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Original article

Polymorphisms of the *TRPV2* and *TRPV3* genes associated with fibromyalgia in a Korean population

Dong-Jin Park[1], Seong-Ho Kim[2], Seong-Su Nah[3], Ji Hyun Lee[4], Seong-Kyu Kim[5], Yeon-Ah Lee[6], Seung-Jae Hong[6], Hyun-Sook Kim[7], Hye-Soon Lee[8], Hyoun Ah Kim[9], Chung-Il Joung[10], Sang-Hyon Kim[11] and Shin-Seok Lee[1]

Abstract

Objective. Researchers continue to gather evidence that transient receptor potential vanilloid (TRPV) channels contribute towards pain signalling pathways. However, it is unknown whether polymorphisms of the *TRPV* gene are associated with FM. For the first time, we investigated the association between the polymorphisms of the *TRPV2* and *TRPV3* genes, FM susceptibility and the severity of the symptoms.

Methods. A total of 409 patients with FM and 423 controls were enrolled from 10 medical centres that participated in the Korean nationwide FM survey. The alleles and genotypes at three positions [rs3813768(C > G), rs8121(C > T) and rs1129235(C > A)] in the *TRPV2* gene and two positions [rs7216486 (G > A) and rs395357(C > T)] in the *TRPV3* gene were genotyped.

Results. The frequencies of the alleles and genotypes of individual *TRPV2* and *TRPV3* genes were not significantly associated with FM susceptibility. However, the GTA haplotype of *TRPV2* showed a defence against FM susceptibility (P = 0.035). In addition, polymorphisms of *TRPV3* were associated with symptom severity in FM patients. The single nucleotide polymorphism rs395357 of *TRPV3* was associated with the scores of the Brief Fatigue Inventory (P = 0.017) in FM patients. Furthermore, haplotypes of *TRPV3* were associated with the Brief Fatigue Inventory and the 36-item Short-Form Health Survey mental health summary scores (P = 0.036).

Conclusion. This study was the first to evaluate the associations of *TRPV* gene polymorphisms with FM. Our results suggest that certain *TRPV2* haplotypes may have a protective role against FM and that some genotypes and haplotypes of *TRPV3* contribute towards the symptoms of FM.

Key words: fibromyalgia, transient receptor potential vanilloid, polymorphism

BASIC SCIENCE

Rheumatology key messages

- Polymorphisms of the *TRPV2* and *TRPV3* genes are associated with the risk of FM and sensitivity to pain.
- *TRPV2* haplotypes are associated with a protective role against FM.
- Genotypes and haplotypes of *TRPV3* are related to symptom severity in FM.

[1]Department of Rheumatology, Chonnam National University Hospital & Medical School, Gwangju, [2]Department of Internal Medicine, Inje University Haeundae Paik Hospital, Busan, [3]Department of Internal Medicine, Soonchunhyang University, College of Medicine, Cheonan, [4]Department of Internal Medicine, Maryknoll Medical Center, Busan, [5]Department of Internal Medicine, Catholic University of Daegu, School of Medicine, Daegu, [6]Department of Internal Medicine, School of Medicine, Kyung Hee University, [7]Department of Internal Medicine, Soonchunhyang University Seoul Hospital, [8]Department of Internal Medicine, Hanyang University College of Medicine and the Hospital for Rheumatic Diseases, Seoul, [9]Department of Allergy and Rheumatology, Ajou University Hospital, Ajou University School of Medicine, Suwon, [10]Department of Internal Medicine, Konyang University Medical School, Daejeon and [11]Department of Internal Medicine, School of Medicine, Keimyung University, Daegu, Korea

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Correspondence to: Shin-Seok Lee, Department of Rheumatology, Chonnam National University Hospital & Medical School, 42 Jebong-ro, Dong-gu, Gwangju 501-746, Republic of Korea.
E-mail: shinseok@chonnam.ac.kr

Introduction

FM is a prevalent disease that is reported in 1–5% of the general population, with a greater prevalence among females [1]. FM is a medical syndrome characterized by chronic widespread pain and other related symptoms, including fatigue, insomnia, cognitive disturbance, joint stiffness and affective distress [2]. To date, proof of a causative pathway that would explain the development of FM has not been established. FM is currently considered to be a result of the interaction of numerous factors, including psychological, genetic, neurobiological and environmental [3, 4].

Recent studies have expanded our knowledge regarding the association between genetic factors and FM. A number of studies have reported progress in the area of FM genetics, including association or genome-wide association studies, and have suggested that certain genes influence sensitivity to pain and also increase the risk of developing FM. In these studies, FM has been associated with polymorphisms of genes involved in the serotoninergic, dopaminergic and catecholaminergic systems [3, 5, 6]. However, the pathophysiology and symptoms of FM have not, until now, been fully described by known genetic factors. Because several ion channels are involved in the detection of painful thermal, mechanical and chemical stimuli, dysfunctional ion channels have been proposed as a possible risk factor associated with susceptibility to FM [7, 8]. For example, a study suggested that the *SCN9A* gene-encoded dorsal root ganglia (DRG) sodium channel polymorphism was associated with symptom severity in FM [8].

There is emerging evidence that transient receptor potential vanilloid (TRPV) channels contribute to pain hypersensitivity. TRPV is one of the transient receptor potential (TRP) channels, which are a large group of ion channels comprising six protein families. The TRP channels are responsible for the detection of a wide range of noxious chemical, mechanical and thermal stimuli [7]. Among several TRP channels, TRPV1–TRPV4 mainly respond to heat detection, and therefore these TRPV channels are known as thermoTRPs. ThermoTRPs are expressed in pain pathways such as the DRG neurons [9]. DRG neurons play a key role in pain perception and are involved in the transduction of noxious stimuli into electric impulses at the peripheral terminals [8]. Interestingly, a recent genome-wide linkage scan study has suggested the *TRPV2* gene as a potential candidate gene for FM [10].

However, the question of whether polymorphisms of the *TRPV* gene are associated with FM remains unanswered. Therefore we adopted a case–control study design, employing a large sample comprised entirely of ethnically homogeneous Koreans, to investigate the association between *TRPV2* and *TRPV3* gene polymorphisms and FM susceptibility, and to determine the clinical differences within FM patients in the presence of *TRPV2* and *TRPV3* gene polymorphisms.

Patients and methods

Study design and population

We performed a nationwide FM cohort study (the Korean Nationwide FM Survey) among the Korean population. The cohort was established to evaluate genetic susceptibility and the clinical manifestations and outcomes of FM. The survey targeted a prospective cohort of FM patients recruited from the outpatient rheumatology clinics of 10 medical centres. In this study, a cross-sectional design was employed to identify the genetic factors associated with FM susceptibility and the severity of symptoms. As mentioned previously [11], we enrolled 409 FM patients (382 females and 27 males) with a mean age of 48.1 years (S.D. 10.9) from the outpatient rheumatology clinics of 10 medical centres. All patients were diagnosed as having FM, according to the classification criteria for FM proposed by the ACR in 1990 [2], at the time of the initial diagnosis. The mean symptom duration before diagnosis was 8.5 years (S.D. 8.3) and the mean duration after diagnosis was 1.9 years (S.D. 3.0). We also recruited 423 healthy controls (397 females, 25 males) with a mean age of 45.5 years (S.D. 12.5). Healthy controls were selected randomly, without matching for age or sex, from among the individuals visiting the general health examination clinics at each medical centre. The controls had no history of FM or chronic pain.

This research complied with the Declaration of Helsinki, and informed consent was obtained from all enrolled participants. Exactly the same informed consent form and study protocol were provided to the independent Institutional Review Board/Ethics Committee (IRB/EC) at each medical centre and each IRB/EC reviewed the appropriateness of the protocol and risks and benefits to the study participants. Ultimately the IRB/EC at each medical centre independently approved this study without revision of the informed consent form or study protocol.

Measures

The patients were interviewed at the time of enrolment to determine their demographic characteristics, including age, gender, BMI, symptom duration and tender point counts and scores. In addition, peripheral venous blood was sampled and then stored in EDTA-coated tubes. The patients had been treated for FM based on the clinical judgment of their rheumatologist. Concomitant medications were tricyclic antidepressants, selective serotonin reuptake inhibitors, serotonin-norepinephrine reuptake inhibitors, pregabalin, gabapentin, NSAIDs, tramadol, acetaminophen, benzodiazepine and muscle relaxants. Although not shown as a table, the current medications used by the patients with FM did not differ between the *TRPV2* and *TRPV3* gene polymorphisms.

Tender points were assessed by thumb palpation according to the standardized tender point examination protocol [12]. The number of tender points was counted at 18 specific sites on the body, and this number ranged between 0 and 18. The intensity at each tender point was assessed by tender point scores as follows: 0, no

tenderness; 1, light tenderness (confirming answer when asked); 2, moderate tenderness (spontaneous verbal response); and 3, severe tenderness (moving away). The possible total score of the tender points ranged from 0 to 54.

We further undertook comprehensive clinical assessments of the FM patients using a self-report questionnaire and semi-structured questionnaires. The Korean version of the FM Impact Questionnaire (FIQ) was used to assess the functional abilities of FM [13], and the Brief Fatigue Inventory (BFI) and the Beck Depression Inventory (BDI) were used to assess the severity of fatigue and depression, respectively [14, 15]. The 36-item Short-Form Health Survey (SF-36) is a generic health survey that measures the physical and mental health status of patients [16]. Therefore the SF-36 was used to assess the quality of life of the FM patients. In addition, the severity of anxiety was measured using the State-Trait Anxiety Inventory 1 (STAI-1) and STAI-2 [17].

Genotyping of the *TRPV2* and *TRPV3* gene polymorphisms

The assay reagents for rs3813768(C > G), rs1129235(C > A) and rs8121(C > T) in the *TRPV2* gene and for rs395357 (C > T) and rs7216486 (G > A) in the *TRPV3* gene were designed by Applied Biosystems (Waltham, MA, USA). The reagents consist of TaqMan MGB probes (FAM and VIC dye-labelled). The reaction in 10 μl was optimized to work with 0.125 μl 40X reagents, 5 μl 2X TaqMan Genotyping Master mix (Applied Biosystems) and 2 μl 50 ng genomic DNA. The PCR conditions were as follows: one cycle at 95 °C for 10 min, 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The PCR was performed using ABI Plus (Applied Biosystems). The samples were read and analysed using the software included with the instrument. The reference sequence for the *TRPV2* gene was based on the sequence of human chromosome 17p11.2, and that for the *TRPV3* gene was based on the sequence of human chromosome 17p13.2. Primer sequences used for TaqMan probe genotyping of the *TRPV2* and *TRPV3* genes are summarized in Table 1.

Statistical analysis

Statistical analysis was performed using SPSS software (SPSS version 21; IBM, Armonk, NY, USA). P-values <0.05 were considered to indicate statistical significance. The Hardy–Weinberg equilibrium was tested for each *TRPV2* and *TRPV3* polymorphism. Genotype and haplotype frequencies of the *TRPV2* and *TRPV3* single nucleotide polymorphisms (SNPs) were compared between the FM patients and controls using a Fisher's exact test or a Pearson's chi-squared test. Logistic regression analysis was performed to evaluate the association of each *TRPV2* and *TRPV3* genotype and haplotype with FM susceptibility. An analysis of covariance, adjusted for age and sex, was used to explore the differences in the clinical measurements of the FM patients according to each of the *TRPV2* and *TRPV3* genotypes and haplotypes. Combined allele analysis was performed using PHASE software (version 2.1.1; Department of Statistics, University of Washington, Seattle, WA, USA) to construct haplotype structures and estimate their frequencies. We also performed a permutation test for the null hypothesis that the patients with FM and the healthy controls are random draws from a common set of haplotype frequencies (number of permutations performed = 10 000).

Results

Genotype and allele differences of the *TRPV2* and *TRPV3* genes and their associations with clinical measurements

Genotyping of the *TRPV2* and *TRPV3* SNPs was successfully performed in all enrolled subjects except for two patients and three controls with *TRPV2* rs3813768, one patient and one control with *TRPV2* rs8121, three patients and one control with *TRPV2* rs1129235, one patient and one control with *TRPV3* rs7216486 and one patient and one control with *TRPV3* rs395357. Except for the rs1129235 of *TRPV2* (P = 0.0417), the genotype distributions of the *TRPV2* and *TRPV3* SNPs were consistent with the Hardy–Weinberg equilibrium in both the patients and the controls.

The allele and genotype frequencies of the SNPs of both *TRPV2* and *TRPV3* were not significantly different between the FM patients and controls. However, although it was statistically insignificant, patients with the CG genotype rs3813768 of *TRPV2* were less likely to have FM following an age- and sex-adjusted model [odds ratio (OR) = 0.771 (95% CI 0.575, 1.0376), P = 0.086]. In comparison, patients with the AA genotype of *TRPV2* rs1129235 were more likely to have FM after adjustment for age and sex (OR = 1.668 (95% CI 0.919, 3.028), P = 0.093] (Table 2).

Within the FM cohort, patients with the CC genotype of *TRPV3* rs395357 had more severe fatigue symptoms, as measured by the BFI, than did the other genotypes (P = 0.017). However, we could not find any association between *TRPV3* genotypes and individual components of the BFI. Furthermore, no associations were observed between clinical measurements and other *TRPV2* and *TRPV3* SNPs (Table 3).

Haplotype frequencies and clinical measurements in patients with FM and healthy controls

In the haplotype analysis, using *TRPV2* SNPs, there were four frequent haplotypes (CTA, GCC, GTA and CCC) with a frequency >1% in the patients and controls. These haplotypes showed significantly different distributions between the FM patients and the controls (P = 0.0002; Table 4). Among these haplotypes, the GTA haplotype was found more frequently in FM after age- and sex-adjusted analysis [OR = 0.637 (95% CI 0.418, 0.969), P = 0.035; Table 5]. However, the clinical symptoms, assessed using the FIQ, BFI, physical component summary, mental component summary (MCS), BDI, STAI-1 and STAI-2 scores, did not significantly differ among the patients with haplotypes of *TRPV2* (Table 6).

Table 1 Primer sequences used for TaqMan probe genotyping of the *TRPV2* and *TRPV3* genes

Region	Primers	Primer sequences (5′ → 3′)
TRPV2 rs3813768	Forward	GTTTTCAGGTTGGAGACATTAGATG
	Reverse	AGGCCAAGAAGATGGCTCTGAGGCG
TRPV2 rs8121	Forward	CATTCTGCCACTGCTGCAGATCGAC
	Reverse	GGGACTCTGGCAATCCTCAGCCCCT
TRPV2 rs1129235	Forward	AGGCTGTGCTGAACCTTAAGGACGG
	Reverse	GTCAATGCCTGCATTCTGCCACTGC
TRPV3 rs7216486	Forward	ACATGCGCTTCACAAAGTCATTCTG
	Reverse	GTCTTGAAGTCCTCGGCCACGGTCA
TRPV3 rs395357	Forward	ACTTGGAGTTCTGCTGGATGTTCAG
	Reverse	TCACCCAGGCCTATGGTGAGCTTGA

A: adenine; C: cytosine; G: guanine; T: thymine.

On the other hand, the haplotypes of the *TRPV3* gene did not show a different distribution between the FM patients and the controls (P = 0.820; Table 4). Although three frequent haplotypes (AT, AC and GC) were not associated with FM susceptibility, FM patients with these haplotypes showed a difference in the BFI and MCS scores among the cases (P = 0.036; Table 6).

Discussion

To our knowledge, our study is the first investigation into the association of *TRPV* SNPs with FM. In the present study we showed that although the allele and genotype frequencies were not significantly different between the FM patients and the controls, the GTA haplotype of the *TRPV2* gene has a protective role against FM susceptibility and some genotypes and haplotypes of the *TRPV3* gene contribute towards the symptoms of FM. Our study suggests that *TRPV2* and *TRPV3* gene polymorphisms may be one of the genetic factors associated with the development and symptom severity of FM in a Korean population.

The TRP channel family is known to mediate pain perception and processing in various cell types in the peripheral and central nervous systems and is involved in several processes, including neuron activation and neurotransmitter release, relevant to chronic pain pathophysiology [7, 18]. After the molecular identification of TRPV1, also known as the capsaicin receptor, additional TRP family members have been discovered in DRG, including TRPV2, TRPV3, TRPV4, TRP ankyrin 1 (TRPA1) and TRP melastin 8 (TRPM8) [18]. These channels are expressed in sensory fibres and are emerging as sensory transducers that may participate in the processing of pain sensations [7, 9]. The TRP channels are responsible for the detection of a wide range of noxious chemical, mechanical and thermal stimuli [9]. Among several TRP channels, thermoTRPs are also expressed in pain pathways such as the DRG neurons [9]. Because DRG neurons play a key role in the nociceptive sensory pathway, researchers have been interested in the potential role of TRP channels in chronic pain disorders.

The role of thermoTRPs in the nociceptive pain pathway has been demonstrated in a number of studies. Extensive research with modified animals and pharmacological agents have confirmed that these TRP channels are involved in the generation and transduction of pain, and thus represent promising targets for novel analgesic agents [7, 18]. Studies of *TRPV1* gene knockout mice suggest that the channel contributes to the detection of painful stimuli by nociceptive neurons [19]. The *TRPV1* gene knockout mice showed impaired detection of painful levels of heat, indicating little thermal hypersensitivity in the setting of inflammation. The expression of *TRPV2* also affects mechanical and thermal hyperalgesia, as demonstrated in a rat model of cisplatin-induced peripheral neuropathy [20]. The *TRPV3* gene knockout mice have deficits in response to heat perception [21]. The *TRPV4* gene knockout mice presented a reduced sensitivity to pressure exerted on the tail and an impaired threshold to noxious stimuli and the conduction velocity of myelinated nerves responding to stimuli [22]. Moreover, there has been experimental and clinical evidence to substantiate TRP channels as attractive drug targets, and a number of molecules targeting TRP channels have already advanced to clinical trials. Topical capsaicin, targeting TRPV1, has been used for decades for the treatment of chronic painful disorders such as diabetic neuropathy and post-herpetic neuralgia [23]. Recently a selective antagonist for TRPV3, GRC15300, has already entered into clinical trials after substantiation of its efficacy in inflammatory and neuropathic pain models [24].

The role of the *TRP* gene in the pathogenesis of chronic pain disorder was also evaluated by several investigations. Armero *et al.* [25] showed that the Met315Met *TRPV1* genotype is more frequent in female patients with neuropathic pain in a Caucasian population. Binder *et al.* [26] evaluated the associations of TRP channel polymorphism, including TRPV1, TRPM8 and TRPA1, with the somatosensory function in patients with neuropathic pain. Although no genetic variants of the TPR channels were

Table 2 Genotype and allele analyses of *TRPV2* and *TRPV3* genes in FM patients and healthy controls

Marker[a]	Genotype/ allele	Control, *n* (%)	FM, *n* (%)	P-value[b]	OR (95% CI), P-value[c]	OR (95% CI), P-value, adjusted by age, sex[c]
TRPV2 rs3813768	C/C	234 (55.7)	253 (62.0)	0.183	1	1
	C/G	160 (38.1)	134 (32.8)		0.775 (0.579, 1.036), 0.085	0.772 (0.575, 1.037), 0.086
	G/G	26 (6.2)	21 (5.1)		0.747 (0.409, 1.364), 0.342	0.813 (0.443, 1.493), 0.505
	C	628 (74.8)	640 (78.4)	0.088	1	
	G	212 (25.2)	176 (21.6)		0.815 (0.648, 1.023), 0.078	0.831 (0.659, 1.047), 0.116
TRPV2 rs8121	C/C	21 (5.0)	19 (4.6)	0.971	1	1
	C/T	138 (32.7)	133 (32.5)		1.065 (0.548, 2.071), 0.852	1.088 (0.556, 2.129), 0.806
	T/T	263 (62.3)	257 (62.8)		1.080 (0.567, 2.056), 0.815	1.068 (0.557, 2.046), 0.844
	C	180 (21.3)	171 (20.9)	0.880	1	
	T	664 (78.7)	647 (79.1)		1.026 (0.810, 1.298), 0.833	1.004 (0.791, 1.275), 0.971
TRPV2 rs1129235	C/C	31 (7.3)	20 (4.9)	0.293	1	1
	A/C	137 (32.5)	128 (31.4)		1.448 (0.786, 2.669), 0.235	1.556 (0.837, 2.892), 0.162
	A/A	254 (60.2)	259 (63.6)		1.581 (0.878, 2.846), 0.127	1.668 (0.919, 3.028), 0.093
	C	199 (23.6)	168 (20.6)	0.167	1	
	A	645 (76.4)	646 (79.4)		1.186 (0.940, 1.497), 0.150	1.193 (0.943, 1.511), 0.142
TRPV3 rs7216486	A/G	32 (7.6)	28 (6.8)	0.782	1	1
	A/A	390 (92.4)	381(93.2)		1.116 (0.659, 1.890), 0.682	1.083 (0.637, 1.840), 0.768
	G	32 (3.8)	28 (3.4)	0.786	1	
	A	812 (96.2)	790 (96.6)		1.112 (0.663, 1.864), 0.687	1.080 (0.642, 1.816), 0.772
TRPV3 rs395357	C/C	48 (11.4)	40 (9.8)	0.649	1	1
	C/T	167 (39.6)	172 (42.2)		1.236 (0.772, 1.979), 0.378	1.237 (0.767, 1.996), 0.382
	T/T	207 (49.1)	196 (48.0)		1.136 (0.715, 1.805), 0.589	1.103 (0.689, 1.765), 0.684
	C	263 (31.2)	252 (30.9)	0.944	1	
	T	581 (68.8)	564 (69.1)		1.013 (0.823, 1.247), 0.902	0.992 (0.802, 1.226), 0.939

[a]Missing data were excluded from the analyses: *TRPV2* rs3813768 (two patients and three controls), *TRPV2* rs8121 (one patient and one control), *TRPV2* rs1129235 (three patients and one control), *TRPV3* rs7216486 (one patient and one control), *TRPV3* rs395357 (one patient and one control). [b]Value was determined by Fisher's exact test or chi-square test. [c]Logistic regression analyses were used to calculate the OR (95% CI). TRPV: transient receptor potential vanilloid; A: adenine; C: cytosine; G: guanine; T: thymine.

Table 3 Least-squares means (95% CIs) of responses in patients with FM, according to genotype

Position	Genotype	*n*[a]	Tender point number	Tender point count	FIQ	BFI	PCS	MCS	BDI	STAI-1	STAI-2
TRPV2	CC	253	13.72 (12.76, 14.68)	25.06 (21.83, 28.3)	60.43 (56.06, 64.79)	6.60 (4.97, 8.23)	37.52 (35.77, 39.28)	33.16 (30.35, 35.96)	18.37 (15.89, 20.85)	50.26 (47.39, 53.12)	50.56 (47.9, 53.21)
rs3813768	CG	134	13.95 (12.84, 15.06)	27.38 (23.63, 31.13)	59.89 (55, 64.79)	6.36 (4.55, 8.16)	37.38 (35.42, 39.35)	32.18 (29.03, 35.33)	18.17 (15.38, 20.95)	49.83 (46.61, 53.05)	49.48 (46.51, 52.45)
	GG	21	12.69 (10.72, 14.67)	23.19 (16.54, 29.84)	56.28 (47.06, 65.51)	8.54 (5.17, 11.91)	39.39 (35.68, 43.10)	30.94 (25, 36.87)	16.36 (11.12, 21.6)	50.27 (44.23, 56.31)	51.19 (45.59, 56.79)
P-value[b]			0.491	0.295	0.676	0.471	0.585	0.654	0.754	0.959	0.693
TRPV2 rs8121	CC	19	14.73 (12.45, 17.02)	25.64 (17.92, 33.36)	60.93 (50.6, 71.26)	9.35 (5.57, 13.12)	38.00 (33.84, 42.15)	28.51 (21.88, 35.14)	18.42 (12.56, 24.29)	52.17 (45.42, 58.92)	53.43 (47.19, 59.67)
	CT	133	13.49 (12.44, 14.54)	25.90 (22.34, 29.45)	59.56 (54.87, 64.26)	6.52 (4.79, 8.25)	37.38 (35.49, 39.27)	33.13 (30.12, 36.14)	17.87 (15.20, 20.54)	49.22 (46.15, 52.3)	49.04 (46.20, 51.88)
	TT	257	13.76 (12.78, 14.73)	25.38 (22.08, 28.68)	60.04 (55.6, 64.48)	6.60 (4.95, 8.26)	37.82 (36.04, 39.6)	32.56 (29.71, 35.4)	18.31 (15.78, 20.83)	50.66 (47.75, 53.57)	50.95 (48.26, 53.63)
P-value[b]			0.552	0.954	0.958	0.324	0.88	0.397	0.941	0.531	0.232
TRPV2	CC	20	14.81 (12.63, 16.98)	27.07 (19.72, 34.42)	63.05 (53.23, 72.86)	9.15 (5.54, 12.76)	38.28 (34.30, 42.25)	27.25 (20.99, 33.51)	19.20 (13.59, 24.8)	54.02 (47.57, 60.47)	54.85 (48.90, 60.80)
rs1129235	CA	128	13.27 (12.18, 14.35)	24.92 (21.25, 28.59)	59.41 (54.60, 64.21)	6.45 (4.67, 8.24)	37.32 (35.38, 39.27)	33.62 (30.56, 36.68)	17.61 (14.87, 20.36)	49.19 (46.02, 52.36)	48.60 (45.69, 51.51)
	AA	259	13.80 (12.84, 14.77)	25.78 (22.51, 29.05)	59.68 (55.31, 64.05)	6.54 (4.9, 8.19)	37.80 (36.03, 39.56)	32.71 (29.92, 35.49)	18.31 (15.81, 20.82)	50.28 (47.4, 53.16)	50.78 (48.13, 53.44)
P-value[b]			0.317	0.804	0.773	0.34	0.834	0.151	0.801	0.345	0.081
TRPV3	GA	28	13.49 (11.68, 15.3)	23.37 (17.27, 29.48)	65.58 (57.34, 73.82)	6.07 (3.04, 9.1)	36.01 (32.69, 39.33)	29.55 (24.25, 34.86)	18.69 (13.99, 23.38)	50.75 (45.25, 56.25)	53.20 (48.20, 58.21)
rs7216486	GG	381	13.71 (12.82, 14.59)	25.75 (22.76, 28.73)	59.51 (55.56, 63.47)	6.73 (5.25, 8.21)	37.74 (36.15, 39.34)	32.82 (30.27, 35.37)	18.09 (15.83, 20.34)	50.07 (47.47, 52.67)	50.04 (47.64, 52.45)
P-value[b]			0.801	0.412	0.122	0.647	0.273	0.196	0.788	0.795	0.185
TRPV3	CC	40	13.47 (11.79, 15.14)	23.41 (17.80, 29.03)	59.92 (52.46, 67.38)	9.90 (7.16, 12.64)	38.85 (35.85, 41.84)	29.51 (24.74, 34.28)	19.96 (15.68, 24.24)	51.72 (46.73, 56.72)	51.09 (46.51, 55.67)
rs395357	CT	172	13.68 (12.65, 14.71)	24.55 (21.09, 28.00)	61.00 (56.38, 65.62)	6.83 (5.11, 8.54)	37.78 (35.92, 39.63)	31.81 (28.86, 34.77)	18.44 (15.82, 21.07)	50.37 (47.33, 53.4)	50.66 (47.85, 53.47)
	TT	196	13.74 (12.75, 14.73)	26.81 (23.49, 30.12)	58.90 (54.46, 63.34)	6.10 (4.48, 7.72)	37.37 (35.59, 39.15)	33.79 (30.95, 36.63)	17.62 (15.1, 20.14)	49.68 (46.77, 52.59)	49.75 (47.05, 52.46)
P-value[b]			0.944	0.249	0.631	0.017	0.586	0.117	0.496	0.68	0.724

All values were given as mean (95% CI) unless otherwise indicated. [a]Missing data were excluded from analyses: *TRPV2* rs3813768 (two patients and three controls), *TRPV2* rs8121 (one patient and one control), *TRPV2* rs1129235 (three patients and one control), *TRPV3* rs7216486 (one patient and one control), *TRPV3* rs395357 (one patient and one control). [b]P-values for analyses of covariance adjusted for age and sex. FIQ: Fibromyalgia Impact Questionnaire; BFI: Brief Fatigue Inventory; PCS: Physical Component Summary; MCS: Mental Component Summary; BDI: Brief Depression Inventory; STAI-1: State-Trait Anxiety Inventory-1; STAI-2: State-Trait Anxiety Inventory-2; TRPV: transient receptor potential vanilloid; A: adenine; C: cytosine; G: guanine; T: thymine.

Table 4 Estimates of haplotype frequencies in FM patients and healthy controls

Gene	Combined alleles	All subjects, % (S.E.)	Controls, % (S.E.)	FM, % (S.E.)	P-value[b]
TRPV2[a]	CTA	70.41 (0.13)	67.96 (0.18)	72.99 (0.19)	0.0002
	GCC	16.59 (0.13)	17.12 (0.18)	16.03 (0.17)	
	GTA	6.46 (0.13)	7.81 (0.17)	5.03 (0.19)	
	CCC	3.81 (0.13)	3.36 (0.18)	4.28 (0.18)	
	CTC	1.74 (0.05)	3.13 (0.04)	0.26 (0.11)	
	GCA	0.44 (0.04)	0.49 (0.03)	0.40 (0.08)	
	CCA	0.37 (0.04)	0.13 (0.03)	0.63 (0.08)	
	GTC	0.19 (0.05)	0.0 (0.01)	0.38 (0.10)	
TRPV3[a]	AT	66.18 (0.17)	65.76 (0.23)	66.62 (0.18)	0.8203
	AC	30.15 (0.17)	30.42 (0.23)	29.87 (0.18)	
	GT	2.86 (0.17)	3.10 (0.23)	2.60 (0.18)	
	GC	0.82 (0.17)	0.72 (0.23)	0.91 (0.18)	

[a]Missing data were excluded (*TRPV2*, $n = 19$; *TRPV3*, $n = 25$). [b]P-values for permutation test of the null hypothesis that cases and controls are random draws from a common set of haplotype frequencies (number of permutations = 10 000). TRPV: transient receptor potential vanilloid; A: adenine; C: cytosine; G: guanine; T: thymine.

Table 5 Combined allele frequencies and ORs in FM patients and healthy controls

Gene	Combined allele	Controls, *n* (%)	FM, *n* (%)	Crude OR (95% CI)	P-value[a]	Age- and sex-adjusted OR (95% CI)	P-value[a]
TRPV2	CTA	569 (70.9)	579 (74.3)	1 (reference)		1 (reference)	
	GCC	145 (18.1)	129 (16.6)	0.874 (0.671, 1.138)	0.318	0.883 (0.676, 1.153)	0.361
	GTA	63 (7.8)	39 (5.0)	0.608 (0.401, 0.922)	0.019	0.637 (0.418, 0.969)	0.035
	CCC	26 (3.2)	32 (4.1)	1.210 (0.712, 2.055)	0.482	1.208 (0.707, 2.067)	0.489
TRPV3	AT	547 (65.4)	527 (66.5)	1 (reference)		1 (reference)	
	AC	259 (31.0)	241 (30.4)	0.966 (0.781, 1.194)	0.748	0.993 (0.801, 1.230)	0.947
	GT	30 (3.6)	24 (3.0)	0.830 (0.479, 1.439)	0.508	0.850 (0.489, 1.478)	0.565

[a]Computed for the estimated coefficient of each haplotype in the logistic regression. [b]Computed by Pearson's chi-squared test. Missing data were excluded (*TRPV2*, $n = 19$; *TRPV3*, $n = 25$). Among eight haplotype structures of the *TRPV2* gene, four haplotypes with a frequency of at least 1% in both patients and controls are presented; the total frequency of the other haplotype structures was 11 (1.4%) for controls and 1 (0.1%) for patients. Among the four haplotype structures of the *TRPV3* gene, three haplotypes with a frequency of at least 1% in both patients and controls are presented; the total frequency of the other haplotype structures was 2 (0.2%) for controls and 4 (0.5%) for patients. Logistic regression models were used to calculate the OR. TRPV: transient receptor potential vanilloid; A: adenine; C: cytosine; G: guanine; T: thymine.

found to differ between the neuropathic pain patients and controls, they found that *TRP* polymorphisms contributed significantly to the modulation of somatosensory function in neuropathic pain patients. Carreno *et al.* [27] carried out a case–control genetic association study to identify SNPs in *TRP* genes that may increase the genetic susceptibility to migraine. From a total of 14 *TRP* genes with a known brain expression, the SNPs of *TRPV1* rs222741 and *TRPV3* rs7217270 were associated with migraine in the Spanish cohort.

However, *TRP* gene polymorphisms have not yet been investigated within the context of FM patients. Because the TRP channel appears to play a role in the development of peripheral and central sensitization, it is worth evaluating the role of the *TRP* gene in the development of FM. Notably, a recent genome-wide linkage scan study, the FM Family Study, suggested the *TRPV2* gene as a potential candidate gene for FM [10]. In that study, Arnold *et al.* [10] suggested the linkage of FM to the chromosome 17p11.2-q11.2 region that coincides with the map coordinates for the serotonin transporter gene (*SLC6A4*) and *TRPV2* genes.

In the present study, the allele and genotype frequencies of individual *TRPV2* and *TRPV3* SNPs were not significantly different between the FM patients and the controls. However, certain haplotypes of the *TRPV2* and *TRPV3* genes were found to be associated with FM. In particular, the GTA haplotype of the *TRPV2* gene showed protective tendencies against FM susceptibility. The haplotype represents a set of alleles of a group of closely linked individual SNPs that are usually inherited as a unit [28]. The importance of identifying haplotypes

Table 6 Numbers of haplotypes and least-squares means (95% CIs) of responses in patients with FM

Gene	Combined allele	*n*[a]	Tender point number	Tender point count	FIQ	BFI	PCS	MCS	BDI	STAI-1	STAI-2
TRPV2	CTA	579	13.65 (12.97, 14.32)	25.22 (22.97, 27.47)	60.18 (57.19, 63.18)	6.52 (5.39, 7.66)	37.38 (36.16, 38.59)	33.03 (31.11, 34.95)	18.16 (16.44, 19.87)	50.11 (48.13, 52.09)	50.28 (48.45, 52.10)
	GCC	129	13.49 (12.54, 14.45)	25.14 (21.94, 28.34)	60.71 (56.48, 64.93)	6.94 (5.36, 8.52)	37.21 (35.49, 38.92)	31.72 (29.01, 34.43)	17.40 (14.99, 19.81)	49.91 (47.10, 52.71)	49.68 (47.11, 52.26)
	GTA	39	13.18 (11.58, 14.77)	27.37 (22.03, 32.70)	53.78 (46.86, 60.71)	7.19 (4.61, 9.77)	39.25 (36.44, 42.06)	32.05 (27.60, 36.50)	18.89 (14.88, 22.91)	50.79 (46.22, 55.36)	50.36 (46.14, 54.59)
	CCC	32	13.74 (12.00, 15.48)	25.15 (19.33, 30.97)	59.84 (52.12, 67.57)	8.11 (5.17, 11.04)	36.87 (33.74, 40.01)	33.90 (28.94, 38.86)	21.11 (16.69, 25.53)	51.77 (46.67, 56.86)	51.13 (46.42, 55.85)
	P-value[b]		0.926	0.876	0.292	0.668	0.553	0.720	0.467	0.906	0.940
TRPV3	AT	527	13.75 (13.09, 14.40)	26.28 (24.07, 28.48)	59.28 (56.35, 62.22)	6.28 (5.19, 7.37)	37.52 (36.34, 38.7)	33.41 (31.53, 35.30)	17.79 (16.12, 19.46)	49.80 (47.88, 51.72)	49.82 (48.04, 51.60)
	AC	241	13.59 (12.80, 14.39)	24.22 (21.56, 26.88)	60.65 (57.10, 64.21)	7.78 (6.45, 9.10)	38.14 (36.71, 39.57)	31.09 (28.81, 33.38)	18.92 (16.89, 20.95)	50.83 (48.48, 53.17)	50.90 (48.74, 53.06)
	GT	24	13.30 (11.55, 15.05)	23.49 (17.61, 29.37)	65.48 (57.34, 73.61)	6.01 (3.03, 8.98)	36.25 (32.98, 39.51)	29.45 (24.23, 34.67)	18.81 (14.20, 23.43)	51.22 (45.79, 56.65)	54.16 (49.24, 59.08)
	P-value[b]		0.818	0.168	0.247	0.036	0.418	0.036	0.446	0.574	0.140

All values were given as mean (95% CI) unless otherwise indicated. [a]Missing data were excluded from the analyses. [b]P-values from an analysis of covariance adjusted for age and sex. FIQ: Fibromyalgia Impact Questionnaire; BFI: Brief Fatigue Inventory; PCS: Physical Component Summary; MCS: Mental Component Summary; BDI: Brief Depression Inventory; STAI-1: State-Trait Anxiety Inventory 1; STAI-2: State-Trait Anxiety Inventory 2; TRPV: transient receptor potential vanilloid; A: adenine; C: cytosine; G: guanine; T: thymine.

has increased in evaluating the genetic background of certain diseases. Previous studies have revealed that certain haplotypes are associated with FM susceptibility and also pain sensitivity. In FM, the most widely investigated haplotype is the ACCG haplotype of the catechol-O-methyltransferase (*COMT*) gene. Diatchenko *et al.* [29] revealed that specific haplotypes of the *COMT* gene, consisting of SNPs rs6269, rs4633, rs4818 and rs4680, were strongly associated with low, average or high pain sensitivity and the ACCG haplotype of the *COMT* gene was defined as a high pain-sensitivity haplotype. In accordance with this result, other studies have shown the ACCG haplotype of the *COMT* gene to be more frequent in FM patients, and also associated with greater pain sensitivity [5, 30]. FM patients with the ACCG haplotype had a higher FIQ score than those with other haplotypes [30]. Studies have also indicated an association between FM and haplotypes. Kim *et al.* [11] showed that certain guanosine triphosphate cyclohydrolase 1 haplotypes, rather than individual SNPs, were associated with FM susceptibility and pain sensitivity. Additionally, such an association between FM susceptibility and haplotypes was observed in a study of the adrenal receptor gene [31]. In our study, we also suggested that the haplotype of *TRPV2* may be associated with susceptibility to FM.

In this study, although the SNPs and haplotypes of *TRPV3* were not associated with the presence of FM, some genotypes and haplotypes of *TRPV3* contributed to symptom severity within the FM cohort. *TRPV3* SNP rs395357 was associated with the severity of fatigue in FM, and haplotypes of *TRPV3* influence the differences in the scores of BFI and MCS in FM patients. Fatigue is one of the common symptoms in FM patients and contributes to a greater degree of disability in FM [32, 33]. Fatigue is thought to be a result of the interaction of environmental and biologic factors [34, 35]. Also, as with FM, researchers take into account genetic effects on the development of fatigue. However, the genetic background of the pathogenesis of fatigue remains to be determined. As mentioned above, because TRP ion channels regulate the physiologic signalling pathways in the CNS, several studies have considered *TRP* genes as contributing to the development of fatigue. Light *et al.* [36] showed an atypical upregulation of TRPV1 expression following exercise in chronic fatigue syndrome (CFS). They also found positive relationships between post-exercise pain and fatigue and increases in TRPV1. Additionally, Marshall-Gradisnik *et al.* [37], in a recent pilot study, suggested that TRP ion channels may contribute to the aetiology of CFS. In that study, a number of *TRP* SNPs (*TRPM3*, *TRPA1* and *TRPC4*) were associated with the presence of CFS, compared with non-fatigued controls. In this context, the association we demonstrated between *TRP* mutation and fatigue symptoms in FM patients is understandable. In our study, we first described how the SNPs and haplotypes of *TRPV3* could explain the severity of fatigue in FM patients.

Interestingly, haplotypes of the *TRPV3* gene were related to the MCS of the SF-36 among FM patients.

Researchers have tried to identify a candidate gene associated with impaired quality of life in certain diseases [38]. Raszeja-Wyszomirska *et al.* [39] demonstrated that SNPs of the TNF receptor-associated factor 1 and complement 5 (*TRAF1-C5*) genes were associated with the MCS of the SF-36 in primary biliary cirrhosis. Genetic polymorphisms associated with impaired quality of life were also evaluated in irritable bowel syndrome and cancer patients [40, 41]. Our study suggested the *TRPV3* polymorphism as the mechanism underlying the impaired quality of life in patients with FM. However, the factors affecting the quality of life are diverse; therefore the results should be interpreted carefully. Clearly, further studies are needed to understand the biologic and genetic mechanisms underlying the complex nature of quality of life.

This study had several limitations. It was mainly focused on susceptibility to FM and was performed using a case–control design, like most SNP studies. In addition, we were unable to investigate the associations between genetic variation and treatment outcome in FM patients. Recently, researchers have paid attention to the effects of SNPs on disease progression and outcome. One study revealed that genetic variation is associated with an improved response to the anti-TNF agent in patients with RA [42]. Likewise, Zhang *et al.* [43] revealed that the *COMT* gene haplotype contributes to the individual variation of postoperative fentanyl consumption in patients who underwent radical gastrectomy. Therefore, further prospective studies are needed to evaluate the effect of the *TRPV2* and *TRPV3* polymorphisms on the clinical outcome in FM patients. Finally, although not shown in the table, the CTC haplotype protected against susceptibility to FM [crude OR = 0.113 (95% CI 0.034, 0.377), $P < 0.001$; age- and sex-adjusted OR = 0.083 (95% CI 0.025, 0.276), $P < 0.001$]. However, because only 3 of 396 FM patients have the CTC haplotype, the clinical significance of this result is difficult to judge. The haplotype, which has a prevalence of $<1\%$, could be an incidental mutation rather than a loss-of-function, gain-of-function or neutral mutation. Nevertheless, the roles of the CTC haplotype in FM should be investigated further.

In summary, we conducted the first investigation to evaluate the association of *TRPV2* and *TRPV3* with FM in a Korean population. Polymorphisms of both the *TRPV2* and *TRPV3* genes were associated with FM but affected the progression of FM in different ways. In the present study, the polymorphism of *TRPV2* influenced the susceptibility to FM, while the polymorphism of *TRPV3* contributed towards symptom severity in FM. Further prospective studies with large populations are needed to verify these results.

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References

1 Jones GT, Atzeni F, Beasley M *et al*. The prevalence of fibromyalgia in the general population: a comparison of the American College of Rheumatology 1990, 2010, and modified 2010 classification criteria. Arthritis Rheumatol 2015;67:568–75.

2 Wolfe F, Smythe HA, Yunus MB *et al*. The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160–72.

3 Maletic V, Raison CL. Neurobiology of depression, fibromyalgia and neuropathic pain. Front Biosci (Landmark Ed) 2009;14:5291–338.

4 Sommer C, Hauser W, Burgmer M *et al*. [Etiology and pathophysiology of fibromyalgia syndrome]. Schmerz 2012;26:259–67.

5 Martinez-Jauand M, Sitges C, Rodriguez V *et al*. Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. Eur J Pain 2013;17:16–27.

6 Buskila D, Sarzi-Puttini P. Biology and therapy of fibromyalgia. Genetic aspects of fibromyalgia syndrome. Arthritis Res Ther 2006;8:218.

7 Wang H, Woolf CJ. Pain TRPs. Neuron 2005;46:9–12.

8 Vargas-Alarcon G, Alvarez-Leon E, Fragoso JM *et al*. A SCN9A gene-encoded dorsal root ganglia sodium channel polymorphism associated with severe fibromyalgia. BMC Musculoskel Dis 2012;13:23.

9 Levine JD, Alessandri-Haber N. TRP channels: targets for the relief of pain. Biochim Biophys Acta 2007;1772:989–1003.

10 Arnold LM, Fan J, Russell IJ *et al*. The fibromyalgia family study: a genome-wide linkage scan study. Arthritis Rheum 2013;65:1122–8.

11 Kim SK, Kim SH, Nah SS *et al*. Association of guanosine triphosphate cyclohydrolase 1 gene polymorphisms with fibromyalgia syndrome in a Korean population. J Rheumatol 2013;40:316–22.

12 Okifuji A, Turk DC, Sinclair JD, Starz TW, Marcus DA. A standardized manual tender point survey. I. Development and determination of a threshold point for the identification of positive tender points in fibromyalgia syndrome. J Rheumatol 1997;24:377–83.

13 Kim YA, Lee SS, Park K. Validation of a Korean version of the Fibromyalgia Impact Questionnaire. J Korean Med Sci 2002;17:220–4.

14 Yun YH, Wang XS, Lee JS *et al*. Validation study of the Korean version of the Brief Fatigue Inventory. J Pain Symptom Manage 2005;29:165–72.

15 Richter P, Werner J, Heerlein A, Kraus A, Sauer H. On the validity of the Beck Depression Inventory. A review. Psychopathology 1998;31:160–8.

16 Ware JE Jr, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473–83.

17 Kim JT, DG S. A standardization study of State-Trait Anxiety Inventory in Korea. N Med J 1978;21:1223–9.

18 Cortright DN, Krause JE, Broom DC. TRP channels and pain. Biochim Biophys Acta 2007;1772:978–88.

19 Caterina MJ, Leffler A, Malmberg AB *et al*. Impaired nociception and pain sensation in mice lacking the capsaicin receptor. Science 2000;288:306–13.

20 Hori K, Ozaki N, Suzuki S, Sugiura Y. Upregulations of P2X(3) and ASIC3 involve in hyperalgesia induced by cisplatin administration in rats. Pain 2010;149:393–405.

21 Moqrich A, Hwang SW, Earley TJ *et al*. Impaired thermosensation in mice lacking TRPV3, a heat and camphor sensor in the skin. Science 2005;307:1468–72.

22 Suzuki M, Mizuno A, Kodaira K, Imai M. Impaired pressure sensation in mice lacking TRPV4. J Biol Chem 2003;278:22664–8.

23 Derry S, Moore RA. Topical capsaicin (low concentration) for chronic neuropathic pain in adults. Cochrane Database Syst Rev 2012;9:CD010111.

24 Khairatkar Joshi NM, Thomas A. The TRPV3 receptor as a pain target: a therapeutic promise or just some more new biology? Open Drug Disc J 2010;2:89–97.

25 Armero P, Muriel C, Lopez M, Santos J, Gonzalez-Sarmiento R. [Analysis of TRPV1 gene polymorphisms in Spanish patients with neuropathic pain]. Med Clin 2012;139:1–4.

26 Binder A, May D, Baron R *et al*. Transient receptor potential channel polymorphisms are associated with the somatosensory function in neuropathic pain patients. PLoS One 2011;6:e17387.

27 Carreno O, Corominas R, Fernandez-Morales J *et al*. SNP variants within the vanilloid TRPV1 and TRPV3 receptor genes are associated with migraine in the Spanish population. Am J Med Genet B 2012;159B:94–103.

28 International HapMap Consortium. A haplotype map of the human genome. Nature 2005;437:1299–320.

29 Diatchenko L, Nackley AG, Slade GD *et al*. Catechol-O-methyltransferase gene polymorphisms are associated with multiple pain-evoking stimuli. Pain 2006;125:216–24.

30 Vargas-Alarcon G, Fragoso JM, Cruz-Robles D *et al*. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis Res Ther 2007;9:R110.

31 Vargas-Alarcon G, Fragoso JM, Cruz-Robles D *et al*. Association of adrenergic receptor gene polymorphisms with different fibromyalgia syndrome domains. Arthritis Rheum 2009;60:2169–73.

32 Jason LA, Taylor RR, Kennedy CL. Chronic fatigue syndrome, fibromyalgia, and multiple chemical sensitivities in a community-based sample of persons with chronic fatigue syndrome-like symptoms. Psychosom Med 2000;62:655–63.

33 Granzow B. Common characteristics of chronic fatigue syndrome, fibromyalgia and multiple chemical sensitivity. Deut Med Wochenschr 1999;124–1224.

34 Fernadez-Sola J, Padierna ML, Xarau SN, Mas PM. Chronic fatigue syndrome and multiple chemical hypersensitivity after insecticide exposition. Med Clin 2005;124:451–3.

35 Brown MM, Jason LA. Functioning in individuals with chronic fatigue syndrome: increased impairment with co-occurring multiple chemical sensitivity and fibromyalgia. Dyn Med 2007;6:6.

36 Light AR, Bateman L, Jo D *et al*. Gene expression alterations at baseline and following moderate exercise in patients with chronic fatigue syndrome and fibromyalgia syndrome. J Intern Med 2012;271:64–81.

37 Marshall-Gradisnik SM, Smith P, Brenu EW, *et al*. Examination of single nucleotide polymorphisms (SNPs) in transient receptor potential (TRP) ion channels in chronic fatigue syndrome patients. Immunol Immunogenet Insights 2015;7:1–6.

38 Sprangers MAG, Thong MSY, Bartels M *et al*. Biological pathways, candidate genes, and molecular markers associated with quality-of-life domains: an update. Qual Life Res 2014;23:1997–2013.

39 Raszeja-Wyszomirska J, Wunsch E, Kempinska-Podhorodecka A *et al*. *TRAF1-C5* affects quality of life in patients with primary biliary cirrhosis. Clin Dev Immunol 2013;2013:510547.

40 Jun SE, Kohen R, Cain KC, Jarrett ME, Heitkemper MM. TPH gene polymorphisms are associated with disease perception and quality of life in women with irritable bowel syndrome. Biol Res Nurs 2014;16:95–104.

41 Schroecksnadel K, Fiegl M, Prassl K *et al*. Diminished quality of life in patients with cancer correlates with tryptophan degradation. J Cancer Res Clin 2007;133:477–85.

42 Plant D, Prajapati R, Hyrich KL *et al*. Replication of association of the PTPRC gene with response to anti-tumor necrosis factor therapy in a large UK cohort. Arthritis Rheum 2012;64:665–70.

43 Zhang F, Tong JB, Hu J *et al*. COMT gene haplotypes are closely associated with postoperative fentanyl dose in patients. Anesth Analg 2015;120:933–40.