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Genetic variation in neuroendocrine genes associates with somatic symptoms in the general population: Results from the EPIFUND study

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Abstract

Objective: Functional somatic syndromes commonly occur together, share a genetic component and are associated with numerous somatic symptoms. This study aimed to determine if genetic variation in two neuroendocrine systems, the serotoninergic system and the hypothalamic-pituitary-adrenal (HPA) axis, was associated with the number of reported somatic symptoms. **Methods:** This population-based cohort study (Epidemiology of Functional Disorders) recruited participants from three primary care registers in the northwest of England. Somatic symptoms, anxiety, depression, and pain were assessed using the Somatic Symptoms Checklist, Hospital Anxiety and Depression scales, and body manikins, respectively, via a postal questionnaire. Tag Single Nucleotide Polymorphisms (SNPs) (r^2>0.8) were selected for serotoninergic system genes (*TPH2*, *SLC6A4* and *HTR2A*) and HPA axis genes (*CRH*, *CRHR1*, *CRHBP*, *MC2R*, *POMC*, *NR3C1*, and *SERPINA6*) and genotyped using Sequenom technology. Negative binomial regression was used to test for association between SNPs and the number of somatic symptoms. Stepwise-regression was used to identify independent effects and adjustments were made for anxiety, depression, and pain. **Results:** A total of 967 subjects were successfully genotyped for 143 (87%) SNPs. Multiple SNP associations with the number of somatic symptoms were observed in *HTR2A* and *SERPINA6* as well as two SNPs in *TPH2*. Stepwise regression identified two effects in *HTR2A* and a single effect in *TPH2* which were independent of anxiety, depression, and pain. A single effect was also identified in *SERPINA6* but was no longer significant when adjusted for pain. **Conclusion:** This study finds association of SNPs in *HTR2A*, *SERPINA6*, and *TPH2* with somatic symptoms implicating them as potentially important in the shared genetic component to functional somatic syndromes, although replication is required.

Keywords: (max 6); Somatisation; Polymorphism; Genetics; Serotonin; HPA

Introduction

Functional somatic syndromes, which are characterized by medically unexplained symptoms, share common features including overlapping symptoms, a female preponderance, and frequent comorbid depression and anxiety. These syndromes, which include, among others, fibromyalgia (FM) [chronic widespread pain (CWP)], chronic fatigue syndrome (CFS), and irritable bowel syndrome (IBS), also have distinct differences and whether they should be considered as discrete entities has been widely debated [1].

Familial aggregation of FM has been repeatedly demonstrated [2–4], and a twin study of CWP estimated heritability to be approximately 50% [5]. Twin studies have also reported a genetic component to CFS [6,7] and IBS [8,9]. Not all twin studies of IBS, however, have reported a genetic component [10,11], and others have found that the observed genetic component is explained by a genetic influence on associated psychiatric disorder or a tendency to report multiple bodily symptoms [12,13].

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In order to try and elucidate if these syndromes share common genetic risk factors, Kato et al. carried out a population-based twin study of functional (CWP, CFS, IBS, and recurrent headache) and psychiatric disorders (major depression and generalized anxiety disorder) [14]. Although a specific genetic component to each functional syndrome was observed, the findings also implied that there are genetic factors influencing pain sensitization and psychiatric disorders which are shared across these functional somatic syndromes.

Reporting of somatic symptoms is common across these frequently co-occurring syndromes [15] and somatic symptoms have been shown to predict new-onset IBS [16] and CWP [17] independently of other psychosocial factors. Therefore, when looking for genetic risk factors for these functional somatic syndromes, somatic symptoms may act as a suitable marker. Alternatively, a genetic predisposition to somatization itself, which has been reported [13,18], may be an important factor in whether or not individuals develop a functional syndrome/s.

We have previously conducted candidate gene studies of the primary stress response system (hypothalamic-pituitary-adrenal axis (HPA) and the serotoninergic system with musculoskeletal pain due to the altered functioning of these systems in FM [19–22]. We reported evidence of genetic association in the serotonin receptor 2A gene (*HTR2A*), the serotonin biosynthesis gene (*TPH2*) [23], and the corticosteroid binding globulin gene (*SERPINA6*) [24] with CWP and the extent of pain in a population-based cohort, EPIFUND. Both of these neuroendocrine systems may also be important in somatization. HPA axis function has been investigated in IBS and CFS; however, the results are inconclusive [25]. The mechanism is unknown, but selective serotonin reuptake inhibitors appear to be effective in somatization [26,27]. Consequently, the aim of this study was to determine if genetic variation in these neuroendocrine pathways is associated with somatic symptoms in the general population.

Methods

Subjects

Subjects included in this analysis were participants in EPIFUND (Epidemiology of Functional Disorders), a prospective population-based cohort study with data collected at 3 time-points over a 4-year period. Subjects aged 25–65 years old were recruited from three primary care registers in the North-West of England.

Ascertainment of somatic symptoms

The Somatic Symptoms Checklist, a seven-item validated screening test for life-time somatization [28], was used to determine a score for the number of somatic symptoms in each subject at baseline via a postal questionnaire. The score was determined for each individual using 5-items, troubled breathing, frequent pain in fingers or toes, frequent vomiting (when not pregnant), loss of voice, and loss of memory giving a total somatic symptoms score ranging from 0–5. Two further items were excluded from the somatic symptoms score. Frequent trouble with menstrual cramps was excluded so that scores were comparable between genders and the item on difficulty swallowing was excluded due to a high proportion of missing answers.

Ascertainment of anxiety, depression and pain

Anxiety and depression were assessed at baseline using the Hospital Anxiety and Depression (HAD) scale [29]. The HAD questionnaire contains seven items on anxiety and seven items on depression in the last week which are answered on a four-point Likert scale (0–3) with total scores ranging from 0 to 21. Higher scores indicate an increased likelihood of having an anxiety or depressive disorder.

Subjects completed a detailed pain questionnaire at all three time-points which asked whether they had experienced aches or pains lasting for one day or longer in the past month. The location of any pain reported was then shaded on body manikins (right and left sides, front and back). A coding schedule [30], which divides the body into 29 sites was used to determine the total number of pain sites [from 0 (no pain) to 29 (pain in all sites)] at each time-point. Each individual's highest number of pain sites reported at any of the three time-points was considered as their maximum number of pain sites (i.e., 0–29 painful sites).

Genetic analysis

DNA samples, using buccal swab sampling, were obtained with informed consent from subjects participating in the second follow up with informed consent. Pair-wise tagging single nucleotide polymorphisms (SNPs) ($r^2 \geq 0.8$) with a minor allele frequency (MAF) greater than 5% were selected for HPA axis genes (*CRH*, *CRHBP*, *CRHR1*, *POMC*, *MC2R*, *NR3C1*, and *SERPINA6*) and serotoninergic system genes (*HTR2A*, *SLC6A4*, and *TPH2*) and their 10-kb flanking regions using Tagger and HapMap CEPH data [31] implemented in Haploview version 3.32 [32]. Genotyping of all SNPs was carried out using Sequenom MassARRAY technology following the manufacturer's instructions (www.sequenom.com).

Quality control thresholds for sample and assay quality were set to 90%. Deviation from Hardy-Weinberg Equilibrium (HWE) was tested in all samples passing QC, with SNPs showing a significant deviation ($P \leq .01$) being excluded from the analysis. Allele frequencies were also checked for consistency with HapMap frequencies. Linkage disequilibrium (LD) between SNPs was examined by pair-wise comparisons of r^2 and D′ using Haploview version 3.32 [32].

Statistical analysis

The distribution of the somatic symptoms scores is positively skewed, and the data is over-dispersed. Therefore,

Table 1
Characteristics of the study population

	Somatic Symptoms Score							
	0	1	2	3	4	5	Total	*P*
n (%)	513	280	125	35	11	3	967	–
% female	50.3	67.1	66.4	74.3	72.7	100	58.5	<.001
Age, mean (SD)	49.4 (10.2)	49.3 (10.1)	49.8 (10.9)	50.5 (8.5)	49.8 (9.6)	41.3 (12.2)	49.5 (10.2)	.891
HAD anxiety score, median (IQ range)	4 (2–7)	5 (3–8)	7 (4–10)	10 (7–13)	12 (9–13)	17 (10–17)	5 (3–8)	<.001
HAD depression score, median (IQ range)	2 (1–4)	3 (1–6)	4 (2–7)	7 (3–10)	10 (5–12)	8 (6–14)	3 (1–5)	<.001
Max number of pain sites, median (IQ range)	4 (1–7)	6 (3–10)	8 (4–13)	13 (8–19)	21 (12–23)	19 (17–28)	5 (2–9)	<.001

negative binomial regression analysis was used to test for association between SNPs and the somatic symptoms score using an additive model. Results are reported as the proportional change in somatic symptoms score with 95% confidence intervals (95% CI) for each copy of the minor allele. Where multiple significant SNP associations were observed in the same gene, forward stepwise regression was used to determine independent effects. The results were adjusted for HAD anxiety and depression scores and the maximum number of pain sites. Analysis was conducted in STATA version 9.2, and p values of less than .05 were considered statistically significant.

Results

Subjects

DNA samples were obtained from 1189 subjects of which 195 (16%) were excluded as they did not meet sample quality control criteria. Of the remaining 994 subjects, 967 had complete somatic symptoms data and were included in the analysis. The distribution of the somatic symptoms score in these 967 subjects is detailed in Table 1 along with information on demographic characteristics and related comorbidities. Fifty-three percent of subjects reported 0 somatic symptoms, 29% reported one, and 18% reported two or more. The percentage of female subjects significantly increased with the increasing number of somatic symptoms (P<.001), but age did not significantly differ with somatic symptoms score (P=.891). Increasing number of painful sites, HAD depression, and anxiety scores were significantly associated with increasing somatic symptoms score (P<.001 for all comparisons).

Genotyping

Of the 166 Tag SNPs selected, 23 failed genotyping or the SNP assay quality control threshold. The remaining 143 SNPs were in HWE (P>.01) and had frequencies consistent with HapMap. Coverage of HapMap SNPs (r^2<0.8) within the genes and their 10-kb flanks ranged from 60% to 100%.

Genetic association analysis

In *HTR2A*, 10 SNPs spanning 5′ to intron 2 showed significant associations with the somatic symptoms score (Fig. 1). With the exception of rs2274639, these SNPs are in a block of relatively high D′, suggesting that limited recombination has occurred between them. Stepwise regression analysis showed that the multiple associations were explained by two effects; each copy of the minor allele of rs9567746 was associated with a 20% increase in the number of somatic symptoms [proportional change=1.20 (1.02–1.41) P=.024] and each copy of the minor allele of rs2274639 was associated with a decrease in the number of somatic symptoms [proportional change=0.75 (0.59–0.96) P=.024]. Both these associations remained significant after adjusting for HAD depression and anxiety scores and the maximum number of pain sites; rs9567746 [proportional

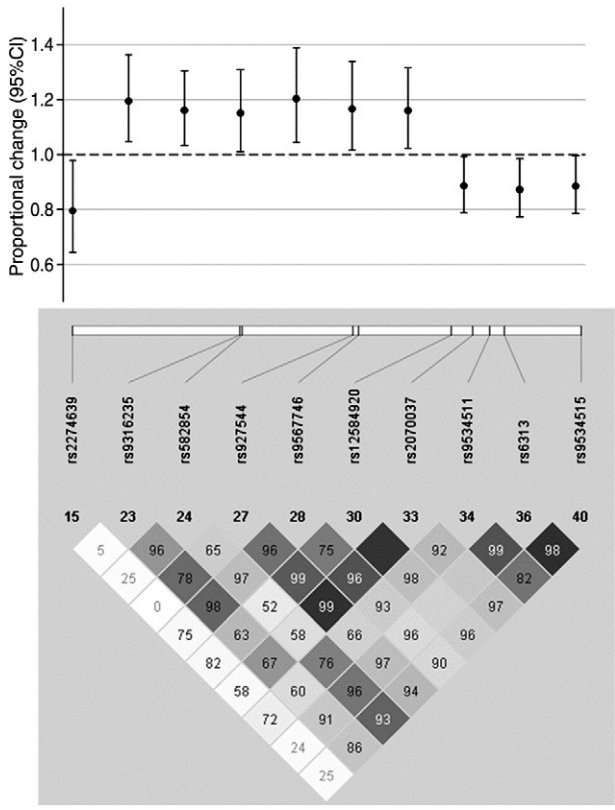

Fig. 1. Significant associations with somatic symptoms score in *HTR2A*. Proportional change in somatic symptoms score with 95% CI for significantly associated SNPs in *HTR2A*. Pair-wise LD [colored by r^2 (white=0 black=1] and numbered by D′ (no number where D′=1) between associated SNPs is shown.

change=1.17 (1.03–1.33) *P*=.015] and rs2274639 [proportional change=0.81 (0.67–0.98) *P*=.03].

In *SERPINA6*, 7 SNPs spanning the gene showed evidence of association with somatic symptoms score (Fig. 2). The SNPs are in a region of high D′, so there has been limited recombination between these SNPs. Stepwise regression showed that the associations are explained by a single effect driven by rs746530, which was associated with a 19% increase in the number of somatic symptoms for each copy of the minor allele [proportional change=1.19 (1.05–1.34) *P*=.006]. Adjusting for HAD anxiety and depression scores did not change the result [proportional change=1.16 (1.04–1.30) *P*=.009]; however, the association was alleviated and no longer significant after adjusting for the maximum number of pain sites (proportional change=1.09 (0.98–1.21) *P*=.130).

In *TPH2*, each copy of the minor allele of rs4565946 and rs7305115 were associated with a decrease and increase in the number of somatic symptoms respectively; for rs4565946 proportional change=0.87 (0.77–0.98) *P*=.019 and for rs7305115 proportional change=1.14 (1.01–1.28) *P*=.029. Moderate LD (r^2=0.54) exists between these two SNPs and stepwise regression found the association at rs4565946 explained the association at rs7305115. Adjustment for HAD anxiety and depression scores and the maximum number of pain sites did not affect this association; rs4565946 proportional change=0.88 (0.79–0.98) *P*=.016.

No associations were observed between SNPs in *CRH*, *CRHBP*, *CRHR1*, *POMC*, *MC2R*, *NR3C1* and *SLC6A4* and somatic symptoms score.

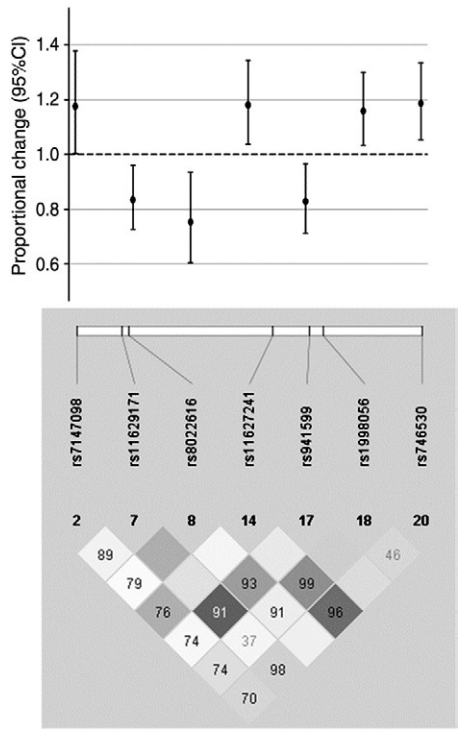

Fig. 2. Significant associations with somatic symptoms score in *SERPINA6*. Proportional change in somatic symptoms score with 95% CI for significantly associated SNPs in *SERPINA6*. Pair-wise LD [colored by r^2 (white=0 black=1) and numbered by D′ (no number where D′=1)] between associated SNPs is shown.

Discussion

To our knowledge this is the first report of a genetic association study of reporting multiple somatic symptoms. Associations were observed between SNPs in the *serotonin receptor 2A* gene (*HTR2A*) and the serotonin biosynthesis gene (*tryptophan hydroxylase*, *TPH2*) with the number of somatic symptoms reported independently of co-morbid anxiety, depression and pain. The *corticosteroid binding globulin* gene, *SERPINA6,* was also associated with the number of somatic symptoms but the finding became non-significant after adjusting for pain.

In this study the minor allele (T) of a synonymous SNP, rs6313 (T102C), in *HTR2A* was associated with a decrease in the number of somatic symptoms. A previous study reported increased somatization in FM subjects with the TT genotype compared to CT/CC [33]. The C allele, however, has been associated with an increased risk of, FM [34], CFS [35] and Temporomandibular joint disorder [36]. It is important to note that other studies did not confirm these findings [33,37] and that all studies had modest sample sizes. Neither of the SNPs in *TPH2*, which we have reported to be associated with the number of somatic symptoms or the other tag SNPs genotyped have previously been associated with functional syndromes. However, Smith et al (2008) found that SNPs in complete LD (r^2=1) with rs7305115, which was associated with the number of somatic symptoms in this study, could be used to distinguish subclasses of CFS [35].

The two *TPH2* SNPs associating with somatic symptoms are different SNPs to those which showed association with pain outcomes in our previous study [23], however, they lie within the same haplotype block suggesting that variation in this region of the gene may be important in susceptibility to both somatization and pain. The same can be said of the findings for *SERPINA6* and *HTR2A.* For both genes, multiple SNPs which were associated with somatic symptoms here were previously associated with CWP and/or the number of pain sites reported e.g. an association of an increase in the number of somatic symptoms co-occurs with an increased risk for CWP and/or an increase in the number of pain sites reported.

A major limitation of this study is that the data on somatic symptoms was collected via a postal survey so we were unable to assess whether there was any underlying pathology, which might account for the bodily symptoms reported. The somatic symptoms checklist was originally validated as a screening test for somatization disorder [28] and we have previously shown it to be a robust outcome

predictor for pain [38] and was therefore utilized in the baseline phase of the EPIFUND study to assess somatic symptoms. A Somatic Symptoms Checklist score of 3 or more (of 7 items) indicates probable somatization disorder [28] and we found that 18% of our population scored 2 or more on our brief (5 item) questionnaire. This suggest that the participants in our study would correspond approximately to the populations studied by Barsky and Kroenke [39,40], who used a self-administered questionnaire (PHQ-15) to detect probable somatization without measuring whether symptoms were explained by underlying organic disease or not . However, in the analysis reported here, a count of the number of somatic symptoms was used as the outcome of interest. This approach allows all the available data to be used which increases the statistical power of the study and avoids using arbitrary cut-offs.

The associations reported here have modest effect sizes and significance and may represent false positives as a result of multiple testing. In order to correct for this we used matrix spectral decomposition methodology proposed by Li and Ji [41]. This method determines the number of independent tests (n=123), accounting for LD between SNPs, resulting in a p-value cut off of P=.0004. None of our results reach this level of significance; however, this approach may be too stringent and result in false negatives if applied. Independent replication of our findings in other large cohorts is essential to determine whether the observed associations are false positives or true susceptibility loci for somatization. Future studies should seek to validate these findings but also to use different measures of somatization such as the PHQ15 to determine the validity of the different measures in genetic studies.

In addition this study only investigated a small number of candidate genes. As the pathophysiology of somatization is poorly understood, a large-scale genome-wide association study would be a more appropriate method to identify genes involved in somatization and to identify genes which are shared between and unique to functional somatic syndromes. A further limitation of this study is that ethnicity was not determined; however, subjects were recruited from geographic areas that are predominantly white Caucasian. In view of these limitations this should be regarded as a preliminary report but as the first of its kind it makes an important contribution.

In conclusion, we report associations between somatic symptoms and genetic variation in two neuroendocrine systems; the serotoninergic system and HPA axis. Our findings do not appear to be explained by co-morbid symptoms of depression or anxiety, which correlate with a high number of bodily symptoms [42]. Replication of these findings in large independent cohorts is required to determine if the reported associations are valid. Our findings are in keeping with results from our previous genetic association studies of pain which adds weight to the body of evidence which suggests that there is a shared genetic component to functional somatic syndromes.

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