A common risk variant in CACNA1C supports a sex-dependent effect on longitudinal functioning and functional recovery from episodes of schizophrenia-spectrum but not bipolar disorder

Urs Heilbronner, Dörthe Malzahn, Jana Strohmaier, Sandra Maier, Josef Frank, Jens Treutlein, Thomas W. Mühleisen, Andreas J. Forstner, Stephanie H. Witt, Sven Cichon, Peter Falkai, Markus M. Nöthen, Marcella Rietschel, Thomas G. Schulze

Institute of Psychiatric Phenomics and Genomics, Ludwig-Maximilians-University Munich, Germany
Department of Genetic Epidemiology, University Medical Center, Georg-August-University, Göttingen, Germany
Department of Genetic Epidemiology in Psychiatry, Central Institute of Mental Health, Mannheim, Germany
Institute of Neuroscience and Medicine (INM-1), Research Centre Jülich (FZJ), Jülich, Germany
Institute of Human Genetics, University of Bonn, Germany
Division of Medical Genetics, University Hospital Basel, University of Basel, Switzerland
Department of Psychiatry and Psychotherapy, Ludwig-Maximilians-University Munich, Germany
Department of Psychiatry and Psychotherapy, University Medical Center, Georg-August-University, Göttingen, Germany
Department of Genomics, Life & Brain Center, University of Bonn, Bonn, Germany

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Abstract
Sex is a powerful modulator of disease susceptibility, course and outcome. The gene CACNA1C is among the best replicated vulnerability genes of bipolar disorder and schizophrenia. The aim of the present study was to investigate whether sex and a variant in CACNA1C (rs10774035 as a proxy for the well-acknowledged risk variant rs1006737) influence psychosocial adaptation in a
large German patient sample with schizophrenia-spectrum (n=297) and bipolar (n=516) disorders. We analyzed Global Assessment of Functioning (GAF) scores, retrospectively collected for different time points during disease course. We investigated whether CACNA1C sex-dependently modulates longitudinal GAF scores and recovery from episodes of psychiatric disturbance in the above mentioned disorders. Psychosocial recovery was measured as difference score between the current GAF score (assessing the last remission) and the worst GAF score ever during an illness episode. Covariate-adjusted association analyses revealed a sex × rs10774035 genotype interaction on longitudinal GAF and recovery from illness episodes only in schizophrenia-spectrum but not in bipolar disorders. In schizophrenia-spectrum affected males, rs10774035 minor allele (T) carriers had higher GAF scores at three time points (premorbid, worst ever, current). In contrast, females carrying rs10774035 minor alleles had impaired recovery from schizophrenia-spectrum episodes. These results encourage further investigations of gene × sex interactions and longitudinal quantitative phenotypes to unravel the rich variety of behavioral consequences of genetic individuality.

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1. Introduction

Acute episodes of schizophrenia-spectrum (SZspec) and bipolar disorders (BDs) are characterized by a steep decline in overall psychosocial functioning. Both disorders can have a chronic course characterized by recurring episodes of intense behavioral symptoms. There is, however, great inter-individual variation in the severity of impairment over time, ranging from a single mild episode to chronic impairment without recovery between episodes. Disease severity can be assessed comprehensively by quantifying overall psychosocial functioning on a single global continuum provided by the Global Assessment of Functioning (GAF) scale (DSM-IV Axis V). This approach allows for comparison between disorders and is also more sensitive to changes over time than symptom-specific assessments (Endicott et al., 1976). Changes in psychosocial functioning over time can be used as a measure of outcome or functional recovery from episodes of mental disorders (Jobe and Harrow, 2005; Lieberman et al., 2008).

The sex-specific genetic architecture of mental disorders has gained attention lately (Ober et al., 2008), sparked by a finding in the Reelin gene that increases the risk to develop SZspec in females but not males (Shifman et al., 2008). Subsequent investigation by Goes et al. (2009) also implicated Reelin as a sex-specific genetic risk factor in BDs, pointing to a sex-specific cross-disorder vulnerability locus. Similarly, evidence for sex-specific effects of CACNA1C has recently been established (Strohmaier et al., 2013). CACNA1C, coding for an L-type voltage-gated calcium channel subunit, is among the most intensively studied genes involved in the etiology of both BDs and SZspec (Bhat et al., 2012; Green et al., 2010). Whereas CACNA1C as susceptibility locus for SZspec and BDs has been identified hypothesis-free by case-control genome-wide association studies (GWAS) its full behavioral consequences have yet to be elucidated. Therefore, the present study probes the modulating role of CACNA1C variants on longitudinal outcome within SZspec and BD patients on a continuous measure of disease course within cases. We took advantage of a large German sample (n=813) with measures on the degree of psychosocial adaptation at different disease stages. Given that there are well-known sex-specific differences in the course of both SZspec (Gur et al., 1996; Leung and Chue, 2000) and BDs (Azorin et al., 2013; Hendrick et al., 2000; Kawa et al., 2005; Kessing, 2004), we hypothesized sex to exert a modulating influence on the putative effects of CACNA1C on longitudinal course of global functioning levels and on recovery from episodes of psychiatric disturbance. Specifically, we investigated the sex-dependent association between psychosocial adaptation, including recovery from illness episodes, and the CACNA1C single nucleotide polymorphism (SNP) rs10774035. In European populations, this SNP is in strong linkage disequilibrium (LD) with SNP rs1006737 (r²=1.000 and D’=1.000, SNAP database http://www.broadinstitute.org/mpg/snap/), for which genetic associations with SZspec and BDs have been shown. In line with other studies (e.g. Bigos et al., 2010), we thus investigated the effects of CACNA1C rs1006737 via a proxy SNP.

2. Experimental procedures

2.1. Participants

813 adult inpatients aged between 17 and 80 years with a life-time diagnosis of BD (n=516, thereof 443 with bipolar I disorder, 71 with bipolar II disorder and 2 with bipolar disorder not otherwise specified) or SZspec (n=297, thereof 232 with schizophrenia, 49 with schizoaffective disorder, and 16 with schizophreniform disorder) according to DSM-IV criteria and a minimum illness duration of 6 months were included in this study. Diagnoses were established by the German version of the Structured Clinical Interview for DSM-IV-TR Axis I Disorders (Diagnostic and Statistical Manual of Mental Disorders, 4th ed, text revision, SCID-I (First et al., 2002; Wittchen and Fydrich, 1997). All patients were recruited from consecutive hospital admissions at the Central Institute of Mental Health, Mannheim, and the Department of Psychiatry, University of Bonn, Germany, for the purpose of psychiatric genetic studies. The study protocol was approved by the local ethics committees, and
in accordance with the Declaration of Helsinki. Informed written consent was obtained prior to study participation from all participants.

### 2.2. CACNA1C polymorphism

The C/T (minor allele) polymorphism rs10774035 is in strong LD with the G/A (minor allele) polymorphism rs1006737 for European populations (SNAP database; http://www.broadinstitute.org/mpg/snap/, \( r^2 = 1.000, D^2 = 1.000 \)) and was directly genotyped fulfilling standard quality control criteria (Rietschel et al., 2012). Both SNPs are located in an intron directly flanking standard quality control criteria (Rietschel et al., 2012). Both SNPs are located in an intron of the CACNA1C gene. (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=10774035). Observed minor allele frequencies (MAFs) for rs10774035 were similar in both diagnostic groups and sexes, with overlapping 95% confidence intervals indicating no differences at the 5% significance level (see Table 1). In addition to the strong LD with rs1006737, the genotyped variant rs10774035 is located in a DNase I hypersensitive region, according to RegulomeDB (http://regulomedb.org/snp/chr12/2368673). DNase I hypersensitivity indicates a remodeled chromatin state related to transcriptional activity, as these have lost their condensed structure and are therefore accessible to regulatory proteins, e.g. transcription factors.

### 2.3. Phenotypes

For each subject, we analyzed longitudinal GAF scores (DSM-IV Axis V), retrospectively collected for different time points during illness course (see below), as principal dependent variable. Historically, the GAF scale was developed from the Global Assessment Scale (GAS) which assesses general psychosocial functioning on a health-illness continuum ranging from 1 to 100 (Endicott et al., 1976). Briefly, GAS values of 100-91 correspond to superior psychosocial functioning, whereas values of 10-1 are used to describe the low end of this continuum in which an individual needs constant supervision to avoid damage to itself or others or to ensure minimal personal hygiene. The GAS was initially developed by Luborsky (1962) as Health-Sickness Rating Scale, revised by Endicott et al. (1976) under the name GAS of which a modified version was included in the DSM-III-R (Cooper and Michels, 1988) and, with minimal changes, also in the DSM-V as GAF scale (Axis V). GAF information was assessed for three time points during the individual disease course:

- **GAF1**: the premorbid GAF, defined as the highest GAF score present before illness onset
- **GAF2**: the worst GAF score ever, i.e. the lowest GAF value ever present during an illness episode
- **GAF3**: the current GAF (pre-admission GAF) defined as the GAF score right before the “current” episode for which the patient received clinical treatment at the time of interview

All assessments were performed by board-certified psychiatrists or psychologists or psychiatry/psychology trainees at advanced stages in their postgraduate education. GAF1 and GAF2 were retrospectively assessed based on a multitude of sources to increase the validity of this retrospective assessment, including a structured diagnostic interview with the patient, a thorough examination of clinical records, and family interview method, whenever possible. The difference score of GAF3 and GAF2 (“current status” (last remission) minus “worst ever”) served as a measure for the degree of recovery from episodes of psychiatric disorder. We analyzed all participants for whom information on rs10774035 genotype, age, duration of illness and GAF scores were complete.

<table>
<thead>
<tr>
<th>Sample size (n)</th>
<th>S2</th>
<th>S3</th>
<th>Statistic (df) p</th>
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<tr>
<td><strong>CACNA1C SNP rs10774035</strong></td>
<td></td>
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<td>CACNA1C</td>
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<tr>
<td>MAF± 95% CI</td>
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<tr>
<td>MAF± 95% CI (females)</td>
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<td>0.34±0.04</td>
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<tr>
<td>MAF± 95% CI (males)</td>
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<td>0.33±0.04</td>
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<tr>
<td>HWE p</td>
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<tr>
<td>Sex (% female)</td>
<td>45.1</td>
<td>55.6</td>
<td>7.91 (1) 0.005</td>
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<tr>
<td><strong>Duration of illness</strong></td>
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<tr>
<td>Females</td>
<td>10.7±10.2</td>
<td>16.6±11.0</td>
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<tr>
<td>Males</td>
<td>9.6±9.2</td>
<td>17.4±13.7</td>
<td>6.81 (388.75) &lt;0.001</td>
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<td><strong>Age at onset</strong></td>
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<tr>
<td>Females</td>
<td>27.0±8.9</td>
<td>28.1±11.7</td>
<td>1.11 (332.96) 0.2662</td>
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<td><strong>Premorbid GAF</strong></td>
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<td>90.6±9.8</td>
<td>24946 &lt;0.001</td>
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<td>83.8±11.4</td>
<td>91.8±8.9</td>
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<td><strong>Worst ever GAF</strong></td>
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<tr>
<td>Females</td>
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<td>29.1±9.1</td>
<td>24325 &lt;0.001</td>
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<tr>
<td>Males</td>
<td>25.2±9.1</td>
<td>29.3±9.7</td>
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<td>78.3±15.3</td>
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<td><strong>Recovery (GAF differential)</strong></td>
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<td>49.5±16.6</td>
<td>25535.5 &lt;0.001</td>
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<tr>
<td>Males</td>
<td>35.6±14.8</td>
<td>48.9±16.1</td>
<td>27492 &lt;0.001</td>
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</table>
2.4. Statistical analyses

We used the software R (version 3.0.2, www.r-project.org). All p-values reported are two-sided. BD and SZspec samples were analyzed separately as well-known sex-specific differences in the course of SZspec and BD may yield different interaction patterns with sex. GAF values covary with sex and age at illness onset (premorbid GAF) or with sex and duration of illness (worst ever and current GAF, see Gade et al. (2015). We therefore adjusted GAF scores by linear regression for age at illness onset or duration of illness, separately for females and males. The GAF linear regression residuals at all three time points were jointly analyzed by non-parametric longitudinal rank-sum test LNPT (Malzahn et al., 2010; R package nparLD: Noguchi et al., 2012). The LNPT statistic resembles a heteroscedastic, two-way repeated measures ANOVA with two-level factor sex and three-level factor CACNA1C rs10774035 genotype. When a significant sex × genotype interaction was found, subsequent analyses tested rs10774035 main effects separately in males and females. Sex-stratified analyses also considered the recovery phenotype (GAF3 minus GAF2). Latter was adjusted for sex, duration of illness, and premorbid GAF (as recovery correlates with premorbid levels of psychosocial adaptation, data not shown). When analyzing recovery in males and females, we took advantage of a non-parametric maximum test (Marcus, 1982) that accounts for the unknown genetic mode of inheritance (Konietschke et al., 2012; R package nparcomp). Note that LNPT and nparcomp are both robust against potential variance heterogeneity between genotype groups or time points and remain valid and powerful also for non-normally distributed target variables.

3. Results

Table 1 displays descriptive statistics of and statistical comparisons between both diagnostic samples. Clinically expected differences are a lower percentage of females in SZspec compared to BDs, earlier age of onset of SZspec in males but not in females compared to BDs, and consistently lower GAF scores throughout all three time points as well as worse recovery in SZspec compared to BDs for men and women. Patients with BDs had a longer duration of illness at the time of observation compared to the SZspec patients. All other statistical test results are covariate-adjusted (including adjustment for sex, age at illness onset, duration of illness) and displayed in Table 2 (as well as in Figures 1 and 2).

3.1. Longitudinal analysis of covariate-adjusted residual GAF scores

LNPT detected no sex × CACNA1C interaction in the BD sample (p = 0.870). In contrast, sex × CACNA1C interaction was significant in the SZspec sample (p = 0.012, see Table 2). Figure 1 illustrates that the CACNA1C rs10774035 minor (T) allele had a beneficial main effect on longitudinal residual GAF scores in SZspec affected males (p = 0.018). In SZspec affected males, the rs10774035 TT genotype was associated with elevated median residual GAF scores at all three time points, compared to the CC and CT genotype groups. In contrast, SZspec affected females displayed an opposing - but non-significant - tendency towards reduced premorbid and current median residual GAF scores in female T allele carriers (LNPT on residual GAF1, 3: p = 0.125). However, residual GAF2 scores behaved differently (LNPT on residual GAF1, 2, 3: p = 0.482, i.e. greatly increased p-value when including residual GAF2).

3.2. CACNA1C rs10774035 effects on covariate-adjusted residual recovery from illness episodes

As reported above, CACNA1C rs10774035 genotype affected residual GAF scores in SZspec males consistently at all three time points. Correspondingly, residuals of the recovery phenotype were not significantly affected by rs10774035 in SZspec males (p = 0.966). In contrast, the rs10774035 minor (T) allele significantly reduced residuals of the recovery phenotype in SZ spec females (p = 0.042) consistent with a dominant mode of inheritance (see Figure 2). The associations of CACNA1C rs10774035 with outcome in SZspec and non-association with outcome in BDs were also found when additionally adjusting GAF scores for diagnostic subcategory and when only analyzing the largest diagnostic subgroups (schizophrenia, bipolar I disorder), respectively (data not shown).

4. Discussion

We investigated sex-dependent effects of CACNA1C rs10774035 genotype, a proxy SNP for the widely studied
$CACNA1C$ risk variant rs1006737, in SZspec and BDs. We used GAF scores as dependent variables, investigating the course of psychosocial adaptation with a special emphasis on recovery from illness episodes. In the following, we discuss results of the present study in light of the effects of $CACNA1C$ in case-control and population-based studies. For reasons of clarity, we refer to the T allele of rs10774035 (proxy SNP investigated in the present study) and the A allele of rs1006737 collectively as $CACNA1C$ minor allele. Across sexes, it is well known that polymorphisms in the $CACNA1C$ gene confer susceptibility to SZspec and BD (Cross-Disorder Group of the Psychiatric Genomics Consortium, Genetic Risk Outcome of Psychosis (G. R. O. U. P) Consortium; Dao et al., 2010; Ferreira et al., 2008; Green et al., 2010; Hamshere et al., 2013; Liu et al., 2011; Moskvina et al., 2009; Nyegaard et al., 2010; Sklar et al., 2011, 2008; Wray et al., 2012). However, the investigation of sex-specific genetic effects underlying disease risk has shown that the genetic basis for many diseases differs in males and females (Goes et al., 2009; Liu et al., 2012; Ober et al., 2008; Shifman et al., 2008). Such differential effect in females and males have been shown for $CACNA1C$ (Dao et al., 2010; Strohmaier et al., 2013; Witt et al., 2014) and an association in one sex may drive the association in a sample in which sexes are pooled. Given $CACNA1C$’s role in the etiology of both SZspec and BDs, we hypothesized longitudinal course of these disorders to be differentially affected between sexes. Our analyses of GAF levels for three time points in the disease course revealed evidence for sex-specific effects only in the SZspec sample. In line with the view of schizophrenia being a neurodevelopmental disorder (Murray and Lewis, 1987; Woods, 1998) this may be indicative of an early interplay between the sex-specific regulatory genome (Ober et al., 2008), a polymorphic $CACNA1C$ gene and pathophysiology in ontogeny. In SZspec affected males, the $CACNA1C$ minor allele had a main effect on the level of longitudinal GAF regardless of time point investigated, with the homozygous genotype having a beneficial effect (see Figure 1). Our finding may thus be considered out of line with results in the population-based study of Strohmaier et al. (2013), where, in males, the minor allele was associated with lower resilience phenotypes. In the present study, recovery in SZspec affected males was not influenced by $CACNA1C$ genotype. In marked contrast, $CACNA1C$ rs10774035 genotype influenced longitudinal course, particularly functional recovery from episodes, in SZspec affected females. Here, the $CACNA1C$ minor allele was found to have a detrimental effect, consistent with a dominant model of inheritance. However, in females, Strohmaier et al. (2013) found the $CACNA1C$ minor allele to be associated with resilience phenotypes in the general population and thus again in contrast to the present study. These conflicting results of our study and that of Strohmaier et al. (2013) may be explained by acknowledging the fact that different phenotypes were investigated in different study samples and that the mechanisms underlying these phenotypes may play a unique role in health and disease. Alternatively, these results may be an example of the so-called flip-flop phenomenon (Lin et al., 2007), pointing to the explanation that the $CACNA1C$ locus is correlated with a causal variant. The $CACNA1C$ gene codes for the pore-forming alpha1C subunit of the voltage-dependent L-type calcium channel (CaV1.2) (Bhat et al., 2012). Sequence variation in intronic regions of the gene, such as the polymorphisms investigated herein, are thought to influence not functional channel properties but rather expression levels and RNA splicing. In support of this, Bigos et al. (2010) have found greater $CACNA1C$ expression in carriers of the minor allele. Importantly, calcium ions themselves play a crucial role in regulating gene expression (e.g. West et al., 2001). An explanation of the seemingly incongruent role of rs1006737/rs10774035 in psychiatric

![Figure 1](image_url)  
**Figure 1** $CACNA1C$ rs10774035 affects longitudinal course of functioning in SZspec disorders. Boxplots of covariate-adjusted GAF score data for males (left) and females (right) with SZspec disorders. The data are grouped according to $CACNA1C$ rs10774035 genotype (white: CC, light shade: CT, dark shade: TT) and time point of measurement (premorbid, worst ever, current). Sample sizes are identical at all three time points (CC/CT/TT: males \(n=67/77/19\); females \(n=54/60/20\)). $p$-values of covariate-adjusted nonparametric longitudinal analyses (LNPT: joint analysis of the three time points) are given.
disease may thus stem from the fact that \textit{CACNA1C} is a major regulator of downstream processes itself via the regulation of intracellular calcium concentration.

Besides \textit{rs}1006737, \textit{CACNA1C} \textit{rs}1024582 and \textit{rs}2007044 have recently been implicated as putative pathogenic loci by GWAS (Cross-Disorder Group of the Psychiatric Genomics Consortium, Genetic Risk Outcome of Psychosis (G. R. O. U. P) Consortium; Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Both loci are in high LD with \textit{rs}1006737 (\textit{rs}1024582: $r^2=0.933$, $D'=1.000$; \textit{rs}2007044: $r^2=0.788$, $D'=1.000$). Data on \textit{rs}2007044 were available for our sample and associated similarly but slightly weaker (data not shown) compared to the \textit{rs}1006737 proxy \textit{rs}10774035.

Overall, our results suggest that molecular genetic studies using longitudinal and/or sex-specific analyses of quantitative traits underlying mental disorders have the potential to unravel complex genetic interactions. To our knowledge, this is also the first study that relates a genetic polymorphism to improvement following episodes of psychiatric disease. However, the above mentioned differences compared to the study of Strohmaier et al. (2013) emphasize the need for an independent replication.

We used the GAF scale to assess the course of psychosocial adaptation. This instrument is an easy-to-use method that needs relatively little information (Dworkin et al., 1990; Endicott et al., 1976). It correlates well with both symptom ratings (Skodol et al., 1988) and psychosocial variables (Startup et al., 2002). Moreover, the GAF scale has been shown to be a sensitive instrument in multiple-rater longitudinal studies (Dworkin et al., 1990).

Our approach mimics a longitudinal design. While we consider this to be a particular strength of our study, the retrospective measurement of GAF values (psychosocial adaptation) is certainly a limitation. Although earlier studies caution against the use of retrospective assessment when investigating psychosocial variables (Henry et al., 1994), it should be emphasized that, in the present study, raters maximized reliability by integrating information from different sources (patient interview, medical records, medication history).
relative) and did not solely rely on the recollection of study participants.

A further limitation of the present study is also the lack of systematically collected information on medication. Psychotropic medication is known to influence GAF ratings (Jones et al., 1995), and medication adherence is an important predictor of disease outcome (e.g. Ascher-Svanum et al., 2006; Verdoux et al., 2000). However, the lack of sex-specific differences in medication adherence (Lacro et al., 2002) argues against confounding of the sex

genotype interactions studied herein.

Finally, the sample size, while not large in comparison with studies on diagnostic categories, is the largest to date for studying genetics of longitudinal global functioning in SZspec and BDs. Our study underscores the need for studies investigating the complex interplay between sex and confirmed risk variants on longitudinal disease characteristics.

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Author contributions

Conception and study design: UH, MMN, MR, TGS
Sample collection, phenotyping, phenotypic quality control: JS, SM, JF, JT, SHW, MR, TGS
Genotyping, genotypic quality control: JF, JT, TWM, AJF, SC
Statistical analysis: UH, DM
Interpretation of data: all authors
Writing the manuscript: UH, DM, MR, TGS
Critical review of the manuscript: all authors.

Conflict of interest

The authors have no competing financial interest to disclose.

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