Null Results in Brief

No Association between Polymorphisms in the *Histone Deacetylase* Genes and the Risk of Lung Cancer

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Introduction

Although cigarette smoking is the major cause of lung cancer, only a fraction of smokers develop lung cancer during their lifetime, and this suggests that genetic and epigenetic factors are of importance in determining an individual's susceptibility to lung cancer.

Histone deacetylases (HDAC) mediate changes in chromatin structure by removing acetyl groups from the core histones, and they play an important role in the regulation of gene expression (1). HDACs are involved in cell cycle progression, differentiation, and apoptosis, and their deregulation is associated with several cancers (1, 2). Several candidate polymorphisms in the *HDAC* genes have been deposited in public databases (http://www.ncbi.nlm.nih.gov/SNP). Although the functional effects of these polymorphisms have not been elucidated, we have hypothesized that some of these variants and particularly their haplotypes may have an effect on HDAC activities and thus modulate the susceptibility to lung cancer. To test this hypothesis, a case-control study was conducted to evaluate the association between the *HDAC* genotypes/haplotypes and the risk of lung cancer.

Among the candidate polymorphisms, we focused on the amino acid substitution variants and the variants in intron-exon junction (20 bp of introns on each side of each exon) and proximal promoter (1 kb upstream of each gene) regions in *HDAC1* to *HDAC8* genes. In the present study, we evaluated the association among six polymorphisms [IVS1+15A > C (Genbank accession no. NT_029289) in *HDAC3*; −632G > A, IVS4+8G > A, and IVS16-19G > A (Genbank accession no. NT_022173) in *HDAC4*; and IVS3-4A > G and Asp^{593}Glu (818690G > C, Genbank accession no. NT_010783) in *HDAC5*] and lung cancer because the other 17 candidate polymorphisms [Gly^{378}Ala (2769239G > C, Genbank accession no. NT_004511) in *HDAC1*; Arg^{94}Ile and Arg^{230}Ser (18449244G > T and 18439803A > T, respectively; Genbank accession no. NT_025741) in *HDAC2*; −791C > A, −639C > T, −488C > T, −31G > T, IVS2-12C > A, and Arg^{265}Pro (2170423G > C) in *HDAC3*; Val^{785}Met (179747G > A) in *HDAC4*; −492A > G, 5529G > T, Arg^{137}Glu (824603G > A), and IVS20-3T > C in *HDAC5*; Val^{701}Ala and Thr^{994}Ile (11526538T > C and 11531594C > T, respectively; Genbank accession no. NT_079573) in *HDAC6*; and −392T > G (Genbank accession no. NT_011669) in *HDAC8*] were not detected in a preliminary study that consisted of 25 lung cancer cases and 25 healthy controls.

Materials and Methods

This case-control study included 432 lung cancer patients and 432 healthy controls. These subjects have been previously studied with respect to *DNA methyltransferase3B, Xeroderma Pigmentosum group C*, and *vascular endothelial growth factor* polymorphisms, and details of the study population have been described elsewhere (3-5). All the cases and controls were ethnic Koreans. Polymorphism genotyping was determined using a PCR-RFLP assay. The PCR primers were designed based on the Genbank reference sequences, respectively.

The PCR primers for IVS1+15A > C in *HDAC3*; −632G > A, IVS4+8G > A, and IVS16-19G > A in *HDAC4*; and IVS3-4A > G and Asp^{593}Glu in *HDAC5* were 5′-ACTACGGTGAGGAAACAGCG-3′ (forward) and 5-′AGGCTATGGGTCAATGCCAG-3′(reverse); 5′-CCTAATTCGCGCTTAGAGGGTA-3′ (forward) and 5′-GCGGACCTACACCCCACA-3′ (reverse); 5′-GCTTCCACTCAATGACTTTGG-3′ (forward) and 5′-ACGAGCACATCAAGGTGAAGC-3′ (reverse); 5′-TGTAGCTTCGGGTGGAAACT-3′ (forward) and 5′-ACAGGCTGATGCCACAACAA-3′ (reverse); 5′-CCTTCCTGTCTCTTCTGCGC-3′ (forward) and 5′-CTCAGCGAACAGGAGCTGCT-3′ (reverse); and 5′-GAGGAGGAGGACGAGGAAGT-3′ (forward) and 5′-GGAGAGGGAAAGACAAGGGC-3′ (reverse), respectively. The PCR reactions were done in a 20-μL reaction volume containing 100 ng genomic DNA, 10 pmol/L of each primer, 0.2 mmol/L deoxynucleotide triphosphates, 10 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, and 1 unit of Taq polymerase (Takara Shuzo Co., Otsu, Shiga, Japan). The PCR annealing temperatures were 58°C for IVS1+15C > A in *HDAC3*; 60°C for −632G > A, IVS4+8G > A, and IVS16-19G > A in *HDAC4*; and IVS3−4A > G in *HDAC5*; and 61°C for Asp^{593}Glu in *HDAC5*, respectively. The PCR products were digested overnight with the appropriate restriction enzymes

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Table 1. Genotype/haplotype frequencies of *HDAC* polymorphisms and adjusted ORs for lung cancer

Gene	Polymorphism	Genotype*	Case no./control no.	Adjusted† OR (95% confidence interval)
HDAC3	IVS1+15A > C	1/1	182/189	1.0
		1/2	205/193	1.07 (0.80-1.43)
		2/2	45/50	0.90 (0.56-1.44)
HDAC4	−632G > A	1/1	370/365	1.0
		1/2	58/65	0.94 (0.64-1.38)‡
		2/2	4/2	
	IVS4+8G > A	1/1	345/363	1.0
		1/2	83/67	1.29 (0.91-1.84)‡
		2/2	4/2	
	IVS16-19G > A	1/1	204/200	1.0
		1/2	187/199	0.95 (0.71-1.26)
		2/2	41/33	1.28 (0.77-2.13)
HDAC5	IVS3-4A > G	1/1	1/1	
		1/2	87/83	1.5 (0.75-1.48)§
		2/2	344/348	1.0
	$Asp^{593}Glu$	1/1	11/9	
		1/2	107/96	1.19 (0.87-1.62)§
		2/2	314/327	1.0
HDAC4	Haplotype‖,¶	111	456/466	0.97 (0.80-1.78)
		112	252/259	1.00 (0.81-1.23)
		121	84/66	1.27 (0.90-1.78)
		211	54/66	0.84 (0.58-1.23)
		Others**	18/7	—
HDAC5	Haplotype††,¶	11	41/36	1.17 (0.73-1.85)
		12	48/49	0.96 (0.63-1.45)
		21	687/701	0.90 (0.71-1.15)
		22	88/78	1.15 (0.83-1.59)

*Wild-type allele is denoted by 1 and the polymorphic allele by 2.
†Adjusted for age, gender, and pack-years of smoking.
‡Dominant model for the variant allele (1/2 + 2/2 versus 1/1).
§Dominant model for the wild-type allele (1/1 + 1/2 versus 2/2).
‖The order of *HDAC4* polymorphisms is as follows: −632G > A, IVS4+8G > A, and IVS16-19G > A.
¶OR and 95% confidence interval for each haplotype compared to all the other haplotypes combined are shown.
**Other haplotypes were excluded from analysis.
†† The order of *HDAC5* polymorphisms is as follows: IVS3-4A > G and $Asp^{593}Glu$.

(New England BioLabs, Beverly, MA) at 37°C. The restriction enzymes for IVS1+15A > C in *HDAC3*; −632G > A, IVS4+8G > A, and IVS16-19G > A in *HDAC4*; and IVS3-4A>G and $Asp^{593}Glu$ in *HDAC5* were *Hae*II, *Bst*Z17I, *Hin*p1I, *Bsm*AI, *Bst*UI, and *Taq*I, respectively. The digested PCR products were resolved on 6% acrylamide gel or 1.2% agarose gel. For quality control, the genotyping analysis was done ''blind'' with respect to the case/control status. About 10% of the samples were randomly genotyped again by a different author, and the results were 100% concordant. To confirm the genotyping results, selected PCR-amplified DNA samples (n = 2, respectively, for each genotype) were examined by DNA sequencing, and the results were also 100% concordant.

Hardy-Weinberg equilibrium was tested with a goodness-of-fit χ^2 test with 1 degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies among the subjects. The haplotypes and their frequencies were estimated based on a Bayesian algorithm using the Phase program (6) which is available at http://www.stat.washington.edu/stephens/phase.html. Unconditional logistic regression analysis was used to calculate odds ratios (OR) and 95% confidence intervals, with adjustment for possible confounders (gender as a nominal variable; age and pack-years, as continuous variables). Multiple logistic regression analyses were done to analyze the association between the genotypes/haplotypes and the risk of lung cancer after stratifying the subjects according to age (median age, ≤61 and/or >61 years), gender, smoking status, cigarette consumption (median pack-years,<40 and/or ≥40 pack-years), and the histologic types of lung cancer. All analyses were done using Statistical Analysis Software for Windows, version 8.12 (SAS Institute, Cary, NC).

Results

The genotype distributions of the six polymorphisms among the controls were in Hardy-Weinberg equilibrium. The six polymorphisms, the *HDAC4* haplotypes, and the *HDAC5* haplotypes exhibited no apparent relationship with the risk of lung cancer (Table 1). When the analyses were stratified by age, gender, smoking status, and pack-years of smoking, no significant association was found between the *HDAC* polymorphisms/haplotypes and the risk of lung cancer. Moreover, the *HDAC* polymorphisms and their haplotypes exhibited no apparent relationship with any of the histologic types of lung cancer (data not shown).

Statistical Power

For the *HDAC3* IVS1+15A > C polymorphism, this study had 80% power (two-sided test of significance, α = 0.05) to detect an OR of >1.50 (assuming a risk effect) or <0.67 (assuming a protective effect) for carriers of the AC genotype, and an OR of >2.06 or< 0.55 for carriers of the CC genotype relative to the carriers of the AA genotype. This study had 80% power to detect an OR of >1.82 or <0.61 (for *HDAC4* −632G > A) and an OR of >1.72 or <0.63 (for *HDAC4* IVS4+8G > A) for the carriers of at least one polymorphic allele relative to the carriers of the homozygous wild-type allele. For the *HDAC4* IVS16-G > C polymorphism, this study had 80% power to detect an OR of >1.50 or <0.67 for carriers of the GC genotype and an OR of >2.28 or <0.53 for carriers of the CC genotype relative to the carriers of the GG genotype. This study had 80% power to detect an OR of >1.57 or <0.60 (for *HDAC5*

IVS3-4A > G) and an OR of >1.52 or <0.63 (for *HDAC5* $Asp^{593}Glu$) for the carriers of at least one wild-type allele relative to the carriers of the homozygous polymorphic allele.

Study Limitations

The limitations of the current study are the same as in other case-control studies, primarily, a selection and information bias, as has been described in previous studies by the current authors (3-5).

Conclusions

This is the first case-control study of HDAC polymorphisms in relation to lung cancer. Our results suggest that HDAC polymorphisms or their haplotypes do not significantly affect the susceptibility to lung cancer. However, because genetic polymorphisms often vary between different ethnic groups, additional studies are needed to clarify the association of HDAC polymorphisms with lung cancer in diverse ethnic populations.

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