# The rs3771863 single nucleotide polymorphism of the *TACR1* gene is associated to a lower risk of sicca syndrome in fibromyalgia patients

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## ABSTRACT

**Objective.** *Fibromyalgia (FM) has been associated with affective spectrum disorders and other chronic pain disorders, which tend to co-occur in individuals and co-aggregate among families. The objective of our study was to investigate the genetic risk factors associated with the presence of related symptoms and with disease severity in subjects affected with FM.*

**Methods.** *Two independent cohorts of subjects diagnosed with FM according to the 1990 ACR criteria were studied. A genetic array composed of 320 single nucleotide polymorphisms (SNPs) was analysed in a discovery cohort comprised by 564 patients, and the most suggestive variants were genotyped in a replication cohort, comprised by 397 subjects. The associated conditions and related symptoms analysed were: the presence of depression, sleep disorders, headache, myofascial syndrome, irritable bowel syndrome, chronic fatigue syndrome, vertiginous syndrome, chronic cystitis, and sicca syndrome. FM severity was assessed by the Fibromyalgia Impact Questionnaire and the Hospital Anxiety and Depression Scale.*
*Analyses were adjusted by elapsed time from pain onset, and a meta-analysis was performed to pool the results.*

**Results.** *Minor allele of the rs3771863 SNP from the* TACR1 *gene showed a significant association with a lower risk of sicca syndrome (pooled and adjusted OR 0.56, [95%CI 0.42–0.76], p=0.00022).*

**Conclusion.** *Our findings indicate a role of the TACR1 gene in the development of sicca syndrome in subjects affected with FM.*

## Introduction

Fibromyalgia (FM) is a condition characterised by chronic widespread pain associated with multiple symptoms, including fatigue, sleep disturbances, cognitive dysfunction, and depressive episodes (1, 2). FM has been associated with a family of related disorders, known as affective spectrum disorders (ASD), that includes a number of psychiatric (such as generalised anxiety disorder, major depressive disorder, and posttraumatic stress disorder) and medical disorders (such as irritable bowel syndrome, migraine, and temporomandibular disorder) (3, 4). All these conditions share physiologic abnormalities and genetic risk factors, which may be central to their aetiology, and usually co-occur in individuals and co-aggregate among families (5).

The complex pathophysiology of FM involves an interplay among numerous factors, including sensory abnormalities, central nervous system dysfunction, abnormalities in the neuroendocrine and autonomic nervous systems, genetic factors, psychosocial variables, and environmental triggers (6).

Regarding the genetic factors, FM has a strong familial component: first-degree relatives of individuals with fibromyalgia display an 8-fold greater risk of developing this condition, compared with the general population (4, 7). Moreover, family members of individuals with FM are much more sensitive to pain than controls and, as previously mentioned, are more likely to have co-occurring ASD (7, 8). Twin studies suggest that approximately half of the risk of developing chronic widespread pain is genetic while the other half is environmental (9, 10).

Many candidate gene association studies have been designed and carried out to identify the genes associated with FM. Given the nature of such studies, much of the research has been subjected to several study design limitations,

such as a small sample size and, therefore, a low statistical power. As a consequence, inconsistent findings have been observed regarding many of the polymorphisms analysed.

Most of the genetic studies have been focused on a small number of relevant biological pathways. So far, the most studied pathways have been the catecholaminergic and the serotonergic neurotransmission (11-14), in which genetic variants located in genes coding for neurotransmitter receptors, transporters, and catabolic enzymes, such as the catechol-O-methyltransferase and monoamine oxidase, were analysed. Also, different genes belonging to the hypothalamic-pituitary-adrenal axis (15, 16) have been studied, as well as nociceptive pathways (17, 18), cytokines (19, 20), and nitric oxide synthase [*NOS* (21)] polymorphisms. Recently, an array of 3295 single nucleotide polymorphisms (SNPs) distributed in about 350 genes was carried out to further identify genetic markers associated with FM (22). Those genes were implicated in the transmission and perception of pain, in mediate peripheral and central inflammatory responses, and / or in influence mood and affective states associated with chronic pain conditions. Moreover, in the last year, two gene wide association studies (GWAS) were published (23, 24), that confirmed the strong genetic component of this condition and the probable role that the central nervous system plays in FM genetic susceptibility.

Despite the large number of published articles analysing polymorphisms as risk factors for FM, the study of the genetic markers associated with disease severity or with the presence of other coexisting comorbidities, and therefore, potentially useful as predictors of disease outcome, is relatively an unexplored field. Having this in mind, the objective of our study was to investigate the genetic association between an array of 320 SNPs, and the presence of related symptoms and syndromes, and disease severity (assessed by different scales and questionnaires) in two independent cohorts of subjects diagnosed with FM, using a discovery and replication design.

## Methods

### *Patients*

For the discovery part of the study, 564 patients that fulfilled the 1990 American College of Rheumatology criteria for FM (25), were recruited from 15 rheumatology clinics throughout the country. All subjects were Spanish Caucasian women, diagnosed with FM after 18 years old, without any other inflammatory rheumatic disease, or serious psychiatric illness, or any other condition associated with limited physical and functional capacity.

For the replication part of the study, in order to confirm suggestive results from the discovery analysis, 397 subjects from the to the Fibromyalgia and Chronic Fatigue Syndrome Spanish Genetic and Clinical Data Bank of Foundation FF (*www.laff.es/es/Banco*) and the Spanish Bank of DNA (Salamanca, Spain) (*www.bancoadn.org/*) were included. This is a Spanish cohort of patients diagnosed with one of both condition (FM and / or chronic fatigue syndrome), recruited from the specialised fibromyalgia units of the *Hospital Clinic* (Barcelona, Spain), *Hospital del Mar* (Barcelona, Spain), *Hospital Gregorio Marañón* (Madrid, Spain), the chronic pain management unit of the *Hospital General* (Guadalajara, Spain), and the Chronic Fatigue unit, *Hospital Vall de Hebron* (Barcelona, Spain). We decided to include only Caucasian patients fulfilling the 1990 diagnosis criteria of the American College of Rheumatology and not fulfilling chronic fatigue syndrome diagnostic criteria (26), in order to gather a more homogeneous population. Subjects diagnosed before the age of 18, those with any other inflammatory rheumatic disease, those associated with limited physical and functional capacity, and/or those with serious psychiatric conditions were excluded.

At the time the subjects were included in the cohorts, demographic and clinical data was collected and recorded following a standard protocol of questionnaires and physical examination, including an assessment of FM severity. A DNA sample was also extracted.

This study was conducted in compliance with the Declaration of Helsinki, and the protocol for this study was approved by the ethics committee of clinical research at the Hospital Clinico San Carlos (Madrid, Spain).

### *Variables*

The presence of related symptoms and syndromes was assessed by direct questioning and review of clinical records in the presence of the patient, requiring its previous diagnosis by their regular rheumatologist. Clinical definitions for related symptoms and disease severity were established as previously described.

In the discovery cohort, two different kind of main variables were considered. On the one hand, we analysed the presence or absence of related symptoms as dichotomous variables: chronic widespread pain, depression, sleep disorder [including insomnia and non-restorative sleep (27)), headache, myofascial pain syndrome (28), irritable bowel syndrome (29), vertiginous syndrome, painful bladder syndrome (30), and sicca syndrome (presence of symptoms of dry eyes and mouth, not satisfying Sjögren syndrome diagnosis criteria (31); we excluded the sicca syndrome that appeared as an adverse event of medication). On the other hand, we analysed the FM severity (continuous variables), assessed by the Fibromyalgia Impact Questionnaire (FIQ) (32), and the Hospital Anxiety and Depression Scale (HADS) (33). In the replication cohort, we studied those main variables that showed a significant association in the discovery cohort.

### *Genotyping*

For the discovery analysis, saliva samples were collected for DNA isolation using Oragene kits (DNA Genotek Inc), following the manufacturer's instructions. The discovery cohort was tested for 320 SNPs located in 22 loci (Table S1), selected based on their previous association with FM or related symptoms, such as depression and fatigue. Samples were genotype using the Illumina GoldenGate genotyping assays (Illumina), following the manufacturer's protocol (chip design and genotyping was funded and performed by Progenika Biopharma, who generously shared the genotypic data).

For the replication analysis, the most suggestive SNPs were selected. DNA was isolated from 10 ml EDTA tubes (Becton Dickinson) and processed using the QIAamp Blood Mini Kit (QIAGEN) following the manufacturer's instructions. Subjects were genotyped using TaqMan Assays-on-Demand from Applied Biosystems, following the manufacturer's protocol and analysed using the ABI 7900HT Fast Real-Time PCR System (Applied Biosystems). 10% of the samples were randomly re-genotyped to assess the reproducibility of the assay. No differences were observed with the previous results.

*Statistical analysis*

Continuous variables were described using median and inter-quartile rank; dichotomous variables were described using proportions.

Genotype data obtained from the Illumina GoldenGate assay was quality filtered using the following criteria: success call rate per individual >0.95, and success call rate per SNP >0.95, minor allele frequency >0.01, Hardy-Weinberg equilibrium *p*-value >0.001.

According to whether the dependent variable was dichotomous or continuous, distribution of genotypic frequencies was analysed using logistic or linear regression models, respectively, as implemented in PLINK. The odds ratio (OR), with 95% confidence intervals (95% CIs), was used to assess the strength of association between genotypes and the main dichotomous variables. We chose an additive pattern of effect of each SNPs on the main variables. Elapsed time from pain onset to inclusion in the study was introduced as a covariate term in the regression models. In order to select those SNPs to replicate, we decided to perform a first *p*-value adjustment by the number of main variables analysed in the discovery cohort: chronic widespread pain, depression, sleep disorder, headache, myofascial pain syndrome, irritable bowel syndrome, vertiginous syndrome, painful bladder syndrome, sicca syndrome, FIQ, Anxiety and Depression components of the HADS. Therefore, we multiplied the obtained *p*-values from each SNP by 12, and those adjusted *p*-values lower than 0.05 were select for replication.

In order to pool the results from the discovery and replication cohorts, we performed a meta-analysis to account for variations due to different genotyping platforms, hospital of origin, and region of sample procurement. The between-population heterogeneity was assessed by using the Cochran's test (for dichotomous outcomes) or Durbin's test (for continuous outcomes) and by calculating the $I^2$ statistic (percentage of total variation across studies that is due to heterogeneity rather than chance). Fixed or random effects models were used according to the absence or presence of heterogeneity, respectively, and analysis were adjusted by the elapsed time from pain onset. Significance of the pooled OR or Beta coefficient was determined by the Z test, and 95% CIs were calculated.

LD within the genotyped loci was analysed using the data from the CEU population released by the 1000 Genome Project, using SNAP (Broad Institute, Cambridge, MA, US; http://www.broadinstitute.org/mpg/snap/) (34).

In order to generate a *p*-value threshold in the join analysis to retain an experiment-wide type I error of 0.05, we decided to carry out a second *p*-value adjustment based in the linkage disequilibium (LD) between neighboring SNPs. Pooled *p*-values were multiplied by number of effectively independent SNPs tested in our array, calculated using spectral decomposition (35).

Post-hoc calculation of the statistical power was performed using the CaTS software (Power calculator for Genome Wide Studies) (36), and association analysis were performed using Stata 10 (STATA Corporation, College Station, Texas), Plink v1.07 (http://pngu.mgh.harvard.edu/purcell/plink/) (37) and GWAMA v1.4 (http://www.well.ox.ac.uk/gwama) (38).

**Table I.** Clinical and demographic characteristics of the discovery and replication cohorts.

| | Discovery (n= 536) | Replication (n=395) | *p*-value |
|---|---|---|---|
| Women, n (%) | 536 (100) | 384 (97.22) | 0.001 |
| Age of pain onset, years, x (IQR) | 39 (31-45) | 38 (31-46) | 0.93 |
| Age at inclusion, years, x (IQR) | 51 (44-57) | 50 (42-56) | 0.18 |
| Elapsed time with pain, years, x (IQR) | 9 (5-18) | 8 (4-14) | 0.0006 |
| With family members affected, n (%) | 74 / 531 (13.94) | 43 (10.89) | 0.16 |
| Trigger points, x (IQR) | 16 (14-18) | 15 (12-17) | <0.001 |
| HADS, depression, x (IQR) | 10 (6-13) | 8 (5-12) | <0.001 |
| HADS, anxiety, x (IQR) | 13 (9-16) | 11 (7-14) | <0.001 |
| FIQ, x (IQR) | 73.58 (63.08-82.99) | 69.97 (55.98-79.77) | <0.001 |
| Chronic widespread pain, n (%) | 534 (99.81) | 358 (90.63) | <0.001 |
| Depression, n (%) | 354 (66.42) | 42 (10.63) | <0.001 |
| Sleep disorder, n (%) | 490 (91.59) | 270 (68.35) | <0.001 |
| Headache, n (%) | 363 (67.85) | 266 (67.34) | 0.73 |
| Myofascial pain syndrome, n (%) | 379 (70.84) | 329 (83.29) | <0.001 |
| Irritable bowel syndrome, n (%) | 232 (43.45) | 240 (60.76) | <0.001 |
| Chronic fatigue syndrome, n (%) | 249 (46.72) | 0 (0) | <0.001 |
| Vertiginous syndrome, n (%) | 211 (39.59) | 166 (42.13) | 0.44 |
| Painful bladder syndrome, n (%) | 128 (23.97) | 112 (28.35) | 0.13 |
| Sicca syndrome, n (%) | 223 (41.84) | 76 (19.24) | <0.001 |

Age at inclusion: Age when the patient was included in the study and clinical data was gathered, x: Median; IQR: Inter Quartile Rank; FIQ: Fibromialgia Impact Questionnaire; HADS: Hospital Anxiety and Depression Scale.

## Results

The demographic and clinical characteristics of the subjects from the discovery and replication cohorts that were analysed are included in Table I. Despite the fact that both cohorts consisted in Spanish FM patients of Caucasian origin, selected with the same inclusion and exclusion criteria, we observed significant differences regarding the distribution of most clinical characteristics.

*Discovery study*

536 subjects from the discovery cohort, with a minimum set of demographic and

**Table II.** Summary of the selected polymorphisms from the discovery cohort, the variables that were associated with, and the results of the replication analysis.

| Main Variable | Discovery Cohort | | | | | | Replication Cohort | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SNP | MAF | OR [95% CI] | *p* | OR* [95% CI] | *p** | MAF | OR [95% CI] | *p* | OR* [95% CI] | *p** |
| Sleep disturbances | rs4760750 | 0.40 | 0.41 [0.26 – 0.66] | $2.10x10^{-4}$ | 0.41 [0.286 – 0.66] | $2.20 x10^{-4}$ | 0.40 | 1.16 [0.85 – 1.58] | 0.35 | 1.16 [0.85 – 1.58] | 0.36 |
| Sleep disturbances | rs4760816 | 0.40 | 0.44 [0.28 – 0.71] | $6.00x10^{-4}$ | 0.45 [0.28 – 0.71] | $6.20 x10^{-4}$ | 0.40 | 1.17 [0.86 – 1.59] | 0.32 | 1.16 [0.86 – 1.59] | 0.33 |
| Sleep disturbances | rs2171363 | 0.40 | 0.45 [0.28 – 0.71] | $7.00x10^{-4}$ | 0.45 [0.28 – 0.71] | $7.20 x10^{-4}$ | 0.41 | 1.20 [0.88 – 1.63] | 0.25 | 1.20 [0.88 – 1.63] | 0.26 |
| Sicca syndrome | rs174696 | 0.21 | 0.55 [0.396 – 0.77] | $4.80x10^{-4}$ | 0.55 [0.39 – 0.78] | $8.00 x10^{-4}$ | 0.20 | 1.48 [0.99 – 2.22] | 0.055 | 1.47 [0.98 – 2.20] | 0.064 |
| Sicca syndrome | rs10171225 | 0.24 | 0.58 [0.43 – 0.80] | $7.80x10^{-4}$ | 0.64 [0.46 – 0.89] | 0.0078 | 0.23 | 0.88 [0.58 – 1.32] | 0.53 | 0.86 [0.57 – 1.30] | 0.47 |
| Sicca syndrome | rs3771863 | 0.19 | 0.55 [0.39 – 0.78] | $9.20x10^{-4}$ | 0.55 [0.38 – 0.79] | 0.0013 | 0.18 | 0.60 [0.35 – 1.02] | 0.061 | 0.60 [0.35 – 1.03] | 0.063 |
| Vertigo | rs2422148 | 0.30 | 0.61 [0.47 – 0.81] | 5.80x10-4 | 0.61 [0.46 – 0.80] | $4.70 x10^{-4}$ | 0.28 | 1.33 [0.96 – 1.82] | 0.085 | 1.33 [0.96 – 1.83] | 0.083 |
| Vertigo | rs2216307 | 0.26 | 0.62 [0.46 – 0.84] | 0.0018 | 0.63 [0.47 – 0.85] | 0.0023 | 0.28 | 1.02 [0.72 – 1.43] | 0.92 | 1.02 [0.72 – 1.43] | 0.92 |
| | SNP | MAF | β [95% CI] | *p* | β* [95% CI] | *p** | MAF | β [95% CI] | *p* | β* [95% CI] | *p** |
| HADS Depression | rs12654778 | 0.38 | -1.07 [-1.67 – -0.46] | $7.00x10^{-4}$ | -1.09 [-1.70 – -0.47] | $5.90x10^{-4}$ | 0.40 | -0.36 [-1.07 – 0.35] | 0.32 | -0.35 [-1.06 – 0.36] | 0.33 |
| HADS Depression | rs10434128 | 0.14 | 1.31 [0.47 – 2.14] | 0.0022 | 1.29 [0.46 – 21] | 0.0025 | 0.15 | 0.29 [-0.67 – 1.24] | 0.56 | 0.31 [-0.64 – 1.26] | 0.52 |

*Analysis adjusted by elapsed time from pain onset to inclusion in the study. (OR): odds ratio; [95% CI]: 95% confidence interval; MAF: Minor allele frequency.

clinical data, were analysed. A genetic array composed of 320 selected SNPs was genotyped, and after quality control, 297 polymorphisms remained and were used in the association analysis.

Using the spectral decomposition analysis, we calculate that the required experiment-wide significance threshold to maintain a type I error rate at 5% was $2.60x10^{-4}$. Taking this into account, we observed that the rs4760750 tryptophan hydroxylase 2 (*TPH2*) polymorphism showed a significant association with the presence of sleep disturbances (Table II), even after adjusting by the elapsed time from pain onset (adjusted OR 0.41, [95%CI 0.286–0.66], $p=2.20x10^{-4}$).

*Replication study and meta-analysis*

The replicated SNPs are listed in Table II, and the pooled results with the previous from the discovery cohort are shown in Table III.

We first analysed the heterogeneity between samples, observing a high heterogeneity for most of the variants [s4760750, rs4760816 and rs2171363 from the *TPH2* gene; rs174696 from the catechol-O-methyltransferase (*COMT*) gene; and rs2422148 and rs2216307 from the tachykinin receptor 1 (*TACR1*) gene]. Only 4 variants showed a low-medium heterogeneity [rs10171225 from the sodium channel, voltage-gated, type IX, alpha subunit (*SCN9A*), rs3771863 from the *TACR1* gene, rs12654778 from the adrenoceptor beta 2 (*ADRB2*), and rs10434128 from the nuclear receptor subfamily 3, group C, member 2 (*NR3C2*)]. It is important to point out that no heterogeneity ($I^2 = 0\%$) was observed for the rs3771863 SNP: as we showed in Table II, this polymorphism had similar effects in both cohorts regarding the risk of sicca syndrome.

When we pooled the data from both cohorts, only the rs3771863 variant from the *TACR1* gene showed a significant association with its main variable in the fixed effects analysis, after adjusting by elapsed time from pain onset: its minor allele was associated to a lower risk of sicca syndrome (OR 0.56, [95%CI 0.42–0.76], $p=2.20x10^{-4}$).

When analysing the LD structure of *TACR1*, we observed that the rs3771863 variant was not in LD with any of the other 36 SNPs genotyped from the same gene.

Taking into account the phenotypic heterogeneity between the discovery and the replication cohort, we decided to perform a post-hoc calculation of the statistical power. Based on the observed results from our study (prevalence of sicca syndrome of 20% in FM subjects, an OR of 0.56, and a minor allele frequency of 19%), the joint analysis showed a 93% power to detect significant differences.

The SNP rs10171225 also showed a significant association with the presence of sicca syndrome in the fixed effects bivariate analysis, although after

**Table III.** Meta-analysis (fixed and random effects models) of the selected polymorphisms from the discovery cohort, the variables that were associated with, and the between-population heterogeneity assessment.

| Main Variable | | Fixed-Effects Model | | | | Random-Effects Model | | | | Heterogeneity | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | SNP | OR [95% CI] | *p* | OR* [95% CI] | *p** | OR [95% CI] | *p* | OR* [95% CI] | *p** | pC | $I^2$ | pC* | $I^{2*}$ |
| Sleep disturbances | rs4760750 | 0.85 [0.66 – 1.10] | 0.21 | 0.85 [0.65 – 1.10] | 0.21 | 0.70 [0.25 – 1.94] | 0.49 | 0.70 [0.26 – 1.92] | 0.49 | 0.00031 | 92% | 0.00034 | 92% |
| Sleep disturbances | rs4760816 | 0.87 [0.67 – 1.12] | 0.28 | 0.87 [0.67 – 1.12] | 0.27 | 0.73 [0.28 – 1.88] | 0.52 | 0.73 [0.29 – 1.87] | 0.52 | 0.00068 | 91% | 0.00071 | 91% |
| Sleep disturbances | rs2171363 | 0.88 [0.68 – 1.15] | 0.35 | 0.88 [0.68 – 1.14] | 0.35 | 0.74 [0.28 – 1.95] | 0.55 | 0.74 [0.28 – 1.95] | 0.55 | 0.00055 | 92% | 0.00058 | 92% |
| Sicca syndrome | rs174696 | 0.83 [0.64 – 1.07] | 0.15 | 0.84 [0.64 – 1.09] | 0.18 | 0.90 [0.34 – 2.37] | 0.83 | 0.89 [0.34 – 2.33] | 0.82 | 0.00021 | 93% | 0.00033 | 92% |
| Sicca syndrome | rs10171225 | 0.57 [0.42 – 0.76] | 0.00015 | 0.72 [0.56 – 0.93] | 0.011 | 0.57 [0.42 – 0.76] | 0.00015 | 0.72 [0.55 – 0.96] | 0.023 | 0.80 | 0% | 0.28 | 15% |
| Sicca syndrome | rs3771863 | 0.68 [0.53 – 0.87] | 0.0023 | 0.56 [0.42 – 0.76] | 0.00022 | 0.70 [0.47 – 1.04] | 0.075 | 0.56 [0.42 – 0.76] | 0.00022 | 0.13 | 57% | 0.79 | 0% |
| Vertigo | rs2422148 | 0.86 [0.69 – 1.05] | 0.14 | 0.85 [0.69 – 1.05] | 0.14 | 0.90 [0.42 – 1.91] | 0.78 | 0.89 [0.42 – 1.92] | 0.78 | 0.00037 | 92% | 0.00031 | 92% |
| Vertigo | rs2216307 | 0.77 [0.62 – 0.96] | 0.022 | 0.77 [0.62 – 0.97] | 0.025 | 0.79 [0.49 – 1.28] | 0.34 | 0.79 [0.50 – 1.27] | 0.34 | 0.033 | 78% | 0.037 | 77% |
| | | β [95% CI] | *p* | β* [95% CI] | *p* | β [95% CI] | *p* | β* [95% CI] | *p** | pD | $I^2$ | pD* | $I^{2*}$ |
| HADS Depression | rs12654778 | 0.47 [0.29 – 0.74] | 0.0013 | 0.468 [0.29 – 0.74] | 0.0012 | 0.48 [0.24 – 0.96] | 0.039 | 0.48 [0.23 – 0.98] | 0.045 | 0.13 | 55% | 0.12 | 58% |
| HADS Depression | rs10434128 | 2.3 [1.27 – 4.45] | 0.0069 | 2.38 [1.27 – 4.45] | 0.0067 | 2.28 [0.84 – 6.19] | 0.11 | 2.29 [0.88 – 5.98] | 0.09 | 0.11 | 60% | 0.13 | 57% |

*Analysis adjusted by elapsed time from pain onset to inclusion in the study. (OR): odds ratio; [95% CI]: 95% confidence interval; pC: Cochran's test *p*-value; pD: Durbin's test *p*-value; $I^2$: Inconsistency (percentage of total variation across studies that is due to heterogeneity rather than chance).

adjusting by elapsed time from pain onset, the significance was lost.

## Discussion

In the present study, we analysed in subjects diagnosed with FM, the association of different genetic variants with the presence of various co-morbid symptoms and syndromes, and with the severity of the disease. We observed that the rs3771863 SNP form the *TACR1* gene was significantly associated with the presence of sicca syndrome. To our knowledge, this is the first study to investigate the genetic factors associated with the presence of different symptoms and syndromes in FM.

The sicca syndrome is characterised by ocular and oral dryness of no apparent cause, and lack of autoimmune abnormalities, such as the presence of autoantibodies, or a pathologic labial salivary gland biopsy. As we pointed out before, FM patients have a higher prevalence of this condition (39), and in turn, the sicca syndrome is associated to impairment in quality of life and psychological status, closely related to the presence of pain and fatigue symptoms (40), both related to autonomic dysregulation (41). Moreover, autonomic dysfunction has been suggested to be associated with some of the FM symptoms as fatigue, morning stiffness, sleep disorders, sicca symptoms and intestinal irritability (42).

*TACR1* gene is located on the 2p11 region of chromosome 2. This receptor is widely expressed at both the central and the peripheral level and it is present in neurons, vascular endothelial cells, muscle and different types of immune cells, such as lymphocytes, macrophages, and dendritic cells (43). *TACR1* belongs to the family 1 of G protein-coupled receptors, sharing the same structural motif. Four functional isoforms have been identified, with likely different signal transduction pathways and affinities for its ligands (43). Endogenous ligands, such as substance P, human hemokinin 1 and endokinins A and B (44), are synthesised in neurons of the CNS, in capsaicin-sensitive primary afferent neurons and capsaicin-insensitive intrinsic neurons of the gastrointestinal tract, immune cells, and in different peripheral organs such as heart, skeletal muscle, skin, placenta, and adrenal glands (45). The activation of the tachykinin receptors has been implicated in a wide variety of biological actions, such as the modulation of pain perception, regulation of emotional behavior, including stress response, motivation and reward/aversion circuits, modulation of smooth muscle motility, visceral sensitivity, induction of neurogenic inflammation, activation of the immune system, regulation of haematopoiesis, and regulation of endocrine secretion (45).

Taking into account the pleiotropic functions mediated by this receptor, different genetics variants located throughout the gene have been associated to different conditions. In two independent studies, SNPs of this gene were significantly associated to alcohol dependence (AD) (46), and to AD severity (47). Also, it has been associated with bipolar disorder (48), and with attention deficit hyperactivity disorder (49). However, no association was observed with the presence of FM, although the study analysed only one SNP in a relatively small sample (50). *TACR1* has been associated to a lower recurrence rate of endometriosis (51), and with paediatric slow transit constipation (52).

Regarding the variant significantly associated to sicca syndrome in our study, rs3771863, its minor allele has been previously related to AD severity (47), and with paediatric slow transit constipation (52). No association with bipolar disorder was observed (48). To the best of our knowledge, its association with sicca syndrome has not been previously assessed.

Taking into account that the rs3771863 variant is located in the first intron of the *TACR1* gene, it is likely that this variant has no effect in the function or structure of the receptor. Hence we performed an *in silico* analysis to assess the potential functional role of this SNP or others in high LD ($r^2$>0.8). In the RegulomeDB (53) (http://regulome.stanford.edu/results), no data was collected regarding rs3771863. However, 3 SNPs in high LD (rs6725947, rs3771868 and rs12328129; $r^2$>0.89) were located in transcription factor-binding sites. Moreover, we assessed if rs3771863 or any other SNPs in high LD had been analysed in a recently published expression quantitative trait locus (eQTL) meta-analysis performed in peripheral blood samples (54) (http://genenetwork.nl/bloodeqtlbrowser/). Unfortunately, none of this variants was studied. Finally, using miRBASE (55) (http://www.mirbase.org/search.shtml), we observed that no miRNA was described to originate from the location of rs3771863 or any of the other polymorphisms in high LD.

There are two described spliced variants of the *TACR1* gene, translating into two different receptors, with different affinity for Substance P and different signaling pathways (56). Although rs3771863 is not located in the area where the splice takes place, it is possible the existence of other spliced variant not described so far, in which this SNPs may play a role.

In the last years, several studies have been published exploring the association between polymorphisms and FM severity, assessed by different methods and questionnaires. Two of these genes were also analysed in our work: *COMT* and 5-hydroxytryptamine (serotonin) receptor 2A, G protein-coupled (*HTR2A*). Regarding the *COMT* gene, it has been observed an association between different SNPs and the FIQ score, the tender point count, the pain threshold, and functional status (12), although these observations were not always replicated (11). On the other hand, the *HTR2A* gene has also been associated with the tender point count, pain threshold and extension, and functional status (13). As with the previous gene, results were not always replicated (14). Regarding our study, the variants analysed from both genes did not show association with FM severity, measured with the FIQ or the HADS.

One limitation of our study was that we only assessed the presence or absence of the subjective symptoms of the sicca syndrome (dry eyes and dry mouth), and therefore no quantitative measurement of their severity was performed. Also, in these patients, no minor salivary gland biopsy was performed as part of a standardised protocol, but performed when the patient's rheumatologist deemed necessary.

Unfortunately such data was not collected in our study.

Another limitation of our study was the clinical heterogeneity observed between the discovery and the replication cohorts. It is well known that the presence of heterogeneity reduces both the statistical power and the observed risks attributed to the susceptibility alleles or genotypes (57). Taking into account that FM is a very heterogeneous disease and that the diagnosis of these associated symptoms are mostly clinical, it is expected to find some degree of heterogeneity when comparing two different cohorts. It is certain that the existing heterogeneity is reducing the studies' power to a certain extent, although the associated variant rs3771863 showed low between-population genetic heterogeneity. Notwithstanding, the protective effect regarding sicca syndrome that we observed could represents a spurious association. Further studies will be necessary to confirm this relationship and address the role played by this variant and the *TACR1* gene in the pathophysiology of the sicca syndrome.

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## References

1. BENNETT RM, JONES J, TURK DC *et al*.: An internet survey of 2,596 people with fibromyalgia. *BMC Musculoskelet Disord* 2007; 8: 27.
2. GIACOMELLI C, SERNISSI F, SARZI-PUTTINI P, DI FRANCO M, ATZENI F, BAZZICHI L: Fibromyalgia: a critical digest of the recent literature. *Clin Exp Rheumatol* 2013; 31 (Suppl. 79): S153-7.
3. HUDSON JI, ARNOLD LM, KECK PE JR, AUCHENBACH MB, POPE HG JR.: Family study of fibromyalgia and affective spectrum disorder. *Biol Psychiatry* 2004; 56: 884-91.
4. ARNOLD LM, HUDSON JI, HESS EV *et al*.: Family study of fibromyalgia. *Arthritis Rheum* 2004; 50: 944-52.
5. HUDSON JI, MANGWETH B, POPE HG *et al*.: Family study of affective spectrum disorder. *Arch Gen Psychiatry* 2003; 60: 170-7.
6. BRADLEY LA: Pathophysiology of fibro- myalgia. *Am J Med* 2009; 122: S22-30.
7. BUSKILA D, NEUMANN L, HAZANOV I, CARMI R.: Familial aggregation in the fibromyalgia syndrome. *Semin Arthritis Rheum* 1996; 26: 605-11.
8. KATO K, SULLIVAN PF, EVENGÅRD B, PEDERSEN NL: Chronic widespread pain and its comorbidities: a population-based study. *Arch Intern Med* 2006; 166: 1649-54.
9. KATO K, SULLIVAN PF, EVENGÅRD B, PEDERSEN NL: Importance of genetic influences on chronic widespread pain. *Arthritis Rheum* 2006; 54: 1682-6.
10. MARKKULA R, JÄRVINEN P, LEINO-ARJAS P, KOSKENVUO M, KALSO E, KAPRIO J: Clustering of symptoms associated with fibromyalgia in a Finnish twin cohort. *Eur J Pain* 2009; 13: 744-50.
11. 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.
12. VARGAS-ALARCÓN G, FRAGOSO J-M, CRUZ-ROBLES D *et al*.: Catechol-O-methyltransferase gene haplotypes in Mexican and Spanish patients with fibromyalgia. *Arthritis Res Ther* 2007; 9: R110.
13. NICHOLL BI, HOLLIDAY KL, MACFARLANE GJ *et al*.: 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.
14. BONDY B, SPAETH M, OFFENBAECHER M *et al*.: The T102C polymorphism of the 5-HT2A-receptor gene in fibromyalgia. *Neurobiol Dis* 1999; 6: 433-9.
15. HOLLIDAY KL, NICHOLL BI, MACFARLANE GJ *et al*.: Genetic variation in the hypothalamic-pituitary-adrenal stress axis influences susceptibility to musculoskeletal pain: results from the EPIFUND study. *Ann Rheum Dis* 2010; 69: 556-60.
16. VARGAS-ALARCÓN G, FRAGOSO J-M, CRUZ-ROBLES D *et al*.: Association of adrenergic receptor gene polymorphisms with different fibromyalgia syndrome domains. *Arthritis Rheum* 2009; 60: 2169-73.
17. HOLLIDAY KL, NICHOLL BI, MACFARLANE GJ *et al*.: Do genetic predictors of pain sensitivity associate with persistent widespread pain? *Mol Pain* 2009; 5: 56.
18. VARGAS-ALARCON G, ALVAREZ-LEON E, FRAGOSO J-M *et al*.: A SCN9A gene-encoded dorsal root ganglia sodium channel polymorphism associated with severe fibromyalgia. *BMC Musculoskelet Disord* 2012; 13: 23.
19. SU S-Y, CHEN JJ-H, LAI C-C *et al*.: The association between fibromyalgia and polymorphism of monoamine oxidase A and interleukin-4. *Clin Rheumatol* 2007; 26: 12-6.
20. YIGIT S, INANIR A, TEKCAN A *et al*.: Association between fibromyalgia syndrome and polymorphism of the IL-4 gene in a Turkish population. *Gene* 2013;
21. ALAŞEHIRLI B, DEMIRYÜREK S, ARICA E *et al*.: No evidence for an association between the Glu298Asp polymorphism of the endothelial nitric oxide synthase gene and fibromyalgia syndrome. *Rheumatol Int* 2007; 27: 275-80.
22. SMITH SB, MAIXNER DW, FILLINGIM RB *et al*.: Large candidate gene association study reveals genetic risk factors and therapeutic targets for fibromyalgia. *Arthritis Rheum* 2012; 64: 584-93.
23. ARNOLD LM, FAN J, RUSSELL IJ *et al*.: The fibromyalgia family study: a genome-wide linkage scan study. *Arthritis Rheum* 2013; 65: 1122-8.
24. 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.
25. WOLFE F, SMYTHE HA, YUNUS MB *et al*.: The American College of Rheumatology 1990 Criteria for the Classification of Fibromyalgia. Report of the Multicenter Criteria Committee. *Arthritis Rheum* 1990; 33: 160-72.
26. FUKUDA K, STRAUS SE, HICKIE I *et al*.: The chronic fatigue syndrome: a comprehensive approach to its definition and study. International Chronic Fatigue Syndrome Study Group. *Ann Intern Med* 1994; 121: 953-9.
27. SPAETH M, RIZZI M, SARZI-PUTTINI P: Fibromyalgia and sleep. *Best Pract Res Clin Rheumatol* 2011; 25: 227-39.
28. GERWIN RD: A review of myofascial pain and fibromyalgia--factors that promote their persistence. *Acupunct Med* 2005; 23: 121-34.
29. FORD AC, BERCIK P, MORGAN DG, BOLINO C, PINTOS-SANCHEZ MI, MOAYYEDI P: Validation of the Rome III Criteria for the Diagnosis of Irritable Bowel Syndrome in Secondary Care. *Gastroenterology* 2013;
30. BOGART LM, BERRY SH, CLEMENS JQ: Symptoms of interstitial cystitis, painful bladder syndrome and similar diseases in women: a systematic review. *J Urol* 2007; 177: 450-6.
31. VITALI C, BOMBARDIERI S, JONSSON R *et al*.: Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. *Ann Rheum Dis* 2002; 61: 554-8.
32. RIVERA J, GONZÁLEZ T: The Fibromyalgia Impact Questionnaire: a validated Spanish version to assess the health status in women with fibromyalgia. *Clin Exp Rheumatol* 2004; 22: 554-60.
33. HERRERO MJ, BLANCH J, PERI JM, DE PABLO J, PINTOR L, BULBENA A: A validation study of the hospital anxiety and depression scale (HADS) in a Spanish population. *Gen Hosp Psychiatry* 2003; 25: 277-83.
34. JOHNSON AD, HANDSAKER RE, PULIT SL, NIZZARI MM, O'DONNELL CJ, DE BAKKER PI: SNAP: a web-based tool for identification and annotation of proxy SNPs using HapMap. *Bioinformatics* 2008; 24: 2938-9.
35. NYHOLT DR: A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am J Hum Genet* 2004; 74: 765-9.
36. SKOL AD, SCOTT LJ, ABECASIS GR, BOEHNKE M: Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006; 38: 209-13.
37. PURCELL S, NEALE B, TODD-BROWN K *et al*.: PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; 81: 559-75.
38. MÄGI R, MORRIS AP: GWAMA: software for genome-wide association meta-analysis. *BMC Bioinformatics* 2010; 11: 288.
39. DINERMAN H, GOLDENBERG DL, FELSON DT: A prospective evaluation of 118 patients with the fibromyalgia syndrome: prevalence of Raynaud's phenomenon, sicca symptoms, ANA, low complement, and Ig deposition at the dermal-epidermal junction. *J Rheumatol* 1986; 13: 368-73.
40. CHAMPEY J, CORRUBLE E, GOTTENBERG J-E *et al*.: Quality of life and psychological status in patients with primary Sjögren's syndrome and sicca symptoms without autoimmune features. *Arthritis Rheum* 2006; 55: 451-7.
41. NEWTON JL, FRITH J, POWELL D *et al*.: Autonomic symptoms are common and are associated with overall symptom burden and disease activity in primary Sjögren's syndrome. *Ann Rheum Dis* 2012; 71: 1973-9.
42. MARTÍNEZ-LAVÍN M, HERMOSILLO AG: Autonomic nervous system dysfunction may explain the multisystem features of fibromyalgia. *Semin Arthritis Rheum* 2000; 29: 197-9.
43. PENNEFATHER JN, LECCI A, CANDENAS ML *et al*.: Tachykinins and tachykinin receptors: a growing family. *Life Sci* 2004; 74: 1445-63.
44. SEVERINI C, IMPROTA G, FALCONIERI-ERSPAMER G *et al*.: The tachykinin peptide

family. *Pharmacol Rev* 2002; 54: 285-322.
45. PATACCHINI R, LECCI A, HOLZER P *et al*.: Newly discovered tachykinins raise new questions about their peripheral roles and the tachykinin nomenclature. *Trends Pharmacol Sci*. 2004; 25: 1-3.
46. SENEVIRATNE C1, AIT-DAOUD N, MA JZ, CHEN G, JOHNSON BA, LI MD: Susceptibility locus in neurokinin-1 receptor gene associated with alcohol dependence *Neuropsychopharmacology* 2009; 34: 2442-9.
47. BLAINE S, CLAUS E, HARLAAR N, HUTCHISON K: TACR1 genotypes predict fMRI response to alcohol cues and level of alcohol dependence. *Alcohol Clin Exp Res* 2013; 37 (Suppl. 1): E125-30.
48. PERLIS RH, PURCELL S, FAGERNESS J *et al*.: Family-based association study of lithium-related and other candidate genes in bipolar disorder. *Arch Gen Psychiatry* 2008; 65: 53-61.
49. YAN TC, MCQUILLIN A, THAPAR A *et al*.: NK1 (TACR1) receptor gene "knockout" mouse phenotype predicts genetic association with ADHD. *J Psychopharmacol* 2010; 24: 27-38.
50. ABLIN JN, BAR-SHIRA A, YARON M, ORR-URTREGER A: Candidate-gene approach in fibromyalgia syndrome: association analysis of the genes encoding substance P receptor, dopamine transporter and alpha1-antitrypsin. *Clin Exp Rheumatol* 2009; 27 Suppl. 56): S33-8.
51. RENNER SP, EKICI AB, MAIHÖFNER C *et al*.: Neurokinin 1 receptor gene polymorphism might be correlated with recurrence rates in endometriosis. *Gynecol Endocrinol* 2009; 25: 726-33.
52. GARCIA-BARCELO M, KING SK, MIAO X *et al*.: Application of HapMap data to the evaluation of 8 candidate genes for pediatric slow transit constipation. *J Pediatr Surg* 2007; 42: 666-71.
53. BOYLE AP, HONG EL, HARIHARAN M *et al*.: Annotation of functional variation in personal genomes using RegulomeDB. *Genome Res* 2012; 22: 1790-7.
54. WESTRA H-J, PETERS MJ, ESKO T *et al*.: Systematic identification of trans eQTLs as putative drivers of known disease associations. *Nat Genet* 2013; 45: 1238-43.
55. KOZOMARA A, GRIFFITHS-JONES S: miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* 2011; 39: D152-7.
56. TULUC F, LAI JP, KILPATRICK LE, EVANS DL, DOUGLAS SD: Neurokinin 1 receptor isoforms and the control of innate immunity. *Trends Immunol* 2009; 30: 271-6.
57. MANCHIA M, CULLIS J, TURECKI G, ROULEAU GA, UHER R, ALDA M: The impact of phenotypic and genetic heterogeneity on results of genome wide association studies of complex diseases. *PLoS One* 2013; 8: e76295.