Contributions of Aryl Hydrocarbon Receptor Genetic Variants to the Risk of Glioma and PAH-DNA Adducts

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The aryl hydrocarbon receptor (AHR) gene is involved in the response to polycyclic aromatic hydrocarbon (PAH) exposure. To investigate the hypothesis that the genetic variants in the AHR gene might be a causal genetic susceptibility to PAH-DNA adduct formation and glioma risk, we conducted a case-control study of 384 glioma cases and 384 cancer-free controls to explore the association between six common single-nucleotide polymorphisms of the AHR gene and glioma risk. Using PAH-DNA adducts as biomarkers, we then evaluated the association between PAH-DNA adduct levels and glioma risk based on a tissue microarray including 11 controls and 77 glioma patients. We further explored the contributions of the glioma risk–associated AHR polymorphisms to the levels of PAH-DNA adducts in glioma tissues based on 77 glioma patients. We found that PAH-DNA adduct staining existed in normal brain tissues and grades I–IV gliomas, and the staining intensity was significantly associated with the glioma grade. Two AHR polymorphisms (rs2066853 and rs2158041) demonstrated significant association with glioma risk. Intriguingly, we also found statistically significant associations between these two variants and PAH-DNA adduct levels in glioma tissue. These data suggest the contributions of AHR rs2066853 and rs2158041 to glioma risk and the PAH-DNA adduct levels, which shed new light on gene-environment interactions in the etiology of glioma. Further studies with a larger sample size and ethnically diverse populations are required to elucidate the potential biological mechanism for, as well as the impact of, the susceptibility to glioma due to genetic variants of AHR.

Key Words: aryl hydrocarbon receptor (AHR); polymorphism; glioma; PAH-DNA adducts.

Approximately 22,000 new cases of primary malignant brain tumors and 13,140 deaths from these cancers were projected to occur in the United States during 2010. Glioma, the most common and deadly brain tumor in adults, derives from glia cells in the brain (van Meir *et al.*, 2010) and has a poor prognosis, with an overall median survival of only 10–15 months (Linnebank *et al.*, 2008; Sanson *et al.*, 2009; Weller *et al.*, 2009). Gliomas are thought to develop from interactions between genetic and environmental risk factors. To our knowledge, the only well-documented environmental risk factor for gliomas is ionizing radiation, and several genetic variants linked to an increase in the risk of glioma have been explored by recent scientific research (Kyritsis *et al.*, 2010; Shete *et al.*, 2009). However, in spite of the years of intensive research, the etiology of glioma has not been fully elucidated (Ohgaki and Kleihues, 2009).

Polycyclic aromatic hydrocarbons (PAHs) are a family of compounds released during incomplete combustion or pyrolysis of organic matter. The extensive sources of human exposure include tobacco smoke, air pollution, and occupational settings. Previous studies have indicated that PAH exposure increases the risks of developing lung cancer, bladder cancer, prostate cancer, and breast cancer (Armstrong and Gibbs, 2009; Rybicki *et al.*, 2004; Santella *et al.*, 2000; Zeegers *et al.*, 2001). In addition, recent experiments demonstrate that paternal or maternal exposure to PAHs increases the risk of childhood cancer in offspring, including brain tumors (Cordier *et al.*, 2004; Perera *et al.*, 2005). In spite of this strong evidence of carcinogenicity, few studies have reported an association between exposure to PAHs and glioma.

The aryl hydrocarbon receptor (AHR), a ligand-activated transcription factor and the regulator for cytochrome P450 enzymes (Nebert *et al.*, 2004), mediates the toxic effects of a variety of environmental chemicals including PAHs (Xue and Warshawsky, 2005) and plays an important role in carcinogenesis (Shimizu *et al.*, 2000). The activation of AHR by PAHs results in a wide range of cell cycle perturbations, a diminished

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capacity for DNA replication and an inhibition of cell proliferation (Dietrich and Kaina, 2010; Puga *et al.*, 2002). Recently, an accumulating body of evidence has indicated that genetic variants in AHR not only lead to substantial differences in sensitivity to biochemical and toxic effects in laboratory animals (Celius and Matthews, 2010; Nebert, 1989) but are also involved in an increased cancer risk in humans, including breast cancer, lung cancer, and non-Hodgkin's lymphoma (Long *et al.*, 2006; Ng *et al.*, 2010; Zhang *et al.*, 2009). However, a direct demonstration of the contribution of AHR polymorphisms to the toxic effects of PAHs and brain tumor risk is still lacking.

Because the AHR gene is involved in the response to PAH exposure, we hypothesized that the genetic variants in the AHR gene may increase the risk of glioma upon exposure to PAHs. The purpose of this study is to investigate the possible contributions of AHR polymorphisms to glioma risk and PAH-DNA adduct formation in glioma tissues and to provide more information about gene-environment interactions in the etiology of glioma.

MATERIALS AND METHODS

Subjects and sample collection. A total of 447 patients with diagnosed glioma at the Department of Neurosurgery of Jiangsu Province Hospital (the First Affiliated Hospital of Nanjing Medical University) and Beijing Tiantan Hospital Neurosurgery Center from 2005 to 2010 were recruited, and 408 patients (87%) agreed. Control subjects were recruited from cancer-free traumatic brain injury patients and healthy people (from the health examination centers) in the same two hospitals during the same time period of case recruitment and were frequency matched to cases based on age and sex. Four hundred healthy controls and 11 traumatic brain injury patients were enrolled. Each participant read and signed the informed consent in accordance with the requirements of the institutional review board of each participating institution. Detailed information on diet, weight, height, smoking habits, and drinking status were collected by trained interviewers using a structured questionnaire. After the interview, an approximate 5 ml of venous blood sample was collected from each participant and stored at −70°C until used for DNA extraction. Finally, 384 glioma patients and 384 cancer-free controls, whose DNA samples were available and adequate, were included in our work (Table 1).

Among the subjects, the brain tissues of grade I–IV glioma patients ($n = 90$) and traumatic brain injury controls ($n = 11$) were obtained after preliminary screening during surgical resection of the lesion for constructing a tissue microarray (TMA). Histological diagnosis and grading of tumors were performed in compliance with the WHO criteria (World Health Organization, 2007).

The study was approved by the Ethics Review Board of the Nanjing Medical University. All of the studies involving human subjects were conducted in full compliance with the government policies and the Declaration of Helsinki.

TMA and immunohistochemistry. Two separate cores taken from paraffin-embedded specimens of 90 glioma cases and 11 normal brain tissues were used to construct TMAs (collaborating with Shanghai Biochip, Shanghai, China). Excluding some severely hemorrhaged, necrotic, and poorly stained areas in glioma tissues, the brain tissues of grade I–IV glioma patients ($n = 77$, $n_{Grade\ I} = 15$, $n_{Grade\ II} = 32$, $n_{Grade\ III} = 21$, $n_{Grade\ IV} = 9$) and traumatic brain injury controls (cancer-free normal brain tissue, $n = 11$) in a TMA were adequate for immunohistochemistry (IHC) analysis.

The immunohistochemical assay for PAH-DNA adducts was performed as described previously (Rundle *et al.*, 2000). PAH-DNA adducts of TMA sections were reacted with a benzo(a)pyrene diol epoxide (BPDE)-DNA (5D11) monoclonal antibody (sc-52625; Santa Cruz, 1:100 dilution) and a horseradish peroxidase–conjugated secondary antibody. Before performing the IHC experiment of the glioma TMA, we have validated the antibody specificity in

TABLE 1
Distribution of Selected Characteristics Between Glioma Cases and Control Subjects

Variables	Case ($n = 384$)	Control ($n = 384$)	p*
Age, y (mean ± SD)	62.4 ± 10.8	61.5 ± 12.1	0.277
Gender, no. (%)			
Male	222 (57.8)	217 (56.5)	0.715
Female	162 (42.2)	167 (43.5)	
Smoking status, no. (%)			
Never	228 (59.4)	218 (56.8)	0.738
Former	70 (18.2)	72 (18.7)	
Current	86 (22.4)	94 (24.5)	
Pack-years (mean ± SD)	32.7 ± 25.1	30.3 ± 27.7	0.209

*p values were derived from the χ^2 test for categorical variables (gender and smoking status) and t-test for continuous variables (age and pack-years).

preliminary experiments by incubating the antibody with DNA extracted from BPDE-treated cells. The antibody with or without the blocking DNA adducts is then used to stain glioma tissue (Supplementary fig. 1). By this way, we confirmed that the 5D11 monoclonal antibody is specific and could be used in the TMA IHC tests. TMA sections were deparaffinized and washed with PBS, treated with 100 µg/ml RNase at 37°C for 1 h, washed with PBS and treated with proteinase K (10 µg/ml at room temperature) for 7 min and then washed. Samples were incubated with 4 N HCl for 10 min to denature the DNA followed by 50mM Tris-base for 5 min at room temperature. After blocking with 5% normal goat serum in Tris buffer (45 min at 37°C), samples were then incubated with mouse monoclonal antibody raised against BPDE-I-G (dilution 1:50; overnight at 4°C). After washing with PBS, samples were incubated with biotinylated anti-mouse antibody (dilution 1:200, at 37°C for 45 min). Endogenous peroxidase was blocked by treating the slides with 0.3% H_2O_2 in methanol for 15 min at room temperature. Staining was detected with a EnVision DAB kit (Dako), and sections were counterstained with hematoxylin (Sigma).

TMA digital images were managed and analyzed by the Aperio ScanScope and the Aperio TMA Lab software (Aperio Technologies). The Aperio analysis package includes algorithms for nuclear, membrane, micrometastasis, and staining intensity quantification, and the ability to run ImagePro Plus macros on entire digital slides. Staining intensity was independently scored by two observers. An average staining score was determined for tissue cores of each case. Histopathologic diagnosis was made independently of immunohistochemical analysis to eliminate bias in scoring staining intensity. Staining intensity from the nuclei of cancer cells was graded on a 0–3 scale, in which 0 indicated no staining, 1 indicated weak, 2 indicated moderate, and 3 indicated intense. The staining index was computed as follows: staining index = staining intensity × positive area (Han *et al.*, 2008).

AHR polymorphisms selection and genotyping. Six single-nucleotide polymorphisms (SNPs) of the AHR gene were selected according to the HapMap data (HapMap Data Rel 24/Phase II Nov08, on NCBI B36 assembly, dbSNP b126) and the pair-wise option of the Haploview 4.0 software. All six SNPs passed testing for the Hardy-Weinberg equilibrium (HWE) on the basis of pair-wise linkage disequilibrium (LD) with the r^2 threshold of 0.8 and minor allele frequency ≥ 0.05 to capture all of the common SNPs (Table 2). Among the six variants, rs2066853 G > A, located within an exon, is a nonsynonymous polymorphism, which leads to an amino acid change from an arginine to a lysine. The remaining five tag SNPs were located within introns. Supplementary figure 2 presents an LD map for the six SNPs within the AHR gene region in CHB HapMap samples in the current study. The colors of the diamonds represent the r^2 values. Blocks connecting pairs of SNPs are shaded according to the strength of the LD between the SNPs, from 0.0 (white) to 1.0 (red), as measured by the squared correlation statistic (r^2 within the diagonal boxes).

Genotyping of AHR polymorphisms was performed using the OpenArray Real-Time PCR System (Applied Biosystems, Foster City, CA) according to

the manufacturer's protocols, and laboratory personnel were blinded to case-control status (Gu *et al.*, 2011). Meanwhile, a random 5% of the cases and controls were genotyped repeatedly, and the final concordance rate was 100%. To confirm genotyping results, we then examined several selected PCR-amplified DNA samples (*n* = 2, for each genotype) with DNA sequencing, and these results were also consistent.

Statistical analyses. All statistical analyses were performed with STATA version 10.0 (Stata Corporation, College Station, TX). The Pearson chi-squared test was used to assess the differences between cases and controls with regard to categorical variables, such as gender and smoking status, and compared observed SNP genotype frequencies with those expected under conditions of HWE. Student's *t*-test was used to test for continuous variables, including age and pack-years.

Unconditional multivariate logistic regression analysis was used to examine associations between genetic polymorphisms and glioma risk by estimating odds ratios (ORs) and 95% confidence intervals (95% CI). A score test of linear trend was conducted for each SNP using a two-sided likelihood ratio test. To correct for multiple comparison testing, we applied the false discovery rate (FDR) method to the *p* values to reduce the potential for spurious findings. Because the PAH-DNA adduct staining index was not normally distributed, the Kruskal-Wallis one-way ANOVA by ranks was used to compare the PAH-DNA adduct staining index of the three genotypes of AHR. A two-tailed *p* value of less than 0.05 was considered statistically significant.

RESULTS

PAH-DNA Adduct Levels in Normal Brain and Glioma Tissues

By detecting the distribution and staining intensity of PAH-DNA adducts in the TMA analysis using IHC, we found that PAH-DNA adduct staining existed in normal brain and gliomas of grades I–IV, and the staining intensity was significantly associated with the glioma grade (as shown in Fig. 1A). In the TMA IHC analysis, PAH-DNA adduct staining was observed in 15 of 23 (65.2%) pilocytic astrocytomas (Grade I), 32 of 35 (91.4%)
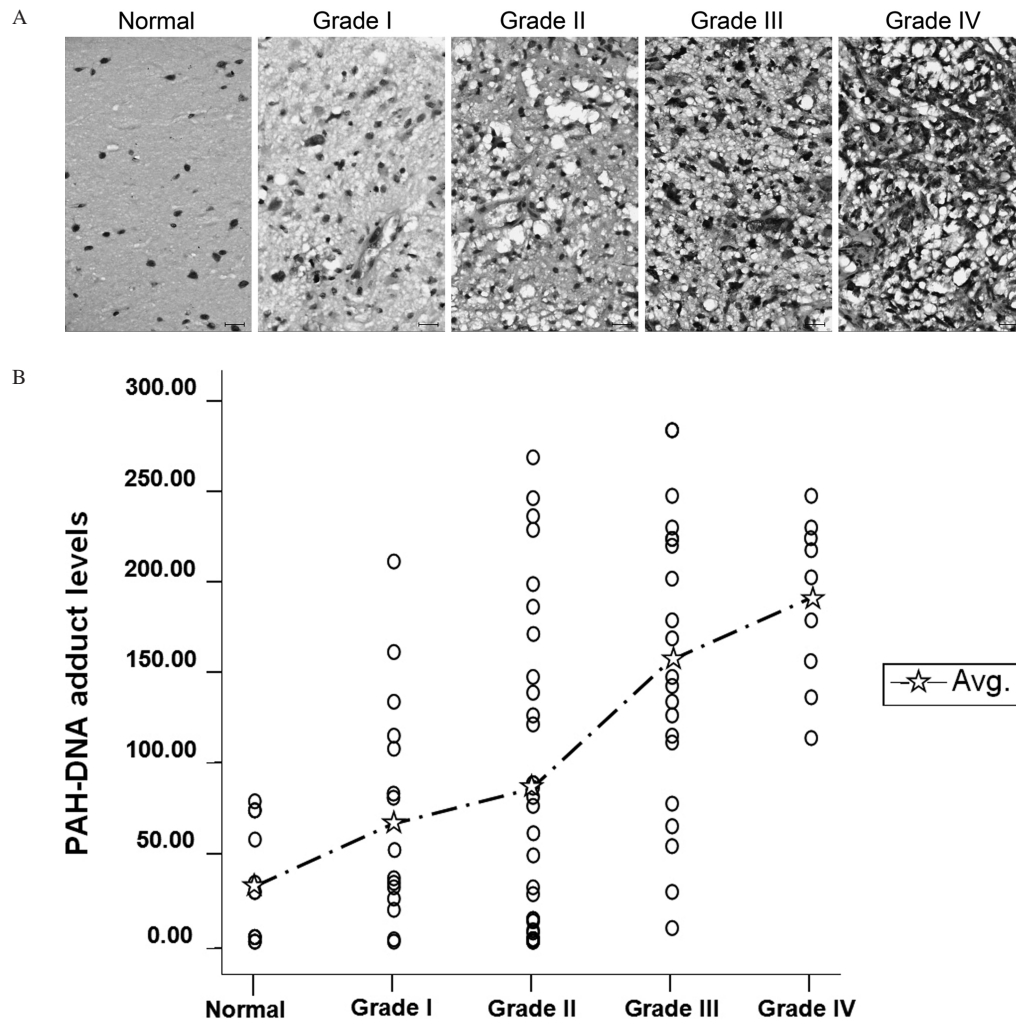

FIG. 1. The distribution and staining intensity of PAH-DNA adducts in the TMA analysis using IHC and the association with the glioma grade. (A) Representative immunoperoxidase staining using 5D11 monoclonal antibody for PAH-DNA adducts of paraffin-embedded TMA; the adducts were detected in normal brain and gliomas of grades I–IV, and the staining intensity was significantly associated with the glioma grade. (B) The PAH-DNA adducts staining intensity index increased with increasing glioma grades (with a mean staining index value of 31.363 for normal, 66.146 for Grade I, 85.647 for Grade II, 156.597 for Grade III, and 190.714 for Grade IV). The Avg. indicates the mean values.

diffuse astrocytomas (Grade II), 21 of 21 (100%) anaplastic astrocytomas (Grade III), and 9 of 11 (81.8%) glioblastoma multiforme (GBM) (Grade IV). PAH-DNA adducts were detected in gliomas with substantial (over 10-fold) interindividual variability and primarily exhibited an endonuclear localization. The staining intensity index increased with increasing glioma grades (with a mean staining index value of 31.363 for normal, 66.146 for Grade I, 85.647 for Grade II, 156.597 for Grade III, and 190.714 for Grade IV), which indicated the PAH-DNA adduct levels were elevated with an accompanied increase in tumor grade (Fig. 1B).

AHR Polymorphisms and Glioma Risk

Distribution of data on age, gender, and smoking status for the cases and controls are shown in Table 1. Three hundred eighty-four healthy controls frequency matched to the 384 glioma cases by age, gender, and smoking status ($p = 0.277$ for age, $p = 0.715$ for gender, and $p = 0.738$ for smoking status) are included. Table 2 shows the information on six genotyped SNPs of AHR, including the location, the minor allelic frequencies (MAF), HWE test, and genotyped rates.

The genotype and allele of AHR polymorphisms in the glioma cases and controls and their associations with the risk of adult glioma are shown in Table 3. All of the individuals of missing genotypes were excluded with a genotype failure rate of no more than 0.034. Two AHR polymorphisms (rs2066853 and rs2158041) demonstrated significant association with the risk of glioma as analyzed by the dominant model (variant-containing genotypes vs. common homozygote). Compared with rs2066853 GG homozygotes, carriers with the A allele exhibited more than a 1.39-fold increased risk of glioma occurrence (adjusted OR = 1.39; 95% CI = 1.04–1.85), and the risk increased significantly with an increasing number of variant alleles (AA homozygotes, adjusted OR = 1.81; 95% CI = 1.16–2.84; P-trend = 0.001). Similarly, individuals with the rs2158041 A allele showed a significant association with the risk of glioma compared with the GG homozygotes (adjusted OR = 1.71; 95% CI = 1.06–2.76; P-trend = 0.023), which indicated that the two polymorphisms contributed to glioma

risk. As to the other polymorphisms, no significant differences were found between the cases and the controls.

AHR Polymorphisms and PAH-DNA Adduct Levels in Glioma

Among the glioma patients, 77 brain tumor samples were available to evaluate the PAH-DNA adduct levels in glioma among different genotype groups of patients. The elevated PAH-DNA adduct levels in glioma correlated with rs2066853 risk genotypes (Fig. 2) and rs2158041 risk genotypes (Fig. 3). Associations between three patient groups with different genotypes of rs2066853 and the values of brain tissue PAH-DNA adduct levels based on 31 patients with GG genotype, 36 patients with GA genotype, and 10 patients with AA genotype were shown in Figure 2. The elevated PAH-DNA adduct levels were observed in the patients carrying

TABLE 2
Information on Genotyped SNPs of AHR Gene Evaluated in This Study

SNP ID	Nucleotide change	Function	MAF*	p value for HWE test	% Genotyped rate
rs2066853	G > A	Exon	0.367	0.243	98.3
rs1476080	A > C	Intron	0.400	0.492	98.6
rs2158041	G > A	Intron	0.256	0.063	98.7
rs2106728	A > G	Intron	0.167	0.866	97.9
rs713150	C > G	Intron	0.381	0.502	97.9
rs6960165	A > G	Intron	0.233	0.280	97.7

Note. MAF, minor allelic frequency; HWE, Hardy-Weinberg equilibrium.
*Minor allele frequency in the Chinese (CHB, Han Chinese in Beijing, China) population, as reported in dbSNP database.

TABLE 3
Genotype and Allele of AHR Polymorphisms in Cases and Controls and Their Associations With Risk of Adult Glioma

Genotypes	Cases n (%)	Controls n (%)	p value[a]	OR (95% CI)[b]
rs2066853				
GG	154 (40.8)	188 (49.2)	0.019	1.00 (reference)
GA	162 (43.0)	153 (40.1)		1.27 (0.93–1.73)
AA	62 (16.4)	41 (10.7)		**1.81 (1.16–2.84)**
GA/AA	224 (59.4)	194 (50.8)		**1.39 (1.04–1.85)**
P for trend				**0.001**
rs1476080				
AA	118 (31.2)	125 (33.2)	0.423	1.00 (reference)
AC	172 (45.5)	179 (47.5)		0.99 (0.72–1.38)
CC	89 (23.5)	74 (19.6)		1.24 (0.83–1.85)
AC/CC	261 (69.0)	253 (67.1)		1.07 (0.79–1.45)
P for trend				0.268
rs2158041				
GG	182 (48.9)	211 (55.7)	0.063	1.00 (reference)
GA	138 (37.1)	134 (35.4)		1.22 (0.89–1.66)
AA	51 (13.7)	34 (9.0)		**1.78 (1.10–2.86)**
GA/AA	189 (50.8)	168 (44.3)		**1.33 (1.00–1.77)**
P for trend				**0.023**
rs2106728				
AA	225 (60.5)	221 (58.8)	0.787	1.00 (reference)
AG	130 (34.9)	133 (35.4)		0.94 (0.70–1.28)
GG	17 (4.6)	21 (5.6)		0.78 (0.40–1.52)
AG/GG	147 (39.5)	154 (41.0)		0.92 (0.69–1.23)
P for trend				0.551
rs713150				
CC	162 (42.7)	167 (43.8)	0.437	1.00 (reference)
CG	157 (41.4)	166 (43.6)		0.96 (0.70–1.30)
GG	60 (15.8)	48 (12.6)		1.27 (0.82–1.96)
CG/GG	217 (57.3)	214 (56.2)		1.03 (0.77–1.37)
P for trend				0.395
rs6960165				
AA	199 (52.2)	207 (54.3)	0.634	1.00 (reference)
AG	154 (40.4)	142 (37.3)		1.15 (0.85–1.55)
GG	28 (7.3)	32 (8.4)		0.92 (0.54–1.59)
AG/GG	182 (47.8)	174 (45.7)		1.10 (0.83–1.47)
P for trend				0.820

Note. Data in boldface represent $p < 0.05$.
[a]FDR corrected p value.
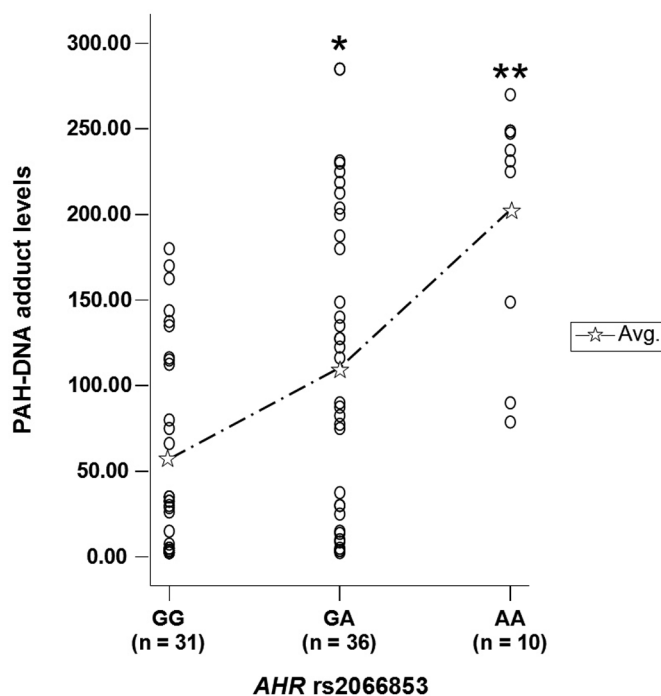[b]Adjusted for age, smoking, and drinking status.

FIG. 2. Association between three patient groups with different genotypes of rs2066853 and the values of brain tissue PAH-DNA adducts level based on 31 patients with GG genotype, 36 patients with GA genotype, and 10 patients with AA genotype. The Avg. indicates the mean values. Significant difference measured by Kruskal-Wallis tests. *$p < 0.05$ compared with the GG groups, **$p < 0.01$ compared with the GG groups.

FIG. 3. Association between three patient groups with different genotypes of rs2158041 and the values of brain tissue PAH-DNA adducts level based on 38 patients with GG genotype, 29 patients with GA genotype, and 10 patients with AA genotype. The Avg. indicates the mean values. Significant difference measured by Kruskal-Wallis tests. *$p < 0.05$ compared with the GG groups.

either one copy (mean ± SD, 110.49±89.72 for GA carriers; $p = 0.022$) or two copies (mean ± SD, 202.62±70.14 for AA carriers; $p < 0.001$) of the risk-associated rs2066853 A allele. In contrast, in patients not carrying the risk-associated allele (with the GG genotype), the adduct levels were significantly decreased (mean ± SD, 57.18±59.82). In addition, associations between three patient groups with different genotypes of rs2158041 and the values of brain tissue with PAH-DNA adduct levels based on 38 patients with GG genotype, 29 patients with GA genotype, and 10 patients with AA genotype were also evaluated and are shown in Figure 3. Similarly, a gradual increase in PAH-DNA adduct levels in glioma was found in the three rs2158041 subgroups, and the increase was statistically significant. Compared with the rs2158041 GG-common homozygotes, rs2158041 AA-genotype carriers showed a significantly higher PAH-DNA adduct level (mean ± SD; 201.38±63.48 for AA homozygotes vs. 78.62±70.69 for GG homozygotes; $p \leq 0.001$).

DISCUSSION

PAH-DNA Adducts Are Associated With Glioma Development Risk

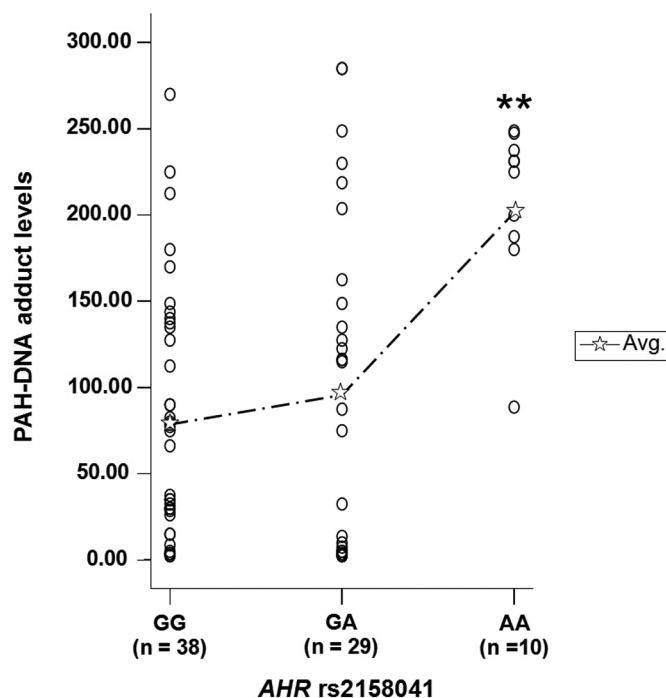An estimated two thirds of all cancer cases are linked to environmental causes. PAHs are very widespread environmental contaminants, and a number of PAHs and their derivatives have been recognized as major culprits causing an increased incidence of cancer (lung, skin, breast, and urinary cancers). However, until this study, the association of PAHs exposure and the risk of glioma was still unclear.

Given the carcinogenicity of these PAHs and their presence in ambient air from a range of sources, a toxicological endpoint is required to investigate the potential health impact of PAHs in cancer risk. However, finding the appropriate toxicological endpoint to determine the exact PAHs exposure level and its effects *in vivo* is difficult. Accordingly, in spite of hundreds of PAHs that have been identified in atmospheric particles, only 33 PAHs are available for toxicological endpoint information, which possibly depends on the relatively rapid and complex metabolism and bodily distribution process of PAHs in mammals.

Herein, PAH-DNA adducts were measured as an endpoint biomarker to evaluate the effects of internal exposure to PAHs on glioma risk due to some great advantages to traditional exposure assessment (Godschalk *et al.*, 2003; Ken-Dror, 2005). (1) PAH-DNA adducts smooth the extreme variability in exposure, which is typical for environmental toxicants, and may integrate exposure over longer periods of time. (2) More important, PAH-DNA adducts not only account for all exposure routes but also account for interindividual differences in uptake, elimination, distribution, metabolism, and repair among

exposed individuals. (3) Many scientific studies have justified the application of DNA adduct measurements as biomarkers in exposure assessment (Castano-Vinyals *et al.*, 2004; Gyorffy *et al.*, 2008). In this study, although the 5D11 antibody was produced in response to the adducts of the carcinogenic intermediate of BPDE-DNA, it cross-reacts with various affinities to other structurally related PAHs; hence, the terminology "PAH-DNA" is commonly used (Rybicki *et al.*, 2004).

In our case-control study, cigarette consumption had no effect on glioma development (Table 1), whereas PAH-DNA adduct levels were significantly associated with glioma grade. This inconsistency indicates that smoking is not the primary factor in PAH-DNA adduct formation and that environmental exposure (likely including ambient pollution, other inhaled products of organic combustion, and diet) may have a larger effect on PAH-DNA adduct levels than smoking (Jia *et al.*, 2011). Our results agree with a previous study that found no correlation between PAH-DNA adduct formation and smoking status (Pratt *et al.*, 2011).

By detecting the staining intensity of PAH-DNA adducts in TMA, we found a significant association between DNA adduct formation and the risk of glioma. Furthermore, the PAH-DNA adduct staining intensity correlated with the tumor grade, suggesting PAH-DNA adduct formation may play a role in glioma carcinogenesis. The same findings were also reported in other cancers (Rybicki *et al.*, 2006; Santella *et al.*, 2000). Nonetheless, how PAHs and their derivatives cross the blood-brain barrier and the causal associations between PAH exposures, PAH-DNA adduct formation, and glioma risk remain to be established.

AHR Polymorphisms and Their Contributions to Cancer Susceptibility and DNA Adduct Formation

Since the discovery of the AHR, studies have determined that it can be activated by a wide range of classes of compounds including PAHs (Denison and Nagy, 2003; Ramadoss *et al.*, 2005). AHR polymorphisms exist in the human population that will result in either forms of AHRs with higher binding affinities or forms that are more functionally efficient, thereby making their carriers more sensitive to environment compounds exposure (Maier *et al.*, 1998).

Because of the wide distribution of AHR pathway proteins in human tissue and its potential role in carcinogenesis, we speculated that abnormalities in AHR action could contribute to glioma carcinogenesis. In the current study, we asked two questions: (1) whether AHR gene polymorphisms are associated with an increased risk of glioma and (2) whether polymorphisms in AHR result in altered PAH-DNA adduct formation, thereby affecting the rate of glioma carcinogenesis. In this study, six tag SNPs of the AHR gene, rs2066853, rs1476080, rs2158041, rs2106728, rs713150, and rs6960165, were evaluated. Only two of them, the rs2066853 and rs2158041 variants, were associated with glioma risk.

The rs2066853 polymorphism is located in the transactivation domain and leads to a nonsynonymous amino acid substitution

in the AHR protein, which has been demonstrated to influence its function. Previous study observes that the AHR-R554K variant displays an increased ability to induce the transcription of CYP1A1 activity in lymphocytes compared with common genotype after induction with 3-methylcholanthrene (Smart and Daly, 2000). However, conflicting evidence also exists. Some studies show that the R554K change does not alter the ability of AHR to regulate CYP1A1- or CYP1B1-driven transcription (Celius and Matthews, 2010). Previous results showed no associations of this variant and human breast cancer risk (Le Marchand *et al.*, 2005; Long *et al.*, 2006; Sangrajrang *et al.*, 2009; Zhang *et al.*, 2011). The lack of associations between this variant and non-Hodgkin's lymphoma (De Roos *et al.*, 2006; Ng *et al.*, 2010) and lung cancer risk (Cauchi *et al.*, 2001; Kawajiri *et al.*, 1995) has also been reported. However, the Lys/Lys genotype of rs2066853 (Arg554Lys) conferred a statistically significant higher risk of lung cancer in heavy smokers (Chen *et al.*, 2009). In this study, we found that the variant genotype of the rs2066853 positively correlated to glioma risk ($p = 0.019$).

Additionally, AHR rs2158041, an intronic polymorphism in the AHR gene, was also associated with an increased glioma risk. This finding is consistent with recent results in lung cancer research that demonstrate that the heterozygous genotype of rs2158041 was significantly associated with an increased risk of lung cancer compared with the common genotype (Chen *et al.*, 2009). The molecular mechanism remains unclear. Perhaps this intronic polymorphism potentially influences the alternative spicing of the gene products (Hirose *et al.*, 2008), or it might be in LD with other causal loci or genes. To identify additional SNPs that could be associated with glioma risk that may be in high LD with this SNP, we screened all of the common variants (with MAF > 0.05) within an approximately 50-kb-long region surrounding this site (~25 kb upstream and ~25 kb downstream of these loci) based on the CHB HapMap data resource. We found that rs2158041 is in LD with SNP rs6461312 ($r^2 = 0.825$) which is located ~5.0 kb downstream of stop codon of AHR. Therefore, it is likely that the rs6461312 SNP near the 3' region of the AHR gene may be the causal variant. However, the exact location and biological functions of the real causal SNPs in AHR are of great interest and warrant further investigation.

Intriguingly, we also found statistically significant associations between these two variants (rs2066853 and rs2158041) and PAH-DNA adduct levels in glioma tissue. Together, these findings show that AHR is an important gene known to regulate enzymes that metabolize PAHs and that AHR gene polymorphisms may increase susceptibility to PAH-DNA adduct formation and glioma risk. Notwithstanding, multiple studies in experimental models and a number of epidemiological investigations have demonstrated an association between DNA adduct formation and an increased cancer risk; our study provides the first direct epidemiological evidence for the contribution of PAH-DNA adducts to glioma risk and also proposes the point for the first time that AHR genetic variants influence PAH-DNA adduct levels in glioma.

Further studies are needed to elucidate two mechanisms: (1) the mechanism by which retrospective parental exposure to PAHs affects the development of brain tumors in offspring and (2) the manner in which PAHs and their derivatives cross the blood-brain barrier and form PAH-DNA adducts. These questions are of great interest and warrant further investigation.

In conclusion, in this case-control study in a Chinese population, we identified possible contributions of AHR rs2066853 and rs2158041 to glioma risk and the PAH-DNA adduct levels in glioma tissue. Although the etiology of glioma remains elusive, our findings are undoubtedly helpful to shed further light on gene-environment interactions conferring susceptibility to glioma and to better understand other cancers caused by PAHs. However, ongoing and future research with a large sample size and ethnically diverse populations is required to elucidate the impact of genetic variants of AHR on the susceptibility to glioma and their potential biological mechanism.

SUPPLEMENTARY DATA

Supplementary data are available online at http://toxsci.oxfordjournals.org/.

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