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Potassium Ion Channel Protein (KCNH) Levels in Patients with Fibromyalgia Syndrome

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ABSTRACT

Although there is not yet full clarity of the pathogenesis of fibromyalgia syndrome (FM), central sensitization is considered to be responsible. The purpose of this study was to measure the plasma levels of potassium ion channel proteins (human KCNH2, KCNH6 and KCNH7) in FM patients and healthy control subjects. The study sample includes 76 newly diagnosed FM patients and 79 healthy individuals. Venous blood samples were taken to measure the plasma levels of KCNH2, KCNH6 and KCNH7. Pain severity in FM patients was assessed using a visual analog scale (VAS). Bioinformatics analysis was performed using the STRING v 11 Protein interaction tool. Age, gender and body mass index were seen to be similar in both groups. In comparisons between FM and control groups, KCNH2 plasma levels was found to be significantly lower in the FM group. No significant correlation was found between plasma levels of KCNH2, KCNH6 and KCNH7 protein levels and VAS score of patients with FM. The KCNH2 protein had a high homology score with 9 proteins. The plasma levels of KCNH2 FM patients were found to be lower than those of the healthy control subjects, no difference was determined in respect of the plasma levels of KCNH6 and KCNH7. These results may be of use in guiding future studies on the pathogenesis of FM.

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Introduction

Fibromyalgia Syndrome (FM) is a chronic musculoskeletal system disease characterized by widespread pain and high levels of sensitivity to pain (1). The pathogenesis of FM has not been fully clarified as yet, but environmental, genetic and immunological factors, as well as central and peripheral mechanisms, are thought to have a role in the pathogenesis (2). Previous studies have attributed FM pathogenesis to central sensitization, suggesting that the central nervous system (CNS) may have a role when there are abnormalities in the processing and/or perception of pain (3, 4).

Central sensitization is defined as the erroneous and abnormal response of the CNS to a peripheral stimulus due to neuronal hyperexcitability and hypersensitivity in the central nervous (5). Some chemokines, cytokines and neuromodulators are known to function during postsynaptic and presynaptic processing of a peripheral nociceptive stimulus in the CNS (2). It has been suggested that hyperalgesia and allodynia develop due to increases or inhibition loss in membrane excitability and synaptic activity through reductions or increases in the inhibitor and excitatory neurotransmitters (3-6).

Membrane and neuronal excitability are controlled through ion channels. Of those ion channels, voltage-gated potassium (Kv) ion channels have a role in the repolarization and depolarization of the membrane and have an important function particularly in the

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control of neuronal excitability (**7**).

The structure of Kv ion channels is tetrameric and each subunit consists of transmembrane sections (S1-S6). Kv channels can be classified in 12 subfamilies (Kv 1-12). The Ether-à-go-go (EAG) family constitutes the majority of potassium channels and comprises Kv 10-12 (8). The EAG ion channel family includes the three subtypes of EAG (ether-gidon-Kv10), ERG (EAG-related gene, Kv11) and ELK (EAG-like Kv12). Of those, the molecular link of ERG (Kv11) with human metabolism has been previously demonstrated (9). Human ERG (hERG) has been divided into three subtypes of hERG1 (Kv11.1, KCNH2), hERG2 (Kv11.2, KCNH6) and hERG3(Kv11.3, KCNH7) and is particularly expressed in the brain, neurons and muscle tissues (10, 11). Previous studies have reported that the Kv11 family expressed in the human central nervous system could play a significant role in nervous system functions (12).

The majority of previous studies that have investigated the relationship between pain and ion channels have focussed on depolarising ion channels, and few studies have examined K+ channels, which have significant roles in control and axonal stimulation (13). Some previous clinical studies have proposed that impaired K^+ channel activity is one of the mechanisms responsible for hyperalgesia and allodynia, (14,15) while other studies have claimed that the use of K^+ channel activators could be promising in the treatment of neuropathic and chronic pain in the future (16,17).

FM is characterized by chronic pain, and it was therefore concluded that K^+ channel proteins, which have been previously shown to play a potential role, and the hERG family, which is widely expressed especially in the central nervous system, may be important in the pathogenesis of FM. In literature, no previous study could be found that has evaluated the levels of K^+ channel proteins in FM. Therefore, the aimed of this study was to compare the plasma levels of KCNH2, KCNH6 and KCNH7 proteins in FM patients and healthy control subjects.

Materials and methods

Study design

This cross-sectional study was conducted between September 2018 and December 2019. The study included a total of 76 patients newly diagnosed with FM according to the criteria of the American College of Rheumatology (2010) (18) and 79 healthy individuals. The exclusion criteria for FM patients were defined as the presence of inflammatory rheumatic disease (lupus, rheumatoid arthritis, seronegative spondyloarthritis, Behçet's disease, etc.), malignancy, a known history of systemic disease (cardiac, endocrine, psychiatric or neurological disease, etc.), regional pain syndrome (lumbar pain, osteoarthritis, myofascial pain, etc.) or the use of any medication other than simple analgesics. The control group was formed of subjects recruited from hospital personnel with no known disease and not using any medication.

A record was made of the demographic data of all the study participants and the visual analog scale (VAS) scores of the FM patients.

This study was approved by the local research and ethics committee Sivas Cumhuriyet University Ethics Committee (Dated: 17.01.2018; Approval Number: 2018-01/33). Each participant signed an informed consent form according to the ethical principles of the Declaration of Helsinki.

Visual Analogue Scale (VAS)

This is a 10-cm scale marked at equal intervals from 0-10 where 0 indicates no pain and 10 indicates intolerable pain. The patients were instructed to state the level of pain felt in the previous week by marking the scale and the result was recorded.

Measurement of the levels of KCNH2, KCNH6 and KCNH7 protein levels

Venous blood samples of all the participants were withdrawn into 4 ml sterile citrate tubes. After centrifugation of the samples at 1000 g for 15 min, the plasma obtained was stored at -80°C until assay of the KCNH2, KCNH6 and KCNH7 levels. KCNH2, KCNH6 and KCNH7 levels were measured with enzyme-linked immunosorbent assay kits (Biont, catalogue numbers: YLA4243HU, YLA4242HU and YLA4244HU, respectively) according to the manufacturer's instructions (Shanghai YL Biotech Co., Ltd, Shanghai, China).

STRING v 11 analysis of KCNH2, KCNH6 and KCNH7 proteins

STRING available online at (https://string-db.org/) has been used to interpret the interaction of KCNH2, KCNH6 and KCNH7 proteins with the proteins of Homo sapiens as organism involved in the biological activity.

Statistical analysis

The statistical assessments were performed using SPSS vn. 22 software. The normal distribution of data was checked using the Kolmogorov-Smirnov test. Data showing normal distribution were compared with the Student's t-test, and data not showing normal distribution with the Mann-Whitney U-test and Spearman's correlation test. A Chi-square test was applied in the evaluations of categorical data. Continuous data were stated as mean/median ± standard deviation (SD) values, and categorical variables as number (n) and percentage (%). The level of statistical significance was considered as $p<0.05$. Taking the 3.6% prevalence rate of FM in Turkey (19) and the total population of the province into consideration, the study included 76 patients to provide a study power of 90.54% in a 95% confidence interval.

Results and discussion

The FM patient group and control group were similar in respect of age and gender. The demographic data are shown in Table 1. In the comparison between the FM patients and the control group in terms of plasma levels of KCNH2, KCNH6, and KCNH7 proteins, the KCNH2 protein levels in the FM group were determined to be significantly lower than in the control group ($p<0.001$). No statistically significant difference was observed between the groups in respect of plasma levels of KCNH6 and KCNH7 proteins ($p>0.05$) (Table 2). The plasma levels of KCNH2, KCNH6 and KCNH7 proteins were not seen to be statistically significantly correlated with the VAS scores of the patients with FM ($p=0.061$, $p=0.153$, $p=0.146$, respectively) (r=0.179, r=0.119 and r=0.167, respectively).

Results of protein-protein interaction analysis

In this study, we determined the protein levels of KCNH2, KCNH6 and KCNH7 from the Potassium voltage-gated channel subfamily in FM patients and controls by the ELISA method. We performed STRING network analysis to determine the functional interactions of these proteins in cellular processes. Protein-protein interactions of each KCNH2, KCNH6 and KCNH7 protein were examined separately. KCNH2 is associated with Potassium and Sodium voltage-gated channel subfamily as expected, and also with Heat shock protein HSP 90-alpha (HSP90AA1), Heat shock protein family A member 4 (HSPA4), Potassium voltage-gated channel subfamily E member 2 (KCNE1) and Vascular endothelial growth factor receptor 1 (VEGFR1, FLT1) proteins (Figure 1). KCNH6 is associated with the Potassium voltage-gated channel subfamily, as well as SIGMAR1 and EBP; protein-protein interactions are common (Figure 1). KCNH7 is related to the Potassium voltage-gated channel subfamily and Grancalcin (GCA). In the study, Multiple Proteins STRING network analysis was performed together with KCNH2, KCNH6 and KCNH7 proteins from the Potassium voltage-gated channel subfamily. Protein-protein interactions with 13 proteins in the first shell (KCNE2, KCNE1, KCNQ1, HSP90AA1, SCN5A, FLT1, KCND3, KCNA5, KCNJ2, SIGMAR1, KCNH2, KCNH6, KCNQ1) were in the range of 0.999-0.800 homology score. At the second shell level, it was observed that it had protein-protein interactions with 31 proteins. (Figure 1) (Table 3).

Table 1. Comparison of the demographic data of the groups

	Controls (n=79) n (%)	Patients (n=76) n (%)	*p*-Value
Gender			
Female	74 (94)	69 (91)	0.510
Male	5 (6)	7 (9)	0.470
Age (years)			
Mean±SD	42.9±5.8	44.9±9.2	0.073
BMI (kg/m^2)	25.1±3.89	25.6±4.24	0.497

BMI: Body mass index; SD: standard deviation; n: Number of patients

Table 2. Comparison of plasma KCNH2, KCNH6 and KCNH7 protein levels between controls and patients

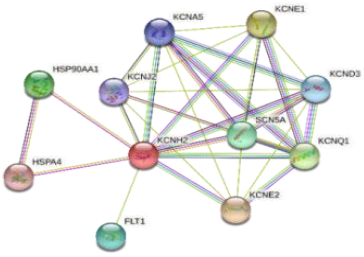
	Controls(n=79)	Patients(n=76)	*p*-Value
KCNH2 (pg/ml)	100 (90-115)	58.89 (47.1-83.5)	0.001*
KCNH6 (pg/ml)	40 (32.4-49.8)	34.4 (29.3-51.1)	0.126
KCNH7 (pg/ml)	86 (69.6-98.4)	86.5 (70.7-113.7)	0.367

*P<0.05 value was regarded as significant; Results are given median 1st and 3rd quartiles; n: Number of patients. Mann-Whitney u test was used.

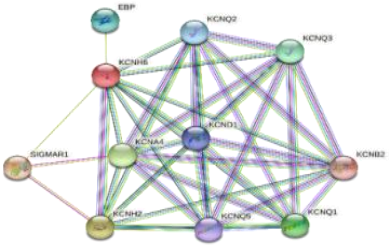
Table 3. Predicted Functional Proteins Associated with KCNH2, KCNH6 and KCNH7 Proteins.

Proteins	Proteins Associated	Predicted functional proteins	Homology score
KCNH2	KCNE2	Potassium voltage-gated channel subfamily E member 2	0.995
KCNH2	KCNE1	Potassium voltage-gated channel subfamily E member 1	0.993
KCNH2	KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	0.987
KCNH2	HSP90AA1	Heat shock protein HSP 90-alpha	0.982
KCNH2	SCN5A	Sodium channel protein type 5 subunit alpha	0.960
KCNH2	FLT1	Vascular endothelial growth factor receptor 1	0.949
KCNH2	KCND3	Potassium voltage-gated channel subfamily D member 3	0.920
KCNH2	KCNA5	Sodium channel protein type 5 subunit alpha	0.903
KCNH2	KCNJ2	Inward rectifier potassium channel 2	0.883
KCNH6	SIGMAR1	Sigma non-opioid intracellular receptor 1	0.876
KCNH6	KCNH2	Potassium voltage-gated channel subfamily H member 2	0.861
KCNH6	KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	0.815
KCNH7	KCNQ1	Potassium voltage-gated channel subfamily KQT member 1	0.803

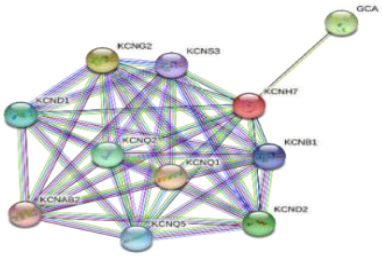
KCNH2



KCNH6



KCNH7



KCNH2, KCNH6 and KCNH7

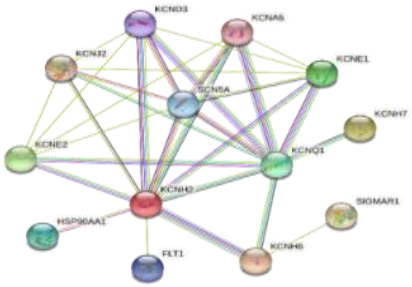


Figure 1. Individual interactions of each KCNH2, KCNH6 and KCNH7 protein are analyzed in the STRING database. The predicted functional proteins with KCNH2, KCNH6 and KCNH7 included KCNE2, KCNE1, KCNQ1, HSP90AA1, SCN5A, FLT1, KCND3, KCNA5, KCNJ2, SIGMAR1, KCNH2, KCNH6 and KCNQ1. Each line has features. [Red line-indicates the presence of gene fusions evidence; Green line- neighborhood evidence; Blue line-gene co-occurrence evidence; Purple line experimental evidence; Yellow line- textmining evidence; Light blue line- protein homology evidence; Black line- coexpression evidence.].

In the comparisons of KCNH2, KCNH6, and KCNH7 protein levels between the FM patient group and the healthy control group, the results of this study showed that the plasma level of KCNH2 protein was lower in the FM group compared to that of the healthy control group. However, the plasma levels of KCNH6

and KCNH7 proteins of patients with FM were similar to those of healthy individuals. In addition, no correlation was determined between plasma KCNH2, KCNH6, and KCNH7 protein levels and the VAS scores of patients with FM. To the best of our knowledge, this is the first study in the literature to have presented these results.

Potassium channels are one of the major ion channel families. In mammals, there are known to be four different K^+ ion channels based on their structural and functional characteristics. Kv is one of the best-documented channels. K^+ channels participate in numerous cellular functions (20). As Kv channels function in many various cellular events, there is an increasing number of studies that have investigated the function of the K^+ ion channel in different disease groups, primarily cancer and cardiac diseases (21, 22). In addition, the role of Kv channels in the pathogenesis of pain has been demonstrated in some previous clinical studies. K^+ channels repolarise neuronal membranes, limit the formation and firing rate of the action potential, and thereby prevent nerve excitability. As the K^+ channels are distributed over the nociceptive neuronal membrane, reduced K^+ channel transmission can cause over-sensitivity to pain.[13] It has been suggested that the reduced K^+ channels function in the nociceptive pathway could be responsible for several types of pain (23). Ishikawa et al. (24) reported that changes occurred in the expression of the Kv channels in the dorsal root ganglia after axotomy. Similarly, a reduction has been shown in the expression of Kv channel mRNA after a peripheral nerve lesion (25). In another study by Takeda et al. (26), the reduction in the expression of Kv channel was considered to be responsible for allodynia in patients with temporomandibular joint pathology. Likewise, another study has shown that the elimination of Kv channels may have a role in the development of pain (27). In addition to the above-mentioned studies, some studies in the literature have shown that K^+ channel modulators/openers may have a good analgesic effect (17). In particular, the positive effect on pain of retigabine as a Kv channel activator and its structural analogue, flupirtine, has been shown. There are also case reports that have shown the positive effect of flupirtine in patients with myofascial pain syndrome and FM (28,29). In the current study, lower plasma levels of KCNH2 were also determined in the patients with FM compared to the control group. The results of this study support the previous studies in the literature that have investigated the association between K^+ ion channels and pain. While KCNH2 is widely expressed in the central nervous system, including the brain and ganglions, KCNH6 and KCNH7 are expressed in specific areas of the central nervous system (11). Therefore, it can be considered that the synthesisation of KCNH2 in FM patients could be affected more than KCNH6 and KCNH7.

As a result of the STRING analysis performed within the scope of the study, we determined that the KCNH2, KCNH6 and KCNH7 proteins have protein-protein interactions in the range of 0.999-0.800 homology score with 13 different proteins. Among these proteins, KCNH2 and proteins with homology scores of 0.982 HSP90AA1 and 0.949 FLT1 (VEGFR-1) were of great importance for us. Previously, Karadağ et al. found significantly higher serum FLT1 (VEGFR-1) levels in FM patients in a study they conducted (1). In another study, high FLT1 (VEGFR-1) levels were shown in some inflammatory rheumatic diseases that cause damage to the vascular system. In addition, high FLT1 (VEGFR-1) levels are thought to induce the functioning and release of macrophages, which play a role in vasculitis and angiopathy. (30,31). The HSP90AA1 protein is a protein found in all cells. It acts as a molecular chaperone, also aids in the proper folding of several target proteins and is known to help maintain protein structures and regulate cell death pathways during cellular stress (32). In addition, the importance of the HSP90AA1 protein in inflammation and pain signaling is known (33). In a study conducted with FM patients, extremely high gene expressions and protein levels of HSP90AA1 were detected (34). In this context, we can say that KCNH2, which has a high homology score with HSP90AA1 and FLT1 (VEGFR-1), may be a biomarker in FM patients. In addition, these three proteins and 11 other proteins with high homology scores can be evaluated in FM patients. Limitations of this study can be considered to be the relatively small number of male FM patients and male healthy subjects, the relationship between disease duration and Kv ion channel proteins and the levels of other Kv ion channel proteins that could not be evaluated.

Conclusions

Plasma levels of KCNH2 were observed to be lower in the FM patients compared to the healthy control group. However, the plasma levels of KCNH6 and KCNH7 proteins of the FM patients were not different from those of the healthy control group. The results of this study may contribute to the clarification of the pathogenesis of FM and may be of guidance for future studies in the investigation of the treatment and/or pathogenesis of FM. There is also a need for more comprehensive studies to evaluate the levels of the KCNH family and other Kv channel functions in FM.

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Interest conflict

None to report.

Author's contribution

A.T supervised the project. A.K and E.H provided patient and control samples. A.T, C.Z, E.O, and S.A designed and performed the experiments. Y.S STRING analysis and interpretation of data. The article was written with the support of A.T, C.Z, A.K, E.H, E.O, S.A, and Y.S.

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