

Research Article

Cemile Zontul, Ayca Tas, Emrullah Hayta and Yavuz Silig*



The relationship between *ASIC3* gene polymorphism and fibromyalgia syndrome

<https://doi.org/10.1515/tjb-2023-0101>

Received May 4, 2023; accepted August 24, 2023;

published online October 19, 2023

Abstract

Objectives: Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterized by widespread body pain over a long period, the cause of which is not yet clearly known. FMS patients usually have high pain sensitivity. We aimed to investigate whether rs4148855 and rs2288646 polymorphisms of *acid-sensing ion channel 3 (ASIC3)*, one of the factors contributing to pain, cause a predisposition to FMS in the Turkish population.

Methods: *ASIC3* gene rs4148855 and rs2288646 polymorphisms in DNA samples obtained from blood samples of 175 patients with FMS and 176 healthy individuals were analyzed by real-time polymerase chain reaction (RT-PCR) using a hydrolysis probe. Statistical data were obtained by chi-square (χ^2) test and logistic regression analysis.

Results: No significant association was found between *ASIC3* gene rs4148855 and rs2288646 polymorphisms and the Turkish population's FMS group and control group ($p>0.05$).

Conclusions: As a result, no significant association was found between the genotype and allele distributions of *ASIC3* polymorphism (rs4148855 and rs2288646) in patients with FMS compared to controls in the Turkish population. Further

studies are needed to elucidate the relationship between ion channels and FMS to elucidate the mechanisms of FMS.

Keywords: fibromyalgia syndrome; single nucleotide polymorphism; acid-sensing ion channel 3; Turkish population

Introduction

Fibromyalgia syndrome (FMS) is a chronic pain syndrome characterized by long-lasting widespread body pain and tenderness in at least 11 of 18 specific areas identified. FMS is associated with fatigue, sleep disturbance, somatic syndromes, psychological disorders, disability and impaired quality of life [1]. The prevalence of FMS in the general population is 2.9–4.7 %. Although FMS can be seen in all ages and genders, it usually affects women aged 40–60 [2, 3]. Gender and advanced age are risk factors for FMS [4]. Physical and mechanical traumas and stress factors in the social environment are influential in the emergence of FMS [5]. Although the etiology of FMS has been attributed to infection, genetic and autoimmune factors, the mechanism is not fully known. Micro-muscle trauma, immunologic, psychological, neuro-hormonal, central nervous system, and environmental factors have been suggested to play a role in the development of the disease [6, 7]. It has been suggested that widespread chronic pain in FMS patients is caused by genetic factors affecting peripheral and central pain mechanisms [8]. The classification of FMS as neuropathic pain and its connection with pathophysiology is becoming increasingly important [9]. Despite many studies on FMS, its pathophysiology still needs to be clarified. In addition, according to genetic studies, there is a predisposition to FMS. Several pain-related genes have been identified, and there is evidence that many polymorphisms are associated with FMS [10].

ASIC3 are sodium channels activated by protons from outside the cell and are a member of acid-sensitive channel proteins structurally related to epithelial sodium channel proteins [11]. *ASIC3* is predominantly expressed in both the central and peripheral nervous systems and is activated by cellular proteins [12–14]. They are the most sensitive acid

*Corresponding author: Yavuz Silig, Department of Biochemistry, Faculty of Medicine, Sivas Cumhuriyet University, Sivas, Türkiye,

E-mail: ysilig@cumhuriyet.edu.tr. <https://orcid.org/0000-0002-0562-7457>

Cemile Zontul, Department of Chemistry and Chemical Processing

Technologies Services, Yıldızeli Vocational School, Sivas, Türkiye,

E-mail: cemilezontul@cumhuriyet.edu.tr. [https://orcid.org/0000-0002-](https://orcid.org/0000-0002-1436-5145)

1436-5145

Ayca Tas, Department of Nutrition and Dietetics, Faculty of Health Sciences, Sivas Cumhuriyet University, Sivas, Türkiye,

E-mail: aycatas@cumhuriyet.edu.tr. <https://orcid.org/0000-0002-7132-1325>

Emrullah Hayta, Department of Physical Therapy and Rehabilitation,

Faculty of Medicine, Acibadem University, Istanbul, Türkiye,

E-mail: emrullah.hayta@acibadem.com. <https://orcid.org/0000-0001-9460-9404>

sensors with high expression in peripheral nerves [15, 16]. In studies on rats, peripheral ASIC3 channels are the primary sensors of acidic pain in inflammatory conditions [17]. ASIC3 regulates myocardial ischemic pain, muscle pressure reflex, and possibly unstable autonomic regulation. Acidosis in muscle tissue is implicated in chronic muscle pain [12, 18]. ASIC3 ion channels activate muscle pain receptors and cause chronic muscle pain [19, 20].

Therefore, in our study, we examined the relationship between *ASIC3* gene polymorphisms (rs4148855 and rs2288646), which is one of the subunits of ASICs, which is important in pain perception, and there is no study in this area in our country, and FMS. This gene is located in the 7q35-36.1 region of the chromosome and is also localized in the intron region of ASIC3 in the first SNP rs4148855 we investigated. Nucleotide polymorphism occurs with the conversion of – in this 110 bp (base pair) long region to GTC. The other one, the SNP rs2288646 region, is also located in the exon region of ASIC3, and a single nucleotide polymorphism occurs when the adenine (A) base in this 106bp-long region converts to the guanine (G) base. As a result of this polymorphism, the presence of the G allele in the exon region resulted in a splicing-reducing effect, and the presence of the A allele resulted in a splicing-enhancing impact [21].

In addition, genetic variants of *ASIC3*, rs4148855 and rs2288646, were found to be associated with diseases such as pain, hypertension, anxiety and insulin resistance.

Materials and methods

Study population

This study consisted of 175 patients with FMS and 176 healthy controls admitted to Sivas Cumhuriyet University Faculty of Medicine, Department of Physical Medicine and Rehabilitation, and Sivas Numune Hospital, Physical Therapy Clinic. Patients who had no previous history of FMS and were diagnosed with FMS for the first time were included in the study, and the patients were from the same ethnic group and geographical region. The control group consisted of individuals admitted to the same hospitals, were similar to the selected patient group in terms of age and gender, were not diagnosed with FMS, and did not have any other disease. The diagnosis of FMS was made by a physician according to the American College of Rheumatology classification criteria [22]. Various questions such as name, surname, age, gender, height, weight, occupation, sleep disturbance, fatigue, headache, morning fatigue, dry mouth, leg numbness, dry eyes, difficulty concentrating, feeling of swelling in soft tissues, family history of FMS were filled in the questionnaire in question-answer form. Our study was approved by Sivas Cumhuriyet University Clinical Research Ethics Committee (Date: 12.05.2015, Decision no: 2015-05/01) and was conducted by the Declaration of Helsinki.

DNA isolation

A 4 mL blood sample was collected from healthy control subjects and FMS patients in sterile citrated blood tubes. Genomic DNA isolation was performed manually using the precipitation method at high salt concentrations when the blood samples reached the laboratory [23].

ASIC3 genotyping

ASIC3 polymorphisms (rs4148855 and rs2288646) were determined by real-time polymerase chain reaction (RT-PCR) using hydrolysis probes (GT0196, Qiagen-Rs 2288646; GT0195, Qiagen-Rs 4148855).

Statistical analysis

The relationship between fibromyalgia and *ASIC3* gene polymorphism, age, gender, height, weight, occupation, sleep disturbance, fatigue, headache, morning fatigue, dry mouth, leg numbness, dry eyes, difficulty concentrating, soft tissue swelling, and family history of fibromyalgia was determined using the Chi-square (χ^2) test (bias value $\alpha=0.05$). Risk estimates for fibromyalgia occurrence were determined by applying a logistic regression test. The degree of association was described as 95 % CI (confidence interval) OR (Odds Ratio). Statistical data analysis was performed using the Statistical Package for the Social Sciences (SPSS) program (Version 22).

Results

Demographic data and statistical analysis of the people included in the study

Demographic information and statistical findings of patients diagnosed with FMS and healthy individuals are given in Table 1. According to the data, 10 (5.7 %) of the patients were male, and 165 (94.3 %) were female. Among the controls, 10 (5.1 %) were male, and 166 (94.9 %) were female. The mean ages of patients and controls were 40.33 ± 16.30 and 39.69 ± 17.27 years for males and 43.89 ± 9.76 and 44.05 ± 8.87 years for females, respectively.

Analysis of patients and controls for *ASIC3* polymorphisms (rs4148855, rs2288646)

Statistical analysis of patients and controls in terms of *ASIC3* (rs2288646, rs4148855) polymorphisms is given in Table 2. Accordingly, 169 (96.6 %) patients had AA, 6 (3.4 %) AG, and 0 (0 %) GG genotypes in terms of rs2288646. Among controls, 170 (96.6 %) had AA, 4 (2.3 %) AG, and 2 (1.1 %) GG genotypes. When the patients and controls were evaluated by the χ^2 method in terms of rs4148855, no statistically significant difference was observed (χ^2 : 2.40, p: 0.310). In terms of

Table 1: Demographic information of patients with FMS and healthy group.

	Control group, n (%)	FMS group, n (%)	p-Value ^a	χ^2	OR (95 % CI)	Adjusted OR (95 % CI) ^b
Number of individuals	176	175	–	–	–	–
Gender			–	–	–	–
Male	10 (5.1)	10 (5.7)				
Female	166 (94.9)	165 (94.3)				
Age			–	–	–	–
Range	22–61	20–62				
Average age						
Male	39.69 ± 17.27	40.33 ± 16.30				
Female	44.05 ± 8.87	43.89 ± 9.76				
Family history of fibromyalgia			–	–	–	–
Present	0 (0.0)	78 (44.5)				
Male	0 (0.0)	3 (3.90)				
Woman	0 (0.0)	75 (96.1)				
Fatigue			0.001	21.94	6.77 (2.75–16.50)	6.86 (2.79–16.85)
Present	142 (81.1)	169 (96.5)				
Male	6 (4.20)	10 (5.90)				
Female	136 (95.8)	159 (94.1)				
Headache			0.001	29.63	3.83 (2.33–6.29)	3.87 (2.35–6.43)
Present	100 (56.8)	146 (83.4)				
Male	2 (2.00)	8 (5.50)				
Female	98 (98.0)	138 (94.5)				
Morning fatigue			0.001	68.09	12.83 (6.34–25.96)	13.23 (6.50–26.92)
Present	99 (56.5)	165 (94.2)				
Male	4 (4.10)	10 (6.10)				
Female	95 (95.9)	155 (93.9)				
Dry mouth			0.001	21.31	2.86 (1.82–4.51)	2.20 (1.84–4.64)
Present	91 (52.0)	132 (75.4)				
Male	1 (1.10)	7 (5.30)				
Female	90 (98.9)	125 (94.7)				
Leg numbness			0.001	31.63	3.57 (2.27–5.62)	3.8 (2.37–6.10)
Present	79 (44.8)	131 (74.8)				
Male	1 (1.30)	4 (3.10)				
Female	78 (98.7)	127 (96.9)				
Dry eye			0.001	13.73	2.31 (1.48–3.61)	2.28 (1.45–3.58)
Present	47 (26.8)	80 (45.7)				
Male	1 (2.10)	4 (5.00)				
Female	46 (97.9)	76 (95.0)				
Difficulty concentrating			0.001	37.98	4.3 (2.69–7.09)	4.6 (2.80–7.67)
Present	89 (50.6)	143 (81.7)				
Male	3 (3.40)	3 (2.10)				
Female	86 (96.6)	140 (97.9)				
Swelling in soft tissues			0.001	20.76	2.71 (1.76–4.18)	3.02 (1.90–4.76)
Present	74 (42.2)	116 (66.2)				
Male	2 (2.70)	3 (2.60)				
Female	72 (97.3)	113 (97.4)				

^ap-Values were calculated using the χ^2 test. ^bAdjusted for age, job, gendering status.

rs4148855, 75 (40.9 %) patients had –/GTC, 88 (50.3 %) –/–, and 12 (6.9 %) patients had GTC/GTC genotypes. Regarding rs4148855, 89 (50.6 %) of the controls had –/GTC, 72 (40.9 %) had –/–, and 15 (8.5 %) had GTC/GTC genotype. No statistically significant difference was observed when the patients and controls were evaluated by the χ^2 method regarding rs2288646 (χ^2 : 3.12, p: 0.210).

Comparison of *ASIC3* polymorphism (rs4148855 and rs2288646) genotypes and allele frequencies in study groups

The statistical analysis of the association of rs4148855 polymorphism of the *ASIC3* gene with genotype and allele distributions in FMS patients and healthy individuals in the

Table 2: Analysis of *ASIC3* (rs2288646 and rs4148855) genotypes of the study groups.

Rs2288646	GG	AG	AA
Patient	0 (0.00)	6 (3.4)	169 (96.6)
Control	2 (1.1)	4 (2.3)	170 (96.6)
χ^2		2.40	
p		0.210	

Rs4148855	GTC/GTC	-/GTC	-/-
Patient	12 (6.9)	75 (42.9)	88 (50.93)
Control	15 (8.5)	89 (50.6)	72 (40.09)
χ^2		3.12	
p		0.210	

χ^2 , chi-square test. p<0.05 determined as statistically significant

control group is given in Table 3. According to these data, the genotypes of 176 individuals in the control group in terms of rs4148855 were found to be 15 GTC/GTC, 89 -/GTC, 72 -/-. Genotypes of 175 individuals in the FMS patient group were determined as 12 GTC/GTC, 75 -/GTC, 88 -/-. In the analysis of *ASIC3* (rs4148855) polymorphism, it was not found statistically significant when FMS patients and controls were evaluated in terms of GTC and - allele (χ^2 : 2.50 p: 0.114, Raw OR: 1.29 95 % CI: 0.93–1.81). In terms of FMS patients and controls *ASIC3* (rs4148855), no statistically significant correlation was found in the analysis performed with the χ^2 method according to GTC/GTC and -/GTC status (χ^2 : 0.01 p: 0.901, Crude OR: 1.05 % 95 % CI: 0.46–2.49). No statistically significant correlation was found in the analysis performed with the χ^2 method according to GTC/GTC and -/- status (χ^2 : 1.03 p: 0.309, Raw OR: 1.15 % 95 % CI: 0.67–3.47). No

statistically significant correlation was found in the analysis performed with the χ^2 method according to GTC/GTC and -/GTC+ -/- status (χ^2 : 0.34 p: 0.055, Raw OR: 1.27 % 95 % CI: 0.58–2.79).

The statistical analysis of the relationship between rs2288646 polymorphism of the *ASIC3* gene and genotype and allele distributions of FMS patients and healthy individuals in the control group is given in Table 4. In the statistics of *ASIC3* (rs2288646), the genotypes of 176 individuals in the control group were determined as 2 GG, 4 as AG, and 170 as AA. Genotypes of 175 individuals in the FMS patient group were found to be 0 as GG, 6 as AG, and 170 as AA. In the analysis of *ASIC3* polymorphism with the χ^2 method, no statistically significant correlation was found when FMS patients and controls were evaluated in terms of G and A alleles (χ^2 : 0.28 p: 0.597, Raw OR: 1.33 % 95 % CI: 0.42–4.37). When patients and controls were evaluated in terms of *ASIC3* (rs2288646), the analysis of GG and AG using the χ^2 method was not significant (χ^2 : 2.40 p: 0.121 Raw OR: 0.67 % 95 % CI: 0.38–1.17). The analysis of GG and AG with the χ^2 method was not found significant (χ^2 : 1.98 p: 0.160 Raw OR: 0.99 % 95 % CI: 0.97–1.00). The analysis of GG and AG + AA using the χ^2 method was insignificant (χ^2 : 2.00 p: 0.157 Raw OR: 0.99 % 95 % CI: 0.97–1.00).

Gene variants between *ASIC3* polymorphism (rs4148855) and fibromyalgia syndromes

In patients with Fibromyalgia given in Table 5, *ASIC3* polymorphism (rs4148855) with sleep disturbance, fatigue,

Table 3: Genotype and allele frequency distribution of rs4148855 polymorphism of *ASIC3* gene in the study groups.

Rs4148855 (GTC/GTC/-/-)	Control group (n=176) (%)	Patient group (n=175) (%)	χ^2	p-Value	OR (95 % CI)	Adjusted OR (95 % CI)
GTC	119 (33.8)	99 (28.2)	Reference			
-	233 (66.2)	251 (71.8)	2.50	0.114	1.29 (0.93–1.81)	-
Codominant						
GTC/GTC	15 (8.52)	12 (6.85)	Reference			
-/GTC	89 (50.57)	75 (42.85)	0.01	0.901	1.05 (0.46–2.39)	1.02 (0.45–2.34)
-/-	72 (40.90)	88 (50.29)	1.03	0.309	1.15 (0.67–3.47)	1.18 (0.78–1.80)
Dominant						
GTC/GTC	15 (8.52)	12 (6.86)	Reference			
GTC/- + -/-	161 (91.48)	163 (93.14)	0.34	0.055	1.27 (0.58–2.79)	1.10 (0.74–1.64)
Recessive						
GTC/GTC + GC/-	104 (59.09)	87 (49.71)	Reference			
-/-	72 (40.91)	88 (50.29)	3.11	0.078	1.46 (0.96–2.23)	1.20 (0.97–1.48)
Overdominant						
GTC/GTC + -/-	87 (49.43)	100 (57.14)	Reference			
-/GTC	89 (50.57)	75 (42.86)	2.09	0.148	0.73 (0.48–1.12)	0.74 (0.48–1.13)

n, number of individuals. p<0.05 determined as statistically significant. χ^2 , chi-square test; OR, odds ratio; CI, confidence interval.

Table 4: Genotype and allele frequency distribution of rs2288646 polymorphism of *ASIC3* gene in the study groups.

Rs 2288646, GG/AA	Control group (n=176) (%)	Patient group (n=175) (%)	χ^2	p-Value	OR (95 % CI)	Adjusted OR (95 % CI)
G	8 (2.27)	6 (1.71)	Referans			
A	344 (97.73)	344 (98.29)	0.28	0.597	1.33 (0.42–4.37)	–
Codominant						
GG	2 (1.14)	0 (0)	Referans			
AG	4 (2.27)	6 (3.43)	2.40	0.121	0.67 (0.38–1.17)	–
AA	170 (96.59)	169 (96.57)	1.98	0.160	0.99 (0.97–1.00)	–
Dominant						
GG	2 (1.13)	0 (0)	Referans			
AG + AA	174 (98.87)	175 (100)	2.00	0.157	0.99 (0.97–1.00)	–
Recessive						
GG + AG	6 (3.40)	6 (3.43)	Referans			
AA	170 (96.60)	169 (96.57)	0.00	0.992	0.99 (0.31–3.14)	0.99 (0.55–1.77)
Overdominant						
GG + AA	172 (97.73)	169 (96.57)	Referans			
AG	4 (2.27)	6 (3.43)	0.42	0.515	1.53 (0.42–5.51)	1.46 (0.40–5.30)

n, number of individuals. $p < 0.05$ determined as statistically significant. χ^2 , chi-square test; OR, odds ratio; CI, confidence interval.

Table 5: *ASIC3* polymorphism (rs4148855) genotype distributions in terms of fibromyalgia syndromes.

FMS syndromes		ASIC3 polymorphism (rs4148855) n (%)			χ^2	p-Value
		GTC/GTC	–/GTC	–/–		
Sleeping disorder	Yes	17 (63.0)	88 (53.7)	96 (60.0)	1.71	0.42
	No	10 (37.0)	76 (46.3)	64 (40.0)		
Fatigue	Yes	27 (100.0)	143 (87.2)	141 (88.1)	3.83	0.14
	No	0 (00.0)	21 (12.8)	19 (11.9)		
Headache	Yes	19 (70.4)	109 (66.5)	118 (73.8)	2.05	0.35
	No	8 (29.6)	55 (33.5)	42 (26.3)		
Morning fatigue	Yes	17 (63.0)	116 (70.7)	131 (81.9)	7.74	0.02
	No	10 (37.0)	48 (29.3)	29 (18.1)		
Dry mouth	Yes	18 (66.7)	103 (62.8)	102 (63.8)	0.15	0.92
	No	9 (33.3)	61 (37.2)	58 (36.3)		
Leg numbness	Yes	14 (51.9)	97 (59.1)	100 (62.5)	1.21	0.54
	No	13 (48.1)	67 (40.9)	60 (37.5)		
Dry eye	Yes	10 (37.5)	59 (36.0)	58 (36.3)	0.01	0.99
	No	17 (63.0)	105 (64.0)	102 (63.8)		
Difficulty concentrating	Yes	18 (66.7)	105 (64.0)	109 (68.1)	0.61	0.73
	No	9 (33.3)	59 (36.0)	51 (31.9)		
Swelling in soft tissues	Yes	14 (51.9)	87 (53.0)	89 (55.6)	0.27	0.87
	No	13 (48.1)	77 (47.0)	71 (44.4)		
Family history of fibromyalgia	Yes	7 (25.9)	38 (23.2)	33 (20.6)	0.53	0.76
	No	20 (74.1)	126 (76.8)	127 (79.4)		

n, number of individuals. $p < 0.05$ determined as statistically significant. χ^2 , chi-square test

headache, dry mouth, numbness in legs, dry eyes, difficulty concentrating, swelling in soft tissues and familial history of fibromyalgia were statistically evaluated by χ^2 method. No significant difference was found between them. However, when morning fatigue was analyzed by the χ^2 method, a meaningful relationship was obtained between gene variants. (χ^2 : 7.74, p : 0.02).

Gene variants between *ASIC3* polymorphism (rs2288646) and fibromyalgia syndromes

In patients with Fibromyalgia given in Table 6, no significant difference was found between gene variants when the *ASIC3* polymorphism (rs2288646) and clinical parameters were evaluated statistically by the χ^2 method.

Table 6: *ASIC3* polymorphism (rs2288646) genotype distributions in terms of fibromyalgia syndromes.

FMS syndromes		<i>ASIC3</i> polymorphism (rs2288646) n (%)			χ^2	p-Value
		AA	AG	GG		
Sleeping disorder	Yes	1 (50.0)	6 (60.0)	194 (57.2)	0.07	0.96
	No	1 (50.0)	4 (40.0)	145 (42.8)		
Fatigue	Yes	2 (100.0)	9 (90.0)	300 (88.5)	0.28	0.86
	No	0 (00.0)	1 (10.0)	39 (11.5)		
Headache	Yes	0 (00.0)	8 (80.0)	238 (70.0)	5.15	0.07
	No	2 (100.0)	2 (20.0)	101 (29.8)		
Morning fatigue	Yes	2 (100.0)	7 (70.0)	255 (75.2)	0.80	0.66
	No	0 (00.0)	3 (30.0)	84 (24.8)		
Dry mouth	Yes	2 (100.0)	9 (90.0)	212 (62.5)	4.31	0.11
	No	0 (00.0)	1 (10.0)	127 (37.5)		
Leg numbness	Yes	2 (100.0)	7 (70.0)	202 (59.6)	1.77	0.41
	No	0 (00.0)	3 (30.0)	137 (40.4)		
Dry eye	Yes	0 (00.0)	4 (40.0)	123 (36.3)	1.19	0.54
	No	2 (100.0)	6 (60.0)	216 (63.7)		
Difficulty concentrating	Yes	1 (50.0)	7 (70.0)	224 (66.1)	0.29	0.86
	No	1 (50.0)	3 (30.0)	115 (33.9)		
Swelling in soft tissues	Yes	0 (00.0)	5 (50.0)	185 (54.6)	2.45	0.29
	No	2 (100.0)	5 (50.0)	154 (45.4)		
Family history of fibromyalgia	Yes	0 (00.0)	4 (40.0)	74 (21.8)	2.43	0.29
	No	2 (100.0)	6 (60.0)	265 (78.2)		

n, number of individuals. $p < 0.05$ determined as statistically significant. χ^2 , chi-square test

Discussion

FMS is known to cause widespread skeletal-muscular pain, fatigue, sleep disturbance, depression, headache, morning fatigue, and bowel dysfunction [2, 24]. In Turkey, the prevalence of FMS among 20–64 year-olds was 3.6 % [25]. In this study, the age range of patients diagnosed with fibromyalgia (n=175) was 20–62 years. The age ranges of other studies conducted in Turkey, and our current study is similar. FMS symptoms are usually observed in women and are found in the age range of 30–50 years [26]. In our study, 165 of 175 FMS patients were female. There are biomarker studies for FMS, but no specific biomarker has been identified yet [10]. According to a study, high serum prolidase activities were observed in FMS [27]. In research by Taş et al., the relationship between FMS and potassium ion channels was investigated, and determined that the level of potassium voltage-gated channel subfamily H member 2 (KCNH2) was low in plasma [28]. In a study by Koçak et al., serum cathepsin S (CatS) and cystatin C (CysC) levels were evaluated in FMS, and both were found to be elevated [29]. In addition, a significant correlation was observed for calcitonin gene-related peptide (CGRP), calcitonin receptor-like receptor (CLR), and receptor component protein (RCP) levels in FMS [30]. In a study conducted by Zontul et al. within the scope of artificial intelligence with FMS, classification was performed with support vector machines and achieved high success [31].

Today, it is suggested that the susceptibility to chronic pain and the response to pain sensitivity are related to the individual's genetic structure. In recent genetic studies, the fact that pain varies from person to person is explained by genetic polymorphism [7]. *ASIC3* is expressed in peripheral sensory neurons and is essential in pain perception and acid-induced hyperalgesia [32]. Our study examined the relationship between the rs4148855 and rs2288646 polymorphisms of the *ASIC3* gene, one of the subunits of ASICs, which is essential in pain perception and which has not been studied in the Turkish population, and FMS. Yu-Linko et al. investigated the relationship between *ASIC3* gene polymorphisms (rs2288646 and rs4148855) and blood pressure in a Taiwanese population. They found that individuals carrying the rs2288646-A allele (AA + AG genotype) were associated with high blood pressure [33]. In another study, the relationship between the rs1042717 polymorphism of the beta-2 adrenergic receptor gene between patients with FMS and healthy individuals was examined, and no significant association was found [34]. In a study by Kaydok et al., the relationship between estrogen receptor 1 (ESR1) gene polymorphisms (rs2228480 and rs2295190) and FMS was investigated. As a result of the evaluation of the relationship between rs2295190 polymorphism and FMS, it was found that patients with CG and GG genotypes had a lower risk of FMS than CC genotypes. No significant difference was observed between rs2228480 polymorphism and FMS [35]. It

has been reported that polymorphism of transient receptor potential vanilloid 2 (TRPV2), an ion channel in FMS, makes FMS more susceptible. TRPV3 has been suggested to increase the occurrence of FMS symptoms [36]. In a study examining polymorphisms of the sodium voltage-gated channel alpha subunit 9 (SCN9A) gene, which encodes voltage-gated sodium channel, in FMS, rs6754031 polymorphism was found to be significantly significant [37]. In a study by Yu et al., an FMS mouse model was created, electroacupuncture was applied, and the ASIC3 receptor was examined. It was found that mechanical hyperalgesia decreased in the mechanism involving ASIC3. It was also found that hyperalgesia and nociceptive signaling decreased in mice in which the *ASIC3* gene was silenced [38]. Hung et al. suggested that ASIC3 is activated by lysophosphatidylcholine (16:0) in chronic hyperalgesia caused by repeated and intermittent sound stress [39]. In a mouse model of FMS, therapeutic ultrasound treatment activated ASIC3 in muscle afferents and produced an analgesic effect [40]. *ASIC3* expression levels have been reported to be increased in patients with chronic fatigue syndrome [41].

This study determined the genotype distribution of *ASIC3* gene (rs4148855 and rs2288646) polymorphisms in patients with FMS and healthy control subjects by RT-PCR. In terms of rs2288646, 169 (96.6 %) FMS patients were found to have GG, 6 (3.4 %) AG, and 0 (0 %) AA genotypes. Among the controls, 170 (96.6 %) had GG, 4 (2.3 %) AG, and 2 (1.1 %) AA genotypes. Our study observed no statistically significant difference between *ASIC3* gene (rs4148855 and rs2288646) polymorphisms in patients with FMS and control subjects.

In this study, the number of male patients with FMS was not the same as the number of female patients. When *ASIC3* gene polymorphisms are evaluated, the effect of these gene polymorphisms on protein level cannot be investigated due to the need for a project budget. These are the limitations of our study.

Conclusions

In the Turkish population, no significant association was found between the genotype and allele distributions of *ASIC3* polymorphism (rs4148855 and rs2288646) in patients with FMS compared to controls. Further studies are needed to elucidate the relationship between ion channels and FMS to elucidate the mechanisms of FMS.

Acknowledgments: The authors thank to Dr Ziyet Cinar for her assistance with the statistical analysis.

Research ethics: The study was approved by Sivas Cumhuriyet University Clinical Research Ethics Committee (Date: 12.05.2015,

Decision no: 2015-05/01) and was conducted by the Declaration of Helsinki.

Informed consent: Informed consent was obtained from all individuals included in this study.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Research funding: This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University (CUBAP) under Project number T-656.

References

- Pagliai G, Giangrandi I, Dinu M, Sofi F, Colombini B. Nutritional interventions in managing fibromyalgia syndrome. *Nutrients* 2020;12: 2525.
- Yılmaz H, Uğurlu H, Sallı A. Fibromiyalji Sendromlu Hastalarda Kas Performansı. *Romatizma/Rheumatism* 2007;22:43–7.
- Marcus DA, Dawn A. Analgesics and opioids. Chronic pain: a primary care guide to practical management. Totowa, NJ: Humana Press; 2009: 349–65 pp.
- McBeth J, Jones K. Epidemiology of chronic musculoskeletal pain. *Best Pract Res Clin Rheumatol* 2007;21:403–25.
- Gormsen L, Rosenberg R, Bach FW, Jensen TS. Depression, anxiety, health-related quality of life, and pain in chronic fibromyalgia and neuropathic pain patients. *Eur J Pain* 2010;14:127–e1.
- Yunus MB. Towards a model of pathophysiology of fibromyalgia: aberrant central pain mechanisms with peripheral modulation. *J Rheumatol* 1992;19:846–50.
- Reisine S, Fifield J, Walsh SJ, Feinn R. Do employment and family work affect the health status of women with fibromyalgia? *J Rheumatol* 2003; 30:2045–53.
- Barcak O, Karkucak M, Capkin E, Karagüzel G, Dilber B, Dedeoglu S. Prevalence of generalized joint hypermobility and fibromyalgia syndrome in the children population of Trabzon: a Turkish study. *Turk Fiz Tip ve Rehabil Derg-Turk J Phys Med Rehabil* 2015;61:6–11.
- Kato K, Sullivan PF, Evengård B, Pedersen NL. Importance of genetic influences on chronic widespread pain. *Arthritis Rheum* 2006;54:1682–6.
- D'Agnelli S, Arendt-Nielsen L, Gerra MC, Zatorri K, Boggiani L, Baciarello M, et al. Fibromyalgia: genetics and epigenetics insights may provide the basis for the development of diagnostic biomarkers. *Mol Pain* 2019;15:1–12.
- Babinski K, Lê KT, Séguéla P. Molecular cloning and regional distribution of a human proton receptor subunit with biphasic functional properties. *J Neurochem* 1999;72:51–7.
- Yagi J, Wenk HN, Naves LA, McCleskey EW. Sustained currents through ASIC3 ion channels at the modest pH changes that occur during myocardial ischemia. *Circ Res* 2006;99:501–9.
- Cheng CF, Yang KC, Kuo TB, Chen CC. S29-3 loss of Asic3 results in abnormal cardiac autonomic regulation and increased cardiac ischemia susceptibility in mice. *Int J Cardiol* 2007;122:47.
- Tan ZY, Lu Y, Whiteis CA, Benson CJ, Chapleau MW, Abboud FM. Acid-sensing ion channels contribute to the transduction of extracellular acidosis in rat carotid body glomus cells. *Circ Res* 2007; 101:1009–19.

15. Law LAF, Sluka KA, McMullen T, Lee J, Arendt-Nielsen L, Graven-Nielsen T. Acidic buffer-induced muscle pain evokes referred pain and mechanical hyperalgesia in humans. *Pain* 2008;140:254–64.
16. Birdsong WT, Fierro L, Williams FG, Spelta V, Naves LA, Knowles M, et al. Sensing muscle ischemia: coincident detection of acid and ATP via interplay of two ion channels. *Neuron* 2010;68:739–49.
17. Lingueglia E. Acid-sensing ion channels (ASICs) in pain. *Biol Aujourd'hui* 2014;208:13–20.
18. Waldmann R, Champigny G, Bassilana F, Voilley N, Lazdunski M. Molecular cloning and functional expression of a novel amiloride-sensitive Na^+ channel. *J Biol Chem* 1995;270:27411–14.
19. Kellenberger S, Schild L. International Union of Basic and Clinical Pharmacology. XCI. structure, function, and pharmacology of acid-sensing ion channels and the epithelial Na^+ channel. *Pharmacol Rev* 2015;67:1–35.
20. Jensen JE, Cristofori-Armstrong B, Anangi R, Rosengren KJ, Lau CHY, Mobli M, et al. Understanding the molecular basis of toxin promiscuity: the analgesic sea anemone peptide APETx2 interacts with acid-sensing ion channel 3 and hERG channels via overlapping pharmacophores. *J Med Chem* 2014;57:9195–203.
21. Fairbrother WG, Yeh RF, Sharp PA, Burge CB. Predictive identification of exonic splicing enhancers in human genes. *Science* 2002;297:1007–13.
22. Wolfe F, Clauw DJ, Fitzcharles MA, Goldenberg DL, Häuser W, Katz RL, et al. Revisions to the 2010/2011 fibromyalgia diagnostic criteria. *Semin Arthritis Rheum* 2016;46:319–29.
23. MWER S, Dykes D, Polesky H. A simple salting-out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1988;16:1215.
24. Smith HS, Barkin RL. Fibromyalgia syndrome: a discussion of the syndrome and pharmacotherapy. *Am J Therapeut* 2010;17:418–39.
25. Topbas M, Cakirbay H, Gulec H, Akgol E, Ak I, Can G. The prevalence of fibromyalgia in women aged 20–64 in Turkey. *Scand J Rheumatol* 2005;34:140–4.
26. Busch AJ, Overend TJ, Schachter CL. Fibromyalgia treatment: the role of exercise and physical activity. *Int J Clin Rheumatol* 2009;4:343–80.
27. Ozturk A, Agbektas T, Tas A, Gul MA, Karadag A, Silig Y. The relationship between serum prolidase activity and histone H3 protein levels and fibromyalgia. *Eur Rev Med Pharmacol Sci* 2022;26:9098–106.
28. Tas A, Hayta E, Karadag A, Zontul C, Ozmen E, Aydin S, et al. Potassium ion channel protein (KCNH) levels in patients with Fibromyalgia Syndrome. *Cell Mol Biol* 2021;67:451–7.
29. Kocak I, Hizmetli S, Tas A, Karadag A, Zontul C, Silig Y. High levels of cathepsin S and cystatin C in patients with fibromyalgia syndrome. *Int J Rheum Dis* 2020;23:966–9.
30. Korucu RU, Karadağ A, Taş A, Özmen E, Hayta E, Siliğ Y. Serum calcitonin gene-related peptide and receptor protein levels in patients with fibromyalgia syndrome: a cross-Sectional study. *Arch Rheumatol* 2020;35:463–7.
31. Zontul C, Hayta E, Zontul M, Taş A, Silig Y. Destek Vektör Makineleri ile Fibromiyalji Sendromu Sınıflaması. *Acta Infologica* 2017;1:2602–3563.
32. Giesecke T, Gracely RH, Williams DA, Geisser ME, Petzke FW, Clauw DJ. The relationship between depression, clinical pain, and experimental pain in a chronic pain cohort. *Arthritis Rheum* 2005;52:1577–84.
33. Friese MA, Craner MJ, Etzensperger R, Vergo S, Wemmie JA, Welsh MJ, et al. Acid-sensing ion channel-1 contributes to axonal degeneration in autoimmune inflammation of the central nervous system. *Nat Med* 2007;13:1483–9.
34. Çakiroğlu GŞ, Hizmetli S, Siliğ Y, Karadağ A, Hayta E, Öztaltın B, et al. Comparison of beta-2 adrenergic receptor gene polymorphisms between patients with fibromyalgia syndrome and healthy controls. *Arch Rheumatol* 2020;35:328.
35. Kaydok E, Ozmen E, Sari I, Cakiroglu GS, Tas A, Silig Y, et al. A significant association between rs2295190 polymorphism of the ESR1 gene and fibromyalgia syndrome. *Cell Mol Biol* 2022;68:182–7.
36. Park DJ, Kim SH, Nah SS, Lee JH, Kim SK, Lee YA, et al. Polymorphisms of the TRPV2 and TRPV3 genes associated with fibromyalgia in a Korean population. *Rheumatology* 2016;55:1518–27.
37. Vargas-Alarcon G, Alvarez-Leon E, Fragoso JM, Vargas, Martinez A, Vallejo M, et al. A SCN9A gene-encoded dorsal root ganglia sodium channel polymorphism associated with severe fibromyalgia. *BMC Musculoskel Disord* 2012;13:1–5.
38. Yen LT, Hsieh CL, Hsu HC, Lin YW. Targeting ASIC3 for relieving mice fibromyalgia pain: roles of electroacupuncture, opioid, and adenosine. *Sci Rep* 2017;7:46663.
39. Hung CH, Lee CH, Tsai MH, Chen CH, Lin HF, Hsu CY, et al. Activation of acid-sensing ion channel 3 by lysophosphatidylcholine 16: 0 mediates psychological stress-induced fibromyalgia-like pain. *Ann Rheum Dis* 2020;79:1644–56.
40. Han DS, Lee CH, Shieh YD, Chang KV, Lin SH, Chu YC, et al. Involvement of ASIC3 and substance P in therapeutic ultrasound-mediated analgesia in mouse models of fibromyalgia. *J Pain* 2023;24:1493–505.
41. Light AR, White AT, Huguen RW, Light KC. Moderate exercise increases expression for sensory, adrenergic, and Immune genes in chronic fatigue syndrome patients but not in normal subjects. *J Pain* 2009;10:1099–112.