Neuro-Oncology

Partitioned glioma heritability shows subtype-specific enrichment in immune cells

--Manuscript Draft--

Manuscript Number:	N-O-D-20-00607R2
Full Title:	Partitioned glioma heritability shows subtype-specific enrichment in immune cells
Short Title:	Glioma heritability and immune cells
Article Type:	Basic and Translational Investigations
Keywords:	glioma; genetic architecture; autoimmune disease; allergies; heritability
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Manuscript Region of Origin:	UNITED STATES
Abstract:	Background: Epidemiological studies of adult glioma have identified genetic syndromes and 25 heritable risk loci that modify individual risk for glioma, as well increased risk in association with exposure to ionizing radiation and decreased risk in association with allergies. In this analysis we assess whether there is shared genome-wide genetic architecture between glioma and atopic/autoimmune diseases. Methods: Using summary statistics from a glioma GWAS meta-analysis, we identified significant enrichment for risk variants associated with gene expression changes in immune cell populations. We also estimated genetic correlations between glioma and autoimmune, atopic, and hematologic traits using LDscore regression, which leverages genome-wide SNP associations and patterns of linkage disequilibrium Results: Nominally significant negative correlations were observed for glioblastoma and primary biliary cirrhosis (r_g =-0.26, p=0.0228), and for non-glioblastoma gliomas and celiac disease (r_g =-0.32, p=0.0109). Our analyses implicate CD4+/CD8+ T cells (p_{min} =0.0034), and dendritic cells (p_{min} =0.00072) in mediating both glioblastoma and non-glioblastoma genetic predisposition, with glioblastoma-specific associations identified in NK (p_{min} =0.0012) and stem cells (p_{min} =0.0031). Conclusions: This analysis identifies putative new associations between glioma and autoimmune conditions with genomic architecture that is inversely correlated with that of glioma and that T cells, NK cells, and myeloid cells are involved in mediating glioma predisposition. This provides further evidence that increased activation of the acquired immune system may modify individual susceptibility to glioma.
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Partitioned glioma heritability shows subtype-specific enrichment in immune cells

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5489/6500 words

3/6 tables and figures

49/50 references

Conflict of interest

There are no conflicts of interest to report

Author contributions

QTO, JE, JB, YH, KMW, CIA and MLB designed the analysis. GLIOGENE Consortium provided the data. QTO and JE analyzed the data. QTO wrote the paper. QTO, JE, JB, YH, BM, RSH, MM, KMW, CIA, and MLB provided feedback and approved the final version of the paper.

Importance of the Study (62/150 words)

This analysis identifies anti-correlation between the genetic architecture of glioma and autoimmune conditions, as well as significant enrichment of the heritability of these tumors in variants affecting immune-cell specific gene expression. The findings of this research support prior studies which find acquired immune traits modify risk for glioma, and may identify specific immune cells and pathways that are implicated in this association.

Abstract (249/250 words)

Background: Epidemiological studies of adult glioma have identified genetic syndromes and 25 heritable risk loci that modify individual risk for glioma, as well increased risk in association with exposure to ionizing radiation and decreased risk in association with allergies. In this analysis we assess whether there is shared genome-wide genetic architecture between glioma and atopic/autoimmune diseases.

Methods: Using summary statistics from a glioma genome-wide association studies (GWAS) meta-analysis, we identified significant enrichment for risk variants associated with gene expression changes in immune cell populations. We also estimated genetic correlations between glioma and autoimmune, atopic, and hematologic traits using LDscore regression, which leverages genome-wide single nucleotide polymorphism (SNP) associations and patterns of linkage disequilibrium

Results: Nominally significant negative correlations were observed for glioblastoma and primary biliary cirrhosis (r_g=-0.26, p=0.0228), and for non-glioblastoma gliomas and celiac disease (r_g=-0.32, p=0.0109). Our analyses implicate dendritic cells (GB p_{HM}= 0.0306 and non-GB p_{HM}=0.0186) in mediating both glioblastoma and non-glioblastoma genetic predisposition, with glioblastoma-specific associations identified in natural killer (NK) (p_{HM}=0.0201) and stem cells (p_{HM}=0.0265).

Conclusions: This analysis identifies putative new associations between glioma and autoimmune conditions with genomic architecture that is inversely correlated with that of glioma and that T cells, NK cells, and myeloid cells are involved in mediating glioma predisposition. This provides further evidence that increased activation of the acquired immune system may modify individual susceptibility to glioma.

Keywords: Glioma; genetic architecture; autoimmune disease; allergies; heritability

Key Points

- There is an anti-correlation between the genetic architecture of glioma and autoimmune conditions.

- There is significant enrichment of the heritability of glioma in variants affecting immune-cell specific gene expression.

- This work support prior studies which have found acquired immune traits modify risk for glioma.

INTRODUCTION

Glioma is the most commonly occurring primary malignant brain tumor in the United States, with an average of ~10,000 cases diagnosed per year [1]. Though uncommon, these tumors cause significant morbidity and mortality. While ionizing radiation exposure has been shown to increase risk of glioma, most cases cannot be attributed to an underlying exposure and the vast majority of cases occur in people without significant family history of cancer [2,3]. Allergies and other atopic conditions have been consistently shown to decrease risk of glioma in case control studies, though Mendelian randomization studies assessing the causality of this association have not been significant [4-6]. Reported history of any auto-immune condition has also been shown to decrease risk of these tumors [7,8]. This suggests that increased immune activation protects against glioma development. Due to the rarity of many auto-immune traits, they have often been pooled in glioma association studies, making it difficult to determine the nature of the association with specific autoimmune conditions.

Complete blood count (CBC) values (*e.g.* red and white blood cell counts and proportions, platelet count, and hemoglobin), cytokines, and other blood and immune cell factors are frequently dysregulated in cancer (including glioma) due both to tumor and treatment effects [9]. Prior genome-wide association studies (GWAS) have identified multiple loci that modify these hematologic traits and indicate these loci have pleiotropic effects on immune phenotypes [10-12]. While leukocyte traits associated with increased systemic inflammation have been shown to be prognostic in glioma and multiple CBC values have been previously associated with cancer prognosis, including in glioblastoma (GB), there has been no evaluation of how heritable variation in these traits contributes to glioma etiology [13,14]. The majority of glioma case-control studies have collected blood samples after diagnosis, where blood composition may already be dysregulated due to systemic immunosuppression caused by the tumor or by steroidal treatments.

A prior GWAS meta-analysis in glioma identified 25 independent loci associated with glioma in European ancestry populations, most of which have relatively small effect sizes [15] and none of which have been previously identified as being associated with autoimmune disease. These associations are estimated to account

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for 30% of the glioma heritability, leaving 70% of the variation in genetic risk in unaccounted for [16,17]. A recent GWAS completed in *IDH1/2* mutant adult diffuse glioma identified in *D2HGDH*, a region known to be associated with allergy [18]. The power of GWAS methods to detect additional common variants of small effect sizes and resolve this "missing heritability" is limited by sample size and sample resources, particularly in rare diseases such as glioma. However, many risk loci identified by GWAS are pleiotropic, and are implicated in risk for multiple complex diseases [19-21].

LD Score regression is a ~~novel~~ statistical methodology that uses genome-wide SNP association data and patterns of linkage disequilibrium (LD) to estimate heritability and correlations between traits, while minimizing the effect of confounding and population stratification [22]. It can also be extended to estimate the polygenic contribution of specific gene annotations (*e.g.* cell type–specific transcriptomic networks) to heritability in genome-wide association studies. In this analysis, we used LD score regression to partition glioma heritability by cell type and to estimate the genetic correlation between glioma and atopic, auto-immune, and hematologic traits in a set of 12,455 European-ancestry cases and 18,169 European-ancestry controls from eight case control studies.

METHODS

Data sources and quality control

Glioma data were generated from a meta-analysis conducted using eight prior glioma GWAS studies, for all glioma, glioblastoma (GB), and non-GB glioma (See **Supplementary Tables 1** for an overview of study characteristics) and betas, standard errors, and p values were used to generate z-scores for use in ~~LDSC~~LD score regression. These studies were previously combined as part of the meta-analysis presented in Melin, et al,[15] and do not include overlapping samples.   We obtained summary statistics (including betas, standard errors, and p values) for 13 immune- and atopy-related traits from the prior case-control studies and the UK biobank and complete blood count values from the UK biobank (**Supplementary Table 2**). Quality control of phenotypes and genotypes is documented in prior publications and documentation.[23-30] We filtered the data to

include autosomal SNPs with minor allele frequency (MAF) of at least 1%. The SNPs for glioma and UK biobank datasets were imputed and filtered to have imputation quality >0.9. Since glioma is heterogeneous with distinct pathways of gliomagenesis and genetic susceptibility, we performed all analyses in all glioma, as well as for GB and non-GB glioma separately.

Estimation of heritability and genetic correlation between traits

Pairwise genetic correlation between these traits was generated using LDSC (https://github.com/bulik/ldsc) using 1,000 Genomes Project European (EUR) samples as a reference for patterns of genome-wide LD.[22] Briefly, LD score regression is a method that regresses summary statistics from GWAS on the LD score, or the strength with which each individual variant tags other variants in the genome. This method allows for calculation of genetic correlation without bias due to population stratification or cryptic relatedness. We used LDSC v1.0.1 to estimate genetic correlation between traits (r_g), the 95% confidence intervals (95% CI) and p values for each pairing, and heritability for each trait. LD Score regression intercept, estimated lambda (λ, an estimate of genetic inflation), maximum χ^2 statistic, and intercept of genetic covariance. The ratio of regression intercept to χ^2 statistic, which estimates the proportion of inflation in χ^2 statistic that is not due to polygenic heritability. In the absence of inflation, this ratio should be equal to zero. Due to the complex and unusual genetic architecture of the leukocyte antigen complex (HLA) region (chromosome 6 from 29mb-33mb), the LDSC method excludes SNPs within this region from all analyses. SNPs were limited to those included in HapMap. After QC, 1,290,028 SNPs were used from glioma datasets. Total number of SNPs used in each pairwise analysis varied due to variation in array used by study and study-specific imputation quality scores. P-values were corrected using the Benjamini-Hochberg procedure, and associations were considered significant at p<0.05. Glioma heritability estimates we converted to the liability scale using an overall lifetime risk of malignant brain tumor of 0.58%, with lifetime risk of GB estimated to be 0.32% and non-GB estimated to be 0.26%.[31] Autoimmune disease heritability estimated from case-control studies were also adjusted to liability scale using prior published prevalence estimates (**Supplementary Table 2**). Heritability for binary

traits from UK was adjusted to the liability scale using sample prevalence, which approximates prevalence of these conditions in the population.

Partitioned heritability is an extension of the LD score regression method described by Finucane, et al that uses genotyping and gene expression reference datasets to identify cell types which are significant enriched for variants contributing to the heritability of a trait.[32,33] Cell-type specific heritability was estimated for glioma and glioma subtypes using LDSC v1.0.1 using provided reference data from GTex and ImmGen,[34,35] and further details of this data preparation are provided in prior publications.[32] All available tissue types and immune cell types were used. Briefly, these reference datasets (previously prepared by Finucane and colleagues, available at http://data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/) were generated using a matrix of normalized gene expression values across genes, for which a t-statistic was calculated for specific expression in the specific tissue or cell type. Briefly, Finucane, et al. then ranked Ggenes were then ranked by these t-statistic and the top 10% of genes with the highest t-statistic to be the gene set for each tissue or cell type, which includes genes that are specifically expressed and genes that are weakly specifically expressed. LD score regression is then used to evaluate contribution of the genome annotation to trait heritability. Specific tissue and immune cell samples were grouped into broader categories per Finucane, et al and minimum p value was selected for samples within these categories.[32,33] Proportion of heritability due to the specific class of tissue or cell type was defined as the proportion of SNP-heritability in the category divided by the proportion of total SNPs. P values within immune tissue types were combined using the harmonic mean p-value (p_{HM})[36] using r R package harmonicmeanp.[37] Associations were considered statistically significant at p<0.05.

RESULTS

Estimation of glioma heritability

We estimated that the overall liability-scaled heritability for glioma was 6.69% (95%CI: 6.67%-6.71%), while estimates for GB and non-GB were 5.62% (95%CI: 5.58%-5.67%) and 6.45% (95%CI: 6.40%-6.50%), respectively.

Cell-type partitioned heritability

In order to identify tissue and cell types where function was likely to be affected by variants associated with risk for glioma, heritability partitioned by tissue and cell type was estimated using GTex and ImmGen reference data (Supplementary Tables 3-5). Across all tissue types, heritability for glioma was significantly enriched in CNS ($p_{HM}= 0.0221$), (**Figure 1A, Supplementary Table 3**). Within GTex brain regions only, there was significant enrichment for GB in cortex ($p_{HM}=0.0061$), **Figure 1B, Supplementary Table 4**) that was not observed in non-GB ($p_{HM}=0.2498$). There was heritability enrichment in multiple immune cell types overall and for both GB and non-GB (**Figure 1C, Supplementary Table 5**). There was a marginally significant association between non-GB only and B cells ($p_{HM}=0.0600$), with the strongest association in B2 cells ($p_{HM}=0.0276$) and transitional B cells ($p_{HM}=0.0489$). There was no significant enrichment with myeloid cells in GB ($p_{HM}=0.1491$) or non-GB ($p_{HM}= 0.0936$), though there was specific enrichment in dendritic cells in both GB ($p_{HM}= 0.0306$) and non-GB ($p_{HM}=0.0186$). Natural killer (NK) cells were significantly associated with GB ($p_{HM}=0.0201$). There was a marginally significant association with T cells for GB ($p_{HM}= 0.0944$), with enrichment of association in γδ T cells in GB ($p_{HM}=0.0399$). Among cell types included in a broad 'other' category, we also identified stem cell specific enrichment in GB ($p_{HM}=0.0265$). See **Supplementary Table 5** for all cell-specific coefficients and p values.

Genome-wide genetic correlations with autoimmune, hematologic and atopic traits

We evaluated genome-wide correlations between the genetic architecture of glioma and multiple atopic, allergic and autoimmune conditions, as well as hematologic traits. In general, λ estimates were higher than intercepts, suggesting that signal was driven by polygenicity rather than population stratification. Ratios were elevated for many traits obtained from case-control studies, including celiac disease, lupus, multiple sclerosis, and ulcerative colitis, suggesting that population stratification may have a significant impact on observed results. After correcting for multiple testing no associations were significant. A nominally significant negative genetic correlation was identified between glioma and primary biliary cirrhosis (PBC, $r_g=-0.26$, p=0.0228) (**Figure 2, Supplementary Table 6**). When correlations were estimated by broad glioma subtypes, the scale

and direction of associations were generally consistent in both GB and non-GB, though most associations were not statistically significant. There was a negative correlation with celiac disease in non-GB only (r_g=-0.32, p=0.0109). No associations remained significant after FDR adjustment of p values.

~~As a sensitivity analysis, and~~ Due to the high variability in the HLA region, LDSC excludes this by default. There is significant ~~due to the~~ enrichment for germline variation associated for autoimmune disease within ~~the HLA~~this region~~,~~ As a sensitivity analysis, genetic correlation was also estimated for these traits and glioma with a reference including the HLA region. Several phenotypes (particularly lupus and type 1 diabetes) showed significant increases in their SNP-based heritability estimates (**Supplementary Table 2**). The direction and scale of genetic correlation did not change substantially for traits other than lupus and type 1 diabetes (**Supplementary Table 7**).

There was a positive correlation between glioma and mean platelet volume (r_g=-0.09, p=0.0337) (**Supplementary Table 4**), mostly driven by the effect of non-GB (r_g=-0.12, p=0.0096). There was also a negative correlation observed between non-GB and lymphocyte percentage (r_g=-0.11, p=0.0312). We did not find any significant associations between GB and any hematologic traits. Although epidemiologic studies have repeatedly observed inverse associations between glioma and atopic conditions, glioma was not correlated with either eosinophil count (r_g=0.01, p=0.9060) or eosinophil percentage (r_g=0.02, p=0.6137), nor was it correlated with atopic traits including hay fever, asthma and eczema (**Supplementary Table 6**). A weak inverse correlation was observed between GB and eczema in the anticipated direction (r_g=-0.25, p=0.0991). No associations remained significant after FDR adjustment of p values. In general, λ estimates were higher than intercepts, suggesting that signal was due to polygenicity as compared to solely population stratification. The intercept for LD score regression has been observed to increase linearly with sample size,[38] which may lead to artificial inflation of these statistics in these continuous traits with very large sample sizes.

DISCUSSION

LD score regression is a ~~novel~~ hypothesis-generating approach that we used to identify 1) cell types that mediate glioma predisposition and 2) traits with correlated or anti-correlated genomic architecture. ~~LD score regression~~This methodology enables researchers to identify traits that are correlated with their phenotype of interest (e.g. glioma) which are otherwise not amenable to study in case-control designs due to the rarity of such traits (*e.g.* the inverse association of PBC with glioma). When based on validly constructed GWAS estimates, LD score regression can also ~~eliminate~~ minimize many sources of bias from case-control studies related to differential participation rates and recall biases.[39] ~~In~~ While this analysis ~~, the approach~~ did not identify shared genomic architecture underlying allergic/atopic traits and glioma risk, ~~but~~ it did identify associations with specific autoimmune phenotypes that varied by ~~tumor grade~~GB status.

We identified significant heritability enrichment for glioma in multiple brain regions as well as immune cell types. This supports previous evidence that conditions affecting individual immune function may be associated with risk of glioma [7,8,40]. Gliomagenesis also involves significant manipulation of the immune system, and is associated with local and global immunosuppression that 'sequesters' T cells to the bone marrow and 'reprograms' other immune cells within the tumor microenvironment.[41] Increased prevalence of markers associated with dysregulated immune function are strongly associated with increased tumor growth and decreased glioma survival, suggesting that innate immune function influences risk and prognosis and leading to a current focus on immune-stimulating treatments for this disease.[42-46] Together, these factors demonstrate the significant role of immune function in both glioma susceptibility and patient prognosis.

This analysis identified significant negative correlations between glioma and several auto-immune traits, with the strongest associations identified with primary biliary cirrhosis (PBC) and celiac disease. The estimate of r_g for multiple sclerosis was similar in scale to both PBC and celiac disease in GB and non-GB, respectively, but this association was not statistically significant. Most of these traits have not been investigated in association with glioma previously. While prior research has suggested a protective association between long-

term diagnosis of (primary type 2 or adult onset) diabetes and glioma, [47] we did not find a correlation between the genetic architecture of type 1 diabetes and glioma. There is an increasing body of scientific literature documenting the biological connections between the gut and the brain. The permeable tissue of the digestive system is the most direct way for compounds to enter the blood stream, and if small enough, these compounds can cross the blood brain barrier. The gut microbiome has been shown to significantly affect neuropsychiatric disorders, as well as response to cancer immunotherapies.[48,49] Celiac disease and PBC are organ-specific autoimmune diseases, in contrast to systematic autoimmune conditions such as rheumatoid arthritis and lupus. The autoimmune component of celiac disease arises when gliadin (a component of gluten) peptides cross the intestinal epithelial barrier and activate CD4+ T-lymphocytes, stimulating expansion of B cells that secrete anti-gliadin and auto-antibodies.[50] Gliadin also activates antigen presenting cells (APCs, or dendritic cells) and intestinal epithelial cells, leading to expansion of CD8+ T-lymphocytes. Immune cell ~~type~~ type-specific heritability analyses found a significant enrichment for GB and non-GB heritability in dendritic cells. PBC is also a T-cell mediated autoimmune disease which primarily affects biliary epithelial cells, and biopsy specimens from patients with this condition show significant invasion of CD4+ and CD8+ T cells.[51] While it is not clear what role NK cells play in the pathogenesis of PBC, these cells have been found in increased quantity in blood and liver specimens of PBC patients. Our analysis found significant GB-specific enrichment for heritability in NK cell. NK cells are an essential component of tumor surveillance in the healthy innate immune system, as they do not need to be activated and will respond to cells based on absence of appropriate MHC receptor. While the primary damaging immune response in autoimmune diseases is usually B- and/or T-cell mediated, both quantitative and qualitative variation in NK cells has been observed in multiple autoimmune conditions.[52] NK cells are currently under investigation for potential role in cancer immunotherapy, as their ability to perform their function in the absence of a 'danger' signal makes them less susceptible to the immunosuppressive tumor environment present in GB.[53] GB-specific enrichment was also identified in stem cells (p_{HM}=0.0157). Stem-like behavior is observed in the subpopulation of glioma cells referred to as glioma stem cells, which exert significant control over the glioma immune microenvironment.[54]

LD score regression identified few genetic correlations with the hematologic factors, with the exception

of mean platelet volume and lymphocyte percentage. These values all have the potential to be markers of

underlying immune function, and further research is necessary to determine their association with glioma risk.

The lack of consistent association across related phenotypes and lack of statistical significance after FDR

correction, also suggests that these may not be genuine associations. Estimates of heritability for glioma were

notably lower than previously estimated using GCTA [55]. Heritability estimates for autoimmune diseases and

allergy/atopy traits estimated by this analysis are generally also lower than what has previously been

reported.[56,57] Heritability estimates in LDSC are known to be biased downwards when data has been previously

corrected for genomic control, as the glioma data used in this analysis has been based on estimated principal

components. Heritability estimates may also be biased when cases and control are not from the same source

population, as is the case in several included glioma GWAS (**Supplementary Table 1**). Despite adjustment to

the liability scale, heritability estimates from case-control studies may be inflated as compared to cohort studies

such as the UK biobank. As a result, the reported heritability estimates may be lower than 'true' heritability for

these traits. Using GCTA to estimate genome-wide heritability has been shown to result in inflated estimates,

particularly when there are measurement errors in the underlying phenotype,[58] which may explain our lower

heritability estimates using LD score regression when compared to previous estimates using GCTA. Molecular

characterization of glioma has demonstrated that the presence or absence of *IDH1/2* mutation differentiates two

distinct lineages of glioma, which were pooled in glioma GWAS data.[59] While the majority of GB (~95%) are

IDH1/2 wild type and non-GB are *IDH1/2* mutant (80%),[59] these groups likely contain non-trivial phenotypic

misclassification. T~~In addition to potential lack of precision in these phenotypes limiting power to detect~~

~~associations, the smaller~~ Relatively small -sample sizes within some phenotypes ~~within each group~~ (particularly

for cases with autoimmune traits ~~from~~ in the UK biobank) may also lead to some tests being underpowered.

~~Program-specific recommendations for~~ LDSC ~~suggestion~~ recommends a minimum sample size of 3,000 for

estimates of heritability, and 5,000 cases for partitioning heritability. Glioma data meet ~~these threshold~~this

threshold, but do not substantially exceed it.

This analysis uses ~~a novel approach~~LD score regression to identify significant enrichment for glioma heritability in immune cell types and novel inverse associations between several auto-immune traits and glioma risk. A strength of ~~using LD score regression to identify shared genetic architecture between distinct phenotypes is~~this methodology is that it does not require having collected prior information on medical history. This method has been shown to be robust under multiple different genetic architecture, as well as when the underlying summary statistics used may be biased.[60] This method requires that a trait have a sufficient portion of risk that is attributable to genetic factors, and is not appropriate for traits which are entirely affected by environment. Prior glioma case-control studies are inconsistent in how these data were collected and which diseases were included on questionnaires, and this approach allows for use of all glioma GWAS regardless of questionnaire data. Both glioma and specific autoimmune diseases are uncommon, and as a result very large sample sizes would be necessary to have adequate power to identify these associations with traditional case-control study designs. There are also several limitations to this approach. Summary statistics are used and therefore it is not able to conduct sensitivity analyses using specific groups of controls within each study, and the sharing of these controls between glioma subsets may bias results. The LD score regression method excludes the HLA region, which is known to be enriched for risk variants in atopic and autoimmune disease as well as a punitive haplotype association with non-GB glioma,[61] and may be a significant source of shared heritability between these traits.

While this analysis does not identify a genetic basis for the previously identified protective effect of atopic traits on glioma risk, it does suggest that the genomic architecture of glioma predisposition may manifest in part via its activity in immune cells. This is consistent with prior research demonstrating the protective effect of allergy and atopic disease on glioma risk, as well as the systematic immune suppression that occurs in the context of glioma. Atopic traits had low heritability, suggesting that the protective effect due to these traits may be influenced mostly by environmental factors. Autoimmune conditions, on the other hand, did show association with glioma and are more strongly influenced by intrinsic (*e.g.* genetic) factors than extrinsic (*e.g.* environmental) exposures, much like glioma. Further studies are necessary in order to confirm these

associations and to identify the mechanism through which increased immune activation may reduce risk of glioma.

ACKNOWLEDGEMENTS

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Figure Key

Figure 1. –log10(p) for individual specific tissue sample and harmonic mean p within tissue categories for A) GB and B) non-GB -based on GTex reference data (dotted line indicates p<0.05)

Figure 2. log10(p) by individual ~~indvidiual~~ cell line sample and harmonic mean p within immune cell category for A) GB and B) non-GB- based on ImmGen reference data (dotted line indicates p<0.05)

Figure 3. Estimated heritability for autoimmune and atopic phenotypes, and genetic correlation between atopic and auto-immune phenotypes and glioma subgroups, dotted line indicates p<0.05

Partitioned glioma heritability shows subtype-specific enrichment in immune cells

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5489/6500 words

3/6 tables and figures

49/50 references

Conflict of interest

There are no conflicts of interest to report

Author contributions

QTO, JE, JB, YH, KMW, CIA and MLB designed the analysis. GLIOGENE Consortium provided the data.

QTO and JE analyzed the data. QTO wrote the paper. QTO, JE, JB, YH, BM, RSH, MM, KMW, CIA, and

MLB provided feedback and approved the final version of the paper.

Importance of the Study (62/150 words)

This analysis identifies anti-correlation between the genetic architecture of glioma and autoimmune conditions, as well as significant enrichment of the heritability of these tumors in variants affecting immune-cell specific gene expression. The findings of this research support prior studies which find acquired immune traits modify risk for glioma, and may identify specific immune cells and pathways that are implicated in this association.

Abstract (249/250 words)

Background: Epidemiological studies of adult glioma have identified genetic syndromes and 25 heritable risk loci that modify individual risk for glioma, as well increased risk in association with exposure to ionizing radiation and decreased risk in association with allergies. In this analysis we assess whether there is shared genome-wide genetic architecture between glioma and atopic/autoimmune diseases.

Methods: Using summary statistics from a glioma genome-wide association studies (GWAS) meta-analysis, we identified significant enrichment for risk variants associated with gene expression changes in immune cell populations. We also estimated genetic correlations between glioma and autoimmune, atopic, and hematologic traits using LDscore regression, which leverages genome-wide single nucleotide polymorphism (SNP) associations and patterns of linkage disequilibrium

Results: Nominally significant negative correlations were observed for glioblastoma and primary biliary cirrhosis (r_g=-0.26, p=0.0228), and for non-glioblastoma gliomas and celiac disease (r_g=-0.32, p=0.0109). Our analyses implicate dendritic cells (GB p_{HM}= 0.0306 and non-GB p_{HM}=0.0186) in mediating both glioblastoma and non-glioblastoma genetic predisposition, with glioblastoma-specific associations identified in natural killer (NK) (p_{HM}=0.0201) and stem cells (p_{HM}=0.0265).

Conclusions: This analysis identifies putative new associations between glioma and autoimmune conditions with genomic architecture that is inversely correlated with that of glioma and that T cells, NK cells, and myeloid cells are involved in mediating glioma predisposition. This provides further evidence that increased activation of the acquired immune system may modify individual susceptibility to glioma.

Keywords: Glioma; genetic architecture; autoimmune disease; allergies; heritability

Key Points

- There is an anti-correlation between the genetic architecture of glioma and autoimmune conditions.

- There is significant enrichment of the heritability of glioma in variants affecting immune-cell specific gene expression.

- This work support prior studies which have found acquired immune traits modify risk for glioma.

INTRODUCTION

Glioma is the most commonly occurring primary malignant brain tumor in the United States, with an average of ~10,000 cases diagnosed per year [1]. Though uncommon, these tumors cause significant morbidity and mortality. While ionizing radiation exposure has been shown to increase risk of glioma, most cases cannot be attributed to an underlying exposure and the vast majority of cases occur in people without significant family history of cancer [2,3]. Allergies and other atopic conditions have been consistently shown to decrease risk of glioma in case control studies, though Mendelian randomization studies assessing the causality of this association have not been significant [4-6]. Reported history of any auto-immune condition has also been shown to decrease risk of these tumors [7,8]. This suggests that increased immune activation protects against glioma development. Due to the rarity of many auto-immune traits, they have often been pooled in glioma association studies, making it difficult to determine the nature of the association with specific autoimmune conditions.

Complete blood count (CBC) values (*e.g.* red and white blood cell counts and proportions, platelet count, and hemoglobin), cytokines, and other blood and immune cell factors are frequently dysregulated in cancer (including glioma) due both to tumor and treatment effects [9]. Prior genome-wide association studies (GWAS) have identified multiple loci that modify these hematologic traits and indicate these loci have pleiotropic effects on immune phenotypes [10-12]. While leukocyte traits associated with increased systemic inflammation have been shown to be prognostic in glioma and multiple CBC values have been previously associated with cancer prognosis, including in glioblastoma (GB), there has been no evaluation of how heritable variation in these traits contributes to glioma etiology [13,14]. The majority of glioma case-control studies have collected blood samples after diagnosis, where blood composition may already be dysregulated due to systemic immunosuppression caused by the tumor or by steroidal treatments.

A prior GWAS meta-analysis in glioma identified 25 independent loci associated with glioma in European ancestry populations, most of which have relatively small effect sizes [15] and none of which have been previously identified as being associated with autoimmune disease. These associations are estimated to account

for 30% of the glioma heritability, leaving 70% of the variation in genetic risk in unaccounted for [16,17]. A recent

GWAS completed in *IDH1/2* mutant adult diffuse glioma identified in *D2HGDH*, a region known to be

associated with allergy [18]. The power of GWAS methods to detect additional common variants of small effect

sizes and resolve this "missing heritability" is limited by sample size and sample resources, particularly in rare

diseases such as glioma. However, many risk loci identified by GWAS are pleiotropic, and are implicated in

risk for multiple complex diseases [19-21].

LD Score regression is a statistical methodology that uses genome-wide SNP association data and

patterns of linkage disequilibrium (LD) to estimate heritability and correlations between traits, while

minimizing the effect of confounding and population stratification [22]. It can also be extended to estimate the

polygenic contribution of specific gene annotations (*e.g.* cell type–specific transcriptomic networks) to

heritability in genome-wide association studies. In this analysis, we used LD score regression to partition

glioma heritability by cell type and to estimate the genetic correlation between glioma and atopic, auto-immune,

and hematologic traits in a set of 12,455 European-ancestry cases and 18,169 European-ancestry controls from

eight case control studies.

METHODS

Data sources and quality control

Glioma data were generated from a meta-analysis conducted using eight prior glioma GWAS studies,

for all glioma, glioblastoma (GB), and non-GB glioma (See **Supplementary Tables 1** for an overview of study

characteristics) and betas, standard errors, and p values were used to generate z-scores for use in LD score

regression. These studies were previously combined as part of the meta-analysis presented in Melin, et al,[15] and

do not include overlapping samples.   We obtained summary statistics (including betas, standard errors, and p

values) for 13 immune- and atopy-related traits from the prior case-control studies and the UK biobank and

complete blood count values from the UK biobank (**Supplementary Table 2**). Quality control of phenotypes

and genotypes is documented in prior publications and documentation.[23-30] We filtered the data to include

autosomal SNPs with minor allele frequency (MAF) of at least 1%. The SNPs for glioma and UK biobank

datasets were imputed and filtered to have imputation quality >0.9. Since glioma is heterogeneous with distinct

pathways of gliomagenesis and genetic susceptibility, we performed all analyses in all glioma, as well as for GB

and non-GB glioma separately.

Estimation of heritability and genetic correlation between traits

Pairwise genetic correlation between these traits was generated using LDSC

(https://github.com/bulik/ldsc) using 1,000 Genomes Project European (EUR) samples as a reference for

patterns of genome-wide LD.[22] Briefly, LD score regression is a method that regresses summary statistics from

GWAS on the LD score, or the strength with which each individual variant tags other variants in the genome.

This method allows for calculation of genetic correlation without bias due to population stratification or cryptic

relatedness. We used LDSC v1.0.1 to estimate genetic correlation between traits (r_g), the 95% confidence

intervals (95% CI) and p values for each pairing, and heritability for each trait. LD Score regression intercept,

estimated lambda (λ, an estimate of genetic inflation), maximum χ^2 statistic, and intercept of genetic covariance.

The ratio of regression intercept to χ^2 statistic, which estimates the proportion of inflation in χ^2 statistic that is

not due to polygenic heritability. In the absence of inflation, this ratio should be equal to zero. Due to the

complex and unusual genetic architecture of the leukocyte antigen complex (HLA) region (chromosome 6 from

29mb-33mb), the LDSC method excludes SNPs within this region from all analyses. SNPs were limited to those

included in HapMap. After QC, 1,290,028 SNPs were used from glioma datasets. Total number of SNPs used in

each pairwise analysis varied due to variation in array used by study and study-specific imputation quality

scores. P-values were corrected using the Benjamini-Hochberg procedure, and associations were considered

significant at p<0.05. Glioma heritability estimates we converted to the liability scale using an overall lifetime

risk of malignant brain tumor of 0.58%, with lifetime risk of GB estimated to be 0.32% and non-GB estimated

to be 0.26%.[31] Autoimmune disease heritability estimated from case-control studies were also adjusted to

liability scale using prior published prevalence estimates (**Supplementary Table 2**). Heritability for binary

traits from UK was adjusted to the liability scale using sample prevalence, which approximates prevalence of these conditions in the population.

Partitioned heritability is an extension of the LD score regression method described by Finucane, et al that uses genotyping and gene expression reference datasets to identify cell types which are significant enriched for variants contributing to the heritability of a trait.[32,33] Cell-type specific heritability was estimated for glioma and glioma subtypes using LDSC v1.0.1 using provided reference data from GTex and ImmGen,[34,35] and further details of this data preparation are provided in prior publications.[32] All available tissue types and immune cell types were used. Briefly, these reference datasets (previously prepared by Finucane and colleagues, available at http://data.broadinstitute.org/alkesgroup/LDSCORE/LDSC_SEG_ldscores/) were generated using a matrix of normalized gene expression values across genes, for which a t-statistic was calculated for specific expression in the specific tissue or cell type. Briefly, Finucane, et al. then ranked genes by these t-statistic and the top 10% of genes with the highest t-statistic to be the gene set for each tissue or cell type, which includes genes that are specifically expressed and genes that are weakly specifically expressed. LD score regression is then used to evaluate contribution of the genome annotation to trait heritability. Specific tissue and immune cell samples were grouped into broader categories minimum p value was selected for samples within these categories.[32,33] Proportion of heritability due to tissue or cell type was defined as the proportion of SNP-heritability in the category divided by the proportion of total SNPs. P values within immune tissue types were combined using the harmonic mean p-value (p_{HM})[36] using R package harmonicmeanp.[37] Associations were considered statistically significant at $p < 0.05$.

RESULTS

Estimation of glioma heritability

We estimated that the overall liability-scaled heritability for glioma was 6.69% (95%CI: 6.67%-6.71%), while estimates for GB and non-GB were 5.62% (95%CI: 5.58%-5.67%) and 6.45% (95%CI: 6.40%-6.50%), respectively.

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Cell-type partitioned heritability

In order to identify tissue and cell types where function was likely to be affected by variants associated with risk for glioma, heritability partitioned by tissue and cell type was estimated using GTex and ImmGen reference data (Supplementary Tables 3-5). Across all tissue types, heritability for glioma was significantly enriched in CNS (p_{HM}= 0.0221), (**Figure 1A, Supplementary Table 3**). Within GTex brain regions only, there was significant enrichment for GB in cortex (p_{HM}=0.0061), **Figure 1B, Supplementary Table 4**) that was not observed in non-GB (p_{HM}=0.2498). There was heritability enrichment in multiple immune cell types overall and for both GB and non-GB (**Figure 1C, Supplementary Table 5**). There was a marginally significant association between non-GB only and B cells (p_{HM}=0.0600), with the strongest association in B2 cells (p_{HM}=0.0276) and transitional B cells (p_{HM}=0.0489). There was no significant enrichment with myeloid cells in GB (p_{HM}=0.1491) or non-GB (p_{HM}= 0.0936), though there was specific enrichment in dendritic cells in both GB (p_{HM}= 0.0306) and non-GB (p_{HM}=0.0186). Natural killer (NK) cells were significantly associated with GB (p_{HM}=0.0201). There was a marginally significant association with T cells for GB (p_{HM}= 0.0944), with enrichment of association in γδ T cells in GB (p_{HM}=0.0399). Among cell types included in a broad 'other' category, we also identified stem cell specific enrichment in GB (p_{HM}=0.0265). See **Supplementary Table 5** for all cell-specific coefficients and p values.

Genome-wide genetic correlations with autoimmune, hematologic and atopic traits

We evaluated genome-wide correlations between the genetic architecture of glioma and multiple atopic, allergic and autoimmune conditions, as well as hematologic traits. In general, λ estimates were higher than intercepts, suggesting that signal was driven by polygenicity rather than population stratification. Ratios were elevated for many traits obtained from case-control studies, including celiac disease, lupus, multiple sclerosis, and ulcerative colitis, suggesting that population stratification may have a significant impact on observed results. After correcting for multiple testing no associations were significant. A nominally significant negative genetic correlation was identified between glioma and primary biliary cirrhosis (PBC, r_g=-0.26, p=0.0228) (**Figure 2, Supplementary Table 6**). When correlations were estimated by broad glioma subtypes, the scale

and direction of associations were generally consistent in both GB and non-GB, though most associations were not statistically significant. There was a negative correlation with celiac disease in non-GB only (r_g=-0.32, p=0.0109). No associations remained significant after FDR adjustment of p values.

Due to the high variability in the HLA region, LDSC excludes this by default. There is significant enrichment for germline variation associated for autoimmune disease within this region As a sensitivity analysis, genetic correlation was also estimated for these traits and glioma with a reference including the HLA region. Several phenotypes (particularly lupus and type 1 diabetes) showed significant increases in their SNP-based heritability estimates (**Supplementary Table 2**). The direction and scale of genetic correlation did not change substantially for traits other than lupus and type 1 diabetes (**Supplementary Table 7**).

There was a positive correlation between glioma and mean platelet volume (r_g=-0.09, p=0.0337) (**Supplementary Table 4**), mostly driven by the effect of non-GB (r_g=-0.12, p=0.0096). There was also a negative correlation observed between non-GB and lymphocyte percentage (r_g=-0.11, p=0.0312). We did not find any significant associations between GB and any hematologic traits. Although epidemiologic studies have repeatedly observed inverse associations between glioma and atopic conditions, glioma was not correlated with either eosinophil count (r_g=0.01, p=0.9060) or eosinophil percentage (r_g=0.02, p=0.6137), nor was it correlated with atopic traits including hay fever, asthma and eczema (**Supplementary Table 6**). A weak inverse correlation was observed between GB and eczema in the anticipated direction (r_g=-0.25, p=0.0991). No associations remained significant after FDR adjustment of p values. In general, λ estimates were higher than intercepts, suggesting that signal was due to polygenicity as compared to solely population stratification. The intercept for LD score regression has been observed to increase linearly with sample size,[38] which may lead to artificial inflation of these statistics in these continuous traits with very large sample sizes.

DISCUSSION

LD score regression is a hypothesis-generating approach that we used to identify 1) cell types that mediate glioma predisposition and 2) traits with correlated or anti-correlated genomic architecture. This

methodology enables researchers to identify traits that are correlated with their phenotype of interest (e.g. glioma) which are otherwise not amenable to study in case-control designs due to the rarity of such traits (*e.g.* the inverse association of PBC with glioma). When based on validly constructed GWAS estimates, LD score regression can also minimize many sources of bias from case-control studies related to differential participation rates and recall biases.[39] While this analysis did not identify shared genomic architecture underlying allergic/atopic traits and glioma risk, it did identify associations with specific autoimmune phenotypes that varied by GB status.

We identified significant heritability enrichment for glioma in multiple brain regions as well as immune cell types. This supports previous evidence that conditions affecting individual immune function may be associated with risk of glioma [7,8,40]. Gliomagenesis also involves significant manipulation of the immune system, and is associated with local and global immunosuppression that 'sequesters' T cells to the bone marrow and 'reprograms' other immune cells within the tumor microenvironment.[41] Increased prevalence of markers associated with dysregulated immune function are strongly associated with increased tumor growth and decreased glioma survival, suggesting that innate immune function influences risk and prognosis and leading to a current focus on immune-stimulating treatments for this disease.[42-46] Together, these factors demonstrate the significant role of immune function in both glioma susceptibility and patient prognosis.

This analysis identified significant negative correlations between glioma and several auto-immune traits, with the strongest associations identified with primary biliary cirrhosis (PBC) and celiac disease. The estimate of r_g for multiple sclerosis was similar in scale to both PBC and celiac disease in GB and non-GB, respectively, but this association was not statistically significant. Most of these traits have not been investigated in association with glioma previously. While prior research has suggested a protective association between long-term diagnosis of (primary type 2 or adult onset) diabetes and glioma, [47] we did not find a correlation between the genetic architecture of type 1 diabetes and glioma. There is an increasing body of scientific literature documenting the biological connections between the gut and the brain. The permeable tissue of the digestive

system is the most direct way for compounds to enter the blood stream, and if small enough, these compounds can cross the blood brain barrier. The gut microbiome has been shown to significantly affect neuropsychiatric disorders, as well as response to cancer immunotherapies.[48,49] Celiac disease and PBC are organ-specific autoimmune diseases, in contrast to systematic autoimmune conditions such as rheumatoid arthritis and lupus. The autoimmune component of celiac disease arises when gliadin (a component of gluten) peptides cross the intestinal epithelial barrier and activate CD4+ T-lymphocytes, stimulating expansion of B cells that secrete anti-gliadin and auto-antibodies.[50] Gliadin also activates antigen presenting cells (APCs, or dendritic cells) and intestinal epithelial cells, leading to expansion of CD8+ T-lymphocytes. Immune cell type-specific heritability analyses found a significant enrichment for GB and non-GB heritability in dendritic cells. PBC is also a T-cell mediated autoimmune disease which primarily affects biliary epithelial cells, and biopsy specimens from patients with this condition show significant invasion of CD4+ and CD8+ T cells.[51] While it is not clear what role NK cells play in the pathogenesis of PBC, these cells have been found in increased quantity in blood and liver specimens of PBC patients. Our analysis found significant GB-specific enrichment for heritability in NK cell. NK cells are an essential component of tumor surveillance in the healthy innate immune system, as they do not need to be activated and will respond to cells based on absence of appropriate MHC receptor. While the primary damaging immune response in autoimmune diseases is usually B- and/or T-cell mediated, both quantitative and qualitative variation in NK cells has been observed in multiple autoimmune conditions.[52] NK cells are currently under investigation for potential role in cancer immunotherapy, as their ability to perform their function in the absence of a 'danger' signal makes them less susceptible to the immunosuppressive tumor environment present in GB.[53] GB-specific enrichment was also identified in stem cells (p_{HM}=0.0157). Stem-like behavior is observed in the subpopulation of glioma cells referred to as glioma stem cells, which exert significant control over the glioma immune microenvironment.[54]

LD score regression identified few genetic correlations with the hematologic factors, with the exception of mean platelet volume and lymphocyte percentage. These values all have the potential to be markers of

underlying immune function, and further research is necessary to determine their association with glioma risk. The lack of consistent association across related phenotypes and lack of statistical significance after FDR correction, also suggests that these may not be genuine associations. Estimates of heritability for glioma were notably lower than previously estimated using GCTA [55]. Heritability estimates for autoimmune diseases and allergy/atopy traits estimated by this analysis are generally also lower than what has previously been reported.[56,57] Heritability estimates in LDSC are known to be biased downwards when data has been previously corrected for genomic control, as the glioma data used in this analysis has been based on estimated principal components. Heritability estimates may also be biased when cases and control are not from the same source population, as is the case in several included glioma GWAS (**Supplementary Table 1**). Despite adjustment to the liability scale, heritability estimates from case-control studies may be inflated as compared to cohort studies such as the UK biobank. As a result, the reported heritability estimates may be lower than 'true' heritability for these traits. Using GCTA to estimate genome-wide heritability has been shown to result in inflated estimates, particularly when there are measurement errors in the underlying phenotype,[58] which may explain our lower heritability estimates using LD score regression when compared to previous estimates using GCTA. Molecular characterization of glioma has demonstrated that the presence or absence of *IDH1/2* mutation differentiates two distinct lineages of glioma, which were pooled in glioma GWAS data.[59] While the majority of GB (~95%) are *IDH1/2* wild type and non-GB are *IDH1/2* mutant (80%),[59] these groups likely contain non-trivial phenotypic misclassification. T Relatively small sample sizes within some phenotypes (particularly for cases with autoimmune traits in the UK biobank) may also lead to some tests being underpowered. LDSC recommends a minimum sample size of 3,000 for estimates of heritability, and 5,000 cases for partitioning heritability. Glioma data meet this threshold, but do not substantially exceed it.

This analysis uses LD score regression to identify significant enrichment for glioma heritability in immune cell types and novel inverse associations between several auto-immune traits and glioma risk. A strength of this methodology is that it does not require having collected prior information on medical history.

This method has been shown to be robust under multiple different genetic architecture, as well as when the underlying summary statistics used may be biased.[60] This method requires that a trait have a sufficient portion of risk that is attributable to genetic factors, and is not appropriate for traits which are entirely affected by environment. Prior glioma case-control studies are inconsistent in how these data were collected and which diseases were included on questionnaires, and this approach allows for use of all glioma GWAS regardless of questionnaire data. Both glioma and specific autoimmune diseases are uncommon, and as a result very large sample sizes would be necessary to have adequate power to identify these associations with traditional case-control study designs. There are also several limitations to this approach. Summary statistics are used and therefore it is not able to conduct sensitivity analyses using specific groups of controls within each study, and the sharing of these controls between glioma subsets may bias results. The LD score regression method excludes the HLA region, which is known to be enriched for risk variants in atopic and autoimmune disease as well as a punitive haplotype association with non-GB glioma,[61] and may be a significant source of shared heritability between these traits.

While this analysis does not identify a genetic basis for the previously identified protective effect of atopic traits on glioma risk, it does suggest that the genomic architecture of glioma predisposition may manifest in part via its activity in immune cells. This is consistent with prior research demonstrating the protective effect of allergy and atopic disease on glioma risk, as well as the systematic immune suppression that occurs in the context of glioma. Atopic traits had low heritability, suggesting that the protective effect due to these traits may be influenced mostly by environmental factors. Autoimmune conditions, on the other hand, did show association with glioma and are more strongly influenced by intrinsic (*e.g.* genetic) factors than extrinsic (*e.g.* environmental) exposures, much like glioma. Further studies are necessary in order to confirm these associations and to identify the mechanism through which increased immune activation may reduce risk of glioma.

ACKNOWLEDGEMENTS

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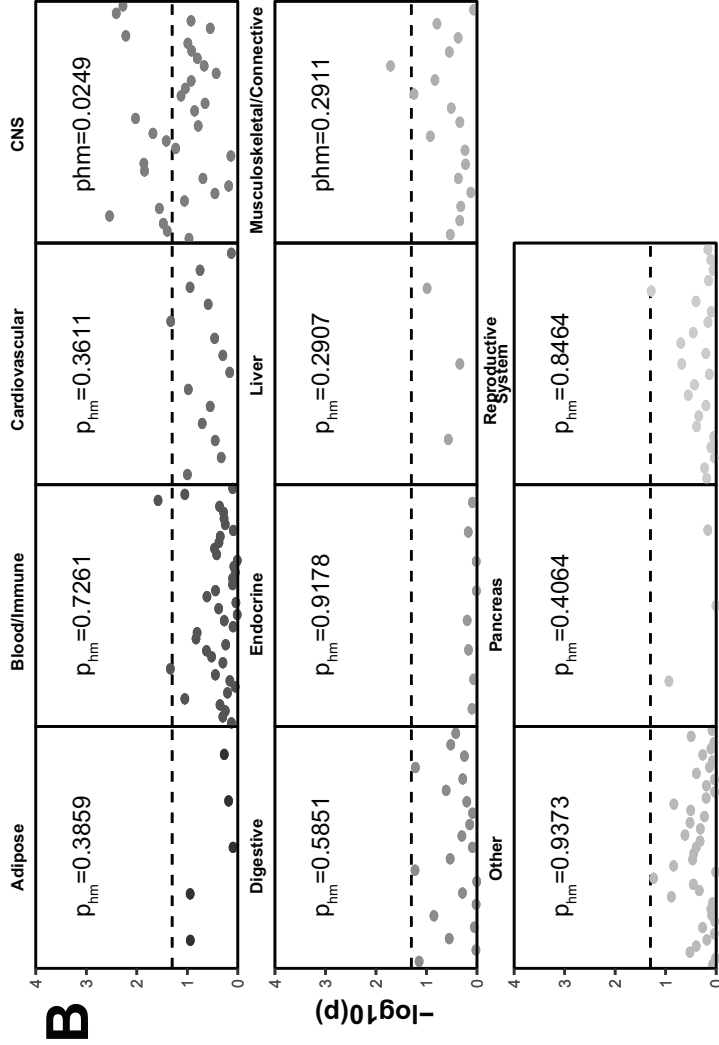
Figure Key

Figure 1. –log10(p) for individual specific tissue sample and harmonic mean p within tissue categories for A) GB and B) non-GB based on GTex reference data (dotted line indicates p<0.05)

Figure 2. log10(p) by individual cell line sample and harmonic mean p within immune cell category for A) GB and B) non-GB based on ImmGen reference data (dotted line indicates p<0.05)

Figure 3. Estimated heritability for autoimmune and atopic phenotypes, and genetic correlation between atopic and auto-immune phenotypes and glioma subgroups, dotted line indicates p<0.05
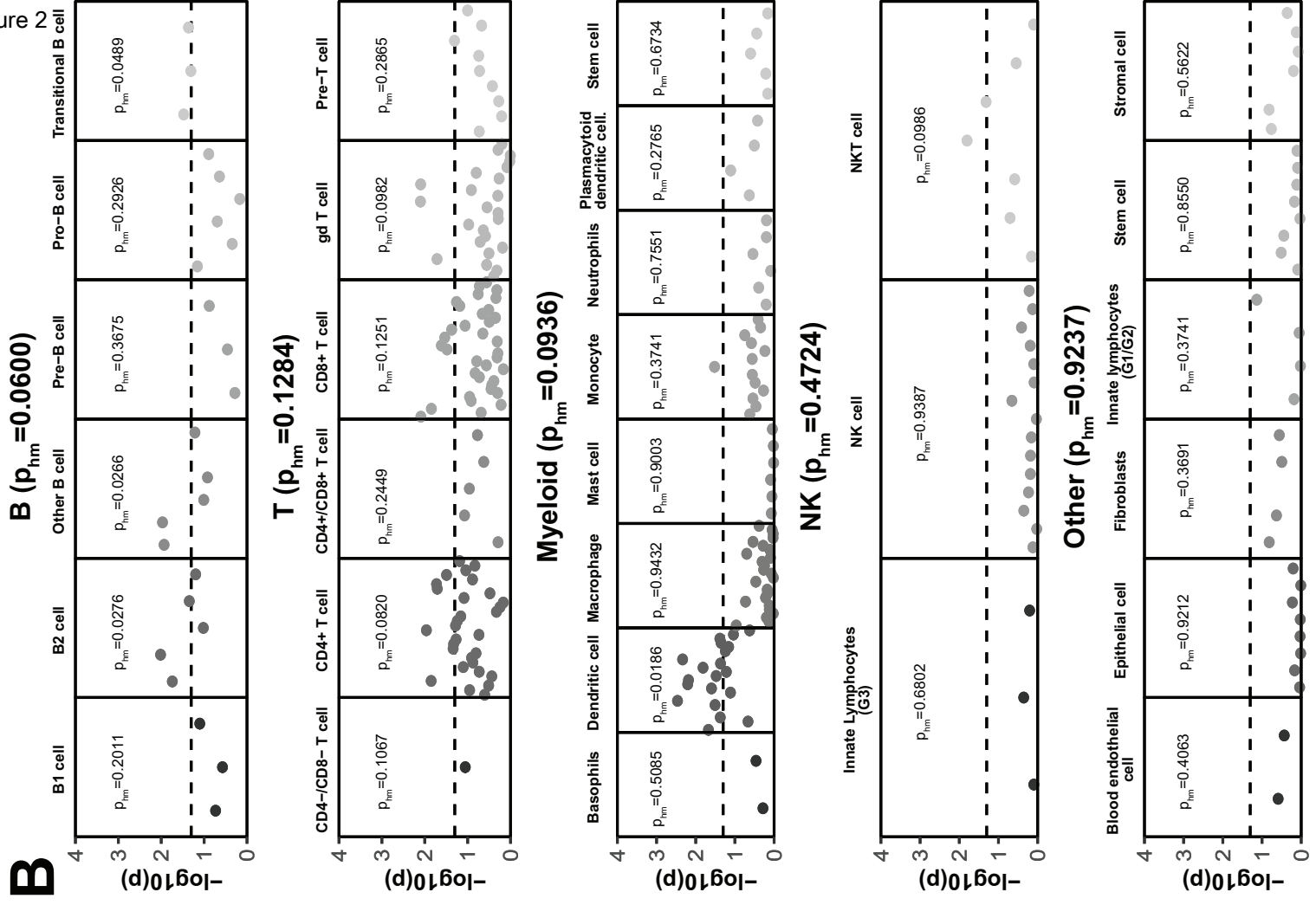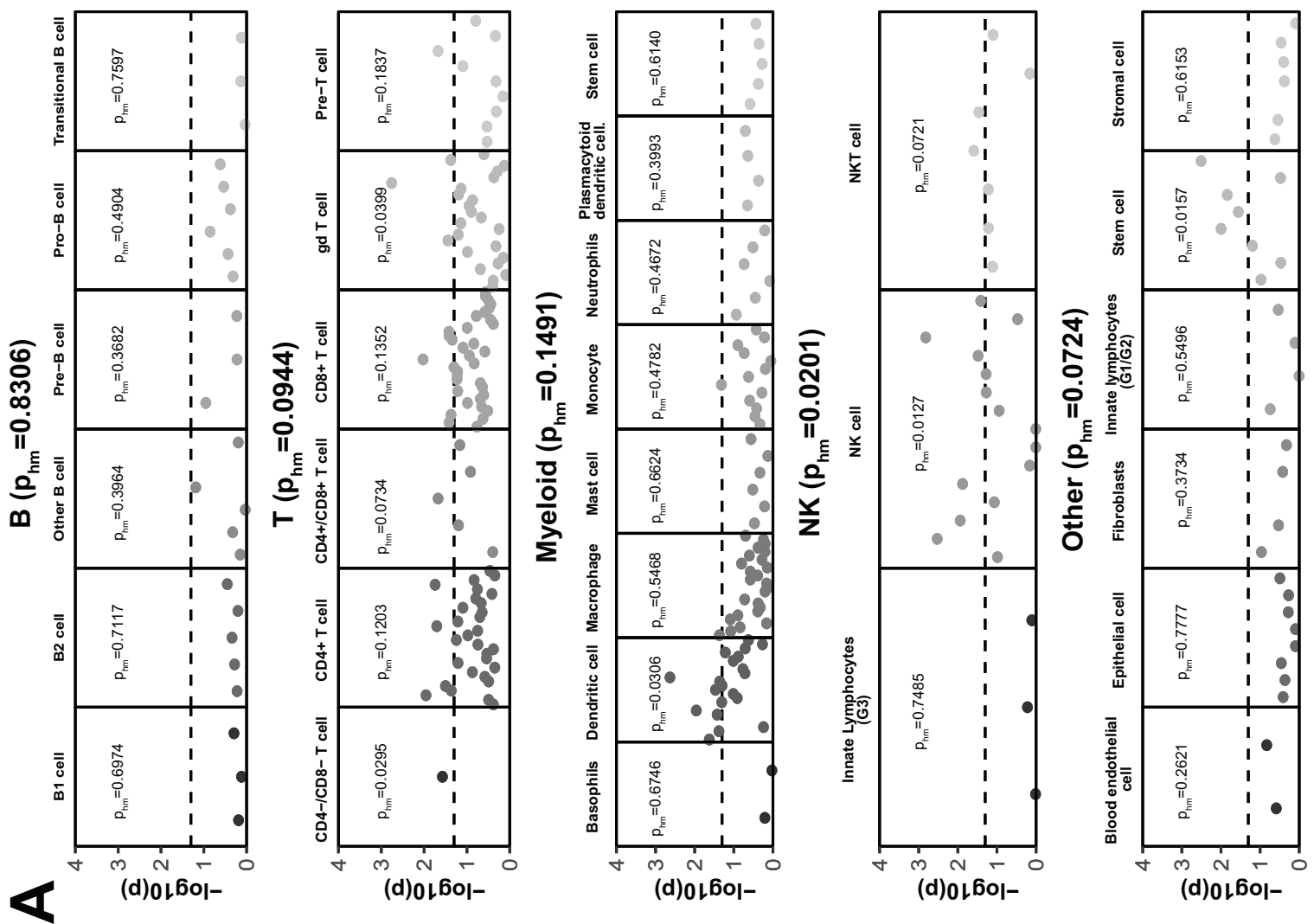
Figure 1

Figure 2

Figure 3