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A Novel Approach to Exploring Potential Interactions among Single-Nucleotide Polymorphisms of Inflammation Genes in Gliomagenesis: An Exploratory Case-Only Study

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Abstract

Background—Despite extensive research on the topic, glioma etiology remains largely unknown. Exploration of potential interactions between single-nucleotide polymorphisms (SNPs) of immune genes is a promising new area of glioma research. The case-only study design is a powerful and efficient design for exploring possible multiplicative interactions between factors that are independent of one another. The purpose of our study was to use this exploratory design to identify potential pair wise SNP-SNP interactions from genes involved in several different immune-related pathways for investigation in future studies.

Methods—The study population consisted of two case groups: 1224 histological-confirmed, non-Hispanic white glioma cases from the U.S. and a validation population of 634 glioma cases from the U.K. Polytomous logistic regression, in which one SNP was coded as the outcome and the other SNP was included as the exposure, was utilized to calculate the odds ratios of the likelihood of cases simultaneously having the variant alleles of two different SNPs. Potential interactions were examined only between SNPs located in different genes or chromosomes.

Results—Using this data-mining strategy, we found 396 significant SNP-SNP interactions among polymorphisms of immune-related genes that were present in both the U.S. and U.K. study populations.

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Conclusion—This exploratory study was conducted for the purpose of hypothesis generation, and thus has provided several new hypotheses that can be tested using traditional case-control study designs to obtain estimates of risk.

Impact—This is the first study, to our knowledge, to take this novel approach to identifying SNP-SNP interactions relevant to glioma etiology.

Introduction

While there are very few established risk factors for glioma, several studies have suggested the involvement of inflammation-related genetic and immunological factors in gliomagenesis (1-8). For example, a history of asthma and allergies, as well as higher Immunoglobulin E (IgE) levels, has been associated with a protective effect against glioma development and prognosis (8, 9). Single-nucleotide polymorphisms (SNPs) that increase asthma risk appear to also be associated with decreased glioma risk (1-4, 6, 8). While results from such studies provide some support for the role of immunological and genetic factors in gliomagenesis, the relationship between these factors and how they work together to influence glioma risk are topics that warrant further clarification.

The failure of case-control studies to identify more risk factors for glioma development may be due, in part, to the fact that most of these studies focus on the main effects of certain genetic or immunological factors, instead of also examining the interactions between these factors (8, 9). Gliomagenesis is likely an intricate process, involving the interplay between several different immunological pathways (8). Thus, it is possible that any single factor alone does not exert more than a modest effect on glioma risk. If this is the case, studies that focus on examining interactions between risk factors, rather than determining what influence each risk factor may have independently, would be the key to identifying important predictors of gliomagenesis. However, case-control studies often lack adequate power to detect such interactions, as glioma is a rare disease and studies of glioma etiology are often plagued with small sample sizes (9).

The case-only study design has been used to examine the role of gene-environment interactions, especially in the etiology of rare diseases (10-14). However, this exploratory study design is equally efficient for investigating potential gene-gene interactions and has the advantage of having better power for the detection of such interactions than a traditional case-control design (15, 16). The purpose of the current study was to utilize the case-only study design to explore the role of pairwise SNP-SNP interactions for genes involved in several different immune-related pathways (Table 1), first among a population of 1224 non-Hispanic white glioma cases from the U.S. and then among a validation population of 634 Caucasian glioma cases from the U.K.

Materials and Methods

Study Population

The U.S. study population consisted of 1224 cases ascertained through The University of Texas M.D. Anderson Cancer Center between 1990 and 2008. Glioma cases were histopathologically-confirmed (ICD-O codes 9380-9384, 9390-9411, 9420-9451, and 9505), non-Hispanic white adults (>18 years old). After written informed consent was obtained, interviews were conducted using structured questionnaires, and an approximately 20-ml venous blood sample was collected from each participant. These samples were used to obtain DNA for genotyping. Other detailed information on the study population is available elsewhere (17). Study protocol was approved by the M.D. Anderson Cancer Center Institutional Review Board.

The validation population included 634 cases from the U. K. recruited between September 2000 and February 2004 in studies contributing to the INTERPHONE Study (17, 18). This study was an international multicenter case-control study of primary brain tumors (PBTs) coordinated by the International Agency for Research on Cancer. In the U. K. individuals with a PBT were recruited through neurosurgery, neuropathology, oncology and neurology centers in the Thames regions of Southeast England and Northern U.K., including central Scotland, the West Midlands, West Yorkshire and the Trent area. Cases had a histologically-confirmed glioma (ICD-O-2 codes 9380-9384, 9390-9411, 9420-9451 and 9505; ICD10 code C71) with no prior history of brain tumors. Individuals with self-reported non-western-European ancestry were excluded from these analyses in order to minimize population stratification. More details on the U.K. studies are provided elsewhere (17, 18).

Genotyping and SNP Selection

Genotyping was performed using the Illumina Human 610 Quad SNP Chip according to manufacturer's instructions (Illumina, San Diego, USA). Samples were excluded when fewer than 95% of genotypes were called overall. For quality assurance purposes, duplicate samples were genotyped in the same batches.

Because previous research has implicated the involvement of inflammatory pathways in gliomagenesis (1-9, 19), we attempted to compile a comprehensive list of the key signaling pathways that participate in the inflammatory response using the "interactive graphic models of molecular and cellular pathways" tool available on the Biocarta pathway maps website (20) and the Cancer Genome Anatomy Project website (21). The 28 sub-pathways of interests are listed in Table 1.

Once these inflammation-related pathways were identified, a list of candidate genes (n=204) involved in these pathways was then compiled using both the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (22) and Biocarta pathway maps (20). SNPs of the candidate genes were extracted from the Illumina Human 610 Quad SNP Chip, resulting in a total of 5304 SNPs that were considered for inclusion in this study, and were tested to identify the tagSNPs. Tag-SNPs are SNPs that can represent a region of the genome with high linkage disequilibrium [LD] ($r^2 > 0.8$). Tag-SNPs were determined using Haploview (23), which left a total 3454 remaining SNPs. Finally, all SNPs with <5% minor allele frequency or a genotype call rate <95% in the study population were eliminated from the analysis, as well as all X chromosome SNPs. After removal of these SNPs, there were 3310 SNPs left for analysis, the majority of which were intronic.

Statistical Analysis

Genotypes for each SNP were classified based on the number of variant alleles (i.e. 0, 1, or 2). The potential interaction between each pair of SNPs was analyzed using polytomous logistic regression in which the genotype (number of variant alleles) for one SNP was modeled as the "exposure" and the genotype for the other SNP served as the "outcome". Such an analysis makes no assumptions about the underlying genetic model and simply aims to assess whether the cases had a significantly higher likelihood of simultaneously having the variant alleles of both polymorphisms. In other words, a significant association would imply that the variant alleles of two SNPs were present among the cases more often than expected (and therefore, more often than among non-affected individuals). Here, our focus was on reporting interactions between SNPs of either *different chromosomes* or *different genes* on the same chromosome, rather than between SNPs of the *same gene*. This is because of the concern that two SNPs located in close proximity to each other may be less likely to be separated during recombination, which would artificially inflate the odds ratios obtained from the regression analyses.

Because there were almost 5.5 million potential pairwise interactions examined in this exploratory study, the Benjamini and Hockberg method (false discovery rate) was used to calculate corrected p-values for the purpose of determining statistical significance (24). All analyses were first conducted in the U.S. study population and then repeated in the U.K. validation population. A post-hoc analysis was conducted using an entropy-based approach to further evaluate pairwise SNP-SNP interactions in these populations (25). The results from this post-hoc analysis [not shown] were highly consistent with the results obtained from the polytomous logistic regression analyses. All statistical analyses were conducted using original code written in R version 2.8.1 and Matlab version 7.70 (R2008b; The Mathworks, Natick, MA).

Results

In the U.S. study population (n=1224), 761 cases (62.2%) were male and 463 (37.8%) were female. The average age was 47 (± 13) years. This distribution of sex and age are usually seen in populations of glioma cases (8, 9, 26), indicating that this case group is relatively representative of the overall population of glioma patients seen in the U.S. The U.K. study population (n=634) had a relatively similar sex and age distribution. There were 402 male cases (63.4%) and 232 (36.6%) female cases, with an overall average age of 46 (± 12) years.

Using the Benjamini and Hockberg p-value correction to determine statistical significance, interactions were considered significant if they had a p-value of less than 3.05×10^{-4} in the U.S. dataset and 2.44×10^{-4} in the U.K. dataset. Excluding interactions between SNPs in the same genes, a total of 2347 significant interactions were present in the U.S. dataset, and 1494 were observed in the U.K. dataset (Table 2). Among the 2347 total significant interactions found in the U.S. dataset, 1489 were between SNPs on different chromosomes, whereas 858 were between SNPs in different genes on the same chromosomes. Among the 1494 total significant interactions identified in the U.K. dataset, 1008 were between SNPs on different chromosomes, whereas 486 were between SNPs in different genes on the same chromosomes. There were 396 significant interactions in common between the U.S. and U.K. datasets, all of which were interactions on different genes but the same chromosomes. The median LD (r^2) between the SNP-SNP pairs involved in these 396 significant interactions was 0.03. Due to the large number of significant interactions detected and the fact that the biological functions of the vast majority of examined SNPs are unknown, our findings were reported and interpreted based on the genes (rather than the specific SNPs) implicated by the results.

The genes most frequently present among all statistically significant SNP-SNP interactions were the same in the U.S. population as in the U.K. population (Table 3).

The pairs of genes involved in the five most statistically significant interactions between SNPs on different chromosomes in the U.S. and U.K. datasets are given in Table 4. None of the significant interactions between SNPs on different chromosomes were observed in both populations. However, there were 396 significant interactions detected in both study populations between SNPs of different genes on the same chromosomes. Of these, 110 interactions were found to be highly significant (p-values $< 10^{-16}$) in both groups. Table 5 displays the genes pairs most commonly involved in these 110 interactions, with the most interactions occurring between the SNPs of the *STAT1* and *STAT4* genes.

Discussion

For diseases of unknown etiology, the use of data mining strategies provides a practical way to generate new hypotheses. Our study is the first to utilize the case-only design to explore

potential SNP-SNP interactions among glioma cases in order to supply new avenues of investigation into the elusive etiology of this disease. The case-only study design is an efficient and powerful design for identifying possible multiplicative interactions between factors that are independent of each other (i.e. SNPs not in linkage disequilibrium and/or SNPs on different genes) (15, 16). Here we found 926 significant pairwise interactions among polymorphisms of immune-related genes in a population of 1224 U.S. glioma cases, and 606 significant interactions in the validation population of 634 U.K. cases (excluding interactions between SNPs in the same gene), with 396 significant interactions in common between both populations. We then determined which genes were most commonly present among all significant interactions and selected the interactions with the smallest p-values for further investigation in a future case-control study. It is unknown whether the interactions found here truly represent biological relationships, or if they are due to statistical fluctuations in the data. However, they provide promising new hypotheses to examine in future studies on glioma etiology, a topic that has remained enigmatic despite much prior research.

The fact that the most common genes among the significant interactions were the same between both case groups lends credibility to their potential involvement in the process of gliomagenesis (Table 3). The top five genes most frequently involved in the statistically significant SNP-SNP interactions were *MAP3K7*, *TLR4*, *CRADD*, *PRKCA*, and *SYK*. The protein products of all these genes have functions that are directly relevant to carcinogenesis. For example, among a wide variety of other functions, protein kinase C- α (*PRKCA*) acts as a receptor for phorbol esters, a class of tumor promoters. Its involvement in mediating tumor growth and progression is well established, and *PRKCA*, as well as other members of the protein kinase C family, have served as therapeutic targets for cancer treatment (27). Another example is the *TLR4* gene product. *TLR4* is a fundamental regulator of the innate immunity and can induce the production of pro-inflammatory cytokines (28), which may be particularly relevant to glioma etiology given the prior observations that atopic conditions tend to be inversely associated with glioma risk (29, 30). SNPs in the *TLR4* gene have previously been associated with prostate cancer in certain populations (28, 31). Given the functions of the protein products and the interrelated pathways through which they act, the potential joint impact of SNPs in these genes would not be particularly surprising, especially if the polymorphisms affect either the amount or the functional efficiency of the expressed proteins.

Although none of the most significant interactions between SNPs on *different chromosomes* were replicated in the validation population, 396 significant interactions between SNPs in *different genes* on the *same chromosome* were identified in both study populations. Of these 396 replicated interactions, 110 were highly significant with p-values less than 10^{-16} . Many of the gene pairs involved in these highly significant interactions act in concert with each other, either through the same pathway or in complementary pathways. In fact, the most frequently implicated interactions were between SNPs of the *STAT1* and *STAT4* genes, both of which code for transcription factors involved in the JAK-STAT pathway. The JAK-STAT pathway is a pleiotropic signal transduction pathway responsible for numerous cellular functions, including immune development and cell cycle control (32). It is the key mechanism through which cytokines and growth factors are regulated. Genetic mutations leading to the over-activation of the JAK-STAT pathway are associated with the development of atopic diseases (32), which are known to be inversely associated with glioma risk (2, 5, 8, 9). Furthermore, this pathway is suspected to play a role in the development of various other types of cancer, including leukemias, lymphomas, and head and neck cancers (33, 34).

Currently, the biological function of the majority of the SNPs included in these analyses is unknown. Thus, the specific mechanisms that explain how the interactions reported here actually work to influence glioma risk cannot be completely elucidated until the functions of these polymorphisms are determined. However, by examining the pathways through which the implicated genes may be linked, we can begin to see the value of investigating the hypotheses generated by this exploratory study. Gliomagenesis is an extremely complex process involving interchange between multiple biological processes and pathways (8, 9). Thus far glioma research has focused on examining the main effects of single genetic factors on disease risk without resulting in any major breakthroughs on etiology, other than the two risk factors already established. Perhaps this is a sign that the focus in this area of research needs to shift toward newer methods of hypothesis generation and testing for interactions rather than solely main effects. Because our study is the first of its kind, we are unable to corroborate our results with findings from previous research, but the purpose of this analysis was simply to provide new hypotheses for future investigation.

A limitation of this study is that despite controlling for multiple comparisons, the results presented in Table 3 may partly be driven by the number of SNPs analyzed per gene, given that some of these genes (i.e. *MAP3K7*) had more genotyped tag-SNPs than average. However, there were other “large” genes in our dataset that did not yield many significant interactions and thus, were not included in this table (e.g. *F13A1* with 107 genotyped SNPs). Consequently, we do not believe that these results were entirely attributable to the number of SNPs in these genes. Nonetheless, these results should be interpreted with caution.

A commonly cited limitation of using the case-only design for the detection of pairwise multiplicative interactions is that it assumes absolute independence between the factors being examined. For this reason, we did not consider interactions between SNPs in strong linkage disequilibrium or within the same gene. Furthermore, this exploratory study was conducted for the purpose of hypothesis generation, not hypothesis testing. A thorough investigation of the interactions reported here should be conducted in a large case-control study. However, use of the case-only design in this study has allowed us to have increased precision and thus, to provide several new hypotheses that can now be tested utilizing a more traditional approach.

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Table 1**Immune-Related Pathways of Interest Used to Determine Relevant Genes**

Pathways of Interest^a
Cells and molecules involved in local acute inflammatory response
Chaperones modulating interferon signaling pathway
CTL mediated immune response against target cells
CXCR4 signaling pathway
Cytokine network
Cytokines and inflammatory response
Dendritic cells in regulating TH1 and TH2 development
IFN alpha signaling pathway
IFN gamma signaling pathway
IGF-1 signaling pathway
IL 17 signaling pathway
IL 18 signaling pathway
IL 2 signaling pathway
IL 3 signaling pathway
IL 4 signaling pathway
IL 5 signaling pathway
IL 6 signaling pathway
IL10 anti-inflammatory signaling pathway
IL2 receptor beta chain in T cell activation
IL7 signal transduction
IL12 and Stat4 dependent signaling pathway in Th1 development
NF-kB signaling pathway
Nitric oxide signaling pathway
NO ₂ -dependent IL 12 pathway in NK cells
Th1/Th2 differentiation
TNF/Stress related signaling
Toll-Like receptor pathway
IL22 soluble receptor signaling pathway

^aThis list consists of all inflammation-related pathways identified from the “interactive graphic models of molecular and cellular pathways” tool available on the Biocarta pathway maps website (www.biocarta.com/genes/allpathways.asp) and the Cancer Genome Anatomy Project website (<http://cgap.nci.nih.gov/Pathways>).

Table 2

Number of significant interactions by population

	U.S. Population	U.K. Population	Significant Interactions in Common
Between SNPs on same gene	31092	23629	22706
Between SNPs on different genes, same chromosome	858	486	396
Between SNPs on different chromosomes	1489	1008	0
Total	33439	25123	23102

Note. Significance determined by FDR corrected p-value cut-offs: 3.05×10^{-4} in U.S. population; 2.44×10^{-4} in U.K. population

Table 3

Top five genes most frequently present among statistically significant SNP-SNP interactions in U.S. and U.K. study populations

Gene	Protein Product	Relevant Function of Protein Product ^a	Number of SNPs in Gene	Number of Significant Interactions Involving Gene (%) ^b	
				U.S.	U.K.
<i>MAP3K7</i>	Mitogen-activated protein kinase kinase kinase 7	Involved in mediating TGF- β signal transduction pathway; involved in regulating gene transcription and apoptosis	209	3226 (9.6)	2488 (9.9)
<i>TLR4</i>	Toll-like receptor 4	Important activator of innate immune response; can trigger production of inflammatory cytokines or type I interferons	100	2255 (6.7)	1621 (6.4)
<i>CARD10</i>	Death-domain containing protein	Induces apoptosis	82	1445 (4.3)	1057 (4.2)
<i>PRKCA</i>	Protein kinase C- α	One of family of receptors for phorbol esters, a class of tumor promoters	104	1408 (4.2)	1014 (4.0)
<i>SYK</i>	Spleen tyrosine kinase	Involved in regulating BCR-stimulated responses	83	1396 (4.2)	1000 (4.0)

^a Protein product function information obtained from NCBI Entrez Gene database (<http://www.ncbi.nlm.nih.gov/sites/entrez>) by using gene symbol as search term.

^b Percent is calculated by dividing the number of interactions the gene is involved in by total number of significant interactions in each study population.

Table 4

Gene pairs involved in top 5 most significant interactions between SNPs on different chromosomes in the U.S. and U.K. study populations

Gene 1	Gene 2	P^a
<i>U.S. Study Population</i>		
<i>IL5RA</i>	<i>SMAD3</i>	3.26×10^{-7}
<i>PTK2</i>	<i>HNF1A</i>	3.94×10^{-7}
<i>MAP3K7</i>	<i>GNAQ</i>	1.55×10^{-6}
<i>LRPPRC</i>	<i>F13A1</i>	1.70×10^{-6}
<i>PTK2B</i>	<i>CRADD</i>	2.07×10^{-6}
<i>U.K. Study Population</i>		
<i>CD2</i>	<i>PIK3C2G</i>	1.96×10^{-7}
<i>TXN</i>	<i>PIK3C2G</i>	9.19×10^{-7}
<i>PPP3CA</i>	<i>MAP3L7</i>	9.63×10^{-7}
<i>F13A1</i>	<i>IL7</i>	1.16×10^{-6}
<i>MAP3K7</i>	<i>ITGAL</i>	1.29×10^{-6}

^aP-values of associations from polytomous logistic regression analyses for most significant SNP-SNP interactions, not for the gene pairs themselves.

Table 5

Most frequent gene pairs involved in the 110 most significant interactions in common between the U.S. and U.K. study populations^a

Gene 1 ^b	Gene 2	Number of SNPs in Gene 1	Number of SNPs in Gene 2	Frequency of Interactions
<i>STAT1</i>	<i>STAT4</i>	11	27	40
<i>IL1A</i>	<i>IL1B</i>	6	11	29
<i>IFNG</i>	<i>IL22</i>	5	12	11
<i>IFNAR1</i>	<i>IFNAR2</i>	11	13	9
<i>IL22RA2</i>	<i>IFNGR1</i>	16	31	5

^a $p < 10^{-16}$; total n=110

^b All significant interactions in common between the U.S. and U.K. study populations are between SNPs in different genes on the same chromosomes.