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Gene-physical activity interaction with pain, fatigue, and resilience in women with fibromyalgia

Interacción entre genes y actividad física con dolor, fatiga y resiliencia en mujeres con fibromialgia

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A todos mis familiares, los de sangre y los hechos gracias a mi estilo de vida…

To all my relatives, the blood ones and those made from my lifestyle…

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Que la Tesis Doctoral titulada "Interacción entre genes y actividad física con dolor, fatiga y resiliencia en mujeres con fibromialgia" que presenta D. **Fernando Estévez López** al superior juicio del Tribunal que designe la Universidad de Granada, ha sido realizado bajo mi dirección durante los años 2015-2018, siendo expresión de la capacidad técnica e interpretativa de su autora en condiciones tan aventajadas que le hacen merecedora del Título de Doctor, siempre y cuando así lo considere el citado Tribunal.

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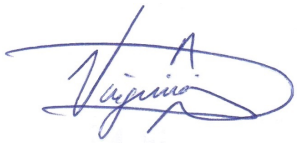

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Garantizamos, al firmar esta Tesis Doctoral, que el trabajo ha sido realizado por el doctorando bajo la dirección de los directores de la tesis y hasta donde nuestro conocimiento alcanza, en la realización del trabajo, se han respetado los derechos de otros autores al ser citados, cuando se han utilizado sus resultados o publicaciones.

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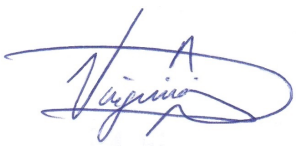

Fdo. Virginia A. Aparicio García-Molina

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Fdo. Fernando Estévez López

En Granada, a 1 de febrero de 2018

El doctorando D. Fernando Estévez López ha realizado la presente Tesis Doctoral Internacional como beneficiario de un contrato predoctoral para la formación de doctores (código BES-2014-067612) en la convocatoria del año 2014 y en el marco del Programa Estatal de Promoción del Talento y su Empleabilidad del Plan Estatal de Investigación Científica y Técnica y de Innovación 2013-2016 del Ministerio de Economía y Competitividad, por Resolución de 16 de marzo de 2015 de la Secretaría de Estado de Investigación, Desarrollo e Innovación (BOE-A-2015-20154778, publicado el 30 de abril de 2015).

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Research projects and funding

The present Doctoral Thesis was performed as a result of the cross-sectional study of **the al-Ándalus project** (http//www.alandalusfibromialgia.com), which received the following funds:

I. Fibromyalgia: follow-up and genetic modulation. Effects of physical exercise and hydrotherapy on pain and health-related quality of life (DEP2013-40908-R). Principal investigator: Manuel Delgado Fernández. Date: 2014-2016 (3 years). Funder: The Spanish Ministry of Economy and Competitiveness, The Government of Spain (Plan Nacional I+D+i).

II. Physical activity in women with fibromyalgia: effects on pain, health and quality of life (DEP2010-15639). Principal investigator: Manuel Delgado Fernández. Date: 2011-2013 (3 years). Funder: The Spanish Ministry of Science and Innovation, The Government of Spain (Plan Nacional I+D+i).

III. Levels of physical activity, physical fitness, health, and quality of life in the southern Spanish population with fibromyalgia: effects of physical exercise and genetics (CTCD-201000019242-TRA). Date: 2011-2013 (3 years). Funder: The Government of southern Spain, Spain (Consejería de Turismo, Comercio y Deporte, Junta de Andalucía).

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Abbreviations

ACR, American College of Rheumatology

ACT, arm curl test

ANCOVA, analysis of covariance

ANOVA, analysis of variance

BCCG, Box-Cox Cole and Green

BCPE, Box-Cox power exponential

BMI, body mass index

BST, back scratch test

CI, confidence interval

CRF, cardiorespiratory fitness

CSR, chair sit-and-reach test

CST, 30-s chair stand test

FIQ, Fibromyalgia Impact Questionnaire

FLEX, flexibility

FM, fibromyalgia

GAMLSS, Generalized Additive Model for Location, Scale and Shape

ICC, Intraclass Correlation Coefficient

IFIS, International FItness Scale

HRQoL, health-related quality of life

MCS, mental component scale of the Short Form-36 Health Survey

MDC, minimal detectable change

MMSE, Mini Mental State Examination

MS, muscular strength

NO, normal

NS, not significant

PCS, physical component scale of the Short Form-36 Health Survey

RPE, rating of perceived exertion

SD, standard deviation

SE, standard error

SEM, standard error of measurement

SF-36, Short Form-36 Health Survey

SP-AG, speed-agility

SS, symptom severity

VAS, visual analogue scale

WPI, Widespread Pain Index

6 MWT, six minutes walk test

SUMMARY

People with fibromyalgia often experience chronic pain and other non-painful symptoms (e.g., fatigue symptoms). This disease has not cure yet. Thus, adaptation to the disease is strived for. In the dynamic process of adaptation, people's strengths play a crucial role. Family aggregation suggests, but does not conclude, genetic susceptibility to fibromyalgia. In fibromyalgia, lifestyle, and specifically physical activity plays a key role in the treatment and development of the disease. However, previous research did not consider the interplay of genetic and lifestyle factors. From a clinical and public health perspective, to understand the interplay between genetics and physical activity on the mains phenotypes of fibromyalgia is of interest.

The overall aim of the present Doctoral Thesis was to enhance the understanding of the genetics of fibromyalgia, paying attention to the potential gene-gene and gene-lifestyle (i.e., time spend on physical activity and sedentary behaviour) interactions, and their association with pain, fatigue, and resilience. To do so, 64 polymorphisms of 34 fibromyalgia candidate-genes were studied in a well-characterised sample of southern Spanish women.

The findings of the present Doctoral Thesis informed that susceptibility to fibromyalgia and its core symptoms (i.e., pain, fatigue, and low resilience) may have a genetic component, which is mostly related to dysfunctions in neurotransmission. Moreover, the sodium voltage-gated channel alpha subunit 9 (SCN9A) gene was the one that showed the most robust associations with the phenotype of fibromyalgia. The SCN9A gene encodes the Na(v)1.7 sodium channel, which suggests that blocking the gain-of-function of this type of channel may lead to beneficial effects on living with fibromyalgia. Furthermore, the interaction gene-lifestyle was related to the phenotype of fibromyalgia. In conclusion, an active lifestyle may help to modulate an unfavourable genetic predisposition to pain, fatigue, and resilience.

Información en Castellano

Resumen

La fibromialgia, enfermedad caracterizada por dolor crónico y fatiga, no tiene cura. Por ello, es muy importante conseguir adaptarse a la enfermedad. Dado que hay agregación familiar en la fibromialgia, se ha sugerido que hay susceptibilidad genética a esta enfermedad. El estilo de vida de las personas con fibromialgia (por ejemplo, la actividad física) juega un papel clave en la fibromialgia. Sin embargo, la interacción entre factores genéticos y comportamentales aún no ha sido estudiada. Este conocimiento podría suponer los cimientos para ofrecer un cuidado más individualizado.

El objetivo general de esta Tesis Doctoral ha sido mejorar el conocimiento de la genética de la fibromialgia, prestando espacial atención a las interacciones gen-gen y gen-factores ambientales (i.e., actividad física y comportamiento sedentario). Para ello, se analizaron 64 polimorfismos de 34 genes candidatos en una muestra bien definida de mujeres Andaluzas (sur de España).

Los hallazgos de la presente Tesis Doctoral indican que la susceptibilidad a la fibromialgia y sus síntomas principales (e.g. dolor, la fatiga y la baja resiliencia) pueden tener un componente genético que, sobre todo, está relacionado con alteraciones en la neurotransmisión. El gen SCN9A fue el que se asoció de forma más robusta con el fenotipo de la fibromialgia. Este gen codifica un el canal de sodio Na(v)1.7, lo que sugiere que bloqueando este tipo de canal podría ser beneficioso en las personas con fibromialgia. Además, la interacción gen-estilo de vida se asoció con el fenotipo de la fibromialgia. En conclusión, la actividad física puede modular una predisposición genética a tener niveles altos de dolor y fatiga, así como bajos de resiliencia.

General introduction and aims

Fibromyalgia: the challenge

Fibromyalgia is a common disease of unknown aetiology whose cardinal symptom is chronic widespread musculoskeletal pain [1]. In Spain, the estimated point prevalence of this disease is 2.4% [2]. The prevalence of fibromyalgia is around nine times higher in women [3]. This disease lacks of objective signs that are analysable in a specific laboratory test [1].

Fibromyalgia is a challenge for several reasons. To the individuals, this disease usually has a burden that negatively impacts on people's daily living [4]. To the economy, people with fibromyalgia often require more resources from the health care system [5]. To the society, given that the signs of fibromyalgia are invisible (e.g., pain, fatigue, and depression), society often has negative responses to people with fibromyalgia by, for instance, accusation of malingering, disbelieving, and lack of understanding [6–8]. To researchers and clinicians, predisposing, triggering, and perpetuating factors related to fibromyalgia and its symptoms are not consensually determined yet [9]. This paucity on the knowledge may be, at least, in part responsible for the lack of a treatment that universally, uniformly, and relevantly helps to reduce the impact of the disease in long-term [10].

Fibromyalgia includes painful and other symptoms

Fibromyalgia is a heterogeneous population; i.e., the clinical picture is highly variable among people [11]. Even within a person, symptoms of fibromyalgia usually fluctuates between days [12,13]. The first diagnostic criteria, launched by the American College of Rheumatology in 1990, were based on the presence of (i) widespread pain for at least 3 months and (ii) tenderness measured by a physical examination [1]. As time passed, fibromyalgia has been recognised as a disease that is related to pain and non-pain symptoms [14,15]. Accordingly, updated version of the diagnostic criteria had been proposed [14–17]. The modified 2011 preliminary criteria (also known as fibromyalgia

research or epidemiological criteria) includes an assessment of several symptoms in addition to pain; e.g., fatigue, waking unrefreshed, and depression [16,18].

Whatever the version is, all the diagnostic criteria for fibromyalgia include a compulsory presence of chronic pain [1,14]. It is assumed that hyperactivity of the central nervous system is a key player in processing stimuli that usually are not painful as painful [19,20]. Potential mechanisms include, but at not limited to, aberrations in the pathways related to pain facilitation and inhibition [21].

In addition to pain, high levels of fatigue are often in fibromyalgia [22]. Up to 82% of people with fibromyalgia report severe fatigue [22]. Fatigue has been recently recognised as a core symptom of fibromyalgia by being included in its diagnostic criteria [17]. Despite its importance, research in fibromyalgia has widely focused on pain while the study of fatigue has been omitted [23].

Physical activity, sedentary behaviour, and fibromyalgia

Physical activity is defined as any bodily movement produced by skeletal muscles that result in energy expenditure above basal metabolic rate [31]. Sedentary behaviour is activity performed while awake that is done in a seated or lying position and does not increase energy expenditure substantially [31]. Ample evidence suggests that physical activity and sedentary behaviours are powerful markers of health in fibromyalgia [32–35]. For instance, higher self-reported physical activity and lower self-reported sedentary behaviours are related to better processing of pain by the central nervous system [36,37]. Thus, an active lifestyle is advisable to people with this disease, indeed the management of fibromyalgia involves a stepped approach that begins with physical exercise therapy [38].

Some considerations on the assessment of physical function in fibromyalgia should be done. The epidemiological study of physical function widely relies on self-reported assessment [39], which is the best way for accounting what the person experiences and

perceives. However, self-reported assessments are imperfect when estimating physical activity and sedentary behaviours in fibromyalgia: people with this disease tend to report higher levels of physical activity and lower levels of sedentary behaviour than the estimations made by accelerometers [40–42]. Consequently, the association of these two behaviours, based on self-reports, with health outcomes may be biased in fibromyalgia. Thus, research that uses accelerometers may be informative.

Psychological resilience and fibromyalgia

Acute pain is adaptive in the signalling of injury or illness in order to attract attention to specific bodily areas that need to be cared [43]. Chronic pain, however, supposes a stressor [44] that may lead to substantially increasing the allostatic load; i.e., the psychological and physiological burden of maintaining homeostasis [45]. Given that the impact that chronic pain has in people depends on their characteristics and skills, psychological variables are involved in adaptation to leaving with chronic diseases [46], such as in fibromyalgia [43,47–50].

Individual differences are key on keeping a positive functioning in fibromyalgia [43,47–50]. Under stressful circumstances, such as living with fibromyalgia, resilience is the ability of maintaining a positive functioning [51] while vulnerability is the susceptibility of being fragile [52]. Overall, increased levels of resilience and reduced of vulnerability are associated with better adaptation to fibromyalgia [43,47,50]. Although traditionally, research has been conducted in vulnerability or people's weaknesses, to identify factors that are related to higher resilience or people's assets is meaningful [43].

Genetics of fibromyalgia

Family aggregation suggests genetic susceptibility to fibromyalgia [24]. Most of the candidate-genes of fibromyalgia are related to neurotransmitters [25]. For instance, the most extensively studied gene is the catechol-O-methyltransferase (COMT), which

participates in degrading catecholamines and several other neurotransmitters and, therefore, in modulating pain perception by the central nervous system. Findings of the previous literature regarding COMT gene and susceptibility to fibromyalgia are inconclusive [26,27]. Therefore, research has aimed at identifying new candidate genes; see [25,28]. For instance, the sodium voltage-gated channel alpha subunit 9 (SCN9A) gene has been recently proposed as part of the pathogenesis of fibromyalgia [29]. Mutations of the SCN9A gene may be related to upregulation of the sodium channels and, consequently, to hyperreactivity to nociceptive stimulus [30].

Limitations of the previous fibromyalgia candidate-genes studies

Although inspiring, previous candidate-gene studies had some caveats. Firstly, it is common that a substantial number of studies focused on identifying new candidate-genes while omitting the inclusion of those genes that were suggested by the past literature. To test new genes is valuable because may help to better understand the pathology of fibromyalgia. However, to replicate previously identified candidate-genes is imperative [53,54]. Secondly, the phenotype was most of the times characterised by pain-related outcomes without considering other important phenotypes such as fatigue or resilience. Thirdly, gene-gene interactions are likely present in fibromyalgia [55] and can help in several ways as, for instance, by unravelling potential mechanisms of the disease [56]. For the first time, Tour and colleagues has recently conducted an cutting-edge study in which emerged an additive association of the opioid receptor μ1 and serotonin transporter 5-HTT genes with pain modulation [55]. Fourthly, fibromyalgia is related to a modulation of a genetic predisposition by environmental factors (e.g., physical activity and sedentary behaviour) [57–60]. However, the study of fibromyalgia has not considered the interplay of such factors [25]. The understanding of this interaction might help to tailor the general advice of engaging in physical activity while reducing sedentary behaviour according to the

genotype of people with fibromyalgia. Fifthly, although the diagnosis of fibromyalgia is usually inaccurate [61,62], previous studies did not always corroborated it.

The al-Ándalus project: an ambitious proposal comes true

To overcome common caveats of the past literature, the al-Ándalus project included 64 single nucleotide polymorphisms of 34 candidate-genes of fibromyalgia susceptibility, symptoms, or potential mechanisms. This project was conducted in a representative sample of women with fibromyalgia from Andalusia (southern Spain). A complete battery of assessments was performed in order to characterise not only the phenotype of pain but also the one of fatigue and resilience. Furthermore, physical activity and sedentary behaviour were objectively measured. The inclusion of a large sample, several genes, and the measurement of physical activity and sedentary behaviours allowed us to analysing the singular association of fibromyalgia candidate-genes, as well as the gene-gene, gene-physical activity, and gene-sedentary behaviour additive associations (i.e., interactions) with fibromyalgia-related outcomes.

Aims

The **overall aim** of the investigations summarised in this thesis was to enhance the understanding of the genetics of fibromyalgia, paying attention to the potential gene-gene and gene-environment (i.e., physical activity and sedentary behaviour) interactions in women with fibromyalgia. To do so, 64 polymorphisms of 34 fibromyalgia candidate-genes were studied in a well-characterised sample of southern Spanish women.

The **specific aims** of the separate studies were the following:

- To call the attention on the importance of including objective measures of physical function in chronic pain diseases; e.g., fibromyalgia (**Study I**).
- To compare the genotype frequencies of candidate-genes in a sample of Spanish women with and without fibromyalgia; i.e., study of the genetic susceptibility to fibromyalgia (**Study II and Study III**).
- To analyse the singular association of candidate-genes, as well as the gene-gene, gene-physical activity, and gene-sedentary behaviour interactions with pain- (**Study IV**), fatigue- (**Study V**), and resilience- (**Study VI**) related outcomes in fibromyalgia.

Objetivos

El **objetivo general** de esta Tesis Doctoral ha sido mejorar el conocimiento de la genética de la fibromialgia, prestando especial atención a las interacciones gen-gen y gen-factores ambientales (i.e., actividad física y comportamiento sedentario). Para ello, se analizaron 64 polimorfismos de 34 genes candidatos en una muestra bien definida de mujeres Andaluzas (sur de España).

Los **objetivos específicos** de los estudios fueron los siguientes:

- Llamar la atención sobre la importancia de medir la función física de forma objetiva en enfermedades caracterizadas por dolor crónico como, por ejemplo, fibromialgia (**Estudio I**).
- Comparar las frecuencias del genotipo de genes candidatos en una muestra de mujeres con y sin fibromialgia; i.e., estudio de susceptibilidad a la fibromialgia (**Estudios II y III**).
- Analizar la asociación individual de genes candidatos, así como las interacciones gen-gen, gen-actividad física y gen-comportamiento sedentario, con dolor (**Estudio IV**), fatiga (**Estudio V**) y resiliencia (**Estudio VI**) en fibromialgia.

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Materials and methods

The present doctoral thesis in a nutshell

The present Doctoral Thesis is part of the cross-sectional study of the al-Ándalus project (http://www.alandalusfibromialgia.com/). Data collection took part in two waves (i) November 2011 to January 2013 and (ii) September 2015 to September 2016. The study was conducted in the eight provinces of Andalusia (southern Spain): Almería, Cádiz, Córdoba, Granada, Huelva, Jaén, Málaga, and Sevilla. Table 1 provides an overview of the methods of each study included in the present Doctoral Thesis.

Table 1. Overview of the methods of the Doctoral Thesis

Study	Design	Participants [a]	Independent variable	Dependent variable
I	Letter to the editor	n/a	n/a	n/a
II	C-C	313 FM cases, 111 controls	Group: cases vs. controls	Genotype: 3 polymorphisms of 2 genes
III	C-C	314 FM cases, 112 controls		Genotype: 61 polymorphisms of 33 genes
IV [b]	C-S	274 FM	Genotype: 64 polymorphisms of 34 genes	Pain-related outcomes
V [b]	C-S	276 FM		Fatigue-related outcomes
VI [b]	C-S	276 FM		Resilience-related outcomes

n/a, not applicable; C-C, cases vs. controls design; C-S, cross-sectional design.

[a] All the participants were women; [b] Studies IV to VI included age, body fat, and drugs (analgesics and antidepressants) consumption as confounders.

Participants

The al-Ándalus project aimed at recruiting a geographically representative sample of women with fibromyalgia from Andalusia (southern Spain). We used as a reference the database of Spanish Association of Rheumatology, as well as the Census of the eight provinces of Andalusia (Andalusian population by province according Multi-territorial Information System of Andalusia (http://www.juntadeandalucia.es/institutodeestadisticaycartografia/sima/index2.htm).

The level of accuracy (*k*) was set as a fraction of the standard deviation (*SD*) of the population (accuracy=*k***SD*). Following the common practice in clinical studies, we selected a *k* of 10-50%. Therefore, for a confidence interval of 95% (95% CI), a sample consisting of 240 women was required to an accuracy of 11% [1]. The recruitment of the participants was facilitated by local fibromyalgia associations in the eight provinces of Andalusia (southern Spain).

The inclusion criteria for people with fibromyalgia were: a certified diagnosis of fibromyalgia by a rheumatologist and meeting the 1990 American College of Rheumatology (ACR) criteria on examination [2]. The inclusion criteria for the non-fibromyalgia participants (i.e., controls) were neither to have a diagnosis of fibromyalgia nor to fulfil the 1990 ACR criteria. General exclusion criteria were: male gender, self-report of having an acute or terminal illness, having severe cognitive impairment as determined by as score less than 10 on the Mini-Mental State Examination (MMSE) [3,4], and incomplete study evaluations.

Ethical considerations

The Ethics Committee of the Hospital Virgen de las Nieves (Granada, Spain) approved the present study (registration number: 15/11/2013-N72). The ethical guidelines of the Declaration of Helsinki (modified in 2000) were followed. All participants provided written informed consent before taking part in the study.

Procedure

The assessments were conducted (i) either in morning or afternoon sessions, according to the participants' convenience, (ii) at the University facilities or at fibromyalgia associations, and (iii) over three consecutive days. On day 1, the participants were interviewed using the MMSE and they completed sociodemographic and clinical data (including current pain intensity). Then, a saliva sample was collected (for genotyping purposes), and measurements of body composition (including body fat) and tender points were done. Subsequently, the participants received several questionnaires to be completed at home on day 2. On day 3, the participants returned the questionnaires and they received the accelerometer to be worn for nine consecutive days.

Measures related to genotyping

Samples were genotyped for a total of 64 single nucleotide polymorphism of 34 candidate-genes that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms (table 2). As described elsewhere [5,6], we collected buccal mucosa cells and performed DNA non-organic extraction (proteinase K and salting-out) procedures. Afterwards, we conducted a spectrophotometric quantification (NanoDrop 2000c, ThermoFisher, Waltham, Massachusetts, USA). All the samples were standardised to 50 ng/μL and they had an A260/230 ratio between 1.7 and 1.9. The samples in low concentration values were processed by Genomiphi™ V2 DNA Amplification Kit (Sigma Aldrich St. Louis, Missouri, USA). Until being processed, all the

samples were stored at -20º C. The OpenArray® genotyping was performed according to the manufacturer's protocol using Accufill™ system liquid robot that adds 1.2µl of genomic DNA sample and 3.8µl of the following reagents: 2×TaqMan® Universal polymerase chain reaction (PCR) Master Mix, No AmpErase® UNG, 20×Primer and TaqMan® Probe (FAM™ dye) mix, and sterile-filtered water. Table 3 shows the manufacturer thermal cycling conditions.

Plates include a no template control for each polymorphism in the analysis, and each plate has a total of 48 samples. Tables 4 and 5 provide further details about the TaqMan® OpenArray® custom assay designs and the 64 analysed polymorphisms, respectively. We performed a TaqMan™ OpenArray™ Genotyping Plate, Custom Format 64 QuantStudio™ 12K Flex. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Table 2. Rationale for the inclusion of the 64 single nucleotide polymorphisms in the present study

Gene Polymorphisms	**Previous studies on fibromyalgia susceptibility**	**Previous studies on fibromyalgia symptoms**	**Previous studies on fibromyalgia-related mechanisms or symptoms**
ADRA1A			
rs574584	[7]	[7]	
rs1048101	[7]	[7]	
rs1383914	[7]	[7]	
ADRB2			
rs1042713	[7]	[7]	
rs1042714	[7]	[7]	
ADRB3			
rs4994	[7]	[7]	
APOE			
rs429358			Alzheimer's Disease [8] Dementia [9] Cognitive performance [10]
BDNF-AS			
rs6265			Major depression [11]
rs7124442			Major depression [12]
CHMP1A			
rs6860	[13]		
CNR1			
rs806377			Happiness [14]
COMT			
rs4633	[15–17]	[16,17]	
rs4680	[15,17–23]	[15–18,20–22,24–27]	
rs4818	[15–18]	[16–18]	
rs6269	[16]	[15,16]	
rs165599	[16,17]	[16,17]	
rs2097903	[16]	[16]	
CREB1			
rs2254137			Cognitive performance [28]
CRHR1			
rs242940			Resilience [29]
rs7209436			Resilience [30]
DRD3			
rs6280	[27]	[27]	
DRD4			
rs1800443			Response to drug therapy [31]
rs1800955			Personality [32]
FKBP5			
rs1360780			Resilience and vulnerability [33]
rs3800373			Resilience and vulnerability [33]
rs9296158			Resilience and vulnerability [34]
rs9470080			Resilience and vulnerability [33]
GABRB3			
rs4906902	[35]		
GBP1			

rs7911	[35]		
GCH1			
rs841	[36]		
rs752688	[36]		
rs3783641	[36]		
rs4411417	[36]		
GPX1			
rs1050450			Oxidative stress [37]
HTR2A			
rs6311	[38]		
rs6313	[38]		
MAOA			
rs6323			Major depression [39]
rs1137070			Major depression [39]
MTHFR			
rs1801133	[40]		
MYT1L			
rs11127292	[41]		
NOS3			
rs1799983			Cardiovascular risk [42]
OPRM1			
rs1799971		[43]	
P2RX7			
rs2230912			Mood disorders [44]
PCLO			
rs2522833			Major depression [45]
SCN9A			
rs573542	[7]	[7]	
rs4371369	[46]	[46]	
rs4387806	[46]	[46]	
rs4453709	[46]	[46]	
rs4597545	[46]	[46]	
rs6746030	[46]	[46]	
rs6754031	[46]	[46]	
rs7607967	[46]	[46]	
rs12620053	[46]	[46]	
rs12994338	[46]	[46]	
rs13017637	[46]	[46]	
SERPINA1			
rs28929474			Granulomatosis with polyangiitis [47]
SLC6A4			
rs25531	[48,49]	[49]	
rs25532	[48,49]	[49]	
SOD2			
rs4880			Oxidative stress [50]
SNAP25			
rs3746544	[51]	[51]	
TAAR1			
rs8192619	[35]		
TACR1			
rs3771863	[52]		
TXNRD1			

rs4964728	Longevity [53]
rs7310505	Longevity [53]

Acronyms of genes as follows: ADRA1A, adrenoceptor alpha 1A; ADRB2, adrenoceptor beta 2; ADRB3, adrenoceptor beta 3; APOE, apolipoproteine E; BDNF-AS, brain-derived neurotrophic factor antisense RNA; CHMP1A, charged multivesicular body protein 1A; CNR1, cannabinoid receptor 1; COMT, catechol-O-methyltransferase; CREB1, CAMP responsive element binding protein 1; CRHR1, corticotrophin-releasing hormone receptor 1; DRD3, dopamine receptor D3; DRD4, dopamine receptor D4; FKBP5, FK506 Binding Protein 5; GABRB3, gamma-aminobutyric acid type A receptor beta 3 subunit; GBP1, guanylate binding protein 1; GCH1, GTP cyclohydrolase 1; GPX1, glutathione peroxidase 1; HTR2A, 5-hydroxytryptamine receptor 2A; MAOA, Monoamine oxidase A; MTHFR, methylene tetrahydrofolatereductase; MYT1L, myelin transcription factor 1 like; NOS3, nitric oxide synthase 3; OPRM1, opioid receptor μ1; P2RX7, purinergic receptor P2X 7; PCLO, piccolo presynaptic cytomatrixprotein; SCN9A, sodium voltage-gated channel alpha subunit 9; SERPINA1, serpin family A member 1; SLC6A4, solute carrier family 6 member 4; SOD2, superoxide dismutase 2; SNAP25, synaptosome associated protein 25; TAAR1, trace amine associated receptor 1; TACR1, tachykinin receptor 1; TXNRD1, thioredoxinreductase 1.

Table 3. Thermal cycling conditions

	AmpliTaq Gold® enzyme activation	**Polymerase chain reaction**
	HOLD CYCLE	(40 cycles)
		Denature - Anneal/Extend
Time	10 min	15 sec -1 min
Temperature	95 °C	92 °C-60 °C

Table 4. TaqMan™ OpenArray™ custom assay designs of candidate gene polymorphisms included in the present study

Gene	Polymorphism	Custom assay
ADRA1A	rs574584	C___2315104_10
	rs1048101	C___2696454_30
	rs1383914	C___2696575_1_
ADRB2	rs1042713	C___2084764_20
	rs1042714	C___2084765_20
ADRB3	rs4994	C___2215549_20
APOE	rs429358	C___3084793_20
BDNF-AS	rs6265	C__11592758_10
	rs7124442	C__27833027_10
CHMP1A	rs6860	C___7519204_10
CNR1	rs806377	Self designed
COMT	rs4633	C___2538747_20
	rs4680	C__25746809_50
	rs4818	C___2538750_10
	rs6269	C___2538746_1_
	rs165599	C___2255335_10
	rs2097903	C__16114953_10
CREB1	rs2254137	C__11514151_20
CRHR1	rs242940	C___2544836_10
	rs7209436	C___1570087_10
DRD3	rs6280	C____949770_10
DRD4	rs1800443	C___7470708_20
	rs1800955	C___7470700_30
FKBP5	rs1360780	C___8852038_10
	rs3800373	C__27489960_10
	rs9296158	C___1256775_10
	rs9470080	C_____92160_10
GABRB3	rs4906902	C__11300465_10
GBP1	rs7911	Self designed
GCH1	rs841	C___9866639_10
	rs752688	C___9866644_10
	rs3783641	C__25800745_10
	rs4411417	C__11164699_10
GPX1	rs1050450	Self designed
HTR2A	rs6311	C___8695278_10
	rs6313	C___3042197_1_
MAOA	rs6323	Self designed
	rs1137070	C___8878813_20
MTHFR	rs1801133	C___1202883_20
MYT1L	rs11127292	C___8971225_10
NOS3	rs1799983	C___3219460_20
OPMR1	rs1799971	Self designed

P2RX7	rs2230912	C__15853715_20
PCLO	rs2522833	C___2553139_10
SCN9A	rs573542	C____903247_10
	rs4371369	C____372246_20
	rs4387806	C__27943991_10
	rs4453709	C____259382_20
	rs4597545	C____518820_10
	rs6746030	C__29330435_10
	rs6754031	C__29108389_10
	rs7607967	C____372249_10
	rs12620053	C__31157449_10
	rs12994338	C__30668947_10
	rs13017637	C__30668948_10
SERPINA1	rs28929474	C__34508510_10
SLC6A4	rs25531	Self designed
	rs25532	Self designed
SOD2	rs4880	C___8709053_10
SNAP25	rs3746544	C__27494002_10
TAAR1	rs8192619	C__25961904_10
TACR1	rs3771863	C__27498949_10
TXNRD1	rs4964728	C__31582257_20
	rs7310505	C__29227804_10

Acronyms of genes as follows: ADRA1A, adrenoceptor alpha 1A; ADRB2, adrenoceptor beta 2; ADRB3, adrenoceptor beta 3; APOE, apolipoprotein E; BDNF-AS, brain-derived neurotrophic factor antisense RNA; CHMP1A, charged multivesicular body protein 1A; CNR1, cannabinoid receptor 1; COMT, catechol-O-methyltransferase; CREB1, CAMP responsive element binding protein 1; CRHR1, corticotrophin-releasing hormone receptor 1; DRD3, dopamine receptor D3; DRD4, dopamine receptor D4; FKBP5, FK506 Binding Protein 5; GABRB3, gamma-aminobutyric acid type A receptor beta 3 subunit; GBP1, guanylate binding protein 1; GCH1, GTP cyclohydrolase 1; GPX1, glutathione peroxidase 1; HTR2A, 5-hydroxytryptamine receptor 2A; MAOA, Monoamine oxidase A; MTHFR, methylenetetrahydrofolate reductase; MYT1L, myelin transcription factor 1 like; NOS3, nitric oxide synthase 3; OPRM1, opioid receptor μ1; P2RX7, purinergic receptor P2X 7; PCLO, piccolo presynaptic cytomatrix protein; SCN9A, sodium voltage-gated channel alpha subunit 9; SERPINA1, serpin family A member 1; SLC6A4, solute carrier family 6 member 4; SOD2, superoxide dismutase 2; SNAP25, synaptosome associated protein 25; TAAR1, trace amine associated receptor 1; TACR1, tachykinin receptor 1; TXNRD1, thioredoxin reductase 1.

Table 5. Further details of the single nucleotide polymorphisms included in the present study

Polymorphism	Chromosome details	Biological function	cDNA position	Protein position	Aa	SIFT	Polymorphism Phenotype	Clinical significance
ADRA1A								
rs574584	8:26866167-26866167	Upstream gene variant						
rs1048101	8:26770511-26770511	Missense	1475	347	C/R	Tolerated (0.71)	Benign (0)	
rs1383914	8:26865532-26865532	Upstream gene variant						
ADRB2								
rs1042713	5:148826877-148826877	Missense	1633	16	G/R	Tolerated (0.17)	Benign (0.143)	Drug response, Risk factor
rs1042714	5:148826910-148826910	Missense	1666	27	E/Q	Tolerated (0.47)	Benign (0.008)	Risk factor,
ADRB3								
rs4994	8:37966280-37966280	Missense	686	64	W/R	Tolerated (1)	Benign (0)	Risk factor
APOE								
rs429358	19:44908684-44908684	Missense	499	130	C/R	Tolerated (1)	Benign (0)	Pathogenic, Association
BDNF-AS								
rs6265	11:27658369-27658369	Missense Val66Met	864	74	V/M	Tolerated (0.2)	Possibly damaging (0.791)	Benign, Risk factor, Protective
rs7124442	11:27655494-27655494	3 prime UTR variant						
CHMP1A								
rs6860	16:89644712-89644712	3 prime UTR variant						

CNR1								
rs806377	6:88149004-88149004	Upstream gene variant						
COMT								
rs4633	22:19962712-19962712	Synonymous,NMD transcript	351	62	H			Benign
rs4680	22:19963748-19963748	Missense, NMD transcript	637	158	V/M	Tolerated (0.07)	Benign (0.019)	Benign, Drug response
rs4818	22:19963684-19963684	Synonymous,NMD transcript	573	136	L			Benign
rs6269	22:19962429-19962429	Intron variant, NMD transcript variant						
rs165599	22:19969258-19969258	Downstream gene variant						
rs2097903	7:10642782-10642782	Intron variant, Non coding transcript variant						
CREB1								
rs2254137	2:207579304-207579304	Intron variant						
CRHR1								
rs242940	17:45815234-45815234	Intron variant						
rs7209436	17:45792776-45792776	Intron variant						
DRD3								
rs6280	3:114171968-114171968	Missense	456	9	G/S	Tolerated low confidence (1)	Benign (0.003)	
DRD4								
rs1800443	11:639830-639830	Missense	593	194	V/G	Deleterious (0)	Unknown (0)	Benign
rs1800955	11:636784-	Upstream gene variant						

	636784	
FKBP5		
rs1360780	6:35639794-35639794	Intron variant
rs3800373	6:35574699-35574699	3 prime UTR variant
rs9296158	6:35599305-35599305	Intron variant
rs9470080	6:35678658-35678658	Intron variant
GABRB3		
rs4906902	15:26774621-26774621	Upstream gene variant
GBP1		
rs7911	1:89052437-89052437	3 prime UTR variant
GCH1		
rs841	14:54843774-54843774	Non coding transcript exon variant, Non coding transcript variant
rs752688	14:54844851-54844851	Intron variant, Non coding transcript variant
rs3783641	14:54893421-54893421	Intron variant, Non coding transcript variant
rs4411417	14:54853845-54853845	Intron variant, Non coding transcript variant
GPX1		
rs1050450	3:49357401-49357401	Downstream gene variant
HTR2A		
rs6311	13:46897343-46897343	Upstream gene variant

rs6313	13:46895805-46895805	Synonymous	234	34	S			
MAOA								
rs6323	X:43731789-43731789	Synonymous	1014	297	R			
rs1137070	X:43744144-43744144	Synonymous	1533	470	D			
MTHFR								
rs1801133	1:11796321-11796321	Missense	788	263	A/V	Deleterious (0.02)	Probably Damaging (0.999)	Uncertain significance, Not provided, Benign, Drug response
MYT1L								
rs11127292	2:2026171-2026171	Intron variant						
NOS3								
rs1799983	7:150999023-150999023	Missense	1251	298	D/E	Tolerated (1)	Benign (0.001)	Pathogenic, Risk factor
OPMR1								
rs1799971	6:154039662	Missense	118A>G	Asn40Asp	Tolerated (0.05)	Benign (0.138)		
P2RX7								
rs2230912	12:121184393-121184393	3_prime_UTR_variant,NMD_transcript_variant	1323	460	Q/R			
PCLO								
rs2522833	7:82824392-82824392	Missense	14778	4814	S/A	Tolerated (0.54)	Unknown (0)	
SCN9A								
rs573542	8:26866301-26866301	Upstream gene variant						

rs4371369	2:166260145-166260145	Intron variant						
rs4387806	2:166294304-166294304	Intron variant						
rs4453709	2:166269944-166269944	Intron variant, Non coding transcript variant						
rs4597545	2:166293988-166293988	Intron variant						
rs6746030	2:166242648-166242648	Missense	3822	1161	W/R	Tolerated (1)	Benign (0)	
rs6754031	2:166298928-166298928	Intron variant						
rs7607967	2:166256826-166256826	Intron variant						
rs12620053	2:166301776-166301776	Intron variant						
rs12994338	2:166303519-166303519	Intron variant						
rs13017637	2:166303436-166303436	Intron variant						
SERPINA1								
rs28929474	14:94378610-94378610	Missense	1373	366	E/K	Tolerated (0.07)	Probably damaging (0.997)	Pathogenic, Other
SLC6A4								
rs25531	17:30237328-30237328	Coding for the serotonin transporter. Upstream gene variant						
rs25532	17:30237152-30237152	Upstream gene variant						
SOD2								
rs4880	6:159692840-159692840	Missense	158	16	V/A	Tolerated (0.93)	Benign (0)	Benign, Drug Response, Risk factor

SNAP25					
rs3746544	20:10306436-10306436	3 prime UTR variant			
TAAR1					
rs8192619	6:132645209-132645209	Synonymous	795	265	C
TACR1					
rs3771863	2:75192588-75192588	Intron variant			
TXNRD1					
rs4964728	12:104255955-104255955	Intron variant			
rs7310505	12:104260770-104260770	Intron variant			

Aa, amino acid. Acronyms of genes as follows: ADRA1A, adrenoceptor alpha 1A; ADRB2, adrenoceptor beta 2; ADRB3, adrenoceptor beta 3; APOE, apolipoprotein E; BDNF-AS, brain-derived neurotrophic factor antisense RNA; CHMP1A, charged multivesicular body protein 1A; CNR1, cannabinoid receptor 1; COMT, catechol-O-methyltransferase; CREB1, CAMP responsive element binding protein 1; CRHR1, corticotrophin-releasing hormone receptor 1; DRD3, dopamine receptor D3; DRD4, dopamine receptor D4; FKBP5, FK506 Binding Protein 5; GABRB3, gamma-aminobutyric acid type A receptor beta 3 subunit; GBP1, guanylate binding protein 1; GCH1, GTP cyclohydrolase 1; GPX1, glutathione peroxidase 1; HTR2A, 5-hydroxytryptamine receptor 2A; MAOA, Monoamine oxidase A; MTHFR, methylenetetrahydrofolate reductase; MYT1L, myelin transcription factor 1 like; NOS3, nitric oxide synthase 3; OPRM1, opioid receptor μ1; P2RX7, purinergic receptor P2X 7; PCLO, piccolo presynaptic cytomatrix protein; SCN9A, sodium voltage-gated channel alpha subunit 9; SERPINA1, serpin family A member 1; SLC6A4, solute carrier family 6 member 4; SOD2, superoxide dismutase 2; SNAP25, synaptosome associated protein 25; TAAR1, trace amine associated receptor 1; TACR1, tachykinin receptor 1; TXNRD1, thioredoxin reductase 1.

Measures related to physical activity and sedentary behaviour

Triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA) were used to objectively measure physical activity and sedentary behaviour. Activity counts were measured at a rate of 30 hertz and stored at an epoch length of 60 second [54,55]. Participants wore the accelerometer on the hip up to 9 days; the first and last days were excluded from the analyses. A total of 7 continuous days with a minimum of 10 valid hours per day was required for being included in the study analysis. Sedentary behaviour and physical activity were calculated based upon recommended vector magnitude cut point [54,55]: <200 and ≥2690 count per minute for sedentary behaviour and moderate to vigorous physical activity, respectively. We used the manufacturer software (Actilife™ v.6.11.7 desktop) for data download, reduction, cleaning and analyses purposes. Further details are available elsewhere [56,57]. In the present study, we used binary data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of moderate to vigorous physical activity in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value.

Measures related to confounders

Socio-demographic and clinical data. The participants filled out an initial questionnaire that included questions about date of birth, marital status, educational level, and presence/absence of acute or terminal illness (such as cancer, stroke, recent cardiomyopathy, severe coronary disease, schizophrenia, or any other disabling injury).

Body fat (%) was measured using a portable eight-polar tactile-electrode impedanciometer (InBody R20, Biospace, Seoul, South Korea). During the assessment, the participants were barefoot and they wore only underwear and no metal objects.

Consumption of drugs. The consumption of analgesics and antidepressants was registered as binary variables (yes/no).

Measures related to phenotype

Tenderness. A standard pressure algometer (FPK 20; Wagner Instruments, Greenwich, CT, USA) was used to assess the 18 fibromyalgia tender points according to the 1990 ACR criteria for the diagnosis [2]. An algometer score was calculated as the sum of the minimum pain-pressure values obtained for each tender point.

The revised fibromyalgia impact questionnaire (FIQ). The 'pain rating' question from was used to assess perceived pain intensity (on a 0 to 10 numeric rating scale) in the context of the past 7 days [58].

The 36-item short form health survey (SF-36). The SF-36 is a generic tool for the assessment of health-related quality of life. For this study, only the 'bodily pain' dimension was used, which assesses the perception of pain in the context of the past 4 weeks. The scores range from 0 to 100, with higher scores indicating lower bodily pain [59].

Visual analogue scale (VAS). A 100-mm VAS, annotated with the words 'no pain' and 'maximum of pain' at the appropriate ends was used to assess perceived pain intensity. The distance between the beginning of the line representing 'no pain' and the pen mark expressing the patients' perception of pain was measured [60].

The Pain catastrophizing scale (PCS). The PCS is a 13-item questionnaire in which patients are asked to reflect on past painful experiences and indicate their thoughts or feelings about pain, on a 5-point scale. For this study, the total score (ranging from 0 to 52) was used, where higher scores represent a more negative appraisal of pain [61].

The Chronic pain self-efficacy scale (CPSS). The CPSS measures efficacy expectations for coping with pain. It contains 19 items grouped into 3 subscales (ranging 0-100): pain

management, coping with symptoms, and physical function. The total score is the sum of the three subscales (ranging 0-300), where higher scores indicate higher self-efficacy [62].

The Multidimensional Fatigue Inventory (MFI) [63]. The MFI is a 20-item questionnaire that evaluates five dimensions of fatigue: general fatigue, physical fatigue, reduced motivation, reduced activity, and mental fatigue. Each fatigue dimension is assessed with four items on a 5-point Likert-scale ranging from 1= 'yes, that is true' to 5 = 'no, that is not true'. Thus, the MFI scores for each fatigue dimension range from 4 to 20, where higher scores indicating more fatigue. The time frame of the fatigue scales is 'lately'.

The *Positive and Negative Affect Schedule* (PANAS) [64] is a 20-item questionnaire that assesses positive affect and negative affect (i.e., the affective components of subjective well-being). The PANAS consists of 10 positive and 10 negative adjectives answered on a 5-point Likert scale ranging from 1 = 'very slightly or not at all' to 5 = 'extremely'. The 2-factor structure (positive affect, negative affect) is also appropriate for people with fibromyalgia [65]. The PANAS scores range from 10 to 50 for both subscales, where higher scores reflect more positive affect or negative affect. An 'in general' time frame was asked to participants.

The *Satisfaction with Life Scale* (SWLS) [66] is a 5-item questionnaire that assesses the perceived global life satisfaction (i.e., the cognitive component of subjective-well-being) on a 5-point Likert-scale ranging from 1 = 'strongly disagree' to 5 = 'strongly agree'. Thus, the SWLS scores range from 5 to 25, where higher scores reflect more cognitive well-being. The time frame of the SWLS is 'in general'.

The *Emotional Regulation subscale of the Trait Meta-Mood Scale* (TMMS) [67] is 8-item scale that assesses one's perceived skills to regulate mood and repair negative emotional experiences on a 5-point Likert-scale ranging from 1 = 'strongly disagree' to 5 = 'strongly

agree'. Thus, the scores range from 8 to 40, where higher scores reflect greater emotional regulation.

The Life Orientation Test-Revised (LOTR) [68] is a 10-item questionnaire that assesses dispositional optimism (sum of the 1st, 4th, and 10th items) and pessimism (sum of the 3rd, 7th, and 9th items) on a 5-point Likert-scale ranging from 0 = 'totally disagree' to 4 'totally agree'. The 2nd, 5th, 6th, and 8th items are fillers. The scores range from 0 to 12, where higher scores reflect greater either optimism or pessimism.

Statistical analyses

Studies II and III

The Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD=r^2>0.5) were checked for all the polymorphisms using SNPStats v3.0.1 [69]. Using SPSS for Mac v.20.0 (IBM, Armonk, NY, USA), we computed Pearson's χ^2 and logistic regression to analyse the differences between fibromyalgia and controls on polymorphisms genotype frequency. Significance was set at p<0.05. In the Study II, additionally, one-way analysis of variance (ANOVA) was performed to compare pain levels between all the genotypes of the rs4680, rs4818 (catechol-O-methyltransferase, COMT, gene) and rs6860 (charged multivesicular body protein 1A, CHMP1A. gene) polymorphisms.

Studies IV to VI

The Hardy-Weinberg equilibrium (HWE; p>0.01) and linkage disequilibrium (LD; r^2>0.5) were evaluated with 'genetics' R package [70]. We used general linear models using Gaussian error distributions to analyse the singular associations of polymorphisms with phenotypes (i.e., pain-, fatigue-, and resilience-related outcomes); age, body fat (%), and the consumption of analgesics and antidepressants were entered as covariates as they are potential confounders of the associations under study [71]. Interactions between

polymorphisms of different genes as well as between polymorphisms and physical activity or sedentary behaviour were assessed using the same models but including the following interaction terms in separate models: gene*gene, gene*physical activity, or gene*sedentary behaviour.

Five genetic models (i.e., dominant, recessive, log-additive, codominant, and overdominant) were used for all analyses. Exceptionally, when the minor allele frequency was lower than 0.1, we analysed only the dominant model of those polymorphisms due to their low number of minor homozygotes. For each polymorphism, P-values were computed using the likelihood ratio test between a model with the polymorphism or interaction term and the null model. Given that some of studied polymorphisms were in LD, we considered as significant those associations with either P-values lower than the Bonferroni's correction or with P- and false discovery rate (FDR) values lower than 0.05.

All analyses were performed in the R environment 3.4.1. Gene-phenotype associations along with gene-to-gene interactions were assessed with the 'SNPassoc' package [72]. We developed our own script (available upon request) to study the gene-environment interactions.

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Results

STUDY I: Assessment of physical function: considerations in chronic pain populations (Pain, 2018, 58(7):1397)

We read with interest the study by Karayannis et al. [4] inspired in a common clinical practice: to assume a strong relationship between pain interference and physical function. Using an elegant design, they observed that the moderate concurrent correlation between pain interference and physical function did not seem to extent to longitudinal changes. Therefore, they concluded that pain interference is not an appropriate surrogate or proxy of physical function in a large sample (n = 389) composed by a mixture of non-cancer chronic pain populations.

We agree with Karayannis et al. [4] about the psychometrical appropriateness of the physical function item bank of the National Institute of Health-Patient Reported Outcome Measurement Information System (PROMIS®) in comparison to other patient-reported outcome measures. Different chronic pain conditions might impose a distinct burden on patients' physical function. However, the PROMIS assesses generic aspects of physical function. In accordance with the Initiative on Methods, Measurements, and Pain Assessments in Clinical Trials (IMMPACT) and the Outcome Measures in Rheumatology (OMERACT) initiative, physical function is better assessed by a combination of generic and disease-specific measures [5]. Given that Karayannis et al. [4] recruited a mixture sample of chronic pain populations, further research testing the prospective association of pain interference and physical function in specific chronic pain subpopulations using specific-condition physical function questionnaires is welcome. By doing so, a corroboration of pain interference as an inappropriate surrogate of self-reported physical function related to specific chronic pain conditions might be provided.

It must be also noted that physical function is measurable by either patient-reported outcomes [5], as Karayannis et al. [4] did, or performance-based measures (e.g., the 6 min

walk test). Advantages and disadvantages of both approaches have been described elsewhere [5]. In people with non-cancer chronic pain there is discordance between patient-reported and performance-based status [2,3,7]. People who experience chronic pain tend to report more impaired physical function than they are indeed able to perform [1–3]. A potential reason behind this finding is that patients' beliefs have an influence on perceived physical function [2,6]. It seems that self-reports and performances of physical function provide unique yet related information in chronic pain populations. Therefore, the interesting findings obtained by Karayannis et al. [4] suggesting a lack of prospective association between pain interference and self-reported physical function should not be extended to performed physical function without empirical corroboration.

In summary, Karayannis et al. [4] designed an interesting longitudinal study including a large sample size of people with chronic pain conditions to test whether pain intensity might be used as a surrogate measure of physical function. Their findings suggest that researchers and clinicians should assess both domains specifically because the prospective association between them is not significant. Based on the limitations of their study, Karayannis et al. [4] indicated that replication of their findings is required. To do so, we believe that it is of interest to include a battery of physical function assessments including generic and specific-population patient-reported outcomes as well as performance-based measures in specific subpopulations of chronic pain (e.g., chronic low back pain, fibromyalgia, rheumatoid arthritis). Nevertheless, we do recognise the inspiring and well-conducted study by Karayannis et al. [4], which may have implications for clinical practice and healthcare policies.

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STUDY II: The TT genotype of the rs6860 polymorphism of the charged multivesicular body protein 1A gene is associated with susceptibility to fibromyalgia in southern Spanish women (Rheumatology International, *In press*)

In 2012, Barbosa et al. [1] analysed the association between the genotype frequencies of the rs4680 and rs4818 polymorphisms of the catechol-O-methyltransferase (COMT) gene. Of note is that, in the abstract, they mentioned the rs6860 polymorphism when it should have been rs4680. Indeed, the rs6860 polymorphism is not part of the COMT gene.

The rs4680 and rs4818 polymorphisms are related to the COMT gene, which is the most widely studied gene in fibromyalgia. The rs4680 polymorphism codes for valine by methionine substitution at codon 158. The rs4818 promotes a silent mutation based on the substitution of guanine by cytosine. The rs6860 polymorphism is located in 3'-UTR region, which contains binding sites for regulatory proteins and microRNAs that may affect the charged multivesicular body protein 1A (CHMP1A) gene.

In the present study, we measured the rs4680 and rs4818 polymorphisms of the COMT gene and the rs6860 polymorphism of the CHMP1A gene in 313 women with fibromyalgia and 111 healthy women (control group). We observed similar frequencies of the rs4680 and rs4818 polymorphisms genotype, while the TT genotype and the T allele of the rs6860 polymorphism were more frequent in the case group compared to the control group (Table 6). Additionally, the level of pain in the past 7 days was assessed with the revised fibromyalgia impact questionnaire [2]. One-way analysis of variance indicated that, in fibromyalgia, pain was similar for all the genotypes of the rs4680, rs4818, and rs6860 polymorphisms (p = 0.41, 0.47, and 0.60, respectively).

In the present study, the association of the rs4680 and rs4818 polymorphisms of the COMT gene with fibromyalgia, either susceptibility or pain, previously observed by

Barbosa et al [1] in Brazil, did not emerge among Spanish women. Thus, our findings agree with a recent meta-analysis concluding that these polymorphisms are not associated with having fibromyalgia [3]. The present study identified that a genetic marker of multivesicular body sorting of proteins to the interiors of lysosomes, the rs6860 polymorphism of the CHMP1A gene, is associated with fibromyalgia susceptibility in southern Spanish women. The latter finding is in line with previous studies in animal models [4] and in humans [5].

Oezel et al. [4] observed mitochondrial dysfunction and autophagy in blood mononuclear cells of animal models with fibromyalgia. In humans, Cordero et al. [5] suggested that massive mitophagy may promote cell-bioenergetics imbalance affecting cells functioning of people with fibromyalgia. Additionally, there are several and complex molecular pathways in mitophagy/autophagy processes. Recent data highlight the relevant role of deubiquitination. For instance, the deubiquitinating enzymes USP30 and USP35 usually are important regulators of the PARK2-mediated mitophagy [6]. Deubiquitination occurs mostly in the early steps of autophagy. The VPS34/Beclin 1-based signaling complexes, which are relevant for the full formation of the pre-autophagic structures, are regulated by several deubiquitinases as USP10 and USP13, among others [7]. Moreover, given its location in the 3'-UTR, the rs6860 polymorphism may also interact with other genes promoting the expression of either long non-coding RNA (lncRNA) [8] or microRNAs [9].

The findings of the present study show (i) a lack of association of the rs4680 and rs4818 polymorphisms of the COMT gene with fibromyalgia, either susceptibility or pain, and (ii) that an increased frequency of the TT genotype and the T allele of the rs6860 polymorphism of the CHMP1A gene may confer genetic susceptibility to fibromyalgia, but not to the pain levels experienced by the patients, in female southern Spanish population. Given the high heterogeneity of fibromyalgia [10], the lack of an independent replication sample is a limitation to the present study. Our findings are in agreement with the available

evidence of mitochondrial dysfunction and excessive autophagy in fibromyalgia [4, 5]. However, other possibilities related to the function of rs6860 polymorphism should not be discarded. Therefore, future research replicating our findings and describing in depth the role of the rs6860 and other CHMP1A-gene polymorphisms in fibromyalgia population is warranted.

Table 6. Genotype and allele frequencies of the rs4680, rs4818, and rs6860 polymorphisms in fibromyalgia and control participants

Frequency	FM, *n*	(%)	Control, *n*	(%)	Odds ratio (95% CI, lower to upper	*p*-value
rs4680 (COMT gene)						
Genotype						
AA	73	(23.7)	23	(20.7)	0.87 (0.64 to 1.18)	0.36
AG	151	(49.0)	53	(47.8)		
GG	84	(27.3)	35	(31.5)		
Allele						
A	297	(48.2)	99	(44.6)	0.86 (0.64 to 1.18)	0.39
G	319	(51.8)	123	(37.0)		
rs4818 (COMT gene)						
Genotype						
CC	108	(36.5)	40	(37.0)	0.95 (0.69 to 1.29)	0.74
CG	140	(47.3)	47	(43.5)		
GG	48	(16.2)	21	(19.4)		
Allele						
C	356	(60.1)	127	(58.8)	0.95 (0.69 to 1.30)	0.75
G	236	(39.9)	89	(41.2)		
rs6860 (CHMP1A gene)						
Genotype						
CC	56	(17.9)	25	(22.5)	1.43 (1.04 to 1.97)	0.03
CT	157	(50.2)	64	(57.7)		
TT	100	(31.9)	22	(19.8)		
Allele						
C	269	(43.0)	114	(51.4)	1.40 (1.03 to 1.90)	0.03
T	357	(57.0)	108	(48.6)		

Logistic regression analyses were conducted to calculate the odds ratio and 95% Confidence Interval (95% CI). CHMP1A, charged multivesicular body protein 1A; COMT, catechol-O-methyltransferase; A, Adenine; C, Cytosine; G, Guanine; T, Thymine; FM, Fibromyalgia.

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STUDY III: Identification of candidate genes associated with fibromyalgia susceptibility in southern Spanish women: the al-Ándalus project (Journal of Translational Medicine, *In press*)

ABSTRACT

Background. Candidate-gene studies on fibromyalgia susceptibility often include a small number of single nucleotide polymorphisms (SNPs), which is a limitation. Moreover, there is a paucity of evidence in Europe. Therefore, we compared genotype frequencies of candidate SNPs in a well-characterised sample of Spanish women with fibromyalgia and healthy non-fibromyalgia women.

Methods. A total of 314 women with a diagnosis of fibromyalgia (cases) and 112 non-fibromyalgia healthy (controls) women participated in this candidate-gene study. Buccal swabs were collected for DNA extraction. Using TaqMan™ OpenArray™, we analysed 61 SNPs of 33 genes related to fibromyalgia susceptibility, symptoms, or potential mechanisms.

Results. We observed that the rs841 and rs1799971 GG genotype was more frequently observed in fibromyalgia than in controls (p=0.04 and p=0.02, respectively). The rs2097903 AT/TT genotypes were also more often present in the fibromyalgia participants than in their control peers (p=0.04). There were no differences for the remaining SNPs.

Conclusions. We identified, for the first time, associations of the rs841 (guanosine triphosphate cyclohydrolase 1 gene) and rs2097903 (catechol-O-methyltransferase gene) SNPs with higher risk of fibromyalgia susceptibility. We also confirmed that the rs1799971 SNP (opioid receptor μ1 gene) might confer genetic risk of fibromyalgia. Further studies are needed to confirm or refute our findings.

BACKGROUND

Family aggregation suggests genetic susceptibility to fibromyalgia [1]. Variations in neurotransmitter-related genes increase susceptibility to fibromyalgia via hypersensitivity to peripheral painful stimulus by the central nervous system (CNS) [2]. Catechol-O-methyltransferase (COMT), the most widely studied gene in fibromyalgia, is involved in degrading catecholamines and several other neurotransmitters and, therefore, in modulating pain perception by the CNS. The association between COMT single nucleotide polymorphisms (SNPs) and fibromyalgia susceptibility is controversial [2,3].

Guanosine triphosphate cyclohydrolase 1 (GCH1) and opioid receptor μ1 (OPRM1) are candidate neurotransmitter-related genes that may confer fibromyalgia susceptibility [4,5]. GCH1 gene participates in the synthesis of dopamine and serotonin. In Korean individuals, the rs841 SNP was associated with discomfort with a tender point examination [4]. OPRM1 gene encodes μ-opioid receptor that bids opiates. In the Turkish population, a study identified that rs1799971 SNP is associated with fibromyalgia susceptibility [5]. However, whether GCH1 and OPRM1 genes are associated with fibromyalgia susceptibility in the Caucasian population is unknown.

Candidate-gene studies on fibromyalgia susceptibility often include a small number of SNPs (e.g., [4,5]), which is a limitation [6]. Additionally, there is a paucity of evidence in Europe. The present candidate-gene study compared genotype frequencies of candidate SNPs in a well-characterised sample of Spanish women with fibromyalgia (cases) vs. healthy non-fibromyalgia women (controls). We, therefore, analysed SNPs that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms.

MATERIALS AND METHODS

Participants

The participants were recruited mainly via fibromyalgia associations from Andalusia (southern Spain). The fibromyalgia patients invited to non-fibromyalgia acquaintances with similar sociodemographic characteristics to participate in the study as controls. All participants signed an informed consent form. The Ethics Committee of the *Virgen de las Nieves* Hospital (Granada, Spain) approved the study. We followed the ethical guidelines of the Declaration of Helsinki.

The fibromyalgia participants had been previously diagnosed with fibromyalgia by a professional rheumatologist and met the 1990 American College of Rheumatology (ACR) criteria for fibromyalgia, which was further confirmed by a tender point examination [7]. Controls neither had a medical diagnosis of fibromyalgia nor fulfilled the 1990 ACR criteria.

Genetic analysis

The participants were genotyped for 61 SNPs (table 2) that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms (table 3). As described elsewhere [8,9], we collected buccal mucosa cells and we performed DNA non-organic extraction (proteinase K and salting-out) procedures. Afterwards, we conducted a spectrophotometric quantification (NanoDrop 2000c, ThermoFisher). All the samples were standardised to 50 ng/μL and they had an A260/230 ratio between 1.7 and 1.9. The samples in low concentration values were processed by Genomiphi™ V2 DNA Amplification Kit (Sigma Aldrich). Until being processed, all the samples were stored at -20º C. The OpenArray® genotyping was performed according to the manufacturer's protocol using Accufill™ system liquid robot that adds 1.2μl of genomic DNA sample and 3.8μl of the following reagents: 2×TaqMan® Universal PCR Master Mix, No AmpErase®

UNG, 20×Primer and TaqMan® Probe (FAM™ dye) mix, and sterile-filtered water. The table 4 shows the manufacturer thermal cycling conditions.

Plates include a NTC for each SNP in the analysis, and each plate has a total of 48 samples. Tables 2 and 5 provide further details about the TaqMan® OpenArray® custom assay designs and the 63 analysed SNPs, respectively. We performed a TaqMan™ OpenArray™ Genotyping Plate, Custom Format 64 QuantStudio™ 12K Flex. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Statistical analysis

The Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD=r^2>0.5) were checked for all the SNPs using SNPStats v3.0.1 [10]. Using SPSS for Mac v.20.0 (IBM, Armonk, NY, USA), we computed Pearson's χ^2 and logistic regression to analyse the differences between fibromyalgia and controls on SNPs genotype frequency. Significance was set at p<0.05.

RESULTS

Table 7 shows the characteristics of the participants included in the present study. Those SNPs that did not fulfil genotyping quality controls were excluded. Through this strict filtering out rule we excluded the following eight SNPs because they did not meet the HWE criterion: the rs6323, rs7911, rs136078, rs806377, rs1050450, rs3746544, rs4411417, rs7124442, and rs12620053. HWE and LD were confirmed for the remaining 52 SNPs, which were, therefore, included in the present study.

The rs841and rs1799971 GG genotypes were more frequently observed in fibromyalgia than in controls (p=0.04 and p=0.02, respectively; Table 8). The rs2097903 AT/TT genotypes were also present in the fibromyalgia participants more often than in

their non-fibromyalgia peers (p=0.04; Table 8). There were no differences for the remaining 51 SNPs (table 6).

DISCUSSION

The present candidate-gene study (including 63 SNPs of 33 genes) is the largest conducted on fibromyalgia susceptibility. We identified, for the first time, that the rs841 (GCH1 gene) and rs2097903 (COMT gene) SNPs were associated with having fibromyalgia. We also confirmed that the rs1799971 SNP (OPRM1 gene) seems to confer genetic risk of fibromyalgia. The COMT gene is involved in neurotransmitters degradation while the GCH1 gene is related to their synthesis. The μ-opioid receptor, which is encoded by the OPRM1 gene, bids both endogenous and exogenous opiates. In line with previous literature [2], our findings suggest that an augmented processing of pain may be involved in the genetic susceptibility to fibromyalgia.

Vargas-Alarcón et al. [3] showed that genotype frequencies of the rs4680, rs4818, and rs6269 SNPs, but not rs2097903, were different between fibromyalgia and control participants. In contrast, we found that the rs2097903 SNP was different between the study cohorts; 78.8% of the patients, and only 69.1% of the controls were TT genotype carriers. Vargas-Alarcón et al. [3] excluded fibromyalgia participants with rheumatic comorbidities and control participants with chronic pain. Of note is that we corroborated the rheumatologist diagnosis of our fibromyalgia participants by fulfilling the 1990 ACR criteria and discarding it in controls. We included fibromyalgia participants with other rheumatic conditions and controls that experienced chronic pain if they did not fulfil the fibromyalgia diagnosis. Therefore, the ecological validity of our findings is high. Moreover, a later genome-wide association study [2] in the Spanish population also failed to replicate the findings by Vargas-Alarcón et al. [3].

GCH enzyme participates in nitric oxide (NO) production. It is widely recognised that NO plays a key role in health and disease by a substantial number of paths [11]. Among other roles, increased NO concentrations often lead to dorsal horn hyperexcitability [12]. Overall, our results are consistent with a previous study in a Korean population in which genotype frequencies of rs841, rs752688, rs4411417, and rs3783641 SNPs in the GCH1 gene were not associated with fibromyalgia susceptibility [4]. However, we found a significant association of the rs841 SNP and fibromyalgia susceptibility with 76.8% of the cases carrying the GG genotype vs. 66.7% of the controls. Interestingly, rs841 was the only SNP correlated to discomfort with a tender point examination in Korean patients [4]. Currently, no study in Caucasian population is available; therefore, future research testing associations of GCH1 gene SNPs, particularly the rs841, with fibromyalgia susceptibility in Caucasians is welcome.

A hypersensitive CNS also appears to have behavioural implications. An active lifestyle is associated with better health status in fibromyalgia [13–15]. However, impaired functioning of the anterior cingulate cortex and amygdala, among other neural areas, may yield to processing harmless movements as painful [16]. The activity of these structures is modulated by μ-opioid receptor availability, which is reduced in fibromyalgia [17]. In agreement with a study in Turkey [5], our findings suggest that the rs1799971 SNP confers fibromyalgia susceptibility, as most of the present patients' sample were GG genotype (55.8%) and most of the controls were AA/AG (57.7%).

Most of the non-significant findings emerged in SNPs of sodium voltage-gated channel alpha subunit 9 (SCN9A) and adrenoceptor alpha 1A (ADRA1A) genes. Genotype frequencies of a SCN9A SNP (rs6754031) were, however, significantly different between fibromyalgia and controls in Mexico [18]. ADRA1A gene activates mitogenic responses and regulates growth and proliferation of many cells. In two subsamples (from Spain and Mexico), a previous study analysed associations of 4 ADRA1A SNPs [19],

showing that only rs1383914 was associated with an increased risk of fibromyalgia susceptibility in the Spanish sample [19].

We did not include a replication sample; therefore, our findings must be interpreted with caution. The unadjusted analyses for multiple comparisons are another limitation, which would yield to non-significant differences in the genotype frequencies of the SNPs. However, to conclude that there is no difference from a statistical point of view would be too stringent. Our findings may be biologically meaningful and informative, and should be further investigated in other populations. Additionally, the unbalanced size of the study cases and controls limits our statistical power. We included, however, more people in the smaller group (cases, n=112) than most of previous studies [20].

CONCLUSIONS

In conclusion, we identified associations of the rs841 (GCH1 gene) and rs2097903 (COMT gene) SNPs with higher risk of fibromyalgia. We also confirmed that rs1799971 SNP (OPRM1 gene) seem to confer higher susceptibility to fibromyalgia. Further studies are needed to confirm or refute the present findings.

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Table 7. Socio-demographic and clinical characteristics of the study samples

	Fibromyalgia (*n*=314)	Controls (*n*=112)
Age, mean (*SD*), years old	52.3 (8.7)	48.2 (7.6)
Education level, *n (%)*		
Unfinished studies	33 (10.5)	6 (5.4)
Primary	157 (50.0)	32 (28.5)
Secondary (and vocational)	88 (28.0)	48 (42.9)
University	36 (11.5)	26 (23.2)
Marital status, *n (%)*		
Married	246 (78.3)	84 (75.0)
Single	24 (7.7)	11 (9.8)
Separated/divorced	29 (9.2)	13 (11.6)
Widow	15 (4.8)	3 (2.7)
Missing data	0 (0.0)	1 (0.9)
Working status, *n (%)*		
Working	82 (26.1)	49 (43.8)
Household	105 (33.5)	38 (33.9)
Incapacity pension or sick leave	63 (20.1)	4 (3.5)
Unemployed	51 (16.2)	14 (12.5)
Others	13 (4.1)	7 (6.3)
Tender points count, mean (*SD*)	16.9 (1.8)	2.8 (3.0)

SD, Standard Deviation.

Table 8. Genotype frequencies of single nucleotide polymorphisms (SNP) in fibromyalgia (FM) and non-fibromyalgia (control) participants

SNP (gene)	Genotype /allele	FM, *n* (%)	Control, *n* (%)	OR (95% CI, lower to upper)	*p*-value
rs841 (GCH1)	AA/AG	72 (23.2)	37 (33.3)	0.61 (0.38 to 0.97)	0.04
	GG	239 (76.8)	74 (66.7)		
	A	76 (12.2)	40 (18.0)	1.58 (1.04 to 2.40)	0.03
	G	546 (87.8)	182 (82.0)		
rs1799971 (OPRM1)	GG	173 (55.8)	47 (42.3)	0.58 (0.38 to 0.90)	0.02
	AA/AG	137 (44.2)	64 (57.7)		
	A	163 (26.3)	78 (35.1)	1.52 (1.09 to 2.11)	0.01
	G	457 (73.7)	144 (64.9)		
rs2097903 (COMT)	AA	64 (21.2)	34 (30.9)	1.66 (1.02 to 2.71)	0.04
	AT/TT	238 (78.8)	76 (69.1)		
	A	274 (45.4)	116 (52.7)	1.34 (0.99 to 1.83)	0.06
	T	330 (54.6)	104 (47.3)		

Logistic regression analyses were conducted to calculate de Odds Ratio (OR) and 95% Confidence Interval (95% CI). COMT, catechol-O-methyltransferase gene; GCH1, GTP cyclohydrolase 1 gene; OPRM1, opioid receptor μ1 gene; A, Adenine; C, Cytosine; G, Guanine; T, Thymine.

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STUDY IV: Genetics, people's behaviour, and pain in women with fibromyalgia: the promising role of avoiding sedentary behaviour. *Submitted*

ABSTRACT

Objectives. To test the individual association of 64 single nucleotide polymorphisms of 34 candidate-genes, the gene-gene interactions, the gene-physical activity, and gene-sedentary behaviour interactions in a well-characterised sample of southern Spanish women with fibromyalgia.

Methods. We extracted DNA from saliva samples in 274 women with fibromyalgia. We measured physical activity and sedentary behaviour by accelerometers, and pain-related outcomes were objectively and subjectively measured; e.g., algometry and the 36-item short form health survey (i.e., bodily pain subscale), respectively. Age, body fat, and analgesics and antidepressants consumption were included were considered as potential confounders.

Results. The rs6311 and rs6313 polymorphisms (HTR2A gene) were individually related to algometer score (P=0.0007, FDR=0.025 and P=0.0017, FDR=0.032, respectively). The rs4818 and rs1799971 polymorphism (catechol-O-methyltransferase –COMT- and OPRM1 genes, respectively) were additively related to pain catastrophizing (P=0.00003, FDR=0.017). The rs1383914 (ADRA1A gene), rs6860 (CHMP1A gene), rs4680 and rs165599 (COMT gene), and rs12994338 (SCN9A gene) polymorphisms along sedentary behaviour showed a joint association with bodily pain (all, $P<0.05$ and $FDR\leq0.02$).

Conclusions. The ADRA1A, CHMP1A, COMT, HTR2A, OPRM1, and SCN9A genes were associated with pain-related outcomes in women with fibromyalgia. Therefore, the present study highlights the relevance of taking account the gene-gene and genotype-sedentary behaviour interactions when studying pain in fibromyalgia. Avoiding sedentary behaviour might be beneficial for reducing pain in women with fibromyalgia, particularly in some genotypes.

INTRODUCTION

People with fibromyalgia experience an hyperactive response to pain by the central nervous system [1,2]. Pain is present in all the proposed diagnostic criteria of fibromyalgia [3]. Therefore, research of this disease has extensively focused on pain as an outcome and in its related mechanisms. It is widely accepted that the experience of pain is promoted by both genetic susceptibility and environmental factors such as people's behaviours [4].

In fibromyalgia, the most often studied gene in relation to pain is the catechol-O-methyltransferase (COMT). By regulating the dopaminergic pathways, the COMT participates in the opioidergenic central processing of pain [5]. Among Spanish women with fibromyalgia, an early study found that the rs4818 polymorphism was related to self-reported pain [6]. For the first time in fibromyalgia, Tour and colleagues have recently conducted an inspiring study in which emerged an additive association of the opioid receptor μ1 (OPMR1) and serotonin transporter 5-HTT (5-HTTLPR) genes with pain modulation [7].

In addition to genotype individual associations and gene-gene interactions, when considering complex phenotypes such as pain, gene-environmental interactions are likely present and can help to better understand the disease (e.g., its unravel underlying mechanisms [8]). Greater levels of physical activity and lower levels of sedentary behaviour are both related to lower pain in fibromyalgia [9,10]. However, no previous research studied the additive association of genotype and physical activity or sedentary behaviour with pain. The understanding of this interaction might help to tailor the general advice of engaging in physical activity while reducing sedentary behaviour according to the genotype of people with fibromyalgia.

To overcome common caveats of the previous literature, the al-Ándalus project included 64 polymorphisms of 34 genes previously investigated in relation to fibromyalgia

susceptibility, symptoms, or potential mechanisms. Moreover, self-reports and a physical examination of pain (i.e., subjective and objective assessments, respectively) along with objectively measurements of physical activity and sedentary behaviour were performed in a representative sample of women with fibromyalgia from southern Spain. Therefore, in the present study we analysed the singular association of 64 polymorphisms of 34 fibromyalgia candidate-genes, as well as the gene-to-gene, gene-physical activity and gene-sedentary behaviour additive associations (i.e., interactions) with pain outcomes in a well-characterised sample of southern Spanish women with fibromyalgia.

MATERIALS AND METHODS

Participants

The participants (i) were recruited mainly via fibromyalgia associations from Andalusia (southern Spain), (ii) had been previously diagnosed with fibromyalgia by a professional rheumatologist and met the 1990 American College of Rheumatology (ACR) criteria for fibromyalgia, which was further confirmed by a tender point examination [11], and (iii) signed an informed consent form. The Ethics Committee of the *Virgen de las Nieves* Hospital (Granada, Spain) approved the study. We followed the ethical guidelines of the Declaration of Helsinki.

Genetic analysis

Samples were genotyped for 64 polymorphisms that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms (table 2). As described elsewhere [12,13], we collected buccal mucosa cells and performed DNA non-organic extraction (proteinase K and salting-out) procedures. Afterwards, we conducted a spectrophotometric quantification (NanoDrop 2000c, ThermoFisher, Waltham, Massachusetts, USA). All the samples were standardised to 50 ng/μL and they had an A260/230 ratio between 1.7 and 1.9. The samples in low concentration values were

processed by Genomiphi™ V2 DNA Amplification Kit (Sigma Aldrich St. Louis, Missouri, USA). Until being processed, all the samples were stored at -20º C. The OpenArray® genotyping was performed according to the manufacturer's protocol using Accufill™ system liquid robot that adds 1.2µl of genomic DNA sample and 3.8µl of the following reagents: 2×TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 20×Primer and TaqMan® Probe (FAM™ dye) mix, and sterile-filtered water. The table 3 shows the manufacturer thermal cycling conditions.

Plates include a NTC for each polymorphism in the analysis, and each plate has a total of 48 samples. The tables 4 and 5 provide further details about the TaqMan® OpenArray® custom assay designs and the 64 analysed polymorphisms, respectively. We performed a TaqMan™ OpenArray™ Genotyping Plate, Custom Format 64 QuantStudio™ 12K Flex. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Measures related to physical activity and sedentary behaviour

Triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA) were used to objectively measure physical activity and sedentary behaviour. Activity counts were measured at a rate of 30 hertz and stored at an epoch length of 60 second [14,15]. Participants wore the accelerometer on the hip up to 9 days; the first and last days were excluded from the analyses. A total of 7 continuous days with a minimum of 10 valid hours per day was required for being included in the study analysis. Sedentary behaviour and physical activity were calculated based upon recommended vector magnitude cut point [14,15]: <200 and ≥2690 count per minute for sedentary behaviour and moderate to vigorous physical activity, respectively. We used the manufacturer software (Actilife™ v.6.11.7 desktop) for data download, reduction, cleaning and analyses purposes. Further details are available elsewhere [16,17]. In the present study, we used binary data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity

recommendations (≥150 min/week of moderate to vigorous physical activity in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value.

Measures related to pain outcomes

Algometry. A standard pressure algometer (FPK 20; Wagner Instruments, Greenwich, CT, USA) was used to assess the 18 fibromyalgia tender points according to the 1990 ACR criteria for the diagnosis [11]. The pain pressure threshold was defined as the average threshold obtained at the 18 tender sites.

The revised fibromyalgia impact questionnaire (FIQ). The 'pain rating' question from was used to assess perceived pain intensity (on a 0 to 10 numeric rating scale) in the context of the past 7 days [18].

The 36-item short form health survey (SF-36). The SF-36 is a generic tool for the assessment of health-related quality of life. For this study, only the 'bodily pain' subscale was used, which assesses the perception of pain in the context of the past 4 weeks. The scores range from 0 to 100, with higher scores indicating lower bodily pain [19].

Visual analogue scale (VAS). A 100-mm VAS, annotated with the words 'no pain' and 'maximum of pain' at the appropriate ends was used to assess perceived pain intensity before the beginning of the fitness assessment. The distance between the beginning of the line representing 'no pain' and the pen mark expressing the patients' perception of pain was measured [20].

The Pain catastrophizing scale (PCS). The PCS is a 13-item questionnaire in which patients are asked to reflect on past painful experiences and indicate their thoughts or feelings about pain, on a 5-point scale. For this study, the total score (ranging from 0 to 52) was used, where higher scores represent a more negative appraisal of pain [21].

The Chronic pain self-efficacy scale (CPSS). The CPSS measures efficacy expectations for coping with pain. It contains 19 items grouped into 3 subscales (ranging 0-100): pain management, coping with symptoms, and physical function. The total score is the sum of the three subscales (ranging 0-300), where higher scores indicate higher self-efficacy [22].

Measures related potential confounders

Socio-demographic and clinical data. The participants self-reported their date of birth, marital status, and educational level.

Body fat (%) was measured using a portable eight-polar tactile-electrode impedanciometer (InBody R20, Biospace, Seoul, South Korea).

Consumption of drugs. The consumption of analgesics and antidepressants was registered as binary variables (yes/no).

Statistical analysis

The Hardy-Weinberg equilibrium (HWE; p>0.01) and linkage disequilibrium (r^2>0.5) were evaluated with 'genetics' R package [23]. We used general linear models using Gaussian error distributions to analyse the singular associations of polymorphisms with phenotypes (i.e., pain outcomes); age, body fat (%), and the consumption of analgesics and antidepressants were entered as covariates as they are potential confounders of the associations under study [24]. Interactions between polymorphisms of different genes as well as between polymorphisms and physical activity or sedentary behaviour were assessed using the same models but including the following interaction terms in separate models: gene*gene, gene*physical activity, or gene*sedentary behaviour.

Five genetic models (i.e., dominant, recessive, log-additive, codominant, and overdominant) were used for all analyses. Exceptionally, when the minor allele frequency was lower than 0.1, we analysed only the dominant model of those polymorphisms due to

their low number of minor homozygotes. For each polymorphism, P-values were computed using the likelihood ratio test between a model with the polymorphism or interaction term and the null model. Given that some of studied polymorphisms were in linkage disequilibrium (online supplementary figure 1), we considered as significant those associations with either P-values lower than the Bonferroni's correction or with p- and false discovery rate (FDR) values lower than 0.05.

All analyses were performed in the R environment 3.4.1. Gene-phenotype associations along with gene-to-gene interactions were assessed with the 'SNPassoc' package [25]. We developed our own script (available upon request) to study the gene-environment interactions.

RESULTS

Table 9 shows the characteristics of the 274 participants included in the present study.

Online supplementary figure 1 presents the linkage disequilibrium values. The rs7124442 (brain-derived neurotrophic factor antisense RNA, BDNF-AS, gene), rs7911 (guanylate binding protein 1, GBP1, gene) rs1050450 (glutathione peroxidase 1, GPX1, gene), rs4411417 (GTP cyclohydrolase 1, GCH1, gene), rs6323, rs1137070 (monoamine oxidase A, MAOA, gene), rs3746544 (synaptosome associated protein 25, SNAP25, gene) polymorphisms did not meet the HWE criteria. A low genotyping rate (i.e., ≤0.90) was observed for the rs9470080 (FK506 binding protein 5, FKBP5, gene), rs4371369, rs4387806, rs6746030, rs12620053 (sodium voltage-gated channel alpha subunit 9, SCN9A, gene), and rs7310505 (thioredoxin reductase 1, TXNRD1, gene) polymorphisms. The remaining 51 polymorphisms were included in the present study. Online supplementary figure 1 presents the linkage disequilibrium values.

Individual association between genotype and phenotype

The individual associations of the rs6311 and rs6313 polymorphisms (5-hydroxytryptamine receptor 2A, HTR2A, gene) and algometer score were significant. Under the overdominant model, figure 1 shows that carriers of the CC/TT genotype had higher pain thresholds (i.e., lower pain) than those with the CT genotype (P=0.0007, FDR=0.025 and P=0.0017, FDR=0.032, respectively). These two polymorphisms were in linkage disequilibrium (D'=0.98). The remaining individual associations between genotype and pain were not significant; online supplementary figures 2-7.

Gene-gene interaction

Figure 2 shows an additive association of the rs4818 and rs1799971 polymorphisms (COMT and OPRM1 genes, respectively) with pain catastrophizing under the codominant model (P=0.00003, FDR=0.017). Carriers of the CC genotype of the rs4818 polymorphism reported similar pain catastrophizing regardless of the genotype of the rs1799971 polymorphism. However, CG rs4818 carriers reported higher catastrophizing when they were GG carriers of the rs1799971 polymorphism than when they were AG carriers. The opposite finding was observed for the GG genotype of the rs4818 polymorphism. Conclusion regarding the AA genotype of the rs1799971 cannot be drawn given the low sample size ($n \leq 10$) in all the genotypes.

The interactions involving the rs1383914 polymorphism (adrenoceptor alpha 1A, ADRA1A, gen) with the rs752688 polymorphism (GTP cyclohydrolase 1, GCH1, gen), and rs1042713 polymorphism (adrenoceptor beta 2, ADRB2, gen) with rs429358 polymorphism (apolipoproteine E, APOE, gen), were significant. However, they were considered as false positives; none or one participant in some of the genotypes. The remaining gene-gene interactions were non-significant, online supplementary figures 8-37.

Gene-environment interaction

Genotype and physical activity were not additively related to any pain outcome (online supplementary figures 38-43). An exception was the interaction of the rs573542 polymorphism, ADRA1A gene, and physical activity with pain self-efficacy (P=0.0003, FDR=0.013). However, only 7 participants with the GA/GG genotype engaged in higher levels of physical activity, which suggest an insufficient statistical power to reach conclusions.

Figure 3 shows that the association of the genotypes of the rs1383914 (ADRA1A gene), rs6860 (charged multivesicular body protein 1A, CHMP1A, gene), rs4680 and rs165599 (COMT gene), and rs12994338 polymorphisms (SCN9A gene) with bodily pain (SF-36) differs according to the levels of sedentary behaviours of the participants (all, $P<0.05$ and $FDR\leq0.02$). Participants that engage in high levels of sedentary behaviours reported a similar pain regardless of their rs4680, rs6860, rs165599, and rs1383914 genotype. However, those who spent low time in sedentary behaviour showed a higher (i.e., better) score on bodily pain only if they were a particular genotype: AA/GG for rs4680, rs6860, rs165599 and AG for rs1383914. Moreover, participants with the CC/TT genotype of the rs12994338 polymorphism showed the worst bodily pain only when they engage in high sedentary behaviour.

The association of the rs25531 polymorphism (solute carrier family 6 member 4, SLC6A4, gene) and sedentary behaviour with acute pain (VAS score) lacked of statistical power yet it was significant (P=0.0002 and FDR=0.003). Only 18 participants were AG genotype, nine in each sedentary behaviour level. The remaining additive associations of genotype and sedentary behaviour with pain outcomes were not significant, online supplementary figures 44-49.

DISCUSSION

The present candidate-gene study including 64 polymorphisms of 34 genes is the most comprehensive on pain outcomes in fibromyalgia until date. We observed that the rs6311 and rs6313 polymorphisms (HTR2A gene) were individually related to algometer score, an objective measure of pain. The present research is unique because of the study of gene-gene and gene-environment interactions. We found significant interactions of the rs4818 and rs1799971 polymorphisms (COMT and OPRM1 genes, respectively) with pain catastrophizing, and of the rs1383914 (ADRA1A gene), rs6860 (CHMP1A gene), rs4680 and rs165599 (COMT gene), and rs12994338 (SCN9A gene) polymorphisms and sedentary behaviour with bodily pain (SF-36).

Although inconsistent across studies, a review concluded that the HTR2A gene is individually associated with susceptibility to fibromyalgia [26]. In our dataset, in comparison to the GT/TT, the GG genotype of the rs6311 and 6313 polymorphisms (HTR2A gene) was related to better algometer scores, i.e., an objective measure of pain threshold. In this line, among people with chronic low back pain, those with the GG genotype the rs6311 and 6313 polymorphisms showed the lowest disability score [27]. Overall, it seems that the GG genotype of the rs6311 and 6313 polymorphisms (HTR2A gene) may buffers the levels of pain experienced by people living with a chronic pain disease.

The HTR2A gene encodes one of the receptors for serotonin. The serotoninergic system has wide-ranging actions throughout the body, including an antinociceptive role in the dorsal horn of the descending tract of the spinal cord. In fibromyalgia, abnormalities in the serotoninergic system are present [28] and serotonin reuptake inhibitors is effective for treating pain [29]. Therefore, alterations in the HTR2A gene may be related to pain-related outcomes, which is consistent with our findings [30].

In the present study, the additive association of the COMT and OPRM1A genes (rs4818 and rs1799971 polymorphisms, respectively) was related to pain catastrophizing. Among carrier of the GG genotype of the rs1799971 polymorphism (OPMR1 gene), those participants with the

CG carriers of the rs4818 polymorphism (COMT gene) showed the highest pain catastrophizing. However, in those carrying the AG genotype of the rs1799971 polymorphisms, GG carriers of the rs4818 reported the highest pain catastrophizing. An inspiring study has recently suggested that opioid and serotonergic mechanisms (i.e., OPMR1 and 5-HTTLPR genes, respectively) are additively related to the modulation of hypoalgesia induced by exercise in women with fibromyalgia [31]. In this line, the present findings suggest that opioids may interact with other neurotransmitters as those regulated by the COMT gene (e.g., adrenaline, noradrenaline, and dopamine) to modulate women with fibromyalgia pain-related cognitions (i.e., catastrophizing). Moreover, this interaction is in agreement with the hypothesis of aberrances on the central nervous system on fibromyalgia [32]. Although the interaction of the COMT and OPMR1 genes had not been explored previously in fibromyalgia, their additive association with postoperative pain has been observed [33].

In fibromyalgia, the common co-occurrence of pain, depression, and fatigue points to the hypothalamic-pituitary-adrenal axis and sympathetic nervous system as potential determinants of the disease onset and prognosis [34–37]. In the central nervous system, the COMT gene modulates the production of catecholamines and other neurotransmitters that binding to adrenergic receptors, some of them modulated by the ADRA1A gene in the sympathetic nervous system. The dorsal root ganglia may be a player in the sympathetically maintaining of pain in fibromyalgia [38]. The hypothesis is that mutations in the SCN9A gene may lead to up-regulation of sodium channels, which drives to hyperexcitability of the dorsal root ganglia and, finally, to increased pain [38]. Other potential mechanisms involved in fibromyalgia pain oxidative stress [39] and excessive autophagy [4, 5], where the CHMP1A gene may play a role [40], via amygdala [39] and mTOR signalling [41] pathways, respectively.

In the present study, while the individual association of genotype and pain did not emerge, we found as significant the additive association of the rs4680, rs165599 (COMT gene), rs1383914 (ADRA1A gene), rs12994338 (SCN9A gene), and rs6860 (CHMP1A gene) polymorphisms and sedentary behaviour with pain (as assessed with the bodily pain subscale of

the SF-36). It is noteworthy that people's behaviours may modulate the activity of these mechanisms [37,42–44]. Fleshner [45] highlighted that sedentary people may be particularly sensitive to stress, which is in line with our findings. Therefore, it seems that, in women with fibromyalgia, to avoid sedentary behaviour may attenuate the genetic predisposition to increased pain.

In fibromyalgia, the general advice to manage the disease is to combine pharmacological and physical exercise treatments [46–48]. However, no universally effective treatment is available for fibromyalgia, which may be a consequence of the heterogeneity observed in this population [49]. Therefore, the characteristics of people with fibromyalgia should be attended. In line with the literature, our results suggest a potential role of the sympathetic nervous system, dorsal root ganglia, and hypothalamic-pituitary-adrenal axis throughout genetic modulation of neurotransmitters and opioids. Accordingly, the challenge would be to better modulate the functioning of these mechanisms potentially involved in fibromyalgia pain. Regarding pharmacological therapy, the present findings suggest that serotonin reuptake inhibitors could be effective analgesic in this profile of patients with such genetic predisposition. Regarding physical programmes, to reduce time spends on sedentary behaviour might be particularly beneficial to reduce pain in women with fibromyalgia that are carriers of a particular genotype as follows: AA/GG for rs6860 (CHMP1A gene), rs4680, rs165599 (COMT gene), AG for rs1383914 (ADRA1A gene), and CC/TT for rs12994338 (SCN9A gene).

The experience of fibromyalgia (as any chronic pain experience) encompasses multiple interacting factors, in addition to biological and behavioural, psychological factors are also crucial [50,51]. However, the present study did not include the interplay of psychological factors with genetics and behaviour of women with fibromyalgia, which is a limitation. Further research testing more sophisticated models (e.g., mobile toy model [50]) is warranted. Although unlikely, we welcome a replication study with an independent sample in order to test the robustness of the present findings. Our sample size may be seen as another limitation, particularly to evaluate the interaction of gene and people's behaviour. Thus, some of our non-significant findings could be

indeed statistically significant in larger samples. However, we consider that compared with previous studies, our sample size is a strength of the present study as it is relatively large (e.g., [6,52]). We also corroborated the rheumatologist' fibromyalgia diagnosis according to the 1990 ACR fibromyalgia criteria [11], and it was representative of the southern Spanish population of women with fibromyalgia [53]. Another strength was the inclusion of 64 polymorphisms of 34 candidate-genes made this study most comprehensive research of genetics and pain-related outcomes in fibromyalgia. Furthermore, we objectively measured physical activity and sedentary behaviour, while pain was objectively and subjectively assessed in order to understand also the experience of living with fibromyalgia. Finally, we adjusted our analyses for multiple comparisons, which support the robustness of our findings.

To conclude, the present candidate-gene study is the most comprehensive on pain outcomes in fibromyalgia until date. For the first time, we identified (i) individual associations of the rs6311 and 6313 polymorphisms (HTR2A gene) with algometer score; i.e., an objective measure of pain; (ii) additive associations of the rs4818 and rs1799971 polymorphisms (COMT and OPRM1 genes, respectively) with pain catastrophizing; and (iii) additive associations of the rs4680, rs165599 (COMT gene), rs1383914 (ADRA1A gene), rs12994338 (SCN9A gene), and rs6860 (CHMP1A gene) polymorphisms and sedentary behaviour with the bodily pain (SF-36). Therefore, the present study highlights the relevance of taking account the gene-gene and genotype-sedentary behaviour interactions when studying pain outcomes in women with fibromyalgia. If corroborated in future (observational and experimental) longitudinal research, our findings might suggest that to avoid sedentary behaviour may beneficial for reducing pain particularly in some genotypes of women with fibromyalgia.

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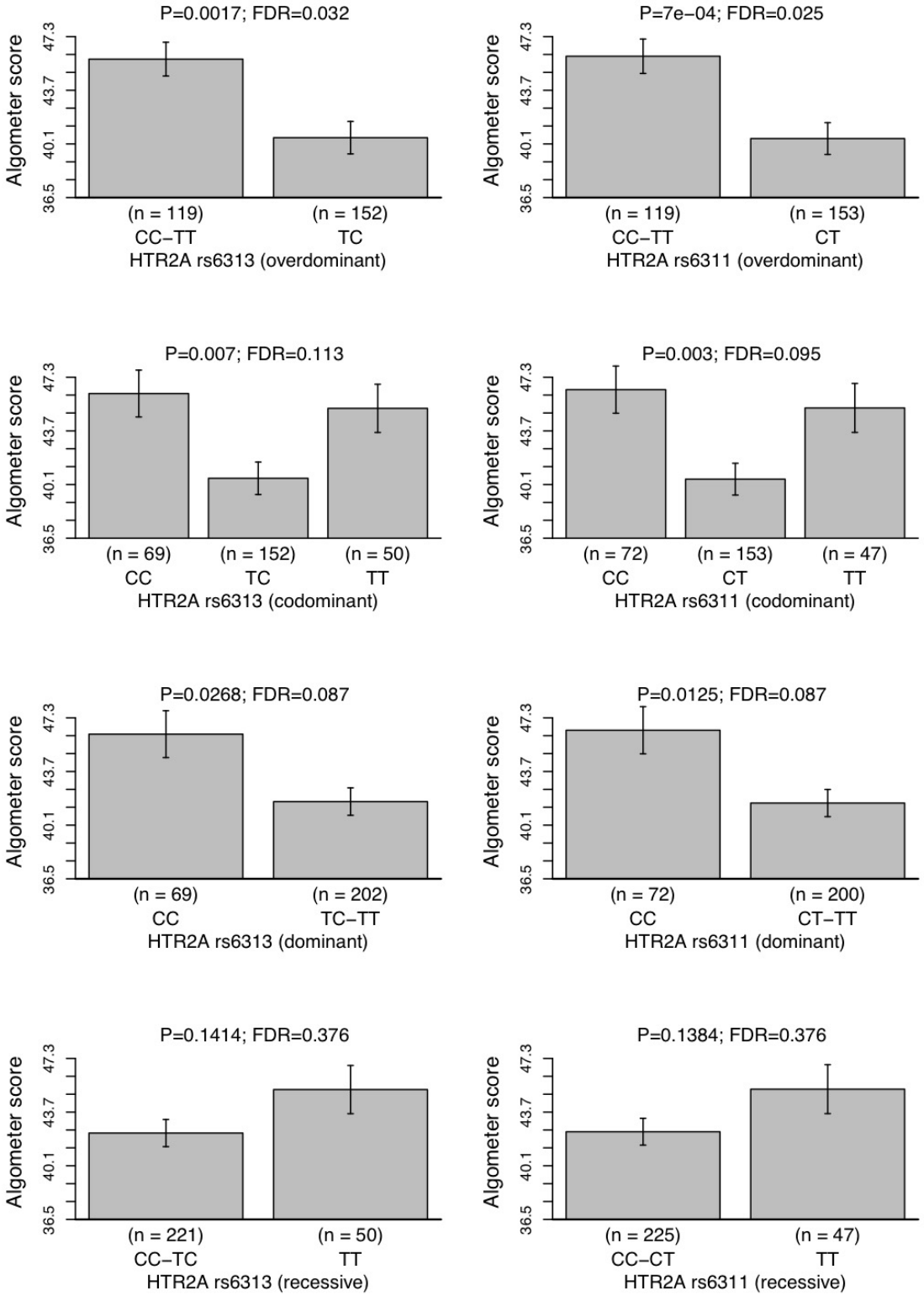

Figure 1. Individual associations of the genotype of the rs6311 and rs6313 polymorphisms (HTR2A gene) with algometer score (algometry).

HTR2A, 5-hydroxytryptamine receptor 2A gene; According to the p- and false discovery rate (FDR) values, the significance was reached only under the overdominant model.

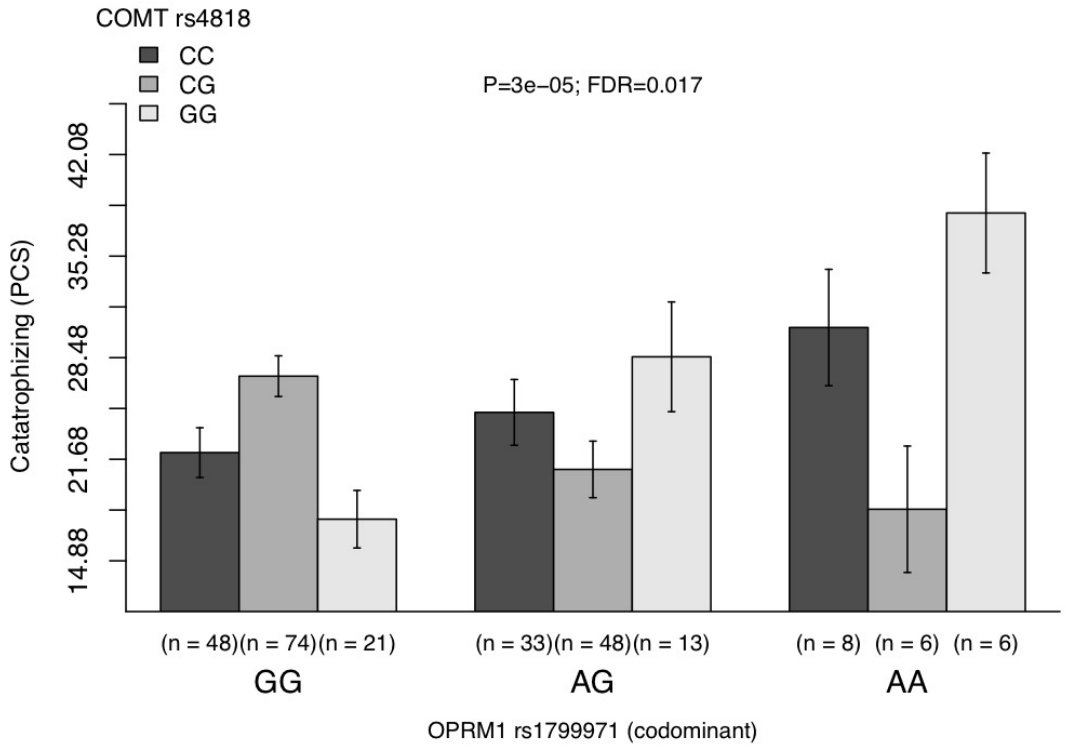

Figure 2. Gene-gene interaction of the rs4818 and rs1799971 polymorphisms with satisfaction with life.

COMT, catechol-O-methyltransferase gene; OPRM1, opioid receptor μ gene; PCS, pain catastrophizing scale (scores range 0-52); According to the p- and false discovery rate (FDR) values, this gene-gene interaction was significant. Given the lack of statistical power ($n \leq 10$ in some groups), conclusions regarding carriers of the AA genotype of the rs1799971 are precluded.

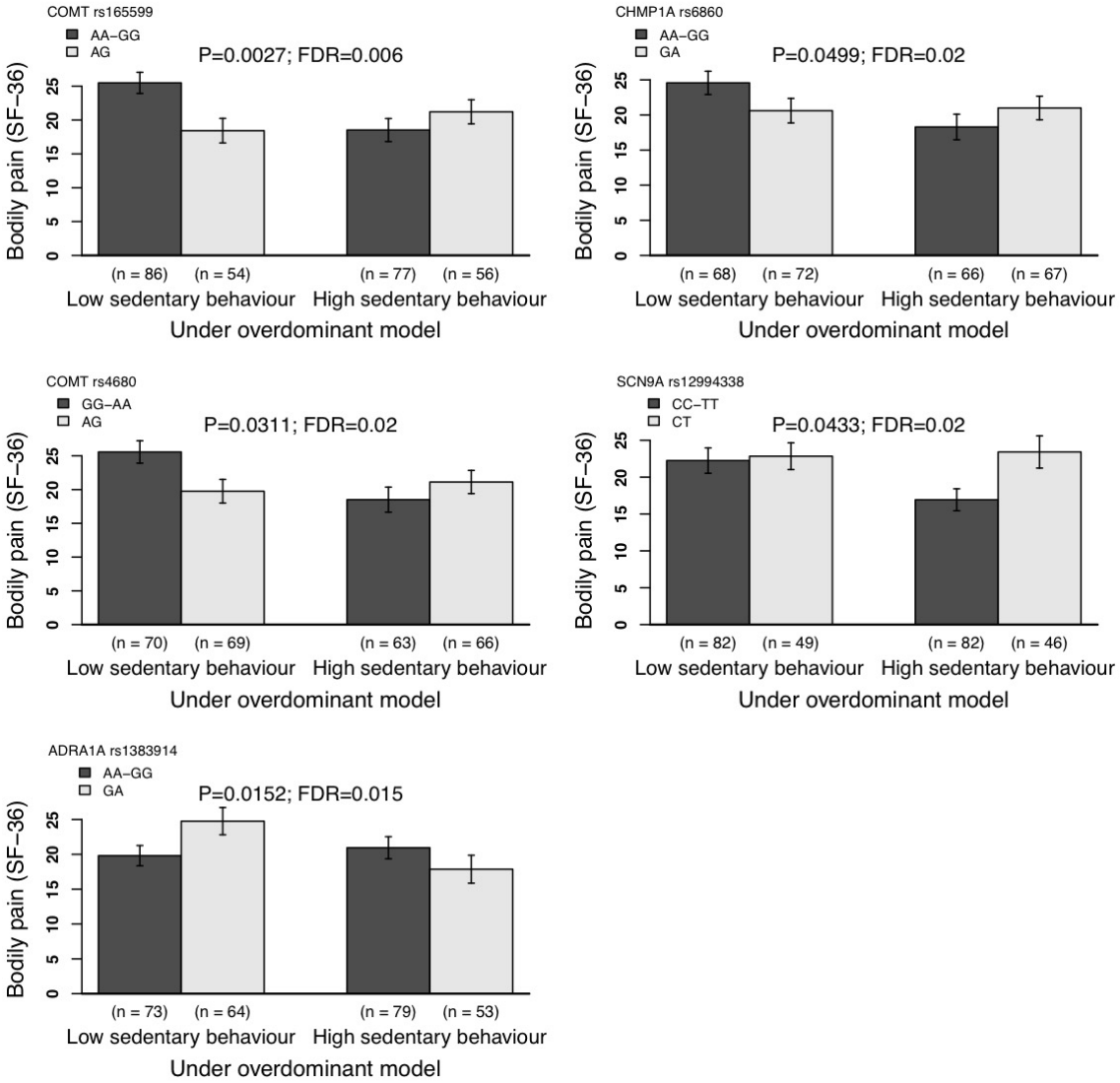

Figure 3. Additive association gene-physical activity or gene-sedentary behaviour with pain outcomes.

ADRA1A, adrenoceptor alpha 1A gene; CHMP1A, charged multivesticular body proteints 1A gene; COMT, catechol-O-methyltransferase gene; SCN9A, sodium voltage-gated channel alpha subunit 9 gene. SF-36, the 36-item short form health survey (scores range 0-100).

Sedentary behaviour was objectively measured using triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA) as dichotomised data (low vs. high) using the mean as the cut-off value.

According to the p- and false discovery rate (FDR) values, all these gene-sedentary behaviour interactions were significant.

Table 9. Characteristics of the participants in the study, *n*=274

	n (%)
Education level	
Unfinished studies	26 (9.5)
Primary	136 (49.6)
Secondary (and vocational)	81 (29.6)
University	31 (11.3)
Marital status	
Married	214 (78.1)
Single	21 (7.7)
Separated/divorced	27 (9.9)
Widow	12 (4.4)
Working status	
Working	73 (26.6)
Household	91 (33.2)
Incapacity pension or sick leave	55 (20.1)
Unemployed	46 (16.8)
Others	9 (3.3)
Drugs consumption (yes vs. no)	
Analgesics (yes)	245 (89.8)
Antidepressants (yes)	145 (52.9)
	Mean (SD)
Age, years old	51.7 (7.7)
Body fat (%)	40.5 (7.6)
Tender points count	16.9 (1.8)
Physical activity (accelerometers, min/week)	
Moderate-to-vigorous physical activity	86.9 (118.9)
Sedentary behaviour	459.1 (108.1)
Pain-related outcomes	
Algometer score (Algometry)	42.8 (13.2)
Pain rating (FIQR, 0-10)	7.3 (1.7)
Visual analogue scale (0-100)	6.5 (2.2)
Pain catastrophizing (PCS, 0-52)	21.2 (24.5)
Pain self-efficacy (CPSS, 0-100)	36.4 (22.9)

SD, standard Deviation; FIQR, revised fibromyalgia impact questionnaire; SF-36, 36-item short form health survey; PCS, pain catastrophizing scale; CPSS, chronic pain self-efficacy scale.

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STUDY V: Additive association of physical activity and the sodium channel protein type 9 subunit alpha and methylene tetrahydrofolate reductase genes with fatigue.

Submitted

ABSTRACT

Objectives. To analyse the single association of 64 single nucleotide polymorphisms of 34 fibromyalgia candidate-genes and the gene-gene and gene-physical activity behaviour interactions with fatigue in southern Spanish women with fibromyalgia.

Methods. We extracted DNA from saliva of 276 fibromyalgia women to analyse polymorphisms of genes related to fibromyalgia susceptibility, symptoms, or potential mechanisms. Accelerometers registered the participants' physical activity and sedentary behaviour levels for 7 consecutive days. Fatigue was self-reported in the Multidimensional Fatigue Inventory. Age, body fat (%), and analgesics and antidepressants consumption were considered as confounders in all the analyses. Based on the Bonferroni's and False Discovery Rate (FDR) values, the significance was interpreted.

Results. AT carriers of the rs4453709 polymorphism (sodium channel protein type 9 subunit alpha, SCN9A, gene) showed the highest reduced motivation. Carriers of the heterozygous genotype of the rs1801133 (methylene tetrahydrofolate reductase, MTHFR, gene) and rs4597545 (SCN9A gene) polymorphisms who spent more time on physical activity reported lower levels of fatigue than those who did not. The homozygous genotype of the rs7607967 polymorphism (i.e., AA/GG genotype; SCN9A gene) appraised their fatigue as lower when they engaged in low time in sedentary behaviour than those that spent high sedentary time.

Conclusions. To carry the AT genotype of the rs4453709 polymorphism (SCN9A gene) was individually related to worse scores on reduced motivation. Additionally, physical activity behaviours and the SCN9A and MTHFR genes were jointly related to fatigue. Thereby, the

potential benefits of following an active lifestyle might be observed more clearly in women with fibromyalgia genetically predispose to higher levels of fatigue.

INTRODUCTION

Fibromyalgia is a common disease characterized by chronic widespread pain and increased sensitivity to painful stimuli [1]. In fibromyalgia, fatigue is markedly prevalent [2]. People living with the disease identify fatigue as one of the main symptoms of fibromyalgia [3]. Therefore, the diagnosis of fibromyalgia is undergoing some changes as, for instance, with the inclusion of fatigue as part of the diagnostic [4].

It is hypothesised that the pathogenesis of fibromyalgia involves a genetic susceptibility that is modulated by environmental factors [5]. Previous research extensively focused on pain-related outcomes [6]. Given that pain and fatigue are often experienced concurrently in fibromyalgia [7], an overlap in their aetiological mechanisms seems plausible [8]. For instance, the catechol-O-methyltransferase (COMT) gene, which has been widely studied because of its association with pain, is linked to fatigue in Spanish and Mexican people with fibromyalgia [9]. Among the environmental exposures, to spend more time on physical activity and less on sedentary behaviour is related to lower fatigue [10–12].

A comprehensive understanding of the singular association of genotype, and the additive association (i.e., interaction) between genes as well as between genes and physical activity behaviour may help to better understand the biological and behavioural mechanisms of fatigue, which is of interests in fibromyalgia. Thus, the present study examined the singular association of 64 single nucleotide polymorphisms of 34 fibromyalgia candidate-genes, as well as the additive associations (i.e., interactions) of gene-gene, gene-physical activity, and gene-sedentary behaviour with fatigue in southern Spanish women with fibromyalgia.

MATERIALS AND METHODS

Participants

The participants (i) were recruited mainly via fibromyalgia associations from Andalusia (southern Spain), (ii) had been previously diagnosed with fibromyalgia by a professional

rheumatologist and met the 1990 American College of Rheumatology (ACR) criteria for fibromyalgia, which was further confirmed by a tender point examination [1], and (iii) signed an informed consent form. The Ethics Committee of the *Virgen de las Nieves* Hospital (Granada, Spain) approved the study. We followed the ethical guidelines of the Declaration of Helsinki.

Genetic analysis

Samples were genotyped for 64 polymorphisms that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms (see, table 2). We collected buccal mucosa cells and performed DNA non-organic extraction (proteinase K and salting-out) procedures. Afterwards, we conducted a spectrophotometric quantification (NanoDrop 2000c, ThermoFisher, Waltham, Massachusetts, USA). See tables 3 to 5 for further details. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Measures related to physical activity and sedentary behaviour

Triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA) were used to objectively measure physical activity and sedentary behaviour for 9 days. In the present study, we used binary data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of moderate to vigorous physical activity in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value.

Measures related to fatigue

The *Multidimensional Fatigue Inventory (MFI)* [13] was used to assess general fatigue, physical fatigue, mental fatigue, reduced activity, and reduced motivation.

Statistical analysis

All analyses were performed in the R environment 3.4.1. The Hardy-Weinberg equilibrium (HWE; p>0.01) and linkage disequilibrium (r^2>0.5) were evaluated with 'genetics' package [14]. Gene-phenotype associations along with gene-gene interactions were assessed with the 'SNPassoc' package [15]. We developed our own script to study gene-environment interactions.

To analyse the singular associations of polymorphisms with phenotypes, we compute general linear models with age, body fat (%), and the consumption of analgesics and antidepressants as covariates. Interactions between polymorphisms of different genes as well as between polymorphisms and physical activity or sedentary behaviour were assessed using the same models but including their interaction terms in separate models. We considered as significant those associations with either P-values lower than the Bonferroni's correction or with p- and false discovery rate (FDR) values lower than 0.05.

RESULTS

Table 10 shows the characteristics of the 276 participants included in the present study.

The rs6323, rs7911, rs806377, rs1050450, rs1137070, rs3746544, rs4411417, and rs7124442 polymorphisms did not meet the HWE criteria. A low genotyping rate (i.e. ≤0.90) was observed for the rs4371369, rs4387806, rs6746030, rs7310505, rs9470080, and rs12620053 polymorphisms. The remaining 50 polymorphisms were included in the present study. Supplementary figure 1 presents the linkage disequilibrium values.

Figure 4 shows that the AT genotype of the rs4453709 (SCN9A gene) showed highest levels of reduced motivation than the AA/TT genotype; overdominant model, P=0.0004, FDR=0.016. The remaining individual associations between genotype and fatigue outcomes were not significant; supplementary figures 2-6.

All the gene-gene interactions were not significant (see supplementary figures 7-31).

Figure 5 shows the significant gene-environment interactions. Among carriers of the CT genotype of the rs1801133 (methylene tetrahydrofolate reductase, MTHFR, gene), those who met the physical activity recommendations showed lower physical fatigue and reduced motivation than those who did not met such physical activity levels; P=0.0002, FDR=0.01 and P=0.0025 and FDR=0.042, respectively. In the participants carrying the CG genotype of the rs4597545 (SCN9A gene), in comparison to those who engage in low levels, the participants that had increased levels of physical activity reported lower mental fatigue; which was corroborated across different models: P=0.0003 and FDR=0.012 for codominant, P=0.0058 and FDR=0.047 for recessive, and P=0.0001 and FDR=0.004 for overdominant. In those participants carrying the AA/GG genotype of the rs7607967 (SCN9A gene), low sedentary behaviour was associated with lower scores on the reduced activity dimension of the MFI (P=0.0012, FDR=0.048 for the overdominant model).

A set of statistically significant additive associations were not interpreted as such given that they lacked of statistical power (i.e., $n \leq 10$ in some genotypes): of the rs1801133 (MTHFR gene) and physical activity with mental fatigue, and of the rs6860 (CHMP1A gene) and physical activity with mental fatigue, both under the recessive model. The remaining additive associations of genotype and people's behaviours with resilience outcomes did not reach the significance; supplementary figures 32-36 and 37-41 for physical activity and sedentary behaviour, respectively.

DISCUSSION

The present study conducted in southern Spanish women with fibromyalgia showed that the genotype of the rs4453709 (SCN9A gene) was individually related to reduced motivation. Gene-gene interactions were not related to the phenotype of fatigue. We observed additive associations of the genotype (i) of the rs1801133 (MTHFR gene) and physical activity levels with physical fatigue and reduced motivation, (ii) of the rs4597545 (SCN9A gene) and physical activity levels with mental fatigue, and (iii) of the rs7607967 (SCN9A gene) and sedentary behaviour levels with the reduced activity dimension of the fatigue phenotype.

It is widely accepted that a chronically hyperactive central nervous system is part of the pathology of fibromyalgia [16,17]. In this disease, it has been recently hypothesised that, the dorsal root ganglion may also be hypersensitive to pain stimuli [18]. In the present study, we found that among the rs4453709 genotype (SCN9A gene), AT carriers reported the highest reduced motivation. The SCN9A gene encodes a specific type of sodium channels (i.e., the Na(v)1.7) that are highly located in the dorsal horn in the spinal cord and in the dorsal root ganglion, the first structure is part of central nervous system while the latter receives afferent information from the peripheral nervous system. Thus, our findings support that the central nervous system is involved in the experience of levels of fatigue in women with fibromyalgia; which does no necessarily precludes a role of the peripheral one [19].

The Na(v)1.7 channels are crucial for pain signalling [20]. It is noteworthy that pain and fatigue often co-exist in fibromyalgia [7] and their mechanistic pathways may be shared [8]. Moreover, the Na(v)1.7 channels are not exclusively related to pain but also in other sensory stimuli such as acid sensing [21] and cough reflex [22]. Therefore, our findings seem to extend the implication of the Na(v)1.7 channels from pain to fatigue in women with fibromyalgia. Sodium channels are key in the generation and conduction of action potentials. Thus, the SCN9A gene by modulating the Na(v)1.7 channels function might be involved in the ample array of symptoms experienced by people with fibromyalgia; e.g., feeling physically, emotionally, and cognitively weak to face activities of daily living. However, this speculation needs to be corroborated in future research.

A number of studies have shown that higher time spent on physical activity and lower in sedentary behaviour are related to a better prognosis of the fibromyalgia [23–25], including fatigue [10–12]. These behaviours may modulate the potential effects that the people's genotype has on their phenotypes [26,27]. Previous research on candidate-genes of fibromyalgia symptoms, however, did not account for these behaviours. In the present study, the heterozygous carriers of the rs1801133 (MTHFR gene) and rs4597545 polymorphisms (SCN9A gene) who met the physical activity recommendations reported lower levels of fatigue than those who did not.

Furthermore, the homozygous participants of the rs7607967 polymorphism (i.e., AA/GG genotype; SCN9A gene) appraised their fatigue as lower when they engage low (i.e., below the sample mean) time in sedentary behaviour than those that spent high time (i.e., above the sample mean). Therefore, we provided early evidence confirming that genotype and behaviours of people with fibromyalgia were additively associated with fatigue-related outcomes.

The MTHFR gene encodes an enzyme that is central in the folate metabolism as a participant on the methionine-homocysteine cycle, which leads to DNA methylation [28]. The folate metabolism is key for feeding other biochemical cycles; its final product is an essential precursor for several neurotransmitters (e.g., serotonin) [29], some of them are related to fatigue (e.g., dopamine [30]). Previous literature suggested that the genotype of the rs1801133 (MTHFR gene) is associated with fatigue in people with migraine [31], and with stiffness and dryness in fibromyalgia [32]. Interestingly, in the present study the association of the rs1801133 (MTHFR gene) genotype and fatigue differs according to the physical activity levels of our participants. Additionally, metabolite abnormalities in the hippocampus of women with fibromyalgia are also related to the clinical picture of this disease [33]. It must to be noted that the hippocampus is a core centre in the appraisal of stress. In line with our findings, physical exercise improve the levels of metabolites [34] as well as the angiogenesis, neurogenesis, and connectivity of the hippocampus [35].

The finding of the present study should be considered in lights of its limitations. Although unlikely, we welcome a replication study with an independent sample in order to test the robustness of the present findings. For some polymorphisms, our sample size was not large enough for testing the gene-people's behaviours interaction. On the other hand, we included a large number of candidate-genes and physical activity and sedentary behaviours were objectively measured for 7 consecutive days. Furthermore, our results were adjusted for multiple comparisons.

In conclusion, we observed an association between the genotype of the rs4453709 polymorphism (SCN9A gene) and reduced motivation. We also found additive associations of

the genotype (i) of the rs1801133 polymorphism (MTHFR gene) and physical activity levels with physical fatigue and reduced motivation, (ii) of the rs4597545 polymorphism (SCN9A gene) and physical activity levels with mental fatigue, and (iii) of the rs7607967 polymorphism (SCN9A gene) and levels of sedentary behaviour with the reduced activity dimension of the fatigue phenotype. Thereby, the benefits of following an active lifestyle might differ between women with fibromyalgia according to their genotype.

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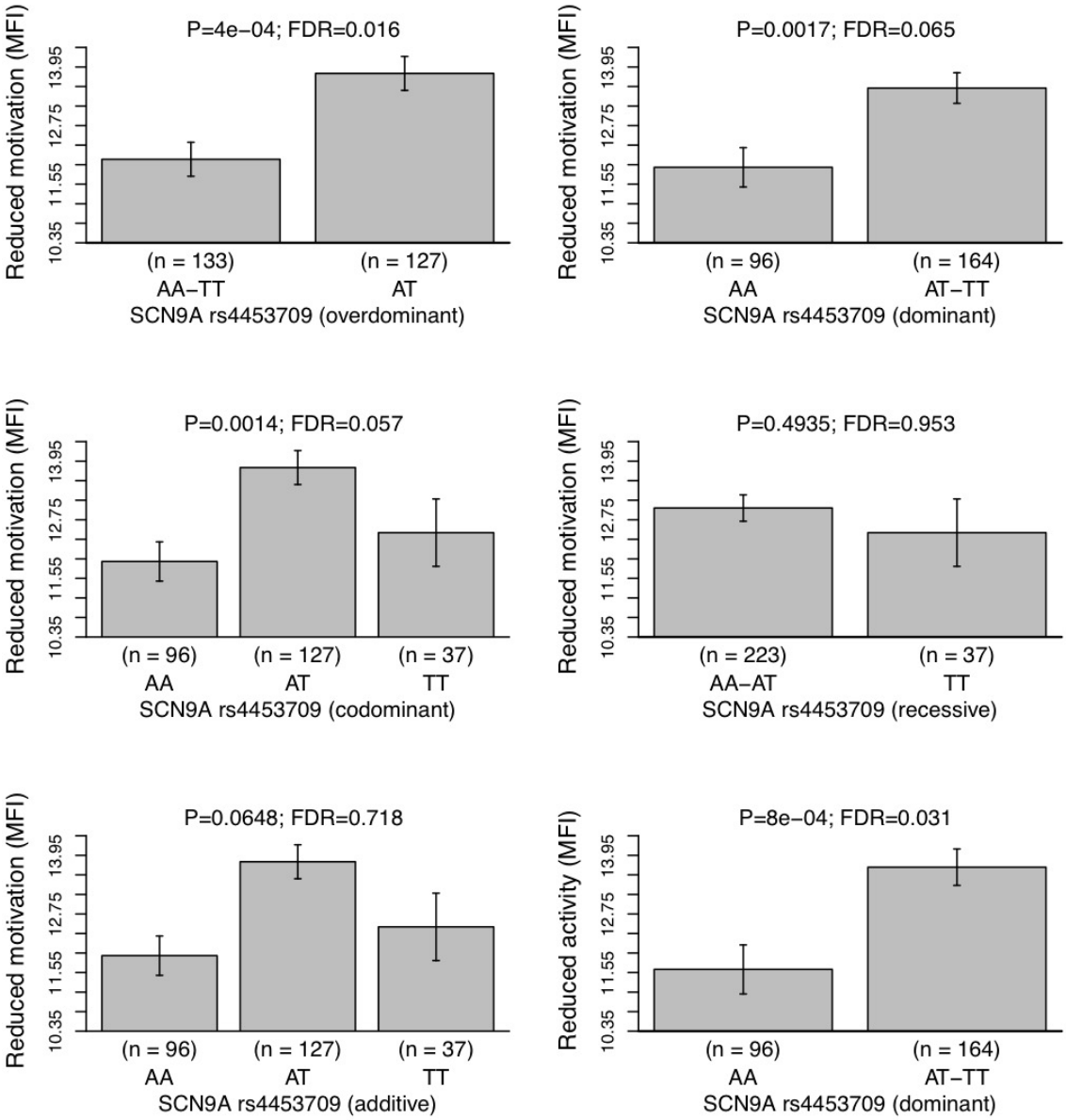

Figure 4. Individual associations of the genotype of the rs4453709 polymorphism (SCN9A gene) with reduced motivation and reduced activity

SCN9A, sodium voltage-gated channel alpha subunit 9 gene; MFI, multidimensional fatigue inventory (MFI, scores range 0-20); According to the p- and false discovery rate (FDR) values, the significance was reached only under the overdominant model for reduced motivation and under the dominant model for reduced activity.

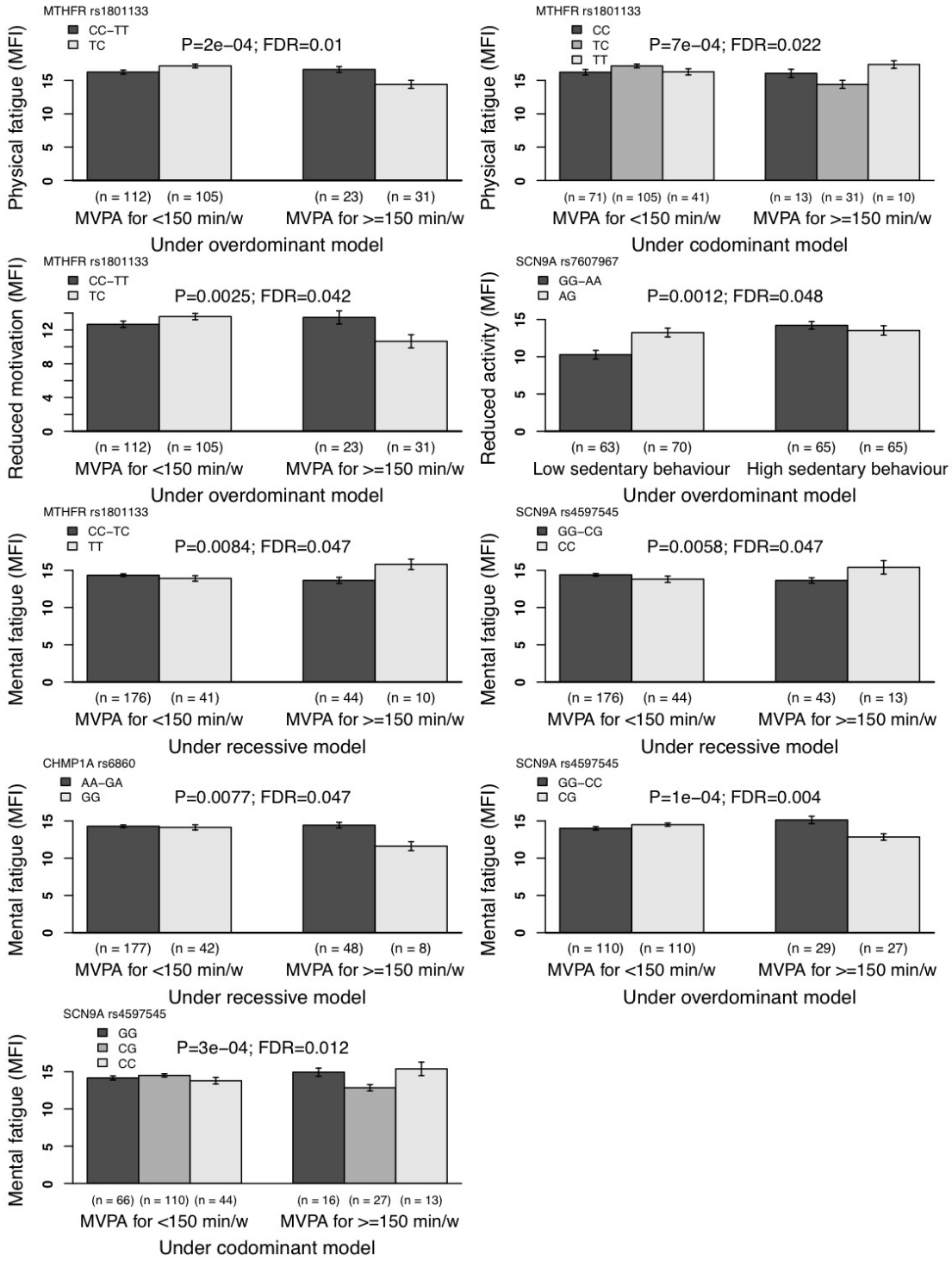

Figure 5. Additive association gene-physical activity or gene-sedentary behaviour with resilience outcomes

CHMP1A, charged multivesicular body protein 1A gene; MTHFR, methylene tetrahydrofosfate reductase gene; SCN9A, sodium voltage-gated channel alpha subunit 9 gene; MVPA, moderate to vigorous physical activity; MFI, multidimensional fatigue inventory (scores range 0-20).

Physical activity and sedentary behaviour were objectively measured using triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA). We dichotomised data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of MVPA in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value.

According to the p- and false discovery rate (FDR) values, all these gene-physical activity or gene-sedentary behaviour interactions were significant. However, the interactions of rs1801133-physical activity with mental fatigue (recessive model) and physical fatigue (codominant model), of the rs6860-physical activity with mental fatigue, and of the rs7607967-sedentary behaviour with reduced activity lacked of statistical power; $n \leq 10$ in some groups.

Table 10. Characteristics of the participants in the study, *n*=276

	n (%)
Education level	
Unfinished studies	26 (9.4)
Primary	139 (50.4)
Secondary (and vocational)	80 (29.0)
University	31 (11.2)
Marital status	
Married	215 (77.9)
Single	21 (7.6)
Separated/divorced	27 (9.8)
Widow	13 (4.7)
Working status	
Working	74 (26.8)
Household	93 (33.7)
Incapacity pension or sick leave	55 (19.9)
Unemployed	45 (16.3)
Others	9 (3.3)
Drugs consumption (yes vs. no)	
Analgesics (yes)	247 (89.5)
Antidepressants (yes)	147 (53.3)
	Mean (SD)
Age, years old	51.8 (7.7)
Body fat (%)	40.4 (7.6)
Tender points count	16.9 (1.8)
Physical activity (accelerometers, min/week)	
Moderate-to-vigorous physical activity	87.0 (119.2)
Sedentary behaviour	459.1 (107.9)
Fatigue (MFI, 4-20)	
General fatigue	18.0 (2.5)
Physical fatigue	16.4 (3.1)
Reduced activity	12.8 (4.9)
Reduced motivation	12.9 (4.0)
Mental fatigue	14.7 (2.4)

SD, standard Deviation; MFI, multidimensional fatigue inventory

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STUDY VI: Gene-gene and gene-environment interactions related to resilience in Spanish women with fibromyalgia: the sodium voltage-gated channel alpha subunit 9 (SCN9A) gene as a marker of resilience. *Submitted*

ABSTRACT

Objectives. To analyse the single associations of 64 single nucleotide polymorphisms of 34 fibromyalgia candidate-genes, and the additive associations (i.e., interactions) of gene-gene and gene-physical activity behaviour with resilience in Spanish fibromyalgia women.

Methods. We collected saliva samples using buccal swabs for DNA extraction to 276 fibromyalgia women. We analysed 64 main SNPs of 34 genes related to fibromyalgia susceptibility, symptoms, or potential mechanisms. Physical activity and sedentary behaviour were measured by accelerometry and resilience by questionnaires. Age, body fat, and analgesics and antidepressants consumption were included were considered as potential confounders.

Results. Participants carrying the AT genotype of the rs4453709 polymorphism (SCN9A gene) reported the lowest optimism [P=0.0005, false discovery rate (FDR)=0.015]. The rs4680 and rs6860 polymorphisms (COMT and CHMP1A genes, respectively) were additively related to satisfaction with life (P=0.00005, FDR=0.045). We observed a significant additive association of the rs4906902 polymorphism (GABRB3 gene) and physical activity with satisfaction with life (P=0.009, FDR=0.038), and of two polymorphism of the SCN9A gene with emotional regulation: rs4597545 (P=0.0017, FDR=0.038) and rs6754031 (P=0.002, FDR=0.042).

Conclusions. We have identified the CHMP1A, COMT, GABRB3, and SCN9A gene-related polymorphisms as potential markers of resilience in fibromyalgia women. Given its individual and additive associations, the SCN9A gene seems to be of particular interests. To engage in an active lifestyle may buffer the negative effects of having a genotype related to poor resilience.

INTRODUCTION

Fibromyalgia is a common disease characterised by the presence of chronic widespread musculoskeletal pain [1]. Probably due to the high heterogeneity observed in this population [2], there is not a consensus on the causes of and the mechanisms involved in fibromyalgia [3]. The available literature suggests that the onset and prognosis of fibromyalgia are related to genetic susceptibility and environmental factors such as people's behaviours [3–5]. In fibromyalgia, however, there is a lack of knowledge on the interplay of genetic and environmental factors [6].

Candidate-gene studies have been mostly focused on depression. However, individual differences are key on the successful adaptation to living with fibromyalgia [7–10]. In the face of stress, resilience is the ability of maintaining a positive functioning while vulnerability is the susceptibility of being fragile. Higher levels of resilience and lower levels of vulnerability are related to better adaptation to fibromyalgia [2,9,10]. To identify candidate-genes of the phenotype of resilience would allow understanding its biological pathways [11].

Literature suggests that a chronically hyperactive brain, where harmless stimuli are signalled as harmful, may be involved in fibromyalgia [12]. In that scenario, several areas and neurotransmitters might play a role in resilience to stress [13]. For instance, the hypothalamic-pituitary-adrenal (HPA) axis and hippocampus play a key role in the response to stress [14,15], including the processing of emotions. The aperture of sodium channels is essential for neurons depolarization, which turns in action potentials [16]. A candidate-gene for modulating the sodium channels located in the hypothalamus is the sodium voltage-gated channel alpha subunit 9 (SCN9A) gene [17–19]. Additionally, the gamma-aminobutyric acid type A receptor beta 3 subunit (GABRB3) gene encodes a protein, expressed at different levels of the brain (e.g., hippocampus), that receives the gamma-aminobutyric acid –the major inhibitory neurotransmitter of the nervous system [20,21].

The previous candidate-gene study of resilience usually presents several limitations. First, even though genes may interact, the additive association of different genes is omitted. In

fibromyalgia, a inspiring research has shown that the opioid receptor μ1 (OPMR1) and serotonin transporter 5-HTT (5-HTTLPR) genes are additively related to pain modulation [22]. Second, people's behaviours are often not considered. However, in the understanding of complex phenotypes such as resilience, gene-environmental interactions may be informative [23]. In this regard, physical activity is a particularly interesting behaviour [24,25]. Therefore, the present study analysed the singular association of 64 single nucleotide polymorphisms of 34 fibromyalgia candidate-genes, as well as the additive associations (i.e., interactions) of gene-gene and gene-physical activity behaviour with resilience outcomes in women with fibromyalgia from southern Spain.

MATERIALS AND METHODS

Participants

The participants (i) were recruited mainly via fibromyalgia associations from Andalusia (southern Spain), (ii) had been previously diagnosed with fibromyalgia by a professional rheumatologist and met the 1990 American College of Rheumatology (ACR) criteria for fibromyalgia, which was further confirmed by a tender point examination [1], and (iii) signed an informed consent form. The Ethics Committee of the *Virgen de las Nieves* Hospital (Granada, Spain) approved the study. We followed the ethical guidelines of the Declaration of Helsinki.

Genetic analysis

Samples were genotyped for 64 polymorphisms that had been previously investigated in relation to fibromyalgia susceptibility, symptoms, or potential mechanisms (table 2). As described elsewhere [26,27], we collected buccal mucosa cells and performed DNA non-organic extraction (proteinase K and salting-out) procedures. Afterwards, we conducted a spectrophotometric quantification (NanoDrop 2000c, ThermoFisher, Waltham, Massachusetts, USA). All the samples were standardised to 50 ng/μL and they had an A260/230 ratio between 1.7 and 1.9. The samples in low concentration values were processed by Genomiphi™ V2 DNA Amplification Kit (Sigma Aldrich St. Louis, Missouri, USA). Until being processed, all the

samples were stored at -20º C. The OpenArray® genotyping was performed according to the manufacturer's protocol using Accufill™ system liquid robot that adds 1.2µl of genomic DNA sample and 3.8µl of the following reagents: 2×TaqMan® Universal PCR Master Mix, No AmpErase® UNG, 20×Primer and TaqMan® Probe (FAM™ dye) mix, and sterile-filtered water. The table 3 shows the manufacturer thermal cycling conditions.

Plates include a NTC for each polymorphism in the analysis, and each plate has a total of 48 samples. The tables 4 and 5 provide further details about the TaqMan® OpenArray® custom assay designs and the 64 analysed polymorphisms, respectively. We performed a TaqMan™ OpenArray™ Genotyping Plate, Custom Format 64 QuantStudio™ 12K Flex. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Measures related to physical activity and sedentary behaviour

Triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA) were used to objectively measure physical activity and sedentary behaviour. Activity counts were measured at a rate of 30 hertz and stored at an epoch length of 60 second [28,29]. Participants wore the accelerometer on the hip up to 9 days; the first and last days were excluded from the analyses. A total of 7 continuous days with a minimum of 10 valid hours per day was required for being included in the study analysis. Sedentary behaviour and physical activity were calculated based upon recommended vector magnitude cut point [28,29]: <200 and ≥2690 count per minute for sedentary behaviour and moderate to vigorous physical activity, respectively. We used the manufacturer software (Actilife™ v.6.11.7 desktop) for data download, reduction, cleaning and analyses purposes. Further details are available elsewhere [30,31]. In the present study, we used binary data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of moderate to vigorous physical activity in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value.

Measures related to resilience

The *Positive and Negative Affect Schedule* (PANAS) [32] is a 20-item questionnaire that assesses positive affect and negative affect (i.e., the affective components of subjective well-being). The PANAS consists of 10 positive and 10 negative adjectives answered on a 5-point Likert scale ranging from 1 = 'very slightly or not at all' to 5 = 'extremely'. The 2-factor structure (positive affect, negative affect) is also appropriate for people with fibromyalgia [33]. The PANAS scores range from 10 to 50 for both subscales, where higher scores reflect more positive affect or negative affect. An 'in general' time frame was asked to participants.

The *Satisfaction with Life Scale* (SWLS) [34] is a 5-item questionnaire that assesses the perceived global life satisfaction (i.e., the cognitive component of subjective-well-being) on a 5-point Likert-scale ranging from 1 = 'strongly disagree' to 5 = 'strongly agree'. Thus, the SWLS scores range from 5 to 25, where higher scores reflect more cognitive well-being. The time frame of the SWLS is 'in general'.

The *Emotional Regulation subscale of the Trait Meta-Mood Scale* (TMMS) [35] is 8-item scale that assesses one's perceived skills to regulate mood and repair negative emotional experiences on a 5-point Likert-scale ranging from 1 = 'strongly disagree' to 5 = 'strongly agree'. Thus, the scores range from 8 to 40, where higher scores reflect greater emotional regulation.

The Life Orientation Test-Revised (LOT-R) [36] is a 10-item questionnaire that assesses dispositional optimism (sum of the 1st, 4th, and 10th items) and pessimism (sum of the 3rd, 7th, and 9th items) on a 5-point Likert-scale ranging from 0 = 'totally disagree' to 4 'totally agree'. The 2nd, 5th, 6th, and 8th items are fillers. The scores range from 0 to 12, where higher scores reflect greater either optimism or pessimism.

Measures related to potential confounders

Socio-demographic and clinical data. The participants filled out an initial questionnaire that included questions about date of birth, marital status, educational level, and presence/absence of acute or

terminal illness (such as cancer, stroke, recent cardiomyopathy, severe coronary disease, schizophrenia, or any other disabling injury).

Body fat (%) was measured using a portable eight-polar tactile-electrode impedanciometer (InBody R20, Biospace, Seoul, South Korea). During the assessment, the participants were barefoot and they wore only underwear and no metal objects.

Consumption of drugs. The consumption of analgesics and antidepressants was registered as binary variables (yes/no).

Statistical analysis

The Hardy-Weinberg equilibrium (HWE; p>0.01) and linkage disequilibrium (r^2>0.5) were evaluated with 'genetics' R package [37]. We used general linear models using Gaussian error distributions to analyse the singular associations of polymorphisms with phenotypes (i.e., pain outcomes); age, body fat (%), and the consumption of analgesics and antidepressants were entered as covariates as they are potential confounders of the associations under study [38]. Interactions between polymorphisms of different genes as well as between polymorphisms and physical activity or sedentary behaviour were assessed using the same models but including the following interaction terms in separate models: gene*gene, gene*physical activity, or gene*sedentary behaviour.

Five genetic models (i.e., dominant, recessive, log-additive, codominant, and overdominant) were used for all analyses. Exceptionally, when the minor allele frequency was lower than 0.1, we analysed only the dominant model of those polymorphisms due to their low number of minor homozygotes. For each polymorphism, P-values were computed using the likelihood ratio test between a model with the polymorphism or interaction term and the null model. Given that some of studied polymorphisms were in linkage disequilibrium (online supplementary figure 1), we considered as significant those associations with either P-values lower than the Bonferroni's correction or with p- and false discovery rate (FDR) values lower than 0.05.

All analyses were performed in the R environment 3.4.1. Gene-phenotype associations along with gene-to-gene interactions were assessed with the 'SNPassoc' package [39]. We developed our own script (available upon request) to study the gene-environment interactions.

RESULTS

Table 11 shows the characteristics of the 276 women with fibromyalgia included in the present study.

The rs7124442 (brain-derived neurotrophic factor antisense RNA, BDNF-AS, gene), rs7911 (guanylate binding protein 1, GBP1, gene) rs1050450 (glutathione peroxidase 1, GPX1, gene), rs4411417 (GTP cyclohydrolase 1, GCH1, gene), rs6323, rs1137070 (monoamine oxidase A, MAOA, gene), rs25532 (solute carrier family 6 member 4, SLC6A4, gene), and rs3746544 polymorphisms (synaptosome associated protein 25, SNAP25, gene) polymorphisms did not meet the HWE criteria. The low genotyping rate (i.e., ≤0.90) was observed for the rs9470080 (FK506 binding protein 5, FKBP5, gene), rs4371369, rs4387806, rs6746030, rs12620053 (SCN9A gene), and rs7310505 polymorphisms (thioredoxin reductase 1, TXNRD1, gene) polymorphisms. The remaining 50 polymorphisms were included in the present study. Online supplementary figure 1 presents the linkage disequilibrium values.

Individual association between genotype and phenotype

Figure 6 shows that the rs4453709 polymorphism (SCN9A gene) was individually associated with optimism under the overdominant model: those participants with the AT genotype showed lower optimism than participants with the AA/TT genotype (P=0.0005, FDR=0.015). The remaining individual associations between genotype and resilience outcomes were not significant (online supplementary figures 2-7).

Gene-gene interaction

Figure 7 illustrates that the rs4680 and rs6860 polymorphisms (catechol-O-methyltransferase and charged multivesicular body protein 1A, COMT and CHMP1A, genes respectively) were additively related to satisfaction with life (P=0.00005, FDR=0.045). The AA genotype of the rs4680 polymorphism was associated with the highest satisfaction with life in carriers of the AA genotype for the rs6860 polymorphism, carriers of the AG genotype of the rs6860 polymorphism reported a similar satisfaction with life regardless of their rs4680 genotype, and carriers of the AG and GG genotypes of the rs4680 and rs6860 polymorphisms, respectively, experienced the poorest satisfaction with life. The remaining gene-gene additive associations with resilience were non-significant, see online supplementary figures 8-37.

Gene-environment interaction

Figure 8 shows four significant gene-environment interactions. Under the overdominant model, the rs4906902 AA/GG genotype (GABRB3 gene) was related to higher satisfaction with life than the AG genotype, but only in those participants with increased levels of physical activity (P=0.0009, FDR=0.038). The rs4597545 CC genotype (SCN9A gene) was associated with higher emotional regulation than the GG or CG genotypes, but only in participants with low levels of sedentary behaviour (P=0.0017, FDR=0.038 and P=0.0004, FDR=0.017 for codominant and additive models, respectively). Under the additive model, the rs6754031 GG genotype (SCN9A gene) was related to higher emotional regulation than the GT or TT genotypes, but only for those participants with low sedentary behaviour (P=0.002, FDR=0.042).

The additive association of the rs6280 polymorphism (dopamine receptor D3, DRD3, gene) and physical activity, and of the rs12994338 polymorphism (SCN9A gene) and sedentary behaviour with pessimism and emotional regulation, respectively, were significant. However, they lacked of statistical power, $n \leq 10$ in some groups. The remaining additive associations of genotype and people's behaviours with resilience outcomes did not reach the significance (online

supplementary figures 38-43 and 44-49 for physical activity and sedentary behaviour, respectively).

DISCUSSION

Although traditionally research has been conducted in vulnerability or people's weaknesses, to understand the genetics of resilience or people's assets is of interest [13], in particular, in chronic diseases such as fibromyalgia. The main findings of present study showed that (i) the rs4453709 polymorphism (SCN9A gene) was individually associated with optimism, (ii) the rs4680 and rs6860 polymorphisms (COMT and CHMP1A genes, respectively) were additively related to satisfaction with life, (iii) the interaction of the rs496902 polymorphism (GABRB3 gene) with physical activity were related to satisfaction with life, and (iv) the interactions of the rs4597545 and rs6754031 polymorphisms (SCN9A gene) and sedentary behaviour were associated with emotional regulation. Taken together, these findings suggest that dysfunction in neurotransmitters, the peripheral nervous system, and the HPA might play a role in adaptation to living with fibromyalgia. An active lifestyle, characterised by engaging in physical activity and avoiding sedentary behaviour, may buffer a genetic predisposition to experiencing lower levels of resilience.

It has been suggested that mesolimbic dopaminergic pathways may be associated to optimism [13]. In the present study, carriers of the AT genotype of the rs4597545 polymorphism (SCN9A gene) reported the lowest levels of optimism, which corroborates that molecular heterosis is common in neurotransmitters-related genes [40]. The SCN9A gene is related to the activity of the Na(v)1.7 sodium channels [19]. In general, activity of the sodium channels is essential for the membrane depolarization of the neurons and, in consequence, for generating and conducting action potentials [16]. Given that these Na(v)1.7 channels are highly expressed in the neurons located at the dorsal root ganglion, sympathetic ganglion and hypothalamic neurons [17–19], our findings suggest that the peripheral nervous system plays a role in the adaptation to living with fibromyalgia.

In fibromyalgia, previous candidate-gene studies failed to take account of the interaction of genotype and environmental factors. In women with fibromyalgia, we provided unique evidence: those how carried the CC genotype of the rs4597545 polymorphism or the GG genotype of the rs6754031 polymorphism (SCN9A gene) and engaged in low sedentary behaviour reported the highest emotional regulation. Moreover, AA/GG carriers of the rs4906902 polymorphism (GABRB3 gene) that met the physical activity recommendations reported the highest satisfaction with life. As previously stated, the SCN9A may modulate the activity of the HPA axis [19]. The GABRB3 gene encodes a protein related to the chloride channel. The GABRB3 protein is expressed at different levels of the brain including, but not limited to, the cerebral cortex, hippocampus, and thalamus [41]. These structures, along with the HPA axis, may be related to resilience [13] and modulated by people's behaviours (e.g., physical activity) [42,43]. Our findings might suggest that the potential benefits on engaging in an active lifestyle depend on the women with fibromyalgia genotype. For instance, to avoid sedentary behaviour might be related to higher emotional regulation in women with the CC, but not with the CG or GG, genotype of the rs4597545 polymorphism (SCN9A gene).

While the individual association of the COMT and CHMP1A related polymorphism with resilience outcomes did not emerge, the present study found a joint association of the rs4680 and rs6860 polymorphisms (COMT and CHMP1A genes, respectively) with satisfaction with life. In young adults, a previous study showed that the rs4680 polymorphism (COMT gene) was individually related to an index of well-being –a score including, but not limited to, satisfaction with life [44]. Dopaminergic and noradrenergic pathways may be involved in this association [45,46]. Given that the CHMP1A may play a role in necroptosis and in the polycomb group of proteins [47], research on this gene has focused mostly on cancer [48]. Notwithstanding, we have shown that the rs6860 polymorphism from the CHMP1A gene is associated with fibromyalgia susceptibility [49]. In the present study, the COMT and CHMP1A gene-gene interaction seemed to draw differences in resilience: women with fibromyalgia carrying the AA genotype in both the rs4680 and rs6860 polymorphisms reported the highest satisfaction with life. The COMT gene has showed epistasis with several other genes [50].

Therefore, further research is warranted to better understand the interaction of the COMT and CHMP1A genes.

Limitations and strengths

A limitation of the present study is that a replication sample was not included. To test the interactions of gene-gene and gene-people's behaviour, larger sample sizes are more adequate to reach sufficient statistical power [51]. Thereby, some of our non-significant results might be indeed statistically significant in studies with a higher statistical power. Yet significant, the additive association of the rs6280 polymorphism (DRD3 gene) and physical activity, and of the rs12994338 polymorphism (SCN9A gene) and sedentary behaviour with pessimism and emotional regulation, respectively, lacked of statistical power ($n \leq 10$) in some groups. Our study has several strengths: the sample is larger than most of previous literature [6] and it was representative of the southern Spanish population of women with fibromyalgia [52]. Additionally, the fibromyalgia diagnosis was corroborated with a physical examination in line with the 1990 ACR fibromyalgia criteria [1]. Other strengths were the inclusion of 64 polymorphisms of 34 candidate-genes and the objective measure of physical activity and sedentary behaviour. Finally, our analyses were adjusted for multiple comparisons, which made our findings more robust.

Conclusions

In southern Spanish women with fibromyalgia, the rs4453709 polymorphism (SCN9A gene) was individually related to optimism. The gene-gene interaction of the rs4680 and rs6860 polymorphisms (COMT and CHMP1A genes, respectively) were additively associated with satisfaction with life. Furthermore, a number of gene-environment interactions emerged: the rs496902 polymorphism (GABRB3 gene) and physical activity were jointly related to satisfaction with life; also, the rs4597545 and rs6754031 polymorphisms (SCN9A gene) and sedentary behaviour were additively associated with emotional regulation.

Overall, our findings might suggest that dysfunction in neurotransmitters, the peripheral nervous system, and the HPA might play a role in adaptation to living with fibromyalgia. The identification of a genotype related to poor resilience in women with fibromyalgia, could help to develop specific targeted therapies for patients with such adverse genotype. For instance, in women with fibromyalgia, increasing physical activity levels and decreasing sedentary behaviour may buffer a genetic predisposition to have higher odds of experiencing lower resilience.

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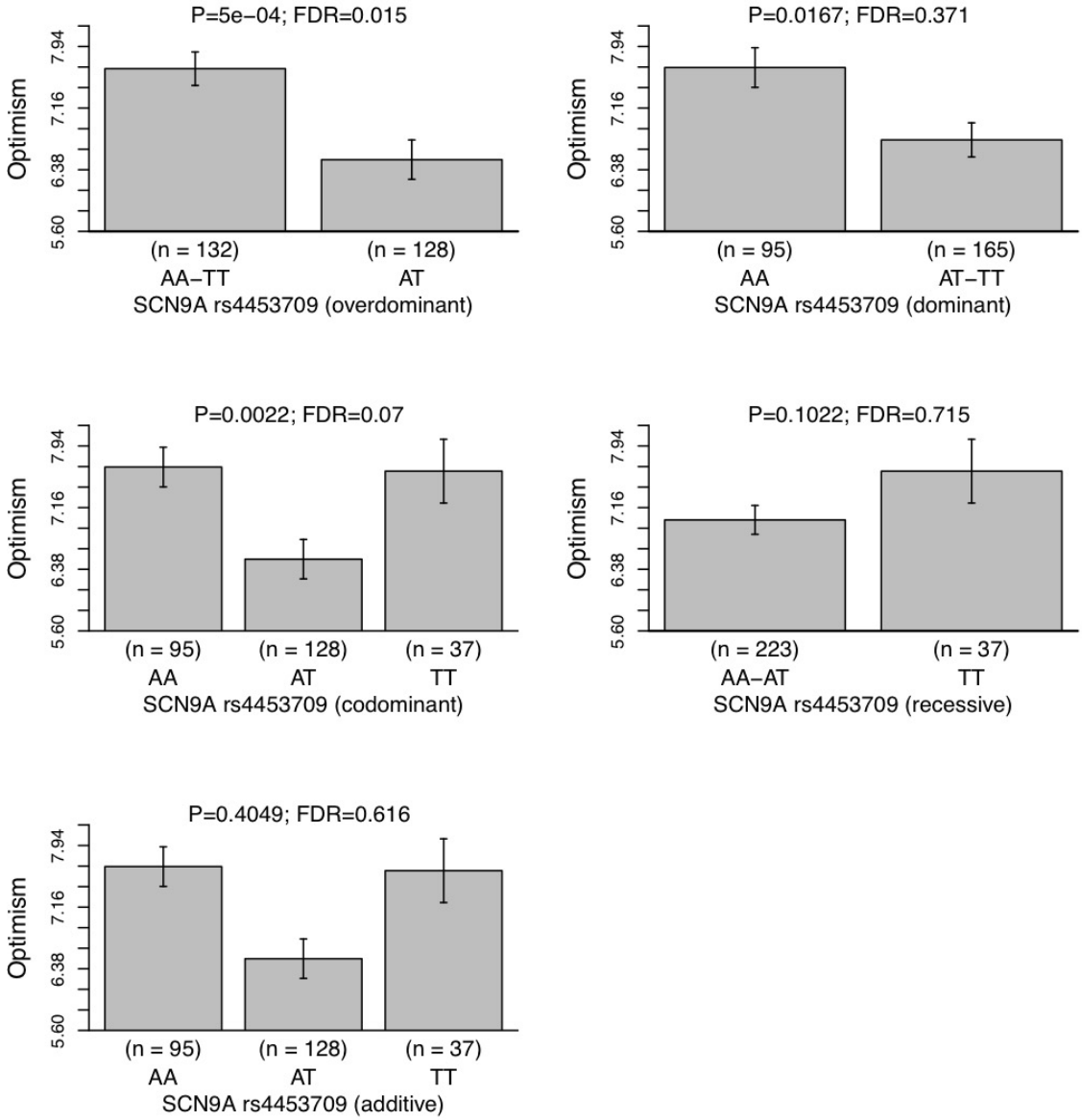

Figure 6. Individual associations of the genotype of the rs4453709 polymorphism (SCN9A gene) with optimism

SCN9A, sodium voltage-gated channel alpha subunit 9 gene; Optimism was assessed by means of the life orientation test-revised (LOTR, scores range 0-12); According to the p- and false discovery rate (FDR) values, the significance was reached only under the overdominant model.

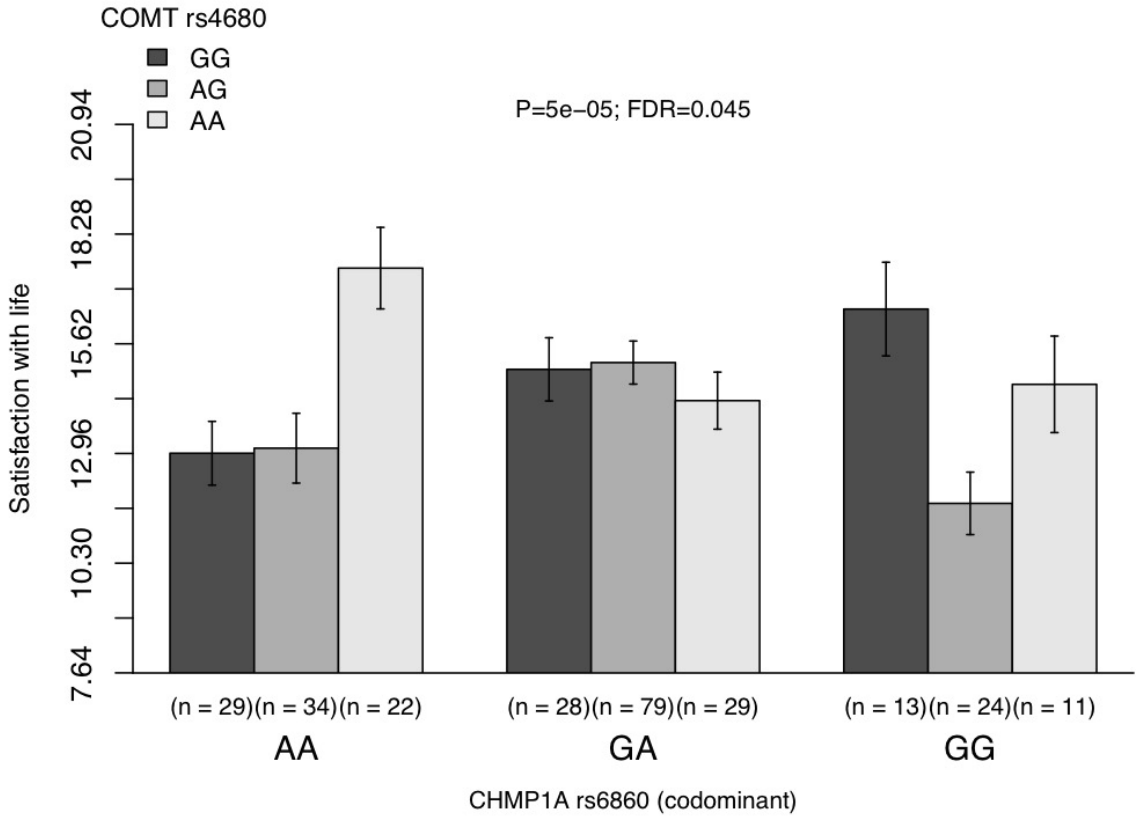

Figure 7. Gene-gene interaction of the rs4680 and rs6860 polymorphisms with satisfaction with life

COMT, catechol-O-methyltransferase gene; CHMP1A, charged multivesicular body protein 1A gene; Satisfaction with life was assessed by means of the satisfaction with life schedule (SWLS, scores range 5-25); According to the p- and false discovery rate (FDR) values, this gene-gene interaction was significant.

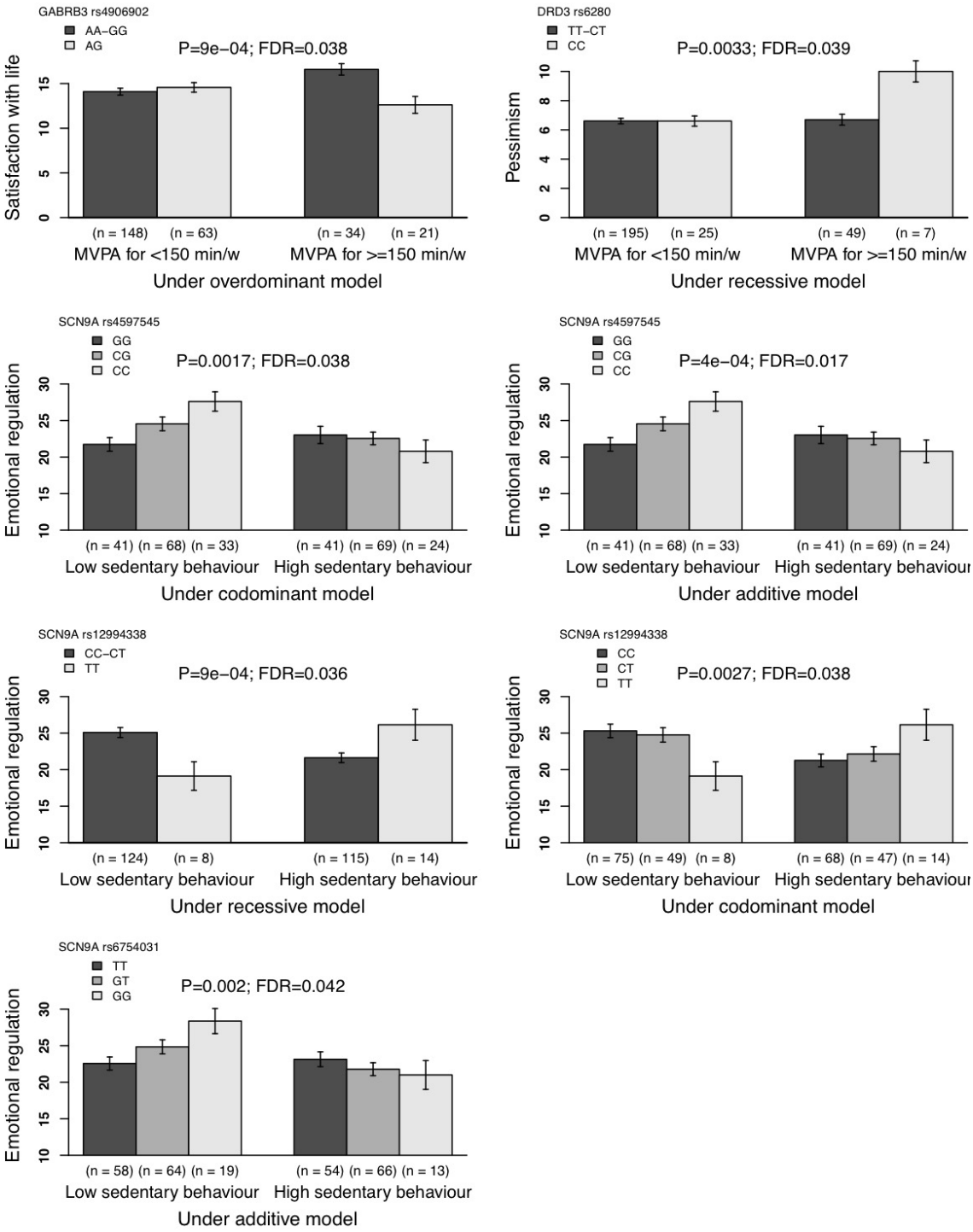

Figure 8. Additive association gene-physical activity or gene-sedentary behaviour with resilience outcomes

GABRB3, gamma-aminobutyric acid type A receptor beta 3 subunit gene; DRD3, dopamine receptor D3; SCN9A, sodium voltage-gated channel alpha subunit 9 gene; MVPA, moderate to vigorous physical activity.

Physical activity and sedentary behaviour were objectively measured using triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA). We dichotomised data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of MVPA in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the mean as the cut-off value (i.e., 459.7 min/week).

Satisfaction with life, pessimism, and emotional regulation were assessed by means of the satisfaction with life schedule (SWLS, scores range 5-25), life orientation test-revised (LOTR, scores range 0-12), and trait-meta mood scale (TMMS-24, scores range 8-40), respectively.
According to the p- and false discovery rate (FDR) values, these gene-physical activity or gene-sedentary behaviour interactions were significant. However, rs6280-physical activity and rs12994338-sedentary behaviour lacked of statistical power; n<10 in some groups.

Table 11. Characteristics of the participants in the study, *n*=276

	n (%)
Education level	
Unfinished studies	26 (9.4)
Primary	138 (50.0)
Secondary (and vocational)	81 (29.3)
University	31 (11.2)
Marital status	
Married	215 (77.9)
Single	21 (7.6)
Separated/divorced	27 (9.8)
Widow	13 (4.7)
Working status	
Working	73 (26.4)
Household	93 (33.7)
Incapacity pension or sick leave	55 (19.9)
Unemployed	46 (16.7)
Others	9 (3.3)
Drugs consumption (yes vs. no)	
Analgesics (yes)	248 (89.9)
Antidepressants (yes)	147 (53.3)
	Mean (SD)
Age, years old	51.8 (7.7)
Body fat (%)	40.5 (7.6)
Tender points count	16.9 (1.8)
Physical activity (accelerometers, min/week)	
Moderate-to-vigorous physical activity	86.6 (118.6)
Sedentary behaviour	459.7 (107.8)
Resilience	
Positive affect (PANAS, 10-50)	23.3 (6.8)
Negative affect (PANAS, 10-50)	23.5 (8.1)
Satisfaction with life (SWLS, 5-25)	14.4 (4.5)
Emotional regulation (TMMS, 8-40)	23.4 (7.5)
Optimism (LOTR, 0-12)	7.1 (2.7)
Pessimism (LOTR, 0-12)	6.7 (2.6)

SD, standard Deviation; PANAS, the positive and negative affect schedule; SWLS, the satisfaction with life schedule; TMMS-24, the trait-meta mood scale; LOTR, the life orientation test-revised

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General discussion

Scientific literature has identified a number of candidate-genes of fibromyalgia, the vast of them are related to neurotransmitters [1–3], which potentially points out the presence of a chronically sensitised central nervous system in the pathology of this disease [3]. For instance, the most widely studied candidate-gene of fibromyalgia is the catechol-O-methyltransferase (COMT). However, previous findings related to the COMT are controversial [3,4]. New genes have been identified recently [2,5]. The sodium voltage-gated channel alpha subunit 9 (SCN9A) gene is associated with fibromyalgia susceptibility in a sample from the Mexican population [6], which has not been analysed in Spaniards. For the first time, the additive relationship of two genes (the opioid receptor μ1 and serotonin transporter 5-HTT, OPRM1 and HTR2A, genes) with pain modulation has been demonstrated in fibromyalgia [7]. Finally, it is well-known that (i) higher time spend on physical activity is correlated with a more favourable fibromyalgia symptomatology [8–10], and (ii) the interplay of genes and physical activity is related to health outcomes in other pathologies [11–13], which had not been tested in fibromyalgia.

The aims of this Doctoral thesis were (i) to call the attention on the importance of including objective measures of physical function in chronic pain diseases; e.g., fibromyalgia (**Study I**), (ii) to compare the genotype frequencies of candidate-genes in Spanish women; i.e., study of the genetic susceptibility to fibromyalgia (**Study II and Study III**), and (iii) to analyse the singular association of candidate-genes, as well as the gene-gene, gene-physical activity, and gene-sedentary behaviour interactions with pain (**Study IV**), fatigue (**Study V**), and resilience (**Study VI)** in Spanish women with fibromyalgia.

Table 12. Summary of the findings provided by the present Doctoral Thesis conducted in women with fibromyalgia (FM)

Gene	Polymorphism	Higher FM susceptibility	Individual association [a]	Gene-PA interaction [a,b]	Gene-SB interaction [a,b]
ADRA1A	rs1383914	TT		AG = ↑ bodily pain	
COMT	rs4680				AA/GG = ↑ bodily pain
	rs165599				AA/GG = ↑ bodily pain
	rs2097903	AT/TT			
CHMP1A	rs6860	TT			AA/GG = ↑ bodily pain
HTR2A	rs6311		GG = ↑ algometer score		
	rs6313		GG = ↑ algometer score		
GABRB3	rs4906902			AA/GG = ↑ life satisfaction	
GCH1	rs841	GG			
MTHFR	rs181133			CT = ↓ physical fatigue & ↓ reduced motivation	
OPRM1	rs1799971	GG			
SCN9A	rs4453709		AA/TT = ↓ reduced motivation & ↑ optimism		
	rs4597545			CG = ↓ mental fatigue	CC = ↑ emotional regulation
	rs6754031				CG = ↑ emotional regulation
	rs7607967				AA/GG = ↓ reduced activity
	rs1299338				CC/TT = ↑ bodily pain

[a] The genotypes that showed the more favourable profile associations with the outcomes are indicated (e.g., GG = ↑ algometer score means that GG carriers showed the highest algometer score, which is a good sign indicating a higher pain threshold). [b] In comparison with the opposite levels of

physical activity behaviour (e.g., the top-right cell indicates that, when compared to those who engage in higher sedentary time, AA/GG carriers that spent less time in such behaviour reported higher bodily pain, which is a good sign since higher scores means lower pain in the questionnaire).

Acronyms of genes as follows: ADRA1A, adrenoceptor alpha 1a; COMT, catechol-O-methyltransferase, CHMP1A, Charged multivesicular body protein 1A, HTR2A, 5-hydroxytryptamine receptor 2A; GABRB3, Gamma-aminobutyric acid type A receptor beta 3 subunit; GCH1, guanosine triphosphate cyclohydrolase 1; MTHFR, methylene tetrahydrofolate reductase; OPRM1, (opioid receptor μ1; SCN9A, sodium voltage-gated channel alpha subunit 9.

Physical activity (PA) and sedentary behaviour (SB) were objectively measured using triaxial accelerometers GT3X+ (Actigraph, Pensacola, Florida, USA). We dichotomised data of physical activity and sedentary behaviour as follows (i) fulfilment (yes vs. no) of the physical activity recommendations (≥150 min/week of MVPA in bouts of, at least, 10 min of length and, (ii) sedentary behaviour (low vs. high) using the sample mean as the cut-off value. Algometer score, bodily pain, emotional regulation, life satisfaction, and optimism were assessed by a physical examination using an algometer, the 36-item short form health survey, the trait meta-mood scale, satisfaction with life scale, and the life orientation test-revised, respectively. Dimensions of fatigue (i.e., physical fatigue, reduced activity, reduced motivation, and mental fatigue) were assessed with the multidimensional fatigue inventory.

Susceptibility to fibromyalgia: Study II and Study III

Table 12 (higher fibromyalgia susceptibility column) summarises the findings of our cases vs. controls studies. In line with previous studies [14], our results confirmed that the GG genotype of the rs1799971 (OPRM1 gene) is more common in women with fibromyalgia than in non-fibromyalgia peers. Additionally, for the first time in the literature, we showed that the genotype AT/TT of the rs2097903 (COMT gene), TT of the rs6860 (CHMP1A gene), GG of the rs841 (GCH1 gene) polymorphisms were associated with higher odds of having fibromyalgia.

Also in Spaniards, Vargas-Alarcón et al. [4] reported that the frequency of the rs2097903 genotype was similar in their fibromyalgia and control samples. As stated in the Chapter III, methodological differences between studies may drive us to reach different results. For instance, Vargas-Alarcón et al. [4] excluded cases with other rheumatic diseases and controls with chronic pain and, in order to increase the ecological validity of our study, we did not. Among Korean people, the genotype of the rs841 polymorphism was related to discomfort with a tender point examination but not to fibromyalgia susceptibility [15]. However, our results showed the opposite among Spanish women. At a first glance, different racial backgrounds may turn in a different genetic susceptibility to fibromyalgia. To confirm or refute this hypothesis, research in independent Asian and Caucasian samples is needed.

Replication studies are important to reach robust conclusions in genetics [16,17]. Due to a typo-error in a previous study, we analysed for the first time the CHMP1A gene in fibromyalgia. Surprisingly, the TT genotype of the rs6860 was related to higher fibromyalgia susceptibility. Since in the case-control study we did not adjust for multiple comparisons, our results should be better understood as preliminary.

Associations of genotype with phenotype (Studies IV to VI)

Table 12 (individual association column) summarises the individual associations between the genotype and the phenotype. In people with chronic low back pain, carriers of the

GG genotype the rs6311 and 6313 polymorphisms (HTR2A gene) showed the lowest disability score [18]. In this line, we observed that the GG genotype of the rs6311 and 6313 polymorphisms was individually related to higher algometer score; i.e., higher pain threshold, which is favourable. Overall, it seems that the GG genotype of the HTR2A gene may buffers the levels of pain experienced by people living with a chronic pain disease.

Mutations of the SCN9A gene have been hypothesised as key players of the hyperreactivity to nociceptive stimulus commonly observed in fibromyalgia [19,20]. In the present Doctoral thesis, the AA/TT genotype of the rs4453709 polymorphism was individually associated with lower fatigue and higher in optimism. From our dataset, also emerged associations between the interaction of the SCN9A genotype-physical activity behaviours and all the outcomes included in the present Doctoral Thesis; i.e., pain, fatigue, and resilience. Thus, the SCN9A gene is the most robust candidate-gene identified in the present research.

In addition to individual associations between genotype and phenotype, gene-gene interactions are likely present in fibromyalgia [7] and can be informative (e.g., signalling potential mechanistic pathways) [21]. For the first time, a gene-gene interaction between the OPRM1 and 5-HTTLPR genes has been demonstrated in fibromyalgia pain modulation [7]. Using the al-Ándalus project data, we found two gene-gene interactions with cognitions related to (i) pain (i.e., catastrophizing), the COMT and OPRM1 and (ii) resilience (i.e., satisfaction with life), the COMT and CHMP1A. Interestingly, the COMT gene was involved in both gene-gene interactions. Thus, the COMT gene seems interact with other genes possibly due its potential for epistasis with several other genes [22].

Genotype-physical activity interactions with phenotype (Studies I and IV to VI)

People with fibromyalgia appraise their physical functioning as poorer than it is observed in objective measurements [23–25]. We do not consider this fact as evidence supporting that people with fibromyalgia are complainers. In chapter I, we have emphasized that both people's

appraisals and observations of their physical functioning are informative. In epidemiology, the common approach is to assess physical activity behaviours using questionnaires because they require fewer resources. Given the paucity of knowledge, in the present Doctoral Thesis, we focused on objective data of physical activity behaviours.

Fibromyalgia is characterised by an array of multidimensional symptoms, which lead to a complex phenotype. Thus, a core-starting point of the present Doctoral Thesis was that sophisticated models are required rather than testing only the individual association genotype-phenotype. Our results corroborated our hypothesis: most of the associations emerged for such an interaction. In the last decade, the al-Ándalus project has demonstrated that higher levels of physical activity and lower of sedentary behaviour are key markers of health in the population, as a whole, of people with fibromyalgia [8,26–34]. Therefore, the interaction of gene-physical activity behaviours indicated that the association of people's behaviours with pain, fatigue, and resilience is particularly high in specific subgroups of people according to their genotype.

Our findings, if confirmed in future prospective research, may be of public health and clinical importance. To engage in physical exercise is advisable in fibromyalgia [35–37]. A EUropean League Against Rheumatism (EULAR) taskforce concluded that physical exercise has strong level of evidence [38]. Given the heterogeneity of people with fibromyalgia [39–41], we appraise the available literature more cautiously. Currently, none treatment alternative has shown to be efficient, universally and in long-term, in fibromyalgia patients [35]. In line with Turk, we consider that clustering people with fibromyalgia into several homogeneous groups, instead of 'one-size-fits-all' approach, may be the most appropriate way to treating the disease [40].

Table 12 (gene- physical activity and sedentary behaviour interaction) summarises the main findings related to the interaction of gene-physical activity behaviours with pain, fatigue, and resilience. Briefly, the candidate gene that along with physical activity behaviours was additively and more robustly related to the outcomes was the SCN9A. If corroborated in

prospective research, the CG genotype of the rs4597545 may be a responder to the effect of physical activity interventions on mental fatigue. Reducing sedentary behaviour may be particularly effective for (i) improving the emotional regulation of the carriers of the CC (rs4597545) and CG (rs6754031) genotypes, and (ii) for lowering a specific dimension of fatigue (i.e., reduced activity) and improving bodily pain among carriers of the AA/GG (rs7607967) and CC/TT (rs1299338) genotypes. Although this speculation is going far from our results, we at least are able to suggest that the characteristics of women with fibromyalgia should be attended when assessing and monitoring the disease.

Potential mechanisms of pathology in fibromyalgia (Studies II to VI)

The present Doctoral Thesis did not focus on determining the pathology mechanisms of fibromyalgia. However, our findings might modestly shed some lights on the disease mechanisms. Most of the candidate-genes identified are directly related to (excitatory and inhibitory) neurotransmitters such as catecholamine (COMT and ADRA1A genes), serotonin (HTR2A gene), gamma-aminobutyric acid (GABRB3 gene), and dopamine (OPRM1 gene) pathways. Moreover, the GCH1 gene is involved in the folate metabolism and, consequently, is indirectly related to several neurotransmitters (e.g., serotonin [42] and dopamine [43]). An exception is the CHMP1A gene, which is involved in oxidative stress [44] and excessive autophagy [4, 5].

The SCN9A gene was the most usually associated with pain, fatigue, and resilience in the present research. Sodium channels are key player on generating action potentials. The SCN9A gene encodes a specific type of these channels, the Na(v)1.7 that are highly expressed in structures of the central nervous system and also in the dorsal root ganglion (it receives afferent sensitive information from the periphery). Thus, impairments in the central and peripheral nervous systems may be present in fibromyalgia [45]. On the basis of our robust findings, we speculate that by modulating the Na(v)1.7 channels, the SCN9A gene is involved in chronically sensitised core alarm system that gives alarm signals including the ample array of fibromyalgia

symptoms (e.g., pain, fatigue, unrefreshed sleep, negative mood). In addition to promote active lifestyles, to find blockers of the Na(v)1.7 may have a positive impact on smoothed out the hyperactivity of the nervous system in fibromyalgia [46].

Limitations and strengths of the present Doctoral Thesis

The main limitations were:

- Lack of an independent replication sample.
- Cross-sectional design, which precludes causality. The al-Ándalus project included (2 and 5 years) follow-up measures and two intervention studies, which will improve the understanding of the associations observed in the present Doctoral Thesis.
- The sample size was not large enough to analyse the gene-gene and gene-physical activity behaviours of some polymorphisms.

The main strengths were:

- The sample was well-characterised (e.g., the research team corroborated the diagnosis of fibromyalgia).
- A large number of candidate-genes were included (i.e., 64 polymorphisms of 34 genes).
- Additionally to the common gene-phenotype associations, we analysed the interaction of gene-gene and gene-physical activity on the core symptoms of fibromyalgia (i.e, pain and fatigue) and on resilience.
- Physical activity and sedentary time were objectively measured.

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Conclusions

CONCLUSIONS

1. Objective measurements and subjective appraisals of physical functioning provide related but distinct information in fibromyalgia. When studying the associations between physical functioning and health status, inferences between different types of assessment may be inappropriate (**Study I**).
2. There is a genetic predisposition to the susceptibility to fibromyalgia, which is higher among the following genotypes of several polymorphisms: TT genotype of the rs6860, CHMP1A gene; AT/TT of the rs2097903, COMT gene; and GG of the rs841 and rs1799971, GCH1 and OPMR1 genes, respectively (**Studies II and III**)
3. The GG genotype of the rs6311 and 6313 (HTR2A gene) was individually associated with higher pain thresholds (**Study IV**), and the AA/TT of the rs4453709 (SCN9A gene) with better scores on reduced motivation and optimism (**Studies V and VI, respectively**).
4. Gene-gene interactions were related to cognitions of pain (COMT and OPMR1, **Study IV**) and well-being (COMT and CHMP1A; **Study VI**).
5. The COMT, GABRB3, MTHFR, and SCN9A genes were, with physical activity behaviours levels, additively related to pain-, fatigue-, and resilience-related outcomes. The individual relationship of the SCN9A and its interaction with physical activity behaviours to pain, fatigue, and resilience is the most robust.

Therefore, the **overall conclusion** of the present Doctoral Thesis was that susceptibility to fibromyalgia, its core symptoms (i.e., pain, fatigue, and low resilience) may have a genetic component, which is mostly related to dysfunctions in neurotransmission. The sodium voltage-gated channel alpha subunit 9, SCN9A, gene was the one that showed the most robust associations with the phenotype of fibromyalgia. The SCN9A gene encodes the Na(v)1.7 sodium channel, which suggests that blocking the gain-of-function of this type of channel may lead to beneficial effects on living with fibromyalgia. Furthermore, an active lifestyle may help to modulate an unfavourable genetic predisposition to pain, fatigue, and resilience. The associations of physical activity behaviours with a favourable phenotype might be more clearly observable for some genotypes of women with fibromyalgia.

Conclusiones

1. Las mediciones objetivas y las subjetivas de la función física proporcionan información diferente en fibromialgia. Al estudiar las asociaciones entre la función física y el estado de salud, no deben hacerse inferencias entre ambos tipos de medidas (**Estudio I**).
2. Existe una predisposición genética a la susceptibilidad a la fibromialgia que es mayor en los siguientes genotipos y polimorfismos: el TT del rs6860 (gen CHMP1A), el AT/TT del rs2097903 (gen COMT) y el GG del rs841 y rs1799971 (genes GCH1 y OPMR1, respectivamente (**Estudios II y III**).
3. El genotipo GG de los polimorfismos rs6311 y rs6313 se asociaron de forma individual con un mayor umbral del dolor (**Estudio IV**) y el AT/TT del rs4453709 (gen SCN9A) con mejores puntuaciones de motivación reducida y optimismo (**Estudio V y VI**, respectivamente).
4. Las interacciones gen-gen se relacionaron con las cogniciones de dolor (genes COMT y OPMR1; **Estudio V)** y bienestar (genes COMT y CHMP1A; **Estudio VI**).
5. Los genes COMT, GABRB3, MTHFR, y SCN9A y los niveles de actividad física se relacionaron conjuntamente con dolor, fatiga y resiliencia. El gen SCN9A, tanto individualmente como aditivamente con el estilo de vida, fue el que monstró una asociación más robusta.

Por lo tanto, la **conclusión general** de la presente Tesis Doctoral indica que la susceptibilidad a la fibromialgia y sus síntomas principales (como el dolor, la fatiga y la baja resiliencia) pueden tener un componente genético que, sobre todo, está relacionado con alteraciones en la neurotransmisión. El gen SCN9A fue el que se asoció de forma más robusta con el fenotipo de la fibromialgia. Este gen codifica un el canal de sodio Na(v)1.7, lo que sugiere que bloqueando este tipo de canal podría ser beneficioso en las personas con fibromialgia. Además, un estilo de vida activo puede modular una predisposición genética a tener niveles altos de dolor y fatiga, así como bajos de resiliencia. La actividad física podría ser particularmente favorable para algunas mujeres con fibromialgia dependiendo de su genotipo.

Online supplementary information