

Lack of association of fragile histidine triad (*FHIT*) polymorphisms with lung cancer in the Korean population

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Abstract The fragile histidine triad (*FHIT*), which was located on chromosome 3p14.2, was currently considered a promising candidate for a tumor suppressor gene. *FHIT* performed a crucial function in the tumorigenesis of lung cancer. The inactivation of *FHIT* via genetic alterations, including the chromosomal deletions and aberrant transcription, are often associated with lung cancer. In this study, the association between *FHIT* and lung cancer development was evaluated in a study of Korean patients. A total of 299 Korean lung cancer patients and 296 control subjects were recruited into this study. Direct DNA sequencing and TaqMan analysis were employed. Logistic regression analyses were conducted in order to characterize

the association between *FHIT* polymorphisms and lung cancer risk. Via direct sequencing in 24 Korean individuals, 27 sequence variants were identified. Eleven of these polymorphisms were selected for a larger scale genotyping ($n = 595$). Our finding indicated that the polymorphisms and haplotypes in the *FHIT* gene are not associated with lung cancer in the Korean population.

Keywords Fragile histidine triad (*FHIT*) · Korean population · Haplotypes · Lung cancer · Single nucleotide polymorphisms

Introduction

Lung cancer is one of the most lethal known malignancies and ranks the second highest in incidence in Korea (Shin et al. 2005). Lung carcinogenesis is a complex multistep process that involves a number of genetic changes in the tumor suppressor gene. The p53 and RB genes are frequently inactivated by genetic alterations including chromosomal deletions and mutations, whereas the p16 gene is inactivated not only as the result of genetic alterations, but also by transcriptional silencing resulting from hypermethylation. The fragile histidine triad (*FHIT*) gene, a recently highlighted gene with regard to these genetic alterations, has also been shown to be inactivated in a large proportion of cases of lung cancer development (Kohno and Yokota 1999; Wistuba et al. 2001). *FHIT*, which is located on chromosome 3p14.2, is currently considered to be a promising candidate for a tumor suppressor gene (Pekarsky et al. 2002). The inactivation of *FHIT* has been determined to occur as the consequence of genetic alterations including the chromosomal deletions and aberrant transcription associated with a variety of cancers, such as colorectal, and lung cancer (Sozzi

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et al. 1997a, b; Yu et al. 2002). In addition, the majority of tumors and tumor-derived cell lines did not express or evidence reduced levels of *FHIT* (Druck et al. 1997; Hadaczek et al. 1998). Recently, Zanasi et al. reported that *FHIT* suppressed the tumorigenesis induced by *N*-nitrosomethylbenzylamine (NMBA) in *FHIT*-knockout mice (Zanasi et al. 2005). Specifically, the inactivation of the *FHIT* gene was profoundly associated with lung carcinogenesis. Sasaki et al. reported that reduced *FHIT* gene expression might occur early and play an important role in lung cancer tumorigenesis, and might also be correlated with the prognosis of lung cancer (Sasaki et al. 2006). Mao et al. reported that *FHIT* may be inactivated in tumor development, as in cases of smoking-associated lung cancer (Mao et al. 1997). However, no studies have yet been conducted to determine whether *FHIT* is associated with the risk of lung cancer risk.

In this study, we have attempted to characterize to the association between *FHIT* polymorphisms and the risk of lung cancer development in the Korean population, using genotype and haplotype analysis. Our finding indicated that *FHIT* polymorphisms and haplotypes are not significantly associated with lung cancer risk in the Korean population.

Materials and methods

Study subjects

The case-control study included 299 lung cancer patients and 296 healthy controls. The clinical characteristics of these groups have been summarized in Table 1. The eligible cases included all patients diagnosed with primary lung cancer at the Korea University Medical Center in Korea from October 2001 to April 2004. Lung cancer patients were recruited from the patient pool at the Genomic Research Center for Lung and Breast/Ovarian cancer, and the control subjects were randomly selected from a pool of healthy volunteers who had previously visited the Cardio-

Table 1 Demographic characteristics among lung cancer cases and controls

	Control	Case	<i>P</i> -value
Number of subjects	296	299	
Age (years)	51.8 ± 13.2	62.3 ± 10.3	<0.0001
Sex			
Male	283	219	<0.0001
Female	16	80	
Smoking status			
Never	90	76	0.332
Current	204	204	
Unknown	5	19	

vascular Genome Center. A detailed questionnaire including questions regarding diet, smoking status, drinking status, lifestyle and medical history were completed by each of the patients and each of the controls with the help of a trained interviewer. With regard to the smoking status of the subjects, any subject who reported smoking at least once on a daily basis was considered to be a smoker for the purpose of this study. The samples of this study were all approved by the Institutional Review Board of the Korea University Medical Center.

Sequencing analysis

We sequenced all exons and their boundaries, in addition to the promoter region of the *FHIT* (OMIM No.601153) gene in 24 Korean DNA samples, using the ABI Prism 3700 DNA analyzer (Applied Biosystems, Foster City, CA). All primer sets for the amplification and sequencing analysis were designed on the basis of GenBank sequences (Supplement Table 1). The primer of the related information can be accessed at <http://www.SNP-genetics.com>. Sequence variants were confirmed by chromatograms.

Genotyping with fluorescence polarization detection

In order to genotype the polymorphic sites, all amplifying primers and probes were designed for the TaqMan system (Supplement Table 2). Primer Express (Applied Biosystems) was used to design both the PCR primers and the MGB TaqMan probes. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. PCRs were conducted using TaqMan Universal Master Mix without UNG (Applied Biosystems) at PCR primer concentrations of 900 nM and TaqMan MGB-probe concentrations of 200 nM. The reactions were carried out in a 384-well format, with a total reaction volume of 5 µl using 20 ng of genomic DNA. The plates were then placed in a thermal cycler (PE 9700, Applied Biosystems) and heated at 50°C for 2 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. The TaqMan assay plates were transferred to a Prism 7900HT instrument (Applied Biosystems) in which the fluorescence intensity in each well of the plate was read. Fluorescence data files from each plate were analyzed using automated software (SDS 2.1).

Statistical analysis

The χ^2 tests were used to determine whether individual variants were in equilibrium at each locus in the population (Hardy–Weinberg equilibrium). Logistic regressions were used for calculating *P* values controlling for age, sex and smoking status as covariate. *P* values and odds ratios were

Table 2 Frequencies of *FHIT* polymorphisms in Korean population

Loci	Position	Amino acid change	rs #	Minor allele frequency	Heterozygosity	HWE ^a	
						Case	Control
-716064A > G	Promoter		rs9862543	0.283	0.405	–	0.059
-715859A > T	Promoter		rs1996958	0.172	0.284	0.992	0.878
-715850Tinsdel	Promoter		novel	0.043	0.083	–	0.977
-715821A > G	Promoter		novel	0.277	0.401	0.902	0.050
-715523T > C	Promoter		rs9858223	0.173	0.287	0.906	0.995
-714809A > T	Promoter		novel	0.146	0.249	–	0.403
-714528G > C	Promoter		rs9880846	0.439	0.492	0.064	0.091
-714473A > G	Promoter		novel	0.042	0.080	–	0.831
-663800T > C	Intron 1		rs17064630	0.479	0.499	–	0.676
-663794C > T	Intron 1		novel	0.021	0.041	–	0.917
-663785C > T	Intron 1		novel	0.383	0.472	0.810	0.520
-663500G > T	Intron 2		novel	0.063	0.117	–	0.744
-663277A > T	Intron 2		rs17064628	0.354	0.457	–	0.993
-285191A > C	Intron 3		rs11130785	0.063	0.117	–	0.744
-285185C > T	Intron 3		novel	0.167	0.278	–	0.327
-285136G > C	Intron 3		rs2205353	0.313	0.430	–	0.744
-285126G > A	Intron 3		rs2205352	0.254	0.379	0.936	0.868
-284843G > T	Intron 4		novel	0.063	0.117	–	0.744
523001A > G	Intron 6		rs13067835	0.233	0.357	0.410	0.754
525722G > A	Intron 7		rs4679627	0.341	0.450	0.855	0.637
525764A > G	Intron 7		rs4679626	0.333	0.444	–	0.759
525812AAAAAinsdel	Intron 7		novel	0.438	0.492	–	0.945
614565T > C	Exon 8	Cys97Arg	rs1385816	0.394	0.477	0.659	0.401
784503G > T	Intron 8		rs17061162	0.022	0.043	–	0.915
784575C > T	Intron 8		novel	0.087	0.159	–	0.648
784627T > A	Intron 8		rs1683356	0.202	0.322	0.341	0.120
784722C > T	Exon 9	Ala141Val	novel	0.003	0.005	0.996	.

^a P-values of deviation from among lung cancer and normal subjects

Bold face means SNPs genotyped in a larger population ($n = 595$) and plain faces were based on the sequencing data ($n = 24$)

obtained using logistic regression. Heterozygosity for each locus with allele frequencies p and q ($q = 1-p$) was derived using $H = 1-p^2-q^2 = 2p(1-p)$. We examined widely used measures of linkage disequilibrium between all pairs of biallelic loci; Lewontin's D' (D') (Hedrick 1987) and r^2 . Haplotypes and their frequencies were inferred using the algorithm developed by Stephens et al. (2001). Statistical methods of ANOVA and logistic regression were performed to analyze the ADJ-BMD levels and genotype distribution. The effective number of independent marker loci in *FHIT* was calculated to correct for multiple testing, using the software SNPSpD (<http://www.genepi.qimr.edu.au/general/daleN/SNPSpD/>), which is based on the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs (Nyholt 2004). The resulting number of independent marker loci was applied to correct for multiple testing. Statistical powers were calculated using Statistical Power

Calculator (http://www.dssresearch.com/toolkit/spcalc/power_p2.asp).

Results

By direct DNA sequencing in 24 individuals, we identified 27 genetic variants with exons and flanking regions of *FHIT* including the promoter regions. Eleven of the identified polymorphisms were selected for larger scale genotyping on the basis of locations, frequency, linkage disequilibrium (LD) and haplotype tagging status (Table 2; Fig. 1). Among 27 polymorphisms, -715859A > T, -715821A > G, -715523T > C, -714528G > C, -663785C > T, -285126G > A, 523001A > G, 525722G > A, 614565T > C, 784627T > A and 784722C > T of *FHIT* were selected and analyzed for a larger scale

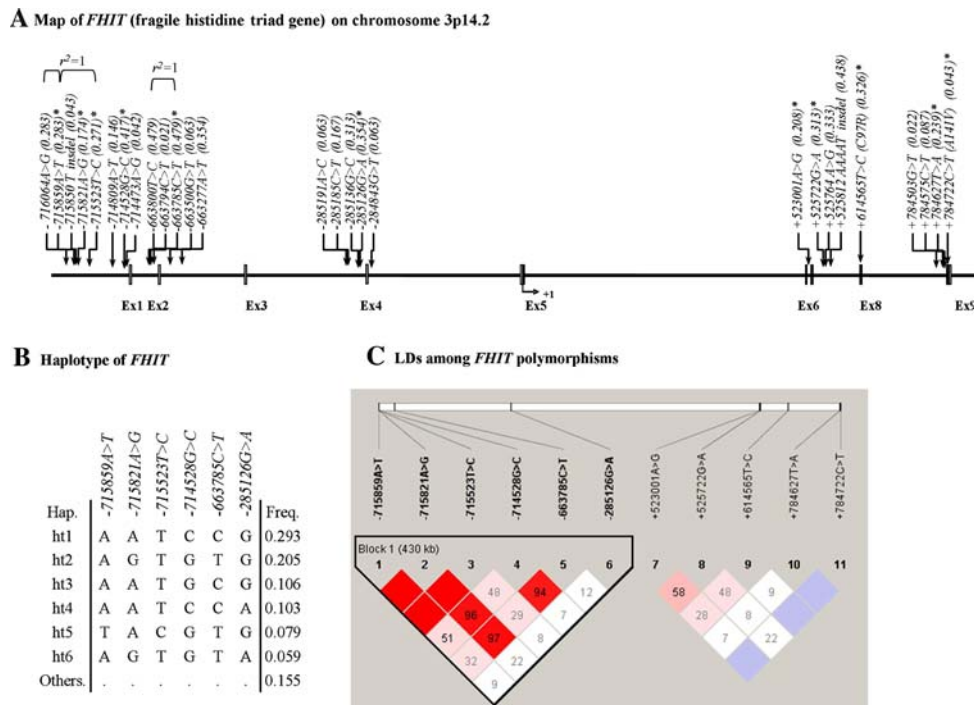


Fig. 1 Gene map, haplotypes and LD coefficients in *FHIT*. **a** Gene Map and SNPs in *FHIT* on chromosome 3p14.2 (reference sequence of *FHIT*: NT_022517.17). Coding exons are marked by black blocks and 5' and 3' UTR by white blocks. Asterisks indicate SNPs that were genotyped in a larger population. The frequencies of SNPs without larger scale genotyping were based on sequencing data ($n = 24$). The first base of the translation site was denoted as nucleotide +1.

b Haplotypes of *FHIT*. Haplotypes with frequency >0.03 are presented. **c** Linkage disequilibrium coefficient (D') among *FHIT* SNPs. Only six SNPs, including -715859 A > T, -715821A > G, -715523T > C, -714528G > C, -663785C > T and -285126G > A, were used for construction of haplotype, because the rest of the polymorphisms were not in strong LD

genotyping ($n = 595$) by considering their allele frequencies, haplotype-tagging status, and LDs among polymorphisms in Korean lung cancer patients and normal controls. Genotype frequencies for cases and controls were in Hardy–Weinberg equilibrium. Linkage disequilibrium coefficients (D') and r^2 among polymorphisms were also calculated (Fig. 1). Only six SNPs, including -715859 A > T, -715821A > G, -715523T > C, -714528G > C, -663785C > T and -285126G > A, were used for construction of the haplotype, because the rest of the polymorphisms were not in strong LD. The allelic frequencies of each polymorphism and haplotype were compared between the patients and controls using logistic regression models (Table 3, Supplement Table 3). Logistic regression analyses revealed that *FHIT* + 525722G > A showed marginal association with the lung cancer risk, but the association was lost after correction using SNPSpD. In the stratified analyses by age, gender and smoking status as well as the analyses of historical types of lung cancer, no significant associations were found between the *FHIT* polymorphisms and lung cancer risk (Supplement Tables 4 and 5).

Discussion

In this study, we hypothesized that *FHIT* polymorphisms were associated with lung cancer risk and that these polymorphisms might play a role as a predictor of lung cancer. We conducted preliminary polymorphism study for *FHIT* by sequencing at 24 samples and, for a further larger scale study, selected 11 polymorphisms of *FHIT* via genotyping a total of 595 samples, including 299 lung patients and 296 controls. Our finding indicated no association between the polymorphisms of the *FHIT* gene and lung cancer risk in the Korean population. When considering the absence of association and the limitation of our study, the small number of study subjects might affect the lack of association.

The tumor suppressor gene *FHIT*, which is located on chromosome 3p14.2, is associated with lung cancer. Several groups have reported that abnormalities of the *FHIT* gene and protein occur very frequently in cases of lung cancer (Fong et al. 1997; Sozzi et al. 1997a, b; Yanagisawa et al. 1996). However, very little research has thus far been focused on polymorphisms of the *FHIT* gene. In 1997,

Table 3 Logistic analysis of *FHIT* polymorphisms with the risk of lung cancer while controlling for age, sex and smoking status as covariates among lung cancer and normal subjects

Loci	Genotype	Case	Control	Crude OR (95% CI)	Crude <i>P</i>	Adjusted OR (95% CI)	<i>P</i>	<i>P</i> _{corr}	Statistical power (%)
-715859A > T	AA	189 (68.2%)	203 (69.3%)						
	AT	79 (28.5%)	83 (28.3%)	1.07 (0.79–1.46)	0.67	0.97 (0.66–1.43)	0.89	NS	8.70
	TT	9 (3.3%)	7 (2.4%)						
-715821A > G	AA	151 (54.1%)	154 (52.9%)						
	AG	108 (38.7%)	104 (35.7%)	0.88 (0.69–1.13)	0.33	0.88 (0.65–1.18)	0.39	NS	17.70
	GG	20 (7.2%)	33 (11.3%)						
-715523T > C	TT	190 (68.4%)	201 (68.8%)						
	CT	80 (28.8%)	82 (28.1%)	1.01 (0.74–1.37)	0.95	0.91 (0.63–1.33)	0.63	NS	5.70
	CC	8 (2.9%)	9 (3.1%)						
-714528G > C	GG	96 (34.4%)	102 (34.8%)						
	CG	121 (43.4%)	127 (43.3%)	1.01 (0.81–1.27)	0.90	1.06 (0.81–1.37)	0.68	NS	6.10
	CC	62 (22.2%)	64 (21.8%)						
-663785C > T	CC	109 (39.5%)	113 (38.7%)						
	CT	128 (46.4%)	130 (44.5%)	0.93 (0.74–1.18)	0.56	0.90 (0.68–1.19)	0.47	NS	11.00
	TT	39 (14.1%)	49 (16.8%)						
-285126G > A	GG	141 (51.5%)	169 (58.1%)						
	AG	109 (39.8%)	108 (37.1%)	1.32 (1.01–1.72)	0.04	1.12 (0.81–1.55)	0.50	NS	40.70
	AA	24 (8.8%)	14 (4.8%)						
<i>ht1</i>	–/–	147 (53.1%)	144 (49.3%)						
	–/ht1	107 (38.6%)	129 (44.2%)	0.95 (0.73–1.24)	0.71	1.00 (0.73–1.37)	1.00	NS	8.40
	ht1/ht1	23 (8.3%)	19 (6.5%)						
<i>ht2</i>	–/–	179 (64.6%)	171 (58.6%)						
	–/ht2	86 (31.1%)	99 (33.9%)	0.78 (0.59–1.02)	0.07	0.83 (0.59–1.15)	0.26	NS	37.20
	ht2/ht2	12 (4.3%)	22 (7.5%)						
<i>ht3</i>	–/–	206 (74.4%)	231 (79.1%)						
	–/ht3	69 (24.9%)	57 (19.5%)	1.22 (0.85–1.76)	0.28	1.15 (0.75–1.78)	0.52	NS	19.00
	ht3/ht3	2 (0.7%)	4 (1.4%)						
<i>ht4</i>	–/–	218 (78.7%)	239 (81.9%)						
	–/ht4	52 (18.8%)	49 (16.8%)	1.23 (0.86–1.77)	0.26	1.31 (0.86–2.00)	0.21	NS	20.10
	ht4/ht4	7 (2.5%)	4 (1.4%)						
<i>ht5</i>	–/–	239 (86.3%)	253 (86.6%)						
	–/ht5	36 (13.0%)	38 (13.0%)	1.06 (0.67–1.67)	0.81	0.98 (0.56–1.70)	0.93	NS	7.30
	ht5/ht5	2 (0.7%)	1 (0.3%)						
+523001A > G	AA	150 (53.8%)	179 (61.3%)						
	AG	114 (40.9%)	102 (34.9%)	1.31 (0.99–1.74)	0.06	1.36 (0.96–1.92)	0.08	NS	36.40
	GG	15 (5.4%)	11 (3.8%)						
+525722G > A	GG	106 (38.3%)	135 (46.2%)						
	AG	134 (48.4%)	133 (45.6%)	1.36 (1.06–1.75)	0.02	1.43 (1.05–1.94)	0.02	NS	49.60
	AA	37 (13.4%)	24 (8.2%)						
+614565T > C	TT	107 (38.6%)	113 (38.8%)						
	CT	123 (44.4%)	127 (43.6%)	0.99 (0.79–1.25)	0.95	0.80 (0.61–1.06)	0.12	NS	5.30
	CC	47 (17.0%)	51 (17.5%)						
+784627T > A	TT	165 (67.4%)	168 (59.2%)						
	AT	68 (27.8%)	108 (38.0%)	0.82 (0.61–1.12)	0.21	0.93 (0.65–1.34)	0.70	NS	21.30
	AA	12 (4.9%)	8 (2.8%)						

Table 3 continued

Loci	Genotype	Case	Control	Crude OR (95% CI)	Crude <i>P</i>	Adjusted OR (95% CI)	<i>P</i>	<i>P</i> corr	Statistical power (%)
+784722C > T	CC	275 (98.9%)	292 (100.0%)						
	CT	3 (1.1%)	0 (0.0%)						
	TT	0 (0.0%)	0 (0.0%)						

Logistic regression models were used for calculating crude and adjusted odds ratios (95% confidential interval) and corresponding *P*-values of codominant model for each SNP site and haplotype. Haplotypes in block 2 were not analyzed because SNPs in haplotype block 2 were not tightly linked

**To achieve the optimal correction for multiple testing of single-nucleotide polymorphisms (SNPs) in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (10.4683) in *FHIT* was calculated using the software SNPSpD (<http://genepi.qimr.edu.au/general/daleN/SNPSpD/>), on the basis of the spectral decomposition (SpD) of matrices of pair-wise LD between SNPs (Nyholt 2004)

Statistical powers were calculated using the Statistical Power Calculator (http://www.dssresearch.com/toolkit/spcalc/power_p2.asp)

Gemma et al. initially reported the discovery of a somatic mutation of codon 61 (threonine change to methionine) in exon 6 in human primary gastric cancer as the result of PCR single-strand conformation polymorphism analysis (Gemma et al. 1997). In 2003, only one group reported an association between *FHIT* polymorphisms and cancer risk. They suggested that codon 98 polymorphism in exon 8 of *FHIT* might be associated with an elevated risk of developing cervical cancer (Jee et al. 2003). Our group elected to investigate a possible association between polymorphisms of *FHIT* and risk of lung cancer. Our data indicated that the distribution of allele genotypes between all lung cancer and control subjects was not significantly different.

In summary, 27 sequence variants of *FHIT* were identified in 24 Korean individuals through direct sequencing. Eleven of these polymorphisms were selected for a larger scale genotyping ($n = 595$). We observed no significant difference in allele genotype distribution between the lung cancer cases and controls. Thus, we have concluded that no significant association exists between these polymorphisms and lung cancer risk in the Korean population.

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