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*NAT2*7* Allele Is a Potential Risk Factor for Adult Brain Tumors in Taiwanese Population

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Abstract

Arylamine *N*-acetyltransferase-2 (NAT2) displays extensive genetic polymorphisms that affect the rates of acetylation of drugs and toxic compounds such as amine carcinogens. The association of *NAT2* polymorphisms with adult brain tumors has been unclear. To investigate whether the *NAT2* genotype is a risk factor of brain tumors, we determined the frequencies of three common polymorphisms in the *NAT2* gene, *NAT2*5 (T341C), NAT2*6 (G590A),* and *NAT2*7(G857A),* in brain tumor patients and in age- and gender-matched control subjects (n = 27 in each group). Genotyping was carried out using PCR-RFLP and subjects were phenotyped as a fast or slow acetylator based on the genotypes. The odds ratio of *NAT2*7* allele frequency was significantly higher in patients with brain tumor than in controls (odds ratio, 6.786; 95% confidence interval, 2.06-22.37; P = 0.003); in the mean time, *NAT2*4/*7* genotype was significantly more common in the patient group than in controls (odds ratio, 6.19; 95% confidence interval, 1.68-22.79; P = 0.0039). The tumors in the patients with *NAT2*7* allele tended to be high-grade astrocytoma or glioblastoma multiforme (P = 0.016). In conclusion, these data suggest that the presence of *NAT2*7* allele might be a potential risk factor for the development of brain tumors in Taiwan. (Cancer Epidemiol Biomarkers Prev 2008;17(3):661–5)

Introduction

The *N*-acetylation polymorphism causes individual variations in the metabolism of xenobiotics with a primary aromatic amine or a hydrazine structure. *N*-acetyltransferase-2 (NAT2), one of the two NAT isoenzymes in humans, is responsible to the well-known inherited individual variation in the ability to acetylate arylamine agents, including polycyclic aromatic hydrocarbon in diesel exhaust and 4-aminobiphenyl and 2-naphthaylamine in cigarette smoke (1). NAT2 is expressed in the liver, colonic, and bladder mucosa. Its role in the etiology of bladder cancer has been suggested in multiple studies (2-4). Slow acetylator phenotypes caused by polymorphisms in *NAT2* gene confer a higher risk of bladder cancer due to the reduced activity in the hepatic *N*-acetylation pathway, which detoxifies compounds such as 4-aminobiphenyl and competes with *N*-oxidation of these compounds by cytochrome *P*4501A2 to reactive hydroxylamines. The relationship between the polymorphism of *NAT2* and other cancers such as lung and colorectal cancer has also been studied (5-11).

Human brain cells also express NAT2 enzymes (12). Although the brain is partially protected from chemical insults by blood-brain barrier, several drugs and pollutants can reach the brain. Therefore, NAT2 activity due to gene polymorphism could affect the risk of developing primary brain tumors. However, previous studies of *NAT2* polymorphisms on brain tumors on Caucasians have been inconclusive. Two studies found no association between NAT2 acetylator status and brain tumors (9, 13). However, the other study reported that a higher number of rapid acetylation phenotype was found in brain tumor patients compared with healthy volunteers (14). Furthermore, the association of *NAT2* polymorphisms with brain tumors in Asian population has not been studied. The present study was to analyze the association between *NAT2* genetic polymorphisms and the risk of brain tumors in Taiwanese population. The results indicated that the patients with *NAT2*4/*7* genotype were significantly at risk of developing brain tumors, although no association was found between the phenotypes of NAT2 and the overall survival. This trend suggests that, like bladder and lung cancer, genetic factors might as well play a role in developing brain tumors in our population.

Materials and Methods

Study Subjects. The baseline characteristics of 27 primary brain tumor patients (19 males and 8 females) from Hsin-Kong Wu Ho Su Hospital are shown in Table 1. Of the 27 tumor samples, 2 were grade 1 astrocytomas, 3 grade 2 astrocytomas, 7 grade 3 astrocytomas, 11 glioblastoma multiforme, 2 oligodendrogliomas, and 2 meningiomas. Peripheral blood samples from 27 age- and gender-matched controls without the history of brain tumors were also collected in Wanfang Hospital after obtaining informed consent. The use of human tissues and record review for research purpose has been

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Table 1. Comparison of brain tumor patients and controls

	Patients	Controls	*P*	Odds ratio (95% confidence interval)
n	27	27	—	
Gender				
Male (%)	19 (70.37)	19 (70.37)	—	
Female (%)	8 (29.63)	8 (29.63)		
Age, mean (SD)	45.56 (20.63)	45.81 (20.10)	0.541	
NAT2 genotypes				
*NAT2*4/*4* (%)	4 (14.81)	7 (25.93)	0.014*	
*NAT2*4/*5* (%)	0 (0)	2 (7.40)		
*NAT2*4/*6* (%)	2 (7.40)	9 (33.33)		
*NAT2*4/*7* (%)	14 (51.85)	4 (14.81)	0.039*	6.19 (1.68-22.79)
*NAT2*5/*7* (%)	1 (3.7)	0		
*NAT2*6/*6* (%)	1 (3.7)	1 (3.70)		
*NAT2*6/*7* (%)	5 (18.52)	2 (7.40)		
*NAT2*7/*7* (%)	0 (0)	2 (7.40)		
*NAT2*7* frequency (%)				
0	7 (25.93)	19 (70.37)		
≧1	20 (74.07)	8 (29.63)	0.003*	6.786 (2.06-22.37)
Acetylator phenotypes				
Fast (%)	20 (74.07)	22 (81.48)	0.513	
Slow (%)	7 (25.93)	5 (18.52)		

*Statistically significant.

approved by the Institutional Review Board in Taipei Medical University-Wanfang Hospital and Shin Kong Wu Ho-Su Hospital.

PCR-RFLP Analysis for Determination of *NAT2* Genotypes. Genomic DNA from paraffin-embedded tissues from brain tumor patients was extracted by commercial kits (DEXPAT). Genomic DNA from peripheral blood mononuclear cells was extracted by proteinase K digestion followed by the conventional phenol/chloroform method. Analyses for mutations at the *NAT2* gene locus were done by the use of RFLP after nested PCR using the scheme shown in Fig. 1. Primer sequences are showed in Table 2. PCR products were subjected to the restriction enzyme digested by *Kpn*I, *Taq*I, and *Bam*HI, respectively. The digested products were resolved by 6% PAGE. *NAT2*4* represents the wild-type allele and the loss of restriction cutting sites, *Kpn*I, *Taq*I, and *Bam*HI, denoted as *NAT2*5 (T341C)*, *NAT2*6 (G590A)*, and *NAT2*7 (G857A)* allele, respectively.

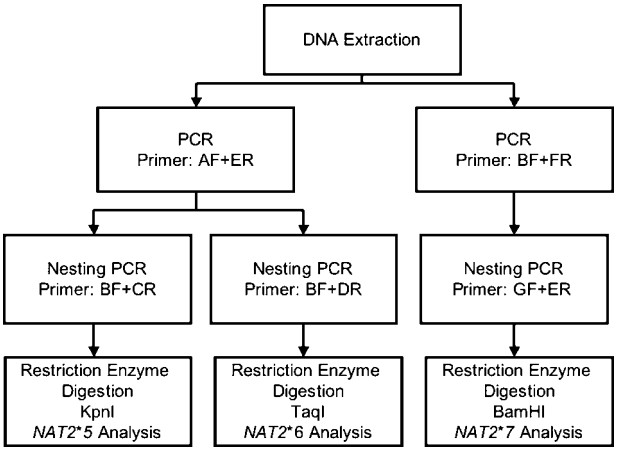

Figure 1. Schema for amplification of *NAT2*5 (T341C)*, *NAT2*6 (G590A)*, and *NAT2*7 (G857A)* by nested PCR.

Individuals with one or two wild-type *NAT2*4* alleles were defined as fast acetylators and those with any two polymorphic alleles as slow acetylators.

Statistical Analysis. The difference in the phenotypes, genotypes, and variant allele frequencies in patients and normal controls were compared by using the χ^2 test. The continuous data was compared by Student's *t* test. The survival analysis was done by log-rank tests (SPSS 13.0 for Windows; SPSS). A two-tailed $P < 0.05$ was considered statistically significant.

Results

The *NAT2* genotypes of the 27 brain tumor patients and the 27 age- and gender-matched normal controls were examined firstly by PCR-RFLP methods and their corresponding phenotypes were determined (Table 1). Representative results are shown in Fig. 2. The comparison of genotypes and phenotypes for the two groups was shown in Table 1. The distributions of genotypes for the two groups were significantly different ($P = 0.014$), with higher percentage of patients with *NAT2*4/*7* genotype than those in controls ($P = 0.0039$; odds ratio, 6.19; 95% confidence interval, 1.68-22.79), suggesting that this genotype might be a potential risk factor for brain tumors. Further analysis showed that the frequency of brain tumor patients with at least one *NAT2*7* allele was significantly higher than the control ones (odds ratio,

Table 2. Primer sequences used in *NAT2* genotyping

Primer name	Sequence
AF	5′-GGAACAAATTGGACTTGG-3′
BF	5′-GCTGGGTCTGGAAGCTCCTC-3′
CR	5′-GAGATGAGAATTAAGAAATT-3′
DR	5′-GGAGACGTCTGCAGGTATGT-3′
ER	5′-TTGGGTGATACATACACAAGGG-3′
FR	5′-TCTAGCATGAATCACTCTGC-3′
GF	5′-TTTAAAACTCTCACTGAGGAAGA-3′

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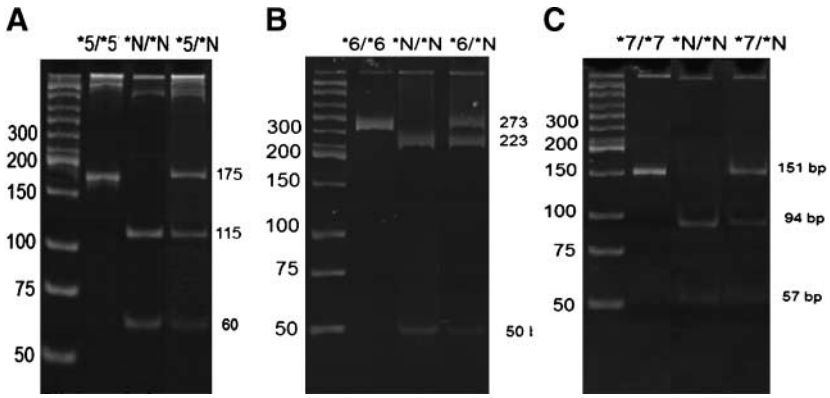

Figure 2. PCR-RFLP analysis for NAT2 genetic polymorphisms. After nested PCR amplification, products were subjected to a digestion with (**A**) *Kpn*I for *NAT2*5* allele and (**B**) *Taq*I for *NAT2*6* allele and *Bam*HI for *NAT2*7* allele. Restriction enzyme-digested products were loaded onto a 6% polyacrylamide gel. Representative results for various polymorphisms.

6.786; 95% confidence interval, 2.06-22.37; $P = 0.003$; Table 2). Stratified by the histology types and pathologic grading of the tumors, patients with *NAT2*7* allele were significantly more likely to have high-grade astrocytomas and glioblastoma multiforme than patients without *NAT2*7* allele ($P = 0.016$; Table 3). However, the presence of *NAT2*7* allele did not affect either the progression-free survival or the overall survival (Table 3).

There was no difference in the distribution of acetylator status between brain tumor patients and normal controls ($P = 0.513$; Table 1). The relationships between acetylator phenotypes and mean age of patients, tumor types, tumor grades, or the survival were further evaluated and none of these tumor characteristics was associated with NAT2 phenotypes (Table 4; Fig. 3). Twenty of 27 (74.07%) patients had fast acetylator phenotypes and 7 (25.93%) had slow acetylator phenotypes. Similar to prior studies on the *NAT2* genotypes in Chinese population, 22 of healthy controls are fast acetylators and 5 are slow acetylators (15).

Discussion

The present study examined the relationship between NAT2 genotypes/phenotypes and the risk of brain tumors. The results indicated that there is no difference in frequencies of phenotypes, the NAT2 acetylator status, between the tumor patients and the control group. The phenotype results are in agreement with studies included patients with gliomas without further classification (9, 13) but in contrast to another study with astrocytoma and meningioma patients (14). However, previous three studies did not further analyze the data of genotypes and allele frequency. Interestingly, the genotype results of the current study indicated, for the first time, that the *NAT2*7* allele, particularly *NAT2*4/*7* genotype, was associated with increased risk of brain tumors in Taiwanese population. Patients with *NAT2*7* allele are also more likely to develop high-grade astrocytoma or glioblastoma multiforme. This finding may further provide the answer to the discrepancy of the phenotypes data between studies. The significant differences in

Table 3. Characteristics of brain tumor patients with and without *NAT2*7* allele

	With *NAT2*7* allele ($n = 20$)	Without *NAT2*7* allele ($n = 7$)	P
Gender			
Male (%)	5 (25.00)	3 (42.86)	0.793
Female (%)	15 (75.00)	4 (57.14)	
Age, mean ± SD	46.00 ± 19.61	44.29 ± 24.96	0.854
Tumor type			
Astrocytoma grade I (%)	2 (10.00)	0	0.016*
Astrocytoma grade II (%)	3 (15.00)	0	
Astrocytoma grade III (%)	6 (30.00)	1 (14.29)	
Glioblastoma multiforme (%)	9 (45.00)	2 (28.57)	
Oligodendroglioma (%)	0	2 (28.57)	
Meningioma (%)	0	2 (28.57)	
Tumor grade			
I (%)	2 (10.00)	2 (28.57)	0.465
II (%)	3 (15.00)	2 (28.57)	
III (%)	6 (30.00)	1 (14.29)	
IV (%)	9 (45.00)	2 (28.57)	
Progression-free survival			
Mean ± SD	465.25 ± 695.27	423.57 ± 870.96	0.311
Range	15-2,800	10-2,390	
Overall survival			
Mean ± SD	764.25 ± 900.99	464.43 ± 884.78	0.494
Range	60-3,195	10-2,461	

*Statistically significant.

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Table 4. Comparison of fast and slow acetylators with brain tumor

	Fast acetylator (n = 20)	Slow acetylator (n = 7)	P
Gender			
Male (%)	15 (75.00)	4 (57.14)	0.373
Female (%)	5 (25.00)	3 (42.85)	
Age, mean ± SD	46.25 ± 21.14	43.57 ± 20.54	0.774
Tumor type			
Astrocytoma grade I (%)	2 (10.00)	0	0.420
Astrocytoma grade II (%)	1 (5.00)	2 (28.57)	
Astrocytoma grade III (%)	5 (25.00)	2 (28.57)	
Glioblastoma multiforme (%)	9 (45.00)	2 (28.57)	
Oligodendroglioma (%)	1 (5.00)	1 (14.29)	
Meningioma (%)	2 (10.00)	0	
Tumor grade			
I (%)	4 (20.00)	0	0.188
II (%)	2 (10.00)	3 (42.86)	
III (%)	5 (25.00)	2 (28.57)	
IV (%)	9 (45.00)	2 (28.57)	
Progression-free survival			
Mean ± SD	537.85 ± 825.96	190.42 ± 160.01	0.309
Range	15-2,800	10-510	
Overall survival			
Mean ± SD	710.65 ± 817.62	617.57 ± 1,143.14	0.229
Range	40-2,800	10-3,195	

phenotypes are only found in more malignant types of astrocytoma and glioblastoma. The results on genotype, allele frequency, and tumor grades all indicated that *NAT2*7* allele, particularly *NAT2*4/*7* genotype, may be a risk factor of brain tumor.

How the presence of a *NAT2*7* allele affects the brain tumor risk is still unclear. However, it is not surprising that certain genotypes are associated with increased risk of developing cancer, but the case of phenotypes is another story. One study from Egypt has shown that *NAT2*5/*5* genotype has been associated with increased risk of schistosomiasis-associated bladder cancer (16). Similarly, an association between bladder cancer and the presence of *NAT1*10* allele has also been reported in some studies (17, 18). In lung cancer studies, although the association between lung cancer and NAT phenotype is weak, patients with *NAT1*14, NAT1*15,* and *NAT2*5B/*6* genotypes have increased cancer risk (19, 20). Likewise, *NAT1*10* allele has been found to be associated with colorectal cancer and head and neck cancer (21, 22). Further studies are required to elucidate whether these nucleotide differences represent altered chromosomal stability in a tissue-specific manner in the presence of genotoxic agents in the environment rather than changes in enzymatic activities.

Because cancer is a multigene disease, another speculation, wherein genotypes could be a risk factor, is that multiple genetic factors might take part in carcinogenesis at different stages of cancer development. Taking lung cancer as an example, although NAT2 acetylator phenotype alone is not associated with an increased risk, *NAT2* genotypes, when combined with other genetic factors such as *NAT1, GSTM1,* or *CYP1,* may increase the cancer risk. Individuals, with combined *NAT1* rapid and *NAT2* slow genotype, seemed to have a significantly higher risk for lung adenocarcinoma (23), or the *NAT2* slow genotype when combined with the *GSTM1-null* genotype may increase the susceptibility to adduct formation, gene mutation, and lung cancer risk (24). Similarly, a combination of NAT2 slow/CYP1A1 rapid acetylator may also predispose higher risk to lung cancer in nonsmoking females (11). Although there is no single polymorphism able to predict the risk of brain tumors, an allelic combination could jointly affect the risk of brain tumors (13, 25). Because the development of diseases is an outcome of interactions between human

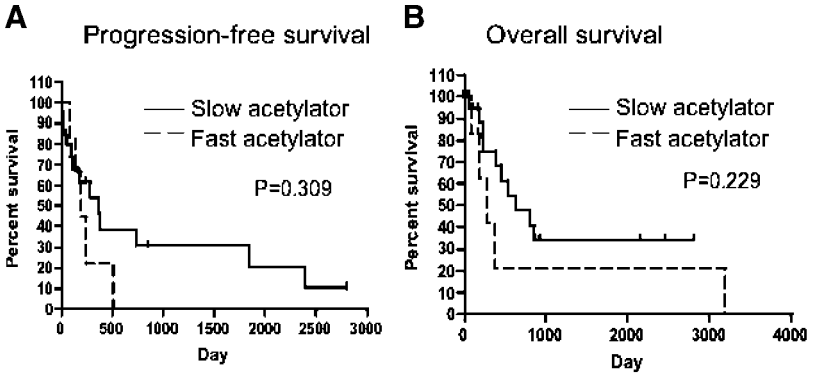

Figure 3. Survival curves in patients with fast and slow acetylator phenotypes. **A.** Progression-free survival. **B.** Overall survival.

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genes and environment, the presence of environmental agents, including toxins, might variably, possibly, and preferentially affect genetic activity among different human populations. It is not surprising that results on the association between NAT2 acetylator phenotypes or genotypes and cancers are often dependent on populations studied. Therefore, we speculate that *NAT2*4/*7* or *NAT2/*7* allele might take part in the complex interactions between environmental agents and cellular activities in Asian genetic background, all of which together impart increased risks for brain tumors. Although our study is somewhat limited by a small sample size, our results strongly suggest that genetic factors might play a role in the development of brain tumors especially for those with high-grade pathology in Taiwanese population and warrant further studies on the pathogenesis of brain tumors.

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