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Published in: Oncotarget

DOI: 10.18632/oncotarget.11575

Publication date: 2016

Document Version
Publisher's PDF, also known as Version of record

Citation for published version (APA):
Fahmideh, M. A., Lavebratt, C., Schüz, J., Röösl, M., Tynes, T., Grotzer, M. A., ... Feychting, M. (2016). Common genetic variations in cell cycle and DNA repair pathways associated with pediatric brain tumor susceptibility. DOI: 10.18632/oncotarget.11575
Common genetic variations in cell cycle and DNA repair pathways associated with pediatric brain tumor susceptibility

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Keywords: genetic association study, pediatric brain tumors, single nucleotide polymorphism, brain neoplasm, susceptibility

Received: June 14, 2016   Accepted: August 15, 2016   Published: August 24, 2016

ABSTRACT

Knowledge on the role of genetic polymorphisms in the etiology of pediatric brain tumors (PBTs) is limited. Therefore, we investigated the association between single nucleotide polymorphisms (SNPs), identified by candidate gene-association studies on adult brain tumors, and PBT risk.

The study is based on the largest series of PBT cases to date. Saliva DNA from 245 cases and 489 controls, aged 7–19 years at diagnosis/reference date, was genotyped for 68 SNPs. Data were analyzed using unconditional logistic regression.

The results showed EGFRrs730437 and EGFRrs11506105 may decrease susceptibility to PBTs, whereas ERCCIrs3212986 may increase risk of these tumors. Moreover, stratified analyses indicated CHAF1Ars243341, CHAF1Ars2992, and XRCCIrs25487 were associated with a decreased risk of astrocytoma subtype. Furthermore, an increased risk of non-astrocytoma subtype associated with EGFRrs9642393, EME1rs12450550, ATMrs170548, and GLTSCRrs1035938 as well as a decreased risk of this subtype associated with XRCC4rs7721416 and XRCC4rs2662242 were detected.

This study indicates SNPs in EGFR, ERCCI, CHAF1A, XRCCI, EME1, ATM, GLTSCR1, and XRCC4 may be associated with the risk of PBTs. Therefore, cell cycle and DNA repair pathways variations associated with susceptibility to adult brain tumors also seem to be associated with PBT risk, suggesting pediatric and adult brain tumors might share similar etiological pathways.
INTRODUCTION

Brain tumors are the most common pediatric solid tumors and the leading cause of cancer mortality in children. High-dose ionizing radiation and rare inherited syndromes are the only established risk factors for brain tumors, causing a small proportion of cases [1]. Thus, brain tumors are considered as multifactorial disorders resulting from progressive accumulation of genetic and epigenetic alterations in concert with environmental exposures. It has been suggested that genetic polymorphisms in four main pathways including DNA repair, cell cycle, metabolism, and inflammation play an important role in brain carcinogenesis [2]. Therefore, candidate gene-association studies of adult brain tumors have mainly focused on these four hypothesized pathways to identify genetic susceptibility factors for adult brain tumors [3–9].

Whereas a considerable number of candidate gene-association studies are available on adult brain tumors, very few and small genetic studies have been performed on brain tumors in children and adolescents [10–13], due to difficulties in collecting a sufficient number of DNA samples. Hence, the role of genetic polymorphisms in pediatric brain tumor (PBT) etiology is largely unknown. However, some studies have found similar genetic mutation patterns for adult and pediatric brain tumor progression within specific histological types [14–18]. These similarities in prognostic factors provide an important starting point for identifying candidate genetic risk factors for PBTs; that might be similar to those involved in adult brain tumorigenesis [19]. Moreover, in our previous genetic association study of PBT risk, we concluded that single nucleotide polymorphisms (SNPs) identified by genome-wide association studies (GWAS) on adult glioma might also be associated with the risk of brain tumors in children [20].

The aim of this study, which represents the largest series of PBT cases to date, was to determine whether the SNPs identified by candidate gene-association studies on adult brain tumors, involved in the four main pathways discussed above, are also related to pediatric brain tumor risk.

RESULTS

We successfully genotyped 63 SNPs in 245 cases and 489 controls. The distributions of allele frequencies in 3 SNPs (rs4444903, rs9288516, and rs61754966) were not in agreement with HWE (p < 0.001) and therefore these SNPs were dropped from analyses. As shown in Table 1, the age and sex distributions were similar in cases and controls.

As Table 2 illustrates, the A alleles of EGFR rs730437 (ORDOM 0.59 [95% CI 0.42–0.83], p = 0.002) and EGFR rs11506105 (ORDOM 0.71 [95% CI 0.51–0.98], p = 0.036) involved in cell cycle pathway were associated with decreased susceptibility to PBTs, whereas the A allele of ERCCI rs3212986 (ORDOM 1.53 [95% CI 1.11–2.09], p = 0.009) involved in DNA repair pathway was associated with an increased risk of these tumors. Moreover, the interactions between these SNPs and covariates including age, sex and country were not significant.

The stratified analyses of two histological subtypes indicated that the protective effect of EGFR rs730437 remained significant in both patients with astrocytoma and non-astrocytoma tumor subtypes (pDOM = 0.018 and pDOM = 0.014, respectively), whereas the risk effect of ERCCI rs3212986 was more evident in patients with astrocytoma subtype (pDOM = 0.002). Moreover, a decreased risk of astrocytoma subtype associated with the C alleles of CHAFIA rs243341 and rs2992 as well as the T allele of XRCCI rs25487 involved in DNA repair pathway was detected (pDOM = 0.040, pDOM = 0.049, and pDOM = 0.033, respectively). In addition, the stratified analyses showed an increased risk of non-astrocytoma tumor subtype associated with the C alleles of EGFR rs9642393, EME1 rs12450550, and ATM rs170548, and the T allele of GLTSCR1 rs1035938 (pREC = 0.021, pDOM = 0.041, and pREC = 0.027, respectively) as well as a decreased risk of this subtype associated with the A allele of XRCCI rs7721416 and the C allele of XRCC4 rs2662242 (DNA repair pathway) (pREC = 0.032 and pREC = 0.024, respectively) (Tables 3 and 4).

Non-significant findings as well as the significant findings with wide confidence intervals are shown in the online appendix Tables S1–S3.

Strong LD (D’ ≥ 0.95) was observed between four genotyped SNPs in EGFR (rs730437, rs11506105, rs4947986, and rs3752651) in which five haplotypes with frequency of > 1% were detected. In the EGFR block, the distribution of haplotypes was suggestively different between PBT patients and controls (χ2 = 8.1, df = 4, p = 0.089) and the most common haplotype (CGGT) had a significant risk effect compared with the other haplotypes combined (OR 1.31 [95% CI 1.05–1.64], p = 0.017) (Table 5). In the astrocytoma subgroup, the CHAFIA block (rs243341, rs105038, rs243356, and rs2992) with three haplotypes with frequency of >1% was found; however, the distribution of haplotypes in this haploblock was not significantly different between patients and controls (χ2 = 2.95, df = 2, p = 0.226). Moreover, in the non-astrocytoma subgroup, two haploblocks including ATM (rs228599, rs664143, rs170548, rs3092993, and rs3092992) and XRCCI (rs7721416 and rs2662242) with, respectively, five and three haplotypes with frequency of >1% were observed; nevertheless, in none of these haploblocks, the distribution of haplotypes was significantly different between patients and controls (χ2 = 1.92, df = 4, p = 0.751 and χ2 = 2.98, df = 2, p = 0.226, respectively).

As described above, 248 testing procedures were performed. When the Bonferroni correction is applied, the reference p value is 0.0002 for an experiment-wide
| Characteristics                        | Cases | Astrocytomas | Non-astrocytomas | Controls |
|----------------------------------------|-------|--------------|------------------|----------|
| No. of participants                   | 245   | 134          | 111              | 489      |
| Sex                                    |       |              |                  |          |
| Males                                  | 136 (56%) | 74 (55%)   | 62 (56%)        | 261 (53%)|
| Females                                | 109 (44%) | 60 (45%)   | 49 (44%)        | 228 (47%)|
| Age-group (at diagnosis/reference date)|       |              |                  |          |
| 7–9 years old                          | 48 (20%) | 28 (21%)   | 20 (18%)        | 112 (23%)|
| 10–14 years old                        | 108 (44%) | 60 (45%)   | 48 (43%)        | 219 (45%)|
| 15–19 years old                        | 89 (36%) | 46 (34%)   | 43 (39%)        | 158 (32%)|
| Country                                |       |              |                  |          |
| Sweden                                 | 106 (43%) | 48 (36%)   | 58 (52%)        | 174 (36%)|
| Norway                                 | 24 (10%) | 15 (11%)   | 9 (8%)          | 62 (13%) |
| Denmark                                | 62 (25%) | 37 (28%)   | 25 (23%)        | 134 (27%)|
| Switzerland                            | 53 (22%) | 34 (25%)   | 19 (17%)        | 119 (24%)|
| Type of tumor (ICCC-3 group III)†     |       |              |                  |          |
| Astrocytoma (IIIb)                     |       |              |                  |          |
| Pilocytic astrocytoma                  | 93    |              |                  |          |
| Sutependymal giant cell astrocytoma    | 5     |              |                  |          |
| Pleomorphic xanhtoastrocytoma           | 4     |              |                  |          |
| Diffuse astrocytoma                    | 13    |              |                  |          |
| Anaplastic astrocytoma                 | 11    |              |                  |          |
| Fibrillary astrocytoma                 | 2     |              |                  |          |
| Glioblastoma                           | 5     |              |                  |          |
| Giant cell glioblastoma                | 1     |              |                  |          |
| Other gliomas (IIId)                   | 20 (8%) |          |                  |          |
| Malignant glioma                       | 11    |              |                  |          |
| Oligoastrocytoma                       | 2     |              |                  |          |
| Oligodendroglia                        | 6     |              |                  |          |
| Anaplastic oligodendroglia             | 1     |              |                  |          |
| Ependymoma (IIIa)                      | 19 (8%) |          |                  |          |
| Subependymoma                          | 2     |              |                  |          |
| Choroid plexus papilloma               | 4     |              |                  |          |
| Choroid plexus carcinoma               | 1     |              |                  |          |
significance level of 0.05, and 0.0004 for a significance level of 0.10; none of the reported associations met these limits. As summarized in Table S4, no significant SNP-SNP interactions were observed ($p < 0.001$).

**DISCUSSION**

This study suggests that SNPs involved in cell cycle and DNA repair pathways previously linked with brain tumors in adults are also associated with pediatric brain tumorigenesis. The results indicate that the A alleles of *EGFR* rs730437 and rs11506105 may decrease susceptibility to PBTs, whereas the A allele of *ERCC1* rs3212986 may increase the risk of these tumors. Moreover, the C alleles of *CHAF1A* rs243341 and rs2992 as well as the T allele of *XRCC1* rs25487 were associated with a decreased risk of astrocytoma subtype. In addition, an increased risk of non-astrocytoma tumor subtype associated with the C alleles of *EGFR* rs9642393, *EME1* rs12450550, and *ATM* rs170548, and the T allele of *GLTSCR1* rs1035938 as well as a decreased risk of this subtype associated with the A allele of *XRCC4* rs7721416 and the C allele of *XRCC4* rs2662242 were detected.

Genetic and epigenetic aberrations involved in cell cycle and DNA repair pathways have been proposed to play a role in PBT pathogenesis and progression [21–25]. Overexpression of the epidermal growth factor receptor (EGFR), which plays an important role in cell growth and development through the cell cycle pathway, is shown to be common and correlated with tumor grade in pediatric brain tumors [26, 27]. The function of genetic variants of *EGFR* is still unclear; however, intronic variations of *EGFR* found in this study may affect EGFR expression and play a role in pediatric brain tumorigenesis.

DNA repair is an important mechanism to maintain genomic stability and its functional failure may lead to carcinogenesis. Somatic variations of DNA repair genes have been identified in PBTs [28–30]; however, no study has investigated the role of DNA repair gene variations in PBT etiology. In this study, we examined the association between PBT risk and 37 SNPs in 17 DNA repair genes, shown to be associated with risk of adult brain tumors. Of these, 9 SNPs in 7 DNA repair genes (*ERCC1*, *CHAF1A*, *XRCC1*, *EME1*, *ATM*, *GLTSCR1*, and *XRCC4*) were also associated with susceptibility to PBTs. A few studies have previously indicated that DNA repair gene alterations may increase the predisposition to cancer formation in children [31, 32]. Moreover, these variations might predict the tumor drug sensitivity and the treatment outcome [33].

The *ERCC1* gene encodes excision repair cross-complementing group 1 which is involved in DNA nucleotide excision repair (NER) pathway [34]. The result of association between the A allele of *ERCC1* rs3212986 and increased risk of PBTs is in line with our finding in a meta-analysis of this polymorphism in adult brain tumors [3]. *XRCC1* which encodes an enzyme named X-ray
cross-complementing group 1, plays an important role in base excision repair pathway (BER) [35]. Chromatin assembly factor 1, subunit A (CHAF1A) is involved in DNA mismatch repair (MMR) during the correction of DNA replication errors [36].

$X_RRC4$ encodes X-ray repair complementing defective repair in Chinese hamster cells 4 that functions in the repair of DNA double-strand breaks (DSBs) produced by ionizing radiation and restriction enzymes [37].

$EME1$ encodes essential meiotic structure-specific endonuclease 1 which forms an endonuclease complex with methyl methanesulfonate-sensitive UV-sensitive 81 protein (MUS81) and plays an important role in DNA repair and maintaining of genome integrity [38]. $ATM$ encodes a protein kinase which is a member of phosphatidylinositol 3-kinase-like kinase (PIKK) family. ATM is a key regulator of cell cycle checkpoint signaling pathways which responds to DNA strand breaks by inducing cell-cycle arrest and hence facilitates DNA repair [39]. $GLTSCR1$ stands for glioma tumor suppressor candidate region 1 and $GLTSCR1$ rs1035938 polymorphism alters a CpG site within the 5’ CpG island of the gene. This germ-line alteration might affect the transcription of $GLTSCR1$ and other candi-date genes in the region [40].

The present study was conducted based on the largest series of pediatric brain tumor cases to date with the purpose to investigate the association between genetic polymorphisms along with four main pathways hypothesized to be involved in brain tumorigenesis and PBT risk. Although investigating the effect of environmental risk factors was not the aim of our study, the fact that we did not take into consideration the possible gene-environment interactions is a limitation of this work. Moreover, due to shortage of large enough pediatric patient materials with access to DNA samples, we were not able to replicate our findings. Therefore, additional studies are necessary to validate these results, explore the mechanisms through which these genetic polymorphisms influence cancer susceptibility, and to investigate gene-environment interactions underling risk of PBTs.

In conclusion, the present study indicates that while the minor alleles of polymorphisms in $ERCC1$ may increase the risk of PBTs, the minor alleles of SNPs in $EGFR$ are associated with decreased susceptibility to these tumors. Furthermore, polymorphisms in $CHAF1A$ and $XRCC1$ are associated with the risk of astrocytoma, whereas SNPs in $EME1$, $ATM$, $GLTSCR1$, and $XRCC4$ are associated with susceptibility to non-astrocytoma subtypes. Therefore, genetic polymorphisms in cell cycle and DNA repair pathways associated with susceptibility

| SNP      | Chr. | Gene | Pathway     | Location (bp) | Minor allele | MAF$^a$ in cases | MAF$^a$ in controls | Model       | OR$^b$   | 95% CI       | P       | CHISQ | Pinter$^c$ |
|----------|------|------|-------------|---------------|--------------|-----------------|-------------------|-------------|---------|--------------|--------|-------|-----------|
| rs730437 | 7    | $EGFR$ | Cell cycle | 55215018      | A            | 0.44            | 0.51              | Dominant     | 0.59    | 0.42–0.83    | 0.002  | 0.435 |
|          |      |       |             |               |              |                 |                   | Recessive    | 0.79    | 0.56–1.15    | 0.23   |       |           |
|          |      |       |             |               |              |                 |                   | Additive     | 0.75    | 0.60–0.93    | 0.009  |       |           |
|          |      |       |             |               |              |                 |                   | Allelic      | 0.016   |              | 5.77   |       |           |
| rs11506105 | 7   | $EGFR$ | Cell cycle | 55220177      | A            | 0.39            | 0.45              | Dominant     | 0.71    | 0.51–0.98    | 0.036  | 0.285 |
|          |      |       |             |               |              |                 |                   | Recessive    | 0.87    | 0.59–1.28    | 0.477  |       |           |
|          |      |       |             |               |              |                 |                   | Additive     | 0.82    | 0.66–1.02    | 0.079  |       |           |
|          |      |       |             |               |              |                 |                   | Allelic      | 0.099   |              | 2.73   |       |           |
| rs3212986 | 19   | $ERCC1$ | DNA Repair | 45912736      | A            | 0.27            | 0.21              | Dominant     | 1.53    | 1.11–2.09    | 0.009  | 0.311 |
|          |      |       |             |               |              |                 |                   | Recessive    | 1.14    | 0.59–2.19    | 0.699  |       |           |
|          |      |       |             |               |              |                 |                   | Additive     | 1.34    | 1.04–1.73    | 0.023  |       |           |
|          |      |       |             |               |              |                 |                   | Allelic      | 0.029   |              | 4.78   |       |           |

$a$: MAF=Minor Allele Frequency  
$b$: OR adjusted for age, sex, and country  
$c$: $P$ value for interactions between SNPs and demographic variables including age, sex and country.
to adult brain tumors seem also to be associated with PBT risk suggesting that pediatric and adult brain tumors might share similar etiological pathways.

**MATERIALS AND METHODS**

**Study subjects and procedures**

This population-based case-control study is based on the Cefalo study, a large international study of brain tumors in children and adolescents conducted in Sweden, Denmark, Norway, and Switzerland. Details of the study methods have been described previously [41]. Briefly, all children aged 7–19 years during the period between 1 April 2004 and 31 August 2008, diagnosed with a primary intracranial brain tumor defined according to the International Classification of Childhood Cancer, third edition (ICCC-3), group III [42], restricted to ICD-O-3 location C71, were considered as case subjects. Cases were subclassified according to the fourth edition of the World Health Organization (WHO) classification of tumors of the central nervous system [43]. Participants with neurofibromatosis or tuberous sclerosis were excluded from analyses. Medulloblastoma cases were excluded here since they will be included in a separate study. Controls were randomly selected from the population registers and matched to the cases by age, sex, and geographic region. A total of 352 (82%) cases and 646 (71%) controls participated in the interviews. The study was approved by the National data protection boards and ethical committees in all participating countries, and written informed consent was obtained from all participants and/or their parents.

Saliva collection and DNA extraction were performed using the Oragene self-collection kit (DNA Genotek, Ottawa,

| SNP      | Chr. | Gene | Pathway   | Location (bp) | Minor allele | MAF\(^a\) in cases | MAF\(^a\) in controls | Model    | OR\(^b\) 95% CI   | P     | CHISQ |
|----------|------|------|-----------|---------------|--------------|---------------------|-----------------------|----------|-------------------|-------|-------|
| rs730437 | 7    | EGFR | Cell cycle| 55215018      | A            | 0.44                | 0.51                  | Dominant | 0.60 0.39–0.91 | 0.018 |        |
|          |      |      |           |               |              |                     |                       | Recessive | 0.84 0.54–1.33 | 0.462 |        |
|          |      |      |           |               |              |                     |                       | Additive  | 0.77 0.59–1.01 | 0.058 |        |
|          |      |      |           |               |              |                     |                       | Allelic   | 0.064          | 3.42  |        |
| rs3212986| 19   | ERCCI | DNA Repair| 45912736      | A            | 0.29                | 0.21                  | Dominant | 1.87 1.26–2.76 | 0.002 |        |
|          |      |      |           |               |              |                     |                       | Recessive | 0.95 0.40–2.25 | 0.914 |        |
|          |      |      |           |               |              |                     |                       | Additive  | 1.49 1.09–2.04 | 0.011 |        |
|          |      |      |           |               |              |                     |                       | Allelic   | 0.012          | 6.36  |        |
| rs243341 | 19   | CHAF1A| DNA Repair| 4405106       | C            | 0.23                | 0.29                  | Dominant | 0.66 0.45–0.98 | 0.040 |        |
|          |      |      |           |               |              |                     |                       | Recessive | 0.62 0.28–1.35 | 0.228 |        |
|          |      |      |           |               |              |                     |                       | Additive  | 0.71 0.52–0.98 | 0.036 |        |
|          |      |      |           |               |              |                     |                       | Allelic   | 0.033          | 4.57  |        |
| rs2992   | 19   | CHAF1A| DNA Repair| 4443046       | C            | 0.23                | 0.29                  | Dominant | 0.67 0.45–0.99 | 0.049 |        |
|          |      |      |           |               |              |                     |                       | Recessive | 0.64 0.29–1.39 | 0.262 |        |
|          |      |      |           |               |              |                     |                       | Additive  | 0.72 0.53–0.99 | 0.046 |        |
|          |      |      |           |               |              |                     |                       | Allelic   | 0.042          | 4.13  |        |
| rs25487  | 19   | XRCC1 | DNA Repair| 44055726      | T            | 0.30                | 0.36                  | Dominant | 0.66 0.44–0.97 | 0.033 |        |
|          |      |      |           |               |              |                     |                       | Recessive | 0.87 0.48–1.59 | 0.652 |        |
|          |      |      |           |               |              |                     |                       | Additive  | 0.77 0.57–1.03 | 0.076 |        |
|          |      |      |           |               |              |                     |                       | Allelic   | 0.077          | 3.13  |        |

\(^a\): MAF=Minor Allele Frequency  \(^b\): OR adjusted for age, sex, and country.
ON, Canada) and the DNA yield was quantitated using PicoGreen (Invitrogen, Carlsbad, CA, USA), according to the standard protocols. This study, in total, included saliva DNA of 245 cases and 489 controls. Distribution of diagnostic subtypes among cases were 134 astrocytoma, 19 ependymoma, 7 intracranial embryonal tumors, 20 other gliomas, 49 other specified intracranial neoplasms, and 16 with unspecified intracranial neoplasms (Table 1).

### Table 4: Summary results for SNPs associated with non-astrocytoma subtype

| SNP     | Chr. | Gene  | Pathway       | Location (bp) | Minor allele | MAF$^c$ in cases | MAF$^c$ in controls | Model  | OR$^b$    | 95% CI      | P     | CHISQ |
|---------|------|-------|---------------|---------------|--------------|------------------|---------------------|--------|-----------|-------------|-------|-------|
| rs730437 | 7    | EGFR  | Cell cycle    | 55215018      | A            | 0.44             | 0.51                | Dominant | 0.56       | 0.36–0.89   | 0.014 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 0.75       | 0.46–1.25   | 0.271 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 0.72       | 0.53–0.97   | 0.030 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.060      |             | 3.54  |       |
| rs9642393 | 7    | EGFR  | Cell cycle    | 55245647      | C            | 0.3              | 0.26                | Dominant | 1.07       | 0.70–1.63   | 0.754 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 2.21       | 1.13–4.35   | 0.021 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 1.23       | 0.89–1.69   | 0.214 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.227      |             | 1.46  |       |
| rs12450550 | 17   | EME1  | DNA Repair    | 48456193      | C            | 0.38             | 0.28                | Dominant | 1.28       | 0.84–1.95   | 0.257 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 2.48       | 1.42–4.33   | 0.001 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 1.42       | 1.06–1.91   | 0.019 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.004      |             | 8.39  |       |
| rs170548 | 11   | ATM   | DNA Repair    | 108234836     | C            | 0.36             | 0.31                | Dominant | 1.57       | 1.02–2.42   | 0.041 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 0.98       | 0.49–1.92   | 0.947 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 1.27       | 0.93–1.72   | 0.135 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.096      |             | 2.78  |       |
| rs1035938 | 19   | GLTSCR1| DNA Repair    | 48183771      | T            | 0.29             | 0.24                | Dominant | 1.15       | 0.75–1.76   | 0.513 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 2.14       | 1.09–4.19   | 0.027 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 1.27       | 0.92–1.74   | 0.145 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.085      |             | 2.96  |       |
| rs7721416 | 5    | XRCC4  | DNA Repair    | 82434993      | A            | 0.41             | 0.47                | Dominant | 0.85       | 0.54–1.35   | 0.494 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 0.51       | 0.27–0.94   | 0.032 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 0.76       | 0.56–1.04   | 0.089 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.083      |             | 2.99  |       |
| rs2662242 | 5    | XRCC4  | DNA Repair    | 82484885      | C            | 0.42             | 0.48                | Dominant | 0.92       | 0.57–1.47   | 0.720 |       |
|         |      |       |               |               |              |                  |                     | Recessive | 0.49       | 0.26–0.91   | 0.024 |       |
|         |      |       |               |               |              |                  |                     | Additive  | 0.78       | 0.57–1.06   | 0.114 |       |
|         |      |       |               |               |              |                  |                     | Allelic   | 0.104      |             | 2.65  |       |

a: MAF=Minor Allele Frequency          b: OR adjusted for age, sex, and country.
Selection of candidate SNPs and genotyping

The PubMed database was searched (up to December 2012) using combinations of the following terms: ‘brain tumor’, ‘single nucleotide polymorphism’, ‘association’, ‘gene’, ‘risk’, ‘case control’, ‘susceptibility’, and ‘polymorphism’ to identify all the published peer-reviewed candidate gene-association studies of brain tumors. All the statistically significant SNPs reported by childhood brain tumor genetic association studies [11–13] as well as the SNPs reported by at least two candidate gene-association studies as being statistically associated with the risk of adult brain tumors [3–9, 44–49] were selected for genotyping.

Of 68 selected SNPs, 63 SNPs were satisfactorily genotyped (success rate ≥ 80%). Genotyping was performed at the Mutation Analysis core Facility (MAF), Clinical Research Centre, Huddinge University Hospital, Stockholm, Sweden, with staff blinded to sample status, using the Sequenom iPLEX Gold platform with matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry. The average success rate was 97% and the concordance rate for duplicate genotyping was 100%.

Statistical analyses

The χ² goodness-of-fit test was used to examine the consistency of allele frequencies with Hardy-Weinberg equilibrium (HWE) among the controls and \( p < 0.001 \) was considered statistically significant. Odds ratios (ORs) and corresponding 95% confidence intervals (CIs) were calculated using unconditional logistic regression modelling to evaluate the association between each SNP and the risk of PBT based on the Cochran–Armitage trend test of additivity (trend) as well as dominant (DOM) and recessive (REC) models, with adjustment for age, sex, and country. We applied the χ² test to compare the allelic frequencies of the genotyped SNPs between cases and controls as well as the Wald test to evaluate the significance of interactions between SNPs and demographic variables including age, sex and country. SNP-SNP interactions were investigated for pairwise combinations of all the SNPs involved in the same pathway and \( p < 0.001 \) was set as the significance level of interaction analyses. Stratiﬁed analyses were performed by astrocytoma alone and the other tumor types combined (including ependymoma, intracranial embryonal tumors (except medulloblastoma), other gliomas, other specified intracranial neoplasms, and unspecified intracranial neoplasm); in order to increase the statistical power. To measure the linkage disequilibrium (LD) between the genotyped SNPs, \( D' \) was calculated. Haploblocks were deﬁned based on the default LD block parameters in Haplovew v4.2. Haplotype analyses were performed for the haplotype blocks harboring the SNPs that were found to be associated with PBTs. Haplotypes with a frequency > 1% were considered in the blocks with different haplotype distribution between cases and controls (\( p < 0.1 \)). Although selection of SNPs for the analyses was based on a priori knowledge from candidate gene-association studies on adults, the possibility of false-positive findings was considered by providing the reference \( p \) value for an experiment-wide signiﬁcance with Bonferroni correction (0.0002 for an experiment-wide signiﬁcance level of 0.05, and 0.0004 for a signiﬁcance level of 0.10). The analyses were carried out using PLINK v1.07 [50] and SAS statistical software version 9.3 (SAS Institute, Inc., Cary, NC, USA).

ACKNOWLEDGMENTS

We gratefully acknowledge collaboration with and support from clinicians and other hospital staff in all countries, as well as assistance from national and local cancer registers with identiﬁcation of patients. We also acknowledge the skillful work of the research nurses, interviewers, and research assistants in all countries. Finally, we acknowledge all those with whom we have collaborated previously within the CEFALO study. Please see Aydin et al. for an exhaustive list [41].

URLs

PLINK: http://pngu.mgh.harvard.edu/~purcell/plink/
SAS: http://www.sas.com/
CONFLICTS OF INTEREST

The authors have declared no conflicts of interest.

GRANT SUPPORT

The Swedish part of the CEFALO study was supported by grants from the Swedish Council for Working Life and Social Research [2004–0504, 2007–0224]; the Swedish Research Council [K2008-70X-15366-04-3]; the Swedish Cancer Society [09 0666]; the Swedish Childhood Cancer Society [PROJ06/050, PROJ09/086]; and the Swedish Radiation Protection Authority [SSI P 1572]. The Danish CEFALO study was supported by the Danish Strategic Research Council [2103-05-0006, 2064-04-0010]. The Swiss part of the CEFALO study was supported by the Swiss Federal Office of Public Health [05.001626]; the Swiss Research Foundation on Mobile Communication [A2006.18]; and the Swiss National Science Foundation [PDFMP3_122873]. The Norwegian CEFALO study was supported by the Research Council of Norway [175163/V40].

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