RESEARCH ARTICLE

Mll3 Genetic Variants Affect Risk of Gastric Cancer in the Chinese Han Population

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Abstract

It is reported that the expression level of MLL3 in gastric cancer tissue highly correlates with tumor progression. However, whether MLL3 genetic variants are associated with the risk of gastric cancer remains unclear. In this study, we conducted a genotyping analysis for MLL3 in 314 cases of gastric cancer and 322 controls from the Chinese Han population. 4 SNPs (rs6943984, rs4725443, rs3800836, rs6464211) were selected for the present analysis. We found 2 SNPs (rs6943984, rs4725443) of MLL3 gene were significantly associated with the risk of gastric cancer : the rs6943984 with the minor allele A and rs4725443 with the minor allele C revealed strong associations with increased gastric cancer risk [*P* <0.001, OR=1.97, 95% CI=1.48~2.64 and *P*<0.001, OR=2.23, 95% CI=1.54~3.24]. Haplotype analysis of the four SNPs showed that haplotype A-T-A-C, G-T-G-C, and G-C-A-C increased the risk of gastric cancer (*P*<0.001, P=0.18, and *P*<0.001, respectively), while haplotype G-T–A-C significantly reduced the risk of gastric cancer (*P*<0.001). We concluded that MLL3 variants are significantly associated with gastric cancer risk. Our results for the first time provided new insight into susceptibility factors of MLL3 gene variants in carcinogenesis of gastric cancer of the Chinese Han population.

Keywords: MLL3 - gastric cancer - genetic variants - Chinese Han

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Introduction

Gastric cancer is a highly aggressive and lethal malignancy. It accounts for 8.6% of new cancer cases worldwide. Of the estimated annual 930,000 new gastric cancer cases, about 700,000 cases died from this disease (Parkin et al., 2002). Despite a general decline in incidence rates of gastric cancer (GC), it remains the fourth most common cancer and one of the leading causes of cancer death worldwide (Siegel et al., 2011). Recent studies indicated that some traditional factors, such as nitrates, smoked fish and salted meats, moldy foods containing aflatoxin, and the infection with Helicobactor pylori (H. pylori), were thought to be involved in the development of gastric cancer (Shin et al., 2011), however the detailed mechanisms of gastric cancer remain unclear. Recent studies suggested that gastric cancer is a multifactor disorder resulting from the interaction between some enviromental factors and the genetic background. The susceptibility of the host genetic factor to malignant disease is a dynamic interactive process, which is thought to be involved in the balance between the host immune response and the malignant cell apoptosis (Kim, 2007). Although the genetic basis of host susceptibility to the development of gastric cancer had not been clearly discovered, several previous study reported that cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) gene promoter polymorphism might significantly increase the risk of gastric cancer development (Hou et al., 2010).

MLL3 is a member of the TRX/MLL gene family and maps to chromosome 7q36.1. It encodes a predicted protein of 4911 amino acids containing two plant homeodomains (PHD), an ATPase alpha/beta signature, a high mobility group, a SET (Suppressor of variegation, Enhancer of zeste, Trithorax) and two FY (phenylalanine tyrosine) rich domains. PHD and SET domains proteins are chromatin regulators and several of them are altered in cancer (Saha et al., 1995). Inactivation of MLL3 in mice results in epithelial tumor formation, suggesting that it functions as a tumor-suppressor gene (Lee et al., 2009). Also, MLL3 has been reported to be frequently deleted in myeloid leukemias (Dohner et al., 1998; Ruault et al., 2002). Moreover, other reports indicate somatic mutations in the MLL3 gene in glioblastoma and pancreatic ductal adenocarcinoma (Balakrishnan et al., 2007). However, the relation between genetic polymorphism of MLL3 and gastric cancer is still unknown.

Here, we described a case-control study that aimed to test the relationship between MLL3 gene polymorphisms and the occurrence of gastric cancer.

Materials and Methods

Subjects

A total of 636 genetically unrelated subjects including 322 normal controls and 314 gastric cancer patients

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Table 1. Characteristics of Study Participants

	Total			Men			Women		
	Case group	Controls	*P*	Case group	Controls	*P*	Case group	Controls	*P*
Number of subjects	314	322		203	214		111	108	
Age (mean±SD, years)	53.1±12.3	53.7±11.7	0.4585	52.4±11.3	52.7±10.6	0.738	54.7±13.1	53.9±10.9	0.577
Smoking (n, %)	265(60.23)	176(39.55)	<0.001	201(66.78)	132(44.75)	<0.001	64(46.04)	44(30.34)	0.006
Alcohol use (n, %)	194(44.09)	188(42.73)	0.734	146(48.50)	144(48.81)	0.904	48(33.09)	44(30.34)	0.891
Family history of cancer (n, %)	40 (12.74)	12 (3.73)	<0.001	24 (11.82)	7 (3.27)	<0.001	16 (14.41)	5(4.63)	<0.001

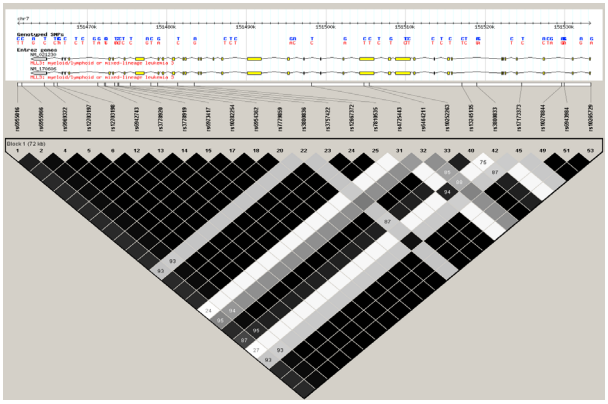


Figure 1. Genetic Variation at Human MLL3 Gene. Using the Haploview 4.2 software and the HapMap phrase II database, we scanned 25 genotyped single-nucleotide polymorphisms (SNPs) in Chinese Han. Linkage disequilibrium (LD) blocks across the locus in Chinese Han. LD blocks derived by solid spline method in Haploview 4.2. LD value shown: r 2 × 100; r 2 colour scheme: r^2 =0: white; 0 < r^2 <1: shades of grey; r^2 =1: black

participated in this study after giving written informed consent. They were recruited from the General Hospital of the People's Liberation Army between March 2009 and May 2012. All subjects were Han Chinese and were raised in Beijing. Histological demonstration of them was confirmed by two pathologists. Individuals that were previously cancerous or had metastasizing cancer from other origins were excluded. For gastric cancer patients, the clinic-pathological vari-ables, including tumor site, tumor area, differentiation grade, depth of tumor infiltration, lymph node metastasis, distant metastasis,and TNM stage, were obtained from the medical records (Table 1). None of the patients had undergone radiotherapy or chemotherapy before surgery. The variables of depth of tumor infiltration, lymphnode metastasis, distant metastasis, and TNM stage were examined and staged according to the American Joint Commission for Cancer Staging in 2002. The controls have no gastrointestinal disorders or personal and familial history of cancers, which was traced back to ≥ 3 generations and laterally to 2nd and 3rd degree relatives. They were obtained from the hospital of patients' routinely healthy examinations.

Genomic DNA samples

The whole blood samples from patients and controls were collected and stored in Vacutainer® tubes (BD Franklin Lakes, NJ) containing anticoagulant of EDTA. Total genomic DNA was extracted from the whole blood according to the phenol/chloro form method. The purity and concentration of the extracted DNA were determined by UV-VIS Spectrophotometer. The extracted DNA was stored at 4°C in TE buffer (10 mmol/L Tris-HCl, 1 mmol/L EDTA, pH 8.0).

Genotyping

There are 8070 SNPs for the human MLL3 gene listed in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/SNP). We also screened the data for the Tag SNPs on the International HapMap Project website (http: //www. hapmap. org/) (Figure 1). There were 4 SNPs were obtained for our study. We designated these SNPs as SNP1 (rs6943984), SNP2 (rs4725443), SNP3 (rs3800836), and SNP4 (rs6464211) which were in order of increasing distance from the 5' end of the gene. Genotyping was performed using the TaqMan® SNP Genotyping Assay (Applied Biosystems Inc.) as described previously (Xie et al., 2009). All assays were conducted blindly by two researchers without the knowledge of case or control status. Additionally, about 10% of the samples were randomly selected and retested, and the results were 100% concordant.

Statistical analysis

Data analysis was performed using the computer software Statistical Package for Social Sciences-SPSS for Windows (version 13.0). Hardy-Weinberg equilibrium was assessed by chi-square analysis. Measurement data are shown as means ±SD, and the differences between the MI patients and the control subjects were assessed by independent - sample T test. Differences in enumeration data between MI patients and control subjects were analyzed using the Chi-square test. Differences in distributions of genotypes, alleles, and haplotypes between MI patients and control subjects were analyzed using the SHEsis platform (Li et al., 2009). The pairwise LD analysis was performed using 4 SNP pairs. We used |D'| values of > 0.5 to assign SNP locations to 1 haplotype block. In the haplotype-based case control analysis, haplotypes with a frequency of < 0.03 were excluded. The frequency distribution of the haplotypes was calculated by performing a permutation test using the bootstrap method. In addition, logistic regression analysis was performed to assess the contribution of the major risk factors. Statistical significance was established at $P < 0.05$.

Results

Study population characteristics

In our experiment, MLL3 genetic polymorphisms were found in 636 Chinese individuals consisting of 314 gastric cancer patients and 322 controls. All these four SNPs were in Hardy-Weinberg equilibrium ($P > 0.05$). There were no statistically significantsignificant differences in age

Table 2. Genotypes and Alleles Distribution of Cases and Control Subjects

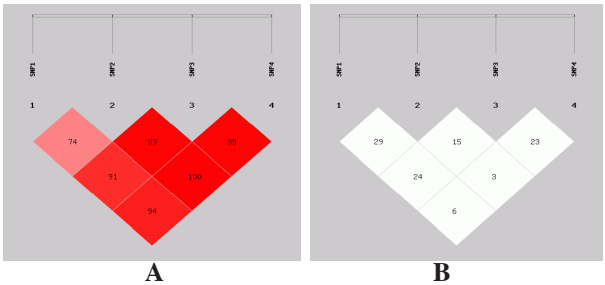
SNP	n	Genotype			*P*	Allele		*P*	OR (95%CL)
SNP1		AA	AG	GG		A	G		
Cases	314	22(0.070)	107(0.341)	185(0.589)	<0.001	151(0.240)	477(0.760)	<0.001	1.97 (1.48~2.64)
Control	322	8(0.025)	73(0.227)	241(0.748)		89(0.138)	555(0.862)		
SNP2		CC	CT	TT		C	T		
Cases	314	8(0.025)	76(0.242)	230(0.732)	<0.001	92(0.146)	536(0.854)	<0.001	2.23 (1.54~3.24)
Control	322	3(0.009)	40(0.124)	279(0.866)		46(0.071)	598(0.929)		
SNP3		AA	AG	GG		A	G		
Cases	314	58(0.185)	150(0.478)	106(0.338)	0.293	266(0.424)	362(0.576)	0.309	0.89 (0.71~1.11)
Control	322	60(0.186)	171(0.531)	91(0.283)		291(0.452)	353(0.548)		
SNP4		CC	CT	TT		C	T		
Cases	314	198(0.631)	97(0.309)	19(0.061)	0.499	493(0.785)	135(0.215)	0.222	1.18 (0.91~1.53)
Control	322	189(0.587)	109(0.339)	24(0.075)		487(0.756)	157(0.244)		

**P* value indicated the global difference of genotypes or alleles between case and control group; OR value refers to the major allele such as G of SNP1, T of SNP2, G of SNP3 and C of SNP4

Table 3. Haplotype Distribution of Patients and Control Subjects

Hplotypes	Hplotypes frequencies		χ^2	*P*	OR	95% CI
	Case (n=314)	Control(n=322)				
A C A C	49.33(0.079)	46.00(0.071)	0.318	0.572	1.128	0.743~1.712
A T A C	91.66(0.146)	42.98(0.067)	22.02	<0.001	2.435	1.664~3.564
A T G C	0.03(0.000)	0.02(0.000)	-	-	-	-
G T A C	82.34(0.131)	202.02(0.314)	58.838	<0.001	0.336	0.253~0.447
G T G C	226.97(0.361)	195.98(0.304)	5.608	0.018	1.327	1.050~1.677
G T G T	125.02(0.199)	157.00(0.244)	3.13	0.076	0.787	0.603~1.027
A T G T	9.98(0.016)	0.00(0.000)	-	-	-	-
G C A C	42.67(0.068)	0.00(0.000)	46.022	<0.001	-	-

Global chi2 is 116.169, while df = 5 (frequency<0.03 in both control & case has been dropped) Fisher's p value is 2.66×10^{-15}



A B

Figure 2. The Patterns of Linkage Disequilibrium in the MLL3 Gene, with Their | D'| (A) and r^2 Values (B)

distribution ($P = 0.459$) between patients and controls by the Mann-Whitney U test or Chi-square test (Table 1). The family cancer history and smoking in case group showed statistically significant difference from the control group ($P < 0.001$, $P<0.001$, respectively). The amounts of alcohol intake were similar between patients and controls.

Association between MLL3 polymorphisms and gastric cancer

We found 2 SNPs (rs6943984, rs4725443) of MLL3 gene were significantly associated with the risk of gastric cancer. Of which, the rs6943984 with the minor allele A and rs4725443 with the minor allele C revealed strong associations with increased gastric cancer risk [$P<0.001$, odds ratio (OR) = 1.97, 95% CI = 1.48-2.64 and $P<0.001$, OR = 2.23, 95% CI = 1.54-3.24] (Table 2).

Figure 2 shows patterns of linkage disequilibrium in the MLL3 gene, with their | D'| and r^2 values. All 4 SNPs are located in 1 haplotype block because all | D'| are beyond 0.5. All 4 SNPs were available for the performance of a haplotype-based case-control study because all of the r^2 values were below 0.5.

In the haplotype-based case-control analysis, haplotypes were established through the use of 4 SNPs (Table 3). The frequency of the A-T-A-C, G-T-G-C, and G-C-A-C haplotypes was significantly higher for gastric cancer patients than for control subjects ($P < 0.001$, $P=0.018$, $P<0.001$, respectively). However, the frequency of G-T-A-C haplotype was lower for gastric cancer patients than for control subjects ($P < 0.001$) (Table 3).

Discussion

In this study, we found genetic polymorphisms of MLL3 were associated with the risk of gastric cancer in Chinese Han population. To the best of our knowledge, this is the first study to analyze the relation between MLL3 genetic polymorphism and gastric cancer in China.

At present, it is generally accepted that gastric cancer is a complex multifactorial and polygenic disorder in which multiple environmental and genetic factors are simultaneously involved (Duell et al., 2012; Zhang et al., 2012).The foundation for human studies examining putative causative genes that may be involved in gastric cancer is based on a candidate gene approach. This involves selecting a functionally relevant gene to study and subsequently investigating its association with the gastric cancer phenotype. Recently, a few studies

into the genetic polymorphisms of MLL3 revealed a positive association with colorectal cancer and pancreatic carcinoma (Balakrishnan et al., 2007; Watanabe et al., 2011). Therefore, the MLL3 gene is thought to be a candidate gene for gastric cancer. In the present study, we genotyped 4 SNPs in MLL3 in Chinese Han subjects, and assessed the association between MLL3 and gastric cancer using a haplotype-based case-control analysis. The AA or AG genotype of rs6943984 and the CC or CT genotype of rs4725443 significantly differed between gastric cancer patients and control subjects, indicating that the risk of gastric cancer is increased in subjects with the A allele of rs6943984 and C allele of rs4725443. In addation, we successfully established haplotypes for the MLL3 gene from the different combination of the 4 SNPs. The frequency of the A-T-A-C, G-T-G-C, and G-C-A-C haplotypes were significantly higher for gastric cancer patients than for control subjects. However, the frequency of G-T-A-C haplotype was lower for gastric cancer patients than for control subjects.

In conclusion, the present results indicate that gastric cancer is associated with the polymorphism rs6943984 and rs4725443 of MLL3.

Acknowledgements

The author(s) declare that they have no competing interests.

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