RHEUMATOLOGY

Rheumatology 2022;61:3180–3191
https://doi.org/10.1093/rheumatology/keab911
Advance Access publication 7 December 2021

Original article

Interplay between genetics and lifestyle on pain susceptibility in women with fibromyalgia: the al-Ándalus project

Fernando Estévez-López[1], Juan M. Guerrero-González[2], Diego Salazar-Tortosa[3], Daniel Camiletti-Moirón[4], Blanca Gavilán-Carrera[4], Virginia A. Aparicio[5], Pedro Acosta-Manzano[6], Inmaculada C. Álvarez-Gallardo[4], Víctor Segura-Jiménez[7,8,9], Alberto Soriano-Maldonado[10], Rinie Geenen[11], Manuel Delgado-Fernández[6], Luis J. Martínez-González[2], Jonatan R. Ruiz[12] and María J. Álvarez-Cubero[2,13]

Abstract

Objectives. It is widely acknowledged that the experience of pain is promoted by both genetic susceptibility and environmental factors such as engaging in physical activity (PA), and that pain-related cognitions are also important. Thus, the purpose of the present study was to test the association of 64 polymorphisms (34 candidate genes) and the gene–gene, gene–PA and gene–sedentary behaviour interactions with pain and pain-related cognitions in women with FM.

Methods. Saliva samples from 274 women with FM [mean (S.D.) age 51.7 (7.7) years] were collected for extracting DNA. We measured PA and sedentary behaviour by accelerometers for a week, pain with algometry and questionnaires, and pain-related cognitions with questionnaires. To assess the robustness of the results, a meta-analysis was also performed.

Results. The rs6311 and rs6313 polymorphisms (5-hydroxytryptamine receptor 2A, *HTR2A*) were individually related to algometer scores. The interaction of rs4818 (catechol-O-methyltransferase, *COMT*) and rs1799971 (opioid receptor μ gene, *OPRM1*) was related to pain catastrophizing. Five gene–behaviour interactions were significant: the interactions of sedentary behaviour with rs1383914 (adrenoceptor alpha 1A, *ADRA1A*), rs6860 (charged multivesicular body protein 1A, *CHMP1A*), rs4680 (*COMT*), rs165599 (*COMT*) and rs12994338 (*SCN9A*) on bodily pain subscale of the Short Form 36. Furthermore, the meta-analysis showed an association between rs4680 (*COMT*) and severity of FM symptoms (codominant model, *P*-value 0.032).

Conclusion. The *HTR2A* gene (individually), *COMT* and *OPRM1* gene–gene interaction, and the interactions of sedentary behaviour with *ADRA1A*, *CHMP1A*, *COMT* and *SCN9A* genes were associated with pain-related outcomes. Collectively, findings from the present study indicate a modest contribution of genetics and gene–sedentary behaviour interaction to pain and pain catastrophizing in women with FM. Future research should examine whether reducing sedentary behaviour is particularly beneficial for reducing pain in women with genetic susceptibility to pain.

CLINICAL SCIENCE

[1]Department of Child and Adolescent Psychiatry/Psychology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands, [2]GENYO (Pfizer-University of Granada-Andalusian Government Centre for Genomics and Oncological Research), Granada, Spain, [3]Department of Ecology and Evolutionary Biology, University of Arizona, Tucson, AZ, USA, [4]Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Cádiz, [5]Department of Physiology, Faculty of Pharmacy, [6]Department of Physical Education and Sport, Faculty of Sport Sciences, University of Granada, [7]Instituto de Investigación Biosanitaria ibs.GRANADA, [8]Hospital Universitario Virgen de las Nieves of Granada, [9]GALENO Research Group, Department of Physical Education, Faculty of Education Sciences, University of Cádiz, Cádiz, [10]Department of Education, Faculty of Education Sciences, and SPORT Research Group (CTS-1024), CERNEP Research Center, University of Almería, Almería, Spain, [11]Department of Psychology, Faculty of Social and Behavioural Sciences, Utrecht University, Utrecht, The Netherlands, [12]PROFITH – "PROmoting FITness and Health Through Physical Activity" – Research Group, Department of Physical Education and Sport, Faculty of Sport Sciences and [13]Department of Biochemistry and Molecular Biology III, Faculty of Medicine, PTS, University of Granada, Granada, Spain

Submitted 4 August 2021; accepted 29 November 2021

Correspondence to: Juan M. Guerrero-González, Genomics Unit, GENYO, PTS Granada, Avenida de la Ilustración 114, 18016 Granada, Spain. E-mail: juan.guerrero@genyo.es

Key words: adrenoceptor alpha 1A gene, charged multivesicular body protein 1A gene, 5-hydroxytryptamine receptor 2A gene, opioid receptor μ1 gene, sodium voltage-gated channel alpha subunit 9 gene

Rheumatology key messages

- Full understanding of FM encompasses interacting biological and environmental factors.
- Gene–gene and genotype–sedentary behaviour interactions may be relevant when studying pain in women with FM.
- Future research should examine reduction of sedentary behaviour in women with genetic susceptibility to pain.

Introduction

FM is a chronic pain condition characterized by widespread pain and other somatic symptoms, fatigue, unrefreshed sleep and cognitive symptoms [1]. Pain is present in all the proposed diagnostic criteria of FM [1]. Therefore, research of this disease has extensively focussed on pain as an outcome and in its related mechanisms. It is widely accepted that pain is promoted by both genetic susceptibility and environmental factors such as people's behaviours. A higher prevalence in women has also been observed, which could be explained by sexually dimorphic dorsal root ganglia inflammation, promoted by stress [2].

In FM, the most often studied gene in relation to pain is the catechol-O-methyltransferase (*COMT*). By regulating the dopaminergic pathways, *COMT* participates in the opioidergic central processing of pain [3]. A relationship between methionine alleles of the *COMT* gene and fatigue has even been suggested, which could be moderated by different levels of fear of pain, establishing an association between psychological and physical factors [4]. Among Spanish women with FM, an early study found an association between rs4818 polymorphism and self-reported pain [5]. Tour and colleagues have recently observed that the interaction of the opioid receptor μ1 (*OPMR1*) and serotonin transporter 5-HTT (*5-HTTLPR*) genes is associated with pain modulation in people both with and without FM [6]. Furthermore, a recent bioinformatics study also proposed *CD38*, glycine amidinotransferase, histidine decarboxylase and Fos proto-oncogene as candidate genes that may be involved in occurrence and development of FM, all of them related to musculoskeletal disease, mental disorder or immune system disease. They also found a wide group of differentially expressed genes and microRNAs that may serve as potential targets for diagnosis and treatment of FM [7].

Greater levels of physical activity (PA) and lower levels of sedentary behaviour are both related to lower pain in FM [8], and physical exercise training is considered important in the treatment of FM [9]. However, no previous research has studied the interaction of genotype and PA or sedentary behaviour with pain. The understanding of this interaction might help to tailor the general advice of engaging in PA while reducing sedentary behaviour according to the genotype of people with FM. A recent study has reported the relevance of two genes (*MRPL4* and *SLC38A*) associated with mitochondrial function or oxidative phosphorylation and glutamate signalling as possible discriminating genes in FM [10].

There are previous reports regarding the impact of physical exercise in FM. Some of them suggest that aerobic exercise reduces autonomic dysfunction, whereas resistance training reduces psychological symptoms such as depression [11, 12], and training focussed on strength improves the symptoms of FM [13]. However, there are few data about genetic variants, PA and their impact in FM. Thus, we have focussed on the search of single nucleotide polymorphisms (SNPs) variants that have a role in pain in FM, taking into account the effect of PA.

Due to the complex nature of the phenotype of pain in FM, our hypothesis was that pain is not only related to individual genotype associations, but also to gene–gene interactions and gene–PA interactions. Therefore, the aims of this study were (i) to analyse the singular association of 64 polymorphisms of 34 FM candidate genes, as well as the gene–gene, gene–PA and gene–sedentary behaviour interactions with pain outcomes in a well-characterized sample of southern Spanish women with FM, and (ii) to test the robustness of previous results by performing a meta-analysis of all the available evidence.

Methods

Participants

The al-Ándalus project aimed at recruiting a geographically representative sample of women with FM from Andalusia (southern Spain) as described elsewhere [14]. Recruiting criteria are available in the Supplementary Materials and Methods, available at *Rheumatology* online.

Genetic analysis

The participants were genotyped for 64 SNPs that had been previously investigated in relation to FM susceptibility, symptoms or potential mechanisms (supplementary Table S1, available at *Rheumatology* online.). As described elsewhere [15, 16], 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). See details about samples preparation in Supplementary Materials and Methods, available at *Rheumatology* online. Supplementary Table S2 (available at *Rheumatology* online) shows the manufacturer thermal cycling conditions.

Plates include an Non Template Control for each SNP in the analysis, and each plate has a total of 48 samples. Supplementary Tables S3 and S4 (available at *Rheumatology* online) provide further details about the TaqMan® OpenArray® custom assay designs and the 64 analysed SNPs, respectively. We performed a TaqMan™ OpenArray™ Genotyping Plate, Custom Format 64 QuantStudio™ 12 K Flex. The data were analysed using the TaqMan® Genotyper Software and downstream analysis using the AutoCaller™ Software.

Measures related to PA and sedentary behaviour

Triaxial accelerometers GT3X+ (Actigraph, Pensacola, FL, USA) were used to objectively measure PA and sedentary behaviour. Activity counts were measured at a rate of 30 Hz and stored at an epoch length of 60 s [17, 18] using a triaxial accelerometer GT3X+ (Actigraph, Pensacola, FL, USA). Participants wore the accelerometer on the hip for up to 9 days; however, the first and last days were excluded from the analyses. A total of seven continuous days with a minimum of 10 valid hours per day was required in order to be included in the study analysis. Participants were instructed to remove the accelerometer while showering or practising water activities. Sleeping time was recorded through a diary and excluded from analyses. Sedentary behaviour and PA were calculated based upon recommended vector magnitude cut point [17, 18]: 0–199 and $\geq$200 counts per min, 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 [19, 20], and detailed information about the treatment of data for this study is described in the Supplementary Materials and Methods, available at *Rheumatology* online.

Measures related to pain outcomes

Measures related to pain outcomes and brief explanations of them are included in the Supplementary Materials and Methods, available at *Rheumatology* online.

Measures related to potential confounders

Measures related to potential confounders and brief explanations of them are included in the Supplementary Materials and Methods, available at *Rheumatology* online.

Procedures

The assessments were conducted over three consecutive days. On day 1, the participants completed sociodemographic and clinical data, and trained researchers measured body composition and tender points. Subsequently, participants received several pain-related questionnaires to be completed at home on day 2. On day 3, participants returned the questionnaires and received the accelerometer to be worn for nine consecutive days.

All participants were provided with and signed written informed consent prior to taking part in the study. The al-Ándalus project protocol was reviewed and approved by the Ethics Committee of the Virgen de las Nieves Hospital (Granada, Spain), registration number: 15/11/2013-N72. The ethical guidelines of the declaration of Helsinki were followed.

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 [21]. Gene–phenotype associations along with gene–gene interactions were assessed with the 'SNPassoc' package [22]. We developed our own script to study gene–environment interactions. For more information about the statistical analysis, see the Supplementary Materials and Methods, available at *Rheumatology* online.

In silico analysis on the functional and structural impact of the SNPs

Information about *in silico* analysis are available in the Supplementary Materials and Methods, available at *Rheumatology* online.

Meta-analysis

This meta-analysis was performed using six selected SNPs analysed in our cohort, located in the *COMT* gene (rs4633, rs4680, rs4818, rs6269, rs165599 and rs20907). See the Supplementary Materials and Methods (available at *Rheumatology* online) for more information.

We used the MetaGenyo platform (http://bioinfo.genyo.es/metagenyo/) [23], which reports statistical data among different genetic variants and pain associated to FM.

Results

Descriptive analysis

Table 1 shows the characteristics of the 274 participants included in the present study. The rs7124442 (brain-derived neurotrophic factor antisense RNA gene, *BDNF-AS*), rs7911 (guanylate binding protein 1 gene, *GBP1*), rs1050450 (glutathione peroxidase 1 gene, *GPX1*), rs4411417 (GTP cyclohydrolase 1 gene, *GCH1*), rs6323 and rs1137070 (monoamine oxidase A gene, *MAOA*), and rs3746544 (synaptosome associated protein 2 gene 5, *SNAP25*) polymorphisms did not meet the HWE criteria. A low genotyping rate (i.e. $\leq$0.90) was

TABLE 1 Characteristics of the participants in the study, $n = 274$

Characteristics	Value
Drugs consumption (yes *vs* no), *n* (%)	
Analgesics (yes)	245 (89.8)
Antidepressants (yes)	145 (52.9)
Age, years, mean (S.D.)	51.7 (7.7)
Body fat (%), mean (S.D.)	40.5 (7.6)
Physical activity (accelerometers), mean (S.D.)	
Moderate-to-vigorous physical activity (min/week)	86.9 (118.9)
Sedentary behaviour (min/day)	459.1 (108.1)
Pain-related outcomes, mean (S.D.)	
Pressure pain threshold (algometry, kg/cm^2)	42.8 (13.2)
Pain rating (FIQR, 0–10)	7.3 (1.7)
Bodily pain (SF-36, 0–100)	21.2 (14.4)
Visual analogue scale (mm, 0–100)	64.5 (21.9)
Pain catastrophizing (PCS, 0–52)	21.2 (24.5)
Pain self-efficacy (CPSS, 0–100)	36.4 (22.9)

FIQR: revised FM impact questionnaire; SF-36: 36-item short form health survey; PCS: pain catastrophizing scale; CPSS: chronic pain self-efficacy scale.

observed for the rs9470080 (FK506 binding protein 5 gene, *FKBP5*), rs4371369, rs4387806, rs6746030, rs12620053 (sodium voltage-gated channel alpha subunit 9 gene, *SCN9A*) and rs7310505 (thioredoxin reductase 1 gene, *TXNRD1*) polymorphisms. The remaining 51 polymorphisms were included in the present study.

Individual association between genotype and phenotype

The individual associations of the rs6311 and rs6313 polymorphisms (5-hydroxytryptamine receptor 2A gene, *HTR2A*) with pressure pain threshold were significant under the overdominant model, but not the other models. Fig. 1 shows that carriers of the CC/TT genotype had higher pain thresholds (i.e. lower pain) than those with the CT genotype [$P = 0.0007$, false discovery rate (FDR) $= 0.025$ and $P = 0.0017$, FDR $= 0.032$]. These two polymorphisms were in linkage disequilibrium (D' $= 0.98$). The remaining individual associations between genotype and pain were not significant (data not shown).

Gene–gene interaction

Fig. 2 shows an interaction of the rs4818 and rs1799971 polymorphisms (*COMT* and *OPRM1* genes, respectively) with pain catastrophizing under the codominant model ($P = 0.00003$, FDR $= 0.017$). Pain catastrophizing for carriers of the CC genotype of the rs4818 polymorphism did not differ for different genotypes 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.

The interactions involving the rs1383914 polymorphism (adrenoceptor alpha 1A gene, *ADRA1A*) with the rs752688 polymorphism (GTP cyclohydrolase 1 gene, *GCH1*), and rs1042713 polymorphism (adrenoceptor beta 2 gene, *ADRB2*) with rs429358 polymorphism (apolipoprotein E gene, *APOE*), 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 (data not shown).

Gene–behaviour interaction

The interaction of genotype and PA was not related to any pain outcome (data not shown). An exception was the interaction of the rs573542 polymorphism, *ADRA1A* gene, and PA with pain self-efficacy ($P = 0.0003$, FDR $= 0.013$). However, only seven participants with the GA/GG genotype engaged in higher levels of PA, which suggests an insufficient statistical power to reach conclusions.

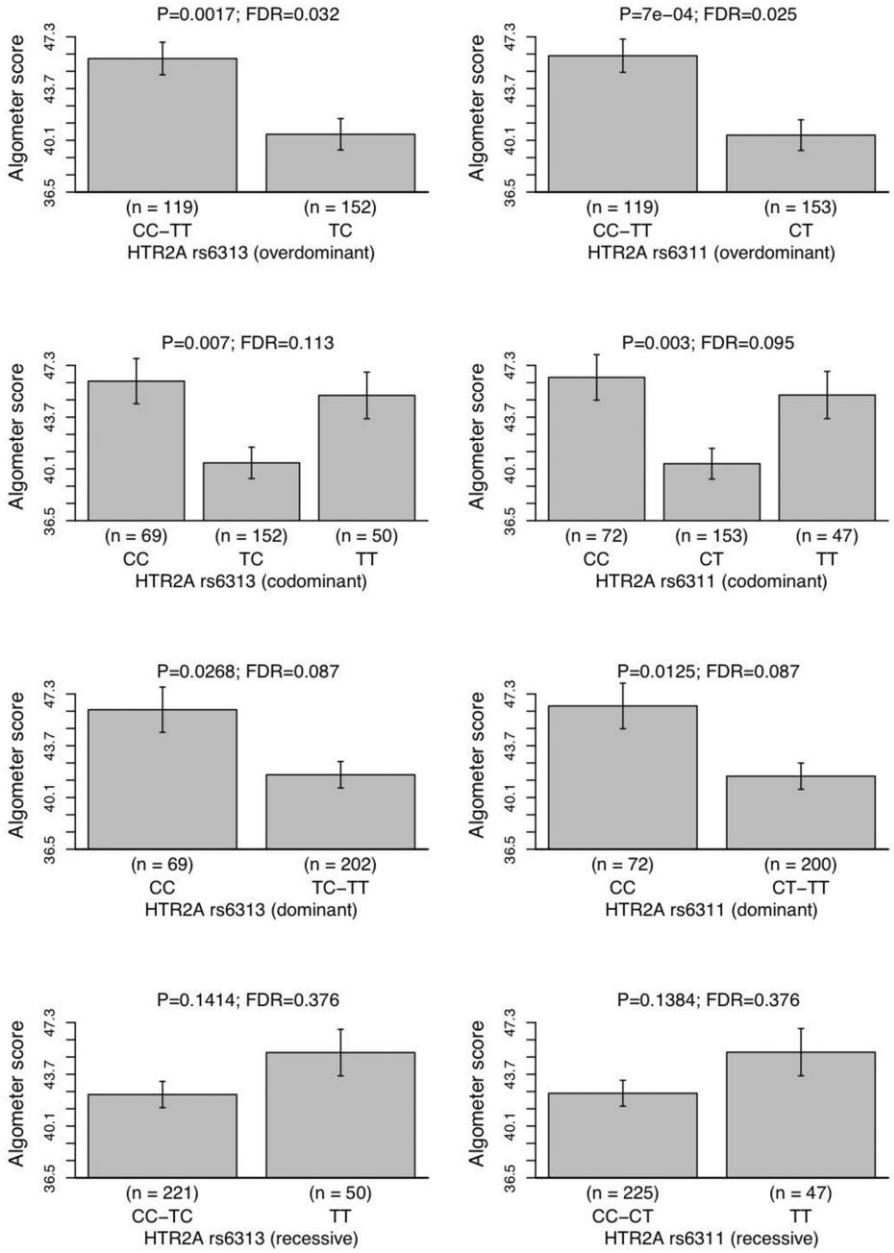
Fig. 3 shows that the associations of the genotypes of the rs1383914 (*ADRA1A* gene), rs6860 (charged multivesicular body protein 1A gene, *CHMP1A*), rs4680 and rs165599 (*COMT* gene), and rs12994338 polymorphisms (*SCN9A* gene) with bodily pain [Short Form 36 (SF-36)] differ according to the levels of sedentary behaviours of the participants (all, $P < 0.05$ and FDR ≤ 0.02). Participants who 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 lower pain on the SF-36 bodily pain scale only if they were a particular genotype: AA/GG for rs4680, rs6860 and rs165599, and AG for rs1383914. Moreover, participants showing the combination of the CC/TT genotype of the rs12994338 polymorphism and high sedentary behaviour showed the worst bodily pain (low scores on the SF-36 bodily pain scale).

The interaction of the rs25531 polymorphism (solute carrier family 6 member 4 gene, *SLC6A4*) and sedentary behaviour on acute pain (visual analogue scale score) lacked statistical power, but was significant ($P = 0.0002$ and FDR $= 0.003$). Only 18 participants were AG genotype, nine in each sedentary behaviour level. The remaining interactions of genotype and sedentary behaviour with pain outcomes were not significant (data not shown).

In silico analysis on the functional impact and clinical effects

Most of the variants were located in introns or upstream and downstream regions, and they did not change the amino acid sequence. Some of them were located in coding regions and produced synonymous variants (rs4633 and rs4818), recognized as benign mutations.

FIG. 1 Individual associations of the genotype of the rs6311 and rs6313 polymorphisms (*HTR2A* gene) with algometry score (algometry, kg/cm^2)



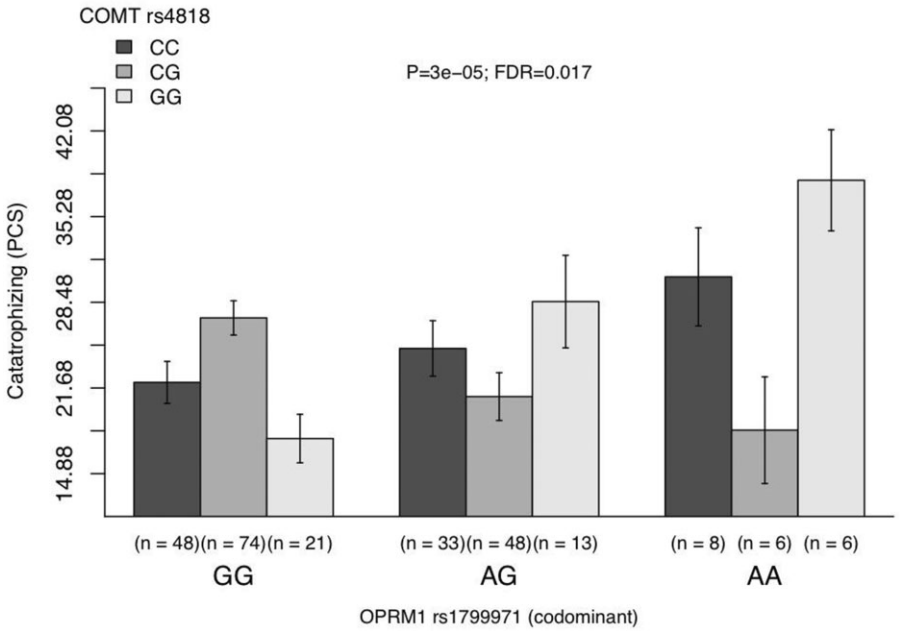
According to the *P*-value and FDR, the significance was reached only under the overdominant model. *HTR2A*: 5-hydroxytryptamine receptor 2A gene; C: cytosine; T: thymine; FDR: false discovery rate.

Interestingly, we found a missense variant (rs4680) that caused a change in the amino acid 158 (Val>Met) of COMT protein. This substitution resulted in a drug response effect involving substances such as nicotine, tramadol, methadone or morphine. See supplementary Table S5, available at *Rheumatology* online, for more detailed information.

Functional analysis was performed using the *COMT* gene and a list of related genes interacting with *COMT*, obtained by the STRING online platform. The resulting list of genes included *COMT*, *CYP1A1*, *CYP1B1*, *DDC*, *DBH*, *PNMT*, *ADH1B*, *ADH6*, *ALDH2*, *MAOA* and *MAOB* (more details in Fig. 4).

The DAVID bioinformatics analysis of these 11 genes showed association with alcohol consumption (72.7% of genes, $P = 1.0 \times 10^{-12}$), drug-related pathways (63.6% of genes, $P = 2.3 \times 10^{-9}$) and oxidoreductase activity (54.5% of genes, $P = 5.3 \times 10^{-8}$). The main related pathway with our list of genes was tyrosine metabolism (72.7% of genes, $P = 5.5 \times 10^{-15}$).

Fig. 2 Gene–gene interaction of the rs4818 and rs1799971 polymorphisms with catastrophizing



According to the *P*-value and FDR, 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. *COMT*: catechol-O-methyltransferase gene; *OPRM1*: opioid receptor μ gene; PCS: pain catastrophizing scale (scores range 0–52); A: adenine; C: cytosine; G: guanine; FDR: false discovery rate.

Meta-analysis

The meta-analysis included a total of 12 studies about several variants in *COMT* gene: rs4633, rs4680, rs4818, rs6269, rs165599 and rs20907. As shown in Table 2, a relationship was observed between rs4680 and pain in FM ($P = 0.032$ and $P = 0.047$ using a codominant and a dominant model, respectively; forest plot shown in Fig. 5). Further information is shown in supplementary Fig. S1, available at *Rheumatology* online. Three of the studies that were about this SNP (Martínez-Jauand [24], Matsuda [27] and Barbosa [32]) did not adapt to HWE ($P < 0.05$), but they were included in the meta-analysis because this assumption of equilibrium was checked in the original articles.

Discussion

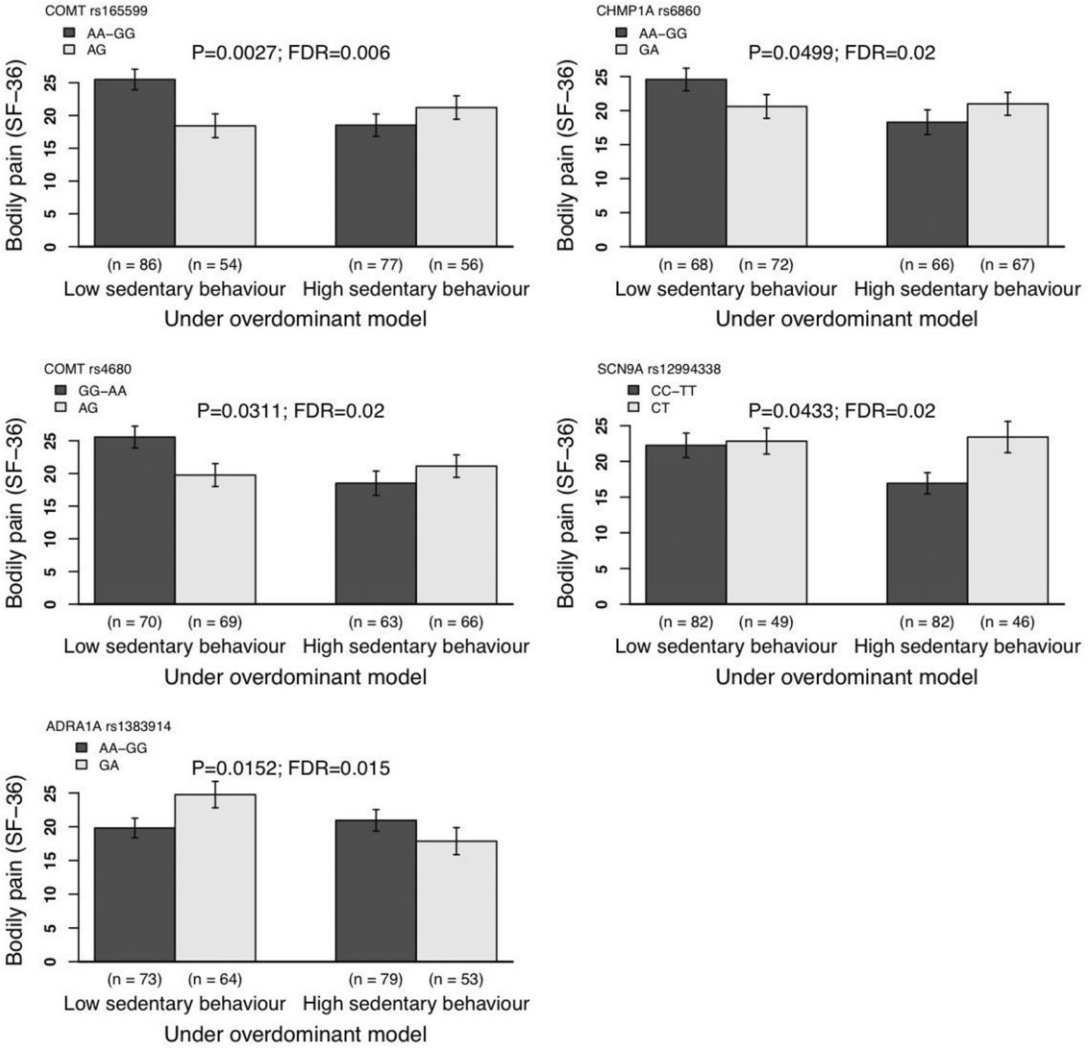
The present candidate gene study including 64 polymorphisms of 34 genes is the most comprehensive on pain outcomes in FM to date. We observed that the rs6311 and rs6313 polymorphisms (*HTR2A* gene) were individually related to pressure pain threshold. The present research is unique because of the study of gene–gene and gene–behaviour interactions. We found significant interactions of the rs4818 and rs1799971 polymorphisms (*COMT* and *OPRM1* genes, respectively) with pain catastrophizing. In addition, we observed gene–sedentary behaviour interaction on bodily pain (SF-36) for several polymorphisms [i.e. the rs1383914 (*ADRA1A* gene), rs6860 (*CHMP1A* gene), rs4680 and rs165599 (*COMT* gene), and rs12994338 (*SCN9A* gene)]. Furthermore, meta-analysis showed association between rs4680 and FM-related pain.

Although inconsistent across studies, a review concluded that the *HTR2A* gene is individually associated with susceptibility to FM [35]. In our dataset, in comparison with the CT/TT, the CC genotype of the rs6311 and rs6313 polymorphisms (*HTR2A* gene) was related to better pressure pain threshold. In this line, among people with chronic low back pain, those with the CC genotype of the rs6311 and rs6313 polymorphisms showed the lowest disability score [36]. Overall, it seems that the CC genotype of the rs6311 and rs6313 polymorphisms (*HTR2A* gene) may buffer the levels of pain experienced by people living with a chronic pain disorder.

The *HTR2A* gene encodes one of the receptors for serotonin. The serotonergic 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 FM, abnormalities in the serotoninergic system are present [37] and selective serotonin reuptake inhibitors are effective for treating pain in some patients [38]. Therefore, the rs6313 variant in the *HTR2A* gene may be involved in pain-related outcomes, which is consistent with our findings [39, 40].

In the present study, the interaction of the rs4818 and rs1799971 polymorphisms (*COMT* and *OPRM1A* genes, respectively) was related to pain catastrophizing.

Fig. 3 Interactions of gene–sedentary behaviour with bodily pain

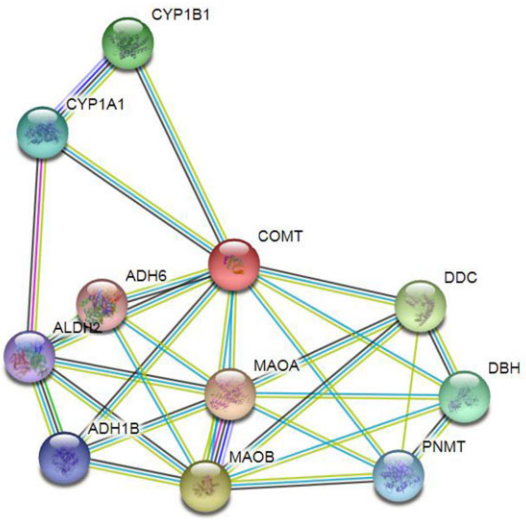


Sedentary behaviour was objectively measured using triaxial accelerometers GT3X+ (Actigraph, Pensacola, FL, USA) as dichotomized data (low *vs* high) using the mean as the cut-off value. According to the *P*-value and FDR, all these gene–sedentary behaviour interactions were significant. ADRA1A: adrenoceptor alpha 1A gene; CHMP1A: charged multivesicular body proteints 1A gene; COMT: catechol-O-methyltransferase gene; SCN9A: sodium voltage-gated channel alpha subunit 9 gene; A: adenine; C: cytosine; G: guanine; T: thymine; SF-36: the 36-item short form health survey (score range 0–100); FDR: false discovery rate.

A conclusion regarding the AA genotype of the rs1799971 cannot be drawn given the low sample size ($n \leq 10$) in all the genotypes. Among carriers 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, while within those carrying the AG genotype of the rs1799971 polymorphism, GG carriers of the rs4818 reported the highest pain catastrophizing. Interestingly, a recent article found a protective effect of G allele of rs4818 for postoperative pain sensibility and duration [41]. An inspiring study has 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 both women with and without FM [6]. 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 pain-related cognitions (i.e. catastrophizing) in women with FM. Moreover, this interaction is in agreement with the hypothesis of aberrances on the CNS in FM [42]. Although the interaction of the *COMT* and *OPMR1* genes had not been explored previously in FM, their additive association with postoperative pain has been observed [43].

In FM, the common co-occurrence of pain and distress points to the hypothalamic–pituitary–adrenal axis

FIG. 4 STRING network with studied genes



ADH1B: alcohol dehydrogenase 1B; *ADH6*: alcohol dehydrogenase 6; *ALDH2*: aldehyde dehydrogenase 2; *COMT*: catechol-O-methyltransferase; *CYP1A1*: Cytochrome P450 Family 1 Subfamily A Member 1; *CYP1B1*: Cytochrome P450 Family 1 Subfamily B Member 1; *DBH*: dopamine beta-hydroxylase; *DDC*: dopa decarboxylase; *MAOA*: monoamine oxidase A; *MAOB*: monoamine oxidase B; *PNMT*: phenylethanolamide *N*-methyltransferase.

and sympathetic nervous system as potential determinants of the disease onset and prognosis [44–47]. In the CNS, the *COMT* gene modulates the production of catecholamines and other neurotransmitters that bind 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 sympathetically maintaining pain in FM [48]. One hypothesis is that mutations in the *SCN9A* gene may lead to up-regulation of sodium channels, which drives hyperexcitability of the dorsal root ganglia and, finally, leads to increased pain [48]. Other potential mechanisms involved in FM are pain oxidative stress [49] and excessive autophagy [50], where the *CHMP1A* gene may play a role [51] via amygdala [49] and *mTOR* signalling [52] pathways.

In the present study, we found significant interactions of sedentary behaviour and the rs4680, rs165599 (*COMT* gene), rs1383914 (*ADRA1A* gene), rs12994338 (*SCN9A* gene) and rs6860 (*CHMP1A* gene) polymorphisms with bodily pain (SF-36). People's physical exercise behaviours may particularly modulate the activity of physiological stress systems [46] and it has been highlighted that sedentary people may be particularly sensitive to stress [53]. Therefore, our results tentatively suggest that, in women with FM, the reduction of sedentary behaviour may attenuate the genetic predisposition to increased pain.

Further clinical-experimental research is warranted in order to shed light on the causality of our findings. If future research confirms the causality of the present associations, evidence-based guidelines could be suggested for the management of pain in FM. For instance, the general advice to manage the disease is to combine pharmacological and physical exercise treatments [9]. However, no universally effective treatment is available for FM, which may be a consequence of the heterogeneity observed in this population [54]. Therefore, it appears useful to take into account the characteristics of people with FM when choosing interventions (precision medicine). Our results suggest a potential role of genetic mechanisms involved in modulation of neurotransmitters and opioids affecting the sympathetic nervous system, dorsal root ganglia and hypothalamic–pituitary–adrenal axis. Accordingly, the challenge would be to better modulate the functioning of these systems potentially involved in FM pain. Regarding pharmacological therapy, the present findings suggest that selective serotonin reuptake inhibitors could be an effective analgesic, particularly in patients with such genetic predisposition. Regarding physical programmes, reducing the amount of time spent in sedentary behaviour might be particularly beneficial to reduce pain in women with FM who are carriers of a particular genotype as follows: AA/GG for rs6860 (*CHMP1A* gene), rs4680 and rs165599 (*COMT* gene), AG for rs1383914 (*ADRA1A* gene), and CC/TT for rs12994338 (*SCN9A* gene).

Full understanding of FM encompasses multiple interacting biological, psychological and social factors [55, 56]. However, the present study only examined the interplay of genetics with PA, and did not consider other biopsychosocial factors, which is a limitation. Also, in FM pain is a symptom that fluctuates from day to day. However, the present cross-sectional study did not allow us to compare our findings with previous research that tested the dynamic association of genotype and daily variations on pain within-person [57]. Thereby, further research testing more sophisticated dynamic network models (e.g. the mobile toy model of pain) [55] is warranted. Moreover, a replication study testing the robustness of the present findings is needed. Compared with previous studies [5], our larger sample size is a strength of the present study. We also corroborated the FM diagnosis according to the 1990 ACR FM criteria, and our sample was representative of the southern Spanish population of women with FM [14]. Another strength was the inclusion of 64 polymorphisms of 34 candidate genes, which made this study the most comprehensive research of genetics and pain-related outcomes in FM until date. Furthermore, we objectively measured PA and sedentary behaviour with accelerometers, while pain was assessed with an algometer and questionnaires. Finally, we adjusted our analyses for multiple comparisons, which support the robustness of our findings.

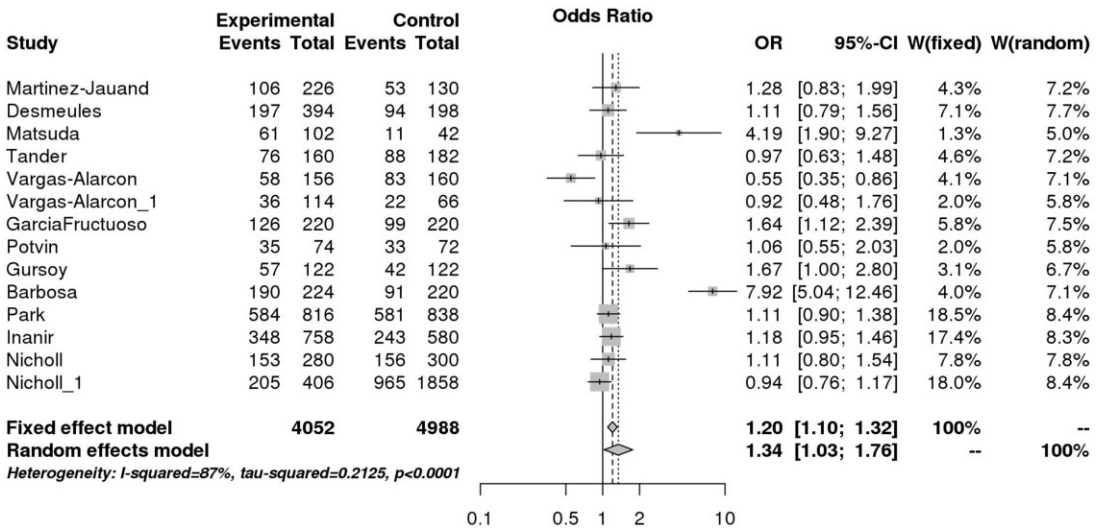
To conclude, the present candidate gene study is the most comprehensive on pain outcomes in FM to date.

Table 2 Summary of the results obtained in present meta-analysis

SNP	Studies/publications	HWE	Significance		OR (95% CI)
rs4633	Martínez-Jauand [24] (Spain)	0.1174	Codominant model	0.986	0.997 (0.688, 1.444)
	Vargas-Alarcón [5] (Spain)	0.6976	Recessive model	0.112	1.204 (0.958, 1.514)
	Vargas-Alarcón [5] (Mexico)	0.8219	Dominant model	0.567	0.801 (0.374, 1.716)
	Park [25] (South Korea)	0.1174			
rs4680	Martínez-Jauand [24] (Spain)	0.0135	Codominant model	**0.032**	1.342 (1.026, 1.756)
	Desmeules [26] (Switzerland)	0.4046			
	Matsuda [27] (Brazil)	0.0007			
	Tander [28] (Turkey)	0.0567			
	Vargas-Alarcón [5] (Spain)	0.8326			
	Vargas-Alarcón [5] (Mexico)	0.8326	Recessive model	0.099	1.355 (0.945, 1.944)
	García-Fructuoso [29] (Spain)	0.8326			
	Potvin [30] (Canada)	0.4046			
	Gürsoy [31] (Turkey)	0.2322			
	Barbosa [32] (Brazil)	0	Dominant model	**0.047**	1.419 (1.005, 2.004)
	Park [25] (South Korea)	0.2070			
	Inanir [33] (Turkey)	0,1585			
	Nicholl [34] (UK)	0.4046			
	Nicholl [34] (European)	0.4046			
rs4818	Martínez-Jauand [24] (Spain)	0.4108	Codominant model	0.305	1.319 (0.777, 2.240)
	Vargas-Alarcón [5] (Spain)	0.4108	Recessive model	0.438	1.271 (0.694, 2.325)
	Vargas-Alarcón [5] (Mexico)	0.4108	Dominant model	0.298	1.594 (0.662 3.839)
	Park [25] (South Korea)	0.8832			
rs6269	Martínez-Jauand [24] (Spain)	0.5121	Codominant model	0.411	1.186 (0.790, 1.781)
	Vargas-Alarcón [5] (Spain)	0.5121	Recessive model	0.494	1.178 (0.736, 1.887)
	Vargas-Alarcón [5] (Mexico)	0.8219	Dominant model	0.487	1.262 (0.655, 2.430)
	Park [25] (South Korea)	0.5121			
rs165599	Vargas-Alarcón [5] (Spain)	0.9225	Codominant model	0.527	0.946 (0.798, 1.123)
	Vargas-Alarcón [5] (Mexico)	0.9225	Recessive model	0.670	0.943 (0.721, 1.234)
	Park [25] (South Korea)	0.9478	Dominant model	0.542	0.915 (0.688, 1.217)
rs20907	Vargas-Alarcón [5] (Spain)	0.9596	Codominant model	0.302	1.214 (0.840, 1.755)
	Vargas-Alarcón [5] (Mexico)	0.9596	Recessive model	0.246	1.364 (0.807, 2.308)
			Dominant model	0.680	1.158 (0.576, 2.329)

HWE: Hardy–Weinberg Equilibrium; OR: odds ratio. Only significant P values appear in bold.

Fig. 5 Forest plot of meta-analysis in rs4680 (*COMT*)



OR: odds ratio.

For the first time, we identified (i) individual associations of the rs6311 and 6313 polymorphisms (*HTR2A* gene) with pressure pain threshold; (ii) interactions of rs4818 and rs1799971 polymorphisms (*COMT* and *OPRM1* genes, respectively) on pain catastrophizing; and (iii) interactions of sedentary behaviour and rs4680, rs165599 (*COMT* gene), rs1383914 (*ADRA1A* gene), rs12994338 (*SCN9A* gene) and rs6860 (*CHMP1A* gene) polymorphisms on bodily pain (SF-36). Therefore, the present study highlights the relevance of taking account of gene–gene and genotype–sedentary behaviour interactions when studying pain outcomes in women with FM. If corroborated in future (observational and experimental) longitudinal research, our findings might suggest that the reduction of sedentary behaviour may be beneficial for reducing pain, particularly in women with FM who have specific genotypes.

Acknowledgements

The authors gratefully acknowledge all participants for their collaboration and enthusiasm. We would like to thank the collaborators in the al-Ándalus project and all the members of the Physical Activity for Health Promotion (PA-HELP; CTS-1018) research group. We would also like to thank Ms Gema Garcia (Genomic unit, Genyo) for her helpful support in DNA extraction and genotyping of the samples. A.S.-M. was supported by the Spanish Ministry of Science, Innovation and Universities (ref. RTI2018-093302-A-I00).

Funding: This work was supported by the Spanish Ministry of Economy and Competitiveness (I+D+i DEP2010-15639, I+D+i DEP2013-40908-R to M.D.-F.; BES-2014-067612 to F.E.-L.), the Spanish Ministry of Education (FPU13/03410 to D.S.-T.; FPU15/0002 to B.G.-C.), the Consejería de Turismo, Comercio y Deporte, Junta de Andalucía (CTCD-201000019242-TRA to M.D.-F.), the Consejería de Salud, Junta de Andalucía (PI-0520-2016 to M.D.-F.) and the University of Granada, Plan Propio de Investigación 2016, Excellence actions: Units of Excellence; Unit of Excellence on Exercise and Health (UCEES). The funders of the present study did not have any role in the study design, data collection and analyses, decision to publish or preparation of the manuscript.

Disclosure statement: The authors have declared no conflicts of interest.

Data availability statement

The data are available upon reasonable request to the first author (F.E.-L., fer@estevez-lopez.com).

Supplementary data

Supplementary data are available at *Rheumatology* online.

References

1 Wolfe F, Walitt BT, Häuser W. What is fibromyalgia, how is it diagnosed, and what does it really mean? Arthritis Care Res (Hoboken) 2014;66:969–71.

2 Martínez-Lavín M. Fibromyalgia in women: somatisation or stress-evoked, sex-dimorphic neuropathic pain? Clin Exp Rheumatol 2021;39:422–5.

3 Zubieta J-K, Heitzeg MM, Smith YR *et al.* COMT val 158 met genotype affects mu-opioid neurotransmitter responses to a pain stressor. Science 2003;299:1240–3.

4 Ferrera D, Mercado F, Peláez I *et al.* Fear of pain moderates the relationship between self-reported fatigue and methionine allele of catechol-O-methyltransferase gene in patients with fibromyalgia. PLoS One 2021;16: e0250547.

5 Vargas-Alarcón G, Fragoso JM, Cruz-Robles D *et al.* Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. Arthritis Res Ther 2007;9:R110.

6 Tour J, Löfgren M, Mannerkorpi K *et al.* Gene-to-gene interactions regulate endogenous pain modulation in fibromyalgia patients and healthy controls-antagonistic effects between opioid and serotonin-related genes. Pain 2017;158:1194–203.

7 Qiu Y, Zhang TJ, Meng LB, Cheng XT, Hua Z. Bioinformatics analysis of gene and microRNA targets for fibromyalgia. Clin Exp Rheumatol 2021;39:21–31.

8 Segura-Jiménez V, Soriano-Maldonado A, Estévez-López F *et al.* Independent and joint associations of physical activity and fitness with fibromyalgia symptoms and severity: the al- Ándalus project. J Sports Sci 2017; 35:1565–74.

9 Macfarlane GJ, Kronisch C, Dean LE *et al.* EULAR revised recommendations for the management of fibromyalgia. Ann Rheum Dis 2017;76:318–28.

10 Lukkahatai N, Walitt B, Deandrés-Galiana EJ, Fernández-Martínez JL, Saligan LN. A predictive algorithm to identify genes that discriminate individuals with fibromyalgia syndrome diagnosis from healthy controls. J Pain Res 2018;11:2981–90. Novdoi: 10.2147/JPR.S169499.

11 Andrade A, Vilarino GT, Serafim TT *et al.* Modulation of autonomic function by physical exercise in patients with fibromyalgia syndrome: a systematic review. PM R 2019; 11:1121–31. Oct

12 Roeh A, Kirchner SK, Malchow B *et al.* Depression in somatic disorders: is there a beneficial effect of exercise? Front Psychiatry 2019;10:141.

13 Andrade A, Dominski FH, Sieczkowska SM. What we already know about the effects of exercise in patients with fibromyalgia: an umbrella review. Semin Arthritis Rheum 2020;50:1465–80.

14 Segura-Jiménez V, Álvarez-Gallardo IC, Carbonell-Baeza A *et al.* Fibromyalgia has a larger impact on physical health than on psychological health, yet both are markedly affected: the al-Ándalus project. Semin Arthritis Rheum 2015;44:563–70. Apr

15 Freeman B, Smith N, Curtis C *et al.* DNA from buccal swabs recruited by mail: evaluation of storage effects on long-term stability and suitability for multiplex polymerase chain reaction genotyping. Behav Genet 2003;33: 67–72.

16 Gómez-Martín A, Hernández AF, Martínez-González LJ *et al.* Polymorphisms of pesticide-metabolizing genes in children living in intensive farming communities. Chemosphere 2015;139:534–40.

17 Aguilar-Farías N, Brown WJ, Peeters G. ActiGraph GT3X+ cut-points for identifying sedentary behaviour in older adults in free-living environments. J Sci Med Sport 2014;17:293–9.

18 Sasaki JE, John D, Freedson PS. Validation and comparison of ActiGraph activity monitors. J Sci Med Sport 2011;14:411–6. Sep

19 Segura-Jiménez V, Álvarez-Gallardo IC, Estévez-López F *et al.* Differences in sedentary time and physical activity between female patients with fibromyalgia and healthy controls: the al-Ándalus project. Arthritis Rheumatol 2015;67:3047–57.

20 Segura-Jiménez V, Borges-Cosic M, Soriano-Maldonado A *et al.* Association of sedentary time and physical activity with pain, fatigue, and impact of fibromyalgia: the al-Ándalus study. Scand J Med Sci Sports 2017;27: 83–92.

21 Warnes G, Gorjanc G, Leisch F, Man M. genetics: Population Genetics. R package version 1.3.8.1. The Comprehensive R Archive Network. 2013. http://cran.r-project.org.

22 González JR, Armengol L, Solé X *et al.* SNPassoc: an R package to perform whole genome association studies. Bioinformatics 2007;23:644–5.

23 Martorell-Marugan J, Toro-Dominguez D, Alarcon-Riquelme ME, Carmona-Saez P. MetaGenyo: a web tool for meta-analysis of genetic association studies. BMC Bioinformatics 2017;18:563.

24 Martínez-Jauand M, Sitges C, Rodríguez V *et al.* Pain sensitivity in fibromyalgia is associated with catechol-O-methyltransferase (COMT) gene. Eur J Pain 2013;17:16–27.

25 Park DJ, Kim SH, Nah SS *et al.* Association between catechol-O-methyl transferase gene polymorphisms and fibromyalgia in a Korean population: a case–control study. Eur J Pain 2016;20:1131–9.

26 Desmeules J, Piguet V, Besson M *et al.* Psychological distress in fibromyalgia patients: a role for catechol-O-methyl-transferase Val158Met polymorphism. Heal Psychol 2012;31:242–9.

27 Matsuda JB, Barbosa FR, Morel LJF *et al.* Serotonin receptor (5-HT 2A) and catechol-O-methyltransferase (COMT) gene polymorphisms: triggers of fibromyalgia? Rev Bras Reumatol 2010;50:141–9.

28 Tander B, Gunes S, Boke O *et al.* Polymorphisms of the serotonin-2A receptor and catechol-O-methyltransferase genes: a study on fibromyalgia susceptibility. Rheumatol Int 2008;28:685–91.

29 García-Fructuoso FJ, Lao-Villadóniga JI, Beyer K, Santos C. Relación entre genotipos del gen catecol O-metiltransferasa y la gravedad de la fibromialgia. Reumatol Clin 2006;2:168–72.

30 Potvin S, Larouche A, Normand E *et al.* DRD3 Ser9Gly polymorphism is related to thermal pain perception and modulation in chronic widespread pain patients and healthy controls. J Pain 2009;10:969–75.

31 Gürsoy S, Erdal E, Herken H *et al.* Significance of catechol-O-methyltransferase gene polymorphism in fibromyalgia syndrome. Rheumatol Int 2003;23:104–7.

32 Barbosa FR, Matsuda JB, Mazucato M *et al.* Influence of catechol-O-methyltransferase (COMT) gene polymorphisms in pain sensibility of Brazilian fibromialgia patients. Rheumatol Int 2012;32:427–30.

33 Inanir A, Karakus N, Ates O *et al.* Clinical symptoms in fibromyalgia are associated to catechol-O-methyltransferase (COMT) gene Val158Met polymorphism. Xenobiotica 2014;44:952–6.

34 Nicholl BI, Holliday KL, Macfarlane GJ *et al.*; European Male Ageing Study Group. No evidence for a role of the catechol-O-methyltransferase pain sensitivity haplotypes in chronic widespread pain. Ann Rheum Dis 2010;69: 2009–12.

35 Lee YH, Choi SJ, Ji JD, Song GG. Candidate gene studies of fibromyalgia: a systematic review and meta-analysis. Rheumatol Int 2012;32:417–26. Feb

36 Yıldız SH. Assessment of pain sensitivity in patients with chronic low back pain and association with HTR2A gene polymorphism. Arch Rheumatol 2017;32:3–9. Mar

37 Rahman A, Underwood M, Carnes D. Fibromyalgia. BMJ 2014;348:g1224.

38 Nüesch E, Häuser W, Bernardy K, Barth J, Jüni P. Comparative efficacy of pharmacological and non-pharmacological interventions in fibromyalgia syndrome: network meta-analysis. Ann Rheum Dis 2013;72:955–62.

39 Sachau J, Bruckmueller H, Gierthmühlen J *et al.* The serotonin receptor 2A (HTR2A) rs6313 variant is associated with higher ongoing pain and signs of central sensitization in neuropathic pain patients. Eur J Pain 2021;25:595–611.

40 Nicholl BI, Holliday KL, MacFarlane GJ *et al.*; European Male Ageing Study Group. Association of HTR2A polymorphisms with chronic widespread pain and the extent of musculoskeletal pain: results from two population-based cohorts. Arthritis Rheum 2011;63: 810–8.

41 Silva EMVM, Lacerda RHW, Farias IL *et al.* COMT rs4818, pain sensitivity and duration, and alveolar bone grafting of oral clefts. Oral Maxillofac Surg 2021;25: 253–6.

42 Docampo E, Escaramís G, Gratacòs M *et al.* Genome-wide analysis of single nucleotide polymorphisms and copy number variants in fibromyalgia suggest a role for the central nervous system. Pain 2014;155:1102–9.

43 Khalil H, Sereika SM, Dai F *et al.* OPRM1 and COMT gene–gene interaction is associated with postoperative pain and opioid consumption after orthopedic trauma. Biol Res Nurs 2017;19:170–9.

44 Thornton LM, Andersen BL, Blakely WP. The pain, depression, and fatigue symptom cluster in advanced breast cancer: covariation with the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. Heal Psychol 2010;29:333–7.

45 Petzke F, Clauw DJ. Sympathetic nervous system function in fibromyalgia. Curr Rheumatol Rep 2000;2: 116–23.

46 Genc A, Tur BS, Aytur YK, Oztuna D, Erdogan MF. Does aerobic exercise affect the hypothalamic-pituitary-adrenal hormonal response in patients with fibromyalgia syndrome? J Phys Ther Sci 2015;27: 2225–31.

47 Geenen R, Bijlsma JWJ. Deviations in the endocrine system and brain of patients with fibromyalgia: cause or consequence of pain and associated features? Ann N Y Acad Sci 2010;1193:98–110.

48 Martinez-Lavin M, Solano C. Dorsal root ganglia, sodium channels, and fibromyalgia sympathetic pain. Med Hypotheses 2009;72:64–6. Jan

49 Li Z, Ji G, Neugebauer V. Mitochondrial reactive oxygen species are activated by mGluR5 through IP3 and activate ERK and PKA to increase excitability of amygdala neurons and pain behavior. J Neurosci 2011; 31:1114–27.

50 Cordero MD, De Miguel M, Moreno Fernández AM *et al.* Mitochondrial dysfunction and mitophagy activation in blood mononuclear cells of fibromyalgia patients: implications in the pathogenesis of the disease. Arthritis Res Ther 2010;12:R17.

51 Estévez-López F, Aparicio VA, Ruiz JR *et al.* 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. Rheumatol Int 2018;38:531–3.

52 Jiménez-Díaz L, Géranton SM, Passmore GM *et al.* Local translation in primary afferent fibers regulates nociception. PLoS One 2008;3:e1961.

53 Fleshner M. Physical activity and stress resistance: sympathetic nervous system adaptations prevent stress-induced immunosuppression. Exerc Sport Sci Rev 2005; 33:120–6.

54 Estévez-López F, Segura-Jiménez V, Álvarez-Gallardo ICIC *et al.* Adaptation profiles comprising objective and subjective measures in fibromyalgia: the al-Ándalus project. Rheumatology (Oxford) 2017;56:2015–24.

55 Da Silva JAP, Geenen R, Jacobs JWG. Chronic widespread pain and increased mortality: biopsychosocial interconnections. Ann Rheum Dis 2018;77:790–2.

56 Bair MJ, Krebs EE. Fibromyalgia. Ann Intern Med 2020; 172:ITC33–ITC48.

57 Finan PH, Zautra AJ, Davis MC *et al.* COMT moderates the relation of daily maladaptive coping and pain in fibromyalgia. Pain 2011;152:300–7.