Significant Association Between Polymorphisms of Wnt Antagonist Genes and Lung Cancer

Meral Yilmaz, PhD, Gonca Donmez, PhD,† Turgut Kacan, MD,‡ Ismail Sari, PhD,§ Nalan Akgül Babacan, MD,‖ Musa Sari, PhD,¶ and Saadettin Kilickap, MD***

Abstract: Further elucidation of the molecular mechanisms underlying lung cancer (LC) is essential for the development of new effective therapeutic agents. Recently, involvement of Wnt antagonists in oncogenesis has been demonstrated in several cancers. The investigation of their contribution to lung carcinogenesis is still under investigation. We aimed to investigate whether there is a susceptibility or preventive effect of Wnt antagonist gene polymorphisms on the development and/or prognosis of LC. We investigated 110 LC patients and 160 controls. Single-nucleotide polymorphisms of Wnt antagonist genes including *DKK2* (rs17037102), *DKK3* (rs3206824), *DKK3* intron4 G/C (rs7396187), *DKK4* (rs2073664), and *sFRP4* (rs1802074) were analyzed using nested polymerase chain reaction and restriction fragment length polymorphism. Results showed that patients with *DKK3* AA compared with controls have a decreased risk of LC (adjusted for smoking habit, body mass index, and familial history) (P = 0.02; odds ratio [OR],0.08; 95% confidence interval [95% CI], 0.01–0.7). It was found that, for sFRP4 polymorphism, patients with GG and GA genotypes versus AA genotype controls showed a decreased risk for LC (P = 0.01; [OR, 0.19; 95% CI, 0.05–0.73 for GG genotype]; [OR = 0.18, 95% CI, 0.04–0.72 for GA genotype]). In addition, a decreased risk of LC was also found for the genotype combination of *DKK3* (rs3206824) GG and *sFRP4* AG + GG (P = 0.004; OR, 0.12; 95% CI, 0.02–0.58). We suggest that these 2 polymorphisms have a protective effect on LC in this study.

Key Words: *DKK* genes, lung cancer, polymorphism, *sFRP4* gene

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Lung cancer (LC) is the most common cancer worldwide and the leading cause of cancer-related death in both men and women.[1] Although important advances are achieved in diagnosis and treatment, the prognosis of LC is still poor.[2] Recent studies have shown that targeted therapy has shown clinical benefits. Thus, further elucidation of the molecular mechanism underlying LC is essential for the development of new effective therapeutic agents.[3–5] As shown in many cancer types, Wnt signaling has emerged as an important pathway in lung carcinogenesis.[5] It was indicated that Wnt signaling is important in non-small-cell LC (NSCLC) cell lines, and decrease in proliferation was determined by inhibition of Wnt. This pathway was modulated by Wnt antagonists such as Dickkopfs (DKKs) and secreted frizzled-related proteins (sFRPs).[6] Wnt antagonists inhibit signaling by directly binding to Wnt ligands or by binding to the low-density lipoprotein receptor–related protein 5 (LRP5)/LRP6 coreceptors. Hirata et al.[7] reported that some polymorphisms in Wnt antagonist genes are associated with renal cancer. Studies investigated the relationship between LC and Wnt antagonist genes in the form of epigenetic approach. No study has shown whether polymorphisms of *DKK2* (Exon3 Arg146Glu-rs17037102), *DKK3* (Exon7 Arg335Gly-rs3206824), *DKK3* (Intron4 G/C-rs7396187), *DKK4* (Exon4 V169V-rs2073664), and *sFRP4* (Exon6 R340K-rs1802074) were related to LC in the Turkish population before. In addition, we did not find any study that investigated these single-nucleotide polymorphisms (SNPs) in LC. Therefore, our study may be basic for studies related with the risk of LC and polymorphisms of Wnt antagonist genes in different populations. Thus, in this study, we aimed to investigate whether there is a susceptibility or preventive effect of Wnt antagonist gene polymorphisms on the development and/or prognosis of LC.

PATIENTS AND METHODS

Patients

We investigated 110 patients who were admitted to the Oncology center of Cumhuriyet University and had been diagnosed as having LC between January 2012 and December 2012 as the patient group. As a control group, 160 healthy, age- and sex-matched, unrelated, hospital-based, voluntary individuals, without hereditary disease and cancer history, were selected. The study was approved by the Ethical Committee of Cumhuriyet University in Sivas, Turkey (decision no. 2011/025). Data about smoking habit, alcohol consumption, family history, and body mass index (BMI) were collected from the groups. A written informed consent was obtained from all individuals.

DNA Extraction From Blood Samples

Four milliliters of peripheral whole-blood samples was collected into EDTA-containing tubes from both groups. DNA was extracted from whole blood by the salting out procedure and stored at −20°C until analyzed.

Genotype Analysis

Nested polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods were used for genotyping analysis of *DKK2* nonsynonymous (exon 3 Arg146Glu) (rs17037102), *DKK3* nonsynonymous (exon 7 Arg335Gly) (rs3206824), *DKK3* intron 4 G/C (rs7396187), *DKK4* synonymous (exon 4 V169V) (rs2073664), and *sFRP4* nonsynonymous exon 6 (R340K) (rs1802074) gene polymorphisms. We selected these polymorphic sites based on previous reports and HapMap data (available at: http://www.hapmap.org/. Accessed January 3, 2009), which were composed of possibly functional SNPs (nonsynonymous and 50 or 30 untranslated region SNPs) or disease-associated SNPs.[7] In addition, we did not obtain any data that described whether these polymorphisms have functional significance for protein activity and/or production. Primer sets for

From the *Departments of Research Centre, †Medical Biology, ‡Medical Oncology, and §Biochemistry, Cumhuriyet University, Faculty of Medicine, Sivas; ‖Department of Medical Oncology, Marmara University, Faculty of Medicine, Istanbul; ¶Department of Biology, Cumhuriyet University, Faculty of Science, Sivas; and **Department of Medical Oncology, Hacettepe University, Faculty of Medicine, Ankara, Turkey.
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Reprints: Meral Yilmaz, PhD, Department of Research Centre, Cumhuriyet University, Faculty of Medicine, 58140 Sivas, Turkey.
E-mail: meralylmz@cumhuriyet.edu.tr.
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amplification of target sites of these genes, PCR and RFLP products, and restriction enzymes were shown in Table 1. Both steps of nested PCR reaction were made in a total volume of 25 μL including 10 pmol of each amplification primer set, 5 nmol of each of the 4 deoxynucleotide triphosphates (Fermentas), 10 mmol/L Tris-HCl (pH 8.3 at 25°C), 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 1 unit of Taq DNA polymerase (Fermentas), and 100 ng genomic DNA for the first PCR step (5 μL PCR product for the second PCR step). Polymerase chain reactions were performed using Gene Amp PCR system 9700 Applied Biosystems thermal cycler (USA). For each nested PCR step, PCR conditions occurred at an initial denaturation step (94°C, 5 minutes) followed by 35 cycles of denaturation step (94°C, 30 seconds), annealing step (52°C, 1 minute for first step; 58°C, 1 minute for second step), extension step (72°C, 30 seconds), and followed by 1 cycle of final extension (72°C, 5 minutes).

For RFLP analysis, 8 μL of PCR products was treated with specific 5U restriction enzyme (Table 1) and 1.5 μL suitable reaction buffer for each SNP in a total reaction volume of 10 mL and then incubated at 37°C overnight. All RFLP products were run on a 3% agarose gel and imaged using UV transilluminator (Fig. 1).

Statistical Analysis

All statistical analyses were made using Statistical Package for Social Sciences 15.0 program (SPSS Inc, Chicago, Ill). Mean age and BMI were calculated by independent *t* test. Distributions of sex and smoking habit, alcohol consumption, and allele-genotype among patient-control groups were evaluated using χ^2 test. In addition, odds ratios (ORs) and 95% confidence interval (95% CI) were also calculated using χ^2 test. The value of $P < 0.05$ was considered statistically significant.

RESULTS

One hundred ten patients with LC and 116 healthy voluntary controls (hospital based) were investigated for the current study. The demographic features of all subjects and clinical characteristics of the patient group were shown in Table 2. The frequencies of smoking habit and family history were significantly higher in patients compared with the controls. In addition, we detected that patients who have first-degree relatives (father and/or mother and/or sister) with a positive cancer history were also higher than controls ($P = 0.0001$). Body mass index was found to be significantly lower in patients than the controls.

Genotype distributions of the 5 polymorphisms among groups were fitted by Hardy-Weinberg equilibrium. Frequencies of genotypes and alleles for these polymorphisms of Wnt antagonist genes including *DKK2* (rs17037102), *DKK3* (rs3206824), *DKK3* (rs7396187), *DKK4* (rs2073664), and *sFRP4* (rs1802074) in patients with LC and controls were shown in Table 3. We found no significant difference between patient-control groups and genotype distributions for the 4 polymorphisms concerning rs17037102, rs3206824, rs7396187, and rs2073664. We found that patients with AA genotype of the *DKK3* exon7 polymorphism compared with controls have a decreased risk of LC (adjusted for smoking habit, BMI, and familial history) (Table 3). A statistically significant difference between genotype distribution of *DKK3* exon7 polymorphism and histological types of LC (small-cell LC [SCLC] and NSCLC) was determined in this study ($P = 0.0001$). In addition, when genotype distributions of *sFRP4* polymorphism in patients with LC were compared with controls, patients with GG and GA genotypes versus AA genotype controls showed a decreased risk for LC (Table 3). However, when the genotype together with other risk factors (smoking

TABLE 1. Presentation of Amplification Primer Sets and Sizes of PCR and RFLP Products and Restriction Enzymes for Wnt Antagonist SNPs

SNPs	Regions	Forward and Reverse Primer Sequences (5′ → 3′)	PCR Products, bp	RE Enzymes	Alleles and Product Size, bp	
DKK2 nonsynonymous (Exon3 Arg146Gln)	rs17037102	1 F:TGGCTTCATATTTCACATCAAGA 1R:TGTGTGGTCTTCCTAGATTCTGC	226	*Ddel*	G	132
		2 F:TGATCATCTCCAGGCATCTG 2R:ATTCTGCCATCCCAAGTCAT	132		A	77 + 55
DKK3 nonsynonymous (Exon7 Arg335Gly)	rs3206824	3 F:GAGGTCCCCGATGAGTATGA 3R:TAGGAAGAAGCCTGGTCAGC	242	*Ddel*	G	210
		4 F:GGTCCCCGATGAGTATGAAG 4R:AGCACACACCTGGGGAAATA	210		A	115 + 95
DKK3 (Intron4 G/C)	rs7396187	5 F:TTCCTTAGGTCCCTAGGTCCA 5R:AGGGCAAAGGAGACTCTTCA	377	*Fnu4HI*	G	245
		6 F:ACAGGGCATGGCAGTTAGAG 6R:CTCTTCACCCAACAGGCATT	245		C	171 + 74
DKK4 synonymous (Exon4 V169V)	rs2073664	7 F:GCCATGGCATTACTGCTTTT 7R:ATTGCTGGTCAATTGGCTTC	384	*EcoNI*	C	224 + 68
		8 F:CTGCGTGCTGTGTCTGTTTT 8R:AACGCTGGAAGATTTCTGGA	292		T	292
sFRP4 nonsynonymous (Exon6 R340K)	rs1802074	9 F:AAGAGAGGCTGCAGGAACAG 9R:TCTGTACCAAAGGGCAAACC	397	*EarI*	G	134 + 112
		10 F:AGAGCGGAGAACAGTTCAGG 10R:TGGCCTTACATAGGCTGTCC	246		A	246

SNP indicates single-nucleotide polymorphism; RE, restriction endonucleases; *DKK2*, Dickkopf 2; *DKK3*, Dickkopf 3; *DKK4*, Dickkopf 4; *sFRP4*, secreted frizzled-related protein 4; rs, SNP identification number; G, guanine; A, adenine; C, cytosine; T, thymine; OR, odds ratio; CI, confidence interval; bp, base pair.

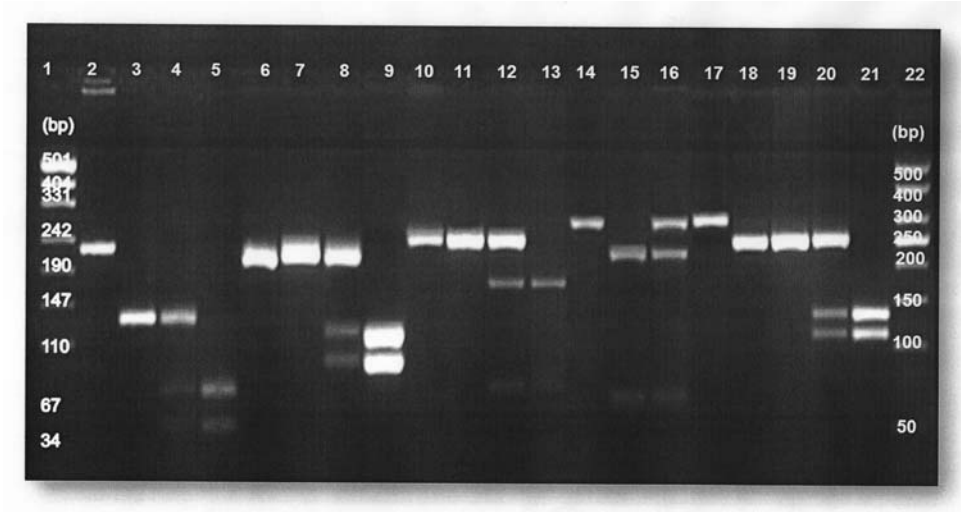

FIGURE 1. Illustration of genotypes of *DKK2*, *DKK3*, *DKK4*, and *sFRP4* gene polymorphisms on 3% agarose gel. 1, pUC19Marker (Fermentas); 2 to 5 for *DKK2* nonsynonymous Exon3 Arg146Glu polymorphism: PCR product (226 bp), GG-wild type (132 bp), GA-heterozygous type (132 + 77 + 55 bp), AA-polymorphic type (77 + 55 bp), respectively; 6 to 9 for *DKK3* nonsynonymous Exon7 Arg335Gly polymorphism: PCR product (210 bp), GG-wild type (210 bp), GA-heterozygous type (210 + 115 + 95 bp), AA-polymorphic type (115 + 95 bp), respectively; 10 to 13 for *DKK3* Intron4 G/C polymorphism: PCR product (245 bp), GG-wild type (245 bp), GC-heterozygous type (245 + 171 + 74 bp), CC-polymorphic type (171 + 74 bp), respectively; 14 to 17 for *DKK4* synonymous Exon4 V169V polymorphism: PCR product (292 bp), CC-wild type (224 + 68 bp), CT-heterozygous type (292 + 224 + 68 bp), TT-polymorphic type (292 bp), respectively; 18 to 21 for *sFRP4* nonsynonymous Exon6 R340K: PCR product (246 bp), GG-wild type (134 + 112) bp, GC-heterozygous type (246 + 134 + 112 bp), CC-polymorphic type (246 bp), respectively; 22, Marker 50 bp (Biomed).

habit, BMI, and familial history of cancer) was evaluated, no statistically significant association between decreased cancer risk and GG genotype of the *sFRP4* polymorphism was defined (Table 3). When controls with GG genotype were compared with patients with AA genotype of *sFRP4* rs1802074 polymorphism, these LC patients have 5 times increased risk of cancer ($P = 0.01$; OR, 5.15; 95% CI, 1.36–19.4). However, when LC patients with AA genotype of the polymorphism were analyzed together with other risk parameters for LC including smoking habit, decreased BMI, and familial history of cancer, a significant association was not found between cancer risk and AA genotype ($P = 0.14$; OR,3.04; 95% CI, 0.68–13.4). The distributions of genotypes did not differ in patients with recurrence of cancer nor in patients with metastasis ($P > 0.05$). In addition, there was no association between genotypes of the 5 polymorphisms and histological type of LC ($P > 0.05$). Besides, there was no statistically significant difference for genotype distributions of these polymorphisms either early stages or advanced stages in NSCLC and SCLC ($P > 0.05$).

Furthermore, gene-gene interaction was analyzed using *DKK3* (rs3206824) and *sFRP4* (rs1802074) polymorphisms between controls and patients. When genotype combinations for both SNPs were investigated, decreased risk of LC was found for only the combination of *DKK3* (rs3206824) GG and *sFRP4* (rs1802074) AG + GG genotypes (Table 4).

Haplotype analysis was performed for 4 possible haplotypes, which were defined by 2 polymorphisms of *DKK3* gene in this study. A linkage was observed for the 2 variants in both patients and controls ($\chi^2 = 29.37$, $P = 0.0001$ for patients; $\chi^2 = 26.45$, $P = 0.0001$ for controls). Because GG haplotype was more common in both groups, GG haplotype was evaluated as a reference haplotype. We did not find a statistically significant difference between patients with LC and controls for the 4 haplotypes in haplotype analysis of 2 SNPs of *DKK3* gene ($P > 0.05$) (Table 4).

In addition, genotype distributions of the 5 SNPs according to clinicopathological parameters in all patients were investigated in this study. We did not observe any significant difference, except genotype distribution of *sFRP4* polymorphism, between males and females.

DISCUSSION

We analyzed 110 patients with LC, and majority of those have NSCLC (91.8%) in this study. We found that squamous cell LC was the most common type of NSCLC. Contrary to our data, the most common form of NSCLC is adenocarcinoma (AC) in most Asian countries and western countries.[1,8] It suggested that smoking habit and lifestyle in eastern countries could be the cause of this.[9,10]

In our study, which is consistent with other studies,[11,12] smoking habit ratio was higher in males than in females and in patients than in controls. Smokers had a significantly higher (~9-fold) risk than nonsmokers in our study for LC. In addition, family history of LC was significantly higher in patients than the control group in the present study. Epidemiological studies have shown that a family history of LC is a predictor of increased risk and affects prognosis.[13] Furthermore, consistent with other studies,[14,15] BMI was significantly lower in patients with LC than in the control group in the current study.

When effects of DKK2 (rs17037102), DKK3 (rs3206824), DKK3 (rs7396187), DKK4 (rs2073664), and sFRP4 (rs1802074) polymorphisms on LC were investigated in this study, we detected that *DKK2* (rs17037102) and *DKK4* (rs2073664) polymorphisms were not associated with LC development. Consistent with our data in LC, Hirata et al.[7] did not find any association with both polymorphisms and renal cancer either. Besides, another *DKK4* gene region (rs3763511) has been analyzed by Alanazi et al.[16] They reported that the SNP did not display any association in overall breast cancer. However, they found a statistically significant association between increased risk of estrogen receptor–negative breast cancer and *DKK4* rs3763511 ($P = 0.009$; OR, 16.7; 95% CI, 0.838–334.06).[16]

TABLE 2. Demographic Features of Both Groups and Characteristics of Patients

Characteristics	Patients, n = 110	Controls, n = 160	*P*	OR (95% CI)
Mean age ± SD, y	60.1 ± 8.9 (35–80)	58.33 ± 8.2 (43–85)		
Mean BMI ± SD, kg/m^2	24.8 ± 4.53 (16.0–40.6)	27.9 ± 4.6 (18.9–42.9)	0.0001*	4.15 (2.4–7.0)
Sex, n (%)				
Male/female	93/17 (84.5/15.5)	130/30 (81.2/18.8)		
Smoker/nonsmoker	95/15 (86.4/13.6)	67/92 (42.1/57.9)	0.0001*	8.69 (4.6–16.3)
Alcohol consumption, n (%)	0.86	1.10 (0.5–2.1)		
Yes/no	18/92 (16.4/83.6)	24/136 (15/85)		
Familial cancer history, n (%)	0.004*	2.91 (1.4–5.9)		
Yes/no	24/86 (21.8/78.2)	14/146 (8.8/91.2)		
Histological type of LC, n (%)				
SCLC	9 (8.2)			
NSCLC	101 (91.8)			
Adenocarcinoma	29 (28.7)			
Squamous carcinoma	44 (43.5)			
Others	28 (27.8)			
Clinicopathological stage of LC				
Tx	9 (8.2)			
T0	3 (2.7)			
T1	3 (2.7)			
T2	7 (6.4)			
T3	17 (15.5)			
T4	64 (58.2)			
Early stage	10 (9.1)			
Advanced stage	100 (90.9)			
Recurrence of cancer				
Yes/no, n (%)	9/101 (8.2/91.8)			
Metastases				
Yes/no, n (%)	69/41 (62.7/37.3)			

*$P < 0.05$ indicates statistically significant.

BMI indicates body mass index; SD, standard derivation; SCLC, small-cell lung cancer; NSCLC, non-small-cell lung cancer.

Another member of DKK family is *DKK3*. A study indicated that reduced *DKK3* expression is related with LC.[17] In another study, Lee et al.[18] indicated that *DKK3* gene is downregulated by promotor hypermethylation in cervical cancer and LC. *DKK3* promotor hypermethylation has also been observed in NSCLC.[19] It has been reported in a different study that GGG/AGG polymorphism at codon 335 in *DKK3* gene was not a contributing factor for the development of cervical cancer. In this study, genotype frequencies of this SNP among the case-control groups in Korea were found similarly by Lee and colleagues.[18] The SNP in REIC (*DKK3*-rs3206824) gene was also investigated in Japan patients with LC. Genotype frequencies of the polymorphisms were defined as 50%, 42.9%, and 7.1% in patients and 51.5%, 38.5%, and 10% in controls, respectively. Kobayashi et al.[19] have reported that there was no statistically significant difference regarding the distribution of GGG and AGG alleles among groups. In our study, frequencies of GG, GA, and AA genotypes for rs3206824 polymorphism were 77.3%, 20.9%, and 1.8% in patients and 76.2%, 21.9%, and 1.9% in controls. Similar to findings of the 2 studies,[18,19] we did not find significant differences between genotype distributions of the *DKK3* gene polymorphism (rs3206824) and patient-control groups ($P > 0.05$) yet. However, when the polymorphism, together with other risk factors such as smoking habit, familial history of cancer, and BMI, was evaluated, we determined a decreased risk (OR,0.08) for LC. In addition, when another polymorphism (rs7396187) in *DKK3* gene was investigated in our study, we did not find any association between LC risk and genotypes of the polymorphism. Besides, we did not determine any association between haplotypes of *DKK3* gene and LC risk. In contrast to our study, Hirata et al.[7] reported that there was a significant difference in the frequencies of the genotypes of both *DKK3* rs3206824 and DKK3 rs7396187 in patients with renal cell carcinoma compared with controls. They also found a significant decrease in the frequency of genotypes of *DKK3* rs7396187 in patients with renal cell carcinoma (for GC genotype, $P = 0.01$; OR,0.57; 95% CI, 0.37–0.88; for CC genotype, $P = 0.02$; OR, 0.45; 95% CI, 0.22–0.92).[7] We did not analyze the expression of *DKK3* gene in samples with LC and association between DKK3 gene polymorphisms and gene expression. We did not find any data regarding the effect of the polymorphism on *DKK3* gene expression in the literature. It has been reported that, in human tumor, *DKK3* protein behaves as a tumor suppressor and plays a tissue-specific function. The codon 335 of *DKK3* protein was located at coiled coil motif in the protein. A coiled coil is a structural motif in proteins. Many coiled coil–type proteins are involved in important biological functions such as the regulation of gene expression. It has been suggested that the exon7 Arg335Gly polymorphism in the gene possibly has various effects on protein function and thus the substitution may alter the activity of *DKK3* protein.[18,19] However, Kobayashi et al.[19] suggested that the amino

TABLE 3. Genotype Frequencies and OR Values for SNPs of DKK Genes and Haplotype Analysis of DKK3 Gene Polymorphisms in Both Groups

			Crude		Adjusted	
Genotypes	**Controls, n (%)**	**Patients, n (%)**	***P***	**OR (95% CI)**	***P***	**OR (95% CI)**
DKK2 nonsynonymous (Exon3 Arg146Gln-c.437 G/A)						
GG	125 (78.1)	75 (68.2)		Reference		Reference
GA	34 (21.2)	34 (30.9)	0.08	1.66 (0.9–2.9)	0.76	1.57 (0.08–28.8)
AA	1 (0.6)	1 (0.9)	1	1.66 (0.1–27)	0.52	2.64 (0.13–51.5)
GA + AA	35 (21.8)	35 (31.8)	0.08	1.66 (0.9–2.8)		
G	284 (88.8)	184 (83.6)		Reference		
A	36 (11.2)	36 (16.4)	0.09	1.54 (0.9–2.5)		
DKK3 nonsynonymous (Exon7 Arg335Gly-c.1003 A/G)						
GG	122 (76.2)	85 (77.3)		Reference		Reference
GA	35 (21.9)	23 (20.9)	0.88	0,94 (0,5–1,7)	0.12	0.20 (0.02–1.52)
AA	3 (1.9)	2 (1.8)	1	0.95 (0,1–5.8)	0.02*	0.08 (0.01–0.7)
GA + AA	38 (23.8)	25 (22.7)	0.88	0.94 (0.5–1.6)		
G	279 (87.2)	193 (87.7)		Reference		
A	41 (12.8)	27 (12.3)	0.89	0.95 (0,56–1,6)		
DKK3 (Intron4 G/C)						
GG	138 (96.2)	90 (81.8)		Reference		
GC	22 (13.8)	19 (17.3)	0.49	1.32 (0.6–2.5)		
CC	0 (0)	1 (0.9)				
GC + CC	22 (13.8)	20 (18.2)	0.39	1.39 (0.7–2.7)		
G	276 (92.6)	199 (90.5)		Reference		
C	22 (7.4)	21 (9.5)	0.42	1.32 (0.7–2.47)		
DKK4 synonymous (Exon4 V169V-57C/T)						
CC	120 (75)	92 (83.6)		Reference		
CT	39 (24.4)	18 (16.4)	0.12	0.6 (0.3–1.1)		
TT	1 (0.6)	0 (0)				
CT + TT	40 (30)	18 (16.4)	0.09	0.58 (0.3–1.0)		
C	241 (85.8)	202 (91.8)		Reference		
T	40 (14.2)	18 (8.2)	0.04	0.53 (0.29–0.96)		
sFRP4 nonsynonymous (Exon6 R340K-c.1019 G/A)						
AA	3 (1.9)	10 (9.1)		Reference		Reference
GA	55 (34.4)	34 (30.9)	0.01*	0.18 (0.04–0.72)	0.24	2.40 (0.54–10.5)
GG	102 (63.8)	66 (60)	0.01*	0.19 (0.05–0.73)	0.77	0.91 (0.47–1.74)
AG + GG	157 (98.2)	100 (90.9)	0.01*	0.19 (0.05–0.71)		
G	259 (80.9)	166 (75.5)		Reference		
A	61 (19.1)	54 (24.5)	0.13	1.38 (0.91–2.09)		

*$P < 0.05$ indicates statistically significant. OR has been adjusted for smoking habit, BMI, familial history.

sFRP4 indicates secreted frizzled-related protein 4; R, arginine; K, lysine; G, guanine; A, arginine.

acid residue 335 located near the C-terminal domain of the protein and the hypermethylation in the promoter of *DKK3* gene caused a decrease in *DKK3* gene expression in human cancer cell lines.

We also investigated the relationship between *sFRP4* polymorphism (rs1802074) and LC in the current study. Functional loss of sFRPs contributes to the activation of Wnt signaling, leading to carcinogenesis.[20] It was reported that *sFRP* expression is reduced by epigenetic inactivation in several cancer types.[21–25] Until now, there is no study investigating the relationship between *sFRP4* SNPs and LC. So, this is the first study in this subject. In this study, we found that GG, GA, and AA genotype frequencies were 60%, 30.9%, and 9.1% in LC patients and 63.8%, 34.4%, and 1.9% in controls, respectively. Our present data are in concordance with a previous study in renal cancer examined by Hirata et al.[7] Hirata et al.[7] found that GG, GA, and AA genotype frequencies were 50%, 33%, and 17% in renal cancer patients and 56%, 39%, and 9% in controls, respectively. Their results indicate a significantly increased frequency of the AA genotype of the rs1802074 SNP in the *sFRP4* gene in patients with renal cancer.[7] In our study, we found that patients with the GG genotype have a protective effect on LC ($P = 0.01$; OR,0.19; 95% CI, 0.05–0.73). However, no statistically significant association between LC risk and AA genotype of *sFRP4* exon6 R340K polymorphism (rs1802074) (adjusted for smoking habit, BMI, and familial history) has been defined (OR,2.40; 95% CI,0.54–10.5; $P = 0.24$). We suggest that the AA genotype of *sFRP4* (rs1802074) polymorphism may be an independent factor from smoking habit, BMI, and familial history for LC.

Next, gene-gene interaction analysis was performed for gene polymorphisms of *DKK3* (rs3206824) and *sFRP4* (rs1802074)

TABLE 4. Gene-Gene Interaction Analysis of *DKK3*-rs3206824 and *sFRP4*-rs1802074 Polymorphisms and Haplotype Analysis for *DKK3* rs3206824 and rs7396187 Polymorphisms

***DKK3* (GG/GA + AA)-*sFRP4* (AA/AG)**					
		Patients, n (%)	**Controls, n (%)**	***P***	**OR (95% CI)**
GG-AA		10 (9.3)	2 (1.3)		Reference
GG-AG/GG		98 (90.7)	155 (98.7)	0.004*	0.12 (0.02–0.58)*
GA/AA-AA		0 (0)	1 (33.3)	—	—
GA/AA-AG/GG		2 (16.7)	2 (50.0)	0.24	0.20 (0.01–2.38)
DKK3 rs3206824 (exon7 G/A)-7396187 (intron G/C) Haplotype					
G	G	92	127		Reference
G	C	3	3	0.69	1.38 (0.27–6.99)
A	G	7	12	0.81	0.80 (0.30–2.12)
A	C	8	8	0.60	1.38 (0.50–3.81)

*$P < 0.05$ indicates statistically significant.

OR indicates odds ratio; CI, confidence interval; G, guanine; A, adenine; C, cytosine; *DKK3*, Dickkopf 3; *sFRP4*, secreted frizzled-related protein 4.

because a decreased risk of LC was determined for 2 gene polymorphisms. A remarkable correlation between GG for *DKK3* (rs3206824) and AG + GG for *sFRP4* (rs1802074) was found in this study ($P = 0.004$; OR,0.12; 95% CI, 0.02–0.58). Although a similar finding was observed in renal carcinoma ($P = 0.04$; OR,0.43; 95% CI, 0.19–0.96), a stronger correlation between GA + AA for *DKK3* (rs3206824) and AG + GG for *sFRP4* (rs1802074) than the combination of GG/AG + GG has been reported by Hirata et al.[7] ($P < 0.0001$; OR,0.19; 95% CI, 0.09–0.45).

The molecular mechanisms of these 5 polymorphisms on LC are unclear. However, we know that accumulating evidence about functional effects of synonymous mutations has been shown that synonymous SNPs can change mRNA folding and can reduce mRNA stability, thereby changing translation.[26–28] In addition, nonsynonymous SNPs present amino aside alter and may affect protein function. So, it is believed generally that nonsynonymous SNPs may have a relationship with cancer susceptibility.[29] A computer program has been evolved to predict the effect of coding nonsynonymous SNPs on protein structure and function. PolyPhen computer program (http://genetics.bwh.harvard.edu/pph/) was used by Hirata et al.[7] According to the program, DKK2 nonsynonymous (rs17037102), DKK3 nonsynonymous (rs3206824), sFRP4 nonsynonymous exon 6 (rs1802074) were judged to be benign. However, it has been reported that the x-ray repair complementing defective repair in Chinese hamster cell 1 gene arginine 399 glutamine (XRCC1 Arg399Gln) has been declared to be affect the survival and prognosis of several cancers as LC and colorectal carcinoma, whereas it is judged as benign by the PolyPhen program.[30,31] In addition, we found similar data with Hirata et al.[7] that these polymorphisms associated with the "odds" of LC are not associated with clinical or pathologic factors. Hirata et al.[7] declared in the study that it is reasonable to consider that the functional role of an SNP as a risk factor is not always the same as that of a prognostic factor because a risk SNP may contribute to the early stage of carcinogenesis of nearly normal cells, whereas a prognostic SNP may be involved in the progression of fully transformed cells. We know that there have been many examples that a risk SNP is of no significance as a prognostic SNP and vice versa.[32,33]

In conclusion, we suggested that these 2 polymorphisms are associated with LC susceptibility in this study. However, any effect of the 5 SNPs on clinicopathologic parameters, including grade, stage, lymph node status, or metastasis was not found among all patient groups and patients with NSCLC and SCLC. We anticipate that significant data will be obtained in the future if gene expression, methylation, and polymorphism analysis of Wnt antagonist genes will be investigated in larger patients groups with LC and LC cell lines.

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