Association between endothelin-1 and fibromyalgia syndrome

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Received March 9, 2017; Accepted August 1, 2017

DOI: 10.3892/mmr.2017.7395

Abstract. Fibromyalgia syndrome (FMS) is characterized by widespread chronic musculoskeletal pain, stiffness and pressure hyperalgesia at soft tissue tender points. Patients with FMS may exhibit a tendency towards cold extremities and cold-induced vasospasm. Endothelin-1 (EDN1) is a potent vasoconstrictor that is mainly produced by endothelial cells. The present study aimed to determine whether plasma expression levels avvnd single-nucleotide polymorphism (SNP; rs1800541) of the *EDN1* gene were associated with FMS and/or any of its clinical variables. Plasma EDN1 levels were assessed by ELISA, and SNP genotypes were determined using polymerase chain reaction-high-resolution melting curve analysis. Patients with the TG genotype and the G allele may have an elevated risk of FMS. In addition, patients with FMS with the TG genotype and/or T allele exhibited higher plasma EDN1 levels compared with healthy controls. EDN1 levels increased significantly in patients with FMS compared with normal controls. In addition, *EDN1* SNP was found to be associated with susceptibility to FMS.

Introduction

Fibromyalgia syndrome (FMS) is a complex disorder that is characterized by chronic widespread pain and muscle tenderness, and may be accompanied by disturbances in sleep, fatigue, anxiety and other clinical manifestations, such as depression, gastrointestinal symptoms and headache (1). FMS affects ≥2% of the adult population, with females significantly more affected than males (2). The etiology of FMS varies among subjects, but may include neurogenic inflammation caused by allergen-induced inflammatory response, bacterial or viral infections, irritants or chemical exposure, as well as oxidative and emotional stressors (3,4). Inflammation often includes increased levels of cytokines, neuropeptides, growth factors and neurotransmitters; however, many of these are abnormal in patients with FMS (5-7).

Although progress has been made in understanding the disease mechanism of FMS, its pathophysiology has not been clearly established. Genetic factors may influence susceptibility to FMS, but no specific gene has been identified. As FMS often occurs in several family members, there may be a genetic component. Several previous studies have reported candidate FMS polymorphisms, such as in the serotoninergic system genotype (8), the catechol-O-methyltransferase gene (9) and the D4 dopamine receptor (10). However, many of the previously identified associations have been weak or inconsistent.

Endothelin 1 (EDN1) is a peptide produced by endothelial and vascular smooth muscle cells and is a potent vasoconstrictor (11). Owing to the vasoconstrictive and hypertrophic actions on blood vessels, EDN1 has been linked to the development of hypertension (12). The human *EDN1* gene is 5.5 kb in length, with 5 exons and 4 introns (13). Patients with FMS express high levels of EDN1 (14,15), have a high prevalence of insulin resistance (16) and may have increased body fat for a given weight (17). These data suggested that the EDN system may be activated in these patients, and the associations between *EDN1* polymorphisms and EDN1 levels with the development of FMS may be more prominent compared with those in the general population. The prevalence of the *EDN1* SNP rs1800541 and its association with EDN1 levels in patients with FMS has not yet been investigated. The T1370G single-nucleotide polymorphism (SNP; rs1800541) is in the *EDN1* promoter region and may affect to EDN1 expression levels, which may be a potential intermediate hypertension phenotype (18).

The present study examined whether the rs1800541 SNP occurs more frequently in patients with FMS than in the local general population without FMS, and whether plasma EDN1 levels may be associated with susceptibility to FMS or the clinical variables.

Materials and methods

Subjects and clinical assessment. This study included a total of 88 patients with FMS (83 female and five male; age,

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Key words: endothelin-1, plasma level, single-nucleotide polymorphism, fibromyalgia syndrome

48.02±11.30 years; weight, 58.63 ± 0.99 kg; mean ± standard error of the mean) and 87 healthy controls (all female; age, 40.87±6.21 years; weight, 57.17±0.84 kg) without a history of FMS or chronic widespread pain (19). Collected data also included height and weight measurements that were used to assess body mass index (BMI). Biospecimens used by the present study were provided by the Biobank of the College of Medicine, Soonchunhyang University (Cheonan, Korea). This study was approved by the Ethics Review Committee of the Biobank of the College of Medicine, Soonchunhyang University (SCHIRB-BIO-150006); written informed consent was received from all patients prior to the study.

Clinical assessment. The presence of tender points was assessed according to the standardized manual tender point survey (20). The number of tender points was counted at 18 specific sites on the body, and the intensity of each tender point was assessed as follows: 0, no tenderness; 1, light tenderness (confirmed answer when asked); 2, moderate tenderness (spontaneous verbal response); and 3, severe tenderness (moving away). Therefore, the possible number of tender points ranged between 0 and 18, and the possible total score ranged between 0 and 54. Clinical disease activity and severity of FMS were assessed using various tools to diagnose FMS. The Korean version of the Fibromyalgia Impact Questionnaire (FIQ) was used to assess functional abilities (21); the Brief Fatigue Inventory (BFI) was used to assess fatigue severity (22); the Beck Depression Inventory (BDI) was used to assess depression severity (23); the Medical Outcomes Study 36-item Short-Form Health Survey, which comprises eight items, including physical health (physical functioning, role-physical, bodily pain and general health) and mental health (vitality, social functioning, role-emotional and mental health) (24), was used to assess quality of life; and the State-Trait Anxiety Inventory (STAI)-1 and STAI-2 was used to assess anxiety (Tables I and II) (25).

Measurement of plasma EDN1 levels. Plasma was extracted from fresh whole blood samples with EDTA. The blood was centrifuged at 13,200 x g for 10 min and stored at -80˚C. Plasma EDN1 levels were determined using an Endothelin ELISA kit (cat no. 583151; Cayman Chemical, Ann Arbor, MI, USA) with 50 μl plasma from each subject, according to the manufacturer's protocol.

Genotyping. DNA was extracted from fresh whole-blood samples (300 μl) using a DNA purification kit (Nanohelix Co., Ltd., Seoul, Korea), according to the manufacturer's protocol. SNPs were identified by polymerase chain reaction-high-resolution melting (PCR-HRM) curve analysis and the SensiFAST HRM Kit (Bioline, Taunton, MA, USA) as previously described (26). The *EDN1* gene primers were: Forward, 5'-CAGAATGACCCGGTGACACT-3' and reverse, 5'-CATTGGCTTTTTCCGCTAGT-3'. Cycling conditions were as follows: Activation of polymerase at 95˚C for 2 min; followed by 43 cycles of 95˚C for 5 sec, 60˚C for 10 sec and 72˚C for 15 sec; and the HRM step at 95˚C for 15 sec; 55˚C for 15 sec; and 95˚C for 5 sec.

Statistical analysis. Hardy-Weinberg equilibrium (HWE) was assessed using SNP Stats (http://bioinfo.iconcologia.net/index.php) and SPSS 18.0 software (SPSS Inc., Chicago, IL, USA). Associations between the SNP and patients with FMS were estimated by computing odds ratios (ORs) and 95% confidence intervals (CIs) by logistic regression analyses with age and sex controlled as covariates. Models assuming co-dominant (where relative disease hazard differs between subjects with one minor allele and those with two minor alleles), dominant (subjects with one or two minor alleles demonstrate the same relative hazard), recessive (individuals with two minor alleles are at increased risk of the disease), or over-dominant inheritance (assumes the heterozygote has the strongest impact and compares major alleles/major alleles + minor alleles/minor allele vs. major alleles/minor allele) were used in the logistic regression analysis for the SNP. Genotypic and allelic frequencies of the SNP were compared between the patients with FMS and controls using the χ^2 test for the case-control association study. The difference between patients with FMS and controls was adjusted for age and sex as covariables. P<0.05 was considered to indicate a statistically significant difference.

Table I. Clinical features of FMS and control groups.

	Control (n=87)		FMS (n=88)		
Parameter	Mean	SE	Mean	SE	P-value
Age	40.87	0.67	48.02	1.20	N/A
Male, n (%)	0 (0.00)		5 (5.68)		N/A
Female, n (%)	87 (100.00)		83 (94.32)		
FIQ	0.00	0.00	59.03	1.98	<0.001
BFI	23.36	1.66	51.92	2.14	<0.001
BDI	27.38	0.61	40.05	1.18	<0.001
PCS	76.44	1.44	45.42	0.19	<0.001
MCS	75.22	1.49	50.33	2.31	<0.001
STAI1	44.21	0.70	42.45	0.71	0.081
STAI2	45.24	0.69	49.31	0.74	<0.001

FMS, fibromyalgia syndrome; N/A, not applicable; FIQ, fibromyalgia impact questionnaire; BFI, brief fatigue inventory; BDI, Beck depression inventory; PCS, physical component summary; MCS, mental component summary; STAI, State-Trait Anxiety Inventory.

Results

The relationship between the SNP and the clinical features of patients with FMS was assessed, including number of tender points, FIQ, BFI, BDI, STAI-I and STAI-II scores (Tables I and II). Most of the parameters were significantly different between the control and FMS group (Table I). None of the parameters assessed in the patients with FMS differed among the SNP genotypes or alleles (Table II).

The *EDN1* SNP T1370G genotype was detected successfully by PCR-HRM analysis in all subjects (n=175, 100%). Distribution of the *EDN1* T1370G genotype was consistent with HWE in the control and FMS subjects (P>0.05). In total, 88 patients with FMS (age, 48.02±11.30 years) were genotyped, of whom 83 (94.3%) were female. A total of 87 healthy control

Table II. Clinical assessments by genotype and allele in patients with FMS.

	FMS genotype/allele				Genotype/allele		
Assessment	TT	TG	GG	P-value	T	G	P-value
n	35	40	13		110	66	
FIQ	61.06±2.85	56.80±3.26	60.42±4.75	0.593	59.51±1.74	58.23±2.36	0.663
BFI	55.29±3.25	49.80±3.12	49.38±6.55	0.447	53.29±1.85	49.64±2.58	0.252
BDI	39.03±1.77	40.68±1.70	40.85±3.87	0.785	39.63±1.00	40.74±1.46	0.531
PCS	45.52±2.72	46.90±2.97	40.64±5.86	0.559	46.02±1.62	44.43±2.42	0.586
MCS	47.95±3.44	52.83±3.29	49.05±7.77	0.612	49.72±1.95	51.34±2.89	0.644
STAI1	43.31±1.11	42.80±1.05	39.08±1.79	0.133	43.13±0.62	41.33±0.83	0.086
STAI2	48.47±1.11	50.23±1.10	48.69±2.26	0.526	49.12±0.64	49.62±0.90	0.652
Tender points	17.34±0.22	16.60±0.40	17.62±0.21	0.139	17.00±0.26	17.07±0.18	0.817
Total score	29.77±1.19	27.70±1.26	27.85±1.52	0.442	27.76±0.86	29.02±0.70	0.259

Data are presented as the mean ± standard error of the mean; unclear or missing data have been excluded. FMS, fibromyalgia syndrome; FIQ, fibromyalgia impact questionnaire; BFI, brief fatigue inventory; BDI, Beck depression inventory; PCS, physical component summary; MCS, mental component summary; STAI, State-Trait Anxiety Inventory.

Table III. Genotype and allele frequencies of endothelin 1 single-nucleotide polymorphisms in healthy control patients and patients with fibromyalgia syndrome.

	Control (n=87)		FMS (n=88)				
Genotype/allele	Freq.	%	Freq.	%	Model	OR (95% CI)	P-value
TT	55	63.20	35	39.80	Co-dominant	3.14 (1.59-6.23)	0.004
TG	20	23.00	40	45.50		1.70 (0.70-4.15)	
GG	12	13.80	13	14.80	Dominant	2.60 (1.41-4.79)	0.002
					Recessive	1.08 (0.46-2.53)	0.850
					Overdominant	2.79 (1.45-5.36)	0.002
T	130	0.75	110	0.62			
G	44	0.25	66	0.38		0.56 (0.36-0.89)	0.014

Freq, frequency; OR, odds ratio; CI, confidence intervals.

patients were genotyped (age, 40.87±6.21 years), which were significantly younger compared with the patients with FMS, and which were all female; therefore, all results were adjusted for age and sex. The average body weight in the patients with FMS was 58.63±0.99 kg, and in the control group was 57.17±0.84 kg, which was not significantly different (P=0.260). The average BMI of the subjects was 23.33±0.36 (FMS) and 22.17±0.43 (control), which was slightly different (P=0.040); however, the BMI score was not identified as related to FMS genotype and plasma EDN1 levels.

Of the patients with FMS, 35 out of 88 (39.80%) had the TT genotype, 40 (45.50%) had TG and 13 (14.80%) had the GG genotype of the *EDN1* SNP. TG and GG genotype frequencies were 23.00 and 13.80% in the control group and 45.50 and 14.80% in the FMS group for the co-dominant model (OR, 3.14 and 1.70; 95% CI, 1.59-6.23 and 0.70-4.15, respectively; P=0.004; Table III), respectively. The GG genotype was associated with an increased risk for FMS. FMS susceptibility was significantly associated in the over-dominant model (TG + GG vs. TT; OR, 2.79; 95% CI, 1.45-5.36; P=0.002; Table III), indicating that absence of the G allele (TT) decreased the risk for FMS compared with the presence of the G allele (GG or TG). Allelic frequency was also associated with susceptibility to FMS (OR, 0.56; 95% CI, 0.36-0.89; P=0.014; Table III). The T allele frequency was lower in the FMS group (62.0%) compared with in the control group (75.0%).

Association between EDN1 gene polymorphisms and plasma EDN1 levels in healthy control patients and patients with FMS. Plasma EDN1 levels increased significantly in patients with FMS compared with patients in the control group (mean ± standard error of the mean; 38.89±5.87 vs. 61.34±8.14 pg/ml; P=0.027; Fig. 1). Plasma EDN1 levels increased significantly in patients with the GG genotype (83.30±21.93 pg/ml) compared with those with the TG genotype (53.91±10.04 pg/ml) or TT genotype (38.50±3.56 pg/ml;

P=0.011; Fig. 2) in all patients. Plasma EDN1 levels in the G allele group (67.27±8.93 pg/ml) was relatively higher than that in the T allele group (42.35±3.15 pg/ml) among all patients (P<0.05; Fig. 3).

The relationship between plasma EDN1 level and EDN1 polymorphism was further compared within the patients. Plasma EDN1 levels in patients with FMS with the TG genotype were significantly higher compared with EDN1 levels in healthy control patients with the TG genotype (68.32±14.41 vs. 25.08±4.51 pg/ml; P=0.006; Fig. 4A). In addition, the level of T-carrier group (TT + TG) in FMS patients was significantly higher compared with healthy controls (57.45±8.05 vs. 31.87±3.85 pg/ml; P=0.002; Fig. 4B). It was also demonstrated that patients with FMS with the T allele exhibited higher levels of EDN1 in the plasma compared with healthy controls (53.50±5.70 vs. 32.91±3.05 pg/ml; P=0.002; Fig. 5). However, the levels of EDN1 expression were not significantly different between controls and FMS patients with G allele (P>0.05).

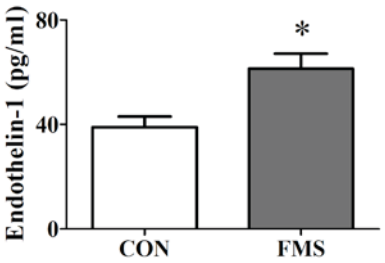

Figure 1. Comparison of plasma endothelin 1 levels between patients with FMS and healthy control patients. Plasma endothelin 1 levels in patients with FMS (n=88) compared with the levels in healthy control patients (n=87), as determined by ELISA. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. CON. CON, control; FMS, fibromyalgia syndrome.

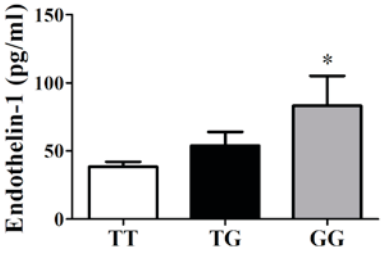

Figure 2. Plasma EDN1 expression levels in all patients containing TT, TG and GG of the *EDN1* gene. The expression levels containing TT (n=90), TG (n=60) and GG (n=25) of the *EDN1* gene, as determined by ELISA. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. TG or TT. EDN1, endothelin-1.

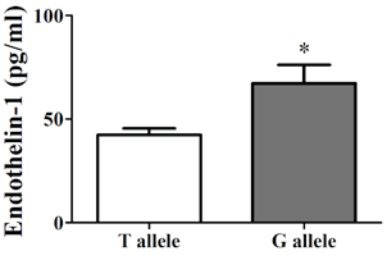

Figure 3. Plasma EDN1 levels in patients with FMS and controls containing the T (n=240) or G alleles (n=110) of the *EDN1* gene, as determined by ELISA. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. T allele. EDN1, endothelin-1; FMS, fibromyalgia syndrome.

Discussion

The present study investigated the association between plasma EDN1 expression levels and the EDN1 SNP in patients with FMS. Genotypic and allelic distributions of EDN1 SNP in patients with FMS were significantly different from those in the healthy control patient group, which suggested an association between EDN1 SNP and FMS. In addition, plasma EDN1 levels in patients with FMS were demonstrated to be higher than those of healthy controls.

Vascular endothelial cells are able to modulate local vascular tone by secreting relaxing factors, such as nitric oxide, and constrictive factors such as EDN1. Other than its direct vasoconstrictive effect, EDN1 may also increase the sensitivity of blood vessels to other circulating vasoconstrictive hormones, including noradrenaline, serotonin and angiotensin II (27). EDN1 expression was previously demonstrated to be increased in certain vascular beds, such as in the heart (28) and the kidney (29), following tissue ischemia. Increased EDN1 production has also been described in other vascular and rheumatologic diseases, including vasospastic syndrome, multiple sclerosis, giant cell arteries and systemic lupus erythematosus (30,31). It has been reported that patients with FMS have significantly higher levels of brachial-ankle pulse-wave velocity (baPWV) compared with healthy controls (32). BaPWV is correlated with disease severity assessed by FIQ (32). Another study reported that plasma EDN1 levels in German patients with FMS were significantly higher compared with those in healthy controls (n=21/group) (31). The present study analyzed plasma EDN1 expression levels and SNPs to validate these results in a relatively large Korean population (n=175 total), and demonstrated that plasma EDN1 expression levels were increased in patients with FMS. In addition, subjects with G allele had higher EDN1 levels compared with T allele. Previous studies suggested a possible effect of EDN1 in distinctive vascular cold-response of patients with FMS (33,34), in which repeated relative ischemia might increase EDN1 level. An elevated EDN1 level, in turn, may further enhance vasospasms.

Elevated tissue or plasma concentrations of EDN1 may occur in a variety of pathological states, such as metastasized prostate and breast cancer cells (35), following cutaneous injury (36). Plasma EDN1 levels may also increase following ischemic injury related to acute respiratory distress syndrome, sepsis and disseminated intravascular coagulation (37,38). EDN1 expression contributes to pain in many of these pathologies; inflammation leads to the release of substances that may excite or sensitize primary afferent nerve fibers and cause pain and/or hyperalgesia (39,40), and EDN1 has been reported to be significantly oversecreted in inflammatory conditions (41). EDN1 levels in synovial fluid are elevated in patients with inflammation-related diseases, such as rheumatoid arthritis (RA), osteoarthritis and gout. Plasma EDN1 levels in patients with active RA were demonstrated to be higher than the levels in patients with non-active RA, whereas EDN1-like immunoreactivity in synovial fluid was revealed to be several-fold higher than in plasma (42).

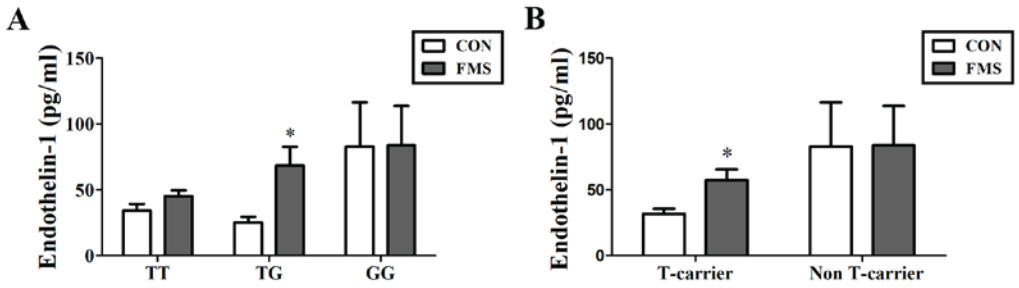

Figure 4. Plasma EDN1 levels and genotypes of *EDN1* in the healthy control patients and patients with FMS. (A) EDN1 levels in control with the genotypes TT (n=55), TG (n=20), and GG (n=12) and FMS patients with *EDN1* genotypes TT (n=35), TG (n=40), and GG (n=13) were determined by ELISA. (B) EDN1 levels in T-carrier control patients (TT + TG; n=130) and non-T-carrier controls (GG; n=44), and T-carrier patients with FMS (n=110) and non-T-carrier patients with FMS (N=66) were determined by ELISA. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. CON. CON, control; EDN1, endothelin-1; FMS, fibromyalgia syndrome.

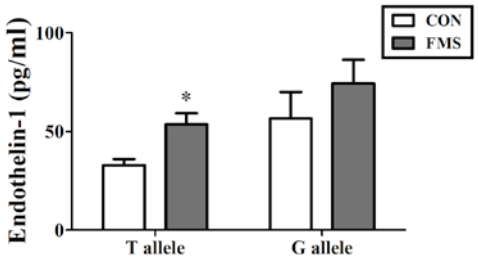

Figure 5. Plasma EDN1 levels and alleles of *EDN1* in healthy control patients and patients with FMS. Plasma levels of EDN1 in the control group with T allele (n=130) or G allele (n=44) and in FMS patients with T allele genotype (n=110) or G allele genotype (n=66) were determined by ELISA. Data are presented as the mean ± standard error of the mean. *P<0.05 vs. CON. CON, control; EDN1, endothelin-1; FMS, fibromyalgia syndrome.

Endothelial dysfunction may induce vascular inflammation by producing vasoconstricting agents, adhesion molecules and growth factors (43,44). Patients with cardiovascular disease exhibit increased tissue expression and plasma levels of inflammatory markers and mediators, including C-reactive protein (CRP) and adhesion molecules, such as intercellular adhesion molecule 1 (ICAM1), selectins and vascular cell adhesion molecule 1 (VCAM1) (45,46). In addition, patients with hypertension have been reported to exhibit increased plasma concentrations of tumor necrosis factor-α (a primary inflammatory cytokine), interleukin 6 (a secondary inflammatory cytokine), ICAM1, VCAM1, selectin E, von Willebrand factor and CRP (47). However, high concentrations of inflammatory mediators may be independent risk factors for the development of hypertension (48,49). Additional studies are required to clarify the relationship between elevated plasma EDN1 levels and the aforementioned markers of vascular inflammation in patients with FMS to draw definite conclusions.

Results from the present study suggested that EDN1 may have a potential effect on disease susceptibility in patients with FMS. However, whether high EDN1 plasma levels contributed to the etiology and how expression affected pain and psychiatric problems related to FMS remains unclear.

The present study, to the best of our knowledge, was the first to demonstrate that patients with the EDN1 TG genotype may have an elevated risk of FMS in the Korean population. In addition, patients with FMS with the EDN1 T allele exhibited significantly higher plasma EDN1 levels compared with healthy controls. The results revealed that patients with the TG genotype were more susceptible to FMS with increased plasma EDN1 levels. Functional analysis of EDN1 SNP rs1800541 in the future may help to clarify the potential biological mechanism of FMS.

In conclusion, plasma EDN1 levels were significantly increased in Korean patients with FMS compared with those in healthy controls. EDN1 SNP was revealed to be associated with susceptibility to FMS. The power of sample size was calculated using a genetic power calculator (http://zzz.bwh.harvard.edu/gpc). In this study, the genetic power was calculated to be 0.5322 for the EDN1 SNP (number of case: 88; control-to-case ratio: 0.988; number of cases for 80% power: 165); it was insufficiently powerful to determine a positive association. Owing to the relatively small number of subjects, these results should be validated by additional studies using larger sample sizes.

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