Association of Adrenergic Receptor Gene Polymorphisms With Different Fibromyalgia Syndrome Domains

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**Objective.** Fibromyalgia (FM) patients have signs of relentless sympathetic hyperactivity associated with hyporeactivity to stress. Adrenergic receptors (ARs) are parts of the sympathetic nervous system that are fundamental for maintenance of homeostasis. We undertook this study to correlate α1A-AR and β2-AR gene polymorphisms with the presence of FM and with different domains of the FM syndrome as measured by the Fibromyalgia Impact Questionnaire (FIQ).

**Methods.** We studied 78 Mexican FM patients and 48 age-matched controls as well as 78 Spanish FM patients and 71 controls. All subjects studied were women. Single-nucleotide polymorphisms (SNPs) of α1A-AR (rs574584, rs1383914, rs1048101, and rs573542), β2-AR (rs1042713 and rs1042714), and β3-AR (rs4994) were analyzed by 5′ exonuclease TaqMan polymerase chain reaction. Polymorphic haplotypes were constructed after linkage disequilibrium analysis.

**Results.** The β2-AR AC haplotype was a risk factor for the presence of FM. This haplotype had an increased frequency in Mexican patients compared with Mexican controls (42.1% versus 30.5%; \( P = 0.04 \)). Similarly, 50.4% of Spanish patients had this haplotype compared with 40.0% of Spanish controls (\( P = 0.05 \)). In Spanish patients, the α1A-AR SNP rs1383914 was associated with the presence of FM (\( P = 0.01 \)), and the α1A-AR SNP rs1048101 was linked with FIQ disability (\( P = 0.02 \)). Mexican patients with the rs574584 GG genotype presented the highest FIQ score compared with Mexican patients with other genotypes (\( P = 0.01 \)), and in Mexicans SNP rs574584 was associated with FIQ morning stiffness (\( P = 0.04 \)) and with FIQ tiredness upon awakening (\( P = 0.02 \)).

**Conclusion.** AR gene polymorphisms are related to the risk of developing FM and are also linked to different domains of the FM syndrome.

Fibromyalgia (FM) is a frequent cause of generalized pain in the community. Two main features define this syndrome: 1) chronic widespread pain and 2) chronic generalized allodynia manifested by tenderness on palpation at specific anatomic sites. These 2 pain-related defining features are accompanied by other multisystem symptoms. According to the American College of Rheumatology (ACR) criteria, other distinctive features of FM include sleep problems, fatigue, paresthesias, headaches, morning stiffness, anxiety, dryness of the eyes and mouth, pseudo–Raynaud’s phenomenon, and irritable bowel (1).

The Fibromyalgia Impact Questionnaire (FIQ) is the instrument most often used to gauge FM severity. The FIQ was designed to define the severity of different FM domains using a numeric scoring system (2).

Diverse groups of investigators have reported that FM patients have alterations consistent with prominent sympathetic dysfunction. Furthermore, mounting evidence suggests that FM is a sympathetically maintained neuropathic pain syndrome (3). Dysautonomia may also explain FM symptoms not related to pain.

Catecholamines (norepinephrine, epinephrine, and dopamine) are the sympathetic neurotransmitters. Catechol-O-methyltransferase (COMT) is the major
catecholamine-degrading enzyme. COMT has recently been implicated in the modulation of pain (4), and some polymorphisms in the gene that encodes this enzyme have been associated with the risk of developing FM in several populations (5–7).

Catecholamines act by binding to adrenergic receptors (ARs). ARs are parts of the sympathetic nervous system that are fundamental for maintenance of homeostasis. ARs are G proteins expressed on virtually every cell type in the body. Such receptors are broadly classified as α-AR and β-AR. The α-AR are more directly related to vasoconstriction, whereas the β-AR increase cardiac output and vasodilation. ARs are subject to many regulatory factors, including desensitization, down-regulation, and internalization. This plasticity may compromise homeostasis (8). Investigations in animals demonstrated that low COMT activity leads to increased pain sensitivity via AR activation (9). The aim of the present study was thus to correlate α-AR and β-AR gene polymorphisms with the presence of FM and with different domains of the FM syndrome.

**PATIENTS AND METHODS**

**Patients.** We studied 2 groups of patients with FM from Mexico and Spain. These groups are essentially the same as those in our previous COMT genetic study (7). For the present investigation we increased the size of the Mexican sample. All subjects studied were women. The criteria for inclusion were diagnosis of FM in accordance with the ACR guidelines (1) and absence of concurrent rheumatic disease. Patients were referred from different private rheumatology practices in Mexico and Spain. Mexican controls were women who considered themselves to be healthy and who denied having chronic pain. Spanish controls were DNA donors at a national DNA bank who in the screening questionnaire denied having chronic pain. Twenty-seven Mexican controls were paramedical personnel; the rest of the Mexican control group consisted of healthy relatives of paramedical personnel or healthy persons accompanying patients to an outpatient clinic for nonrheumatic disease. Patients and controls were matched by age and sex.

This research was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all participants, and the Human Research Committee of the National Institute of Cardiology Ignacio Chávez approved the study. Both patients and controls filled out a validated Spanish translation of the FIQ (10).

**Genotyping.** Genomic DNA from whole blood containing EDTA was extracted by standard techniques (11). Seven different single-nucleotide polymorphisms (SNPs) were studied (Table 1). SNPs of α1A-AR (rs574584, rs1383914, rs1048101, and rs573542) were chosen because of their different location in both promoter and coding gene regions. There is no information about the functional effects of such SNPs. SNPs of β2-AR (rs1042713 and rs1042714) and β3-AR (rs4994) were chosen based on previous studies showing the functional importance of these gene variants (8).

SNPs were analyzed by 5′ exomnuclease TaqMan assay on an ABI Prism 7000 Sequence Detection System according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA). The National Center for Biotechnology Information (Bethesda, MD) SNP database was used to assign SNP numbers.

**Statistical analysis.** Statistical analysis was carried out with Stata 8.0 software for Windows (StataCorp, College Station, TX). A chi-square test was used to evaluate Hardy-Weinberg equilibrium for each polymorphism. In the exploratory analysis, numeric data showed a non-normal (non-Gaussian) distribution ($P > 0.05$ by Shapiro-Wilk statistic); therefore, data were measured as median and interquartile range. Mexican and Spanish groups were examined separately. The Kruskal-Wallis test was used to compare visual analog scale (VAS) scores on the FIQ with SNPs and genotypes in Spanish and Mexican patients. Proportions of patients and controls by SNP were estimated and compared using chi-square or Fisher’s exact tests as required, and presented as absolute frequencies and proportions. Statistical significance was set at an alpha level of less than or equal to 0.05. Pairwise linkage disequilibrium (LD; D’) estimations between polymorphisms and haplotype reconstruction were performed with Haplovew version 3.32 (Broad Institute of Massachusetts Institute of Technology and Harvard University, Cambridge, MA).

**RESULTS**

Table 2 shows demographic data and the genotype distribution of all AR SNPs studied. The observed
and expected frequencies of the different SNPs in both populations were in Hardy-Weinberg equilibrium. When the α1A-AR polymorphism was analyzed, a different distribution of rs1383914 genotype was observed in Spanish patients versus controls ($P < 0.01$). In Mexicans, the distribution of the α1A-AR SNPs did not differ between patients and healthy controls. The distribution of isolated β2-AR and β3-AR polymorphisms was similar in patients and healthy controls in both ethnic groups.

Correlation between the SNPs and the total FIQ score showed that Mexican patients with the rs574584 GG genotype had the highest FIQ score compared with Mexican patients with other genotypes ($P < 0.01$).

LD analysis showed that the 2 β2-AR polymor-
phisms (rs1042713 and rs1042714) had strong LD in both populations (D' = 0.778 for Mexicans and D' = 0.913 for Spaniards). Likewise, LD analysis in the 3 α1A-AR promoter polymorphisms (rs1383914, rs574584, and rs573542) showed LD in both populations. In light of this finding, we analyzed the most frequent haplotypes to determine whether some of these haplotypes could be associated with FM. Three β2-AR haplotypes (AC, GG, and GC) were frequent in Spaniards and Mexicans. The frequency of the AC haplotype was increased in Mexican patients compared with Mexican controls (42.1% versus 30.5%; P = 0.04). Similarly, the prevalence of the same AC haplotype was increased among Spanish patients compared with their controls (50.4% versus 40.0%; P = 0.05) (Table 3). In the case of α1A-AR polymorphisms, the frequency of the AGA haplotype was decreased in Mexican patients compared with Mexican controls (3.4% versus 10.1%; P = 0.02).

**DISCUSSION**

Our previous investigation showed that the COMT gene “high pain sensitivity” haplotype (ACCG) was associated with FM in Spanish populations. Additionally, a significant correlation was found between the COMT SNP rs6269 and pain and fatigue as well as between the COMT SNP rs165599 and disability and morning stiffness in Mexican patients with FM (7).

Based on findings in animal models, Nackley et al (9) proposed that COMT inhibition results in increased pain sensitivity via AR activation. Additional evidence incriminates dysfunctional ARs in the pathogenesis of chronic painful conditions. Diatchenko et al found a linkage in healthy women between a β2-AR haplotype (termed “haplotype 2”) and the presence of low blood pressure, “somatization,” and the risk of developing a chronic painful illness (temporomandibular joint [TMJ] syndrome). In their heuristic model, haplotype 2 is related to high expression of β2-AR in the synaptic cleft and to inefficient internalization of such receptors in response to agonist stimulation. Their longitudinal observation demonstrated that healthy women with this haplotype 2 have a higher risk of developing TMJ syndrome (12).

The β2-AR AC haplotype has the same nucleotides (adenine for rs1042713 and cytosine for rs1042714) described by Diatchenko et al for haplotype 2. This match suggests the possibility that these 2 haplotypes may represent the same genetic variation. In our study, the β2-AR AC haplotype correlated with the presence of FM in Mexican and Spanish populations. These preliminary results raise the possibility that the β2-AR AC haplotype and haplotype 2 could be the same risk factor for developing chronic painful conditions such as FM and TMJ syndrome. There is a clear clinical overlap between these 2 chronic syndromes.

“Somatization” is a controversial term (13). In our opinion somatization means real symptoms not explainable by current medical knowledge. Dysfunctional ARs may provide a biologic explanation for some “somatization” symptoms. Different stress tests have shown that FM patients have orthostatic intolerance (3). Other studies have confirmed the relationship between low blood pressure and pain sensitivity (14,15). In the present investigation we found that morning stiffness, fatigue, and disability were related to different AR polymorphisms. Therefore, dysautonomia may provide a physiologic explanation for somatization.

In summary, our results show the association of AR polymorphisms with the risk of developing FM as well as with different domains of FM syndrome. This association gives further credibility to the dysautonomic model of FM.

Studies in other ethnic groups with a larger sample size are needed in order to verify or amend these preliminary observations. It will also be important to search for a plausible biologic explanation of how AR gene polymorphisms may facilitate some FM symptoms.

**AUTHOR CONTRIBUTIONS**

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Martínez-Lavin had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. 

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**REFERENCES**