Influence of CMTM8 polymorphisms on Lung cancer susceptibility in the Chinese Han population

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Abstract

Background: Lung cancer is the leading cause of cancer-related mortality worldwide and CMTM8 is a potential tumor suppressor gene, which is down-regulated in lung cancer. The objective of this research was to assess the association of CMTM8 genetic polymorphisms with lung cancer risk in Chinese Han population. Methods: To evaluate the correlation between CMTM8 polymorphisms and lung cancer risk, Agena MassArray platform was used for genotype determination among 509 lung cancer patients and 506 controls. Multiple genetic models, stratification analysis and haploview analysis was used by calculating odds ratio (OR) and 95% confidence intervals (CIs). Results: Significant associations were detected between CMTM8 rs6771238 and an increased lung cancer risk ($p < 0.05$). In stratified analysis, rs6771238 was related to increased risk of lung squamous cell carcinoma ($p < 0.05$), rs6771238 was associated with increased risk of lung adenocarcinoma ($p < 0.05$), rs9835916 and rs1077868 were correlated with lung cancer staging ($p < 0.05$), and rs9835916 was correlated with increased risk of lymph node metastasis in lung cancer patients ($p < 0.05$). Additionally, Haplotype analysis illuminated that haplotypes GG and AG were closely correlated with lung cancer staging, and haplotype AG was correlated with increased lung cancer risk among individuals older than 50 years ($p < 0.05$). Conclusions: Our study first reported that the CMTM8 polymorphisms were risk factors for lung cancer in Chinese Han population. These findings also suggested the potential roles of CMTM8 in the development of lung cancer.

Introduction

Lung cancer is the leading cause of cancer-related mortality worldwide. Lung cancer is also a major health problem in China, where 605,946 new cases of lung cancer (416,333 men and 189,613 women) and 486,555 lung cancer-related deaths were reported in 2010 [1, 2]. Despite the advance of therapeutic strategies, the prognosis of lung cancer patients remains poor, and the 5-year survival rate remains less than 10% in most parts of the world [3, 4]. The reason for this lack of improvement may be due to the high invasion and recurrence rate of lung cancer. Lung cancer development appears to result from a complex interaction between environmental exposures and genetic factors. And genetic factors may play a fundamental role in the development of lung cancer [5]. Therefore, novel biomarkers for predicting the tumor progression of lung cancer are urgently needed.

CMTM8 (CKLF-like MARVEL transmembrane domain containing 8), also known as *CKLFSF8*, belongs to the chemokine-like factor gene superfamily, a novel family that was first cloned by the Peking University Human Disease Genomics Research Center in 2003 [6-8]. The encoding product of this family gene has a special structure, which is between chemokines and four transmembrane proteins. Studies have shown that *CMTM8* is a potential tumor suppressor that can participate in various signal transduction pathways to control tumor occurrence and development, thereby affecting tumor formation, development and metastasis [9]. *CMTM8* is widely expressed in many normal human tissues and is often downregulated or absent in multiple solid tumors, including the liver, lung, colon, rectum, esophagus, stomach [10]. The over-

expression of *CMTM8* can inhibit the proliferation, migration, and invasion of carcinoma cells [11]. Hence, *CMTM8* is a potential marker of early tumor detection in many cancers, including lung cancer.

Single nucleotide polymorphism (SNP), as a natural sequence variation, may affect the expression level of *CMTM8*. With respect to genetic variation, SNPs have been utilized in lung cancer research [12, 13]. However, the risk of *CMTM8* polymorphisms on lung cancer has been unexplored to date. Therefore, we performed a case-control study to determine whether *CMTM8* gene region SNPs impact susceptibility to lung cancer in Chinese Han females in Heilongjiang Province (CHB population). We also performed stratification analysis of lung cancer to evaluate the relationship between *CMTM8* polymorphism and different stratification indexes of lung cancer.

Materials And Methods

Ethics statement

The use of human blood sample and the protocol in this study were strictly comply with the criterions of the Declaration of Helsinki and were approved by the Ethics Committee of the Northwest University, Xi'an, China. Written informed consent was received from each participant.

Study participants

The present hospital-based case control study included a total of 509 lung cancer cases and 506 healthy controls. Lung cancer subjects were recruited from the Tumor Hospital of Shaanxi province, China. Controls were taken from the people who visited the hospital for routine check-up with no history of cancer and any diseases associated with vital organs. All lung cancer cases were newly diagnosed and histopathologically confirmed.

Clinical data and demographic information

We use a standardized epidemiological questionnaire including residential region, age, gender, smoking status, alcohol use, ethnicity, education status, and family history of cancer to collect personal data in an in-person interview. 5 ml of venous blood sample was drawn from each subject and used for DNA extraction and genotyping. All volunteers signed an informed consent form explaining the research purpose of the blood withdrawal.

SNPs selection and genotyping

We screened the SNPs of *CMTM8* with over 5% minor allele frequency (MAF) and disease relevance in 1,000 genome (http://www.internationalgenome.org/). In this study, six SNPS (rs9853415, rs6796318, rs6771238, rs9835916, rs1077868, rs6802418) were selected for genotyping based on the potential role of *CMTM8* gene in the occurrence and development of cancer and previous studies on this gene. Genomic DNA was isolated from peripheral whole blood employing the Gold Mag - Mini Whole Blood Genomic DNA Purification Kit (Gold Mag Co. Ltd., Xi'an, China) following the manufacturer's instructions

and quantified by Nano Drop spectrophotometer 2000 C (Thermo Scientific, Waltham, Massachusetts, USA). Polymerase chain reaction (PCR) extension primers were designed for these SNPS by MassARRAY Assay Design 3.0 software (Agena). Primers in this study were listed in **Supplementary Table S1**. SNP genotyping analysis was carried out at Agena MassARRAY RS1000 instrument (Shanghai, China) system according to the standard scheme recommended by the manufacturer, and data were managed and analyzed by Agena Typer 4.0 software [14, 15].

Statistical analyses

Hardy-Weinberg equilibrium (HWE) of each tSNP in control group was tested by Fisher s exact test [5]. Allele frequencies and genotype frequencies for each SNP of case and control subjects were compared using the Chi squared test. Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using unconditional logistic regression analysis with adjustments for age, gender [16-18] . Associations between genotypes and lung cancer risk were tested in different genetic models (co-dominant, dominant, recessive, and log-additive) by SNPStats website software http://bioinfo.iconcologia.net/snpstats/start.htm [19, 20]. All statistical analysis were performed using SPSS statistical package, version 19.0 (SPSS Inc., Chicago, IL, USA). Haploview software version 4.2 was used to analyze the association between haplotypes and the lung cancer [21]. All *p* values in this study were two-sided, and *p*-value of less than 0.05 as the cutoff value for statistical significance.

SNP functional annotation and Gene expression analysis in GEPIA database

HaploReg v4.1 database (https://pubs.broadinstitute.org/mammals/haploreg/haploreg.php) was applied for exploring functional annotations of the candidate SNPs. Through GEPIA database (http://gepia.cancer-pku.cn/index.html) to predict *CMTM8* gene expression in lung tissue.

Results

General characteristics

Basic characteristics of the patient and control groups are depicted in **Table 1**. As shown, 509 lung cancer cases included 155 females and 354 males with a mean age of 58.53 ± 10.12 years. The 506 healthy controls were comprised of 151 females and 355 males with a mean age of 61.43 ± 9.47 years. There were no significant difference between the lung cancer patients and healthy controls in terms of age and gender.

Hardy–Weinberg equilibrium and SNP alleles

The MAF distribution of selected six SNPs among all subjects were summarized in **Table 2**. In our study, the frequency of alleles of each SNP in controls was consistent with the HapMap CHB population. Furthermore, all six SNP locus in the control subjects conformed to Hardy-Weinberg equilibrium ($p > 0.05$). By chi-square test, we found no SNP sites associated with lung cancer risk.

Association of SNPs with lung cancer risk

Four genetic analysis models (co-dominant, dominant, recessive and log-additive) were applied to analyze and assess the association between each variant and lung cancer risks. In **Table 3**, our analysis revealed the genotype "A/C" of rs6771238 was correlated with improved the risk of lung cancer under the co-dominant model (OR = 1.57, 95% CI=1.01 - 2.42, p = 0.044), the genotype "C/A-A/A" of rs6771238 was correlated with an enhanced lung cancer risk in the dominant mode (OR = 1.54, 95% CI = 1.01-2.36, p = 0.047).

Further, we stratified the samples according to pathological classification, clinical stage, lymph node metastasis and other characteristics. Within the subgroups of lung squamous cell carcinoma and lung adenocarcinoma, the genotype "A/C"of rs6771238 (OR = 2.07, 95% CI = 1.08-3.97, p = 0.028) showed an increased risk of lung squamous cell carcinoma in the co-dominant model. The genotype"A/C-A/A"of rs6771238 also was significantly associated with increased lung squamous cell carcinoma risk under the dominant model (OR = 2.07; 95% CI = 1.10-3.89; p = 0.025). Rs6771238 also was significantly correlated with an enhanced lung squamous cell carcinoma risk under log-additive model (OR = 1.90; 95% CI = 1.06 - 3.38; p = 0.030). While the genotype "A/G" of rs6802418 (OR = 1.43, 95% CI = 1.00-2.03, p = 0.049) may increase the risk of lung adenocarcinoma under the co-dominant model (**Table 4**).

Stratified analysis was performed according to clinical stages, it was found the genotype "T/C" of rs9835916, and rs1077868 were significantly correlated with an enhanced the risk of lung cancer staging under the co-dominant model (OR = 1.75; 95 % CI = 1.00-3.05; p = 0.031) and log-additive model (OR = 1.71; 95 % CI = 1.02-2.88; p = 0.043), respectively (**Table 5**).

Stratified subgroup in the case of lymph node metastasis, only rs9835916 was found to be associated with lymph node metastasis risk in patients with lung cancer. For rs9835916, the "T/C" genotype may increase the risk of lymphatic metastasis under the co-dominant model (OR = 2.51).95% CI = 1.42 - 4.44; p = 0.002), the "T/C-C/C" genotype was related to increased lymphatic metastasis risk in the dominant model (OR = 2.39; 95% CI = 1.40 - 4.07; p = 0.001), rs9835916 may increase the risk of lymphatic metastasis based on the additive model (OR = 1.64; 95% CI = 1.11 - 2.41; p = 0.013), allele "C" also may increase the risk of lymphatic metastasis based on the allele model (OR = 1.56; 95% CI = 1.08 - 2.26; p = 0.018) (**Table 6)**.

Association of haplotypes with lung cancer risk

A haplotype-based association study was performed to show the association between *CMTM8* haplotype and risk of lung cancer. Among the subpopulation (staging), two SNPs (rs1077868 and rs6802418) form an LD block (**Fig. 1**). The frequencies' distribution of haplotypes in case and control group is presented in **Table 7.** The haplotypes "GG" and "AG" was found to prominently increase the risk of lung cancer staging (OR=1.71; 95 % CI= 1.02 - 2.88; p = 0.043).

SNP functional evaluation

In order to evaluate the possible function of the six selected variants in the *CMTM8* gene, we performed a bioinformatics analysis using the HaploReg v4.1 database. The results showed that all the variants were predicted to be regulatory SNPs with different biological functions (**Supplementary table S2**).

GEPIA database analysis on gene expression

Furthermore, GEPIA database analyzed the expression of *CMTM8* gene in lung cancer and found that the expression level of *CMTM8* gene in lung adenocarcinoma was lower than that in normal tissues, which indicates that this gene has a certain relationship with the occurrence of lung cancer (**Supplementary Figure S1**)

Discussion

In recent years, a growing number of studies have found that the *CMTM8* gene plays an important role in the tumor s formation, development and metastasis, and the expression of CMTM8 is down-regulated in lung cancer. In this study, we genotyped five polymorphisms of *CMTM8* and evaluated their correlations with the risk of lung cancer in a Chinese Han population. Our results first showed that rs6771238 was associated with increased lung cancer susceptibility in Chinese Han Population. Stratified analysis showed that rs6771238 was related to increased risk of lung squamous cell carcinoma, rs6771238 was associated with increased risk of lung adenocarcinoma, rs9835916 and rs1077868 were correlated with lung cancer staging, and rs9835916 was correlated with increased risk of lymph node metastasis in lung cancer patients. Haplotype analysis illuminated that “GG” and “AG” were closely correlated with lung cancer staging, and “AG” was correlated with increased lung cancer risk among individuals older than 50 years. To our knowledge, this is the first time to explore the association between *CMTM8* gene polymorphism and lung cancer risk in Chinese Han Population.

Human *CMTM8* localizes to chromosome 3p22.3, where other known tumor suppressor genes that are frequently deleted or methylated in tumors reside [22, 23]. *CMTM8* may be silenced or down-regulated in a similar manner during tumorigenesis. Previous studies demonstrate that *CMTM8* induces caspase-dependent and caspase-independent apoptosis in multiple tumor cell lines [6]. Downregulation of *CMTM8* in epithelial cells induces epithelial-mesenchymal transformation (EMT) through MEK-ERK signaling [9]. Overexpression of *CMTM8* can accelerate the rate of epidermal growth factor receptor internalization, attenuates epidermal growth factor receptor mediated signaling, and inhibits tumor cell growth [8]. At present, indications for tumor suppressive function of *CMTM8* gene products have been found in several tumor types. In osteosarcoma, it was confirmed that *CMTM8* was identified as a candidate tumor suppressor gene, which inhibited the EGFR signaling pathway and affected the occurrence of osteosarcoma [8, 24] *CMTM8* underexpression may result in upregulation of EGFR signaling. In bladder cancer, *CMTM8* is also an important tumor suppressor gene and a useful prognostic indicator for patients with bladder cancer [11, 25]. It is inferred that *CMTM8* overexpression blocks c-MET signaling in vivo model of bladder cancer. Studies have also demonstrated that downregulation of *CMTM8* induced epithelial-to-mesenchymal transition-like changes via c-MET/extracellular signal-regulated kinase (ERK)

signaling in HepG2 hepatocellular carcinoma cells (14), thereby affecting the cancer process [9]. In addition, *CMTM8* was negatively correlated with the tumorgenesis and development of clear-cell renal cell carcinoma, and the location and intensity of expression were significantly correlated with prognosis [26].

However, the expression of *CMTM8* in lung cancer is only known to be down-regulated, and other relevant reports are relatively few. In present study, we investigated for the first time the relationship between *CTMT8* and lung cancer susceptibility. Our results suggest that rs6771238 was associated with increased lung cancer susceptibility in Chinese Han population. Stratified analysis showed that rs6771238 was associated with the risk of lung squamous cell carcinoma, rs6771238 was associated with increased risk of lung adenocarcinoma, rs9835916 and rs1077868 were associated with lung cancer stage, and rs9835916 was associated with lymph node risk in lung cancer patients. In addition, it should be noted that the incidence of lung cancer significantly different according to the different layers. Considering the potential function of the selected SNPs in our study and its influence on gene expression, we speculated that SNPs may affect the carcinogenic process by changing the protein expression and this process may be influenced by individual background, thus leading to different outcomes on lung cancer risk.

Nevertheless, there are limitations that need to be noticed in the present study. First, because subjects are enrolled from the same hospital, inherent selection bias and information bias are inevitable problems. Second, our current research is fundamental, and further functional studies and larger population based prospective studies are required to illuminate the genetic factors underlying lung cancer. Despite the limitations mentioned above, our current findings provide scientific evidence for future studies of gene *CMTM8* with the risk of lung cancer.

Conclusion

To sum up, our study revealed a indicated a novel association between CMTM8 polymorphisms and risk of lung cancer among North Indian population. These studies may help elucidate the underlying mechanisms for CMTM8 polymorphisms in lung cancer. More experimental studies of larger sample size and expression studies are necessary to further explore and confirm the role of these variants in increasing lung cancer risk, which will help in better understanding the genetic heterogeneity in complex diseases like lung cancer.

Abbreviations

CMTM8	CKLF-like MARVEL transmembrane domain containing 8
SNP	Single nucleotide polymorphism
HWE	Hardy–Weinberg equilibrium
OR	Odds ratio
95%CI	95% confidence intervals
LD	Linkage disequilibrium
MAF	Minor allele frequency

Declarations

Acknowledgments

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Conflict of Interest

The authors declare that they have no conflict of interest.

Informed consent

Informed consent was obtained from all individual participants included in the study.

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Author Contributions

TBJ and QFL: conceived and designed the experiments;

ZIX and FLN: performed the experiments;

YWL and HYL: analyzed the data;

JFL: contributed reagents/materials/analysis tools;

JMW and YS: drafted the work or revised it critically for important content.

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Figures

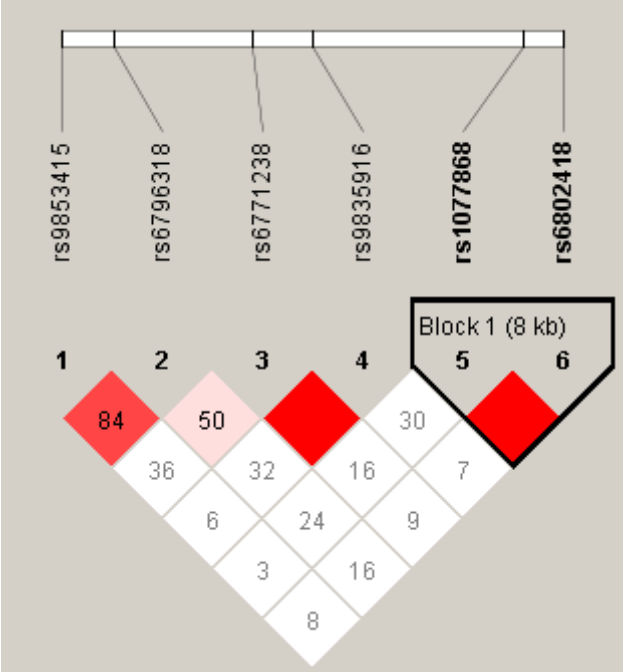

Figure 1

A haplotype-based association study was performed to show the association between CMTM8 haplotype and risk of lung cancer. Among the subpopulation (staging), two SNPs (rs1077868 and rs6802418) form an LD block

Supplementary Files

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