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Relationship Between *CCOMT* Gene Genotypes and Severity of Fibromyalgia

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Background and aim: To determine the possible relationship between *Val158Met* genotypes of the *COMT* gene and the severity of fibromyalgia (FM) syndrome.
Patients and methods: The study included 110 patients aged between 45 and 55 years old diagnosed with FM (ACR, 1990) and 110 samples from control subjects with no pain and no abnormal fatigue (National DNA Bank, Spain). To measure the severity of fibromyalgia, the Fibromyalgia Impact Questionnaire (FIQ) was used. Severe FM was defined as an FIQ of ≥70 and was found in 35.5% of the patients. Polymorphisms were analyzed using standard polymerase chain reaction techniques. All the groups met the Hardy-Weinberg equilibrium.
Results: The frequency of the Met/Met genotype was lower in controls (20.9%) than in patients (34.5%), whereas that of the *Val/Val* genotype was higher in controls (30.9%) than in patients (20.0%), with significant differences (*P*=.048). The mean FIQ values were higher in the *Met/Met* genotypes (71.67) and *Val/Met* genotypes (68.27) and were lower in the *Val/Val* genotype (58.93). Tukey's multiple comparison test indicated that FIQ values presented significant differences when *Met/Met/Val/Val* (Tukey, *P*<.001) and *Val/Val/Val/Met* (Tukey, *P*=.003) were compared.
Conclusions: Our results appear to indicate that the Met/Met genotype is associated with greater severity of FM symptoms.

Key words: Fibromyalgia. Genetics. Catechol O-methyltransferase (COMT). Severity of index. Polymorphism.

Relación entre genotipos del gen *catecol O-metiltransferasa* y la gravedad de la fibromialgia

Fundamento y objetivo: Determinar la posible relación entre los genotipos *Val158Met* del gen *catecol O-metiltransferasa (COMT)* y la severidad del síndrome de fibromialgia (FM).
Pacientes y métodos: El estudio incluyó 110 pacientes de edades comprendidas entre 45 y 55 años diagnosticados de FM (ACR 1990) y 110 muestras de controles sanos sin dolor ni fatiga anormal (Banco Nacional de ADN). Como medida de gravedad de la FM se utilizó el cuestionario de impacto de fibromialgia (FIQ), y se estableció el valor FIQ ≥ 70 para determinar FM severa, criterio que cumplieron el 35,5% de pacientes. Los polimorfismos se analizaron mediante técnicas de reacción en cadena de la polimerasa (PCR) estándar. Todos los grupos cumplían el equilibrio de Hardy-Weinberg.
Resultados: La frecuencia del genotipo *Met/Met* es más baja en controles (20,9%) que en casos (34,5%), mientras que la de *Val/Val* es más alta en controles (30,9%) que en casos (20,0%), y las diferencias son significativas (p = 0,048). Los valores medios de FIQ son más altos en los genotipos *Met/Met* (71,67) y *Val/Met* (68,27) y más bajos en el genotipo *Val/Val* (58,93). El test de Tukey de comparaciones múltiples indica que los valores de FIQ presentan diferencias significativas cuando se compara *Met/Met/Val/Val* (Tukey, p < 0,001) y *Val/Val/Val/Met* (Tukey, p = 0,003).
Conclusiones: Nuestros resultados parecen indicar que el genotipo *Met/Met* está asociado a cuadros clínicos de FM más graves.

Palabras clave: Fibromialgia. Genética. *Catecol O-metiltransferasa (COMT)*. Índice de severidad de la enfermedad. Polimorfismo.

Introduction

Studies done in the past few years have confirmed the clinical observation that there are great individual differences in the sensitivity and tolerability to painful

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sensation. Today, evidence points to the fact that these phenotypic variations are the reflection of genetic differences.[1] Fibromyalgia (FM) is defined, according to the case-classification criteria of the American College of Rheumatology (ACR) of 1990, as a generalized pain syndrome of more than 3 months duration and that, when examining the patient, pain is elicited when a pressure of, or equivalent to 4 kg/cm^2, is applied to characteristic trigger points.[2] It is frequently associated to other processes, such as irritable bowel syndrome, irritable bladder, chronic pelvis pain, headaches, temporomandibular joint dysfunction, restless legs syndrome, and multiple chemical sensitivities.[3] In Spain during the year 2000, at least 2.4% of the population was affected by it, almost exclusively women.[4] Even though the cause is unknown, the presence of an alteration in the perception of pain following a model of "central sensitivization"[5] in which different alterations of the hypothalamus-pituitary-adrenal[6] gland axis would be present and an abnormal regulation of the sympathetic nervous system are found, is widely accepted.[7] The enzyme Cathecol-O-Methyltransferase (COMT) intervenes significantly in the inactivation of cathecolamines, through the transference of a methyl group from S-adenosylmethyonine to dopamine, noradrenaline, and adrenaline, and secondarily in the synthesis of neuropeptides such as dynorphins, beta-endorphins, and enkephalins.[8] The codifying gene for the enzyme has the same name (*COMT*) and is located on chromosome 22, specifically in the 22q11.1-22q11.2 region. This gene (GenBank no.: Z26491) contains a polymorphism on the 158 codon with the substitution of a purine guanine base for adenosine. This one-nucleotide-only transition leads to the replacement of a valine (Val) aminoacid for methyonine (Met) in the enzyme codified by the gene, resulting in the variant named Val158Met (rs4680). It is widely accepted that the genetic variation in the 158 codon of the *COMT* gene is the main cause of alteration of enzyme function in humans. The *COMT* genotypes and alleles are related with the levels of enzymatic activity of the COMT enzyme in such a way that the *Val/Val* genotype leads to maximum activity (COMT H-H), intermediate *Val/Met* (COMT H-L), and *Met/Met* the lowest one (COMT L-L, 3 to 4 times less activity).[9] The distribution of these genotypes in different populations (OMIM www.ncbi.nlm.nih.gov/omim). Both the COMT genotypes as well as the alleles and certain haplotypes have been linked to different illnesses and clinical circumstances, such as anxiety disorders, migraine, addiction to nicotine, and an altered response in the metabolism of some antidepressant drugs, among others, as well as pain sensitivity in healthy persons[1,8] and with FM,[10-12] but seems to have no relationship with to recognized models of neuropathic pain.[13] Familial aggregation of FM has been manifested through many studies and suggests that such an aggregation has been essentially due to genetic factors.[14-16]

The stratification of the severity of FM, its prognosis and the attempts to classify the disease into subgroups, have acquired special relevance[17] due to the everyday use of the classification criteria as criteria for clinical diagnosis[18] and the high prevalence of the syndrome in a context of massive juridical and social vindication. The fibromyalgia impact questionnaire (FIQ) is an auto administered instrument of very rapid application (3-5 min) that constitutes the "gold standard" in the evaluation of the severity of the impact of the syndrome on a particular patient in a given timeframe[19,20]; it takes into account characteristics such as: pain, fatigue, work capacity (external or domestic), limitation, well-being, rigidity, anxiety, and depression, through 10 scales that are evaluated homogenously, independently or in a group, from 0 to 100 (maximum affectation), through a formula that was updated in 2002 even if the patient does not answer to all questions.[19] The present study analyzes the relationship between FM and genotypes *COMT* and develops the hypothesis that determined genotypes that condition low enzymatic activity can have a relationship to the severity of FM measured through the absolute value of FIQ.

Patients and Methods

Cases and Controls

For this case-control study, which complies with all the ethical norms of the Helsinki Declaration of the World Medical Association and its amendments, including Edinburg's in 2000, 110 patients were selected (103 women and 7 men), with ages ranging from 45 to 55 years, diagnosed based on the 1990 ACR criteria,[2] that were successively treated at our department of Rheumatology (Clínica CIMA) and gave written informed consent for the study. DNA from 110 healthy persons without pain or fatigue (based on the questionnaires of the National DNA Bank) were obtained as controls, with the same age and gender characteristics as the National DNA Bank (Universidad de Salamanca, Spain. http://www.bancoadn.org). The National DNA Banks' Ethics committee approved the study on 25/11/2005 and assigned protocol number 0002.

Genotyping

DNA was extracted from peripheral blood cells of patients using a Quiamp (Quiagen-Germany) kit

and, in the case of healthy controls, it was received already extracted from the National DNA Bank. The *COMT Val158Met* genotypes were determined using the polymerase chain reaction amplification technique (PCR) using exhaustive methods.[21] PCR was done on a total reaction volume of 25 µL containing 1 TAQ buffer (Boehringer Mannheim), 2 µCi (α^{33}P) dCPT (New England Biolabs), 200 µM dNTPs (250 µM dATP, dGTP, and dTTP and 25 µM dCPT), 1 µM of initiators (5'-GCC CGC CTG CTG TCA CC-3' and 5'-CTG AGG GGC CTG GTG ATA GTG-3') direct and inverse, respectively, 2 units of Taq ADN polymerase (Boehringer Mannheim) and 100 ng of genomic DNA. PCR was done following standard cycles for denaturalization, aligning, and amplification (5' at 94°C followed by 35 cycles of 30' at 95°C, 30' at 55°C, and 40' at 72°C). For the fragment digestion of the resulting 238 base pairs NlaIII was used as a restriction enzyme (New England Biolabs) for a minimum 3 h at 37°C of the amplified products. The restriction fragments were diluted and separated through electrophoresis in a 3% agarose gel at room temperature, visualized under ultraviolet light and coded according to the length of the PCR product. All detections were done twice to insure reproducibility and minimize any possible mistake, and a negative control was included in each test. Patients were asked to complement the FIQ with its validated version in Spanish[22] and it was quantified by 2 evaluators to minimize any possible mistake, the resulting total of this with the 2002 correction, base don a 0-100 scale, being 100 the maximum affectation. All selected patients complemented the questionnaire and allowed for blood extraction. All blood samples were processed and enough DNA was extracted for study. *COMT* genotypes were tabulated in controls and genotypes and the value of FIQ in cases. The recommended cut point of FIQ≥70 to define the patient as "severely affected" was used.[19]

Statistical Analysis

The genotypical frequency of the groups considered controls, cases, FIQ<70 cases and FIQ≥70 cases were used in the exact tests,[23] allowing for the evaluation of the conformity with the premises of Hardy-Weinberg equilibrium and also in the realization of the exact tests of differentiation between groups.[24] These analysis were done using the Arlequín V.3 software.[25] For the study and comparison of the FIQ values between genotypes, we evaluated in first place, if the FIQ variable follows a normal distribution. Afterwards, descriptive measurements were used as well as comparisons among groups.

These comparisons have been done using ANOVA followed by a Tukey test for multiple comparisons. For the realization of these analyses, SPSS V 12.01 (SPSS Inc., 2003) was used. In all the analysis we considered a significance level of 5%.

Results

Characterization of *COMT Val158Met* Polymorphisms in Controls and Cases

Table 1 shows the frequencies of the genotypes of *COMT Val158Met* polymorphisms in controls and cases, considering the total of individuals and separating them by groups FIQ<70 and FIQ≥70. In all groups and subgroups, frequencies follow the predicted theory by the Hardy-Weinberg equilibrium (control, *P*=.848; cases, *P*=.442; cases FIQ<70, *P*=.477; cases FIQ≥70, *P*=.690). As observed in Table 1, the frequency of *Met/Met* is lower in controls (20.91%) than in cases (34.55%) while the *Val/Val* is higher in controls (30.91%) than in cases (20.00%), being the observed differences statistically significant (Table 2). Differences can also be observed between cases FIQ<70

TABLE 1. Frequencies of *COMT Val158Met* Genotype Polymorphisms in Controls and Cases, Considering All Individuals and Separated According to FIQ<70 and FIQ≥70 Groups*

Genotypes	Controls		Cases		Cases FIQ<70		Cases FIQ≥70	
	N	%	N	%	N	%	N	%
Val/Val	34	30.91	22	20.00	20	28.17	2	5.13
Val/Met	53	48.18	50	45.45	32	45.07	18	46.15
Met/Met	23	20.91	38	34.55	19	26.76	19	48.72

*FIQ indicates fibromyalgia impact questionnaire.

TABLE 2. Results of the Exact Tests of Differentiation (*P*-Values), Applied to Compare the Genotypical Frequencies Among Groups*

	Controls	Cases	Cases FIQ≥70	Cases FIQ<70
Controls	–			
Cases	0.04841	–		
Cases FIQ≥70	0.00020	0.05393	–	
Cases FIQ<70	0.68095	0.36208	0.00421	–

*FIQ indicates fibromyalgia impact questionnaire.

and FIQ≥70 that also resulted significant (Table 2), and the frequency of *Met/Met* is also double in cases with FIQ≥70. The FIQ<70 control/cases comparison shows the similarities in the frequencies of the 2 groups (Table 2), contrary to the comparison of FIQ≥70 control/cases that demonstrate the presence of significant differences in the frequencies of both groups (Table 2).

Relationship Between COMT Genotypes and the FIQ Value

The FIQ value follows a normal distribution, both when analyzed globally and when analyzed for each genotype (Kolmogorov-Smirnov, total: Z=1.242, *P*=.091; *Met/Met:* Z=0.816, *P*=.519; *Val/Met:* Z=1.053, *P*=.217; *Val/Val:* Z=0.642, *P*=.804). The Tables present the descriptive statistics for the FIQ variable, taking into account the different genotypes. As can be observed, the median values of FIQ are higher in the *Met/Met* (71.67) and *Val/Met* (68.27) genotypes and lower in the *Val/Val* (58.00) genotype. The comparison of these between the genotypes show that there are differences in the values of FIQ at least among 2 of the genotypes (ANOVA, F=9.807; *P*<.001) and the usage of the Tukey test in multiple comparisons indicate that the values of FIQ present significant differences when compared to *Met/Met/Val/Val* (Tukey, *P*<.001) and *Val/Val/Val/Met* (Tukey, *P*=.003).
As is observed from the analysis of the confidence intervals (Figure 1), it is improbable that the FIQ for the *Val/Val* genotype has a value >70, while for the *Val/Met* genotypes, particularly *Met/Met* this probability is higher. A wider graphical impression can be obtained when observing Figure 2, which relates the distribution of the FIQ values with the different detected genotypes.

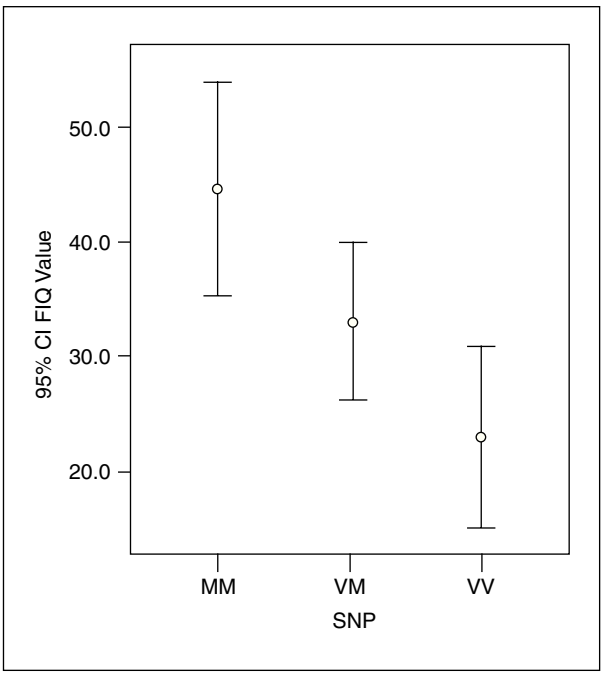

Figure 1. *Graphic representation of the confidence interval (CI) of the median FIQ for each one of the COMT genotypes. SNP indicates single nucleotide polymorphism.*

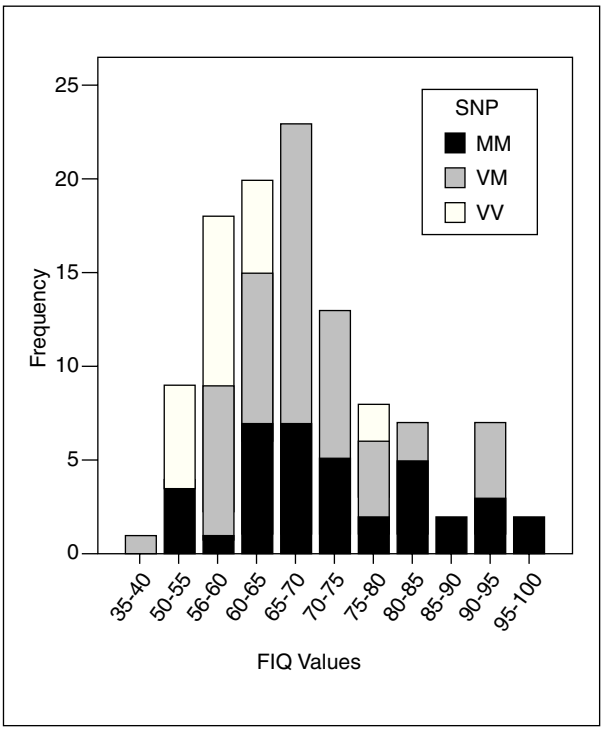

Figure 2. *Distribution of the values of FIQ and detected genotypes. SNP indicates single nucleotide polimorphism.*

Discussion

The high prevalence of FM makes this syndrome a complex sociosanitary problem,[26] one which, independently of the great interest it has generated during the last few years in many groups of investigators, has great gaps in knowledge. Hypothesis have been formulated that try to explain all or part of the physiopathogenic mechanisms of the syndrome that, as everything seems to point at, follows the model of a complex illness.[27] In this context, diverse comorbidities, signs and symptoms have been proposed given the interest in stratifying the impact and severity of the syndrome for each patient. Our work consolidates previous publications[11] in the sense that the homozygous *Met/Met* genotype is significantly more frequent in patients with FM than in controls,

and the magnitude of the association is very solid (*P*=.048).

In second place, a significant relationship (*P*=.004) between the homozygous *Met/Met* polymorphism with high values on the FIQ is information of potential clinical relevance. The increment in the perception of pain mechanism due to the reduction in the enzymatic activity of COMT is not known, although essentially 2 possibilities are suggested, in the first place through the lowering in the production of enkephalins in some regions of the brain, with a lower response to the direct stimuli of substance P, and secondly through the stimulation of adrenergic β_2 receptors as a result of the elevated levels of cathecolamines in the central nervous system (CNS), as occurs in animal models. In any case, future research will be necessary to describe the precise mechanism in humans, though it is postulated that determined haplotypes that include single nucleotide polymorphisms (SNP) of synonymous effect can exert an effect over proteins that go beyond the now known mechanisms of synergy.[1] The limitations of our study are that differences between gender would have been an interesting point to evaluate, something that was not possible due to sample distribution. The value of the statistical significance on the different scales of FIQ, in relation to the detected polymorphisms, and the design of a linear and prospective study to determine the prognostic value of the *Met/Met* genotype with respect to the severity of FM would have also been, in our opinion, interesting proposals.

Conclusions

Our results seem to show that the *Met/Met* genotype is associated to higher FIQ values.

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