant than possessing the genotype at *IL-13* Arg130Gln and *IL-4Ra* Gln576Arg individually (Table 8). Analysis among current-smokers showed similar results with adjusted OR=0.336 (95%CI=0.134-0.842, p=0.020). The interaction between *IL-13* Arg130Gln and *IL-4Ra* Gln576Arg might be a protective factor for glioblastoma (crude OR=0.623, 95%CI=0.392-0.991, p=0.046), as well as a decreased factor for WHO grade III glioma risk significantly (adjusted OR=0.382, 95% CI=0.199-0.736, p=0.004).

5. DISCUSSION

Association of *IL-4Ra* Gln576Arg and *IL-13* Arg130Gln in glioblastoma multiform (GBM) risk

Table 8. Gene –Gene interaction analysis of *IL13* and *IL 4Rα*

	Crude p-value	Crude OR (95%CI)	Adjusted* p-value	Adjusted*OR (95%CI)
Whole	0.045	0.730(0.537-0.993)	0.061	0.739(0.539-1.013)
Smoking status				
never	0.320	0.833(0.580-1.195)	0.361	0.845(0.588-1.213)
ever	0.382	0.657(0.256-1.684)	0.432	0.679(0.258-1.785)
current	0.014	0.322(0.131-0.794)	0.020	0.336(0.134-0.842)
Histology				
Astrocytoma[1]	0.266	0.792(0.526-1.194)	0.227	0.776(0.514-1.171)
Glioblastoma	0.046	0.623(0.392-0.991)	0.135	0.680(0.410-1.127)
Other gliomas[2]	0.252	0.756(0.468-1.220)	0.242	0.745(0.455-1.220)
WHO				
I	0.444	1.346(0.629-2.879)	0.743	1.137(0.528-2.447)
II	0.584	0.890(0.586-1.351)	0.663	0.908(0.589-1.400)
III	0.006	0.423(0.228-0.785)	0.004	0.382(0.199-0.736)
IV	0.054	0.651(0.421-1.007)	0.143	0.706(0.443-1.125)

*:adjusted for age, sex, fmc and smoking status when stratified by histology and WHO, for age, sex, family history of cancer by smoking status, [1]Astrocytomas including diffuse astrocytomas, anaplastic astrocytomas, and other astrocytomas except for glioblastoma, [2]Other gliomas including oligodendrogliomas, ependymomas, medulloblastoma, gliomatosis cerebri or mixed gliomas

susceptibility was first conducted in a Swedish data set by Schwartzbaum *et al.* (23), with *IL-4Ra* Gln576Arg genotype AG and AA were positively associated with GBM compared with GG. However, the association was not confirmed in a larger study including more European countries (24). Meanwhile, a study conducted by Wiemels *et al.* in United States failed to find significant association between these two SNPs and glioma (25), and they state similar insignificant results in glioblastoma cases. The different results comparing with the Swedish study by Schwartzbaum *et al* might due to either small sample size (111 cases in Swedish study) or regional heterogeneity. In our study, no significant associations between *IL-4Ra* Gln576Arg and glioma risk in genotype regression analysis was observed, as well as *IL-13* Arg130Gln. However, several lines of evidence suggest a role for IL-4 and IL-13 cytokines in glioma etiology. Both IL-4 and IL-13 play a central role in allergy by inducing IgE synthesis (31). Their binding to a shared receptor (IL-4Ra) induced activation of Janus tyrosine kinase (32, 33),which eventually activated STAT6 pathway to activate inducible crucial genes for IgE synthesis (19). In addition, they suppress cell proliferation in the normal astrocytic and low-grade astrocytoma cell lines (13, 34), possibly by blocking angiogenesis (35). In *IL-13*, 130Gln substitution results in phosphorylation of STAT6 in monocytes, decreased affinity of IL-13 for IL-13Ra2, and was neutralized less effectively by an IL-13Ra2 decoy (36). The Gln576Arg substitution in *IL-4Ra* occurs immediately adjacent to a tyrosine residue ,Y575, in the cytoplasmic domain and might alter the efficiency of interaction of Y575 with signal intermediates, which could lead to a dysregulation of IL-4 responses (37). Importantly, IL-4Ra polymorphisms might alter IL-13 responses since it is a component of the IL-13 receptor signal transduction system.

Although IL-4Ra and IL-13 were biological functionally interacted, the interaction analysis of *IL-4Ra* Gln576Arg and *IL-13* Arg130Gln has not been reported yet. Thus we did interaction analysis between the two polymorphisms and observed several significant effects. The data suggested that the combination of *IL-4Rα* Arg and *IL-13* Gln was less susceptible to glioma risk comparing with *IL-4Rα* Gln *IL-13* Arg. The effect was enhanced comparing to the two polymorphisms individually, which suggested the interaction effect of these two candidate loci might be more important than single locus in glioma risk. As discussed above, *IL-13* 130Gln results in STAT6 phosphorylation and increases the combination of IL-13 to IL-4Ra by decreasing affinity of IL-13 for IL-13 Rα2, thus IL-4/IL-13 pathway is induced. On the other hand, *IL-4Ra* 576Arg enhanced signaling of IL-4R by increasing IL-4Ra combination with STAT6, which also induced IL-4/IL-13 pathway. Therefore, the substitutions of *IL-4Ra* Gln576Arg and *IL-13* Arg130Gln come to the same effect to enhance *IL-4* and *IL-13* expression (Figure 1). In smoking status stratification, no significant association was indicated in never- or ever-smokers, but the interaction effect was significant in current-smokers. It seemed that smoking might be a part of susceptibility factors. Glioblastoma cells overexpressed IL-13Ra2 and unlike other glia, failed to phosphorylate STAT6 after IL-13 challenge (34). In histology subtypes, the interaction only significantly associated with decreased glioblastoma risk. IL-4 and IL-13 were showed to inhibit low grade gliomas and the effect had not been indicated in higher grade gliomas (34, 38). Our WHO grade stratification analysis indicated the interaction significantly related to less glioma risk in WHOIII, as well as boardline significance in decreasing glioma risk in WHOIV. We did not observe significant interaction in WHOI or WHOII subtypes.

As a part of the IL-4/IL-13 pathway, which is essential for IgE synthesis, genetic variants in the *STAT6* gene has been identified to contribute to the regulation of total serum IgE levels (28). Even though the polymorphisms tested in our study were not located in the coding region, they were very likely to be functionally related to the regulation of serum IgE level. These two SNPs both located in the 3'UTR of *STAT6*, and that polymorphisms in 3'UTR of a gene might influence mRNA stability was shown previously (39).

STAT6 4219A/G has already been tested for association with atopic asthma in two different case-control studies in British and Japanese populations (40). Whereas

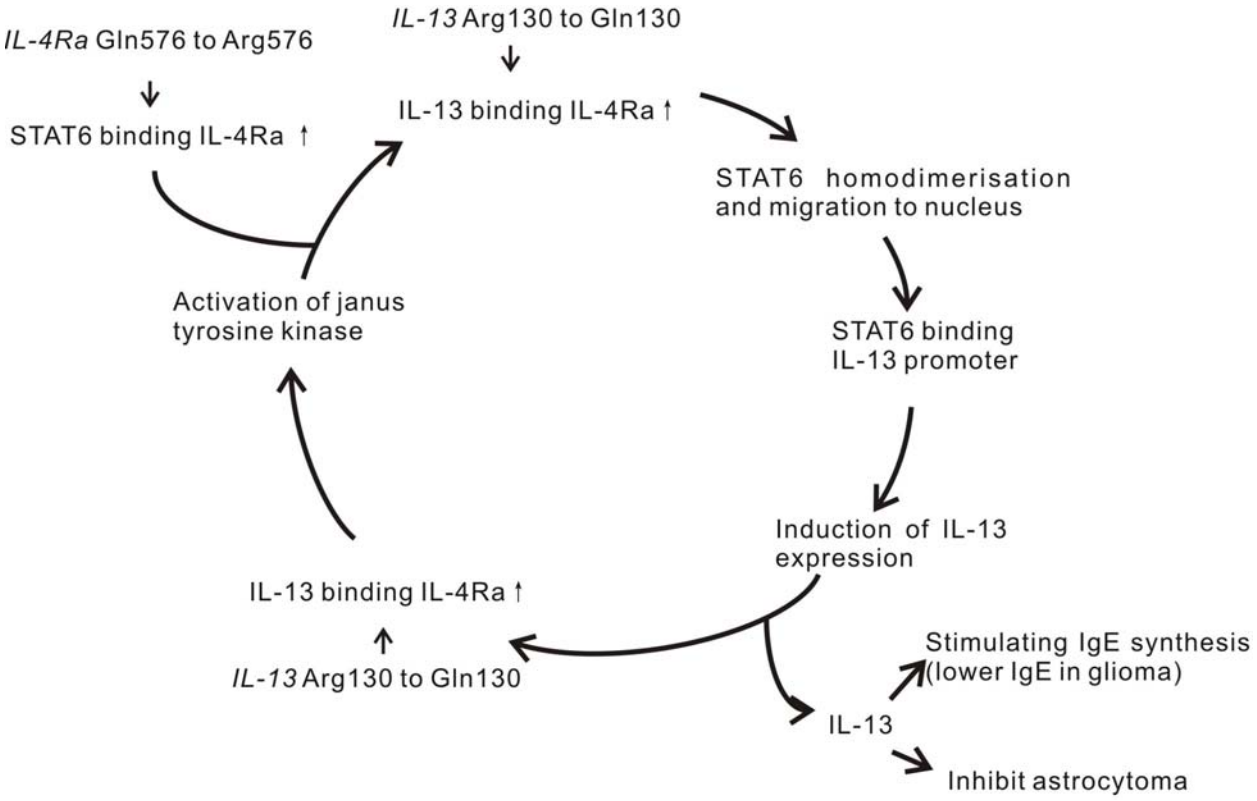

Figure 1. Effect of polymorphisms in *IL-13, IL-4Ra* and *STAT6* in IL-13/IL-4 pathway

strong association was found in the Japanese population (P=0.0043) but no significant association was seen in the British population. To our knowledge, our study is the first time to examine the *STAT6* polymorphism and glioma risk. *STAT6* 4219A/G showed significant association with increased glioma susceptibility risk among never-smokers, but we failed to observe significant associations among ever- and current-smokers. Cigarette smoking is a plausible behavioral exposure that might modulate glioma risk, and no overall association had been reported among either men or women (41, 42). It is possible that the insignificant results in ever- and current- smokers of *STAT6* 4219A/G might attribute to relatively small number of subjects. Specific response of glioblastoma cells and higher grade glioma cells to IL-13 as we discussed above might explain inconsistent results in different histology and WHO subgroups. It may be speculated that in the case of polymorphism, *STAT6* 4219A/G, G allele decreased mRNA stability, thus reducing the amount of *STAT6* mRNA readily available for translation into STAT6. Thereby, STAT6 might be more difficult to be recruited to the IL-4/IL-13 pathway after ligand binding. The activation of intracellular signaling cascade might be blocked and result in the phenomenon that lower IgE level was observed in glioma cells (10). Previous study conducted by Schedel *et al.* in Germany showed G allele in these polymorphisms significantly and consistently contributed to elevated total serum IgE levels (22). As to 4610A/G, no significant association in polymorphism *STAT6* was found in our study.

Subjects recruited in our study were all genetically unrelated, thus all the subjects were included in haplotype construction and regression analysis. Only three haplotypes in our study population were constructed, and it might due to the low frequency of G allele in *STAT6* 4610A/G (0.069). Compared with most frequent haplotype AA (0.498), the other two haplotypes did not indicate significant association with glioma risk. Stratification results showed only in never-smokers, haplotype AG significantly increased susceptibility of glioma risk. This effect was not observed in other subgroups and might suggested that variant allele G of *STAT6* 4610A/G tended to have stronger effect in non-smokers of our study.

Although our study population is one of the largest reported glioma case-control data sets, it is still possible that the sample size was not large enough to identify significant differences in distribution of these SNPs between cases and controls. In spite of importance of IL-4/IL-13 pathway, we were unable to observe overall association of *IL-4Ra, IL-13,* and *STAT6* SNPs in glioma, respectively. Possible reasons might include our failure to simultaneously consider other genes on the IL-4/IL-13 pathway or to identify the specific SNPs involved in glioma susceptibility on *IL-4Ra, IL-13 and STAT6.* It has been reported that at least six SNPs were required to characterize the variability of the *IL-13* gene (43), whereas four were needed to represent that of IL-4Ra(44). We evaluated only one SNP in *IL-4Ra* and one SNP in *IL-13.* We did, however, found significant association between *STAT6*

4219A/G and disease risk after stratification and also a suggestion of an association of *IL-4Ra* and *IL-13* interaction with glioma susceptibility risk. Haplotyple analysis of *STAT6* showed potential relation to glioma among never-smokers. It is becoming clear that SNPs or even individual genes in isolation cannot represent their effect on disease completely, but rather whole genetic pathways must be considered and investigated simultaneously, as well as pathways that perform similar functions. The relative strength of the previous biological functional evidence suggested that further research is needed to evaluate the possible role of the entire IL-4/IL-13 pathway in glioma susceptibility risk.

6.ACKNOWLEDGEMENTS

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Abbreviations: SNP, single nucleotide polymorphism; OR: odds ratio; CI: confidential interval; IL-13:Interleukin-13; IL-4Ra: Interleukin-4 receptor a; STAT6: signal transducer and activator of transcription 6; GBM: glioblastoma multiforme; PCR-LDR: Polymerase chain reaction-ligation detection reaction

Key Words: Glioma, single nucleotide polymorphism, *IL-13*, *IL-4Ra*, *STAT6*

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