The Polymorphism of *EME1* Gene is Associated with an Increased Risk of Lung Cancer: A Case-Control Study from Chinese Population

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Abstract: DNA double-strand breaks (DSBs) can lead to genomic instability and cancer susceptibility if unrepaired. EME1 is one of the key proteins that participate in the recognition and repair of DSBs in humans. We hypothesized that the exonic variants of *EME1* are associated with lung cancer risk. In a two-stage case-control study of 1559 lung cancer patients and 1679 cancer-free controls, we genotyped two exonic variants of *EME1*(Glu69Asp: rs3760413T>G and Ile350Thr: rs12450550T>C) and analyzed their associations with risk of lung cancer. We found that the Asp variant genotypes conferred 1.35-folds risk of lung cancer compared to the Glu/Glu genotype (OR = 1.35, 95%CI = 1.18-1.56, $P = 2.18 \times 10^{-5}$) in both stages. However, the SNP Ile350Thr was not confirmed to be associated with cancer risk in both stages. Moreover, by querying the gene expression database, we further found that the 69Asp variant genotypes confer a significantly lower mRNA expression of EME1 than the Glu/Glu genotype in 260 cases of lymphoblastoid cells (P = 0.013). Our findings suggested that the SNP Glu69Asp of *EME1* is associated with an increased risk of lung cancer, and may be a functional biomarker to predict lung cancer risk in Chinese. Validations in other ethnics are warranted.

Keywords: Lung cancer, *EME1*, exonic variant, functional biomarker.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death in the world. There were over 160,000 people dying of lung cancer during 2010 in the United States [1]. In China, figures from the ministry of health suggested that the mortality rate of lung cancer increased 75.8 percent between 2004 and 2005, compared with the years 1990 to 1992 (http://www.moh.gov.cn/). In Guangzhou city, 4917 patients died of lung cancer between 2000-2002 [2]. Now, lung cancer is one of the major health issues in China and the burden is getting serious [3, 4]. Epidemiological studies of lung cancer have established many etiologic factors including smoking [5], air pollution [6] and various carcinogens [7, 8]. These factors can potentially modify the DNA and cause genomic instability and thus lead to a high risk of lung cancer. DNA double-strand breaks (DSBs) are one of the most serious DNA damage [9, 10]. Unrepaired DSBs can easily lead to chromosomal aberrations, increased genetic instability and ultimately cause cancer development, including lung cancer [11, 12].

In humans, there are two pathways that repair DSBs, which are the homologous recombination (HR) and non-homologous end-joining (NHEJ) [13-15]. In HR repair, the essential meiotic endonuclease 1 homolog 1 (*EME1*) is essential for the endonucleolytic activity of the protein complex (MUS81-EME1). EME1 protein complexes with methyl methanesulfonate-sensitive UV-sensitive 81 (*MUS81*) protein to form a structure-specific endonuclease complex MUS81-EME1 that cleaves nicked Holliday junctions [16], aberrant replication fork structures, D-loops and 3'-flap structure [17, 18]. One crucial role of *EME1* in the maintenance of genomic stability is to provide a stable interaction between the *MUS81* and a DNA substrate [19]. Without the endonuclease activity of *EME1*, *MUS81* had no detectable activity on any kind of DNA damage substrates [20]. *EME1* deficiency can lead to spontaneous genomic instability [21]. Also, haploinsufficiency of *EME1* can spontaneously promote chromosome damages and activate the intra-S-phase checkpoint through the ATM-Chk1/Chk2 pathways, and

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further activate the G2/M checkpoint through the ATM-Chk2 pathway and cause deficient DNA repair [22].

Human *EME1* gene (Gene ID: 146956) spans over 20 kb on chromosome 17q21.33, contains 9 exons and encodes a 570-amino acid protein (NCBI accession no: NP_689676). The EME1 protein consists of a central nuclease domain, two repeats of the helix-hairpin-helix (HhH) motif at C-terminal region, a linker helix, and a flexible intradomain linker that is formed with 36 residues, which is essential for the recognition of DNA [19]. Many single nuclear polymorphisms (SNPs) are observed in the *EME1* gene. Genetic variants might influence the function of gene and thus contribute to susceptibility of human disease. Exonic SNPs are well recognized to harbor the greatest potential on affecting the function of proteins coding by the genes. Therefore, we hypothesized that the exonic variants of *EME1* are associated with lung cancer risk.

To test this hypothesis, we conducted a two-stage case-control study that included a southern Chinese population with a total of 1056 lung cancer cases and 1056 cancer-free controls and an eastern Chinese population with a total of 503 lung cancer cases and 623 controls to examine the role of exonic SNPs of *EME1* in determining the susceptibility of lung cancer in Chinese.

MATERIALS AND METHODS

Study Subjects

The study subjects have been described in previously published studies [23-26]. All the subjects were ethnically Han Chinese. Briefly, in stage I, 1056 lung cancer cases and 1056 sex and age frequency-matched cancer-free controls were recruited from Guangzhou City and surrounding regions in southern China. In stage II, 503 lung cancer cases and 623 sex and age frequency-matched cancer-free controls were enrolled from Suzhou city in eastern China. We did not recruit subjects from other regions of China because we have no resources for gathering samples there. A questionnaire was used to collect information regarding demographic features, including age, sex, smoking status, biomass fuels and family history of cancer. Additional information that was only available for cases such as pathological types or tumor stages was obtained from the medical records. Participants whose BMI were <18.0kg/m^2 were categorized as being underweight, 18.0kg/m^2 to 25.0 kg/m^2 were normal body weight, and >25.0kg/m^2 were overweight. The definitions of smoking status, alcohol use, and family history of cancer have been described elsewhere [27, 28]. Each subject was asked to donate 5 mL of blood after having given a signed informed consent. The study was approved by the institutional review boards of Guangzhou Medical University and Suzhou University.

Genotyping Analysis

There are only two common SNPs with a minor allele frequency (MAF)>0.05 that are Asp69Glu (i.e., rs3760413T>G) and Ile350Thr (i.e., rs12450550T>C) with location in the coding region of *EME1* in Chinese according to the dbSNP database (http://www.ncbi.nlm.nih.gov/). The *EME1* polymorphisms were genotyped using the allele-specific fluorogenic probes on the ABI PRISM 7500 Sequence Detection Systems (Applied Biosystems, Foster City, CA, USA) by the Taqman assay as described previously [29]. For Asp69Glu (T>G), the genomic DNA was amplified in 10μL reaction system by using primers 5'-TGT TTG TGT GAC AGT TTC AGC T-3' (forward) and 5'-TGT CCT CCA GCA CCA GAG TTA TT-3'(reverse), and was detected using the probes FAM-ATT TCT GGG ACA GGT GGT G for the T allele and HEX- ATG TCT GGG ACA GGT GGT G for the G allele. For Ile350Thr (T>C), the primers are 5'-CAC TAT GAA AGG GAA GGA AAC GC -3' (forward) and 5' –TCA CCA GGG CAA ATC CAA AC-3' (reverse), and the probes are FAM- TAA CTG ACA TCA CAG CAA for the T allele and HEX-TAA CTG ACA CCA CAG CA for the C allele. The PCR procedure consists of denaturation at 95°C 10 min, then 40 cycles at 92°C for 15 seconds, 60°C for 1 min. The genotypes were automatically determined by the ABI 7500 Sequence Detection Systems software 2.0.1. 5% samples were randomly selected for sequencing and the results were 100% concordant.

Statistical Analysis

The Chi-square (χ^2) test was used to assess the differences in distributions of age, sex, smoking status, pack-years smoked, biomass fuels, family history of cancer and genotypes of exonic SNPs of *EME1* between cases and control. The Hardy-Weinberg equilibrium (HWE) was tested by a goodness-of-fit chi-square test by comparing the expected genotype frequencies with observed genotype frequencies in cancer-free controls. An unconditional logistic model was used to calculate crude and adjusted odds ratio (OR) and its 95% confidence interval (95% CI) without and with adjustment for age, sex, pack-years smoked,

biomass fuels and family history of cancer. The logistic model was also used for the trend test. A multiple interaction analysis was used to assess the possible interaction between the *EME1* SNPs and selected variables on cancer risk. The multiplicative interaction was suggested when OR 11 > OR 10 × OR 01, in which OR 11 = the OR when both factors are present, OR 01 = the OR when only factor 1 is present, OR 10 = the OR when only factor 2 is present. The statistical power was calculated by using the PS Software (http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize). All analyses were performed using the SAS software (version 9.3; SAS Institute, Cary, NC, USA). All statistical tests were 2-sided and $P < 0.05$ was considered to be statistically significant.

RESULTS

Association between the *EME1* Exonic SNPs and Lung Cancer Risk

The genotype distributions of the *EME1* SNPs, Asp69Glu and Ile350Thr, are summarized in Table **1**. As shown, there were significant deviations in the genotype frequency of both Asp69Glu and Ile350Thr

Table 1: Frequency Distribution of Genotypes in *EME1* and Results of Logistic Regression Analysis for their Associations with Lung Cancer Risk

Genotypes	Cases	Controls [a]	*P* [b]	Crude OR (95%CI)	Adjusted OR (95%CI) [c]
	n (%)	n (%)			
Stage I					
Total no. of subjects	1056	1056			
Glu69Asp (rs3760413T>G)					
Glu/Glu	467(44.2)	539(51.1)	0.007	1.00 (ref.)	1.00 (ref.)
Asp/Glu	507(48.0)	445(42.1)		1.32(1.10-1.57)	1.33(1.11-1.60)
Asp/Asp	82(7.8)	72(6.8)		1.31(0.94-1.85)	1.36(0.96-1.91)
Trend test *P* value				0.004	0.003
Asp/Glu+ Asp/Asp	589(55.8)	517(48.9)		1.32(1.11-1.56)	1.34(1.12-1.59)
Ile350Thr (rs12450550T>C)					
Ile/Ile	909(86.1)	947(89.7)	0.025	1.00 (ref.)	1.00 (ref.)
Thr/Ile	134(12.7)	103(9.7)		1.36(1.03-1.78)	1.36(1.03-1.79)
Thr/Thr	13(1.2)	6(0.6)		2.26(0.85-5.96)	2.58(0.97-6.88)
Trend test *P* value				0.007	0.005
Thr/Ile +Thr/Thr	147(13.9)	109(10.3)		1.41(1.08-1.83)	1.42(1.09-1.85)
Stage II					
Total no. of subjects	503	623			
Glu69Asp (rs3760413T>G)					
Glu/Glu	243(48.3)	352(56.5)	0.008	1.00 (ref.)	1.00 (ref.)
Asp/Glu	241(47.9)	241(38.7)		1.45(1.14-1.85)	1.45(1.13-1.85)
Asp/Asp	19(3.8)	30(4.8)		0.92(0.51-1.67)	0.85(0.46-1.57)
Trend test *P* value				0.040	0.059
Asp/Glu+ Asp/Asp	260(51.7)	271(43.5)		1.39(1.10-1.76)	1.38(1.09-1.75)
Ile350Thr (rs12450550T>C)					
Ile/Ile	410(81.5)	517(83.0)	0.091	1.00 (ref.)	1.00 (ref.)
Thr/Ile	77(15.3)	98(15.7)		0.99(0.72-1.37)	1.03(0.74-1.43)
Thr/Thr	16(3.2)	8(1.3)		2.52(1.07-5.95)	2.55(1.06-6.13)
Trend test *P* value				0.211	0.164
Thr/Ile +Thr/Thr	93(18.5)	106(17.0)		1.11(0.81-1.51)	1.14(0.84-1.56)

[a]The observed genotype frequencies of the two SNPs among the control subjects were all in agreement with the Hardy-Weinberg equilibrium in both stages ($P > 0.O05$ for all).
[b]A χ^2 test for differences in distribution of genotype frequencies between cases and controls.
[c]Adjusted in a logistic regression model that included age, sex, pack-years smoked, biomass fuels and family history of cancer.

SNPs between the cases and controls in the southern Chinese population (P = 0.007 and P = 0.025, respectively). The logistical models showed that compared to the common genotype of Asp69Glu (i.e., Glu/Glu), the Asp/Glu variant genotype conferred a significantly increased risk of lung cancer (adjusted OR = 1.33, 95%CI = 1.11-1.60, P = 0.002), and the Asp/Asp genotype also conferred an increased risk of lung cancer (OR = 1.36, 95%CI = 0.96-1.91), but the effect was not significant (P = 0.083) due to the limited sample size of individuals carrying this low frequency genotype. After combined the two risk genotypes, the Asp variant genotypes (Asp/Glu + Asp/Asp) contributed to 1.34-folds risk of lung cancer in comparison to the Glu/Glu genotype (OR = 1.34, 95%CI = 1.12-1.59, P = 0.001). Meanwhile, compared to the common genotype of Ile350Thr (i.e., Ile/Ile), the Thr/Ile variant genotype was significantly associated with an increased lung cancer risk (OR = 1.36, 95%CI = 1.03-1.79, P = 0.031) and the Thr/Thr genotype was also associated with an increased cancer risk (OR = 2.58, 95%CI = 0.97-6.88), but the effect was also not significant (P = 0.059). After combined these two risk genotypes, the Thr variant genotypes (Thr/Ile + Thr/Thr) were associated with 1.42-folds risk of lung cancer in comparison to the Ile/Ile genotype (OR = 1.42, 95%CI = 1.09-1.85, P = 0.010). Moreover, both significant trends were observed for the SNPs Asp69Glu (P_{trend} = 0.003) and Ile350Thr (P_{trend} = 0.005) in an Asp allele and Thr allele dose-dependent model, respectively.

In stage II of the eastern Chinese population, we only found a significant deviation between cases and controls in the genotype frequency of Asp69Glu (P = 0.008) but not Ile350Thr (P = 0.091). Compared to individuals carrying the Glu/Glu common genotype, individuals carrying the Asp variant genotypes harbored 1.38-folds risk of lung cancer (OR = 1.38, 95%CI = 1.09-1.75, P = 0.009). In addition, the observed genotype frequencies of the two polymorphisms were all in agreement with the Hardy-Weinberg Equilibrium (P > 0.05 for all) in both stages. The distributions of frequency of selected variables of lung cancer cases and controls are presented in Supplementary Table **S1**.

Stratification Analysis

We further combined the two populations to increase the study power in the stratification analysis. Only results for the SNP Asp69Glu were tested and showed because the significant effect of the SNP Ile350Thr was not validated in the eastern Chinese. As shown in Table **2**, the Asp variant genotypes (Asp/Glu + Asp/Asp) conferred 1.35-folds risk of lung cancer compared to the Glu/Glu genotype (OR = 1.35, 95%CI = 1.18-1.56, $P = 2.18 \times 10^{-5}$) in total populations. The increased risk of lung cancer carried by the Asp variant genotypes was still significant in almost all subgroups, except for subgroup of subjects with family history of cancer and subgroup of subjects with pack-years smoked less than 20. However, these may be due to the limited sample size. We also observed a significant interaction between sex and the Asp variant genotypes on lung cancer risk ($P_{interaction}$ = 0.007) with the OR value equaling to 1.83 (95%CI = 1.41-2.37, $P = 5.14 \times 10^{-5}$) in the stratum of females and the OR value equaling to 1.19 (95%CI = 1.01-1.41, P = 0.037) in the stratum of males.

Possible Function of the Asp69Glu by Bioinformatics Analysis

To support the biological plausibility of the variant Asp69Glu, we performed bioinformatics analysis with the Snpinfo software (http://snpinfo.niehs.nih.gov/snpfunc.html). However, the software showed the SNP has no effect on protein structure of EME1. We also queried the SNPexp database (http://app3.titan.uio.no/biotools/tool.php?app=snpexp) to discover the effect of the SNP on EME1 expression, the results show a significant correlation between the Asp69Glu genotypes and mRNA expression levels of EME1 in 260 cases of lymphoblastoid cells in all population under the dominant genetic model (P = 0.013). Cells carrying the Asp variant genotypes expressed significantly lower mRNA levels of EME1 (Asp/Asp: 7.064 ± 0.177; Asp/Glu: 7.094 ± 0.193) than cells carrying the Glu/Glu genotype (7.150 ± 0.162).

DISCUSSION

In the current two-stage case-control study among 1559 lung cancer patients and 1679 cancer-free controls, we investigated the association between *EME1* exonic SNPs and risk of lung cancer, and found that the Asp variant genotypes of the SNP Asp69Glu conferred a significantly increased risk of lung cancer in Chinese. The Asp genotypes also interacted with sex on affecting lung cancer risk. To the best of our knowledge, this is the first study on assessing the association between *EME1* SNPs and lung cancer risk.

There were two published studies reporting association between *EME1* polymorphisms and cancer risk. One was conducted in the Caucasian population

Table 2: Stratification Analysis of the *EME1* Glu69Asp Genotypes by Selected Variables in Lung Cancer Cases and Cancer-Free Controls of Merged Populations

	Patients (*n* = 1559)		Controls (*n* = 1679)		Adjusted OR (95%CI) [a]	
	Asp/Asp *n* (%)	Ile/Thr + Thr/Thr *n* (%)	Ile/Ile *n* (%)	Ile/Thr + Thr/Thr *n* (%)	Asp/Glu+Asp/Asp Vs. Glu/Glu	P_{inter} [b]
Total	710(45.5)	849(54.5)	891(53.1)	788(46.9)	1.35(1.18-1.56)	
Age (years)						
≤ 60	377(46.6)	432(53.4)	463(52.8)	414(47.2)	1.26(1.04-1.53)	0.364
> 60	333(44.4)	417(55.6)	428(53.4)	374(46.6)	1.45(1.19-1.78)	
Sex						
Male	504(46.2)	587(53.8)	601(50.7)	584(49.3)	1.19(1.01-1.41)	0.007
Female	206(44.0)	262(56.0)	290(58.7)	204(41.3)	1.83(1.41-2.37)	
Smoking status						
Never	338(46.0)	397(54.0)	496(54.3)	418(45.7)	1.40(1.15-1.70)	0.599
Ever	372(45.1)	452(54.9)	395(51.6)	370(48.4)	1.33(1.09-1.62)	
Pack-years smoked						
≥20	287(46.0)	337(54.0)	251(52.4)	228(47.6)	1.28(1.01-1.63)	0.563
<20	85(42.5)	115(57.5)	144(50.4)	142(49.6)	1.39(0.96-2.02)	
0	338(46.0)	397(54.0)	496(54.3)	418(45.7)	1.40(1.15-1.70)	
Family history of cancer						
No	626(45.1)	762(54.9)	844(53.6)	732(46.4)	1.41(1.22-1.63)	0.056
Yes	84(49.1)	87(50.9)	47(45.6)	56(54.4)	0.84(0.51-1.38)	
Clinical Stages						
I	89(44.5)	111(55.5)	891(53.1)	788(46.9)	1.41(1.05-1.90)	
II	77(52.4)	70(47.6)	891(53.1)	788(46.9)	1.00(0.71-1.40)	
III	237(48.4)	253(51.6)	891(53.1)	788(46.9)	1.19(0.97-1.46)	
IV	307(42.5)	415(57.5)	891(53.1)	788(46.9)	1.54(1.29-1.84)	
Histological types						
Adenocarcinoma	245(46.5)	282(53.5)	891(53.1)	788(46.9)	1.31(1.07-1.60)	
Squamous cell carcinoma	286(46.5)	329(53.5)	891(53.1)	788(46.9)	1.30(1.08-1.57)	
Large cell carcinoma	32(48.5)	34(51.5)	891(53.1)	788(46.9)	1.16(0.71-1.91)	
Small cell lung cancer	83(43.0)	110(57.0)	891(53.1)	788(46.9)	1.46(1.08-1.97)	
Other carcinomas [b]	64(40.5)	94(59.5)	891(53.1)	788(46.9)	1.66(1.19-2.31)	

[a]ORs were adjusted for age, sex, pack-years smoked, biomass fuels and family history of cancer.
[b]*P* value of a test the multiplicative interaction between Glu69Asp and selected variables on cancer risk were calculated using standard unconditional logistic regression models.

and found that the SNP Ile350Thr was significantly associated with an increased risk of glioblastoma [30]. The other is we previously conducted in southern Chinese, and reported that the SNP Ile350Thr was associated with risk as well as age onset of breast cancer [29]. Here, we still observed the SNP conferred a significant increased risk of lung cancer in southern Chinese. However, this effect was not validated in eastern Chinese, suggesting this SNP might not a common biomarker for lung cancer susceptibility in Chinese. Interestingly, we revealed a novel SNP that is Asp69Glu to be associated with lung cancer risk in both southern and eastern Chinese. Moreover, the SNP can interact with sex on lung cancer risk as that the adverse effect carried by the Asp variant genotypes was more pronounced in females. Genetic variants of *EME1* have been reported to affect breast cancer risk, cancer of which the patients are mostly females. Thus, it is possible that there might be some cross-talks between female special factors and the variant, which needs to be further elucidated. Taken together, we supported that the SNP Asp69Glu might be a genetic biomarker for lung cancer susceptibility of Chinese.

Multiple studies have well established that the EME1 protein plays pivotal roles in DSBs repair and functional deficient of EME1 can promote tumor ignition, growth and progression [31-33]. The EME1 protein consists of a central nuclease domain, two repeats of the helix-hairpin-helix (HhH) motif at the C-terminal region, a linker helix and a flexible intradomain linker. The SNP Asp69Glu is located in the N-terminal region and causes an amino acid change from Glutamic acid to Aspartic acid at codon 69. Results from the bioinformatics analysis show that the SNP has a significant effect on EME1 expression in lymphoblastoid cells, as those individuals carrying the Asp variant genotypes expressed significantly lower EME1 than those carrying the Glu/Glu genotype. This is consistent with our observation of Asp genotypes with increased risk of lung cancer. Once being exposed to the environmental carcinogens that damage DNA, those variant genotypes carriers may exert weaker DNA repair capacity than Glu/Glu genotype carriers and thus, are more susceptible to lung cancer.

In addition, although the association between the SNP Ile350Thr and lung cancer risk was not observed in eastern Chinese, the SNP was significantly associated with lung cancer risk in southern Chinese. We also performed bioinformatics analysis with SNPinfo software and identified that Ile350Thr polymorphism belongs to exonic splicing enhancers (ESE), which mean that this SNP may influence the selective splicing of EME1 protein, and the SNPs3D analysis showed that the Ile350Thr variation is unlikely to be tolerable indicating that the Thr variation hides an unknown biological function that is destructive. Besides, the *EME1* Ile350Thr polymorphism is located in the critical region (residues 221-438) required for DNA-binding and nuclease activity.

Although our study included a relatively large number of subjects, it has several limitations. Our present study was a hospital-based case–control study, restricted to a Chinese Han population, so there may be some bias such as selection bias or information bias. Otherwise, the fact that the genotype frequencies among controls could fit the Hardy-Weinberg disequilibrium law suggested the randomness of subject selection. Also, we have achieved a strong study power (i.e., 98.7%, two-sided test $\alpha = 0.05$) to detect an OR of 1.35 for the Asp variant genotypes (which occurred at a frequency of 46.9% in the total controls) compared with the Glu/Glu genotype. Furthermore, the finding of a significant correlation between the *EME1* Asp69Glu polymorphism and EME1 expression through bioinformatics analysis supports that the above association is biologically plausible. Therefore, it appears that our finding that the Asp variant genotypes that are associated with lung cancer risk is unlikely to be achieved by chance.

In conclusion, our data demonstrated that carriers of the Asp variant genotypes of SNP Asp69Glu had an increased risk of lung cancer in Chinese compared with carriers of the Glu/Glu genotype underlying a biological mechanism that the SNP affects the EME1expression *in vivo*. The SNP Asp69Glu of *EME1* may be a genetic biomarker for susceptibility of lung cancer in Chinese. Validations with larger population-based studies in different ethnic groups are needed.

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ABBREVIATIONS

SNP = Single nucleotide polymorphism

OR = Odds ratio

95%CI = 95% Confidence interval

MAF = Minor allele frequency

PCR = polymerase chain reaction

SUPPLEMENTAL MATERIALS

The supplemental materials can be downloaded from the journal website along with the article.

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