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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 α-AR and β-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 α_{1A}-AR (rs574584, rs1383914, rs1048101, and rs573542), β_2-AR (rs1042713 and rs1042714), and β_3-AR (rs4994) were analyzed by 5′ exonuclease TaqMan polymerase chain reaction. Polymorphic haplotypes were constructed after linkage disequilibrium analysis.**

Results. **The β_2-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 α_{1A}-AR SNP rs1383914 was associated with the presence of FM ($P = 0.01$), and the α_{1A}-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

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Table 1. Characteristics of the SNPs examined*

Gene symbol, RefSNP no.	Location (region)	Chromosome	Position	Variation	SNP type	Amino acid change
ADRB2, rs1042713	Coding	5q31–q32	148186633	A>G	Missense mutation	Arg16Gly
ADRB2, rs1042714	Coding	5q31–q32	148186666	C>G	Missense mutation	Gln27Glu
ADRB3, rs4994	Coding	8p12–p11.2	37942955	T>C	Missense mutation	Trp64Arg
ADRA1A, rs1048101	Coding	8p21–p11.2	26683945	C>T	Missense mutation	Arg347Cys
ADRA1A, rs1383914	Promoter	8p21.2	26778966	G>A	Transition substitution	–
ADRA1A, rs574584	Promoter	8p21.2	26779601	A>G	Transition substitution	–
ADRA1A, rs573542	Promoter	8p21.2	26779735	A>G	Transition substitution	–

* SNPs = single-nucleotide polymorphisms.

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 α_{1A}-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 β_2-AR (rs1042713 and rs1042714) and β_3-AR (rs4994) were chosen based on previous studies showing the functional importance of these gene variants (8).

SNPs were analyzed by 5′ exonuclease 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 Haploview 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

Table 2. Demographic data and genotype distribution of 7 adrenergic receptor SNPs in patients and healthy controls from Mexico and Spain*

	Mexican		Spanish	
	Patients (n = 78)	Controls (n = 48)	Patients (n = 78)	Controls (n = 71)
Age, mean ± SD years	44 ± 12	43 ± 12	47 ± 7	44 ± 9
FIQ score (0–100), mean ± SD	68.7 ± 12.8	3.5 ± 3.1	68.4 ± 11.1	1.6 ± 2.5
SNP, genotype, no. (%)				
rs1042713				
AA	16 (21)	4 (8)	20 (26)	10 (14)
AG	39 (50)	24 (50)	39 (50)	39 (55)
GG	23 (29)	20 (42)	19 (24)	22 (31)
rs1042714				
CC	50 (64)	27 (56)	36 (46)	30 (42)
CG	24 (31)	19 (40)	33 (42)	27 (38)
GG	4 (5)	2 (4)	9 (12)	14 (20)
rs4994				
CC	3 (4)	0 (0)	0 (0)	1 (1)
TC	34 (44)	21 (44)	9 (12)	8 (11)
TT	41 (53)	27 (56)	69 (88)	62 (87)
rs1048101				
CC	19 (24)	13 (27)	13 (17)	9 (13)
CT	37 (47)	19 (40)	38 (49)	42 (59)
TT	22 (28)	16 (33)	27 (35)	20 (28)
rs573542				
AA	65 (83)	43 (90)	75 (96)	63 (89)
AG	11 (14)	4 (8)	3 (4)	8 (11)
GG	2 (3)	1 (2)	0 (0)	0 (0)
rs574584				
AA	67 (86)	39 (81)	74 (95)	63 (89)
AG	9 (12)	8 (17)	4 (5)	8 (11)
GG	2 (3)	1 (2)	0 (0)	0 (0)
rs1383914				
AA	26 (33)	13 (27)	13 (17)	26 (37)
AG	30 (38)	21 (44)	45 (58)	28 (39)†
GG	22 (28)	14 (29)	20 (26)	17 (24)

* All tested individuals were women. SNP = single-nucleotide polymorphism; FIQ = Fibromyalgia Impact Questionnaire.
† $P = 0.01$ versus Spanish patients, by chi-square test.

and expected frequencies of the different SNPs in both populations were in Hardy-Weinberg equilibrium. When the α_{1A}-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 α_{1A}-AR SNPs did not differ between patients and healthy controls. The distribution of isolated β_2-AR and β_3-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 ($P = 0.01$).

The FIQ contains several VAS that measure different FM domains. In Spaniards the rs1048101 polymorphism was associated with disability ($P = 0.02$), whereas in Mexicans the rs574584 polymorphism was associated with morning stiffness ($P = 0.04$) and with tiredness upon awakening ($P = 0.02$).

LD analysis showed that the 2 β_2-AR polymor-

Table 3. Distribution of β_2-adrenergic receptor haplotypes in patients and healthy controls*

	Mexican		Spanish	
Haplotype	Patients	Controls	Patients	Controls
GC	37.8	43.7	17.1	21.5
AC	42.1	30.5†	50.4	40.0‡
GG	17.4	24.8	32.3	36.5
AG	2.7	1.0	0.2	2.0

* Values are the percent of patients or controls with the given haplotype.
† $P = 0.04$ versus Mexican patients.
‡ $P = 0.05$ versus Spanish patients.

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 α_{1A}-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 β_2-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 α_{1A}-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 β_2-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 β_2-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 β_2-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 β_2-AR AC haplotype correlated with the presence of FM in Mexican and Spanish populations. These preliminary results raise the possibility that the β_2-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-Lavín 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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Analysis and interpretation of data. Vargas-Alarcón, Vallejo, Martínez-Lavín.

REFERENCES

1. Wolfe F, Smythe HA, Yunus MB, Bennett RM, Bombardier C, Goldenberg DL, et al. The American College of Rheumatology 1990 criteria for the classification of fibromyalgia: report of the Multicenter Criteria Committee. Arthritis Rheum 1990;33:160–72.
2. Burckhardt CS, Clark SR, Bennett RM. The Fibromyalgia Impact Questionnaire: development and validation. J Rheumatol 1991;18:728–33.
3. Martinez-Lavin M. Biology and therapy of fibromyalgia: stress, the stress response system, and fibromyalgia. Arthritis Res Ther 2007;9:216.
4. Diatchenko L, Slade GD, Nackley AG, Bhalang K, Sigurdsson A, Belfer I, et al. Genetic basis for individual variation of pain perception and the development of a chronic pain condition. Hum Mol Genet 2005;14:135–43.

5. Gursoy S, Erdal E, Herken H, Madenci E, Alasehirli B, Erdal N. Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. Rheumatol Int 2003;23:104–7.
6. Garcia-Fructuoso FJ, Lao-Villadoniga JI, Beyer K, Santos C. Relationship between catechol-O-methyltransferase genotypes and fibromyalgia's severity. Reumatol Clin 2006;2:168–72.
7. Vargas-Alarcon G, Fragoso JM, Cruz-Robles D, Vargas A, Vargas A, Lao-Villadoniga JI, et al. Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis Res Ther 2007;9:R110.
8. Small KM, McGraw DW, Liggett SB. Pharmacology and physiology of human adrenergic receptor polymorphisms. Annu Rev Pharmacol Toxicol 2003;43:381–411.
9. Nackley AG, Tan KS, Fecho K, Flood P, Diatchenko L, Maixner W. Catechol-O-methyltransferase inhibition increases pain sensitivity through activation of both β2- and β3-adrenergic receptors. Pain 2007;128:199–208.
10. Rivera J, Gonzalez T. The Fibromyalgia Impact Questionnaire: a validated Spanish version to assess health status in women with fibromyalgia. Clin Exp Rheumatol 2004;22:554–60.
11. Miller A. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215–7.
12. Diatchenko L, Anderson AD, Slade GD, Fillingim RB, Shabalina SA, Higgins TJ, et al. Three major haplotypes of the β2 adrenergic receptor define psychological profile, blood pressure, and the risk for development of a common musculoskeletal pain disorder. Am J Med Genet B Neuropsychiatr Genet 2006;141B:449–62.
13. Sharpe M, Carson A. "Unexplained" somatic symptoms, functional syndromes, and somatization: do we need a paradigm shift? Ann Intern Med 2001;134:926–30.
14. Maixner W, Fillingim R, Kincaid S, Sigurdsson A, Harris MB. Relationship between pain sensitivity and resting arterial blood pressure in patients with painful temporomandibular disorders. Psychosom Med 1997;59:503–11.
15. Hagen K, Zwart JA, Holmen J, Svebek S, Bovim G, Stovner LJ, the Nord-Trondelag Health Study. Does hypertension protect against chronic musculoskeletal complaints? The Nord-Trondelag Health Study. Arch Intern Med 2005;165:916–22.