Association of Guanosine Triphosphate Cyclohydrolase 1 Gene Polymorphisms with Fibromyalgia Syndrome in a Korean Population

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ABSTRACT. ***Objective.*** Guanosine triphosphate cyclohydrolase 1 (GCH1) is the rate-limiting enzyme in the synthesis of tetrahydrobiopterin, which is an essential cofactor in nitric oxide (NO) production. Polymorphisms in the *GCH1* gene have been implicated in protection against pain sensitivity. The aim of our study was to determine whether single-nucleotide polymorphisms (SNP) in the *GCH1* gene affect susceptibility and/or pain sensitivity in fibromyalgia syndrome (FM).

Methods. A total of 409 patients with FM and 422 controls were enrolled. The alleles and genotypes at 4 positions [rs3783641(T>A), rs841(C>T), rs752688(C>T), and rs4411417(T>C)] in the *GCH1* gene were analyzed. The associations of the *GCH1* SNP with susceptibility and clinical measures in patients with FM were assessed.

Results. The frequencies of alleles and genotypes of the 4 SNP did not differ between patients with FM and healthy controls. Among 13 constructed haplotypes, we further examined 4 (CCTT, TTCT, TTCA, and CCTA) with > 1% frequency in both FM and controls. No associations of *GCH1* polymorphisms with FM-related activity or severity indexes were found, although the number and total score of tender points in patients with FM differed among the 4 haplotypes ($p = 0.03$ and $p = 0.01$, respectively). The CCTA haplotype of *GCH1* was associated with significantly lower pain sensitivity and occurred less frequently than the CCTT haplotype in patients with FM ($p = 0.04$, OR 0.45, 95% CI 0.21–0.96).

Conclusion. Our study provides evidence that certain *GCH1* haplotypes may be protective against susceptibility and pain sensitivity in FM. Our data suggest that NO is responsible for pain sensitivity in the pathogenesis of FM. (First Release Jan 15 2013; J Rheumatol 2013;40:316–22; doi:10.3899/jrheum.120929)

Key Indexing Terms:
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In the synthesis of pain modulators, including nitric oxide (NO) from arginine, serotonin from tryptophan, and biogenic amines from tyrosine[1], 6(R)-t-erythro-5,6,7, 8-tetrahydrobiopterin (BH4) is an essential cofactor. Excess production of BH4 is closely related to increased pain sensitivity[2]. The upregulation of 2 of 3 enzymes for BH4 synthesis in the dorsal root ganglion following sciatic nerve injury[3] clearly implicates BH4 in pain sensitivity.

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Guanosine triphosphate cyclohydrolase (GCH1) is the rate-limiting enzyme in BH4 synthesis and hydrolyzes guanosine triphosphate to form 7,8-dihydroneopterin triphosphate. Variations in the *GCH1* gene are closely associated with pain sensitivity. Tegeder, *et al* demonstrated a reduced pain score following discectomy for radicular back pain in patients with certain GCH1 haplotypes, and showed that increased pain sensitivity due to excess BH4 was dependent on enhanced NO production[2]. Thus, changes in GCH1 enzyme activity can lead to higher BH4 levels and increased NO production, which enhances pain sensitivity. Subsequent studies using diverse stimuli in humans have verified the pain-protective role of specific *GCH1* gene polymorphisms[4,5]. However, Kim and Dionne did not find a relationship between *GCH1* genetic variations and pain sensitivity or analgesic responses in healthy volunteers[6]. In addition, there was no significant effect of *GCH1* gene polymorphism on pain pattern or sensitivity in chronic pancreatitis[7] or chronic widespread pain[8].

Fibromyalgia syndrome (FM) is a complicated disorder characterized by chronic widespread pain, increased tenderness in specific body regions, fatigue, sleep disturbances, cognitive dysfunction, and mood disturbances[9]. Despite progress in understanding the disease mechanism underlying FM, its pathophysiology has not been clearly established. Genetic predisposition, disturbance of neurotransmitters such as serotonin and substance P, central sensitization, and oxidative stress are pathogenic candidates for abnormal pain processing in FM[10].

Oxidative stress and NO, a representative reactive oxygen species participating in diverse processes such as vascular dilatation, neurotransmission, and immune function[11,12], may be involved in the regulation of pain in the pathogenesis of FM. In the processing of pain, substance P and excitatory amino acids (EAA) released from presynaptic afferent terminals induce the activation of the NMDA receptor, which results in increased NO production by NO synthase (NOS), causing hyperexcitation of the dorsal horn[10]. NO was reported to be a potent signaling molecule in pain processing in patients with FM, because the tender point index was positively correlated with NO precursors and byproducts in cerebrospinal fluid (CSF)[13]. Another study demonstrated a significant relationship between serum NO levels and pain scores in patients with FM[14]. These data suggest that NO may be responsible for pain processing in the pathogenesis of FM.

Our working hypothesis was that gene polymorphisms affecting GCH1 activity are closely related to NO production and thereby alter pain sensitivity in FM. We investigated the association between *GCH1* gene polymorphisms and susceptibility and pain in patients with FM.

MATERIALS AND METHODS

Subjects. A total of 409 patients with FM (382 women, 27 men) with a mean age of 48.1 years (SD 10.9) were enrolled from outpatient rheumatic clinics of 10 medical centers that participated in the Korean FM survey. All patients at the time of the initial diagnosis met the classification criteria for FM proposed by the American College of Rheumatology in 1990[15]. Mean duration of symptoms was 8.5 years (SD 8.3), with a mean duration of 1.9 years (SD 3.0) after diagnosis. Medications used at the time of enrollment included selective serotonin reuptake inhibitors (SSRI), serotonin-norepinephrine reuptake inhibitors (SNRI), pregabalin, gabapentin, tricyclic antidepressants, nonsteroidal antiinflammatory drugs (NSAID), tramadol, acetaminophen, benzodiazepine, and muscle relaxants. Based on health surveys for chronic pain, we recruited 422 healthy controls (397 women, 25 men) with a mean age of 45.5 years (SD 12.5) and without a history of FM diagnosis or chronic widespread pain. The Institutional Review Board/Ethics Committee at each medical center approved the protocol for our study. All patients and controls gave informed consent at the time of recruitment.

Clinical assessment. We assessed the presence of tender points according to the standardized manual tender point survey[16]. The number of tender points was counted at 18 specific sites on the body, and the intensity at each tender point was assessed as follows: 0, no tenderness; 1, light tenderness (confirming answer when asked); 2, moderate tenderness (spontaneous verbal response); and 3, severe tenderness (moving away). Thus the possible numbers of tender points ranged from 0 to 18, and the possible total scores ranged from 0 to 54. Clinical markers for disease activity and severity of FM were assessed using the Korean version of the Fibromyalgia Impact Questionnaire (FIQ), for functional abilities assessment[17]; Brief Fatigue Inventory (BFI), for fatigue severity assessment[18]; Brief Depression Inventory (BDI), for depression severity assessment[19]; the 36-item Medical Outcomes Study Short-Form Health Survey (SF-36), comprising 8 items including physical health (physical functioning, role–physical, bodily pain, general health) and mental health (vitality, social functioning, role–emotional, mental health)[20] for quality-of-life assessment; and the State-Trait Anxiety Inventory (STAI)-1 and STAI-2, for anxiety assessment[21].

Genotyping of GCH1 gene polymorphisms. The assay reagents for detecting rs3783641(T>A), rs841(C>T), rs752688(C>T), and rs4411417(T>C) in the *GCH1* gene were designed by Applied Biosystems and included TaqMan MGB polymerase chain reaction (PCR) probes (FAM-labeled and VIC dye-labeled). A 10- l reaction was optimized with 0.125 l of 40× reagents, 5 l of 2× TaqMan Genotyping Master mix (Applied Biosystems), and 2 l (50 ng) of genomic DNA. PCR conditions were as follows: 95°C for 10 min, followed by 40 cycles at 95°C for 15 s and 60°C for 1 min. PCR was performed in an ABI Plus instrument (Applied Biosystems). The samples were read and analyzed using ABI software.

Statistical analysis. Genotype and haplotype frequencies of the *GCH1* SNP were compared between patients with FM and healthy controls using Pearson's chi-squared test. Logistic regression analysis was used to compute OR and 95% CI for the association of each *GCH1* genotype and haplotype with FM susceptibility risk, after adjustment for age and sex. The mean differences in the clinical measures of patients with FM across each of the *GCH1* marker genotypes and haplotypes were assessed by analysis of covariance, after adjustment for age and sex. Statistical power for genetic genotype-phenotype association was estimated as > 80% in case of 0.0125 of alpha (Type 1 error) using the Power for Genetic Association Analyses package. PHASE v2.1.1 software was used for combined allele analysis to construct haplotype structures and estimate their frequencies. The Bayesian method for haplotype reconstruction was described elsewhere[22,23]. Estimates of the sample haplotype frequencies, which can also be used as estimates of the population haplotype frequencies, were obtained from the frequency output file. By using the –c flag, we performed a permutation test for the null hypothesis that patients with FM and healthy controls are random draws from a common set of haplotype frequencies (no. permutations performed = 10,000). Statistical analyses were performed with IBM SPSS Statistics 19.0 (IBM Corp.). Statistical significance was evaluated with a 2-sided significance level of 0.05.

RESULTS

Differences in frequencies of alleles and genotypes for GCH1 polymorphisms. SNP genotyping was successfully performed in all enrolled subjects, except for 1 patient with rs4411417 and 1 patient with rs3783641. The genotype distributions of *GCH1* SNP were consistent with Hardy-Weinberg equilibrium in the controls (p = 0.19 for rs841; p = 0.33 for rs752688; p = 0.16 for rs4411417; p = 0.96 for rs3783641) and in the patients with FM (p = 0.10 for rs841; p = 0.41 for rs752688; p = 0.35 for rs4411417), except for 1 polymorphism (p = 0.02 for rs3783641). The frequencies of all alleles and genotypes for the 4 SNP, rs841(C>T), rs752688(C>T), rs4411417(T>C), and rs3783641(T>A), were similar between patients and controls (p > 0.05 for all; Table 1). In patients, the frequencies of the recessive alleles and heterozygote or homozygote genotypes with a recessive allele did not differ from those for the dominant alleles or wild genotypes of each SNP, after adjustment for age and sex.

Differences in haplotype frequencies for GCH1 polymorphisms. Using the PHASE program, we identified 13 haplotypes based on the SNP data from 408 patients and 421 controls. Table 2 lists 7 *GCH1* polymorphism haplotypes that have > 1% haplotype frequency in the total enrolled subjects. The differences in frequencies among the 7 haplotypes were significant by permutation analysis (p = 0.01).

We examined 4 of the haplotypes with > 1% frequency in patients and controls: CCTT, TTCT, TTCA, and CCTA (Table 3). None of these 4 showed a frequency difference between patients and controls (p = 0.11). However, in a logistic regression analysis adjusted for age and sex, the frequency of the CCTA haplotype was less than that of the CCTT haplotype in patients with FM (p = 0.04, OR 0.45, 95% CI 0.21–0.96). This suggests that the *GCH1*

Table 2. Estimates of haplotype frequencies in patients with fibromyalgia syndrome (FM; n = 408) and healthy controls (n = 421). Statistical package PHASE v2.1.1 was used to construct haplotype structures and estimate frequencies; missing data were excluded (n = 4). Data are percentage ± SE.

Combined Alleles†	All Subjects	Healthy Controls	Patients with FM	p††
CCTT	53.1 ± 0.3	53.8 ± 0.3	52.3 ± 0.3	0.01
TTCT	25.6 ± 0.3	27.3 ± 0.4	23.9 ± 0.3	
TTCA	12.3 ± 0.3	11.8 ± 0.4	12.7 ± 0.3	
CCTA	2.7 ± 0.2	3.4 ± 0.3	1.9 ± 0.2	
CTCT	2.0 ± 0.1	0.2 ± 0.1	4.0 ± 0.1	
TCTT	1.4 ± 0.1	0.9 ± 0.2	1.9 ± 0.2	
CTTT	1.2 + 0.1	1.3 ± 0.1	1.1 ± 0.1	
TCTA	0.6 ± 0.1	0.3 ± 0.2	0.8 ± 0.2	
CCCT	0.4 ± 0.06	0.1 ± 0.03	0.6 ± 0.1	
TTTA	0.3 ± 0.05	0.5 ± 0.09	0.0 ± 0.02	
TTTT	0.2 ± 0.05	0.1 ± 0.08	0.4 ± 0.05	
TCCT	0.1 ± 0.02	0.0 ± 0.02	0.3 ± 0.03	
CTCA	0.1 ± 0.01	0.1 ± 0.01	0.0 ± 0.01	

Among 13 constructed haplotypes, 7 haplotypes with ≥ 1.0% frequency are presented. † Combined alleles are in their physical order along the chromosome: rs841, rs752688, rs4411417, rs3783641. †† p value for permutation test of the null hypothesis that cases and controls are random draws from a common set of haplotype frequencies (no. permutations = 10,000).

Table 1. Genotype and allele analyses of guanosine triphosphate cyclohydrolase 1 single-nucleotide polymorphism in patients with fibromyalgia syndrome (FM; n = 409) and healthy controls (n = 422). Logistic regression models were used to calculate OR.

Position*	Genotype/Allele	Healthy Controls† n (%)	Patients with FM† n (%)	p††	Crude OR (95% CI)	Age- and Sex-adjusted OR (95% CI)
rs841 (C>T)	TT	77 (18.3)	73 (17.9)	0.89	1.00 (reference)	1.00 (reference)
	CT	191 (45.3)	180 (44.0)		0.93 (0.69–1.26)	0.93 (0.68–1.27)
	CC	154 (36.5)	156 (38.1)		0.94 (0.63–1.38)	0.89 (0.60–1.33)
	T	345 (40.9)	326 (39.9)	0.67	1.00 (reference)	1.00 (reference)
	C	499 (59.1)	492 (60.2)		0.96 (0.79–1.17)	0.94 (0.77–1.15)
rs752688 (C>T)	TT	77 (18.3)	76 (18.6)	0.97	1.00 (reference)	1.00 (reference)
	CT	195 (46.2)	191 (46.7)		1.04 (0.76–1.40)	1.00 (0.73–1.37)
	CC	150 (35.6)	142 (34.7)		1.04 (0.71–1.54)	0.97 (0.65–1.45)
	T	349 (41.4)	343 (41.9)	0.81	1.00 (reference)	1.00 (reference)
	C	495 (58.7)	475 (58.1)		1.02 (0.84–1.25)	0.99 (0.81–1.21)
rs4411417 (T>C)	CC	73 (17.3)	75 (18.4)	0.69	1.00 (reference)	1.00 (reference)
	TC	188 (44.6)	189 (46.3)		1.12 (0.83–1.52)	1.11 (0.82–1.51)
	TT	161 (38.2)	144 (35.3)		1.15 (0.78–1.70)	1.09 (0.73–1.63)
	C	334 (39.6)	339 (41.5)	0.41	1.00 (reference)	1.00 (reference)
	T	510 (60.4)	477 (58.5)		1.09 (0.89–1.32)	1.06 (0.87–1.29)
rs3783641 (T>A)	AA	11 (2.6)	16 (3.9)	0.26	1.00 (reference)	1.00 (reference)
	TA	115 (27.3)	95 (23.2)		0.82 (0.60–1.12)	0.80 (0.58–1.11)
	TT	295 (70.1)	298 (72.9)		1.44 (0.66–3.16)	1.65 (0.74–3.67)
	A	137 (16.3)	127 (15.5)	0.68	1.00 (reference)	1.0 (reference)
	T	705 (83.7)	691 (84.5)		0.95 (0.73–1.23)	0.96 (0.74–1.26)

* Calculated from the translation start site. † Missing data were excluded from the analyses (for rs4411417, n = 1; rs3783641, n = 1). †† Pearson's chi-squared test.

Table 3. Combined allele frequencies and OR in patients with fibromyalgia syndrome (FM) and healthy controls. Statistical package PHASE v2.1.1 was used to construct haplotype structures; missing data were excluded (n = 4). Among 13 haplotype structures, the frequencies of 4 major haplotype structures are presented; the total frequency of the other haplotype structures was 29 (3.4%) for controls and 74 (9.1%) for patients. Logistic regression models were used to calculate OR.

Combined Allele*	Healthy Controls n (%)	Patients with FM n (%)	p†	Crude OR (95% CI)	Age- and Sex-adjusted OR (95% CI)
CCTT	460 (54.6)	433 (53.1)	0.11	1.00 (reference)	1.00 (reference)
TTCT	223 (26.5)	187 (22.9)		0.89 (0.71–1.13)	0.88 (0.70–1.12)
TTCA	107 (12.7)	112 (13.7)		1.11 (0.83–1.50)	1.11 (0.82–1.49)
CCTA	23 (2.7)	10 (1.2)		0.46 (0.22–0.98)	0.45 (0.21–0.96)

* Combined alleles are in their physical order along the chromosome: rs841-rs752688-rs4411417-rs3783641.
† Calculated by Pearson's chi-square test.

polymorphism may be pain-protective in the development of FM.

Association between clinical measures and GCH1 polymorphism genotypes and haplotypes. None of the clinical measures assessed in the patients with FM, including FIQ, BFI, PCS, MCS, BDI, STAI-1, and STAI-2 scores, differed significantly among the genotypes or haplotypes of the *GCH1* SNP (Appendix 1 and 2). The frequencies of current medications used for FM treatment, including SSRI, SNRI, tricyclic antidepressants, NSAID, anticonvulsants, tramadol, acetaminophen, benzodiazepine, and muscle relaxants, were also similar among *GCH1* genotypes and haplotypes (data not shown). In addition, genotypes had no association with the number or total score of tender points, although the rs841 genotype showed a tendency toward a difference in the total score of tender points (p = 0.06; Table 4). The association of the 4 major haplotypes with numbers and total scores of tender points showed significant differences (p = 0.03 and p = 0.01, respectively; Table 5).

DISCUSSION

The pathogenesis of FM remains unclear. Nevertheless, possible genetic correlations with specific target molecules related to pain transmission, including components of the serotonergic, catecholaminergic, and dopaminergic pathways, have increased our understanding of the mechanisms of pain regulation in FM[24,25,26,27]. We investigated whether polymorphisms of the *GCH1* gene, a pain-protective gene involved in NO synthesis, could affect susceptibility and/or pain sensitivity in FM. The results of our study demonstrate that certain *GCH1* gene variations are associated with reduced susceptibility to FM and are closely related to tender points noted in the study population. These findings

Table 4. Least-squares means (95% CI) of numbers and total scores of tender points in patients with fibromyalgia syndrome (n = 325) by guanosine triphosphate cyclohydrolase 1 gene single-nucleotide polymorphism genotype.

Position*	Genotype	n†	No. Tender Points	Total Score of Tender Points
rs841 (C>T)	TT	62	14.0 (13.0–15.0)	28.5 (25.1–31.9)
	CT	144	14.4 (13.7–15.1)	28.2 (25.9–30.5)
	CC	119	13.4 (12.7–14.2)	24.5 (22.1–26.9)
p††			0.16	0.06
rs752688 (C>T)	TT	60	14.0 (13.0–15.0)	27.5 (24.1–31.0)
	CT	153	14.4 (13.7–15.0)	28.2 (26.0–30.4)
	CC	112	13.4 (12.7–14.2)	24.7 (22.2–27.2)
p††			0.16	0.11
rs4411417 (T>C)	CC	57	14.1 (13.1–15.1)	28.6 (25.0–32.1)
	TC	155	14.3 (13.7–14.9)	27.7 (25.5–29.8)
	TT	112	13.5 (12.8–14.3)	25.1 (22.6–27.6)
p††			0.28	0.20
rs3783641 (T>A)	AA	14	13.4 (11.3–15.5)	22.3 (15.0–29.6)
	TA	77	13.5 (12.6–14.4)	26.1 (23.0–29.2)
	TT	234	14.2 (13.6–14.7)	27.4 (25.6–29.1)
p††			0.39	0.35

* Calculated from the translation start site. † Missing data were excluded from the analyses: for no. tender points, n = 17; for total score of tender points, n = 17. †† p values from analysis of covariance adjusted for age and sex.

Table 5. Least-squares means (95% CI) of numbers and total scores of tender points in patients with fibromyalgia syndrome by 4 major haplotype structures. Statistical package PHASE v2.1.1 was used to construct haplotype structures; missing data were excluded (n = 2).

Combined Allele*	n†	No. Tender Points	Total Score of Tender Points
CCTT	347	13.9 (13.5–14.3)	26.5 (25.1–28.0)
TTCT	157	14.5 (13.9–15.2)	29.7 (27.6–31.9)
TTCA	93	13.7 (12.9–14.5)	26.3 (23.5–29.2)
CCTA	8	10.7 (7.9–13.5)	17.3 (8.0–26.6)
p††		0.03	0.01

* The combined alleles are in their physical order along the chromosome: rs841, rs752688, rs4411417, rs3783641. † Missing data were excluded from the analyses: for no. tender points, n = 34; for total score of tender points, n = 34. †† p values from analysis of covariance adjusted for age and sex.

implicate NO or its pathway in the regulation of pathogenesis and pain sensitivity in FM.

Potent pain-modulating molecules, including biogenic amines (serotonin and norepinephrine), substance P, and EAA neurotransmitters, influence abnormal pain perception and regulation in FM[13,28,29]. EAA promote NO synthesis, resulting in hyperalgesia in neuropathic pain[30,31]. NO may also be closely associated with pain modulation in FM[13,14]. In a previous report, the tender point index was positively correlated with the levels of the NO precursor arginine and the NO byproduct citrulline in the CSF of patients with FM, suggesting that NO may be a pain transmitter with pronociceptive potency in FM[13]. In another study, serum NO levels were significantly correlated with the visual analog pain score, although serum NO levels were similar between patients with FM and healthy controls[14]. However, data regarding a role of NO in the pathogenesis of FM are inconsistent. In 2 small groups of patients with FM, plasma or serum NO levels were lower than those in healthy controls[32,33]. After treatment of FM patients with 2 different antidepressants, serum NO levels were neither changed nor associated with changes in clinical measures[33]. Further research is needed to identify the role of NO in pain regulation in FM.

GCH1 is the rate-limiting enzyme in the synthesis of BH4, an important regulator of pain sensitivity, and is a cofactor in NOS activation, based on its role in the generation of NO from arginine[1,3]. Genetic variations in the *GCH1* gene were reported to be associated with reduced pain in nociceptive animal models[2]. Healthy volunteers with a pain-protective haplotype based on 15 SNP had significantly lower pain sensitivity[2]. The pain-protective effect of *GCH1* gene variations has been confirmed in subsequent studies[4,5]. One prospective observational study of patients undergoing surgery for lumbar degenerative disc disease showed a significantly improved back pain score and disability index in those with the T allele at rs998259 of the *GCH1* gene[34]. These studies suggest that specific SNP of GCH1 may be significantly associated with pain intensity in diverse clinical conditions. In contrast, investigations found no correlation between *GCH1* SNP and pain sensitivity in chronic pancreatitis[7] and chronic widespread pain[8]. In healthy volunteers, *GCH1* genetic variations contributed negligibly to pain sensitivity or analgesic responses[6]. In our study, there were no significant differences in the frequencies of the alleles or genotypes between patients with FM and healthy controls, based on 4 SNP of the *GCH1* gene: rs3783641(T>A), rs841(C>T), rs752688(C>T), and rs4411417(T>C). In addition, genotype was not associated with the number or total score of tender points. However, the frequency of a proposed pain-protective haplotype, CCTA, was lower than that of CCTT in patients with FM, and the CCTA haplotype was associated with a significantly lower number and total score of tender points. These findings implicate specific *GCH1* haplotypes in protection against susceptibility and pain in FM.

Genetic factors, particularly variations in neurotransmitter-related genes, may predispose individuals to FM. Offenbaecher, *et al* demonstrated a higher frequency of the S/S genotype of the serotonin transporter (5-HTT) promoter region in patients with FM compared with controls[24]. A close association between serotonin transporter gene polymorphism and FM was also reported in 2 distinct ethnic groups, Palestinian Arabs and Jewish Israelis[25]. However, the 5-HT2A receptor gene was not found to be involved in the pathogenesis of FM, despite the marked differential genotypic distribution of its T102C polymorphism between patients with FM and controls[35]. Among 3 catechol-O-methyltransferase polymorphisms (LL, LH, and HH), both LL and LH occurred more frequently in patients with FM than in controls[26]. In addition, Buskila, *et al* reported a significantly lower frequency for a 7-repeat allele in exon III of the dopamine D4 receptor in patients with FM and identified a dopamine D4 receptor polymorphism that was negatively associated with a novelty-seeking personality trait[27]. A close relationship between anxiety-related personality traits and 5-HTT polymorphisms has been identified[25], and FM patients with the S/S genotype of the 5-HTT gene showed higher levels of depression and psychological stress[24]. Thus, disturbances in serotonin, catecholamine, and dopamine expression or activity are likely to be involved in the pathogenesis of FM. Although there is evidence for NO acting as a neurotransmitter in FM, no data regarding FM and molecules required for the synthesis of NO have been reported[13,14].

Although FM has been considered a noninflammatory disease, some studies demonstrated that inflammatory cytokines might contribute to the presentation of clinical phenotypes in FM[36,37,38]. NO also plays a role in the regulation of inflammatory cascades, in addition to modulation of pain[39]. However, there has not been enough evidence about NO as an inflammatory marker in FM. Serum NO levels in FM were similar to those in healthy

controls in the previous Korean population, although interleukin 8 levels of patients with FM were significantly higher than those of controls[38]. It is possible that the role of NO in FM is limited in pain perception, irrespective of the inflammation mechanism in FM. NO is the metabolic byproduct of L-arginine, catalyzed by the NOS. Three isoforms of NOS, including neuronal (nNOS), inducible (iNOS), and endothelial (eNOS), have been identified. Upregulation of the genes encoding nNOS, but not iNOS and eNOS, was identified among BH4-dependent enzymes in the dorsal root ganglion after nerve injury[2]. This also suggests that NO might be mainly associated with pain modulation rather than inflammation in FM.

Our study, to our knowledge the first to investigate an association between FM and the *GCH1* gene, found that 4 GCH1 polymorphisms did not affect the clinical features of FM as assessed by the FIQ, BFI, PCS, MCS, BDI, STAI-1, and STAI-2, and did not affect medications for FM. However, the CCTA haplotype was associated with lower pain sensitivity in patients with FM. In contrast, pain sensitivity in patients with chronic widespread pain was not affected by *GCH1* polymorphisms at rs10483639, rs3783641, or rs8007267[8]. The apparent discrepancy between these study results may be attributable to differences in baseline characteristics of the study populations. Moreover, there were differences in the positions and numbers of the SNP studied.

NO may be a potent pain regulator in the pathogenesis of FM. GCH1 is the rate-limiting enzyme in the production of BH4, which is a cofactor for NO synthesis, and specific *GCH1* gene polymorphisms are related to decreased BH4 synthesis. In our study, the CCTA haplotype of *GCH1* was associated with lower pain sensitivity and occurred less frequently than the CCTT haplotype in patients with FM. These results suggest that *GCH1* polymorphisms might be associated with susceptibility and pain sensitivity in FM.

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REFERENCES

1. Thony B, Auerbach G, Blau N. Tetrahydrobiopterin biosynthesis, regeneration and functions. Biochem J 2000;347:1-16.
2. Tegeder I, Costigan M, Griffin RS, Abele A, Belfer I, Schmidt H, et al. GTP cyclohydrolase and tetrahydrobiopterin regulate pain sensitivity and persistence. Nat Med 2006;12:1269-77.
3. Costigan M, Befort K, Karchewski L, Griffin RS, D'Urso D, Allchorne A, et al. Replicate high-density rat genome oligonucleotide microarrays reveal hundreds of regulated genes in the dorsal root ganglion after peripheral nerve injury. BMC Neurosci 2002;3:16.
4. Tegeder I, Adolph J, Schmidt H, Woolf CJ, Geisslinger G, Lötsch J. Reduced hyperalgesia in homozygous carriers of a GTP cyclohydrolase 1 haplotype. Eur J Pain 2008;12:1069-77.
5. Campbell CM, Edwards RR, Carmona C, Uhart M, Wand G, Carteret A, et al. Polymorphisms in the GTP cyclohydrolase gene (GCH1) are associated with ratings of capsaicin pain. Pain 2009;141:114-8.
6. Kim H, Dionne RA. Lack of influence of GTP cyclohydrolase gene (GCH1) variations on pain sensitivity in humans. Mol Pain 2007;3:6.
7. Lazarev M, Lamb J, Barmada MM, Dai F, Anderson MA, Max MB, et al. Does the pain-protective GTP cyclohydrolase haplotype significantly alter the pattern or severity of pain in humans with chronic pancreatitis? Mol Pain 2008;4:58.
8. Holliday KL, Nicholl BI, Macfarlane GJ, Thomson W, Davies KA, McBeth J. Do genetic predictors of pain sensitivity associate with persistent widespread pain? Mol Pain 2009;5:56.

APPENDIX 1. Least-squares means (95% CI) of clinical assessments in patients with fibromyalgia syndrome (n = 325) by genotypes of guanosine triphosphate cyclohydrolase 1 single-nucleotide polymorphisms.

Position*	Genotype	n†	FIQ	BFI	PCS	MCS	BDI	STAI-1	STAI-2
rs841 (C>T)	TT	62	60.1 (55.4–64.0)	6.7 (5.0–8.4)	35.5 (33.6–37.4)	33.8 (30.8–36.8)	18.5 (15.8–21.2)	49.2 (46.1–52.3)	50.6 (47.8–53.5)
	CT	144	60.5 (57.4–63.6)	6.7 (5.6–7.8)	36.4 (35.2–37.7)	32.5 (30.5–34.4)	19.0 (17.3–20.8)	49.6 (47.6–51.7)	51.9 (50.0–53.8)
	CC	119	58.6 (55.2–62.0)	7.5 (6.2–8.7)	35.6 (34.2–36.9)	35.2 (33.0–37.3)	18.7 (16.7–20.6)	48.1 (45.9–50.3)	50.3 (48.2–52.3)
p††			0.70	0.62	0.59	0.20	0.93	0.58	0.50
rs752688 (C>T)	TT	60	57.1 (52.4–61.9)	7.7 (6.0–9.4)	36.1 (34.2–38.0)	33.1 (30.1–36.1)	18.1 (15.4–20.9)	48.8 (45.7–52.0)	50.9 (48.0–53.8)
	CT	153	61.3 (58.3–64.2)	6.3 (5.2–7.4)	36.2 (35.0–37.4)	33.1 (31.2–35.0)	18.8 (17.1–20.6)	49.5 (47.5–51.5)	51.5 (49.7–53.3)
	CC	112	59.0 (55.6–62.5)	7.5 (6.3–8.8)	35.5 (34.1–36.9)	34.9 (32.7–37.1)	19.1 (17.1–21.1)	48.4 (46.1–50.6)	50.5 (48.4–52.6)
p††			0.31	0.25	0.74	0.43	0.86	0.75	0.79
rs4411417 (T>C)	CC	57	57.2 (52.3–62.1)	6.7 (4.9–8.4)	35.8 (33.8–37.7)	33.7 (30.5–36.8)	17.3 (14.5–20.1)	48.2 (44.9–51.4)	50.0 (47.0–53.0)
	TC	155	61.2 (58.2–64.1)	6.7 (5.7–7.8)	36.3 (35.1–37.5)	32.8 (30.9–34.7)	19.0 (17.3–20.7)	49.6 (47.6–51.5)	51.7 (49.9–53.5)
	TT	112	59.0 (55.5–62.5)	7.5 (6.2–8.8)	35.6 (34.2–37.0)	35.0 (32.8–37.2)	19.3 (17.3–21.3)	48.6 (46.3–50.8)	50.7 (48.6–52.8)
p††			0.34	0.60	0.71	0.34	0.48	0.69	0.58
rs3783641 (T>A)	AA	14	59.7 (49.9–69.6)	8.7 (5.1–12.3)	36.1 (32.1–40.0)	34.5 (28.2–40.8)	20.5 (14.9–26.1)	49.0 (42.5–55.6)	51.4 (45.5–57.4)
	TA	77	59.7 (55.5–63.9)	6.6 (5.1–8.2)	36.6 (34.9–38.3)	33.3 (30.6–36.0)	17.8 (15.4–20.3)	47.8 (45.1–50.6)	50.9 (48.3–53.5)
	TT	234	59.7 (57.3–62.1)	7.0 (6.1–7.9)	35.7 (34.8–36.7)	33.8 (32.3–35.3)	19.0 (17.6–20.4)	49.3 (47.8–50.9)	51.1 (49.6–52.5)
p††			0.99	0.58	0.66	0.92	0.59	0.65	0.98

* Calculated from the translation start site. † Missing data were excluded from the analyses: for rs4411417, n = 1; for FIQ, n = 1; for BFI, n = 5; for BD-I, n = 5; for STAI-1, n = 8; for STAI-2, n = 6. †† p values from 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.

APPENDIX 2. Least-squares means (95% CI) of clinical assessments in patients with fibromyalgia syndrome by 4 major haplotype structures. Statistical package PHASE v2.1.1 was used to construct haplotype structures; missing data were excluded (n = 2).

Combined Allele*	n†	FIQ	BFI	PCS	MCS	BDI	STAI-1	STAI-2
CCTT	347	59.8 (58.0–61.7)	7.0 (6.3–7.7)	35.7 (34.9–36.5)	33.8 (32.6–35.1)	19.1 (18.0–20.2)	49.1 (47.8–50.4)	51.1 (49.9–52.3)
TTCT	157	59.7 (57.0–62.5)	6.7 (5.6–7.7)	36.0 (34.8–37.1)	32.7 (30.9–34.5)	18.6 (16.9–20.2)	50.2 (48.3–52.1)	51.3 (49.6–53.1)
TTCA	93	61.2 (57.5–64.8)	7.0 (5.6–8.3)	35.9 (34.4–37.4)	32.7 (30.3–35.1)	19.0 (16.8–21.1)	48.8 (46.3–51.3)	51.4 (49.2–53.7)
CCTA	8	61.6 (49.1–74.0)	11.8 (7.3–16.3)	38.0 (32.9–43.1)	35.4 (27.3–43.6)	18.6 (11.3–25.9)	46.2 (37.9–54.6)	47.0 (39.3–54.8)
p††		0.92	0.19	0.84	0.66	0.96	0.66	0.76

*Combined alleles are in their physical order along the chromosome: rs841, rs752688, rs4411417, rs3783641. † Missing data were excluded from the analyses: for FIQ, n = 2; for BFI, n = 10; for BDI, n = 10; for STAI-1, n = 16; for STAI-2, n = 12. †† p values from 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.

9. Bennett R. Fibromyalgia, chronic fatigue syndrome, and myofascial pain. Curr Opin Rheumatol 1998;10:95-103.
10. Bradley LA. Pathophysiology of fibromyalgia. Am J Med 2009;122:S22-30.
11. Kigwell BA. Nitric oxide-mediated metabolic regulation during exercise: Effect of training in health and cardiovascular disease. FASEB J 2000;14:1685–96.
12. Akyol O, Zoroglu SS, Armutcu F, Sahin S, Gurel A. Nitric oxide as a physiopathological factor in neuropsychiatric disorders. In Vivo 2004;18:377–90.
13. Larson AA, Giovengo SL, Russell IJ, Michalek JE. Changes in the concentrations of amino acids in the cerebrospinal fluid that correlate with pain in patients with fibromyalgia: Implications for nitric oxide pathways. Pain 2000;87:201-11.
14. Sendur OF, Turan Y, Tastaban E, Yenisey C, Serter M. Serum antioxidants and nitric oxide levels in fibromyalgia: A controlled study. Rheumatol Int 2009;29:629-33.
15. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DI, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia. Report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160-70.
16. 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.
17. Kim YA, Lee SS, Park K. Validation of a Korean version of the Fibromyalgia Impact Questionnaire. J Korean Med Sci 2002;17:220-4.
18. Mendoza TR, Wang XS, Cleeland CS, Morrissey M, Johnson BA, Wendt JK, et al. The rapid assessment of fatigue severity in cancer patients: Use of the Brief Fatigue Inventory. Cancer 1999; 85:1186-96.
19. Richter P, Werner J, Heerlein A, Kraus A, Sauer H. On the validity of the Beck Depression Inventory. Psychopathology 1998;31:160-8.
20. Ware JE, Sherbourne CD. The MOS 36-item short-form health survey (SF-36). I. Conceptual framework and item selection. Med Care 1992;30:473-83.
21. Kim JT, Shin DG. A standardization study of State-Trait Anxiety Inventory in Korea. N Med J 1978;21:1223-9.
22. Stephens M, Smith NJ, Donnelly P. A new method for haplotype reconstruction from population data. Am J Hum Genet 2001;68:978-89.
23. Stephens M, Donnelly P. A comparison of Bayesian methods for haplotype reconstruction from population genotype data. Am J Hum Genet 2003;73:1162-9.
24. Offenbaecher M, Bondy B, de Jonge S, Glatzeder K, Krüger M, Schoeps P, et al. Possible association of fibromyalgia with a polymorphism in the serotonin transporter gene regulatory region. Arthritis Rheum 1999;42:2482–8.
25. Cohen H, Buskila D, Neumann L, Ebstein RP. Confirmation of an association between fibromyalgia and serotonin transporter promoter region (5-HTTLPR) polymorphism, and relationship to anxiety-related personality traits. Arthritis Rheum 2002;46:845–7.
26. Gürsoy S, Erdal E, Herken H, Madenci E, Alaşehirli B, Erdal N. Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. Rheumatol Int 2003;23:104–7.
27. Buskila D, Cohen H, Neumann L, Ebstein RP. An association between fibromyalgia and the dopamine D4 receptor exon III repeat polymorphism and relationship to novelty seeking personality traits. Mol Psychiatry 2004;9:730–1.
28. Russell IJ, Vaeroy H, Javors M, Nyberg F. Cerebrospinal fluid biogenic amine metabolites in fibromyalgia/fibrositis syndrome and rheumatoid arthritis. Arthritis Rheum 1992;35:550-6.
29. Russell IJ, Orr MD, Littman B, Vipraio GA, Alboukrek D, Michalek JE, et al. Elevated cerebrospinal fluid levels of substance P in patients with the fibromyalgia syndrome. Arthritis Rheum 1994;37:1593-601.
30. Garthwaite J, Boulton CL. Nitric oxide signaling in the central nervous system. Annu Rev Physiol 1995;57:683-706.
31. Meller ST, Pechman PS, Gebhart GF, Maves TJ. Nitric oxide mediates the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Neuroscience 1992;50:7-10.
32. Eisinger J, Gandolfo C, Zakarian H, Ayavou T. Reactive oxygen species, antioxidant status and fibromyalgia. J Musculoskelet Pain 1997;5:5–15.
33. Ozgocmen S, Ozyurt H, Sogut S, Akyol O, Ardicoglu O, Yidizhan H. Antioxidant status, lipid peroxidation and nitric oxide in fibromyalgia: Etiologic and therapeutic concerns. Rheumatol Int 2006;26:598-603.
34. Kim DH, Dai F, Belfer I, Banco RJ, Martha JF, Tighiouart H, et al. Polymorphic variation of the guanosine triphosphate cyclohydrolase 1 gene predicts outcome in patients undergoing surgical treatment for lumbar degenerative disc disease. Spine 2010;35:1909-14.
35. Bondy B, Spaeth M, Offenbaecher M, Glatzeder K, Stratz T, Schwarz M, et al. The T102C polymorphism of the 5-HT2A-receptor gene in fibromyalgia. Neurobiol Dis 1999;6:433–9.
36. Wallace DJ, Linker-Israeli M, Hallegua D, Silverman S, Silver D, Weisman MH. Cytokines play an aetiopathogenetic role in fibromyalgia: A hypothesis and pilot study. Rheumatology 2001;40:743–9.
37. Wang H, Moser M, Schiltenwolf M, Buchner M. Circulating cytokine levels compared to pain in patients with fibromyalgia — A prospective longitudinal study over 6 months. J Rheumatol 2008;35:1366–70.
38. Kim SK, Kim KS, Lee YS, Park SH, Choe JY. Arterial stiffness and proinflammatory cytokines in fibromyalgia syndrome. Clin Exp Rheumatol 2010;6 Suppl 63:S71–7.
39. Laroux FS, Pavlick KP, Hines IN, Kawachi S, Harada H, Bharwani S, et al. Role of nitric oxide in inflammation. Acta Physiol Scand 2001;173:113–8.