Original Article

Polymorphisms of DNA Repair Pathway Genes and Cigarette Smoking in Relation to Susceptibility to Large Artery Atherosclerotic Stroke among Ethnic Chinese in Taiwan

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Aim: Cigarette-smoking induced oxidative DNA damage to endothelial cells has been reported to play an etiological role in atherosclerosis development. Individual vulnerability to oxidative stress through smoking exposure and the ability to repair DNA damage, which plays a critical role in modifying the risk susceptibility of large artery atherosclerotic (LAA) stroke, is hypothesized. Thus, we examined the effect of genetic polymorphisms of DNA repair pathway genes and cigarette smoking in relation to risk susceptibility of LAA stroke.

Methods: We enrolled 116 LAA stroke patients and 315 healthy controls from the Armed Forces Taoyuan General Hospital, Taoyuan, Taiwan. Genotyping of polymorphisms of the *OGG1* (*Ser326Cys*), *XRCC1* (*Arg399Gln*), *ERCC2* (*Lys751Gln*), and *ERCC5* (*Asp1104His*) genes was performed and used to evaluate LAA stroke susceptibility.

Results: Of those non-synonymous polymorphisms, the *ERCC2 Lys751Gln* variant was found to be associated with LAA stroke risk (OR: 1.69, 95%CI: 1.02-2.86), and this association was more pronounced in smokers, manifesting a 2.73-fold increased risk of LAA stroke ($p=0.027$). A joint effect on risk elevation of LAA stroke was seen in those patients with *OGG1* and *ERCC2* polymorphisms (OR: 2.75, 95%CI: 1.26-6.00). Moreover, among smokers carrying the *OGG1 Ser326Cys* polymorphism, there was a tendency toward an increased risk of LAA stroke in those patients who had a greater number of high-risk genotypes of *XRCC1*, *ERCC2*, and *ERCC5* polymorphisms ($p_{trend}=0.010$).

Conclusion: The susceptible polymorphisms of DNA repair pathway genes may have a modifying effect on the elevated risk of LAA stroke in smokers among ethnic Chinese in Taiwan.

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Key words; Large artery atherosclerotic stroke, DNA repair genes, Polymorphism, Smoking, Susceptibility

Abbreviations; LAA: Large artery atherosclerotic, BER: Base excision repair, NER: Nucleotide excision repair, ROS: Reactive oxygen species, SNP: Single nucleotide polymorphism, OGG1: 8-oxoguanine glycosylase 1, XRCC1: X-ray repair cross complementing group 1, ERCC2: Excision repair cross complementation group 2, ERCC5: Excision repair cross complementation group 5

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Introduction

Stroke remains the major leading cause of death worldwide, including in the ethnic Taiwanese population. Stroke is believed to be a polygenic multifactori-

al model of etiology caused by the sum of genetic and environmental factors^{1, 2}. Cigarette smoking has been considered as an important risk factor for atherosclerotic vascular diseases, including coronary artery disease (CAD) and cerebrovascular disease (CVD)^{3, 4}. Although the mechanism underlying cigarette smoke-induced cerebrovascular injury regarding to stroke remains unclear, cigarette constituents are known to activate reactive oxygen species (ROS) on DNA damage within the vascular wall, leading to premature vascular aging and endothelial dysfunction^{3, 5, 6}.

A direct causality in which exposure to cigarette smoke results in elevated levels of DNA aberrations in various human cell types, including fibroblasts, lymphocytes and endothelial cells has been shown in earlier reports^{7, 8}. Recently, accumulating evidence from cohort studies revealed that the increased risk of stroke is generally associated with smoking^{9, 10}. The smoking-related oxidized bases, such as 7, 8-dihydro-8-oxoguanine (8-oxo-G) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), are extensively studied DNA destroyers that result in mutagenic guanine base lesions through the action of ROS and lead ultimately to cell tumorigenesis^{11, 12}. In addition, the presence of cigarette smoking-related polycyclic aromatic hydrocarbon (PAH)-DNA adducts has been detected in human mammary artery specimens from smokers¹³, indicating that cigarette smoke can induce chromosomal aberrations in the cardiovascular system. As the cumulative oxidative stress caused by smoking is capable of inducing DNA damage that may correlate with the vascular aging process^{3, 14}, an underlying mechanism is thus hypothesized in which individual vulnerability to oxidative stress through smoking exposure and polymorphisms of DNA repair genes, with accumulated oxidative DNA damage together with deficits in DNA repair enzymes, plays a critical role in modifying the risk susceptibility to stroke among smokers.

Base excision repair (BER) and nucleotide excision repair (NER) pathways are two mainly DNA damage repair systems, which are involved in eliminating and repairing damaged DNA caused by methylating chemicals and ROS. 8-Oxoguanine glycosylase 1 (*OGG1*) enzyme is involved in the excision of 8-oxoguanine, a highly mutagenic compound that can form mismatched base-pairs in DNA. A transversion mutation of the *OGG1* gene with *Ser326Cys* (rs1052133) polymorphism has been known to encode a *N*-glycosylase/DNA lyase with decreased enzyme activity that leads to 8-oxoguanine accumulation^{12, 15}. X-ray repair cross complementing group 1 (*XRCC1*) acts as a scaffolding protein that interacts with poly (ADP-ribose) polymerase, DNA polymerase- β and DNA ligase III in BER¹⁶. Excision

repair cross complementation group 2 (*ERCC2*, also known as *XPD*) encodes an ATP-dependent DNA helicase that interacts with transcription factor BTF2/TFIIH¹⁷ and *ERCC5* (known as *XPG*) genes takes part in incision steps to respond to a wide range of structurally bulky lesions in NER¹⁸. To date, known polymorphisms in *OGG1* (*Ser326Cys*), *XRCC1* (*Arg399Gln*), *ERCC2* (*Lys751Gln*), and *ERCC5* (*Asp1104His*) have been reported to alter the ability of encoded enzymes to repair DNA damage, and those variant alleles have been found in individuals predisposed to increased risk of many kind of cancers and autoimmune disorders¹⁹⁻²².

Although association studies of the polymorphisms of candidate genes contributing to individual susceptibility to stroke disease have been performed^{23, 24}, knowledge of variations in DNA repair enzymes regarding to stroke development is relatively limited. Moreover, extended information remains unclear on DNA repair gene polymorphisms linked to smoking regarding the risk modification of stroke. In this study, we elucidated whether inter-individual differences in these four non-synonymous SNPs in BER (*OGG1 Ser326Cys* and *XRCC1 Arg399Gln*) and NER (*ERCC2 Lys751Gln* and *ERCC5 Asp1104His*) pathway genes might predispose carriers to an increase risk of large artery atherosclerotic (LAA) stroke, especially in the subgroup of smokers among ethnic Chinese in Taiwan.

Methods

Study Population

In this hospital-based case-control study, demographic data were collected from 116 consecutive patients with LAA stroke disease and 315 age- and gender-matched healthy controls. All participants were included upon receipt of their informed consent according to a protocol approved by the Ethics Committee of Institutional Review Board of the Armed Forces Taoyuan General Hospital, Taoyuan, Taiwan. Ischemic stroke (IS) was defined according to the World Health Organization definition as "rapidly developing clinical signs of focal (or global) disturbance of cerebral function lasting more than 24 hours with no apparent cause other than of vascular origin". Any patients with atypical symptoms, including transient ischemic attacks, intracranial hemorrhage, postseizure palsy, brain trauma, or brain tumors, were excluded from the study. All IS patients underwent at least one brain imaging (CT and/or MRI) study. Based on the clinical features examined by brain imaging, cardiac imaging (echocardiography), ultrasonography of ex-

tracranial and intracranial arteries, and angiography (MRA or conventional angiography), these IS patients were subclassified according to the TOAST classification system^{25, 26}. The healthy control group consisted of 315 individuals recruited from the same hospital in the health examination clinic during the same study period, and each of these controls was chosen on the basis that they showed no evidence of any cerebrovascular disorders. No significant differences were observed in socioeconomic status between those who were and were not included.

Data Collection

An experienced research nurse was assigned to administer a systemic questionnaire to both the case and control groups. The information collected included demographic characteristics (ethnic background, residence area, and educational level), medical history (age at diagnosis and family history of stroke, hypertension, diabetes mellitus, and ischemic heart disease), a history of current alcoholism (referred to as alcohol abuse, which was described in the Diagnostic and Statistical Manual of Mental Disorders IV) [American Psychiatric Association, Diagnostic and Statistical Manual of Mental Disorders (4th ed.), American Psychiatric Association, Washington, DC (1994)], a habit of daily cigarette smoking (in this study, all the smokers had consumed more than 10 cigarettes daily for at least 10 years) as well as parameters of hypercholesterolemia (total cholesterol ≥ 200 mg/dl and/or low-density lipoprotein cholesterol ≥ 130 mg/dl) were recorded.

Genotype Analysis

A sample of 10 ml peripheral blood, collected in acetate-citrate dextrose, was obtained from a fasting venous blood sample that was drawn from each patient and the control subject. Genomic DNA was extracted from peripheral blood lymphocytes by a QIAamp DNA blood mini kit (QIAGEN Inc., Chatsworth, CA, USA) following the manufacturer's protocol and was then stored at -20°C for subsequent genotype determination. Those non-synonymous variants of interest were chosen for genotyping based on the following criteria: (i) single nucleotide polymorphism (SNP) resulted in an amino acid substitution for biological considerations; and (ii) minor allelic frequency of the selected allele was observed to be higher than five percent due to consideration of statistical power; and (iii) information on gene single nucleotide polymorphisms (SNPs) in DNA repair pathways were reported among ethnic Chinese population arranged in the HapMap database (<http://www.hapmap.org/>). Genotyping de-

termination of gene variants was carried out using TaqMan Assays-by-Design (Applied Biosystems, Foster City, CA). SNP information was obtained from the public SNP database (<http://www.ncbi.nlm.nih.gov/SNP/index.html>, National Institute of Health, USA). DNA sequences of probes and primers for the genotyping assays were chosen using the TaqMan Assays-by-Design (Applied Biosystems), including the *Arg399Gln* (G-to-A transition, rs25487) in *XRCC1*, *Ser326Cys* (G-to-C transition, rs1052133) in *OGG1*, *Lys751Gln* (A-to-C transition, rs13181) in *ERCC2*, and *Asp1104His* (G-to-C transition, rs17655) in the *ERCC5* gene. Genotype analyses of these variant alleles were performed using the TaqMan assay and PCR amplification. PCR reactions were performed on a GeneAmp PCR System (ABI Prism 7000 Sequence Detection System; Applied Biosystems), using 20 μl containing a 200 nM probe, 900 nM primer set, 1X TaqMan Universal PCR Master Mix (Applied Biosystems), and 50 ng DNA templates. For quality assurance, independent positive and negative control samples were included and analyzed in each 96-well plate in each batch. To ensure that the observed polymorphisms were specific and not the result of experimental variations, all uncertain genotypic results were subjected to a repeating assay for confirmation.

Statistical Analyses

Means and standard deviations or frequencies and percents were calculated for continuous and categorical variables, respectively. The allelic and genotypic frequencies of each polymorphism for these four genes were compared between cases and control subjects using the chi-square test or Fisher's exact test as appropriate. The adjusted odds ratio (OR) and the corresponding 95% confidence interval (95% CI) for the association was estimated considering known risk factors for IS in multivariate logistic regression analysis. Exposure to cigarette smoking was hypothesized to contribute to an elevated risk of LAA stroke. In addition, individuals who carried high-risk alleles, such as the variant allele of *XRCC1 Gln399* and *OGG1 Cys326* of the BER pathway, as well as *ERCC2 Gln751* and *ERCC5 1104His* of the NER pathway, mimicked functional deficits in cells with lower efficacy in response to DNA damage repair in blood endothelial vessel tissues, were thus considered to be higher risk genotypes of LAA stroke development. Further, to examine the joint effect of these variants, we assumed that each variant genotype of the SNPs contributed equally to the increased risk of LAA stroke, and individuals who carried the same number of putative high-risk genotypes would have the same elevated risk sus-

Table 1. Demographic data for LAA stroke patients and healthy controls

Variable [§]	Cases (N=116) No (%)	Controls (N=315) No (%)	OR (95% CI)
Age			
Mean ± SD	72.5 ± 11.0	71.7 ± 6.6	1.37 (0.89-2.16)
Sex			
Men	82 (70.7)	171 (54.3)	1.86 (0.93-3.29)
Women	34 (29.3)	144 (45.7)	
Diabetes mellitus	35 (29.9)	47 (14.9)	2.46 (1.41-4.20)**
Hypertension	78 (66.7)	139 (44.1)	2.60 (1.62-4.17)**
Hypercholesterolemia	51 (43.6)	108 (34.3)	1.50 (0.96-2.38)
Alcoholism	20 (17.1)	41 (13.0)	1.39 (0.75-2.54)
Smoking	42 (36.2)	77 (24.4)	1.75 (1.07-2.84)*

[§] Definitions of hypercholesterolemia, alcoholism, and smoking are described in methods. OR and 95%CI for known risk factors was estimated using multiple logistic regression analysis after considering age and sex. * $p < 0.05$ and ** $p < 0.01$. Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.

ceptibility to LAA stroke. Therefore, the effects of the multiple interactions of genes on LAA stroke risk were evaluated in subjects carrying a combination of susceptible genotypes. All statistical analyses were performed by an experienced statistician. All statistical tests were performed with SPSS version 17.0 based on a 2-tailed probability and $p < 0.05$ was considered significant.

Results

Distributions of the demographics of the cases and healthy controls are summarized in **Table 1**. The patients and healthy controls had a similar average age. Among 116 LAA stroke patients diagnosed with large artery atherosclerosis (mean age: 72.5 ± 11.0 years), 82 were men (mean age: 72.4 ± 11.3 years) and 34 were women (72.6 ± 10.6 years), respectively. A total of 315 healthy controls (mean age: 71.7 ± 6.6 years) were enrolled, including 171 men (mean age: 72.2 ± 6.9 years) and 144 women (mean age: 71.4 ± 5.9 years). Compared to healthy control subjects, an increased LAA stroke risk was found to be associated with known risk factors, including diabetes mellitus (OR: 2.46, 95%CI : 1.41-4.20), hypertension (OR: 2.60, 95%CI: 1.62-4.17), and cigarette smoking (OR: 1.75, 95%CI: 1.07-2.84) using multiple logistic re-

gression analysis (**Table 1**).

Frequencies of genotype polymorphisms for *XRCC1*, *OGG1*, *ERCC2* and *ERCC5* genes between cases and control subjects are shown in **Table 2**. Distributions of the observed allelic and genotypic frequencies of these polymorphisms were in Hardy-Weinberg equilibrium in the controls. Due to the fact that the variant genotypes of these polymorphisms were reported to result in lower enzymatic activities and that a small percentage of subjects had the homozygous variant genotype of these genes in our cohort, individuals carrying heterozygous and homozygous variant genotypes for these genes were grouped and compared to the homozygous wild-type genotype, and such grouping strategy has also been widely used in previous molecular epidemiological studies^{15, 16, 27, 28}. Among 431 subjects collected from the Armed Forces Taoyuan General Hospital, Taoyuan, Taiwan, we found no significant differences in allelic and genotypic frequencies for these four genotypic polymorphisms regarding age at LAA stroke onset (≤ 70 vs. >70 years) or gender ($p > 0.05$). Comparing cases and control subjects, genotype frequencies of either heterozygous or homozygous variants of *XRCC1*, *OGG1*, and *ERCC5* were found higher in the cases; however, the variant genotypes of these genes showed insignificantly increased LAA stroke risk after they were adjusted

Table 2. Genotype frequencies of polymorphisms of DNA repair genes among cases and controls and odds ratio in relation to risk of LAA stroke

Gene and SNP [§]	No. cases (%)	No. controls (%)	aOR (95% CI) [†]	aOR for trend [#]	<i>p</i> for permutation
<i>XRCC1 Arg399Gln</i> (rs25487; <i>G</i> > <i>A</i>)					
<i>Arg/Arg</i>	55 (47.4)	170 (54.0)	1.00 (Ref)	1.19 (0.84-1.69)	0.238
<i>Arg/Gln</i>	49 (42.2)	119 (37.8)	1.37 (0.87-2.17)		
<i>Gln/Gln</i>	12 (10.4)	26 (8.2)	1.43 (0.69-3.05)		
<i>OGG1 Ser326Cys</i> (rs1052133 <i>C</i> > <i>G</i>)					
<i>Ser/Ser</i>	25 (21.5)	86 (27.3)	1.00 (Ref)	1.13 (0.83-1.54)	0.265
<i>Ser/Cys</i>	51 (44.0)	141 (44.8)	1.27 (0.73-2.23)		
<i>Cys/Cys</i>	40 (34.5)	88 (27.9)	1.69 (0.93-3.04)		
<i>ERCC2 Lys751Gln</i> (rs13181; <i>A</i> > <i>C</i>)					
<i>Lys/Lys</i>	87 (75.0)	262 (83.2)	1.00 (Ref)	1.56 (0.97-2.51)	0.062
<i>Lys/Gln</i>	24 (20.7)	48 (15.2)	1.47 (0.85-2.54)		
<i>Gln/Gln</i>	5 (4.3)	5 (1.6)	3.03 (0.87-9.32)		
<i>ERCC5 Asp1104His</i> (rs17655; <i>G</i> > <i>C</i>)					
<i>Asp/Asp</i>	29 (25.0)	108 (34.3)	1.00 (Ref)	1.23 (0.91-1.68)	0.081
<i>Asp/His</i>	61 (52.6)	142 (45.1)	1.61 (0.95-2.67)		
<i>His/His</i>	26 (22.4)	65 (20.6)	1.50 (0.81-2.78)		

[§] The 'rs' number shown is the National Center for Biotechnology Information dbSNP cluster ID for each SNP.

[†] aOR and corresponding 95% confidence interval (95%CI) were calculated by logistic regression, in which a set of dummy variables represented different genotypes of polymorphisms of DNA repair genes. In addition, these statistical analyses were performed considering age, sex, alcohol, cigarette smoking, diabetes mellitus, hypertension, and hypercholesterolemia in the regression model.

[#] The aOR for trend indicates the odds ratio associated with subjects carrying one additional variant allele of individual SNPs, and it was estimated in the logistic regression model, in which the genotype was treated as a continuous variable.

Abbreviations: Ref, reference group.

in the multiple logistic regression model (**Table 2**). The results of genotypic analysis of *ERCC2 Lys751Gln* was important, as it showed that subjects carrying the homozygous variant genotype had an increased LAA stroke risk compared to carriers with the homozygous wild-type genotype, and that carrying one additional risk allele was associated with a 1.56-fold increased OR in LAA stroke risk (95%CI: 0.97-2.51). The possibility of a false positive due to multiple testing is less likely, because our permutation test based on 10,000 random permutations showed that this association was borderline significant ($p=0.062$) (**Table 2**). Additionally, considering subjects who carried the homozygous *ERCC2 Lys751* genotype as a reference, we found an OR of 1.69-fold increase (95%CI: 1.02-2.86, $p=0.047$) for combined heterozygous and homozygous *Lys751Gln* variants in LAA stroke risk.

The modified effect of the variant genotypes of individual genes on risk association between cigarette smoking and LAA stroke are shown in **Table 3**. Smoking habits, as described in Methods, were classified into two groups. Using *Lys751Lys* as a reference, a

greater proportion of the *ERCC2* codon 751Gln variant (*Lys751Gln* and *Gln751Gln*) was seen in the cases, conferring a significantly increased 2.73-fold risk in that subset of smokers (95%CI: 1.12-6.65, $p=0.027$) in the multivariate logistic regression model. When compared to smokers carried the respective wild-type genotypes, a higher frequency of the combined heterozygous and homozygous genotypes of the *Gln399* allele for *XRCC1*, *Cys326* allele for the *OGG1*, and *His1104* allele for *ERCC5* was found in LAA stroke patients than in control subjects; however, the modified effect of these polymorphisms on risk association between smoking and LAA stroke was insignificant (**Table 3**). In addition, we explored the joint effect of these DNA repair genes by determining the association between gene-to-gene interactions and susceptibility to LAA stroke. Using a dummy variable coding scheme and the β estimate from the logistic regression model²⁹, subjects with both polymorphisms of *OGG1 Ser326Cys* and *ERCC2 Lys751Gln* have a 2.75-fold increased OR risk compared to those with respective wild-type genotypes ($p=0.011$) (**Table 4**).

Table 3. Joint effects of genotypic polymorphisms in individual gene and smoking status on risk susceptibility of LAA stroke

Genotype	Non-smoker			Smoker		
	Case/Control	OR (95%CI) [§]	<i>p</i> value	Case/Control	OR (95%CI) [§]	<i>p</i> value
<i>XRCC1 Arg399Gln</i>						
Arg/Arg	34/121	1.00 (Ref) ^b	0.396	21/49	1.00 (Ref)	0.157
Arg/Gln, Gln/Gln	40/117	1.26 (0.74-2.14)		21/28	1.73 (0.81-3.73)	
<i>OGG1 Ser326Cys</i>						
Ser/Ser	14/57	1.00 (Ref)	0.417	11/29	1.00 (Ref)	0.189
Ser/Cys, Cys/Cys	60/181	1.32 (0.68-2.55)		31/48	1.75 (0.76-4.03)	
<i>ERCC2 Lys751Gln</i>						
Lys/Lys	59/197	1.00 (Ref)	0.445	28/65	1.00 (Ref)	0.027
Lys/Gln, Gln/Gln	15/41	1.30 (0.67-2.54)		14/12	2.73 (1.12-6.65)*	
<i>ERCC5 Asp1104His</i>						
Asp/Asp	19/80	1.00 (Ref)	0.325	10/28	1.00 (Ref)	0.168
Asp/His, His/His	55/158	1.35 (0.74-2.45)		32/49	1.82 (0.78-4.35)	

[§] Multivariate analysis of OR and 95% CI, and *p* value was assessed in a logistic regression model after adjustment for age, sex, diabetes mellitus, hypertension, and hypercholesterolemia.

Table 4. Interaction between polymorphisms of the OGG1 Ser326Cys and different DNA repair genes in LAA stroke risk

Gene/polymorphisms		Cases <i>n</i> (%)	Controls <i>n</i> (%)	OR (95%CI) [§]	<i>p</i> value
<i>OGG1</i>	<i>XRCC1</i>				
Ser/Ser	Arg/Arg	12 (10.3)	51 (16.2)	1.00 (Ref)	
Ser/Cys, Cys/Cys	Arg/Arg	43 (37.1)	119 (37.8)	1.58 (0.76-3.27)	0.217
Ser/Ser	Arg/Gln, Gln/Gln	13 (11.2)	35 (11.1)	1.62 (0.65-3.98)	0.301
Ser/Cys, Cys/Cys	Arg/Gln, Gln/Gln	48 (41.4)	110 (34.9)	1.99 (0.96-4.11)	0.062
<i>OGG1</i>	<i>ERCC2</i>				
Ser/Ser	Lys/Lys	17 (14.7)	66 (21.0)	1.00 (Ref)	
Ser/Cys, Cys/Cys	Lys/Lys	70 (60.3)	196 (62.2)	1.42 (0.77-2.60)	0.258
Ser/Ser	Lys/Gln, Gln/Gln	8 (6.9)	20 (6.3)	1.52 (0.57-4.09)	0.406
Ser/Cys, Cys/Cys	Lys/Gln, Gln/Gln	21 (18.1)	33 (10.5)	2.75 (1.26-6.00)	0.011
<i>OGG1</i>	<i>ERCC5</i>				
Ser/Ser	Asp/Asp	5 (4.3)	30 (9.5)	1.00 (Ref)	
Ser/Cys, Cys/Cys	Asp/Asp	24 (20.7)	78 (24.8)	1.77 (0.61-5.12)	0.290
Ser/Ser	Asp/His, His/His	20 (17.2)	56 (17.8)	1.85 (0.62-5.49)	0.267
Ser/Cys, Cys/Cys	Asp/His, His/His	67 (57.8)	151 (47.9)	2.47 (0.91-6.72)	0.075

[§] Multivariate analysis of the adjusted odds ratio and 95% confidence intervals were estimated in a logistic regression model after adjustment for age, sex, diabetes mellitus, hypertension, and hypercholesterolemia.

Considering an increased LAA stroke risk led by the combined effect of these four polymorphisms being the result of decreased availability of active DNA repair enzymes regarding DNA damage, we expected that subjects with a greater number of high-risk genotypes would be even more susceptible to LAA stroke development if they had been exposed to smoking en-

vironments. Based on the stratification of *OGG1 Cys326* polymorphism, we then classified our subjects into two groups according to *XRCC1*, *ERCC2*, and *ERCC5* polymorphisms; those with no more than one high-risk genotype (≤ 1) and those with at least two high-risk genotypes (2-3) in genes that participate in repairing DNA damage, and analyzed LAA stroke risk,

Table 5. Combined effects of DNA repair pathway genes on LAA stroke risk stratified by cigarette smoking

Genotype	Number of high-risk genotypes [§]	Non-smoker			Smoker		
		Case/Control	OR (95%CI) [†]	<i>p</i> value	Case/Control	OR (95%CI) [†]	<i>p</i> value
<i>OGG1</i>	<i>XRCC1/ERCC2/ERCC5</i>						
<i>Ser/Ser</i>	≤ 1	5/32	1.00 (Ref) [#]		5/18	1.00 (Ref) [#]	
<i>Ser/Cys, Cys/Cys</i>	≤ 1	32/106	1.84 (0.66-5.15)	0.248	15/35	1.56 (0.49-4.96)	0.460
<i>Ser/Ser</i>	2-3	9/25	2.23 (0.67-7.59)	0.198	6/11	1.98 (0.48-7.99)	0.346
<i>Ser/Cys, Cys/Cys</i>	2-3	28/75	2.33 (0.82-6.63)	0.114	16/13	4.43 (1.29-15.19)	0.018
<i>p</i> _{trend} =0.138				<i>p</i> _{trend} =0.010			

[§] High-risk genotypes of DNA repair genes are identified as combined genotypes of heterozygous and homozygous variants of the polymorphisms of *XRCC1* (*Arg399Gln*), *ERCC2* (*Lys751Gln*), and *ERCC5* (*Asp1104His*).

[†] A multivariate logistic regression model was estimated after considering age, sex, diabetes mellitus, hypertension, and hypercholesterolemia.

[#] Ref, reference group; risks were estimated using subjects carrying no more than one high-risk genotypes (0-1) as the reference.

because such a definition would provide statistical power to address the question. As a result, when we considered those who carried the homozygous genotype of *OGG1 Ser326* and no more than one risk genotype of *XRCC1*, *ERCC2*, and *ERCC5* polymorphisms as a reference, the combined effect of gene-to-gene interaction on increased LAA stroke risk was shown in smokers who carried *Cys326* variants together with a greater number of high-risk genotypes of *XRCC1*, *ERCC2*, and *ERCC5* (OR: 4.43, 95%CI: 1.29-15.19, *p*=0.018) (Table 5). Moreover, using a dummy variable coding scheme and the β estimate from the regression model^{30, 31)}, a tendency toward an increased LAA stroke risk was statistically significant in smokers who carried *OGG1 Cys326* polymorphisms and greater numbers of high-risk genotypes (*p*_{trend}=0.010) (Table 5).

Discussion

In this study, we attempted to analyze the association between genotype polymorphisms of BER and NER pathway genes and susceptibility in patients with LAA stroke. Support for our hypothesis came from observations that the joint effect of *OGG1*, *XRCC1*, *ERCC2*, and *ERCC5* polymorphisms significantly increased LAA stroke risk, and this association between high-risk genotypes of multiple genes and LAA stroke development was more significant in smokers. In the BER pathway, the *Ser326Cys* variant in the *OGG1* gene was known to correlate with elevated levels of oxidized metabolites, such as 8-oxo-G and

8-OHdG, in lung cancer among smokers³²⁾. The presence of the *Ser326Cys* polymorphism was associated with cancers, diabetes mellitus, and neurodegenerative disorders in molecular epidemiological studies^{22, 33-35)}. In addition, it has been reported that subjects who carry the *OGG1 Ser326Cys* polymorphism are prone to multi-vessel disease in CAD among Chinese in Taiwan³⁶⁾. In this study, the combined genotypes of heterozygous and homozygous *OGG1 Cys326* variant showed insignificantly increased LAA stroke risk when compared to the homozygous *Ser326* genotype. The power calculations in LAA stroke risk of this polymorphism are inconsistent. The major limitation in most molecular association studies is commonly ascribed to differential frequencies of unique SNPs among genetically susceptible individuals. Thus, an investigation of genetic susceptibility with a larger sample size is the best solution to resolve the current discrepancies regarding *OGG1 Cys32* in LAA stroke. On the other hand, after the subgroup stratified by the *Ser326Cys* polymorphism for the *OGG1* gene, the *ERCC2 Lys751Gln* variant genotype showed a significantly elevated risk in association with LAA stroke (Table 4). These findings suggest that *OGG1 Ser326Cys*, although not a genetic marker known to be independently associated with LAA stroke through the BER pathway, may critically and synergistically interact with individual genotypic polymorphisms of the NER pathway regarding increased LAA stroke risk. Moreover, the present case-control study is the first report of an association of DNA repair genes and LAA stroke risk in ethnic Chinese in Taiwan. It is important to

pay attention to further genetic studies among other races, such as different Asian, Caucasian, and African populations, to clarify the role of the polymorphism in the *OGG1* in the investigation of stroke.

Inter-individual variations in decreasing NER capacities were seen in carriers of the *ERCC2 Lys751Gln* and *ERCC5 Asp1104His* polymorphisms and have been reported to correlate with susceptibility to cancer^{37, 38)}. In this study, we found that the homozygous *ERCC2 Gln751* genotype is associated with an increased risk in LAA stroke, and this risk is up to 2.73-fold in smokers carrying the *Gln751* polymorphism of *ERCC2* (Table 3). A growing list of evidence has shown that cigarette smoking can result in high levels of bulky DNA adducts in the rodent heart and aorta^{39, 40)}. It has also been reported that involuntary exposure to smoking environments in those non-smokers is significantly associated with coronary heart disease^{41, 42)}. In addition, the increased lung cancer incidence among smokers influenced by *Gln751* polymorphism has been reported to be the result of accumulated levels of chromosomal aberrations and micronuclei in peripheral cells^{43, 44)}. Given that smokers with mutations in the genes in the NER pathway are defective in their ability to remove DNA cross-links, bulky polycyclic aromatic hydrocarbons (PAH)-DNA adduct, and oxidized base lesions, which may be harmful to endothelial and vascular smooth muscle cells, increased LAA stroke risk due to this polymorphism with relative lower enzyme activity to DNA repair is mechanistically reasonable. Hence, in epistatic analysis, compared to subjects with the *ERCC2 Lys751* wild-type genotype, our data justify the role of the *Gln751* polymorphism in smokers with greater susceptibility to LAA stroke.

Importantly, potentiated by multiple gene effects, cigarette smoking appears to be the predominant risk of LAA stroke; therefore, the assertion that the unique genotype polymorphisms for BER and NER pathways increase LAA stroke risk may be a result of the multiple gene make-up of the smokers. Again, these observations fit well with our aforementioned suggestion that the etiology of stroke is a polygenic multifactorial model caused by a sum of interactions between genetic and environmental factors. In the post-genomics era, it is important to note that the growing list of non-synonymous polymorphisms in the DNA repair genes may provide candidates for further investigations of stroke. Being a heterogeneous group of LAA stroke that reflects a complicated etiology modified by known factors, including hypertension, hyperlipidemia, diabetes mellitus, and obesity^{45, 46)}, genome-wide association studies with a larger sample

size that consider extensive information regarding genetic and environmental factors on modulating LAA stroke risk should contribute to better understanding of the physiology and etiology of LAA stroke development.

Conclusion

Our hospital-based case-control study used a multigenic model of DNA repair pathway genes to evaluate individual vulnerability to oxidative stress through cigarette smoking and investigated the relationship between individuals who carried polymorphisms of DNA repair genes and the risk incidence in smokers with LAA stroke. To the best of our knowledge, this is the first study of its kind. Based on this epistatic analysis, susceptible polymorphisms for BER and NER pathway genes may address the issue of risk assessment for LAA stroke among ethnic Chinese in Taiwan.

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Conflicts of Interest

The authors declare that they have no competing interests.

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