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Genetic variants of *AICDA*/*CASP14* associated with childhood brain tumor

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ABSTRACT. We conducted a hospital-based case-control study in Korea to investigate whether apoptosis- and cell cycle control-related genes are associated with childhood brain tumor. Incident brain tumor cases (N = 70) and non-cancer controls (N = 140), frequency-matched by age and gender, were selected from 3 teaching hospitals in Seoul between 2003 and 2006. Tag single nucleotide polymorphisms (SNPs) (N = 297) in 30 genes related to apoptosis and cell cycle control were selected using a pairwise linkage-disequilibrium-based algorithm. Five tag SNPs in 2 genes (*AICDA* and *CASP14*) remained significant after adjusted multiple tests. The most significant association with childhood brain tumor risk was for IVS1-401G>C in the *AICDA* gene [odds ratio (OR) = 2.8; 95% confidence interval (95%CI) = 1.25-6.46]; the polymorphism *9276A>C of *CASP14* was associated with decreased brain tumor risk (OR = 0.4; 95%CI = 0.19-0.95). We concluded that genetic polymorphisms in *AICDA* and *CASP14* are associated with risk for brain tumor in Korean children.

Key words: Childhood brain tumor; Single nucleotide polymorphism; Apoptosis; Cell cycle control

INTRODUCTION

Childhood brain tumors are the second most common cause of cancer death after leukemia (Korean Statistical Information Service, 2009) (MacGrogan et al., 2004) and the most common solid tumors in children. The incidence of brain tumor among children continues to increase (Reddy, 2001; Kohler et al., 2011; Ostrom and Barnholtz-Sloan, 2011).

Brain tumor is a multi-factorial disorder. Although ionizing radiation is an alleged risk factor, the etiology of childhood brain tumor is mostly unknown (Harder et al., 2008; Shim et al., 2009). The development of brain tumor might be related to the accumulation of genetic variation in important cellular pathways such as apoptosis and cell cycle control (Holland, 2001; Wrensch et al., 2002). However, despite the potential importance of cell cycle control and apoptosis in tumor etiology (Gangwar and Mittal, 2010; Kyritsis et al., 2010), limited studies about the association between genetic polymorphisms in these pathways and brain tumor risk have been conducted (Holland, 2001; Rajaraman et al.,

2007; Gu et al., 2009). The associations between gene alterations related to cell cycle control and apoptosis such as *PTEN*, *CDKN2A*, *MDM2*, *TP53*, and *RB* and brain tumor risk have been found, but the results were restricted to adults (Holland, 2001; Rajaraman et al., 2007). Moreover, these studies genotyped only a limited number of candidate single nucleotide polymorphisms (SNPs).

To comprehensively investigate whether genetic variations in genes related to apoptosis and cell cycle control pathways play a role in brain tumor development, we conducted a hospital-based case-control study in Korean children using 297 tag SNPs representing genetic variation across 30 relevant genes.

MATERIAL AND METHODS

Subjects

Eligible cases (N = 107) were histologically confirmed incident childhood brain tumor diagnosed at 3 teaching hospitals located in Seoul, Korea, between 2003 and 2006. Eligible non-cancer controls (N = 254) were patients without a medical history of childhood tumor recruited from the department of pediatrics, pediatric surgery, pediatric orthopedic surgery, pediatric urology, and pediatric otorhinolaryngology from the same hospitals. For chip analysis, we restricted the subjects to those who had at least 750 ng DNA and a 260/280 ratio of 1.5-2.0. In total, 70 childhood leukemia cases and 140 controls frequency-matched to cases with respect to age (≤4, 5-9, ≥10 years) and gender (male, female) were selected for the genotyping. Among the 70 patients, 34 were medulloblastoma (48.6%), 22 were germinoma (31.4%), 10 were glioma (14.3%), 3 were teratoma (4.3%), and 1 was pineal tumor (1.4%). Informed consent was obtained from all study subjects. The study was approved by the Institutional Review Board for Human Research of Seoul National University Hospital (IRB No. H-0407-128-001).

Information on the characteristics of the child such as birth weight and breast feeding, and parental characteristics such as education status and family history related to brain tumor were collected by trained interviewers using a structured questionnaire (Lee et al., 2009).

SNP selection and genotyping

Genotyping was performed using genomic DNA, which was extracted from peripheral blood with the Gentra Puregene Blood Kit (Gentra, USA). Candidate gene regions were selected based on SNP databases [Cancer Genome Anatomy Project (CGAP) and SNP500 database] (Packer et al., 2004) and the following criteria: minor allele frequency (MAF) >5% in control, and lack of complete or nearly complete linkage disequilibrium (LD) ($r^2 > 0.8$).

A total of 297 tag SNPs among 30 candidate genes related to apoptosis and cell cycle control were selected and genotyped using a GoldenGate™ oligonucleotide pool assay (Illumina®, San Diego, CA, USA). SNPs deemed unusable due to failure of genotyping or monomorphism (72 SNPs), Hardy-Weinberg equilibrium (HWE) $P < 0.001$ (1 SNP), and MAF < 0.05 (10 SNPs) in each case and control were excluded from the analysis. In total, 214 SNPs in

25 gene regions were used in the analysis. Genotype completion rates and concordance rates for all SNPs exceeded 93%.

Statistical analysis

The Fisher exact test was conducted to evaluate genotype distribution for deviation from HWE in the control group.

Based on the global P value computed using the likelihood ratio test with 1 degree of freedom, the association between individual SNP and brain tumor risk was estimated. The statistical significance of the association between each gene region and brain tumor risk was determined using a gene-level min*P* test with P trend value through a permutation based resampling procedure (10,000 times permutation), considering all SNPs in each gene (Chen et al., 2006).

Using unconditional logistic regression analysis, the dominant model-based association between childhood brain tumor risk and statistically associated SNPs by min*P* test was estimated as odds ratios (ORs) and 95% confidence intervals (95%CIs) adjusted for age and gender. The homozygote of the most common allele in the subjects was used as the reference group.

To consider LD between SNPs in selected genes, an LD block was tested using Haploview version 4.2 (www.broad.mit.edu/mpg/haploview).

The significance level was set to min$P < 0.05$ and $P < 0.05$, and all above statistical procedures were conducted using SAS® version 9.2 (Cary, NC, USA).

RESULTS

The distributions of selected characteristics of the brain tumor patients (N = 70) and controls (N = 140) are shown in Table S1. There were no differences in selected characteristics including gender, age, birth weight, breast feeding, parental educational status, and family history of brain tumor between cases and controls.

Among 214 SNPs in 25 gene regions, 14 tag SNPs in 7 genes (*AICDA*, *BCL2L11*, *CASP3*, *CASP7*, *CASP14*, *LMO2*, and *MYC*) were significantly associated with childhood brain tumor ($P_{trend} < 0.05$) (Table 1).

At the gene level, only 2 of 30 gene regions were significantly associated with brain tumor when using a 10,000 times permutation min*P* test for each gene region: activation-induced cytidine deaminase (*AICDA*) gene (min$P = 0.004$) and caspase 14 (*CASP14*) gene (min$P = 0.032$) (Table 1). Four SNPs [IVS1-401G>C (rs12306110), IVS2-462T>C (rs3794318), IVS2+16G>A (rs2518144), and Ex5+143A>C (rs11046349)] of 7 SNPs in the *AICDA* gene and 1 SNP [*9276A>C (rs8110862)] of 8 SNPs in the *CASP14* gene remained significantly after adjusting of multiple tests (Table 2). The most significant association with increased childhood brain tumor risk was found for the IVS1-401G>C in *AICDA* (OR = 2.8; 95%CI = 1.25-6.46). Among significant tag SNPs of *AICDA*, the Ex5+143A>C showed decreased (OR = 0.2; 95%CI = 0.07-0.74), and the IVS2-462T>C and the IVS2+16G>A showed increased brain tumor risk (OR = 2.6; 95%CI = 1.14-5.76 and OR = 2.5; 95%CI = 1.04-6.11, respectively). The genetic variation in *9276A>C of *CASP14* showed decreased risk of brain tumor (OR = 0.4; 95%CI = 0.19-0.95).

Table 1. Polymorphisms in apoptosis- and cell cycle control-related genes and brain tumor risk among Korean children.

Genes	No. of SNPs	Lowest P_{trend} [a]	min$P$ [b]
AICDA	7	0.001	0.004
BAX	4	0.215	0.624
BCL10	7	0.284	0.760
BCL2	51	0.069	0.914
BCL2A1	6	0.058	0.245
BCL2L10	4	0.384	0.756
BCL2L11	10	0.014	0.586
BCL6	8	0.202	0.799
BCL7A	4	0.390	0.790
BCL7C	2	0.414	0.535
CASP10/CASP8	10	0.117	0.649
CASP14	8	0.006	0.032
CASP3	7	0.022	0.088
CASP4/CASP5/CASP1	10	0.080	0.470
CASP6	6	0.121	0.376
CASP7	12	0.017	0.126
CASP8AP2	7	0.673	0.963
CASP9	6	0.083	0.288
CCND1	4	0.072	0.228
LIG3	2	0.329	0.560
LMO2	18	0.015	0.196
MYC	9	0.013	0.088
RIPK1	7	0.461	0.962
RIPK2	3	0.078	0.179
TP53I3	2	0.272	0.439

[a]Likelihood ratio test comparing models with and without terms for each SNP (genotypes coded as 0, 1, and 2) in total SNP. [b]Permutation-based method, case-control status, age, and birth weight were permutated 10,000 times.

Table 2. Childhood brain tumor risk for selected SNPs in *AICDA* and *CASP14* genes.

SNPs	MAF	OR (95%CI)[a]
AICDA		
-997C>G (rs714629)	0.14	0.5 (0.21-1.33)
IVS1-2066T>C (rs2580873)	0.26	1.9 (0.87-4.27)
IVS1-401G>C (rs12306110)	0.19	2.8 (1.25-6.46)
IVS2-462T>C (rs3794318)	0.19	2.6 (1.14-5.76)
IVS2+16G>A (rs2518144)	0.16	2.5 (1.04-6.11)
Ex4+38C>T (rs2028373)	0.48	0.9 (0.42-2.37)
Ex5+143A>C (rs11046349)	0.13	0.2 (0.07-0.74)
CASP14		
-11344T>C (rs714920)	0.28	1.6 (0.75-3.53)
-5786C>T (rs4808901)	0.38	1.5 (0.67-3.35)
*894C>T (rs3181309)	0.38	0.8 (0.33-1.77)
*2935A>T (rs10425745)	0.15	0.9 (0.37-2.34)
*8052C>T (rs16980286)	0.10	0.8 (0.29-2.38)
*9276A>C (rs8110862)	0.41	0.4 (0.19-0.95)
*13181G>T (rs5021087)	0.46	0.5 (0.20-1.08)
IVS4+77T>C (rs3181163)	0.13	0.9 (0.36-2.43)

MAF = minor allele frequency; OR = odds ratio; 95%CI = 95% confidence interval; [a]adjusted for age and gender; rare homozygotes and heterozygotes *vs* common homozygotes.

DISCUSSION

In this study to investigate whether genetic variations in cell cycle control and the apoptosis pathway (297 SNPs in 30 genes) are associated with brain tumor risk, we found

strong evidence that genetic variants in the *AICDA* and *CASP14* gene regions are associated with brain tumor risk in children.

AICDA is a member of apolipoprotein B-editing catalytic polypeptide (APOBEC) family found to be associated with the mechanism of mutagenesis (Marusawa, 2008), and the apoptosis level is affected by AICDA activity through catalytic enzymes such as caspase-9 in B cells (Zaheen et al., 2009; Hsu et al., 2011). AICDA regulates all antigen-induced genetic recombination through DNA deamination ability in the immunoglobulin genes (Honjo et al., 2005; Ramiro et al., 2007; Maul and Gearhart, 2010), and thus, antibody diversity is controlled through AICDA (Maul and Gearhart, 2010). AICDA converts cytosine to uracil through the deamination mechanism of DNA, resulting in nucleotide alterations in target sequences (Perez-Duran et al., 2007; Marusawa, 2008). This means that AICDA can induce somatic hypermutations not only in the immunoglobulin-related but also in the non-immunoglobulin-related genes, and these findings suggest that abnormal AICDA expression could increase tumorigenesis through the accumulation of hypermutations in tumor- and inflammation-related genes (Marusawa, 2008).

CASP14 is a member of the cysteine-aspartic acid protease (caspase) family, which plays a central role in the intrinsic as well as in the extrinsic apoptosis pathway (Koenig et al., 2005). The genetic approach of *CASP14* has been limited, but there are some studies that have found associations between aberrant expression of CASP14 and various tumors such as ovarian, gastric, colon, lung, breast, and cervical. They reported that although the exact cellular function of CASP14 was unknown, low expression of CASP14 was significantly associated with advanced cancer compared to normal status (Koenig et al., 2005; Krajewska et al., 2005).

Some studies examining the association between genetic alterations in apoptosis and cell cycle control and brain tumor risk focused on the adult population (Rajaraman et al., 2007; Wang et al., 2010). Rajaraman et al. (2007) showed that 12 SNPs from 10 genes were involved in apoptosis and cell cycle control, and reported that genetic alterations in *CASP8*, *CCND1*, *CCNH*, and *MDM2* genes were statistically associated with brain tumor risk in the adult population. Although the *CASP8* gene was also examined in the present study, statistically associated SNPs were different, and they were also not in LD. The rs603965 SNP of *CCND1*, also examined in our study, did not show statistical association with risk of brain tumor, in agreement with Rajaraman et al. (2007).

Our study has several limitations, including the small sample size for evaluating the genetic effect, and we did not conduct subtype analysis of brain tumors due to the sample size. Because we used a hospital-based case-control study design, there may have been uncontrolled biases. However, there are strengths as well. To overcome the limitation due to the small sample size and the large number of genes investigated, we performed random permutation (10,000 times) analysis to reduce the probability that these findings were due to chance. Also, the subjects were recruited from a genetically and ethnically homogenous Korean population.

CONCLUSIONS

In conclusion, our results suggest that genetic polymorphisms in *AICDA* and *CASP14* are associated with brain tumor risk in Korean children. Additional studies are needed to replicate these findings with a larger sample size and, more generally, to explore the manner in which these genes may influence the pathogenesis of this poorly understood malignancy.

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Supplementary material

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