Investigation of Established Genetic Risk Variants for Glioma in Prediagnostic Samples from a Population-Based Nested Case-Control Study

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Abstract

Background: Although glioma etiology is poorly understood in general, growing evidence indicates a genetic component. Four large genome-wide association studies (GWAS) have linked common genetic variants with an increased glioma risk. However, to date, these studies are based largely on a case-control design, where cases have been recruited at the time of or after diagnosis. They may therefore suffer from a degree of survival bias, introduced when rapidly fatal cases are not included.

Methods: To confirm glioma risk variants in a prospective setting, we have analyzed 11 previously identified risk variants in a set of prediagnostic serum samples with 598 cases and 595 matched controls. Serum samples were acquired from The Janus Serum Bank, a Norwegian population-based biobank reserved for cancer research.

Results: We confirmed the association with glioma risk for variants within five genomic regions: 8q24.21 (CCDC26), 9p21.3 (CDKN2B-AS1), 11q23.3 (PHLDB1), 17p13.1 (TP53), and 20q13.33 (RTEL1). However, previously identified risk variants within the 7p11.2 (EGFR) region were not confirmed by this study.

Conclusions: Our results indicate that the risk variants that were confirmed by this study are truly associated with glioma risk and may, consequently, affect gliomagenesis. Though the lack of positive confirmation of EGFR risk variants may be attributable to relatively limited statistical power, it nevertheless raises the question whether they truly are risk variants or markers for glioma prognosis.

Impact: Our findings indicate the need for further studies to clarify the role of glioma risk loci with respect to prolonged survival versus etiology. Cancer Epidemiol Biomarkers Prev; 24(5): 1–7. ©2015 AACR.

Introduction

The prognosis for patients with glioma is poor. Given standard care (including surgery, radiation, and chemotherapy), patients with glioblastoma (GBM), the most common glioma subtype, have a 5-year survival rate of less than 10% (1) and the median survival is 12 to 15 months (2–4). One obstacle in the development of treatments and surveillance strategies is that the etiology of the disease is generally poorly understood. The only established environmental risk factor associated with glioma is exposure to moderate to high doses of ionizing radiation (5). Glioma subtypes (6) and Simon and colleagues (7) found rs2736100 (AS1) to display a similar association with all grade tumors (11, 13, 15, 16), though Jenkins and colleagues (17) did not find a significant association with glioma risk for variants within the 7p11.2 (EGFR) region.

The genetic component in glioma etiology has been further substantiated through four genome-wide association studies (GWAS), identifying eight genetic variants within six genomic regions associated with glioma risk (refs. 11–14; see corresponding references in Table 1). The two variants at 9p21.3 (rs4977756 and rs1412829) were found independently, but are in linkage disequilibrium ($D^\prime = 0.76$), whereas the two variants at 7p11.2 (rs11979158 and rs2252586) were found through the same study, but are less frequently co-inherited ($D^\prime = 0.36$). Variants in 20q13.3 (RTEL1), 5p15.33 (TERT), and 9p21.3 (CDKN2B-AS1) are largely shown to be primarily associated with higher grade tumors (11, 13, 15, 16), though Jenkins and colleagues found rs2736100 (TERT) to display a similar association with all glioma subtypes (16) and Simon and colleagues found rs4977756 (CDKN2B) to not be correlated with tumor grade (15). Conversely, variants in 11q23.3 (PHLDB1) and 8q24.21 (CCDC26) are primarily associated with lower grade tumors and oligodendrogloma (11, 13, 15–17).

Further genetic variants that are shown to be associated with glioma risk include a functional variant annotating the 3′ untranslated region of the TP53 gene (rs78378222) (18), and two additional EGFR variants (rs4947979 and rs4947986) that were identified by a candidate gene approach (19).
The presently known glioma risk variants mentioned above (Table 1) have been primarily identified through studies using a case–control design for both the discovery and the replication phases. Case–control studies typically recruit cases to the study at the time of, or subsequent to, diagnosis. This strategy incorporates a risk that cases that rapidly succumb to the disease will not be included in the study, i.e., survival bias. This risk is particularly pronounced when studying diseases with very short survival, such as glioma. For example in pancreatic cancer, known to also have a very poor prognosis, the 5p15.33 (CLPTM1L/TERT) genotype rs401681 is shown to display an association with risk based on postdiagnostic samples but not based on prediagnostic samples acquired from The Janus Serum Bank, a Norwegian population-based biobank reserved for cancer research. The biobank consists of samples collected from 17 of Norway's 19 counties. Donors to the biobank were recruited both from Red Cross Blood Bank donors (1973–1991, men and women, ages 18–65) and various health examination studies, including The Oslo Study (1972–1973, only men, ages 40–49), population-based national health screening programs (1974–1991, men and women, predominantly individuals in their forties), and an investigation of health and life conditions in Troms county (2002–2004). Samples included in this study were collected between 1972 and 2004 from individuals between 18.8 and 74.3 years of age at the time of blood draw (50% of the samples were donated by individuals between 39.9 and 42.8 years of age). The Cancer Registry of Norway was used for identifying cases. The samples were collected at various time points before diagnosis of cases, where the average time between sample collection and diagnosis was 15 years (Table 2). Cases and controls were matched for sex, year of birth (within 15 months), county, and date of sample collection (within 4 months). All samples were analyzed for 11 single-nucleotide polymorphisms, as detailed in Table 1, using cycling temperature capillary electrophoresis, as described by Bjørheim and colleagues (23). The study was approved by the ethical review board at the University of Oslo, Norway.

### Materials and Methods

#### Biological samples

The study was set up as a case–control study nested within the Janus cohort. A total of 1,193 prediagnostic serum samples, from 598 cases and 595 controls (3 of the controls were each matched with two separate cases), were selected from the Janus Serum Bank (21, 22) for analysis. The serum samples were stored at −25°C. The Janus Serum Bank is a Norwegian population-based biobank reserved for cancer research. The biobank consists of samples collected from 17 of Norway’s 19 counties. Donors to the biobank were recruited both from Red Cross Blood Bank donors (1973–1991, men and women, ages 18–65) and various health examination studies, including The Oslo Study (1972–1973, only men, ages 40–49), population-based national health screening programs (1974–1991, men and women, predominantly individuals in their forties), and an investigation of health and life conditions in Troms county (2002–2004). Samples included in this study were collected between 1972 and 2004 from individuals between 18.8 and 74.3 years of age at the time of blood draw (50% of the samples were donated by individuals between 39.9 and 42.8 years of age).

#### Table 1. Included genetic variants, reported to be associated with glioma risk

<table>
<thead>
<tr>
<th>Locus</th>
<th>Cytoband</th>
<th>Gene</th>
<th>Alleles (major/Minor)</th>
<th>MAFa (cases/controls)</th>
<th>Call rate (%)</th>
<th>Refb</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs2736300</td>
<td>5p15.33</td>
<td>TERT</td>
<td>G/T</td>
<td>0.44 (0.48)</td>
<td>96.4</td>
<td>(14)</td>
</tr>
<tr>
<td>rs4947979</td>
<td>7p11.2</td>
<td>EGFR</td>
<td>A/G</td>
<td>0.21 (0.19)</td>
<td>92.3</td>
<td>(19)</td>
</tr>
<tr>
<td>rs4947986</td>
<td>7p11.2</td>
<td>EGFR</td>
<td>C/T</td>
<td>0.24 (0.28)</td>
<td>99.7</td>
<td>(19)</td>
</tr>
<tr>
<td>rs2255285</td>
<td>7p11.2</td>
<td>EGFR (107 kb telomeric)</td>
<td>C/T</td>
<td>0.26 (0.26)</td>
<td>100.0</td>
<td>(13)</td>
</tr>
<tr>
<td>rs1979158</td>
<td>7p11.2</td>
<td>EGFR</td>
<td>T/C</td>
<td>0.18 (0.19)</td>
<td>99.7</td>
<td>(13)</td>
</tr>
<tr>
<td>rs4295627</td>
<td>8q24.21</td>
<td>CCDC26</td>
<td>T/G</td>
<td>0.22 (0.16)</td>
<td>99.2</td>
<td>(14)</td>
</tr>
<tr>
<td>rs4977576</td>
<td>9p21.3</td>
<td>CDKN2B-ASI</td>
<td>A/G</td>
<td>0.15 (0.43)</td>
<td>99.0</td>
<td>(14)</td>
</tr>
<tr>
<td>rs1412829</td>
<td>9p21.3</td>
<td>CDKN2B-ASI</td>
<td>T/C</td>
<td>0.52 (0.43)</td>
<td>99.7</td>
<td>(12)</td>
</tr>
<tr>
<td>rs498872</td>
<td>1q23.3</td>
<td>PHLDB1</td>
<td>G/A</td>
<td>0.37 (0.31)</td>
<td>100.0</td>
<td>(14)</td>
</tr>
<tr>
<td>rs78378222</td>
<td>1p13.1</td>
<td>TP53</td>
<td>A/C</td>
<td>0.04 (0.02)</td>
<td>99.6</td>
<td>(18)</td>
</tr>
<tr>
<td>rs6010620</td>
<td>20q13.33</td>
<td>RETL1</td>
<td>G/A</td>
<td>0.20 (0.27)</td>
<td>99.7</td>
<td>(12, 14)</td>
</tr>
</tbody>
</table>

aMinor allele frequency.  
bReference to the report that originally identified the association with risk.

#### Table 2. Basic characteristics describing the samples included in the separate glioma subtypes analyzed

<table>
<thead>
<tr>
<th>Cases (n)</th>
<th>Age at diagnosisa</th>
<th>Age at samplinga</th>
<th>Sampling before diagnosis (y)a</th>
</tr>
</thead>
<tbody>
<tr>
<td>All cases</td>
<td>594</td>
<td>56.3 (27.7–84.1)</td>
<td>41.4 (18.9–74.5)</td>
</tr>
<tr>
<td>Males</td>
<td>396</td>
<td>56.0 (27.7–84.3)</td>
<td>41.6 (18.9–74.5)</td>
</tr>
<tr>
<td>Females</td>
<td>198</td>
<td>56.7 (32.5–85.2)</td>
<td>41.0 (22.8–70.7)</td>
</tr>
<tr>
<td>All glioma</td>
<td>566</td>
<td>56.4 (27.7–84.1)</td>
<td>41.4 (18.9–74.5)</td>
</tr>
<tr>
<td>Males</td>
<td>378</td>
<td>56.1 (27.7–84.1)</td>
<td>41.6 (18.9–74.5)</td>
</tr>
<tr>
<td>Females</td>
<td>188</td>
<td>56.5 (32.5–85.2)</td>
<td>41.0 (22.8–70.7)</td>
</tr>
<tr>
<td>GBM</td>
<td>376</td>
<td>57.6 (34.7–84.1)</td>
<td>41.7 (19.2–67.2)</td>
</tr>
<tr>
<td>Males</td>
<td>268</td>
<td>57.5 (34.7–84.1)</td>
<td>41.9 (19.2–67.2)</td>
</tr>
<tr>
<td>Females</td>
<td>108</td>
<td>57.6 (38.8–83.2)</td>
<td>41.1 (27.2–66.3)</td>
</tr>
<tr>
<td>Oligo</td>
<td>33</td>
<td>51.8 (27.7–78.3)</td>
<td>41.1 (18.9–66.4)</td>
</tr>
<tr>
<td>Males</td>
<td>24</td>
<td>51.1 (27.7–78.3)</td>
<td>41.1 (18.9–66.4)</td>
</tr>
<tr>
<td>Females</td>
<td>9</td>
<td>54.1 (44.7–64.5)</td>
<td>41.4 (27.4–42.3)</td>
</tr>
<tr>
<td>Ependymoma</td>
<td>28</td>
<td>54.2 (43.3–70.5)</td>
<td>41.4 (28.5–55.3)</td>
</tr>
<tr>
<td>Males</td>
<td>18</td>
<td>53.2 (45.5–70.5)</td>
<td>42.0 (39.9–55.3)</td>
</tr>
<tr>
<td>Females</td>
<td>10</td>
<td>57.0 (43.3–67.2)</td>
<td>40.7 (28.5–51.1)</td>
</tr>
<tr>
<td>Astrocytoma</td>
<td>93</td>
<td>51.1 (27.7–78.9)</td>
<td>41.1 (22.4–74.5)</td>
</tr>
<tr>
<td>Males</td>
<td>56</td>
<td>51.8 (27.7–78.9)</td>
<td>41.2 (22.4–74.5)</td>
</tr>
<tr>
<td>Females</td>
<td>37</td>
<td>48.2 (33.0–72.4)</td>
<td>41.1 (22.8–70.7)</td>
</tr>
</tbody>
</table>

aMedian (min – max).  
bNot including ependymoma.
Figure 1.
Associations between published genetic risk variants and risk of disease, calculated by conditional logistic regression. Gray boxes denote associations that are opposite in direction of those of the original report. Purple confidence intervals denote that both OR and CI have been inverted in the plot, for easier comparison. ORs and P values represent the results of examining associations between genotypes and risk of disease by conditional logistic regression under a log-additive genetic model (accounting for sample matching).
DNA amplification

Five microliter aliquots of serum were transferred into 96-well microplates (Axygen; VWR) and subjected to enzymatic amplification, as described by Ekstrøm and colleagues (24). Serum aliquots were denatured at 95°C for 8 minutes. Then, 40 μL of PCR master mix was added to each well, and the plate was subjected to temperature cycling as described below.

The PCR mix contained 4.5 μL of 10× buffer (Applied Biosystems), 0.8 μL of 10 mmol/L dNTP mix (AmershamBiosciences), and a varying concentration of MgCl2 (Supplementary Table S1). Primers were added to a final volume of 45 μL (Supplementary Table S1). A combination of Taq and Pfu polymerase (both produced in-house) was added to the reaction in varying concentrations (Supplementary Table S1).

Amplification was performed on a DNA Engine Tetrad 2 (Biorad), using the following cycling conditions: denaturation for 5 minutes at 95°C, followed by 44 or 45 cycles of 30 seconds at 95°C, 30 seconds at the annealing temperature, and 60 seconds at 72°C. The cycling was followed by 10 minutes at 72°C for completion of polymerization, 5 minutes at 95°C for double-strand denaturation, 30 minutes at 65°C for heteroduplex formation, and lastly a stepwise temperature decrease of 1°C per minute until a final hold at 12°C (Supplementary Table S1). Primers used for the analysis are shown in Supplementary Table S1. In each primer set, one of the primers was labelled with 6-carboxyfluorescin (6-FAM). All primers were obtained from Integrated DNA Technologies.

Variant detection

Amplified 6-FAM–labelled PCR products were analyzed by denaturant capillary electrophoresis on a MegaACE 1000 DNA Analysis System (GE Healthcare Bio-Sciences AB). The base variants were separated by cycling temperature capillary electrophoresis (CTCE), with separating temperatures listed in Supplementary Table S1, cycled 20 times. The variants were identified by co-analysis with a mutated internal standard, essentially as described by Björnheim and colleagues (23). The assay was run in a 96-well format, where a minimum of two wells per microplate were used for controls, i.e., one serum control (pool of sera from 5 healthy individuals) and at least one negative control without serum template.

Quality control

To investigate potential genotyping errors, we calculated call rates for both samples and genotypes. Samples displaying a call rate of <80% (i.e., where the genotyping had failed at ≥2 SNPs) were removed from further analyses. In addition, the genotype frequency distribution for each SNP among controls was calculated and tested against the Hardy–Weinberg equilibrium. P values of <0.005 were considered indicative of genotyping errors (all SNPs displayed HWE P > 0.005 in this study). All 11 genetic variants included in the study displayed a call rate of >90% (Table 1). Four samples displayed a call rate of <80% (i.e., ≥2 failed genotypes) and were consequently removed from downstream analyses, along with their matched counterparts.

Statistical analyses

Associations between genotypes and risk of disease were examined using conditional logistic regression under a log-additive genetic model (accounting for sample matching). Separate analyses, comparing cases with controls, were performed for the following histologic groups: (i) astrocytoma, (ii) ependymoma, (iii) oligodendroglioma, (iv) GBM, and (v) all gliomas (i.e., including all histologic subgroups except ependymoma). CDK03 codes included in each histologic group are listed in Supplementary Table S2. All analyses were performed using the R software for statistical computing (www.R-project.org).

Results

A total of 594 cases and 591 controls were eligible for inclusion in the study. The characteristics of the study population are summarized in Table 2. With reference to all included cases, the median age at sampling was 41.4 years (range, 19–75), and the median age of diagnosis was 56.3 years (range, 28–84). The complete results of this investigation are summarized in Fig. 1.

Genetic variants in five of the investigated seven regions were confirmed by this study as associated with overall risk of glioma (i.e., all glioma diagnoses, not including ependymomas). The five regions were 8q24.21 (CCDC26; rs4295627), 9p21.3 (CDKN2B-AS1; rs4977756 and rs1412829), 11q23.3 (PHLDB1; rs498872), 17p13.1 (TP53; rs78378222), and 20q13.33 (RTEL1; rs6010620). Within the five confirmed regions, the associations we observed were consistent with previous reports in terms of the directions of the odds ratios (OR), and all associations displayed P < 0.01, except the 17p13.1 (TP53) variant (rs78378222; P = 0.0195). The lowest P values were observed for the variants annotating 9p21.3 (CDKN2B-AS1), at P < 0.0001 (Fig. 1; Supplementary Table S3).

The regions that were not replicated in our data were 7p11.2 (EGFR) and 5p15.33 (TERT). The variant annotating the 5p15.33 (TERT) region (rs2736100) displayed an OR direction that was consistent with previous findings (14), but the P value may only be considered marginally significant [OR, 0.85; 95% confidence interval (CI), 0.71–1.01; P = 0.0655]. Out of the four variants that annotate the 7p11.2 (EGFR) region, two showed no association with overall glioma risk [rs11979158 (OR, 0.91; 95% CI, 0.73–1.13; P = 0.3737) and rs2252586 (OR, 0.96; 95% CI, 0.79–1.16; P = 0.6604)], and the other two (rs4949797 and rs4949796) displayed OR directions opposite of those of the original report (19). These findings thus cannot be considered to positively corroborate previous reports (Fig. 1; Supplementary Table S3).

We further investigated the previously identified glioma risk variants by tumor subtype (Fig. 1; Supplementary Tables S3–S7). Comparing results between analyses of overall glioma and GBM, there were two noteworthy differences. First, the 17p13.1 (TP53) variant rs78378222, which was found associated with overall glioma, was not significantly associated with risk of GBM, although the magnitude of the association was consistent [OR, 1.73; 95% CI, 0.90–3.33; P = 0.1012]. Second, the 7p11.2 (EGFR) variant rs11979158, which was not found associated with overall glioma, displayed a marginally significant association with GBM only [OR, 0.76; 95% CI, 0.57–1.02; P = 0.0670].

Regarding the other histologic subgroups included in the study, only one of the investigated variants displayed an association with oligodendroglioma risk, namely the 8q24.21 (CCDC26) variant rs4295627 (OR, 2.85; 95% CI, 1.17–6.93; P = 0.0214). Both variants annotating 9p21.3 (CDKN2B-AS1) displayed significant association with increased astrocytoma risk [rs4977756 (OR, 1.59; 95% CI, 1.03–2.44; P = 0.0349); rs1412829 (OR, 1.80; 95% CI, 1.14–2.84; P = 0.0113)]. None of the investigated
variants were found to be associated with ependymoma risk. It should, however, be emphasized that the ependymoma sample set was very small (n = 28 cases).

Discussion

We have analyzed prediagnostic serum samples, with matched cases and controls from a population-based cohort, to verify previously reported associations between common genetic variants and glioma risk. Analyses of prediagnostic samples eliminate the risk of survival bias and thereby enable distinction between genetic variants associated with glioma risk (i.e., etiology) and genetic variants associated with prognosis. Our results confirmed the previously reported associations with risk for variants in five of seven regions [8q24.21 (CCDC26), 9p21.3 (CDKN2B-AS1), 11q23.3 (PHLDB1), 17p13.1 (TP53), and 20q13.33 (RTEL1)], indicating that these variants are truly associated with glioma risk and may, consequently, affect gliomagenesis.

All hitherto-presented evidence that the investigated gene variants are associated with glioma risk is largely based on case-control studies, including postdiagnostic samples (11–14, 18, 19), though the study by Rajaraman and colleagues (GliomaScan; ref. 11) included 30% incident cases from cohort studies. Post-diagnostic sampling inherently risks excluding rapidly fatal cases, and thereby introduce a bias toward cases with longer survival. Therefore, based on case-control studies using postdiagnostic samples, it is impossible to know whether identified associations are toward risk of disease or toward prognosis. This issue is particularly pronounced when studying a disease with such short median survival as glioma, and especially GBM, which is the most common glioma subtype. It is thus plausible that the variants that did not replicate in this study are in fact primarily associated with glioma subtype. The study presented here is based on a relatively limited sample size (556); however, it did not appear to be associated with glioma risk overall (P = 0.3737). The latter is in agreement with the corresponding results from GliomaScan (11).

Altogether, based on this study, we could not positively confirm any of the reported associations between common genetic variants annotating 7p11.2 (EGFR) and glioma risk. This could, of course, be due to the original reports being false positives. Alternatively, it may be attributable to the relatively limited statistical power of the current study. Nevertheless, the lack of a positive confirmation of these EGFR gene variants in a prediagnostic setting raises the question whether they may be primarily associated with gliomagenesis.

Moreover, one of the GWAS findings, rs2252586 (located 107 kb telomeric from EGFR), displayed no association with risk in any of the investigated histologic groups. This, however, does not concur with the result of GliomaScan (11), where rs2252286 displayed a significant association with glioma risk when the analysis was restricted to prediagnostic samples from incident cohort cases (n = 556). The other GWAS finding at the same locus (7p11.2; EGFR), rs11979158, displayed what may be considered a marginally significant association with GBM risk (P = 0.0670), though it did not appear to be associated with glioma risk overall (P = 0.3737). The latter is in agreement with the corresponding results from GliomaScan (11).

In this study, variants mapping to two of seven investigated regions were not confirmed, namely 5p15.33 (TERT) and 7p11.2 (EGFR). EGFR is a growth factor receptor that commonly displays somatic aberrations in glioma, including amplification, truncation, and point mutations (25). EGFR protein overexpression has been associated with poor prognosis (26), as have certain EGFR genetic variants (27), demonstrating that they may be important determinants of treatment response and survival. In the present study, we investigated four variants annotating the 7p11.2 (EGFR) region that have previously been identified as associated with glioma risk; two variants were identified through a GWAS (rs2252586 and rs11979158; ref. 13), and two variants were identified through a candidate gene approach (rs4947979 and rs4947986; ref. 19). In terms of linkage disequilibrium, each variant is practically independent of the other variants.

In this study, using prediagnostic samples, the two EGFR gene variants identified through the candidate gene approach, rs4947979 and rs4947986, both displayed ORs that were opposite in direction of those of the original report (19). The association between rs4947986 and decreased risk of overall glioma was significant in this study (P = 0.0456). However, considering that the original report found an association with increased risk, and that we had no hypothesis for an association with decreased risk, we believe this to be a chance finding.

5p15.33 (TERT) was the other region harboring a variant previously associated with risk that was not positively confirmed by this study. The TERT variant rs2736100 displayed consistency with previous reports regarding the direction of the OR and effect size; however, the observed associations with both glioma overall (P = 0.0655) and specifically with GBM (P = 0.0788) may merely be considered marginally significant. TERT is known to be involved in telomere regulation, and certain genetic variants mapping to TERT (including rs2736100) have been linked with relative telomere length in an age-dependent manner (28). This indicates a dynamic change in functionality of TERT variants across a person’s lifespan. The complexity is emphasized further by the fact that there are contradictory results regarding the association between relative telomere length and glioma risk (29, 30). In contrast with our results, providing evidence that rs2736100 (5p15.33, TERT) is accurately linked with risk, are the findings of Rajaraman and colleagues (11). When comparing the strength of rs2736100 (5p15.33; TERT) association with glioma risk between incident cases from cohort studies (prediagnostic samples; n = 556) and cases from case-control studies (post-diagnostic samples; n = 1,300), Rajaraman and colleagues found the association to be stronger in the cohort studies (11), indicating that the association is preferentially with high-grade, rapidly fatal cases. The study presented here is based on a relatively limited number of samples, which makes it difficult to draw decisive conclusions regarding marginally significant findings with modest effect sizes such as this.

As mentioned, it is important to emphasize that the study presented here was relatively small (n = 594 cases), thus limiting the power to detect weak associations. Specifically, it is possible that the association between rs11979158 (7p11.2; EGFR) and risk for GBM (P = 0.0670) could also reach statistical significance in a prediagnostic setting given a larger sample size. Similarly, the lack of a positive confirmation of associations between rs2736100 (5p15.33; TERT) and both glioma overall (P = 0.0655) and GBM (P = 0.0788) is likely attributable to small sample sizes, especially when factoring in previous results indicating an association between rs2736100 and glioma risk among incident cohort cases (11). Furthermore, the 17p13.1 (TP53) variant rs78378222 has previously been associated with risk for both GBM and non–GBM
tumors (31). In this study, we found an association with risk for glioma overall (i.e., all glioma diagnoses, not including ependymoma), but it did not replicate specifically for GBM ($P = 0.1012$). This is a very rare variant (minor allele frequency = 0.03 in this study), and the investigated sample set only included two individuals who were homozygous for the rare allele. Hence, it is likely that the lack of confirmation of rs78378222 specifically for GBM is due to the limited statistical power of the study.

As discussed above, there are disparities comparing the findings of this study with those of the GliomaScan study (11), where associations between genetic variants and glioma risk were also investigated in samples collected before diagnoses (incident cohort cases). Both the allele frequencies and the distribution of histologic subtypes are similar between the studies, though 17% of the incident cohort cases included in the latter study are of unknown histology, making the comparison somewhat uncertain. The numbers of included cases are also similar in both studies (594 for this study and 556 for incident cohort cases in GliomaScan), though GliomaScan included 5-fold more controls. The present study is based on a homogeneous collection of population-based samples, acquired from the Janus Serum Bank, with carefully matched controls, whereas the incident cohort cases in the GliomaScan study originate from 14 different cohort studies from several centers across the world (11). In addition, the time between date of blood draw and diagnosis for incident cases was shorter in GliomaScan (mean 5 years) than in this study (mean 15 years).

Our study shows that the previously reported associations between glioma risk and common gene variants annotating 8q24.21 (CCDC26), 9p21.3 (CDKN2B-AS1), 11q23.3 (PHLDB1), 17p13.1 (TP53), and 20q13.33 (RTEL1) also hold true in a prediagnostic setting. This indicates that they are accurately linked with risk of disease and may thus be important to help understand glioma etiology. These variants were all significantly associated with overall glioma risk ($P < 0.05$). Most of them were also found to be associated specifically with GBM risk, with the exception of rs78378222 (17p13.1; TP53) as mentioned above, and the association between rs988872 (11q23.3; PHLDB1) and GBM risk was marginally significant ($P = 0.0574$). In addition, variants annotating the two regions 9p21.3 (CDKN2B-AS1) and 8q24.21 (CCDC26) displayed associations with risk of astrocytoma and oligodendrogliaoma, respectively. No other variants were specifically linked with these histologic groups. The association between rs4295627 (8q24.21; CCDC26) and oligodendrogliaoma was the most pronounced association observed for this variant, which is in agreement with previous findings linking it primarily with oligodendrogliaoma risk (11, 16).

In this study, we investigated serum samples from the Janus Serum Bank. The samples were stored at $-25^\circ C$ for up to 40 years. The samples were genotyped by means of cycling temperature capillary electrophoresis (23), a PCR-based method that has successfully been applied in previous projects investigating Janus serum samples (24, 32). Because the DNA is expected to be degraded, we applied a primer design that yielded short ampli-


Investigation of Established Genetic Risk Variants for Glioma in Prediagnostic Samples from a Population-Based Nested Case–Control Study

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