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Genome-wide analysis of single nucleotide polymorphisms and copy number variants in fibromyalgia suggest a role for the central nervous system

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ABSTRACT

Fibromyalgia (FM) is a highly disabling syndrome defined by a low pain threshold and a permanent state of pain. The mechanisms explaining this complex disorder remain unclear, and its genetic factors have not yet been identified. With the aim of elucidating FM genetic susceptibility factors, we selected 313 FM cases having low comorbidities, and we genotyped them on the Illumina 1 million duo array. Genotypic data from 220 control women (Illumina 610k array) was obtained for genome-wide association scan (GWAS) analysis. Copy number variants in FM susceptibility were analyzed by array comparative genomic hybridization (aCGH) experiments on pooled samples using the Agilent 2 × 400K platform. No single nucleotide polymorphism (SNP) reached GWAS association threshold, but 21 of the most associated SNPs were chosen for replication in 952 cases and 644 controls. Four of the SNPs selected for replication showed a nominal association in the joint analysis, and rs11127292 (*MYT1L*) was found to be associated to FM with low comorbidities ($P = 4.28 \times 10^{-5}$, odds ratio [95% confidence interval] = 0.58 [0.44–0.75]). aCGH detected 5 differentially hybridized regions. They were followed up, and an intronic deletion in *NRXN3* was demonstrated to be associated to female cases of FM with low levels of comorbidities ($P = .021$, odds ratio [95% confidence interval] = 1.46 [1.05–2.04]). Both GWAS and aCGH results point to a role for the central nervous system in FM genetic susceptibility. If the proposed FM candidate genes were further validated in replication studies, this would highlight a neurocognitive involvement in agreement with latest reports.

1. Introduction

Fibromyalgia (FM) is a highly disabling syndrome affecting 2.9% of the European population [4], mainly women in the fourth decade of life, with a female:male ratio of 21:1 [34]. FM is defined by a low pain threshold and a permanent state of pain, accompanied

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by a constellation of symptoms, including fatigue, sleep disturbances, and cognitive impairment. In the absence of suitable diagnostic tests, FM diagnosis is established by the presence of symptoms for at least 3 months and the exclusion of somatic diseases [44,45].

The mechanisms explaining this chronic pain remain unclear. The most established hypothesis underlying FM etiopathogenesis is the existence of a dysfunction in pain processing. FM patients have been shown to present structural differences in the brain [14,20]. Furthermore, there are several evidences of central sensitization at various levels in the nervous system [12], as well as neurochemical imbalances in the central nervous system leading to a central amplification of pain perception [15,46].

The response to painful stimuli and the FM phenotype have both a genetic component. FM shows family aggregation [5,6] and higher concordance in monozygotic than dizygotic twins (0.29 vs 0.16) [19], while the response to painful stimuli has an estimated heritability of 22% to 55% [29]. However, the exploration of the genetic contribution to pain response and chronic pain states remains scarce [28].

Genetic studies performed so far in FM have not been able to establish a clear genetic association. Most of them have been candidate gene studies, focused on genes related to human leukocyte antigen and neurotransmitters [18,22,48]. So far, 2 studies have attempted to explore the genetic contribution to FM in a genome-wide manner. One of them analyzed over 3200 single nucleotide polymorphisms (SNPs) in 350 genes implicated in pain transmission, in inflammatory responses, and in influencing mood and affective states associated with chronic pain conditions in 496 FM cases and 348 controls. However, the strongest associations did not replicate in independent cohorts [36]. The other one was a linkage scan evaluating 341 markers in 206 affected sibling pairs. They detected a signal in chromosome 17, but no replication analysis was performed [1]. Another recent genome-wide association scan (GWAS) study investigating genetic factors involved in chronic widespread pain [31] identified a region of association in chromosome 5, near *CCT5* and *FAM173B*. These 2 genes also showed a higher RNA expression in mouse models of inflammatory pain.

The aim of this study was to elucidate genetic susceptibility factors for FM. We addressed this objective through 2 main approaches: a genome-wide association study (GWAS) and the evaluation of copy number variants (CNVs) using genotyping data and array comparative genomic hybridization experiments (aCGH). These analyses were performed on a large and very well-characterized cohort of FM patients.

2. Materials and methods

2.1. Samples

FM units of 5 Spanish hospitals participated in the collection of samples. An initial set of 313 samples from women (FM_discovery), characterized by having low levels of psychiatric comorbidities and best fitting the FM diagnosis, was collected at the beginning of the study, and collection continued until an additional set of 1088 women (FM_replication) was achieved (total female FM cohort = 1401). All patients fulfilled the 1990s American College of Rheumatology (ACR) criteria for FM [45] and were selected by the rheumatologists of the units participating in the study. Patients were then evaluated by another set of physicians trained in the assessment of FM patients. They all took the same questionnaires and underwent the same physical examination. A detailed description of the cohort is presented elsewhere [10]. All samples were white and Spanish in origin and had signed informed consent before enrollment. The ethics committees at all recruitment centers approved the project.

We performed cluster analysis on the whole cohort of patients and found that they could be classified into 3 empirical subgroups, which we labeled as follows: FM with low levels of comorbidities and symptomatology (cluster 1), FM with high levels of both symptomatology and comorbidities (cluster 2), and FM with high symptomatology but low levels of comorbidities (cluster 3) [10]. A brief summary of the process is provided in the online Supplementary Data.

Three different control cohorts were used for this study: a cohort of 220 Spanish women (ECHRS) from the Gabriel consortium (http://www.cng.fr/gabriel/index.html) was used in the GWAS analysis (con_ECHRS). In the GWAS replication studies, we genotyped a cohort of 535 female control samples (con_SAL), corresponding to subjects with low levels of pain and fatigue (as assessed by a questionnaire) provided by the National DNA Bank of Salamanca, and a set of 142 female Spanish blood donor samples (con_VH). For the CNV analysis, only the con_SAL set of control samples was used.

A flow chart representing the different cohorts and the analyses performed is provided in Supplementary Fig. 1.

2.2. Whole genome association study

2.2.1. Genotyping

FM women (313 samples, FM_discovery) selected by clinicians for having low levels of psychiatric comorbidities and best fitting the FM diagnosis were genotyped with Illumina 1M-Duo chip. Genotyping was performed in CeGen (Barcelona Node) following the manufacturer's protocol.

Data from 220 general Spanish population samples (Gabriel consortium; http://www.cng.fr/gabriel/index.html) genotyped with Illumina 610 Quad chip were used as the control data set (con_ECHRS).

2.2.2. Quality control

Quality control (QC) was performed with PLINK software [33] (Supplementary Data).

2.2.3. Allelic association

Allelic association analysis was performed with PLINK software (5% of significance level). QQ plots were performed with the WGAViewer software [13], and Manhattan plot and linkage disequilibrium (LD) evaluation were performed with Haploview software [2].

Power analysis was performed with Quanto (http://hydra.usc.edu/gxe/), showing that for SNPs with minor allele frequency (MAF) of $\geqslant$0.05, given our sample size, we had over 80% power to detect associations with odds ratio (OR) of $\geqslant$2.0; however, it showed much lower power to detect associations with smaller ORs (1.2) (Supplementary Data).

2.2.4. Imputation

For GWAS regions showing positive signals, we performed imputation in a window span of 100 kb with Impute v2 (http://mathgen.stats.ox.ac.uk/impute/impute_v2.html) (Supplementary Data).

2.2.5. SNPs annotation and pathway analysis

SNPs showing strongest association were annotated with WGAViewer [13]. The relation to disease of the SNPs and their genomic regions was evaluated with the Decipher database (http://decipher.sanger.ac.uk/). These SNPs were also analyzed with Ingenuity Systems Pathway analysis (IPA) software (http://www.ingenuity.com/) and GeneSet analysis Toolkit v2