Genetic variants of C1orf10 and risk of esophageal squamous cell carcinoma in a Chinese population

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E nvironmental agents can influence tissue integrity, disease development, and related cancer development rate including those of the skin, breast, and gut.1–3 The cellular stress protein response system evolved and plays a key role in minimizing cell injury and maintaining tissue integrity in response to damaging levels of environmental agents.4 As such, the integrity of this system plays a role in modifying progression of diseases associated with aging, DNA or protein damage, and chronic injury. Cells of the normal human esophageal squamous epithelium are under relatively unique environmental stresses including bacterial infestation, viruses, thermal stresses, refluxed acid, oxidizing chemicals, and bile adducts, which contribute to tissue damage, promote aging, and initiate diseases.5–7 To defend these environmental stresses and maintain tissue integrity, a novel type of stress protein system has evolved in mammals.8 Chromosome 1 open reading frame 10 (C1orf10), a novel human gene identified by modified differential display PCR in 2000, is just one of the novel genes within the stress proteins.9,10 It maps to chromosome 1q21 which is commonly suppressed in ESCC by undefined general mechanism, and is suggested to be one member of the EDC.11–13 Besides, molecular characterization of C1orf10 revealed its role as a stress responsive protein, including its upregulated expression by stimulation of heat or acidic reagent,14 the activity as an anti-apoptotic factor which attenuates the death induced by exposure of cells to normally lethal levels of DCA.15 As we know, esophageal cancer is caused by genetic and environmental factors, and stress proteins play important roles in the later process.7 As a member of the stress proteins in esophagus, the role of C1orf10 in stress response suggests that abnormalities of its expression may result in the abnormal response of esophageal epithelium to environmental stimulation which possibly leads to cancer. A previous study has shown that the expression of C1orf10 is either dramatically reduced or absent in esophageal cancer cell lines as well as primary esophageal cancer tissues compared with the corresponding normal esophageal mucosa.9,16 We then hypothesized whether the genetic variants of this gene are associated with ESCC.

Common variants which alter the amount of protein expression, for instance by affecting transcriptional regulation, or subtly alter the activity of protein itself, would be expected to have a quantitative influence on disease activity. In this study, we sought to identify new and functional polymorphisms in the promoter region, coding region, and the untranslated region of the C1orf10 gene. We further carried out a large scale case-control study in the Han Chinese population to examine whether the haplotypes were associated with susceptibility to esophageal cancer.

Materials and Methods

Primer design for scanning the C1orf10 gene. Referring to the human C1orf10 sequence (GenBank accession no. AL135842), four overlapping PCR amplicons were designed to amplify the promoter sequence (2000 bp), whereas three PCR fragments were generated to scan the 5′ UTR, coding region, and 3′ UTR (Table 1). Scanning the C1orf10 gene. Genomic DNA was purified from a panel of 32 unrelated healthy subjects. PCR was carried out in the DNA Engine (Bio-Rad, Hercules, CA, USA) with a 25-µL volume containing about 50 ng genomic DNA, 6 pmol of each primer, 6 µmol of each dNTP, 1.5 mM Mg2+, and 1 U rTaq DNA polymerase with 1× reaction buffer (Takara, Shiga, Japan). Thermal cycles were 95°C for 2 min, then 35 cycles of 95°C for 45 s, followed by extension at 72°C for 7 min. The PCR products were analyzed by DNA sequencing. Subjects for the case-control study. A total of 991 patients with ESCC and 984 healthy controls were recruited in this study. All subjects were unrelated Han Chinese, living in Beijing and the surrounding regions. Patients with ESCC were consecutively recruited between 7 July 2000 and 20 December 2006, at the Cancer Hospital, Chinese Academy of Medical Sciences (Beijing, China). The pathological stage of ESCC at the time of diagnosis was classified by senior pathologists of the hospital on the basis of postoperative histopathological examination or biopsy according to the International Union Against Cancer (UICC) Tumor-Node-Metastasis classification. Tumor grade was classified into low (well differentiated), intermediate (moderately differentiated),
Table 1. Primers used to amplify regions of the human C1orf10 gene for DNA sequencing analysis

| Primer | Sequence (5’-3’) | Orientation | Ref. position | Amplification size | Region |
|--------|-----------------|-------------|--------------|-------------------|--------|
| P1     | CTTCAAACACCAACACAG | Forward     | –2000 to –1983 | 782              | Promoter |
| P2     | TCTCCCTAGCAATCCCTC | Reverse     | –1236 to –1219 |                  |         |
| P3     | TTGGCTCTGAGCTAGGAAAATTC | Forward | –1485 to –1461 | 519              | Promoter |
| P4     | AGCTTACATGAATCAGGGTAAC | Reverse | –991 to –967  |                  |         |
| P5     | TGCTGATTCACACAGGTGGAAC | Forward | –1011 to –990  | 542              | Promoter |
| P6     | AGCAAATCCGCTGTCGAACTC | Reverse | –490 to –469   |                  |         |
| P7     | GTGCAAGTTCAGGATGACACG | Forward | –578 to –558   | 674              | Promoter |
| P8     | GCCTCAGATCCAAAGACCTG | Reverse | +75 to +95     | 361              | Exon 2  |
| P9     | AAGAACCTACAGGACAGAG | Forward | –92 (beginning of exon 2) | 1100          | Exon 3  |
| P10    | AAATCCCCAGTCAAGGC  | Reverse | +86 (beginning of intron 2) |                  |         |
| P11    | GCTCCTGCTGTCAGCCTGT | Forward | –130 (beginning of exon 3) | 1000          | Exon 3  |
| P12    | CCTCCCTGTCGTCAGTCTT | Reverse | –970 (beginning of exon 3) |                  |         |
| P13    | AAGAACCAAGACAGGACAC | Forward | +633 (exon 3)  | 778              | Exon 3  |
| P14    | TGCTCCTCAAAAGTAGGGAC | Reverse | +1800 (exon 3) |                  |         |

Nucleotide positions are referenced relative to the major transcription start-site of the human Chromosome 1 open reading frame 10 (C1orf10) sequence.

Fig. 1. Analysis of the Chromosome 1 open reading frame 10 (C1orf10) –1139G/C polymorphism. (a) Representative gel picture showing PCR-based restriction fragment length polymorphism (PCR-RFLP) analysis of the C1orf10 genotypes in genomic DNAs of study subjects with the restriction enzyme Hha I. M, DNA size markers; subjects 1 and 2, −1139CC genotype; subjects 3 and 4, −1139GC genotype; subjects 5 and 6, −1139GG genotype. (b) Partial DNA sequence of three different allelic PCR products analyzed directly with an automatic sequencer showing a G to C transversion at the nucleotide location at which the arrow points.

and high (poorly differentiated) according to the World Health Organization grade classification. The control subjects were randomly selected from a pool of cancer-free subjects recruited from a nutritional survey conducted in the same region during the same time period as the cases were collected. The selection criteria include no prior history of cancer and frequency matched to the cases by age (±5 years) and sex. At recruitment, informed consent was obtained from each subject, and each participant was then interviewed to collect detailed information on demographic characteristics and lifetime history of tobacco use. This study was approved by the Institutional Review Board of the Chinese Academy of Medical Sciences Cancer Institute.

**Genotype analysis.** The C1orf10 genotypes at the −1139G/C site were determined by PCR-based restriction fragment length polymorphism (PCR-RFLP). The primers used were: −1139F, 5’-GTC TCC GCT TCA CAG AGT GG-3’; −1139R, 5’-GCC ATC CCA GGG GTA AGT GC-3’. PCR was performed with a 12.5-μL mixture reaction containing 50 ng genomic DNA, 0.1 μM of each primer, 0.2 mM of dNTPs, 1.5 mM Mg²⁺, 4% DMSO, 4% 100 × BSA, and 0.5 U rTaq DNA polymerase with 1 × reaction buffer (Takara). The reaction was conducted under the following conditions: an initial melting step of 2 min at 95°C, followed by 35 cycles of 30 s at 95°C, 30 s at 52°C, and 45 s at 72°C, and a final elongation of 7 min at 72°C. The 197 bp PCR products were digested with restriction enzyme Hha I (New England Biolabs, Ipswich, MA, USA) at 37°C overnight and separated on a 3% agarose gel (Fig. 1a) to determine the G/C polymorphism. Due to the Hha I restriction site, the G/G allele produces only one fragment of 197 bp, whereas the C/C allele generates two fragments of 19 and 178 bp, and the G/C heterozygote has all of the three bands. Then, DNA sequencing was done to confirm the result revealed by PCR-RFLP analysis (Fig. 1b). A 10% masked, random sample of cases and controls was tested twice by different persons and all results had 100% concordance.

**Statistical analysis.** χ²-test or unpaired t-test was used to examine differences in demographic variables, smoking, and distributions of genotypes between cases and controls. The associations between C1orf10 genotype and risk of the occurrence of ESCC were estimated by ORs and their 95% CIs, which were calculated by unconditional logistic regression models. The ORs were adjusted for age, sex, and pack-years smoked. We tested the null hypotheses of multiplicative gene-smoking interaction and evaluated departures from the multiplicative joint effect by including main effect variables and their product terms in the logistic regression model. A more-than-multiplicative interaction was suggested when OR1 × OR2 > OR₁ OR₂. The homogeneity test was done to compare the difference between smoking-related ORs among different genotypes or between the product of related ORs and joint effect OR. All analysis was carried out with Statistical Analysis System software (version 9.0; SAS Institute, Cary, NC, USA). Linkage disequilibrium coefficient and haplotype frequencies were estimated using Haplovie and Haplo.stats software, respectively.

**Construction of luciferase reporter plasmids.** To distinguish the different transcriptional activity of two haplotypes in the C1orf10 promoter, we generated two reporter plasmids encompassing –2014 to +32 bp of the C1orf10 gene. The reporter constructs were prepared by amplifying the promoter region from subjects homozygous for −1747G/T, −1139G/C, −1079G/A, −900G/T using −2014F, 5’-GAA CCT AGC CCC ATC AGA AAA GAG TTC A-3’ and +32R, 5’-GAA CCT GAC CCA GGT GGG ATG AAA CA-3’. The PCR product was digested with Nhe I and Xho I and ligated into an appropriately digested pGL3-Basic vector. Besides to test the weather the SNP in the 3’ UTR influence the stability of mRNA, the 3’ UTR of the C1orf10 with 4666G/A were cloned into the Sac I/Xba I digested pS0 vector (http://www.addgene.org/pgvec)?f=c&cmd=findpl&identifier=12178) respectively:17 The
primes used were +4658F, 5′-GGC CGA GCT CCT TCC CGG ACT CCA ATG TC-3′, 5′-GGC CGA GCT CCT TCC CCA ACT CCA ATG TC-3′ and +4990R, 5′-GGC CTC TAG ATT GAT GCA TTA GGG TAG ATG-3′. The PCR was in combination with Pyrobest DNA polymerase (Takara) to ensure high fidelity amplification, and complete DNA sequencing was then performed to confirm the integrity and authenticity of each construct.

Transient transfections and luciferase assays. Human ESCC cells EC9706 were cultured in RPMI-1640 in a humidified, 5% CO₂ incubator at 37°C. For transient transfection experiments, 5 × 10⁵ cells were plated in 96-multwelly plates and grown to 80–90% confluence. Then cells were co-transfected with 200 ng reporter plasmid and 0.6 ng pRL-CMV vector (Luciferase Assay System; Promega, Madison, WI, USA) by 0.5-μL Lipofectamine 2000 per well. The pRL-CMV vector was used as an internal control for transfection efficiency and the pGL3 Basic vector or pIS0 vector without an insert was used as a negative control. Six hours after transfection, the transfection reaction mixture was removed and 20 μl medium with 10% FBS. Luciferase activity was determined using a Dual-Luciferase Reporter Assay System (Promega) according to the manufacturer’s instructions. Fold increase was calculated by defining the activity of empty pGL3 Basic vector or pIS0 vector as 1. Differences were determined by t-test, and P < 0.01 was considered significant.

Results

Identification of genetic variants in the C1orf10 gene in a Chinese population. Fourteen different primers were designed to screen the C1orf10 gene including promoter region, 5′ UTR, coding region, and 3′ UTR from a panel of 32 unrelated healthy Chinese Han individuals. Nucleotide positions are referenced relative to the major transcription start site, and amino acids are designated by the standard 1-letter code.

![Diagram](image.png)

-1747 1139 -1079 -900 G→T G→C G→A G→T
-2016 +1

Promoter 1 2 3
5′ UTR
3′ UTR

Gly480Ser 4666

| Primer Location | 3′UTR | Coding Region | 5′UTR |
|----------------|-------|---------------|-------|
| rs10888486      | 4666G/A | 4666G/A       | 4666G/A |
| rs3829868       | Gly480Ser | Gly480Ser       | Gly480Ser |
| rs4285700       | –900G/T | –900G/T         | –900G/T |

The association between C1orf10 polymorphisms and risk of ESCC. Since close associations between the variations were observed throughout the entire C1orf10 gene, the region sequenced was analyzed as a single LD block for the haplotype inference. Only one SNP (rs3753444) was selected to analyze the association between C1orf10 genotypes and risk of ESCC. The genotype of this SNP could represent the other five SNPs. Since close associations between the variations were observed throughout the entire C1orf10 gene, the region sequenced was analyzed as a single LD block for the haplotype inference. Only one SNP (rs3753444) was selected to analyze the association between C1orf10 genotypes and risk of ESCC. The genotype of this SNP could represent the other five SNPs. The relevant characteristics of study subjects are shown in Table 2. The distributions of age and gender status between patients with ESCC and control subjects were not statistically different (P = 0.991 and 0.667, respectively), suggesting that the frequency matching was adequate. However, as expected, more smokers were presented among cases compared with controls (61.6% vs 37.6%; P < 0.001). In addition, cases had a higher value of 61 pack-years compared with 48.9% among controls (P = 0.002). Of the 991 patients, 98 (9.9%) had stage I, 209 (21.1%) had stage IIA, 126 (12.7%) had stage IIB, and 415 (41.9%) had stage III ESCC, whereas only 65 (6.6%) patients had stage IV disease. In total, 154 (15.5%) patients were classified into well-differentiated ESCC (low grade), and 425 (42.9%) and 144 (14.5%) patients were classified into moderately differentiated (intermediate grade) or poorly differentiated (high grade) ESCC, respectively.

Table 2. Distributions of select characteristics by case-control status

| Variable          | Cases (n = 991) | Controls (n = 984) | P-values * |
|-------------------|----------------|-------------------|------------|
| Sex               |                |                   |            |
| Male              | 797 (80.4)     | 784 (79.9)        | 0.667      |
| Female            | 194 (19.6)     | 200 (20.3)        |            |
| Age (years)       |                |                   | 0.991      |
| ≤ 0               | 213 (21.5)     | 217 (22.1)        |            |
| 51–60             | 355 (35.8)     | 349 (35.5)        |            |
| 61–70             | 332 (33.5)     | 330 (33.5)        |            |
| >70               | 91 (9.2)       | 88 (8.9)          |            |
| Smoking status    |                |                   | <0.001     |
| Non-smoker        | 373 (37.6)     | 546 (55.5)        |            |
| Smoker            | 610 (61.6)     | 370 (37.6)        |            |
| No data           | 8 (0.8)        | 68 (6.9)          |            |
| Smoking lever, pack-years |          |                   | 0.002      |
| ≥22 pack-years    | 249 (40.8)     | 189 (51.1)        |            |
| >22 pack-years    | 361 (59.2)     | 181 (48.9)        |            |
| Tumor stage†      |                |                   |            |
| I                 | 98 (9.9)       |                   |            |
| IIA                | 209 (21.1)     |                   |            |
| IIB                | 126 (12.7)     |                   |            |
| III                | 415 (41.9)     |                   |            |
| IV                 | 65 (6.6)       |                   |            |
| No data           | 78 (7.8)       |                   |            |
| Tumor grade‡      |                |                   |            |
| Low               | 154 (15.5)     |                   |            |
| Intermediate      | 425 (42.9)     |                   |            |
| High              | 144 (14.5)     |                   |            |
| No data           | 268 (27.1)     |                   |            |

*P-value of the difference between cases and controls.
†According to the International Union Against Cancer (UICC) Tumor-Node-Metastasis classification.
‡According to the World Health Organization classification, Low, well-differentiated carcinoma; intermediate, moderately differentiated carcinoma; high, poorly differentiated carcinoma.
The risk of ESCC related to C1orf10 polymorphism was further examined separately by stratification by sex, age, and smoking status (Table 4). In the subgroup above 58 years, compared with subjects with the −1139GG genotype, subjects with the −1139CC genotype manifested significant association with substantially increased risk (OR = 1.70; 95% CI, 1.15–2.52). It was found that among non-smokers, the adjusted OR of esophageal cancer for subjects carrying the −1139CC genotype was 1.28 (95% CI, 0.87–1.90). Among smokers, subjects carrying the −1139CC genotype had an OR of 1.48 (95% CI, 1.00–2.19). Furthermore, the risk of ESCC related to C1orf10 polymorphism was further examined separately by stratification by pack-years (Table 5). Compared with non-smokers carrying the −1139GG genotype, the risk for the presence of both smoking and the −1139CC genotype (OR = 4.63; 95% CI, 2.98–7.17) was greater than the produce of the OR for smoking (OR = 2.91; 95% CI 2.04–4.14) and the OR for the −1139CC genotype (OR = 1.28; 95% CI, 0.87–1.90), with statistical significance in the test for interaction (P = 0.049). These data suggested a potential multiplicative joint effect between smoking and the −1139CC genotype. Moreover, when the risk associated with the polymorphism was further valuated with smoking levels (≤22 and >22 pack-years smoked), it increased consistently with cumulative smoking dose among smokers, in which a multiplicative joint effect between the susceptible genotype and categories of pack-years smoked was also observed. As compared with the reference group, ORs (95% CI) of the −1139CC genotype for non-smokers, smokers who smoked ≤22, and >22 pack-years were 1.28 (0.87–1.90), 3.87 (2.20–6.83), and 5.29 (3.09–9.07), respectively (P < 0.001 for trend test). Thus, there appears to be an interaction between the −1139CC polymorphism and tobacco smoking that contributes to the risk for ESCC in the population. However, no association was found between the C1orf10 polymorphism and the stage and grade of ESCC (data not shown).

### Table 3. Genotype distribution of C1orf10 –1139G/C polymorphism between cases and controls and their association with ESCC

| Genotype | Cases (n = 991) | Controls (n = 984) | OR† (95% CI) |
|----------|----------------|-------------------|--------------|
| −1139 G/C |                |                   |              |
| GG       | 312 (31.5)     | 340 (34.6)        | 1 (ref)      |
| GC       | 482 (48.6)     | 481 (48.9)        | 1.11 (0.90–1.37) |
| CC       | 197 (19.9)     | 163 (16.5)        | 1.34 (1.02–1.76) |

†Data were calculated by unconditional logistic regression in comparison with the reference group carrying the GG genotype and adjusted for sex, age, and smoking status. C1orf10, Chromosome 1 open reading frame 10; CI, confidence interval; OR, odds ratio.

### Table 4. Risk of esophageal cancer related to C1orf10 –1139G/C genotypes by sex, age, and smoking status

| Characters | GG     | OR (95% CI) | GC     | OR (95% CI) | P   | CC     | OR (95% CI) | P   |
|-----------|--------|------------|--------|------------|-----|--------|------------|-----|
| Sex       |        |            |        |            |     |        |            |     |
| Male      | 262/266| 1 (ref)    | 380/386| 1.00 (0.80–1.28) | 0.971| 155/132| 1.24 (0.92–1.68) | 0.162 |
| Female    | 50/74  | 1 (ref)    | 102/95 | 1.75 (1.07–2.86) | 0.025| 42/31  | 1.70 (0.92–3.17) | 0.093 |
| Age       |        |            |        |            |     |        |            |     |
| ≤58 years | 156/166| 1 (ref)    | 251/260| 1.03 (0.77–1.38) | 0.840| 84/89  | 1.02 (0.69–1.50) | 0.873 |
| >58 years | 156/174| 1 (ref)    | 231/221| 1.19 (0.88–1.61) | 0.265| 113/74 | 1.70 (1.15–2.52) | 0.007 |
| Smoking status | |        |        |            |     |        |            |     |
| Non-smoker| 106/185| 1 (ref)    | 192/263| 1.24 (0.91–1.69) | 0.167| 75/98  | 1.28 (0.87–1.90) | 0.209 |
| Smoker    | 203/133| 1 (ref)    | 287/182| 1.05 (0.79–1.41) | 0.726| 120/55 | 1.48 (1.00–2.19) | 0.049 |

†Data were calculated by unconditional logistic regression in comparison with the reference group carrying the GG genotype and adjusted for sex, age, and pack-years within the strata.

### Table 5. Risk of esophageal cancer related to C1orf10 –1139G/C genotypes by smoking status and pack-years smoked

| Characters | GG     | OR (95% CI) | GC     | OR (95% CI) | P   | CC     | OR (95% CI) | P   |
|-----------|--------|------------|--------|------------|-----|--------|------------|-----|
| Smoking status |        |            |        |            |     |        |            |     |
| Non-smoker| 106/185| 1 (ref)    | 192/263| 1.24 (0.91–1.69) | 0.167| 75/98  | 1.28 (0.87–1.90) | 0.209 |
| Smoker    | 203/133| 2.91 (2.04–4.14) | <0.001| 287/182  | 3.34 (2.36–4.72) | <0.001| 120/55  | 4.63 (2.98–7.17) | <0.001 |
| ≤22 pack-years | 93/72  | 2.54 (1.67–3.85) | <0.001| 109/88   | 2.79 (1.84–4.24) | <0.001| 47/29   | 3.87 (2.20–6.83) | <0.001 |
| >22 pack-years | 110/61 | 3.48 (2.27–5.34) | <0.001| 178/94   | 3.85 (2.61–5.67) | <0.001| 73/26   | 5.29 (3.09–9.07) | <0.001 |

†Data were calculated by unconditional logistic regression in comparison with the reference group carrying the GG genotype, and adjusted for sex and pack-years.

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Generation of reporter gene constructs to measure differences in allelic expression between C1orf10 promoter or 3′ UTR variants. To test whether the allelic variations in the promoter region influence the expression level of C1orf10, we generated reporter gene constructs in the context of the regulatory region containing the two haplotypes (T–1747-C–1139-A–1079-T–900 and G–1747-G–1139-G–1079-G–900) (b) EC9706 cells were transiently transfected as described. Fold increase was measured by defining the activity of the empty pGL-3 Basic vector as 1. Data shown are mean fold increase ± SD from three independent transfection experiments, each done in triplicate.

Discussion

In the present study, we sought to identify functional SNPs in C1orf10 in a Chinese population and to investigate their association with the risk of developing ESCC. To summarize, by sequencing the full-length of C1orf10 from a subset of 32 normal Han Chinese subjects, we found six SNPs, four of which (–1747G/T, –1139G/C, –1079G/A, and –900G/T) were in the promoter region, and two of which (Gly480Ser and 4666G/A) were in the exon 3 and 3′ UTR respectively. All the six SNPs have been deposited in the NCBI SNP database. Haplotype analysis showed that the six SNPs covering a region of 7 kb are in linkage disequilibrium. The results of case-control analysis of 991 ESCC patients and 984 controls showed that the –1139CC genotype was associated with increased risk of ESCC. Moreover, we observed a potential multiplicative joint effect between the genotype and smoking, which was associated with even greater risk of ESCC. We observed a more than 4-fold increased ESCC risk associated with the –1139CC genotype among smokers but not non-smokers, suggesting a possible gene–environment interaction between the C1orf10 polymorphism and smoking in the etiology of ESCC in this Chinese population. In addition to tobacco smoking, other environmental factors such as alcohol consumption have been suggested to be associated with risk of ESCC, and this association might also be modulated by C1orf10 genotype. Unfortunately, the information on exposures other than smoking is not available in the present study, which prevents more comprehensive evaluation of the role of gene–environment interaction in ESCC development.

Based on these results and the previous observation of abnormalities in C1orf10 expression in ESCC, we speculated that the
polymorphisms in the promoter region may affect the expression level of the gene; however, the two haplotypes did not show any significant difference by reporter assay. Moreover, the 46666G/A polymorphism in 3′ UTR had no difference on the transcription of reporter gene. Thus, the SNPs in promoter region and 3′ UTR may not contribute to susceptibility of ESCC by influencing expression level of C1orf10. Besides, no significant difference in C1orf10 mRNA expression levels was found when −1139G/C SNP (rs3753444) genotypes were compared by real-time PCR analysis of C1orf10 mRNA in individual esophageal tissues (data not shown). We also noticed Gly480Ser, a SNP which may alter the activity of C1orf10. However, the function of C1orf10 is not clear so far, and not much information is available for discussing potential function of this SNP.

It is very clear that C1orf10 expression in esophageal cancer tissues and cell lines is much lower than that in the corresponding normal esophageal mucosa, whereas the precise function of C1orf10 in ESCC development remains unknown. C1orf10 is involved in heat shock response as one novel member of the tissue-specific and atypical stress response system.(8) Moreover, it might allow cells to tolerate normally lethal levels of DCA and protect from the toxic effect of bile acid as a survival factor.(15) Subsequent studies have also identified C1orf10 as a candidate component of epithelial immunity based on its strong signature of adaptive evolution on DNA sequence of a type that is commonly associated with a coevolutionary arms race with a pathogen.(10) Thus C1orf10 protein expression will presumably help maintain the barrier function in squamous epithelium in response to injury and function as a tumor suppressor. Our data of the case-control analysis showed that the polymorphism of C1orf10 was associated with the risk of ESCC. It is possible that the genetic variants of C1orf10 change the expression level or activity of the gene, and then influence its function in ESCC development. It is also possible that the association between the C1orf10 polymorphism and ESCC might not really relate to the C1orf10 activity, but only to a secondary effect due to the linkage disequilibrium with a yet unidentified, but tightly linked, ESCC locus. In this way the polymorphism of C1orf10 might only serve as a haplotype tag. It is also possible that influence of several tightly linked polymorphisms within or close to the C1orf10 gene lead a combinatory contribu-
tion to the case-control results. In fact, based on the HapMap CHB database (HapMap data, Rel 22/phase II Apr07, on NCBI B36 assembly, dbSNP b126; population: Han Chinese in Beijing, China; MAF are >0.05) in region chr1: 149,129,663.149,214,350, we find that all SNPs reported in this article are located in a region (84 kb) of high LD which contain two known genes: C1orf10 and IFPS. Thus, further studies would be warranted to address these possibilities.

In conclusion, we have found six SNPs that lie in the C1orf10 gene in a Han Chinese population. The six SNPs were strongly linked, spanning a region of 7 kb, and they demonstrated an increased risk of ESCC associated with the −1139CC genotype. These findings suggest a role for genetic determinants of stress protein in the development of esophageal cancer in a Chinese population. Since this is the first report demonstrating the contribution of C1orf10 polymorphism to ESCC, additional studies on ESCC would be warranted in different ethnic populations.

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Abbreviations

C1orf10 Chromosome 1 open reading frame 10
CI Confidence interval
DCA Deoxycholic acid
EDC Epidermal differentiation complex
ESCC Esophageal squamous cell carcinoma
OR Odds ratio
SNP Single nucleotide polymorphism

Disclosure Statement

None.

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