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TNFSF15 is an independent predictor for the development of Crohn's disease-related complications in Koreans

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KEYWORDS
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Abstract

Background: Crohn's disease (CD) is a chronic idiopathic inflammatory bowel disease involving the whole gastrointestinal tract. *TNFSF15* has been proved as a susceptibility gene for CD, but there are few reports about the association between *TNFSF15* single nucleotide polymorphisms (SNPs) and the clinical course of CD.
Aim: To investigate the association between *TNFSF15* genotypes and the clinical course of CD in Koreans.
Methods: A total of 906 CD patients having *TNFSF15* genotype data and clinical information were recruited from CD registry database of a tertiary referral center. The association between five *TNFSF15* SNPs (rs4574921, rs3810936, rs6478108, rs6478109, and rs7848647) and various clinical parameters including stricture, non-perianal penetrating complications, bowel resection, and reoperation was investigated.
Results: Among the five SNPs, rs6478108 CC genotype was associated with the development of stricture and non-perianal penetrating complications during follow-up (HR for stricture = 1.706,

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95% confidence interval 1.178–2.471, $P = 0.005$; HR for non-perianal penetrating complications = 1.667, 95% confidence interval 1.127–2.466, $P = 0.010$), and rs4574921 CC genotype was associated with the development of perianal fistula (HR = 2.386, 95% confidence interval 1.204–4.727, $P = 0.013$) by multivariate analysis. However, there was no significant association of cumulative operation and reoperation rate with 5 SNPs of *TNFSF15*.
Conclusion: In Korean patients with CD, non-risk allele homozygotes of *TNFSF15* SNPs rs6478108 and rs4574921 are independent genetic predictive factors for the development of strictures/non-perianal penetrating complications and perianal fistula, respectively.

1. Introduction

Crohn's disease (CD) is an idiopathic chronic inflammatory bowel disease (IBD). It usually affects the small and/or large intestine and is associated with various complications including stricture, intra-abdominal fistula/abscess, intestinal perforation, and perianal fistula during its clinical course. Although the pathogenesis is still unclear, based on epidemiological, genetic, and immunological studies, the development of CD may be associated with a persistent abnormal immune response triggered by exposure to various environmental factors in genetically susceptible subjects.[1] It has been reported that 140 genes or loci, including *NOD2/CARD15*, *ATG16L1*, *IL23R*, *IBD5*, *TLR4*, *TLR9*, and *TNFSF15*, are related to the risk of CD.[2–11] Some of these genes showed significant associations with the clinical course of CD, mostly in Western studies. *NOD2/CARD15* is one of the most extensively investigated genes in its association with the clinical course of CD and appears to predispose CD patients to frequent ileal involvement, penetrating complications, strictures, and bowel resection.[12–17] In addition to *NOD2/CARD15*, *ATG16L1* single nucleotide polymorphism (SNP) rs2241880 is thought to be associated with ileal or ileocolonic CD.[18] A study in German CD patients revealed that *IL23R* SNP rs1004819 is associated with ileal CD,[19] but in a British study, no such significant association between disease phenotype and *IL23R* variants was found.[20]

TNFSF15 has shown a strong association with the susceptibility of CD in Korean[8] and Japanese[7] studies and also a significant association with susceptibility of CD in Western cohorts.[7,21–23] However, besides a Japanese study that reported a trend for a positive association between *TNFSF15* SNPs and the risk of anal lesions in CD,[24] little is known about the association between *TNFSF15* and the clinical course of CD. Therefore, in this study, we aimed to investigate the association between *TNFSF15* SNPs and the clinical course of CD in Korean patients.

2. Material and methods

2.1. Study population

A total of 906 CD patients were included in this study. Part of the patient cohort had been previously studied.[8] All patients were diagnosed at the IBD center of Asan Medical Center, Seoul, Korea on the basis of clinical, radiologic, endoscopic and histopathologic criteria. The phenotypic subgroups of CD were determined and classified using the Montreal classification[25] with a minor modification. Briefly, CD patients were subgrouped according to age at diagnosis (A1, ≤16 years; A2, 17–40 years; A3, >40 years), disease location (L1, ileum; L2, colon; L3, ileocolon), and disease behavior (B1, inflammatory; B2, stricturing; B3, penetrating). However, the upper gastrointestinal modifier of the Montreal classification system was not included in our classification scheme.

2.2. Collection of clinical data

Patient clinical data were retrieved from the IBD registry database of Asan Medical Center. This IBD registry database was first constructed in 1997, and baseline demographic and clinical information has been prospectively recorded since then. Among the 906 patients, only 20 patients were diagnosed with CD before the construction of the IBD registry. For patients who were referred to us after a diagnosis of CD, the baseline medical information was retrospectively obtained by interviewing patients at the first visit and by reviewing the medical records provided by the referring physicians. When the baseline clinical information was missing from the IBD registry, data was obtained by reviewing medical records or interviewing patients for that information. After registration, all changeable clinical variables including development of complications and data on surgical procedures were prospectively updated at each clinic visit or at least yearly. Clinical data retrieved from the database included age at diagnosis, gender, smoking status at diagnosis, date of diagnosis, date of anti-*TNF* therapy initiation, date of major operations, and date of detection of CD-related complications such as intestinal stricture, intra-abdominal fistulas, intra-abdominal inflammatory masses and/or abscesses, intestinal perforation, and perianal fistula. CD-related complications were categorized as stricture, non-perianal penetrating complications, and perianal fistula. "Stricture" was defined as "a constant luminal narrowing demonstrated by radiologic, endoscopic, or surgical-pathologic methods with prestenotic dilatation or obstructive signs/symptoms." "Non-perianal penetrating complications" consist of intra-abdominal fistulas, intestinal perforation, and inflammatory masses and/or abscesses. Perianal fistula was regarded as an independent form of CD-related complications and was not included in the penetrating complications. Basically, a "major operation" was defined as "bowel resection surgery for CD-related complications." In addition to bowel resection surgery, two bypass surgeries without bowel resection were also regarded as major operations, because they were performed instead of primary bowel resection in an emergency setting for patients with poor medical condition. Surgical drainage of abscess,

simple primary repair of perforation, appendectomy, perianal surgery, and bowel resection related to postoperative complication were not regarded as major operations. "Reoperation" was defined as a repeated major operation. Exposure to anti-*TNF* therapy was divided into "ever" exposed to anti-*TNF* therapy and "never" exposed to anti-*TNF* therapy according to the chronological relationship between anti-*TNF* therapy and the occurrence of each clinical outcome (stricture, non-perianal penetrating complications, perianal fistula, major operation, and reoperation). For example, the patients exposed to anti-*TNF* therapy "before" the development of stricture were regarded as "ever" exposed to anti-*TNF* therapy. On the other hand, not only anti-*TNF* therapy naïve patients but also patients who were exposed to anti-*TNF* therapy "after" the development of stricture were regarded as "never" exposed to anti-*TNF* therapy in the viewpoint of the analysis for stricture.

2.3. Single nucleotide polymorphism genotyping

Genomic DNA was isolated from 5 mL of ethylene diamine tetraacetic acid-anticoagulated venous blood using a standard method with proteinase K and phenol/chloroform extraction. The following seven SNPs were selected for genotyping in the study population: rs4574921, rs10114470, rs3810936, rs6478108, rs6478109, rs4979462, and rs7848647. Genotyping of these SNPs was performed using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry-based system (Sequenom, San Diego, CA) at Analytical Genetics Technology Centre, Princess Margaret Hospital/University Health Network in Toronto, Canada.

2.4. Selection of SNPs based on linkage disequilibrium

According to our previous study, rs4574921, rs10114470, rs3810936, rs6478108, rs6478109, rs4979462, and rs7848647 show positive associations with susceptibility of CD in Koreans.[8,26] Among these, rs3810936, rs6478108, rs6478109, and rs7848647 are four of the five SNPs (rs3810936, rs6478108, rs6478109, rs7848647, and rs7869487) that comprise two common haplotypes of *TNFSF15* and have been reported in previous Japanese[7] and US[27] studies. These were therefore included in our association analysis between *TNFSF15* SNPs and clinical variables of CD patients. In addition, rs4574921 was selected based on the linkage disequilibrium with rs7869487 ($r^2 = 0.63$) and subtype analysis data. However, rs10114470 and rs4979462 were excluded due to high linkage disequilibrium with the other SNPs. In summary, five SNPs (rs4574921, rs3810936, rs6478108, rs6478109, and rs7848647) were selected for statistical analysis.

2.5. Statistical method

The chi-square test or Fisher's exact test were used to analyze the association between *TNFSF15* SNPs and the phenotypes/complications (disease location, disease behavior, stricture, non-perianal penetrating complications, and perianal fistula) at diagnosis of CD. Cumulative incidence rates of major operation, reoperation, stricture, non-perianal penetrating complications, and perianal fistula were calculated using the Kaplan–Meier method. The log-rank test was used to analyze the association between the cumulative incidence data and various factors including *TNFSF15* SNPs. Bonferroni-adjusted *P* values (P_c) of less than 0.05 were regarded as statistically significant in the chi-square test, Fisher's exact test, and log-rank test. Variables showing *P* values less than 0.1 by log-rank test were selected for multivariate analysis using the Cox-proportional hazard model. *P* values less than 0.05 were regarded as having statistical significance in the multivariate analysis. Statistical analysis was performed using SPSS for Windows, version 18.0 (SPSS Inc., Chicago, IL).

2.6. Ethical considerations

This study was approved by the Institutional Review Board of Asan Medical Center, and written informed consent was obtained from all patients.

3. Results

3.1. Demographic and clinical characteristics

The demographic and clinical characteristics of the study population are shown in Table 1. The male-to-female ratio was 2.4:1. The median age at diagnosis was 22.4 years (range, 12.5–73.8), and the median interval between symptom onset and diagnosis was 16.1 months (range, 0–389.2). The median observation period from diagnosis of CD was 86.9 months (range, 0.9–334.8). According to the Montreal classification, ileocolonic (L3) involvement (73.0%) and inflammatory (B1) behavior (79.1%) were the most common clinical phenotypes at diagnosis of CD. At the time of diagnosis, 390 patients (43.0%) had a past or current history of perianal fistula. Anti-*TNF* therapy of this cohort was introduced in 2002, and 256 patients (28.3%) were treated with anti-*TNF* agents during follow-up.

3.2. Association between *TNFSF15* SNPs and phenotypes/complications at diagnosis: cross-sectional analysis

Regardless of Bonferroni adjustment, the frequency of disease location, disease behavior, and CD-related complications at the time of diagnosis were not associated with *TNFSF15* genotypes (Bonferroni-unadjusted $P > 0.05$ in all comparisons, Table 2).

3.3. Association between *TNFSF15* SNPs and stricture during follow-up

A total of 787 (86.9%) patients did not have strictures at the time of CD diagnosis. Of these, strictures developed in 291 (37.0%) patients during the follow-up period. Univariate analysis using the log-rank test revealed that non-risk alleles of all five SNPs were associated with a higher cumulative probability of stricture during follow-up of CD (Fig. 1a–e, Table 3). According to the log-rank test, ileal involvement (L1 or L3) at diagnosis was associated with a higher cumulative probability of strictures compared to L2 location at diagnosis (Fig. 1f, Table 3), and a 1-year or longer interval between symptom onset and diagnosis was associated with a higher

Table 1 Demographic and clinical characteristics of the study population (n = 906).

Clinical variables	
Male, n (%)	636 (70.2%)
Median follow-up period, month (range)	86.9 (0.9–334.8)
Median age at diagnosis, year (range)	22.4 (12.5–73.8)
Median interval between symptom onset and diagnosis, month (range)	16.1 (0–389.2)
Interval between symptom onset and diagnosis, n (%)	
<1 year	384 (42.4%)
≥1 year	522 (57.6%)
Age at diagnosis, n (%)	
≤16 years (A1)	120 (13.2%)
17–40 years (A2)	753 (83.1%)
>40 years (A3)	33 (3.6%)
Location at diagnosis, n (%)	
Ileum (L1)	191 (21.1%)
Colon (L2)	54 (6.0%)
Ileocolon (L3)	661 (73.0%)
Behavior at diagnosis, n (%)	
Inflammatory (B1)	725 (79.1%)
Stricturing (B2)	83 (9.5%)
Penetrating (B3)	103 (11.4%)
Perianal fistula at diagnosis, n (%)	
Absent	516 (57.0%)
Present	390 (43.0%)
Smoking status at diagnosis, n (%)	
Never smoker	624 (69.0%)
Ever smoker	280 (31.0%)
One or more major operations during observation period, n (%)	336 (37.1%)
Exposure to anti-*TNF* therapy during follow-up, n (%)	256 (28.3%)

cumulative probability of stricture development during follow-up (Fig. 1g, Table 3). On the other hand, exposure to anti-*TNF* therapy was strongly associated with a lower cumulative probability of stricture development during follow-up (Fig. 1h, Table 3). With Bonferroni adjustment, rs4574921, rs6478018, rs6478109, rs7848647, location at diagnosis, and exposure to anti-*TNF* therapy remained significant ($P_c < 0.05$, Table 3). Gender, smoking status at diagnosis, history of perianal fistula at diagnosis, and age at diagnosis were not associated with the cumulative probability of stricture development during follow-up (Table 3). A Cox-proportional hazard model revealed that the non-risk allele homozygote of *TNFSF15* rs6478108 was the only independent genetic predictor for the development of strictures in CD patients among the five *TNFSF15* SNPs (HR = 1.706, 95% CI 1.178–2.471, $P = 0.005$, Table 4). L1 (HR = 2.244, 95% CI 1.201–4.192, $P = 0.011$) or L3 location at diagnosis (HR = 1.851, 95% CI 1.032–3.322, $P = 0.039$), 1-year or longer interval between symptom onset and diagnosis (HR = 1.414, 95% CI 1.109–1.803, $P = 0.005$) and no exposure to anti-*TNF* therapy (HR = 3.382, 95% CI 2.143–5.338, $P = 1.66E-7$) were other independent clinical predictors for the development of strictures during follow-up of CD patients (Table 4).

3.4. Association between *TNFSF15* SNPs and non-perianal penetrating complications during follow-up

Among the 801 patients without non-perianal penetrating complications at the time of CD diagnosis, 244 (30.5%) experienced non-perianal penetrating complications during follow-up. According to univariate analysis, the non-risk alleles of the five *TNFSF15* SNPs were significantly associated with a higher probability of non-perianal penetrating complications during follow-up (Fig. 2a–e, Table 3). A log-rank test for the cumulative incidence of non-perianal penetrating complications revealed that age at diagnosis of more than 40 years (A3) and L1 or L3 location at diagnosis were associated with a higher risk of non-perianal penetrating complications during follow-up (Fig. 2f–g, Table 3). Exposure to anti-*TNF* therapy was strongly associated with a lower cumulative probability of non-perianal penetrating complications during follow-up (Fig. 2 h, Table 3). With Bonferroni adjustment, rs4574921, rs6478018, rs6478109, rs7848647, location at diagnosis, and exposure to anti-*TNF* therapy remained significant ($P_c < 0.05$, Table 3). Gender, 1-year or longer interval between symptom onset and diagnosis, smoking status at diagnosis and history of perianal fistula at diagnosis were not associated with the incidence of non-perianal penetrating complications during follow-up (Table 3). According to multivariate analysis using the Cox-proportional hazard model, non-risk allele homozygote of rs6478108 was the only independent risk factor for non-perianal penetrating complications among the five *TNFSF15* SNPs (HR = 1.667, 95% CI 1.127–2.466, $P = 0.010$, Table 4). L1 (HR = 3.922, 95% CI 1.553–9.905, $P = 0.004$) or L3 location at diagnosis (HR = 3.977, 95% CI 1.634–9.681, $P = 0.002$) and no exposure to anti-*TNF* therapy (HR = 3.831, 95% CI 2.337–6.282, $P = 1.01E-7$) were independent clinical predictive factors for non-perianal penetrating complications during follow-up (Table 4).

3.5. Association between *TNFSF15* SNPs and perianal fistulas during follow-up

Among the 516 patients with no history of perianal fistula at diagnosis of CD, 131 (25.4%) experienced new perianal fistula during follow-up. The univariate analysis for the association between genetic and clinical variables and the cumulative probability of developing perianal fistula is summarized in Table 3. The non-risk allele homozygote of rs4574921 (Fig. 3a) and colonic location at diagnosis (Fig. 3f) were associated with a higher cumulative probability of developing perianal fistula during follow-up. Among 256 patients who were exposed to anti-*TNF* therapy during follow-up, only 8 patients were perianal fistula-naïve at the time of anti-*TNF* therapy. Therefore, the effect of anti-*TNF* exposure on perianal fistula could not be assessed. According to multivariate analysis, the non-risk allele homozygote of rs4574921 remained a significant predictive factor for the development of perianal fistula during follow-up of CD (HR = 2.386, 95% CI 1.204–4.727, $P = 0.013$, Table 4). In addition, L2 (HR = 4.905, 95% CI 2.266–10.617, $P = 5.44E-5$) or L3 location at diagnosis (HR = 3.341, 95% CI 1.836–6.078,

Table 2 Association of *TNFSF15* single nucleotide polymorphisms with disease location, behavior at diagnosis, and Crohn's disease (CD)-related complications at the time of diagnosis (chi-square test).

ID	Genotype, n, (%)		Location at diagnosis				Behavior at diagnosis				CD-related complications at diagnosis					
			L1	L2	L3		B1	B2	B3		Stricture		Non-perianal penetrating complication		Perianal fistula	
			n	n	n	*P* value [a]	n	n	n	*P* value [a]	n	*P* value [a]	n	*P* value [a]	n	*P* value [a]
rs4574921	TT	568 (62.7%)	120	37	411	0.672 [b]	445	56	67	0.227 [b]	80	0.243 [b]	66	0.077 [b]	242	0.922
	TC	305 (33.7%)	65	17	223		250	26	29		33		31		133	
	CC [c]	33 (3.6%)	6	0	27		22	4	7		6		8		15	
rs3810936	CC	422 (46.6%)	88	29	305	0.371	330	41	51	0.623	59	0.605	50	0.306	178	0.698
	CT	397 (43.8%)	83	24	290		322	36	39		48		41		171	
	TT [c]	87 (9.6%)	20	1	66		65	9	13		12		14		41	
rs6478108	TT	463 (51.1%)	98	29	336	0.939 [b]	362	49	52	0.749	68	0.326	51	0.761	200	0.970
	TC	371 (40.9%)	80	22	269		298	32	41		44		44		160	
	CC [c]	72 (7.9%)	13	3	56		57	5	10		7		10		30	
rs6478109	GG	460 (51.3%)	96	30	334	0.912 [b]	360	47	53	0.826	66	0.426	52	0.816	200	0.959
	AG	365 (40.7%)	79	21	265		293	32	40		44		43		158	
	AA [c]	72 (8.0%)	13	3	56		57	5	10		7		10		30	
rs7848647	CC	466 (51.6%)	98	30	338	0.858[b]	364	49	53	0.830	68	0.267	52	0.918	201	0.921
	CT	366 (40.5%)	80	21	365		294	32	40		44		43		159	
	TT [c]	71 (7.9%)	12	3	56		57	5	9		6		9		29	

[a] Bonferroni correction was not performed because all *P* values were >0.05.
[b] *P* value by Fisher's exact test.
[c] Non-risk allele homozygote of each *TNFSF15* SNPs.

P = 7.82E−5) was strongly associated with an increased risk of the development of perianal fistula (Table 4).

3.6. Association between *TNFSF15* SNPs and the major operation/reoperation

Of the 906 CD patients, 336 (37.1%) patients had undergone one or more major operation related to CD until the last follow-up. Among these 336, 86 patients had undergone one or more major operation related to CD at the time of or before diagnosis. Therefore, among the 820 patients with no history of major operations related to CD at diagnosis, 250 (30.5%) patients underwent one or more major operation during the observation period. The cumulative probability of major operations during the follow-up period was 16.6% at 1 year, 28.3% at 5 years, 44.1% at 10 years, and 82.8% at 20 years (Appendix A, eFig. 1a). In addition, 87 (25.9%) of the 336 patients underwent repeated surgery due to CD-related complications, and the cumulative probability of reoperation was 3.2% at 1 year, 14.5% at 5 years, 44.0% at 10 years, and 72.0% at 20 years after initial surgery (Appendix A, eFig. 2a). According to the univariate analysis, neither cumulative operation rate nor cumulative reoperation rate was associated with *TNFSF15* SNPs (Appendix A, eFig. 1b–f and eFig. 2b–f). The exposure to anti-*TNF* therapy was significantly associated with both cumulative operation and reoperation rate in CD patients (Appendix A, eFig. 1g and eFig. 2 g).

3.7. Prevalence of major operations related to either stricture or non-perianal penetrating complications in patients with B1 phenotype at diagnosis

Of the 717 patients with an inflammatory phenotype (B1) at diagnosis, 699 (97.5%) had no history of CD-related major operations at the time of diagnosis. Of these 699, 319 patients developed either strictures or non-perianal penetrating complications during follow-up. Among these, three patients underwent bowel resection before the development of stricture or non-perianal penetrating complications due to intractable pain (n = 1), intestinal hemorrhage (n = 1), and unknown cause (n = 1). In addition, of the 319, 167 (52.4%) patients underwent a major operation with or after the development of either stricture or non-perianal penetrating complications, and the median interval between the development of complications and operation was 4.3 months (range 0–125.9) in these patients. The remaining 149 (46.7%) patients were followed without surgery during the median follow-up period of 40.6 months (range, 1.2–180.6).

4. Discussion

In this study, we demonstrated that the non-risk allele homozygote of a *TNFSF15* SNP, rs6478108, could be a genetic predictive factor for the development of stricture and non-perianal penetrating complications and that the non-risk allele homozygote of another *TNFSF15* SNP, rs4574921, could be a predictive factor for the development of perianal fistula during

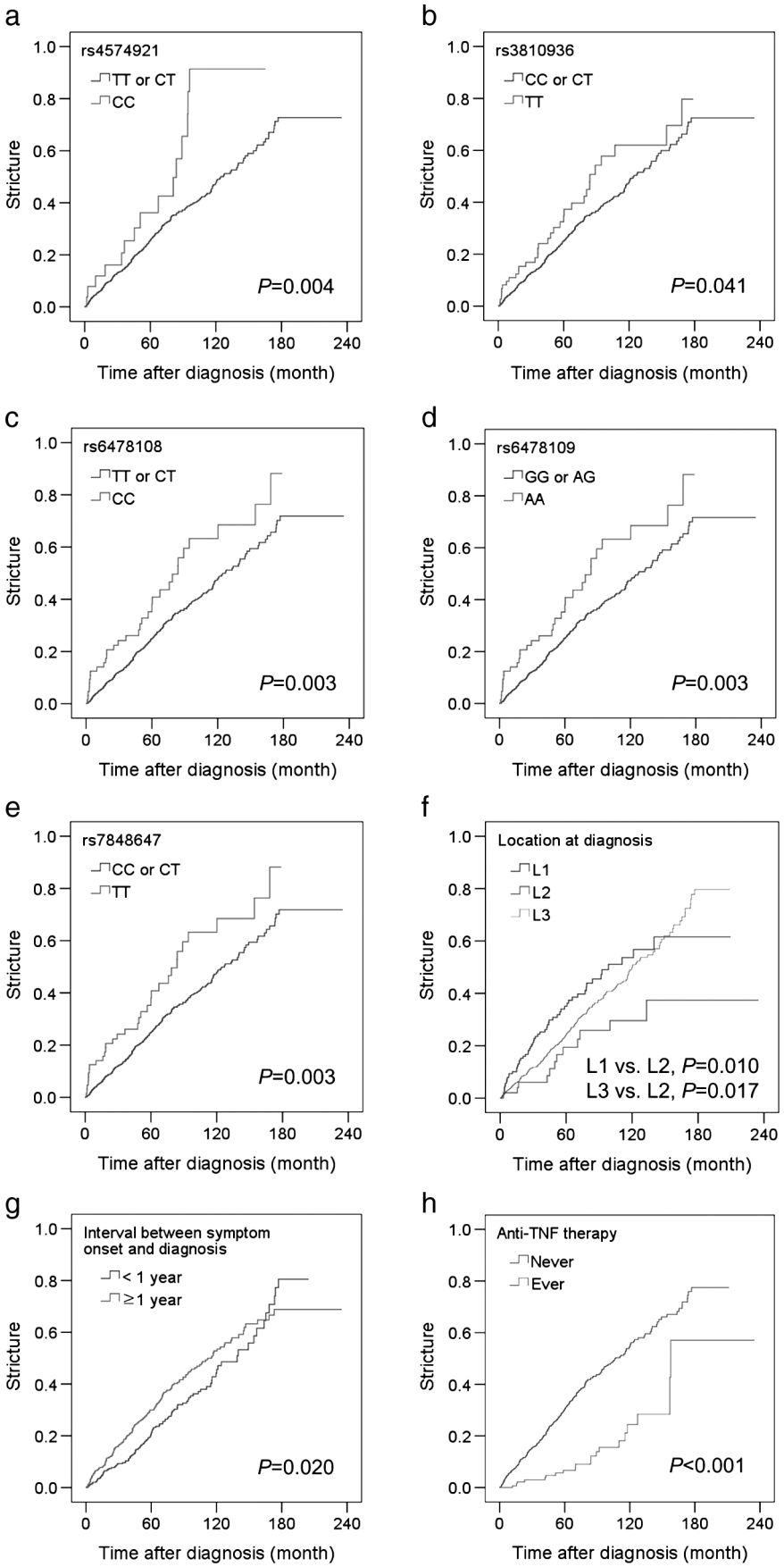


Table 3 Summary of univariate analysis (log-rank tests) for the association between genetic and clinical variables and the cumulative probability of stricture, non-perianal penetrating complications, and perianal fistula during follow-up of Crohn's disease.

Variables	Comparisons	*P* (P_c) value for cumulative probability of stricture	*P* (P_c) value for cumulative probability of non-perianal penetrating complications	*P* (P_c) value for cumulative probability of perianal fistula
TNFSF15 SNPs				
rs4574921	TT or TC vs. CC [a]	0.004 (0.048)	0.002 (0.024)	0.004 (0.040)
rs3810936	CC or CT vs. TT [a]	0.041 (0.492)	0.021 (0.252)	0.232
rs6478108	TT or TC vs. CC [a]	0.003 (0.036)	0.002 (0.024)	0.453
rs6478109	GG or AG vs. AA [a]	0.003 (0.036)	0.002 (0.024)	0.445
rs7848647	CC or CT vs. TT [a]	0.003 (0.036)	0.002 (0.024)	0.442
Clinical variables				
Gender	Male vs. female	0.276	0.412	0.665
Interval between symptom onset and diagnosis	<1 year vs. ≥1 year	0.020 (0.24)	0.096	0.248
Age at diagnosis	≤16 years vs. 17–40 years	0.744	0.911	0.222
	17–40 years vs. >40 years	0.216	0.037 (0.444)	0.179
	≤16 years vs. >40 years	0.223	0.025 (0.3)	0.079 (0.79)
Location at diagnosis	Ileum vs. colon	0.010 (0.12)	0.001 (0.012)	6.0E−6 (6.0E−5)
	Colon vs. ileocolon	0.017 (0.204)	2.48E−4 (0.003)	0.248
	Ileum vs. ileocolon	0.080 (0.96)	0.796	1.60E−5 (1.60E−4)
History of perianal fistula at diagnosis	Absent vs. present	0.939	0.336	Not applicable
Smoking status at diagnosis	Never smoker vs. ever smoker	0.479	0.870	0.317
Exposure to anti-*TNF* therapy	Ever vs. never	7.41E−9 (8.89E−8)	1.21E−9 (1.45E−8)	Not applicable [b]

[a] Non-risk allele homozygote of each SNP.
[b] Only 8 patients were perianal fistula-naïve at the time of anti-*TNF* therapy.

follow-up of CD in Korean patients. We selected four SNPs of *TNFSF15* (rs3810936, rs6478108, rs6478109, and rs7848647), which have been shown to contribute to the *TNFSF15* haplotype in previous Japanese and Western studies.[7,27] In addition, we also selected another SNP, rs4574921, which has low linkage disequilibrium with the other four SNPs. When all SNPs and clinical parameters showing significant association with stricture or non-perianal penetrating complications were considered in multivariate analysis, rs6478108 was the only significant genetic predictive factor for the development of stricture and non-perianal penetrating complications in Korean CD patients during follow-up. The non-risk allele homozygote of *TNFSF15* rs4574921 was associated with a higher cumulative risk for developing perianal fistula in patients who had no perianal fistula at the time of CD diagnosis. Although a previous Japanese study demonstrated a weak association between *TNFSF15* SNPs including rs6478109 and rs7848647 and anal lesions in their cross-sectional observation,[24] neither rs6478109 nor rs7848647 was associated with the risk of developing perianal fistula in our time-dependent observation. To the best of our knowledge, this is the first report demonstrating a positive association between *TNFSF15* and the development of CD-related complications during follow-up in Korean CD patients.

Despite the association between *TNFSF15* SNPs and the development of CD-related complications during follow-up, there was no significant association between *TNFSF15* SNPs and the cumulative operation/reoperation rate in our study. A previous Western study suggested a higher frequency of haplotype B in CD patients who underwent bowel resection surgery.[27] Interestingly, a recent report from the IBDchip European project suggested that a certain SNP of *TNFSF15* (rs4263839) was weakly associated with the need for bowel resection surgery in CD patients (OR = 1.29, 95% CI 1.01–1.65, *P* = 0.0423).[28] We selected four SNPs that compose the "haplotype B", which has been proposed as a risk factor for small bowel resection surgery, and other SNPs (rs6478108,

Figure 1 Univariate analysis of the association of the cumulative probability of stricture with clinical variables and *TNFSF15* SNPs. According to the log-rank tests, all non-risk allele homozygotes of five *TNFSF15* SNPs (rs4574921, rs3810936, rs6478108, rs6478109, and rs7848647) were associated with the development of stricture in Korean CD patients during follow-up (a–e). Among the clinical variables, ileal or ileocolonic location at diagnosis, 1-year or longer interval between symptom onset and diagnosis and no exposure to anti-*TNF* therapy were associated with an increased risk for developing stricture during follow-up (f–h). After Bonferroni correction, rs4574921, rs64780108, rs6478019, rs7848647, disease location at diagnosis, and exposure to anti-*TNF* therapy remained significant ($P_c < 0.05$).

Table 4 Multivariate analysis of risk factors for strictures, non-perianal penetrating complications, and perianal fistulas during follow-up of Crohn's disease.

Candidate risk factors		HR	95% CI	*P* value
For strictures				
Location at diagnosis	Colon	Reference		
	Ileum	2.244	1.201–4.192	0.011
	Ileocolon	1.851	1.032–3.322	0.039
Interval between symptom onset and diagnosis	<1 year	Reference		
	≥1 year	1.414	1.109–1.803	0.005
Exposure to anti-*TNF* therapy	Ever	Reference		
	Never	3.382	2.143–5.338	1.66E−7
TNFSF15 rs6478108 genotype	TT or CT	Reference		
	CC	1.706	1.178–2.471	0.005
For non-perianal penetrating complications				
Location at diagnosis	Colon	Reference		
	Ileum	3.922	1.553–9.905	0.004
	Ileocolon	3.977	1.634–9.681	0.002
Exposure to anti-*TNF* therapy	Ever	Reference		
	Never	3.831	2.337–6.282	1.01E−7
TNFSF15 rs6478108 genotype	TT or CT	Reference		
	CC	1.667	1.127–2.466	0.010
For perianal fistulas				
Location at diagnosis	Ileum	Reference		
	Ileocolon	3.341	1.836–6.078	7.82E−5
	Colon	4.905	2.266–10.617	5.44E−5
TNFSF15 rs4574921 genotype	TT or CT	Reference		
	CC	2.386	1.204–4.727	0.013

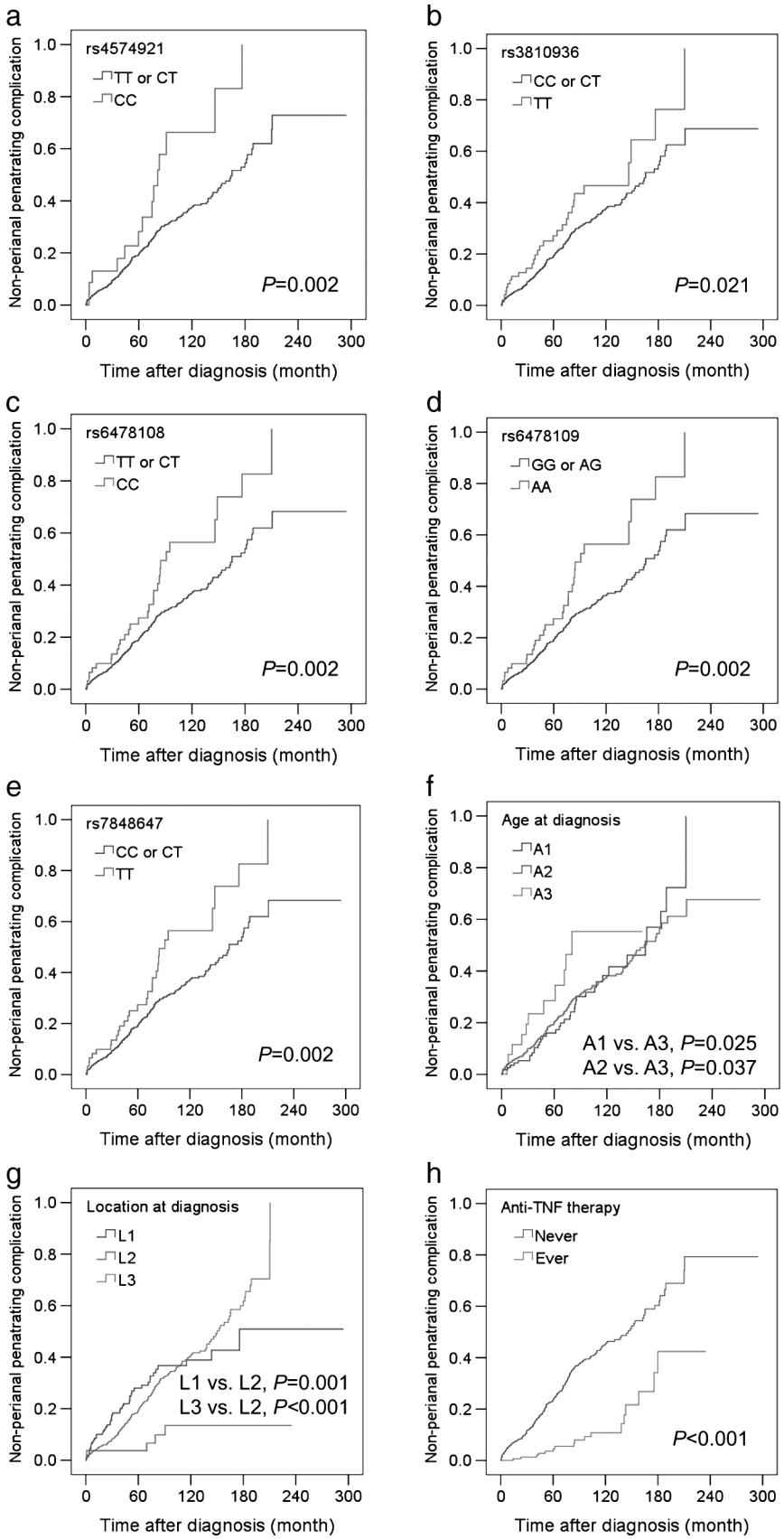
HR = hazard ratio; CI = confidence interval.

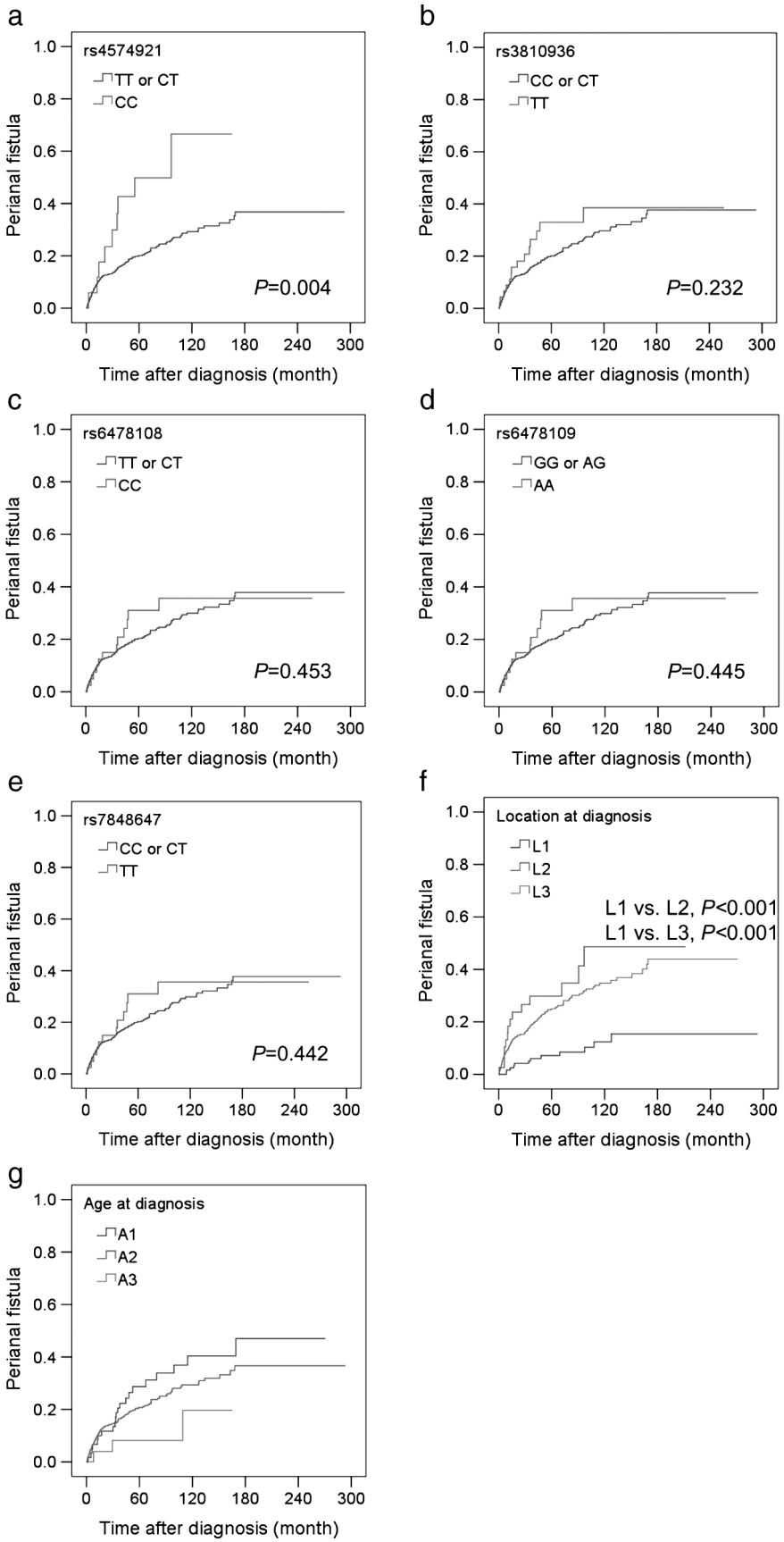
rs6478019, and rs7848647), which have a high linkage disequilibrium with rs4263839 of IBDchip European project ($r^2 \geq 0.95$, Appendix A, eFig. 3). However, we did not identify any significant association between *TNFSF15* SNPs and the need for operations According to the analysis of the clinical course of patients who had B1 phenotype and were operation-naïve at diagnosis, the cumulative incidence of the intestinal complications did not directly influence the cumulative probability of major operations in our analysis, which may explain the negative association between *TNFSF15* SNPs and the incidence of operation or reoperation despite a positive association between *TNFSF15* SNPs and CD-related complications.

We found that ileal involvement (L1 or L3) at diagnosis was an independent predictive factor for the development of stricture and non-perianal penetrating complications in Korean CD patients. These findings are similar to those of previous Western studies on the association between the disease location at diagnosis of CD and the progression of disease behavior. In a hospital-based cohort study on the long-term evolution of disease behavior of CD using the Vienna classification system, the presence of ileal lesions was an independent predictive factor for the development of stricture and penetrating complications during follow-up of CD.[29] In addition, a recent population-based study revealed that CD patients with isolated ileal disease had an increased risk of developing an intestinal complication during follow-up.[30] Another recent population-based study using the Montreal classification also suggested that the probability of progression of disease behavior from inflammatory to stricturing/penetrating disease during follow-up was significantly higher in patients with ileal or ileocolonic involvement at diagnosis of CD.[31]

Furthermore, a 1-year or longer period between symptom onset and diagnosis was related with the development of stricture during follow-up in our study. This may reflect that patients with delayed diagnosis may have a longer duration of disease before diagnosis, making the real disease period long enough to predispose patients to developing stricture after diagnosis. In fact, a recent study suggested that 1-year increments in disease duration could increase the odds ratio for the development of stricture by 1.07.[32]

Figure 2 Cumulative probability of non-perianal penetrating complications according to *TNFSF15* SNPs. According to log-rank tests, all non-risk allele homozygotes of five *TNFSF15* SNPs (rs4574921, rs3810936, rs6478108, rs6478109, and rs7848647) were predictive factors for the development of non-perianal penetrating complications in Korean CD patients during follow-up (a–e). Among the clinical variables, age at diagnosis over 40 years (A3), ileal (L1) or ileocolonic (L3) location at diagnosis and no exposure to anti-*TNF* therapy were associated with an increased risk for developing non-perianal penetrating complications during follow-up (f–h). After Bonferroni correction, rs4574921, rs64780108, rs6478019, rs7848647, disease location at diagnosis, and exposure to anti-*TNF* therapy remained significant ($P_c < 0.05$).





It is notable that anti-*TNF* therapy could reduce the cumulative risk of major operation, reoperation, development of stricture and non-perianal penetrating complications significantly in our cohort. These findings support the disease modifying effect of anti-*TNF* therapy on CD in the previous studies,[28,33–35] although the long-term effect on the clinical course of CD should be investigated more.

There are some limitations in our study. First, the effect of immunomodulators was not considered in our analysis. Therefore, further studies should be conducted to assess whether therapy has an impact on the clinical course of CD patients, especially in those who are genetically predisposed to complications. However, considering the weak effect on the clinical course of immunomodulators compared with biologics,[34] this might not be a major limitation to assess the association between *TNFSF15* SNPs and the clinical course of CD in our study. Second, the retrospective design of this study is a potential limitation. Nevertheless, most of the clinical data were collected by a prospectively built IBD registry database that is updated regularly. Therefore, even with the retrospective design, we believe that we minimized the potential recall bias. Third, because the non-risk allele of each SNP showed low frequency in Korean CD patients, the subgroups of patients with non-risk allele homozygotes of each SNP were considerably small in sample size (Table 2). Therefore, the effect of *TNFSF15* SNPs (rs6478108 and rs4574921) as genetic predictive factors may not be as strong as shown by the hazard ratio when these are applied in clinical practice. Finally, the potential selection bias of this hospital-based cohort study may limit the generalization of our study results, although the demographic characteristics of this study were quite similar to the previous population based cohort study for CD in Korea.[36] Therefore, to determine whether the *TNFSF15* SNPs as predictors for CD-related complication can be generalized to clinical practice, prospective studies based on a larger population should be conducted.

In conclusion, we investigated the association between *TNFSF15* and the clinical course of CD in a Korean population and demonstrated that two *TNFSF15* SNPs, rs6478108 and rs4574921, may be independent genetic predictive factors for the development of stricture/non-perianal penetrating complications and perianal fistula, respectively.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.crohns.2014.04.002.

Conflicts of interest

Suk-Kyun Yang has received a research grant, not related to the topic of the current paper, from Janssen Korea Ltd. For the remaining authors, no competing interests exist.

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Statement of authorship: D.H.Y. designed the study protocol and performed the statistical analysis after collecting both genetic and clinical data. He was involved in updating the IBD registry database for this study and primarily wrote the draft. S.K.Y. constructed the IBD registry database, initiated the study, and developed the study protocol. K.S. constructed the institutional genetic database of IBD patients and was involved in developing the study protocol and writing the manuscript. M.H. was involved in constructing the genetic database of the CD patients by collecting and managing the sequencing data of the CD patients in this study. S.H.P., S.K.P., H.S.L., J.B.K., H.L., K.J.K., and B.D.Y. updated the IBD registry database and were involved in interpreting the subsequent result of the statistical analysis. S.J.M., J.S.B., and J.H.K. were involved in developing the study protocol and writing the manuscript. E.S.S. and C.S.Y. contributed to the development of the study protocol and were involved in the surgeries of CD patients and describing the operation records for updating the surgical variables in the IBD database. I.L. provided and confirmed the pathologic information of the patients and was involved in writing and revising the manuscript.

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Figure 3 Cumulative probability of perianal fistula according to *TNFSF15* SNPs in Crohn's disease patients with no history of perianal fistula at diagnosis. The non-risk allele homozygote of rs4574921 was associated with the development of perianal fistula during follow-up (a). The cumulative probability of perianal fistula was not different according to the genotypes of the other four SNPs (b–e). Colonic involvement (L2 or L3) was associated with a higher cumulative probability of developing perianal fistula (f). Patients diagnosed at 16 years or younger age (A1) showed a trend toward a higher cumulative probability of perianal fistula compared to those diagnosed over 40 years (A3) (g). After Bonferroni correction, rs4574921, and disease location at diagnosis remained significant ($P_c < 0.05$).

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