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Short communication

The association of sex, age and *FKBP5* genotype with common somatic symptoms: A replication study in the lifelines cohort study

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ABSTRACT

Objective: Our aim was to replicate a recent study that reported an association between the rs9470080 CC-genotype and common somatic symptoms in women, but not in men. Additionally, we quantified the genetic contribution to phenotypic variation in common somatic symptom levels.

Methods: We used data from the Lifelines Cohort Study, including 28,299 participants (60.0% female; 44.2% CC-genotype; mean age 42.9 (14.2) years). Common somatic symptoms were measured with the SCL-90 SOM subscale. To assess the association between the rs9470080 genotype and SCL-90 SOM scores we applied similar analyses as the original study, including independent *t*-tests, two-way ANOVAs and a mixed ANOVA. To estimate the proportion of phenotypic variance in SCL-90 SOM scores explained by single nucleotide polymorphisms (SNPs), we used a genomic-relatedness-based restricted maximum-likelihood method.

Results: We could not replicate the original study's findings. We found no association between the rs9470080 genotype and common somatic symptom levels in either female or male participants ($F(1, 8775) = 1.07$, $p = 0.30$ and $F(1,13,903) = 0.01$, $p = 0.93$, respectively). Genome-wide heritability analyses show that 12.1% ($p = 2.1e-08$) of the phenotypic variance in common somatic symptom levels in Lifelines can be explained by SNPs. The genetic contribution to common somatic symptom levels was higher in male participants (SNP-$h^2 = 20.5\%$; $p = 9.1e-08$) than in female participants (SNP-$h^2 = 12.0\%$, $p = 2.8e-05$).

Conclusion: Our findings of significant SNP-h^2 and the sex-specific differences herein, does warrant further sex-stratified research of individual genetic variants associated with common somatic symptoms. Preferably, further research should be performed within the analytic framework of a genome-wide association study.

1. Introduction

Sex is increasingly recognized as a pivotal concept in health research [1,2]. In many diseases, including autoimmune disorders and cardiovascular disease, studies found sex differences in prevalence and presentation [3,4]. Similarly, sex differences are present in the distribution and presentation of common somatic symptoms: women are found to report more numerous, more intense and more frequent somatic symptoms than men [5–7]. Female sex also associates with a worse prognosis of common somatic symptoms [8].

On the one hand, sex differences in the prevalence and longevity of common somatic symptoms are thought to associate with biological attributes, such as differences between male and female anatomy, hormones and genes [7–9]. On the other hand, gender, the psychosocial equivalent of biological sex encompassing the embodiment of different roles, behaviors, identities and relationships of men and women prescribed by social norms, also affects the prevalence of common somatic symptoms. Previous studies show that a sex-by-gender role interaction associates with common somatic symptoms [7,8]. This may point toward a gene-by-environment (GxE) interaction associating with common somatic symptom levels.

A recent study reported that rs9470080 CC-genotype, a single nucleotide polymorphism (SNP) in the FK506-binding protein 5 gene (*FKBP5*), associated with higher levels of common somatic symptoms in female participants, but not in male participants. However, the cohort was small ($N = 1060$), as was the effect size [10]. *FKBP5* is involved in

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the functioning of the HPA-axis [11]. The authors argue that the observed sex difference in common somatic symptoms may be attributed to sex differences in activity of the central nervous system. The authors, however, express the need for further studies to assess the importance of *FKBP5*, and for replication of their study in an independent cohort. We replicated the original study in the Lifelines Cohort Study. We furthermore quantified the contribution of SNPs to phenotypic variation in common somatic symptom levels to establish genome-wide SNP-heritability (SNP-h^2) of common somatic symptom levels in Lifelines.

2. Methods

2.1. Study design

In this study we used data from the Lifelines Cohort Study. Lifelines is a multi-disciplinary prospective population-based cohort study examining in a unique three-generation design the health and health-related behaviors of 167,729 persons living in the North of the Netherlands. It employs a broad range of investigative procedures in assessing the biomedical, socio-demographic, behavioural, physical and psychological factors which contribute to the health and disease of the general population, with a special focus on multi-morbidity and complex genetics. Extensive information on the cohort and recruitment procedures is provided elsewhere [12]. Lifelines is performed according to the principles of the Declaration of Helsinki and is approved by the Medical Ethical Committee of the University Medical Center Groningen (number: 2007/152). For our analyses, we used data from three subsequent measurements. Participants were followed up on average after 13 (min-max: 10–93) and 25 (min-max: 22–92) months.

2.2. Variables

We assessed common somatic symptoms in the past week by the 12-item ordinal Symptom CheckList-90 Somatization subscale (SCL-90 SOM). The SCL-90 SOM refers to how much bother or distress participants experienced in the past 7 days due to somatic symptoms. Symptoms included, but were not limited to headache, dizziness and nausea [8]. The scale is recommended for large-scale studies and has sufficient measurement invariance over time [13,14]. Individual mean SCL-90 SOM scores were calculated for each timepoint.

Lifelines' genotyping, imputation procedures and quality control of genotype data were performed using standard protocols [15]. Participants' age and sex assigned at birth were derived from the municipal databases. As participants' sex refers to sex assigned at birth, we refer to participants as male or female.

2.3. Statistical analyses

For the replication analyses, we implemented a similar analyses pipeline as the original study. That is, we extracted rs9470080 (minor allele frequency [MAF] = 0.33) and dichotomized the genetic variant into a CC- and CT/TT-genotype group. Similarly, we only included participants aged between 18 and 60 years, and grouped age by $\leq$49 years and $\geq$ 50 years.

As per the original study we conducted independent *t*-tests to cross-sectionally analyze the differences in common somatic symptom levels between age groups and sex. To assess the association between rs9470080 genotype and common somatic symptom levels, we applied a two-way ANOVA, adjusted for age groups. The cross-sectional analyses were based on 28,299 genotyped participants without missing data on included variables (60.0% female; 44.2% CC-genotype; 78.9% aged $\leq$49 years). To assess longitudinal data, we conducted a mixed ANOVA. Longitudinal analyses were based on 22,684 genotyped participants (61.3% female; 44.3% CC-genotype; 76.7% aged $\leq$49 years). We adhered to a two-sided α-value of 0.05. Aforementioned analyses were conducted in IBM SPSS v. 25.

Additionally, we conducted a genome-wide heritability analysis in 13,548 unrelated individuals, adjusted for sex and age. As SCL-90 SOM scores were non-normally distributed, we calculated average SCL-90 SOM scores across three time points per individual. Subsequently, we applied rank-based inverse normal transformation to generate a normally distributed trait. Using a genomic-relatedness-based restricted maximum-likelihood method we then estimated the proportion of phenotypic variance in SCL-90 SOM scores that is explained by all common SNPs (i.e. SNP-heritability/SNP-h^2). In this analysis, we did not test for associations of individual SNPs. SNPs with (a) >5% missing data; (b) deviating from the Hardy-Weinberg equilibrium ($p < 1e\text{-}06$) and; (c) with a MAF < 0.01 were excluded from the genome-wide heritability analysis. We restricted our analysis to unrelated individuals (i.e. individuals with <5% degree of relatedness). The analysis was performed using PLINK and GCTA software [16–18]. In compliance with the SAGER guidelines, we report our results stratified by sex [19].

3. Results

We found statistically significant differences in SCL-90 SOM scores between age groups, however these differences were negligible (Table 1). In contrast, we found no significant difference in common somatic symptom levels between rs9470080 genotype groups in male or female participants.

The two-way ANOVA showed that the main effect of female sex, adjusted for age groups, on SCL-90 SOM scores was statistically significant: $F(1, 28{,}294) = 563.0$, $p < 0.001$. The effects of neither the rs9470080 variant ($F(1{,}28{,}294) = 0.40$, $p < 0.53$), nor the sex-by-genotype interaction term ($F(1{,}28{,}294) = 1.05$, $p = 0.31$) were statistically significant. These results indicate that in Lifelines, rs9470080 genotype did not associate with SCL-90 SOM scores, nor did this association differ in strength between female and male participants in the cross-sectional analyses.

As Table 2 shows, we observed no association between rs9470080 genotype and SCL-90 SOM scores across three time points in neither male or female participants ($F(1, 8775) = 1.07$, $p = 0.30$ and $F(1{,}13{,}903) = 0.01$, $p = 0.93$, respectively). Female sex significantly associated with SCL-90 SOM scores ($F(1{,}22{,}679) = 589.7$, $p < 0.001$). The sex-by-genotype interaction term was not statistically significant ($F(1{,}22{,}697) = 0.48$, $p = 0.49$), indicating that no significant sex difference in the association between rs9470080 genotype and common somatic symptom levels was present in longitudinal analyses.

We also assessed to which degree genetic factors contributed to phenotypic variation in SCL-90 SOM scores by means of genome-wide heritability analyses. In contrast to the analysis of a single genetic variant, this analysis estimated the variance in SCL-90 SOM scores explained by all common genetic variants in Lifelines. We observed a significant SNP-h^2 of 12.1% ($N = 13{,}548$, $p = 2.1e\text{-}08$) in SCL-90 SOM scores across three time points in unrelated individuals. Importantly, we observed a significantly higher genetic contribution in male participants (SNP-$h^2 = 20.5\%$, $p = 9.1e\text{-}08$) than in female participants (SNP-$h^2 = 12.0\%$, $p = 2.8e\text{-}05$).

4. Discussion

Despite the larger sample size of our study, we could not replicate the original study's findings. Cross-sectionally, we found significant, yet negligible differences in common somatic symptom levels between age groups in both male and female participants. In neither male nor female participants the symptom levels differed significantly between rs9470080 groups. Longitudinally, we could not corroborate the original study's finding of a significant association between rs9470080 genotype and common somatic symptom levels in female participants. Genome-wide heritability analyses show that 12.1% of the variance in common somatic symptom levels in Lifelines can be explained by common genetic variants, with a higher genetic contribution in male participants

Table 1
Independent *t*-tests to assess mean differences in SCL-90 SOM scores between age groups and rs9470080 genotype groups.

	N	Mean (SD)	DF	t	*p*-value	Mean difference (95% CI)
Age						
Male participants						
≤49 years	8898	1.15 (0.22)	11,318	−3.59	<0.001	−0.02 (−0.03 - -0.01)
≥50 years	2422	1.17 (0.24)				
Female participants						
≤49 years	13,431	1.22 (0.26)	16,977	−7.50	<0.001	−0.04 (−0.05 - -0.02)
≥50 years	3548	1.26 (0.31)				
rs9470080 genotype						
Male participants						
CC-genotype	4971	1.16 (0.23)	11,318	0.21	0.84	0.00 (−0.01–0.01)
CT/TT-genotype	6349	1.15 (0.23)				
Female participants						
CC-genotype	7531	1.23 (0.27)	16,977	−1.26	0.21	−0.01 (−0.01–0.00)
CT/TT-genotype	9448	1.23 (0.27)				

Table 2
Mixed ANOVA with common somatic symptom levels assessed by mean SCL-90 SOM score as an outcome.

	DF	Mean Square	F	*p*-value	Effect size
Between subject effects					
Male participants (*N* = 8778)					
rs9470080 (CT/TT)	1	0.07	1.07	0.30	0.00
Age (≥50 years)	1	0.96	14.1	<0.001	0.00
Female participants (N = 13,906)					
rs9470080 (CT/TT)	1	0.01	0.01	0.93	0.00
Age (≥50 years)	1	4.31	47.3	<0.001	0.03
Total (*N* = 22,684)					
rs9470080 (CT/TT)	1	0.05	0.65	0.42	0.00
Age(≥50 years)	1	4.98	60.6	<0.001	0.03
Sex (female)	1	48.3	589.7	<0.001	0.03
Sex (female) by rs9470080 (CT/TT)	1	0.04	0.48	0.49	0.00
Within subject effects					
Male participants (N = 8778)					
Somatic symptoms	1.35	141.5	4494.5	<0.001	0.34
Somatic symptoms by rs9470080 (CT/TT)	1.35	0.01	0.39	0.596	0.00
Somatic symptoms by age (≥50 years)	1.35	0.15	4.71	0.020	0.01
Female participants (N = 13,906)					
Somatic symptoms	1.36	321.1	8966.7	<0.001	0.39
Somatic symptoms by rs9470080 (CT/TT)	1.36	0.01	0.31	0.65	0.00
Somatic symptoms by age (≥50 years)	1.36	0.01	0.25	0.70	0.00
Total (N = 22,684)					
Somatic symptoms	1.36	421.3	12,341.2	<0.001	0.35
Somatic symptoms by rs9470080 (CT/TT)	1.36	0.01	0.39	0.60	0.00
Somatic symptoms by age (≥50 years)	1.36	0.09	2.51	0.10	0.00
Somatic symptoms by sex (female)	1.36	5.08	148.7	<0.001	0.01
Somatic symptoms by age (≥50 years) by sex (female)	1.36	0.01	0.31	0.65	0.00

As Mauchly's Test of Sphericity showed that the assumption of sphericity was violated, we included a Greenhouse-Geisser adjustment for the degrees of freedom (ε = 0.67 in males, ε = 0.68 in females, ε = 0.68 total).

than in female participants. Sex differences in genetic contribution to disease have been previously reported, for example in depression [20].

The discrepancy in results between the studies could be due to the differing times of follow-up or the different set of somatic symptoms that was assessed. The original study also included symptoms related to sleep, whereas our study focused solely on common somatic symptoms. Possibly, the association reported in the original study could also have been a chance finding as a result of a type I error.

A paucity of studies assessing common somatic symptoms in a genome-wide manner exists [21], with merely one twin-study known to the authors that reported a 7–29% variation in somatic symptoms due to genotype [22]. It is thought that a polygenetic architecture underlies the experience of common somatic symptoms [23]. Therefore, it is highly likely that single SNPs do not associate with common somatic symptom levels, as shown in this study. This means that an interplay between a vast variety of genetic variants results in differing common somatic symptom levels. Therefore, we argue that the clinical relevance of single genetic variants is limited [24]. Nevertheless, our findings of significant SNP-h^2 and the sex-specific differences herein do warrant further investigations of individual genetic variants associated with common somatic symptoms. Such studies, however, should preferably be conducted within the analytic framework of a genome-wide association study and will likely require large sample sizes.

Lastly, although the original study focused on differences in a single SNP, we argue that sex differences in common somatic symptoms are more complex. Sex differences in the prevalence and persistence of common somatic symptoms are likely to be influenced by additional biological factors, including pain processing pathways, and environmental factors such as gender roles [7,8]. Notably, sex-related biological processes and gender may interact and have an effect on health [25].

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Competing interests

The authors have no competing interests to report.

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