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19p13.3-*GADD45B* common variants and 19q13.3-*PPP1R13L* and 19q13.3-*CD3EAP* in lung cancer risk among Chinese

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ABSTRACT

Lung cancer is the most common cause of cancer-related mortality worldwide. The *GADD45* gene family plays important roles in a variety of the responses to cell injury including cell cycle checkpoints, apoptosis, DNA repair and anti-tumor immunity. The 19p13.3-*GADD45B* encoded protein product is involved in apoptosis and inhibiting tumor growth. To evaluate the association of 19p13.3-*GADD45B* common variants and lung cancer risk, the present study containing 544 Chinese lung cancer cases and 550 cancer-free controls was conducted. Three htSNPs (haplotype-tagging single nucleotide polymorphism) (rs7354, rs14384, and rs3783501) covering 95% of the common haplotype diversity in 19p13.3-*GADD45B* and interaction of 19p13.3-*GADD45B* and 19q13.3-*PPP1R13L* and 19q13.3-*CD3EAP* variants and smoking-duration were explored. Genotype and allele frequencies and haplotype distributions of the 19p13.3-*GADD45B* 3 htSNPs were not associated with lung cancer risk after adjustment for smoking status. 19p13.3-*GADD45B* rs7354 was associated with lung cancer risk among ≤20 (years) smokers [C/A-A/A versus CC, OR (95% CI) = 3.20 (1.11−9.20), $P = 0.025$] in a dominant model stratified by smoking duration. MDR (multifactor dimensionality reduction) analyses showed that smoking history as main effect and three-way models (smoking duration, 19p13.3-*GADD45B* rs3783501, 19q13.3-*CD3EAP* rs967591) ($P = 0.001$−0.002) indicated statistically significant association with lung cancer risk. The study identified evidence implicating DNA damage response genes on chromosome 19 in etiology of smoke-exposed lung cancer. In conclusion, our findings demonstrate that 19p13.3-*GADD45B* rs7354 variant and interaction between 19p13.3-*GADD45B* rs3783501 and 19q13.3-*CD3EAP* rs967591 may play a role in association with smoke-exposed lung cancer among Chinese. 19p13.3-*GADD45B* variants should be further evaluated in large prospective studies with molecular pathological annotations of lung cancer.

1. Introduction

Lung cancer is the most common cause of cancer-related mortality worldwide [1]. It is a multifactorial disease with regards to its genetic profile and environmental cause. Cigarette smoking is an important risk factor for lung cancer development. The identification of gene-environment interactions with smoking is a means to identify important biological pathways implicated in smoking-related lung cancer.

The gene *GADD45* (Growth arrest and DNA-damage-inducible) family consists of 3 members: *GADD45A* (Gadd45α/Gadd45), *GADD45B* (Gadd45β/MyD118), *GADD45G* (Gadd45γ/CR6). Transcript levels of these genes are increased following stressful growth arrest conditions and treatment with DNA-damaging agents. Members of the GADD45 gene family play important roles in a variety of the responses to cell injury including cell cycle checkpoints, apoptosis, DNA repair and anti-tumor immunity. *GADD45B* (Gene ID: 4616) is located on chromosome 19p13.3 and contains 3 exons. *GADD45B* is involved in apoptosis and inhibition of tumor growth [2,3, NCBI Gene Database: https://www.ncbi.nlm.nih.gov/gene/4616].

Two adjacent genes on Chr19q13.3, *PPP1R13L/IASPP/RAI* [protein phosphatase 1, regulatory (inhibitor) subunit 13 like)/Inhibitory

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member of the ASPP family/RelA-associated inhibitor] (Gene ID: 10848) and *CD3EAP/ASE-1* [CD3e molecule, epsilon-associated protein/antisense to ERCC1)] (Gene ID: 10849) are involved in apoptosis and rRNA transcription, respectively [4]. We have previously found that the polymorphisms *PPP1R13L* rs1970764 and *CD3EAP* rs967591 were associated with lung cancer risk among both Caucasian Danes and Chinese [4–7].

The three genes (*GADD45B, PPP1R13L* and *CD3EAP*) are assigned to the same chromosome. They are all involved in the NF-kB signaling pathway (NCBI Gene Database: https://www.ncbi.nlm.nih.gov/gene). They may function cooperatively in carcinogenesis. To the best of our knowledge, no study of the association between *GADD45B* SNPs (single nucleotide polymorphism) and lung cancer has been reported yet. In the present work, we aimed to assess the associations of *GADD45B* common variants and to evaluate these interactions between gene-gene and gene-environment in relation to lung cancer risk among Chinese.

2. Materials and methods

2.1. Ethical consideration

The Chinese Administration Office of Human Genetic Resources has approved this protocol. All study participants gave written or oral informed consent.

2.2. Study population

This hospital-based case-control study comprised 1094 subjects, including 544 lung cancer cases and 550 cancer-free controls as previously described [5]. Lung cancer diagnosis was based on standard clinical and histological criteria. Eligible cases were previously untreated (recruited prior to chemotherapy or radiotherapy for cancer). Cancer-free controls were selected from the orthopedics wards in the same region. The controls were matched to the cases by ±3 years age, sex and ethnicity. All subjects were unrelated ethnic Han Chinese. All covariate data were obtained from questionnaires. Stratification criteria were gender, age (10-year intervals) and smoking history (20-year intervals) (Table 1).

2.3. htSNP selection in GADD45B

Three htSNPs (haplotype-tagging single nucleotide polymorphism) of *GADD45B* gene were chosen from the International HapMap Project (http://www.hapmap.org, HapMap Data Rel 27 PhaseII + III, Feb09, on NCBI B36 assembly, dbSNP b26) using the TagSNPs software online and approaches of the algorithm-Tagger-pairwiseTagging on Chr1913.3: 2427135..2429257, eligibility criteria: r^2-cut off of 0.8 and MAF (minor allele frequency)-cut off of 0.05 in CHB samples. Three htSNPs (rs7354, rs14384 and rs3783501) were selected across the *GADD45B* locus, obtaining 95% coverage of the common haplotype diversity in the gene. The information of 3 htSNPs is shown in Table 2. The genotype data regarding *PPP1R13L* rs1970764 and *CD3EAP* rs967591 were used for gene-gene and gene-environment interaction analyses between risk SNPs of Chr19q13.3 sub-region and 19p13.3-*GADD45B* htSNPs (Table 2). The genotyping data of *PPP1R13L* rs1970764 and *CD3EAP* rs967591 were published previously [5].

2.4. DNA extraction and genotyping

Genomic DNA was extracted from 1.5 ml peripheral blood samples using the Puregene DNA Isolation Kit (Gentra Systems, Minneapolis, MN, USA) or FlexiGene DNA kit 250 (Qiagen, Germany).

Genotyping of *GADD45B* rs7354, rs14384, and rs3783501 was conducted employing method of ligase detection reaction coupled with PCR (LDR-PCR) [8]. Primers and probes were synthesized by Shanghai Generay Biotechnology Co. Ltd (P. R. China). Each set of LDR probes was composed of one common probe and two discriminating probes for the two alleles. The sequences (5′- 3′) of primers and probes of *GADD45B* 3 htSNPs are showed in Table 3. The PCR reactions were performed in 15 μl reaction mixtures consisting of 1.5 μl 10 × Taq Buffer with 200 mM $(NH_4)_2SO_4$, 1.5 μl 25 mM $MgCl_2$, 0.3 μl 10 mM dNTP, 0.2 μl 5U/μl Taq polymerase (Fermentas Life Sciences), primer F (10 pmol/μl) 0.25 μl x N and primer R (10 pmol/μl) 0.25 μl x N (multiple PCR x N), and 1 μl DNA. The PCR program was: 94 °C for 2min, 35 cycles of 94 °C for 20s, 60 °C for 20s and 72 °C for 40s, and 72 °C for 3min. LDR was carried out in 10 μl reaction mixture: 1 μl 10 × Taq DNA ligase buffer, 0.125 μl 40U/μl Taq DNA ligase (New England Biolabs, Beverly, MA), and 0.01 μl of each probe (10 pmol/μl) x N (multiple PCR x N). LDR conditions were: 20 cycles of 94 °C for 30s and 60 °C for 3min. LDR products were sequenced using ABI 3730xl by Shanghai Generay Biotechnology Co. Ltd (P. R. China). The call rate of the genotyping was 90.83%.

2.5. Statistical analysis

SNPStats program [9], SHEsis software online [10] and SPSS© v11.5 (SPSS Inc, Chicago, IL, USA) were used for calculation of Hardy-Weinberg equilibrium, allele frequencies, genotype frequencies, haplotype associations, and pair-wise linkage disequilibrium (LD) analysis. Four genetic models (co-dominant model, dominant model, recessive model, and log-additive model) were performed for each single locus case-control association (Table 4). Unconditional logistic regression was applied for determination of adjusted OR, 95% CI (Odd Ratio, 95% confidence interval; adjusted for smoking duration). Haplotypes with frequency <0.03 among both cases and controls were excluded from the analysis. The analyses of smoking duration-SNP and SNP-SNP interactions in relation to lung cancer risk were completed using MDR (multifactor dimensionality reduction) version 3.0.3. dev. Jar [11]. This software (MDR 3.0.3. dev. Jar) is a developmental version which has integrated permutation testing into the main MDR program. The threshold for significance was set at $P < 0.05$.

3. Results

The current study population included 544 lung cancer cases and 550 cancer-free controls (Table 1). There were no statistically significant differences in mean age and gender distribution between cases and controls. However, more cases than controls had a family history of cancer ($P < 0.0001$) and cases had longer smoking history (>20 years) ($P < 0.0001$) than the controls.

The three htSNPs were in Hardy-Weinberg equilibrium among controls (results not shown). The association between the 3 htSNPs in *GADD45B* and lung cancer risk was evaluated using multiple logistic regression analysis with co-dominant, dominant, recessive, and log-additive models. After adjustment for smoking status, the genotype and allele frequencies (Table 4) and haplotype distributions (data not shown) of the *GADD45B* 3 htSNPs were not associated with lung cancer risk. D′ values of pair-wise LD among controls between rs7354 and rs3783501; rs14384 and rs3783501 were 0.924; 0.890; however, the D′ value was 0.457 between rs7354 and rs14384. Rs7354 was associated with lung cancer risk among ≤20 (years) smokers [C/A-A/A versus CC, OR (95% CI) = 3.20 (1.11–9.20), $P = 0.025$] in the dominant model after stratification for smoking duration (Table 5). Smoking history was the main effect in the interaction analysis of 6 attributes ($P < 0.001$ on 1000 permutation