Genetic Polymorphisms of the ß$_2$-Adrenergic Receptor Relate to Guanosine Protein-coupled Stimulator Receptor Dysfunction in Fibromyalgia Syndrome

YANGMING XIAO, WEIJING HE, and I. JON RUSSELL

ABSTRACT. ***Objective.*** To determine the genotype frequencies of ß$_2$-adrenergic receptor (ß$_2$AR) gene polymorphisms (Gly16Arg, Glu27Gln) in patients with fibromyalgia syndrome (FM) by comparison with unrelated healthy controls. We sought any clinical association with these polymorphisms and determined whether the polymorphisms would associate with a biologic guanosine protein-coupled stimulator receptor (Gs) dysfunction in FM.

Methods. Study subjects included 97 clinically characterized patients with FM and 59 controls. The ß$_2$AR polymorphisms at codons 16 and 27 were determined using polymerase chain reaction-restriction fragment length polymorphism. The Gs functions of peripheral blood mononuclear cells (PBMC) were tested using isoproterenol (ISO) as the adrenergic Gs ligand and measuring intracellular cyclic adenosine monophosphate (cAMP) levels.

Results. The frequency of the ß$_2$AR gene polymorphism Gly16Arg in FM (43.5%) was significantly lower than in controls (63.2%), suggesting that this genotype might have some effect on the risk of developing FM. The only clinical association in FM was with sleep dysfunction. Patients with FM who carried the ß$_2$AR polymorphism Arg16Arg also exhibited significantly lower PBMC basal cAMP levels ($p < 0.05$) and lower ISO-stimulated cAMP levels ($p < 0.05$) than FM carrying Gly16Gly or Gly16Arg.

Conclusion. This confirms a relationship between ß$_2$AR polymorphism and FM. It is the first study to demonstrate ß$_2$AR polymorphism-related differences in intracellular cAMP responses of FM PBMC after ß$_2$AR stimulation *in vitro*. These findings may explain some of the differences in responsiveness of FM subgroups to the adrenergic agonist medications currently approved for FM treatment. (J Rheumatol First Release March 15 2011; doi:10.3899/jrheum.101104)

Key Indexing Terms:
ß$_2$-ADRENERGIC RECEPTOR GUANOSINE PROTEIN-COUPLED STIMULATOR RECEPTOR
FIBROMYALGIA SYNDROME SINGLE-NUCLEOTIDE POLYMORPHISMS

Fibromyalgia syndrome (FM) is a relatively common clinical disorder that is more common in women than in men and is characterized by chronic widespread pain plus allodynia at selected musculoskeletal sites[1,2]. In most patients, FM pain is accompanied by a variety of comorbid symptoms including fatigue, headache, dyscognition, morning stiffness, sleep dysfunction, depression, anxiety, irritable bowel syndrome, and irritable bladder[3,4,5]. None of the available medications has been universally successful in controlling all the symptoms of FM, perhaps because of the condition's biological complexity. A better understanding of the pathophysiology and the molecular mechanisms underlying the development and persistence of FM would represent a step toward more effective treatment[6,7].

Although the pathophysiological mechanisms responsible for FM are only partially understood, the findings from several laboratories, including ours, have led to a large body of testable hypotheses. It is currently believed that pain neurotransmission, G-protein couple receptors, and the hypothalamic-pituitary-adrenal axis are all dysfunctional[8,9,10]. About one-third of patients with FM have a close relative who is similarly affected and, strategically, that other person is usually a woman[11]. A family member of a patient with FM is about 8 times more likely to develop FM than is a family member of a patient with rheumatoid arthritis[12]. It was reasonable, therefore, to predict that genetic predisposition to 1 or more biochemical dysfunctions may be important to the development and/or perpetuation of FM. Genetic associations with FM have been sought with polymorphisms of cat-

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Dr. Russell is supported in part by RGK Foundation, Austin, Texas, and Dr. Xiao is supported in part by the San Antonio Area Foundation.

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Accepted for publication January 28, 2011.

echolamine-O-methyl transferase, monoamine oxidases, biogenic amine receptors, α-1-antitrypsin deficiency, peptidases, dopamine receptors, and with the histocompatibility region locus of chromosome 6[13,14]. However, most of the identified associations have not been confirmed in other populations and few if any have been linked to a relevant biological function marker.

The human ß$_2$-adrenergic receptor (ß$_2$AR) is a guanosine (Gs) protein-coupled stimulator receptor that binds catecholamine neurotransmitters (epinephrine, norepinephrine) and raises the concentration of the second messenger, intracellular cyclic adenosine monophosphate (cAMP)[15,16]. The ß$_2$AR is present on skeletal and cardiac muscle cells and on peripheral blood lymphocytes[17,18]. It mediates physiologic responses such as vasodilation and bronchial smooth-muscle relaxation and represents a connection between the sympathetic nervous system and the immune system[17,18]. Alterations in the ß$_2$AR function have been implicated in several psychiatric and psychological disorders, including those associated with chronic pain[14,19]. One study used the Gs protein ligand reagent isoproterenol (ISO) to stimulate intracellular cAMP production and documented a Gs protein receptor dysfunction in FM[20].

The ß$_2$AR gene is located on chromosome 5 q31-32. Although several single-nucleotide polymorphisms (SNP) have been described in this gene[21,22], 2 of them at amino acid position 16 and 27 (Gly16Arg and Glu27Gln) are known to alter Gs protein receptor functions[22,23]. *In vitro*, Gly16 SNP was associated with enhanced ß$_2$-agonist-induced receptor downregulation, while Glu27 SNP seems to protect against this downregulation[24,25].

Our investigation was undertaken with 4 main goals: (1) to identify the relative frequency of ß$_2$AR polymorphisms in FM and control groups; (2) to compare the observed frequencies of those polymorphisms with those expected for the Hardy-Weinberg equilibrium; (3) to identify any FM clinical domains associated with the polymorphisms; and (4) to determine whether any of the polymorphisms would associate with alteration of ß$_2$AR biological function as evidenced by intracellular cAMP second messenger production.

MATERIALS AND METHODS

Subjects. Our study was approved by the Institutional Review Board (IRB) of The University of Texas Health Science Center at San Antonio. The FM subjects were identified from an IRB-approved list of volunteers, who were then contacted by telephone. If a potential study subject agreed to consider participation, an appointment was made, during which a complete description of the study was provided and the subject gave written consent. The FM participants were asked to invite an unrelated control to participate with them.

Each FM participant met the 1990 American College of Rheumatology classification criteria for FM[1]. Patients with concomitant inflammatory rheumatic diseases and patients with other concomitant painful disorders were excluded. The controls were not required to be completely free of musculoskeletal pain symptoms, provided that any discomfort experienced was considered by them to be trivial, that they had not sought medical care because of any pain during the previous 5 years, and that they failed to meet criteria for FM at the screening visit. All of the participants received a stipend.

Clinical measures. To minimize possible confounding variables, subjects were required to discontinue all psychotropic, analgesic, and catecholamine-blocking medication for 2 weeks prior to blood sampling. All medications ingested by subjects during the prior 2 weeks were documented.

On the day of the phlebotomy, comprehensive clinical assessments were performed by self-administered questionnaires and by physical examination. Since some of the self-report instruments that all of the study subjects completed used the word "fibromyalgia," the controls were asked to mentally substitute "Your health status" for the word "fibromyalgia." Self-reported functional measures included the pain visual analog scale, a 10-cm linear scale ranging from no pain = 0 to severe unbearable pain = 10 cm, indicating the average amount of pain experienced within the last 48 hours[26]. Other measures were the Fibromyalgia Impact Questionnaire (FIQ), to assess the overall effect of the FM symptoms on the person's physical function and quality of life[27] (stiffness from the FIQ ranged from no stiffness = 0 to severe stiffness = 10); the Zung Depression Scale (Zung-D), to document the severity of depressive symptoms[28]; the Zung Anxiety Scale (Zung-A)[28]; and a 4-question self-report sleep scale developed and validated by Jenkins, *et al*[29]. The tender point examination was performed by digital palpation to document the total number of symptomatic tender points present, of the anatomically defined 18 typical sites[1]. The tender point index was calculated from the severity scores of discomfort induced by 4 kg of digital palpation pressure at each of the 18 tender points[30]. The average pain perception threshold induced by graded vertical pressure at all 18 tender point sites was determined using a dolorimeter (Pain Diagnostics and Thermography, Great Neck, NY, USA) with a 0.95 cm^2 contact surface[30].

Peripheral blood mononuclear cell (PBMC) preparation. Between 9:00 AM and 11:00 AM, venous blood (30 ml) was drawn from each subject into a sterile glass vacuum tube containing EDTA anticoagulant. Within 30 min after drawing blood, PBMC were isolated using a modification of the Ficoll-Hypaque (Sigma-Aldrich, St. Louis, MO, USA) gradient technique. Peripheral blood was centrifuged at 1200 rpm for 10 min at room temperature. The "buffy coat" was diluted using phosphate buffered saline (PBS) and carefully layered onto the 4 ml of Histopaque (Sigma-Aldrich) before centrifugation at 2000 rpm for 15 min. The PBMC were then aspirated, resuspended in PBS, washed 3 times, and counted.

B$_2$AR genotype determination. The procedures were performed according to Martinez, *et al*[31]. Genomic DNA was extracted from PBMC using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA). The ß$_2$AR genotypes were analyzed using a polymerase chain reaction (PCR)-restriction fragment length polymorphism method. A PCR product that included the region of the ß$_2$AR codons 16 and 27 polymorphisms was generated using the primers 5'-GCC TTC TTG CTG GCA CCC CAT-3' and 5'-CAG ACG CTC GAA CTT GGC CAT G-3'. Amplification of genomic DNA by PCR was performed in a final volume of 50 μl containing about 100 ng of DNA, 25 μl PCR Master Mix, 2× (Promega, Madison, WI, USA), and 20 pmol of each primer. The PCR was performed using denaturation at 94°C for 2 min, followed by 40 cycles at 94°C for 40 s, 64°C for 40 s, and 72°C for 50 s, and then a final extension for 5 min at 72°C. The size of the PCR product generated was 168 base pairs (bp).

For determination of the ß$_2$AR-16 polymorphism, 10 μl of PCR product was digested with 6 U of NcoI (New England BioLabs, Boston, MA, USA) in 10 μl of 2× NEBuffer 3 at 37°C for 2 h. NcoI cuts 22 bp from the 3'-end of both alleles and 18 bp from the 5'-end of the Gly16 alleles.

The ß$_2$AR-27 genotypes were identified using another aliquot of the same PCR product. Ten microliters of the PCR product were digested with 2 U of BbvI (New England BioLabs) in 10 μl of 2× NEBuffer 2 at 37°C for 2 h. BbvI digests only the Gln27 allele to produce 105-bp and 63-bp fragments. After the restriction enzymes action, the fragments were separated using 4% agarose gel electrophoresis.

Measurements of cAMP levels in PBMC. The Gs protein receptor function-

ality was analyzed by determining the intracellular cAMP levels in the PBMC before and after stimulation with the Gs protein receptor ligand reagent, ISO. Samples of 1.0×10^6 PBMC cells each were exposed to 1 mM isobutyl-1-methylxanthine (Sigma-Aldrich), a phosphodiesterase inhibitor, at 37°C for 10 min. The PBMC were then incubated with the ligand ISO (Sigma-Aldrich) at a range of concentrations (0, 10^{-10} M, 10^{-9} M, 10^{-5} M) in PBS buffer at 37°C for 10 min. The reaction was then terminated by centrifugation at 4ºC. The cells were suspended in lysis buffer from the enzyme immunoassay cAMP kit (Amersham Biosciences, UK), which released the intracellular cAMP into the medium for assay. Intracellular cAMP levels were determined using the cAMP assay kit.

Measurements of Gs receptor function in PBMC. We report the PBMC intracellular cAMP levels as the baseline and as the stimulated value. The absolute or net change was derived by subtracting the baseline cAMP from the stimulated value. To evaluate the Gs protein receptor function, we determined the responses to submaximal and maximal doses of ISO for their ability to stimulate increases in cAMP levels compared with basal cAMP levels in PBMC.

Statistical analysis. Allele frequency was calculated by direct counting. Hardy-Weinberg equilibrium analysis was performed for each SNP in each diagnosis group according to the standard 2-allele method: the experimental genotypic distribution was compared with the theoretical distribution estimated from the SNP allelic frequencies and a p value was derived by chi-squared analysis. Haplotypes were constructed using all the SNP data. Pairwise linkage disequilibrium (D' = 0.202) was examined using the Haploview software (www.broad.mit.edu/personal/jcbarret/haploview/). Haplotype frequencies were estimated from genotype data using the partition ligation-expectation maximization algorithm. OR was evaluated for associations between the genotype, allele, haplotype, and FM diagnosis using a multivariate linear regression model. The 95% CI was defined for every statistical deviation. Differences in age and sex between study groups were accommodated by adjusting for age, sex, and ethnicity in a multivariate logistic regression model. We assessed the observed associations between SNP and FM clinical domains using t tests. The results for the PBMC cAMP levels were compared by t test among the FM subgroups defined by each of the 2 $ß_2$AR genotypes. The results were expressed as mean ± SD. All tests were 2-tailed. P values < 0.05 were considered statistically significant.

RESULTS

Demographic characteristics of study subjects. We studied 97 patients with FM and 59 unrelated controls. There was no significant difference in the ethnic background of the 2 groups (Table 1). There were small but statistically significant differences in age and sex by diagnosis group. For the functional studies, we included all patients, while for the genetic studies we excluded the small number of African American and Asian American subjects, analyzing only 92 FM and 57 controls from white or Hispanic ancestry.

Genotype frequency and their associations with FM status. The genotype frequencies of Gly16Arg (rs1042713) and Glu27Gln (rs1042714) are shown in Table 2 and Figure 1. According to the HapMap database (www.hapmap.org), the allele frequencies of these 2 SNP in people of white and Hispanic descent are similar, but among people of Asian and African descent, they are different. Therefore, we excluded those few Asian and African individuals from this analysis to avoid potential confounding of population mixture.

With respect to the Gly16Arg and the Glu27Gln polymorphisms, both the FM and the control populations diverged significantly from the Hardy-Weinberg equilibrium distribution. While we did not find a significant association between the Glu27Gln genotypes and FM diagnosis, heterozygosity at position 16 (Gly/Arg) was enriched in controls compared to FM (63.2% vs 43.5%, respectively), suggesting that this genotype might have some effect on the development of FM. Statistically, those possessing the Gly/Arg heterozygous genotype were associated with a 57% lower risk (OR 0.43, 95% CI 0.20–0.93, p = 0.030) to develop FM compared to those having the Gly/Gly genotype. After adjustment for age and sex, that association remained marginally significant (OR 0.46, 95% CI 0.21–1.02, p = 0.055; Table 2). Consistent with this, in a dominant model analysis we found that those with at least 1 mutant allele (Arg16) were associated with a lower risk trend to develop FM (OR 0.51, 95% CI 0.24–1.06, p = 0.067). When we stratified the subjects by ethnicity, i.e., separating whites and Hispanic Americans, we found this effect to be stronger in whites.

Since these 2 SNP (Gly16Arg, Glu27Gln) are in linkage disequilibrium (D' = 0.202), we next studied the association between haplotypes derived from them and the related risk of FM development. Four haplotypes (Gly16-Glu27, Gly16-Gln27, Arg16-Gln27, and Arg16-Glu27) were signed based on Haploview software. Among them, only haplotype Arg16-Gln27 exhibited a numerically although not statistically lower frequency in FM cases compared to controls (26.3% vs 31.4%), which is consistent with the effect previously described for Arg16. Moreover, we found that those with at least 1 copy of this haplotype were associated with a 47% lower risk of developing FM (OR 0.53, 95% CI 0.27–1.03, p = 0.058).

Table 1. Demographic and clinical characteristics of study subjects: patients with fibromyalgia syndrome (FM) and controls.

Variable	FMS, n = 97, mean ± SD	Controls, n = 59, mean ± SD	p*
Race			0.35
Hispanic Americans	58	35	
Whites	34	22	
Others such as African and Asian American	5	2	
Women, %	90.7	88.1	0.02
Age, yrs	49.8 ± 11.5	43.7 ± 10.2	< 0.001
Body mass index	34.2 ± 8.5	29.8 ± 7.7	< 0.001
Pain (PVAS)	7.0 ± 2.1	1.4 ± 0.9	< 0.001
Jenkins sleep scale	13.9 ± 5.2	4.8 ± 4.6	< 0.001
Zung depression scale	48.8 ± 8.9	32 ± 5.9	< 0.001
Zung anxiety scale	45.3 ± 9.3	28.6 ± 5.1	< 0.001
Tender point index	35.0 ± 5.6	1.3 ± 1.6	< 0.001
Average pain threshold	3.0 ± 0.6	4.9 ± 0.7	< 0.001
FIQ total score	63.3 ± 17.27	18.56 ± 13.07	< 0.001

* p values for the overall comparisons were calculated with the Wilcoxon signed-rank test for continuous variables or Fisher's exact test for categorical variables. VAS: visual analog scale; FIQ: Fibromyalgia Impact Questionnaire.

Table 2. Distributions of Gly16Arg and Glu27Gln genotypes by diagnosis group among patients with fibromyalgia syndrome (FM) and controls.

Genotype	FMS, n = 92 (%)	Controls n = 57 (%)	No Adjustment			Adjustment for Age, Sex		
			OR	95% CI	p	OR	95% CI	p
Gly16Arg								
Gly/Gly	36 (39.1)	14 (24.6)	1.00			1.00		
Gly/Arg	40 (43.5)	36 (63.2)	0.43	0.20–0.93	0.030	0.46	0.21–1.02	0.055
Arg/Arg	16 (17.4)	7 (12.3)	0.89	0.30–2.62	0.830	0.91	0.29–2.86	0.876
Glu27Gln								
Glu/Glu	32 (34.8)	16 (28.1)	1.00			1.00		
Glu/Gln	27 (29.3)	18 (31.6)	0.75	0.32–1.75	0.505	0.87	0.35–2.17	0.769
Gln/Gln	33 (35.9)	23 (40.4)	0.72	0.32–1.60	0.416	0.78	0.33–1.86	0.571

Arg: arginine; Gly: glycine; Glu: glutamic acid; Gln: glutamine.

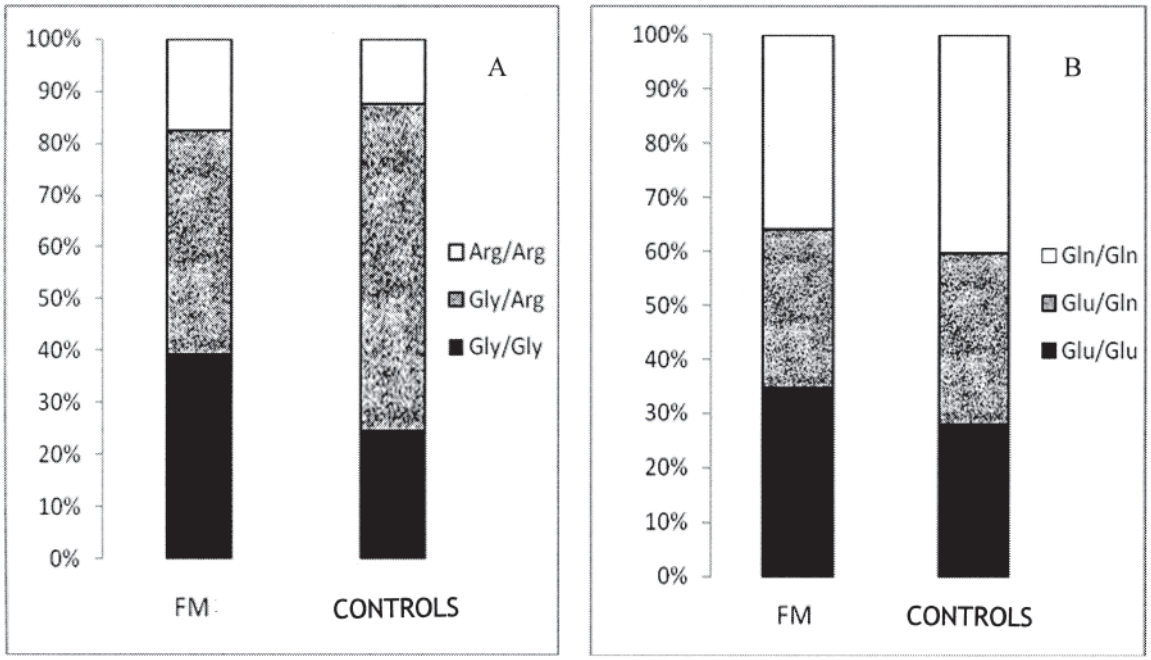

Figure 1. A. ß$_2$-adrenergic receptor (ß$_2$AR) codon 16 (rs1042713) shows a statistically significant difference for the heterozygosity of Gly16Arg (43.5% vs 63.2%) between fibromyalgia syndrome and controls and no difference by diagnosis group for the homozygosity of Arg16 or Gly16. B. ß$_2$AR codon 27 (rs1042714) shows no difference by diagnosis group for Glu27Gln genotype frequency.

Relationships of Gly16Arg phenotypes with FM clinical phenotypes. There was only 1 clinical measure domain phenotype of FM that associated with the genotype polymorphisms (Table 3). Carriers of Gly16Arg heterozygosity exhibited significantly higher scores for the Jenkins sleep scale when compared with carriers of the Arg16 homozygotes in FM (Table 3). Higher scores on the Jenkins sleep scale would indicate more severely dysfunctional sleep.

Relationships of Gly16Arg polymorphism with an FM biological phenotype. Table 4 shows mean cAMP values according to their Gly16Arg genotypes. The patients with FM who were homozygous for Arg16 exhibited significantly lower basal cAMP levels in their PBMC than did the patients with FM who were homozygous for Gly16 or heterozygous for Gly16Arg (Table 4, Figure 2). Consistently, the PBMC from patients with FM who were homozygous for Arg16 also produced significantly less cAMP when stimulated with maximal (10^{-5} M), submaximal (10^{-9} M), or low (10^{-10} M) ISO levels compared to PBMC from patients with FM who were either homozygous for Gly16 or heterozygous for Gly16Arg (Figure 3).

DISCUSSION

We reported a significant decrease in frequency of the het-

Table 3. Clinical measure phenotypes and their associations with 3 ß$_2$-adrenergic receptor-16 genotypically defined subgroups of patients with fibromyalgia syndrome. All data are mean ± SD, compared by Student's unpaired t test.

	Genotypes			p for Comparison		
	Gly16Gly, n = 36	Arg16Arg, n = 17	Gly16Arg, n = 44	Gly16Gly vs Arg16Arg	Gly16Gly vs Gly16Arg	Arg16Arg vs Gly16Arg
PVAS	6.97 ± 2.17	6.67 ± 2.28	7.32 ± 2.09	0.76	0.48	0.39
Jenkins sleep scale	14.03 ± 5.30	11.88 ± 6.23	15.73 ± 4.56	0.23	0.14	0.029
Zung Depression	48.26 ± 8.84	48.04 ± 9.60	49.97 ± 8.41	0.94	0.39	0.47
Zung Anxiety	45.08 ± 9.77	44.49 ± 8.82	46.26 ± 9.88	0.83	0.60	0.50
Tender point index	34.44 ± 5.93	35.29 ± 1.4	35.20 ± 5.76	0.43	0.57	0.93
Average pain threshold	2.94 ± 0.57	2.86 ± 0.42	2.89 ± 0.68	0.55	0.72	0.82
FIQ	62.71 ± 22.46	60.93 ± 17.48	65.12 ± 12.87	0.76	0.58	0.38

Arg: arginine; Gly: glycine; PVAS: pain visual analog scale; FIQ: Fibromyalgia Impact Questionnaire total score.

Table 4. Effects of ß$_2$-adrenergic receptor Gly16Arg genotype on guanosine (Gs) protein functionality in 3 genotypically defined patient subgroups with fibromyalgia syndrome. Gs protein functionality was defined in 2 ways: (1) as the intracellular cyclic adenosine monophosphate (cAMP) second message with the maximally effective stimulation dosage of isoproterenol, (2) and as the absolute increase of the intracellular cAMP second message achieved with the same dosage of isoproterenol. Values are mean ± SD compared by Student's unpaired t test.

	Genotypes			p for Comparison		
	Gly16Gly, n = 36	Arg16Arg, n = 17	Gly16Arg, n = 44	Gly16Gly vs Arg16Arg	Gly16Gly vs Gly16Arg	Arg16Arg vs Gly16Arg
Basal*	5115 ± 3318	3160 ± 1605	4738 ± 3717	0.0056	0.63	0.024
Gs functionality*†						
Stimulated	17541 ± 4840	12790 ± 3904	15602 ± 4761	0.00048	0.077	0.024
Absolute increase	12426 ± 4165	9630 ± 3061	10864 ± 3732	0.0087	0.085	0.19

*Basal intracellular cAMP second message in fmol/10^6 PBMC before stimulation with isoproterenol. † Stimulated: intracellular cAMP second message concentration in fmol/10^6 PBMC achieved with maximal isoproterenol dosage. Absolute increase: difference betwen basal and stimulated intracellular cAMP concentrations in fmol/10^6 PBMC. PBMC: peripheral blood mononuclear cells.

erozygous Gly16Arg genotype in FM compared to controls, suggesting that heterozygosity of Gly16Arg was associated with a lower risk of developing FM. We further observed a heterozygosity advantage in that those with the Gly16Arg genotype exhibited a lower risk for FM compared to those with either the homozygosity Arg16Arg or Gly16Gly.

The ß$_2$AR, which is activated by the endogenous catecholamines norepinephrine and epinephrine, plays a pivotal role in regulation of the activity of the sympathetic nervous system[32]. With the new data from our study relating receptor activation with second message, it seems quite clear that there is a role for ß$_2$AR in the development of FM and in the generation of its symptoms. Support for a role of ß$_2$AR in chronic widespread pain comes from the British Birth Cohort Study[33], in which a history of musculoskeletal pain was associated with SNP and with common ß$_2$AR haplotypes, as we observed. A similar observation was made with ß$_2$AR and the risk of developing chronic facial pain, exemplified by the temporal mandibular pain dysfunction syndrome[34]. Abnormal ß$_2$AR function has also been investigated as a factor relating to depression[20].

In a previous study we observed Gs protein receptor (ß$_2$AR) dysfunction (as defined by production of second message cAMP) *in vitro* with PBMC from patients with FM[35]. This preliminary result, and its relationship to the heterozygous Gly16Arg genotype, supports the proposal of Vargas-Alarcón, *et al*[36], that the ß$_2$AR genetic polymorphism may relate to the adrenergic autonomic nervous system dysfunction documented in FM. In contrast with Vargas-Alarcón, *et al*[36], however, we were unable to document any significant differences in haplotype frequencies for the 2 ß$_2$AR SNP (Gly16Arg, Glu27Gln) between patients with FM and controls among our composite group, nor with the Hispanic American subgroup. Vargas-Alarcón, *et al*[36], found the ß$_2$AR AC haplotype (in essence a combination of Arg16Gly and Gln27Glu) to be associated with a

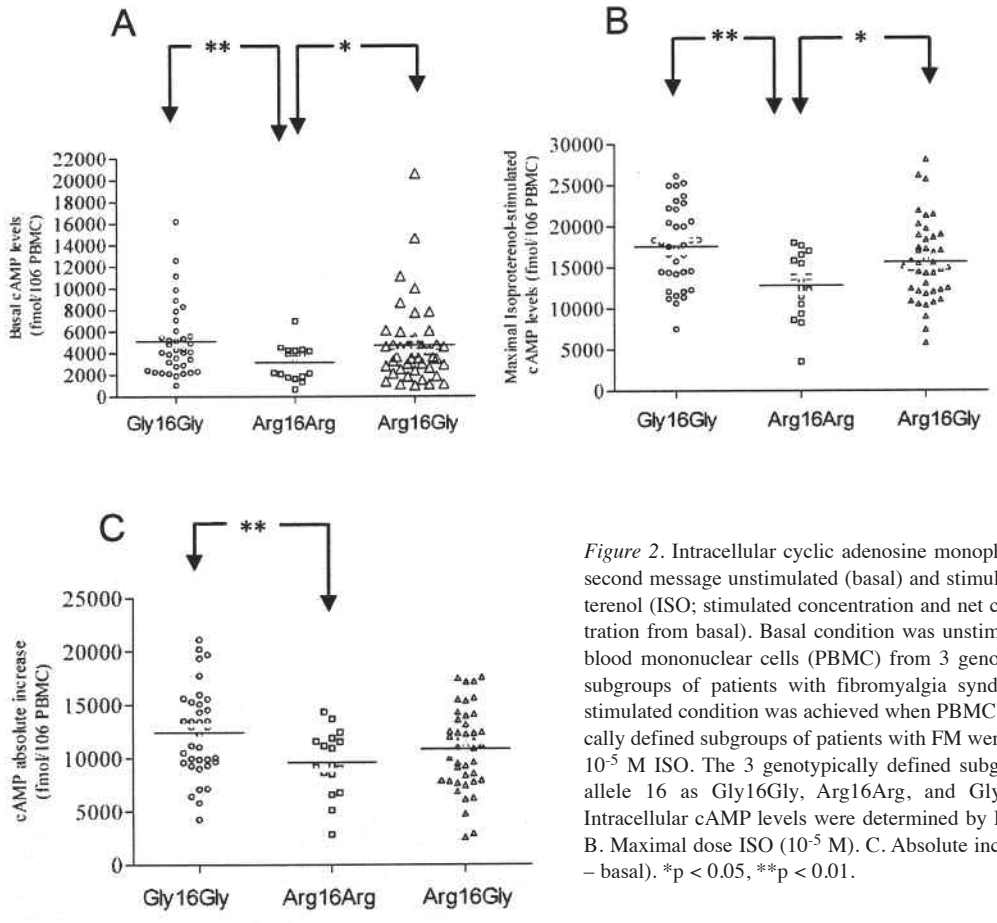

Figure 2. Intracellular cyclic adenosine monophosphate (cAMP) second message unstimulated (basal) and stimulated with isoproterenol (ISO; stimulated concentration and net change in concentration from basal). Basal condition was unstimulated peripheral blood mononuclear cells (PBMC) from 3 genotypically defined subgroups of patients with fibromyalgia syndrome (FM). The stimulated condition was achieved when PBMC from 3 genotypically defined subgroups of patients with FM were stimulated with 10^{-5} M ISO. The 3 genotypically defined subgroups differed at allele 16 as Gly16Gly, Arg16Arg, and Gly16Arg variants. Intracellular cAMP levels were determined by ELISA. A. Basal. B. Maximal dose ISO (10^{-5} M). C. Absolute increase (stimulated – basal). *$p < 0.05$, **$p < 0.01$.

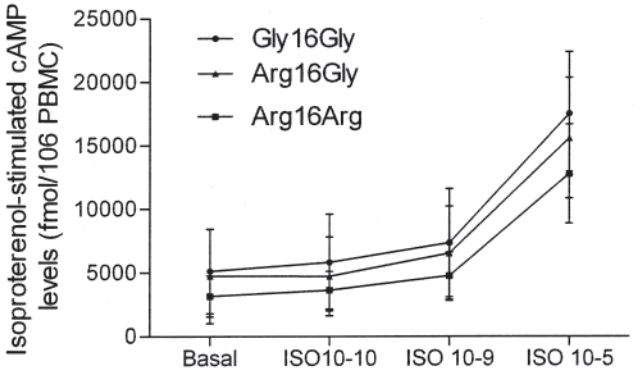

Figure 3. Intracellular cyclic adenosine monophosphate (cAMP) second message levels for peripheral blood mononuclear cells (PBMC) from 3 genotypically defined subgroups of patients with fibromyalgia syndrome (FM) in unstimulated basal condition and in a log-sequence dose response to isoproterenol (ISO). The patient subgroups were homozygous for glycine at allele 16 (Gly16Gly) of the ß$_2$-adrenergic (ß$_2$AR) Gs receptor, or homozygous for arginine at allele 16 (Arg16Arg), or heterozygous for those alleles (Gly16Arg). Patients who were homozygous for Arg16 exhibited significantly lower cAMP levels at basal and when fully stimulated with ISO, compared to PBMC from patients with FM who were homozygous for Gly16 or who were heterozygous for Gly16Arg. The results indicate a significant interaction between ß$_2$AR genotypes and functionality of the Gs receptor protein.

risk of developing FM among both their Spanish and Mexican comparison group cohorts. The reasons for the difference in our findings might be differences in ethnic background, environmental factors, or other confounders.

We appreciate the limitations of our association study, particularly regarding the limited number of subjects studied. Therefore, further studies with more subjects of different ethnic backgrounds will be needed. Nevertheless, it is not likely that our associations are confounded by demographic factors because (1) our association analysis was adjusted for age and sex (we excluded the few subjects in our sample who were of Asian or African ancestry); (2) we repeated 20% of the genotype measurements and got consistent results for all of the repeats, making genotyping error unlikely; (3) the beneficial effect of Arg16 was observed in both the analysis of the single genotype at position 16 alone and in the analysis based on its derived haplotype with the polymorphism at position 27; and (4) the genotyping findings are supported by the biological findings.

Our study shows a significant association of ß$_2$AR with one of the clinical domains characteristic of FM comorbidity, that of dysfunctional sleep. Patients with the heterozygous Gly16Arg polymorphisms exhibited the highest (most abnormal) scores on the Jenkins sleep scale, while lower,

more normal values were seen with the Arg16Arg homozygotes. A conceptually similar finding was noted among the Mexican patients in the Vargas-Alarcón, *et al* study[36], which showed dysfunctional sleep consequences, such as morning stiffness and tiredness on awakening, to be associated with an α1-adrenergic receptor polymorphism in FM, but not with $ß_2$AR in patients with FM. Since dysfunctional sleep has been shown to reduce descending spinal inhibition of pain signals[37], it can be argued that dysfunctional sleep could be at least partially causative of the allodynia that characterizes FM.

Another major finding of our study is that patients with FM homozygous for Arg16 have significantly lower cAMP levels than patients with FM homozygous for Gly16 or heterozygous for Gly16Ag. The implication is that the $ß_2$AR homozygosity Arg16 may fail to adequately activate the receptor or may enhance agonist-mediated desensitization. This is the first FM study demonstrating $ß_2$AR polymorphism-related differences in agonist (ISO)-stimulated PBMC cAMP levels, convincingly showing that the $ß_2$AR polymorphisms represent one of probably many genetic variants involved in the pathophysiology of FM. Several polymorphisms of the $ß_2$AR have been reported to influence the function of the receptor. Specifically, the Arg16 allele was associated with enhanced agonist-induced desensitization that was similar to what we observed in our study[38].

The stimulated $ß_2$AR in these experiments was a Gs protein-coupled stimulator receptor. Activation of that receptor by ISO (a $ß_2$-adrenergic agonist) initiates the guanosine triphosphate (GTP)-dependent activation of intracellular adenylcyclase, which then facilitates conversion of adenosine triphosphate to cAMP, the second message. The quantity of intracellular cAMP generated determines the functional response of the cell. In our study, the homozygous Arg16Arg receptor combination was significantly less effective in creating quantities of intracellular second message than was the Gs receptor associated with the homozygous Gly16Gly, while the heterozygote Gly16Arg was intermediate in this regard. This clearly shows that the $ß_2$AR Gly16Arg-based polymorphisms are functionally different and their different proportions found in FM are likely to have biochemical and clinical consequences.

The $ß_2$AR has been extensively studied for its association with cardiovascular function, asthma, diabetes, pain disorders, and the effects of related drugs[39,40,41]. The $ß_2$AR-16 genotypes have been associated with either decreased or increased agonist-induced desensitization of $ß_2$AR-mediated cAMP responses[42,43]. *In vitro* studies have shown that common variants of the $ß_2$AR involving amino acid substitutions at codons 16 and 27 result in conformational changes that can downregulate and desensitize the response to agonist stimulation[38,44]. Increased desensitization was evident for high concentrations of catecholamines and/or sustained stimulation of the receptor. It has been reported[45] that patients with FM had significantly higher serum levels of norepinephrine. While that finding is controversial[46], elevated endogenous catecholamines might enhance agonist-induced desensitization when the specific genotype, $ß_2$AR Arg16Arg, is present. The frequency of the Arg16Arg genotype in patients with FM (17.4%) was numerically but not statistically higher than in controls (12.3%), so it is unlikely that this difference in prevalence is clinically important.

The management of FM has undergone a dramatic change with the advent of commercial pharmaceutical interest in this disorder[47]. There are at least 3 medications (pregabalin, duloxetine, and milnacipran) approved by the US Food and Drug Administration for the treatment of FM[47,48,49]. Unfortunately, there has been substantial interpatient variability in therapeutic efficacy, such that only about half the patients with FM who are on monotherapy with one of these drugs can expect to achieve a clinically relevant reduction in pain. Both duloxetine and milnacipran are believed to act by increasing synaptic norepinephrine[48,49]. Our study has documented decreased adrenergic agonist-induced cAMP production by the PBMC of patients with FM who were homozygous for Arg16. These findings may help us to understand the variability of responses to adrenergic agonist therapy in genetically defined subgroups of patients with FM. With available technology, it is certainly possible to genotype an individual patient. The question is again raised regarding the ethics of genetic characterization for the purpose of optimizing therapeutic outcomes.

One of the characterizations of FM is that body pain is often accompanied by a myriad of comorbidities that can seem to override the primary complaint on serial clinical visits. It is therefore of interest to examine the concept of compromised $ß_2$AR function with respect to its potential influence on some of the most characteristic comorbidities with FM. In this regard, it must be recalled that most physiological functions result from a balance between 2 opposing influences. With compromised $ß_2$AR tone, the opposing influences might become dominant and symptomatic. For example, it is known that $ß_2$AR relaxes smooth muscle of skeletal muscle arteries, bladder wall, gastrointestinal sphincters, bronchioles, and the nonpregnant uterus, so imbalance might result in painful skeletal muscles, irritable bladder, irritable bowel, poor exercise tolerance, and otherwise unexplained pelvic pain. Our study documents an association with sleep dysfunction. In addition, $ß_2$AR is known to facilitate glycogenolysis to gluconeogenesis and is lipolytic of adipose tissue; failure of these functions is associated with weight gain and even obesity[50]. This concept provides fertile ground for further research in FM.

We have observed a significant difference in the genetic frequency of Gly16Arg between patients with FM and controls. The Gly16Arg polymorphism seems to be associated with an altered risk of developing FM. Knowledge of these

gene polymorphisms may help investigators to sort patients with FM into subgroups for the design of specific pharmacotherapy. Further, we found a genotype-related difference in ISO-induced ß$_2$AR desensitization in PBMC cells from patients with FM, suggesting that the agonist-induced desensitization of cAMP production is genotype Arg16Arg-dependent. Ours is the first study to demonstrate ß$_2$AR polymorphism-related differences in intracellular cAMP levels within FM PBMC before and after ß$_2$AR stimulation. These findings imply that ß$_2$AR polymorphism in FM may influence responses to a variety of ß-adrenergic ligands. This concept may help to explain some of the differences in responsiveness of FM subgroups to the adrenergic agonist medications currently approved for FM treatment. Finally, one could speculate that these findings may directly relate to the adrenergic autonomic nervous system dysfunction documented in FM.

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