Environmental and sex-specific molecular signatures of glioma causation

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Abstract

Background. The relative importance of genetic and environmental risk factors in gliomagenesis remains uncertain.

Methods. Using whole-exome sequencing data from 1105 adult gliomas, we evaluate the relative contribution to cancer cell lineage proliferation and survival of single-nucleotide mutations in tumors by IDH mutation subtype and sex. We also quantify the contributions of COSMIC cancer mutational signatures to these tumors, identifying possible risk exposures.

Results. IDH-mutant tumors exhibited few unique recurrent substitutions—all in coding regions, while IDH wild-type tumors exhibited many substitutions in non-coding regions. The importance of previously reported mutations in IDH1/2, TP53, EGFR, PTEN, PIK3CA, and PIK3R1 was confirmed; however, the largest cancer effect in IDH wild-type tumors was associated with mutations in the low-prevalence BRAF V600E. Males and females exhibited mutations in a similar set of significantly overburdened genes, with some differences in variant sites—notably in the phosphoinositide 3-kinase (PI3K) pathway. In IDH-mutant tumors, PIK3CA mutations were located in the helical domain for females and the kinase domain for males; variants of import also differed by sex for PIK3R1. Endogenous age-related mutagenesis was the primary molecular signature identified; a signature associated with exogenous exposure to haloalkanes was identified and noted more frequently in males.

Conclusions. Cancer-causing mutations in glioma primarily originated as a consequence of endogenous rather than exogenous factors. Mutations in helical vs kinase domains of genes in the phosphoinositide 3-kinase (PI3K) pathway are differentially selected in males and females. Additionally, a rare environmental risk factor is suggested for some cases of glioma—particularly in males.

Key Points

1. Glioma cancer-causing mutations were associated with endogenous rather than exogenous factors.
2. Mutations in the PI3K pathway are differentially selected by sex.
3. A rare environmental risk factor (haloalkanes) is suggested for some cases of glioma.
Glioma is the most common malignant primary brain tumor. The Central Brain Tumor Registry of the United States (CBTRUS)\(^1\) reports incidence rates of 4.67 per 100,000 population. Epidemiological studies have long reported sex-specific differences in glioma risk and outcomes, with males at greater risk of being diagnosed with the disease, as well as being noted to have lower survival rates than for females.\(^2\) Traditionally, gliomas have been classified as grade I-IV based on histology and clinical criteria; recent reports reveal that the incorporation of tumor molecular markers (in particular, the presence or absence of mutations in IDH1/2) into the classification of these tumors improves prognostic ability.\(^3\)

Diagnosis of glioma is associated with significant morbidity and mortality, motivating attempts to discover risk factors through large-scale epidemiology, genetics, and neuropathology collaborations.\(^4\)\(^-\)\(^7\) In addition to rare germline variation associated with Mendelian disorders (e.g., POT1, TP53, NF1/2), common genetic variation also contributes to gliomagenesis. The extent to which such information is clinically useful at a population level is limited, however, as most of the variants conferring significant risk are rare.\(^8\) Numerous environmental exposures have also been explored in epidemiological studies of glioma risk; however—with the exception of ionizing radiation—results have been inconsistent for most factors, and it is unclear if this inconsistency arises due to study limitations or a true absence of environmental risk factors.\(^9\)\(^,\)\(^10\) Deconvolving mutation rate from substitution frequency would guide oncological management and use of targeted therapies, including within glioma.\(^11\)

TCGA and others have identified the most common genetic changes in primary glioma tumors, including mutations in IDH1/2, TERT/ATRX, TP53, and EGFR.\(^5\)\(^,\)\(^6\)\(^,\)\(^12\)\(^,\)\(^13\) The relative importance of each of these drivers and other mutations to tumorigenesis is not well known but can be quantified by the cancer effect size—a metric of the relative overabundance of variants due to their contributions to cellular survival and division. Here, we quantify the cancer effect sizes of glioma single-nucleotide mutations, i.e., the scaled selection coefficient for somatic variants in cancer cell lineages.\(^11\) This metric of the survival and reproductive advantage conferred by mutations in somatic tissue may differ by sex, thus potentially helping explain differences in glioma risk and outcome seen by sex. Additionally, we use mutational signature profiling to associate environmental exposures with the development of glioma.

**Data**

Whole-exome sequencing data from 1105 adult glioma tumor samples were obtained from the National Cancer Institute’s Genomic Data Commons Data Portal\(^15\) and the GLASS Consortium.\(^16\)\(^,\)\(^17\) The dataset includes 436 patients with IDH-mutant (defined as having a mutation in IDH1 R132 or IDH2 R172) glioma (192 female, 244 male) and 669 patients with IDH wild-type glioma (250 female, 419 male). For consistency, all whole-exome TCGA data were first converted to hg19 coordinates using the liftOver function of the R package rtracklayer,\(^18\) and the subset of GLASS whole-genome data that aligned to coding regions were extracted. Data processing, pipelines, and scripts are available at https://github.com/Townsend-Lab-Yale/glioma_CES.

**Methods**

The cancer effect sizes for point mutations were calculated using cancereffectsizeR (https://github.com/townsend-lab-yale/cancereffectsizeR, v0.1.1.9010) as previously described\(^11\) with the exception that the likelihood of the scaled selection coefficient was maximized based on tumor-specific mutation rates, and only COSMIC\(^14\) signatures were used for each tumor type. In summary, the expected frequency \(\mu\) that nucleotide mutations occur before being acted on by selection over the average amount of time elapsed throughout the evolutionary process driving tumorigenesis (from initialization to surgical resection) was defined by calculating the expected frequency that silent mutations occur at the gene level using dNdScv,\(^19\) an R package that calculates the change in non-synonymous divergence (dN) relative to synonymous divergence (dS) informed by mutational covariates (cv). Each possible nucleotide mutation was scaled by a coefficient corresponding to the relative expected frequency within its trinucleotide
context given the specific trinucleotide mutation rates in each tumor. The effect of the trinucleotide context was quantified as the product of the mutational signature weights and their relative trinucleotide mutation rates as detected with deconstructSigs. deconstructSigs weights for tumors with less than 50 substitutions—below which calculating the exact trinucleotide signatures becomes increasingly error-prone—were assessed as n/50 times the trinucleotide weights of that tumor plus (50 – n)/50 times the average trinucleotide weights of the tumors in that tumor type with greater than 50 substitutions, where n is the number of substitutions in that tumor. Defining the rate of substitution, λ, as the frequency at which genetic variants were observed within sequence data, we corrected λ for the fact that one can only observe one substitution per site, even though a flux of mutations at a given rate will generate a Poisson-distributed number of substitutions. 

False-discovery rates represented as Q values were calculated by dndscv as described by Martincorena et al. 

The total cancer effect size contributed by each mutational signature in each tumor, ie, the signature-scaled cancer effect size, was calculated by first determining the relative probability that each mutational signature was responsible for each single nucleotide variant (SNV), given the specific trinucleotide mutation rates in context given the specific trinucleotide mutation rates in each tumor. The effect of the trinucleotide context was quantified as the product of the mutational signature weights and their relative trinucleotide mutation rates as detected with deconstructSigs. deconstructSigs weights for tumors with less than 50 substitutions—below which calculating the exact trinucleotide signatures becomes increasingly error-prone—were assessed as n/50 times the trinucleotide weights of that tumor plus (50 – n)/50 times the average trinucleotide weights of the tumors in that tumor type with greater than 50 substitutions, where n is the number of substitutions in that tumor. Defining the rate of substitution, λ, as the frequency at which genetic variants were observed within sequence data, we corrected λ for the fact that one can only observe one substitution per site, even though a flux of mutations at a given rate will generate a Poisson-distributed number of substitutions.

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Results

As expected, IDH1 variants were estimated to have the highest scaled selection coefficient in IDH-mutant tumors (Figure 1). The gene IDH1 was classified as significantly overburdened with non-synonymous substitutions relative to a neutral expectation, with R132H as the most prevalent variant. Following IDH1 in their scaled selection coefficients are variants in a number of genes known to be frequently mutated in lower-grade glioma (LGG) tumors (primarily IDH-mutant lesions), including IDH2, TP53, CIC, ATRX, PIK3CA, and PIK3R1. In IDH-mutant tumors, high estimated scaled selection coefficients were estimated for several low-prevalence variants located in genes that were not found to be statistically significantly mutated at a gene-wide level for LGG (eg, NRAS Q61R in males and MAX R60Q in females). For IDH wild-type lesions, our analysis confirms well-known gliomagenesis genes for both females and males (eg, EGFR, PIK3CA, TP53, and PTEN), but also identifies low-prevalence mutations that are significant drivers in a small number of patients (eg, the mutation with the highest predicted cancer effect across all wild-type tumors, BRAF V600E). Despite its low prevalence and low gene-wide statistical overburden of mutations,
BRAF V600E is the first and sixth most strongly selected variant in females and males, respectively. Males and females exhibited a similar set of variants as well as similar rank-ordering of variant scaled selection coefficients (Figure 2). Interestingly, however, mutant sites in 2 genes from the phosphoinositide 3-kinase (PI3K) pathway, PIK3CA and PIK3R1, are in different domains for males and females. PIK3CA mutations were only noted in the helical domain (E542K and E545K) for females and primarily in the kinase domain (M1043V and H1047R) in males (Figure 2, Panel D). For PIK3R1, there were 2 variants with high cancer effect for males (N564D and G376R), but none for females (Figure 2, Panel H). While IDH-mutant tumors exhibited few unique recurrent substitutions, all occurring in coding regions, IDH wild-type tumors exhibited many substitutions in non-coding regions (Figure 3).

Across all glioma categories, the predominant molecular signature identified is one that is strongly associated with aging (COSMIC signature #1) (Figure 4A–D). The COSMIC molecular signature #42—attributed to exposure to haloalkanes, a class of chemical agent—was identified in 1/192 (0.5%) female IDH-mutant, 11/244 (4.5%) male IDH-mutant, 48/250 (19.2%) female IDH wild-type, and 93/419 (22.2%) male IDH wild-type tumors. The signature was detected predominantly in IDH wild-type tumors (P = 0.0001) and in males (P = 0.03). We confirmed that substantial numbers of mutations attributed to signature #42 could also be found in these tumors using an alternate signature caller, MutationalPatterns.21 Interestingly, exposure to ultraviolet light (COSMIC signatures #7b,c,d) was identified as a contributing factor in some tumors across all glioma categories.

### Discussion

The relative ranking of the effect sizes of the somatic variants within a tumor indicates the variants that, when successfully targeted by a therapeutic, would have the largest predicted effect on tumor progression.11 This analysis confirms the importance of well-known gliomagenesis genes (including IDH1/2, TP53, PIK3CA, EGFR, and PTEN), identifying and ordering the relative importance of variants within these genes for females and males. In addition to high-prevalence mutations in these well-known gliomagenesis genes, low-prevalence mutations may also be important drivers but may be more difficult to identify—a problem addressed by our methodology, which quantifies cancer effect alongside P value and

![Fig. 2 Cancer effect size of the genes with dndsCV Q < 0.1 and 2 or more substitutions, sorted by IDH mutation status, gene, and then sex. Variant mutations in (A) TP53, with variants, typically exhibiting slightly higher estimated scaled selection coefficients in IDH-mutant males than females and slightly lower estimated scaled selection coefficients in IDH wild-type males than females, (B) EGFR, in which strong selection occurs on variants only in IDH wild-type tumors (C) PTEN, in which strong selection occurs on variants only in IDH wild-type tumors (D) PIK3CA, in which strong selection occurs only on helical variants in females and almost exclusively on kinase domain mutations in males, (E, F) CIC and IDH1, in which strong selection occurs on variants only in IDH-mutant tumors, and (G–L) ATRX, PIK3R1, IDH2, NF1, PTPN11, and TRAT1, showing evidence of additional patterns among IDH wild-type and IDH-mutant tumors of males and females with lower numbers of variants under strong selection.](image-url)
prevalence. A prime example is the *BRAF* variant V600E, which although at low prevalence, exhibited the highest sex-specific scaled selection coefficient among all single-nucleotide variants within *IDH* wild-type tumors. This mitogen-activating protein-kinase variant is frequently reported in other cancers including melanoma and lung cancer. Although in these data, the gene itself was not defined to be statistically significantly overburdened with mutation, the V600E variant showed the largest cancer effect size in female patients with *IDH* wild-type glioma and
ranked as number 6 in terms of cancer effect size in males with this subtype. Although few patients had such a BRAF V600E mutation, its major driver status in patients with the variant implies its importance in glioma classification and prognosis, as well as for treatment, given the successes seen when this mutation has been targeted in melanoma and glioma, with some encouraging data reported. Epidemiological studies have long reported sex-specific differences in glioma risk and outcomes but to date, the molecular basis for such differences is not characterized sufficiently to guide sex-specific treatment. Males are at 50% greater risk of being diagnosed with the disease than are females. A number of population-based projects have attempted to explain sex differences in glioma by examining hormonal and reproductive factors. However, no conclusive determination of the impact of hormone exposure on glioma has been identified. Furthermore, sex differences are noted for glioma across the age spectrum as well as for all subtypes, suggesting that other mechanisms in addition to acute sex hormone actions must be identified to account for the magnitude of sex differences in glioma incidence. Sex-specific differences in survival have also been noted for glioma, with males consistently having significantly lower survival rates than females. Our prior glioma genome-wide association study (GWAS) demonstrated patterns of germline single-nucleotide polymorphisms (SNPs) associated with glioma risk by sex. A recent study of somatic mutations suggests that in patients with high-grade glioma (glioblastoma or GBM), response to standard treatment of surgery, radiation, and/or temozolomide (TMZ) is more effective for females than for males; survival in males was correlated with the expression of cell-cycle regulators, whereas in females, it was correlated with the expression of integrin-signaling pathway components. Sex differences of mutation clonality in glioma evolution have also been noted with sex-specific variation in subclonal mutation number and clonal tendency of cancer genes. These findings strongly suggest that clinically relevant, sex-specific genetic features exist for glioma.

For most of the genes identified in our study, similar importance of variants in each sex was revealed, but some differences are notable. For instance, deregulation of the phosphoinositide 3-kinase (PI3K) pathway contributes to the development and progression of many tumors, including glioma. Somatic mutations in the components of this pathway include those in PIK3CA (encoding the PI3K catalytic subunit p110α), and PIK3R1 (encoding the PI3K regulatory subunit p85α). Activating PIK3CA and PIK3R1 mutations are observed as well as associated with prognosis in a number of malignancies including cancers of the colon, breast, and brain. PIK3CA mutation prevalence has been variably but not consistently reported to vary by sex. Our analysis noted PIK3CA mutations associated with high cancer effect in both males and females, but demonstrated that activating mutations in the helical domain of PIK3CA in IDH-mutant tumors were primarily in females, while activating mutations in the kinase domain were noted in males. A similar pattern of mutations in 13 patients by sex in a hospital-based series of glioma patients can be seen in the dataset of Tanaka et al. Helical and kinase domain mutations trigger gain of function through different mechanisms: the effects of helical domain mutations have been argued to be independent of binding of p85 but require RAS-GTP, whereas the effects of the kinase domain mutations have been argued to be active without RAS-GTP binding and to be highly dependent on the interaction with p85. Helical domain mutations appear to affect enzymatic function via altering transmission of signal to the kinase domain, whereas kinase domain mutations appear to be activating due to a perturbation of the PIK3CA interaction with the cell membrane. These differential functions can impact clinical outcomes: in breast cancer, helical domain mutations are associated with early recurrence and death, compared to optimal prognosis for kinase domain mutations. Progress in the understanding of the etiology of malignant gliomas has led to therapies targeting receptor tyrosine kinases with high potential to improve the therapeutic response while reducing toxicity. Prior reports indicate that while both helical- and kinase domain mutations promote gliomagenesis, patients with helical domain mutations may be more sensitive to combined PI3ki/MEKi treatment and males and females might differentially benefit from such treatment. In these data, PIK3R1 mutations of high cancer effect were seen only in males, regardless of IDH mutation subtype. The differential in mutant PIK3CA/PIK3R1 variants by sex that we have observed should be confirmed in future datasets with a larger sample size.

Challenges to the identification of exposures associated with glioma risk are multifactorial. They include problems common to epidemiologic studies such as confounding, reverse causation, and measurement errors. Moreover, glioma presents tumor-type-specific challenges to risk factor identification: the rare and often rapidly fatal nature of these tumors (making enrollment difficult and causing survivor bias), the pooling of molecularly heterogeneous tumors under the conglomerate diagnosis of glioma, and the rarity of postulated exposures (eg, workplace exposures to toxins). Given the paucity of risk factors for glioma identified through traditional epidemiologic research efforts, we identified mutational processes that act on glioma tumors at the time of diagnosis (and before treatment) by grouping exonic SNVs into COSMIC mutational signatures and thus elucidated exposures that may underlie the development of glioma. This report is the largest effort to define molecular signatures associated with genetic pathways as well as exposure to environmental factors for glioma and the first to do so by sex. Unlike a number of other cancers in which risk is strongly tied to environmental exposure, we find that in most instances, the profile of glioma tumor mutations is associated primarily with endogenous rather than exogenous factors. Cosmic signature 1 was the primary molecular signature identified for glioma regardless of IDH status or sex. This signature is seen in all cancers and is proposed to be a consequence of age-related mutagenesis associated with C→T transitions in CpNpG trinucleotides and correlated with patient age. The underlying proposed biological mechanism is the spontaneous deamination of 5-methylcytosine. Methylation of cytosine, to 5-methylcytosine, is an epigenetic gene regulatory mechanism with implications for aging and disease in all tissues. DNA methylation is an epigenetic modification that occurs when a methyl group is added to the fifth
cytosine base, forming 5-methylcytosine. Methylation to the promoter regions can silence gene function and as a result can turn off tumor suppressor genes.

Our analysis supports the hypothesis that glioma primarily arises as a consequence of endogenous processes associated with aging. However, it also suggests a potential environmental risk factor for some glioma: exposure to haloalkanes. The haloalkane signature of mutations in cancer was discovered through the examination of the genetic changes in the tumors of printing workers with a high rate of cholangiocarcinoma and who were exposed to haloalkanes in Osaka, Japan. Like some gliomas, some cholangiocarcinomas are associated with IDH1/2 mutations. Haloalkanes are a group of chemical compounds derived from alkanes containing one or more halogens. They are widely used commercially including as flame retardants, fire extinguishants (of note given reports of their use as respiratory protective gases), and pharmaceuticals. Some haloalkanes have been demonstrated to be serious pollutants and increased risk of glioma has been found in persons with exposure to such agents. Generally, industrial chemicals have long been suspected as a cause of glioma due to their ability to cross the blood–brain barrier because of their high solubility in fats. Further research should assess whether haloalkanes are a true risk factor for glioma and whether the higher proportion of males vs females exhibiting the signature could be attributed to increased workplace exposure.

An ultraviolet signature underlying mutations in some gliomas was previously indicated and is recapitulated in our analysis. Associations between risk of glioma and melanoma have previously been reported. Identification of a genetic variant in families with both melanoma and glioma, and convergence of melanoma and glioma predisposition on genes involved in telomere maintenance may suggest a common underlying genetic mechanism or predisposition to a common environmental exposure for these 2 cancers. However, the cancer effect of mutations attributed to the ultraviolet signatures—in our analysis—was negligible.

Our results confirm a complex process for gliomagenesis, particularly for IDH wild-type tumors with few common mutations and many located in or near splice sites. Although additional signatures remain to be identified—and less common exposures may be difficult to identify—our results suggest a profile of glioma mutations that are caused primarily by endogenous rather than exogenous factors, limiting options for prevention. Our findings of the potential relevance of haloalkane and possibly ultraviolet exposure could be validated by re-examination of studies that document occupational exposure to sequence cancer tissue and assess molecular mutation signatures in associated glioma tumor specimens compared to controls without documented exposure.

Keywords
cancer effect | glioma | haloalkanes | molecular signatures | mutations

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