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Haplotypes of nine single nucleotide polymorphisms on chromosome 19q13.2-3 associated with susceptibility of lung cancer in a Chinese population

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Abstract

To evaluate the joint effect of nine single nucleotide polymorphisms for three DNA repair genes in the region of chromosome 19q13.2-3 on susceptibility of lung cancer in a Chinese population, we conducted a hospital-based case–control study consisting of 247 lung cancer cases and 253 cancer-free controls matched on age, gender and ethnicity. Associations between the haplotypes and susceptibility of lung cancer were tested. The global test of haplotype association revealed a statistically significant difference in the haplotype distribution between cases and controls (global test: $\chi^2 = 60.45$, d.f. = 15, $P = 2.11\text{E}{-}07$). The two haplotypes were underrepresented among cases (Hap5 defined by *ERCC1*118^{A}–*ERCC2*156^{C}–*ERCC2*312^{G}–*ERCC2*751^{A}–*XRCC1*194^{T}–*XRCC1*206^{A}–*XRCC1*280^{G}–*XRCC1*399^{G}–*XRCC1*632^{G} and Hap12 defined by *ERCC1*118^{G}–*ERCC2*156^{C}–*ERCC2*312^{G}–*ERCC2*751^{A}–*XRCC1*194^{C}–*XRCC1*206^{A}–*XRCC1*280^{G}–*XRCC1*399^{A}–*XRCC1*632^{G}). Three of the haplotypes were overrepresented among cases (Hap3 defined by *ERCC1*118^{A}–*ERCC2*156^{C}–*ERCC2*312^{G}–*ERCC2*751^{A}–*XRCC1*194^{C}–*XRCC1*206^{A}–*XRCC1*280^{G}–*XRCC1*399^{G}–*XRCC1*632^{G}, Hap4 defined by *ERCC1*118^{A}–*ERCC2*156^{C}–*ERCC2*312^{G}–*ERCC2*751^{A}–*XRCC1*194^{C}–*XRCC1*206^{G}–*XRCC1*280^{G}–*XRCC1*399^{G}–*XRCC1*632^{A}, and Hap10 defined by *ERCC1*118^{G}–*ERCC2*156^{A}–*ERCC2*312^{G}–*ERCC2*751^{A}–*XRCC1*194^{T}–*XRCC1*206^{A}–*XRCC1*280^{G}–*XRCC1*399^{G}–*XRCC1*632^{G}). Haplotypes 3 and 10 (cases = 5.7%, controls = 1.0%, OR = 6.56, 95%CI = 1.83–23.54, $P = 0.001$; cases = 13.3%, controls = 5.6%, OR = 2.73, 95%CI = 1.51–4.94, $P = 0.0006$) were the most strongly associated with increased lung cancer risk. There was considerable linkage disequilibrium exists between SNPs both within genes and between genes in the region. The two blocks for solid spine of LD and six htSNPs were found. The haplotype analysis suggested that the biologically effective polymorphisms co-segregate with some of the haplotypes. This result supports the hypothesis that the sub-region is important for lung cancer susceptibility. Haplotype studies using larger study groups will be required to obtain conclusive results.

Keywords: Chromosome 19q13.2-3; DNA repair gene; Single nucleotide polymorphism; Haplotype; Lung cancer; Chinese population

1. Introduction

Lung cancer is one of the most frequent cancers in China and the world. Smoking is considered major risk factor for lung cancer. However, only 1 out of 10 smokers develops lung cancer [1], indicating that genetic susceptibility may be important in disease development.

DNA repair is essential for the maintenance of genetic stability. Mutations in one of the DNA repair genes are one of

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the most common reasons for cancer [2]. Numerous polymorphisms (mainly SNPs) have been identified in various DNA repair genes. Their functional outcome and phenotypic effects are often unknown and the functional significance of DNA repair gene polymorphisms remain to be established. It is highly possible that the individual genetic background modulating the DNA repair capacity may affect the susceptibility of cancer [3]. A recent study of the repair capacity of 244 healthy individuals [4] found a good correlation between genotype and DNA repair phenotypes for polymorphisms in the base excision repair genes *hOGG1* and *XRCC1*, thus providing evidence that some single nucleotide polymorphisms (SNPs) are indeed functional. For polymorphisms with relatively high allele frequencies, even modest effects in terms of odds ratios have large effects in terms of attributable fraction, simply because they are common [5].

At least four pathway of DNA repair operate on specific types of damage DNA, and each pathway involves numerous molecules. BER (base excision repair) operates on small lesions. NER (nucleotide excision repair) pathway repairs bulk lesions. DSBR (double strand break repair) includes the homologous recombination pathway and the non-homologous end-joining repair pathway. MMR (mismatch repair) corrects replication errors [6]. *XRCC1* (X-ray repair cross complementing 1), *ERCC2* (excision repair cross complementation group 2) and *ERCC1* (excision repair cross complementation group 1) involved in DNA repair are all located on chromosome region 19q13.2-3 [7,8]. *XRCC1* is part of the BER. *ERCC2* and *ERCC1* are part of the NER pathway. All three gene products play important roles in repair of DNA damage, and have the potential to be cancer-susceptibility genes.

SNPs are new genetic markers of third generation and new molecular tools for studying the associations between genomic regions and the diseases. SNP maps of high-density offer a superior strategy for unraveling genetic complexity. It has been suggested that use of haplotypes in association studies may have increased power over single-allele studies [9]. Haplotype analyses take into account a number of tightly linked markers, which are much more informative than individual markers. Haplotype analyses can identify unique chromosomal segments likely to harbor disease predisposing genes [10]. The structure of haplotype blocks varies along the chromosomes and between populations [5].

In this paper, we have undertaken an analysis of joint effect of nine SNPs for three genes in the region 19q13.2-3 (Table 1) on susceptibility of lung cancer in a Chinese population. The main aim was to investigate if the haplotypes including these polymorphisms are linked to susceptibility of lung cancer.

2. Materials and methods

2.1. Study population

This hospital-based case–control study consisted of 247 cases with newly diagnosed primary lung cancer and 253 cancer-free controls. All cases were previously untreated (prior to chemotherapy or radiotherapy for cancer). Controls were accrued from non-cancer patients admitted to the bone wards in the same region. Cancer-free status was ensured by Doctor's querying in detail. Cancer-free, randomly selected controls were matched to the cases by age (±3 years) and gender. All subjects were unrelated ethnic Han Chinese. All of the subjects gave written or oral informed consent. The study was approved by the Chinese Administration Office of Human Genetic Resources. Details of the study group are shown in Table 2. Stratification analyses were defined by gender, age (10-year intervals) and smoking history (20-year intervals). Data about smoking history were obtained from questionnaires.

2.2. Genotyping

Genomic DNA was extracted from 1.5 ml peripheral blood samples using the Puregene DNA Isolation kit (Gentra Systems, Minneapolis, MN).

Primer sequences, restriction endonucleases and digested fragments of enzymes for nine cSNPs were used as previously described [11–15]. The PCR primers were synthesized by Takara Biotechnology (Dalian), China. Restriction endonucleases were from NEB (New England Biolabs, Beverly, MA). PCR-RFLP techniques were performed as previously reported [16,17]. The genotyping data of *XRCC1* Arg194Trp, Pro206Pro, Arg280 His, Arg399Gln and Gln632Gln were published previously [15]. 150 of the lung cancer cases and 150 of the controls have previously been genotyped for *ERCC1* Asn118Asn and *ERCC2* Arg156Arg, Asp312Asn, and Lys751Gln polymorphisms [11–14]. However, the genotypes were re-determined for the present study. In repeated genotyping of 20% of the samples, identical genotypes were obtained. The genotyping success rate was 93%, not including ERCC2 Asp312Asn, where the success rate was only 74%. Unfortunately, we did not find the reasons for the failed genotyping. For each individual, the same DNA sample was used for genotyping of all nine SNPs.

2.3. Statistical analysis

χ^2 test or t'-test (equal variances not assumed) was used to evaluate the differences in select demographic variable, family history and smoking history. Hardy–Weinberg equilibrium test, allele frequencies, genotype frequencies, haplotype frequencies and the linkage disequilibrium were calculated using the SHEsis program [18]. To compare the distribution of the genotypes between lung cancer cases and controls and estimate susceptibility between genotypes and lung cancer, χ^2 test, OR (odds ratio) and 95%CI (confidence interval) were

Table 1
The nine cSNPs analyzed, sources of information and current positions on chromosome 19q13.2-3

Gene/SNPs at exon	Reference no. in db SNP of NCBI	Polymorphism	Codon for protein	Current chromosome position (bp)
*XRCC1*exon17	rs3547	G/A	Gln(Q)632Gln(Q)	48739390
*XRCC1*exon10	rs25487	G/A	Arg(R)399Gln(Q)	48747566
*XRCC1*exon9	rs25489	G/A	Arg(R)280His(H)	48748252
*XRCC1*exon7	rs915927	A/G	Pro(P)206Pro(P)	48749067
*XRCC1*exon6	rs1799782	C/T	Arg(R)194Trp(W)	48749414
*ERCC2*exon23	rs13181	A/C	Lys(K)751Gln(Q)	50546759
*ERCC2*exon10	rs1799793	G/A	Asp(D)312Asn(N)	50559099
*ERCC2*exon6	rs238406	C/A	Arg(R)156Arg(R)	50560149
*ERCC1*exon4	rs11615	G/A	Asn(N)118Asn(N)	50615493