Different responses to risperidone treatment in Schizophrenia: a multicenter genome-wide association and whole exome sequencing joint study

Mingzhe Zhao1,2,9, Jingsong Ma3,4,9, Mo Li1,2,9, Wenli Zhu5,9, Wei Zhou1,2, Lu Shen1,2, Hao Wu1,2, Na Zhang1,2, Shaochang Wu6, Chunpeng Fu7, Xianxi Li8, Ke Yang1,2, Tiancheng Tang, Ruoxi Shen1,2, Lin He1,2, Cong Hua1,2,9 and Shengying Qin1,2,9

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Risperidone is routinely used in the clinical management of schizophrenia, but the treatment response is highly variable among different patients. The genetic underpinnings of the treatment response are not well understood. We performed a pharmacogenomic study of the treatment response to risperidone in patients with schizophrenia by using a SNP microarray-based genome-wide association study (GWAS) and whole exome sequencing (WES)–based GWAS. DNA samples were collected from 189 patients for the GWAS and from 222 patients for the WES after quality control in multiple centers of China. Antipsychotic response phenotypes of patients who received eight weeks of risperidone treatment were quantified with percentage change on the Positive and Negative Syndrome Scale (PANSS). The GWAS revealed a significant association between several SNPs and treatment response, such as three GRM7 SNPs (rs141134664, rs57521140, and rs73809055). Gene-based analysis in WES revealed 13 genes that were associated with antipsychotic response, such as GPCR and MAP2K3. We did not identify shared loci or genes between GWAS and WES, but association signals tended to cluster into the GPCR gene family and GPCR signaling pathway, which may play an important role in the treatment response etiology. This study may provide a research paradigm for pharmacogenomic research, and these data provide a promising illustration of our potential to identify genetic variants underlying antipsychotic responses and may ultimately facilitate precision medicine in schizophrenia.

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INTRODUCTION

Schizophrenia is a severe mental disorder with a heterogeneous combination of symptoms. Characteristic symptoms of schizophrenia can be divided into positive symptoms such as delusions and hallucinations, negative symptoms consisting of social withdrawal and affective flattening, as well as cognitive symptoms expressing as a broad set of cognitive dysfunctions. The average lifetime prevalence of schizophrenia is approximately 1% [1]. However, prevalence rates vary geographically by up to fivefold [2]. Schizophrenia accounts for significant health care costs, and is associated with a reduced life expectancy of approximately 15 years on average [3]. Antipsychotic medications are routinely used in the clinical management of schizophrenia, but the efficacy of antipsychotics in alleviating psychotic symptoms with response rates ranging from 66% for first-episode patients to 47% for chronic patients [4]. Patients on antipsychotics often experience a lengthy “trial-and-error” process marked by poorly managed symptoms before the optimal medications and doses are found. Consequently, one-year discontinuation rates may be as high as 74% due to lack of efficacy and tolerability [5]. In addition, this “trial-and-error” approach also creates tremendous social and economic burdens in various ways, as unsuccessful treatments ultimately lead to waste of medical resources and can be a risk to public safety [6, 7]. Therefore, there is a critical need to discover effective predictors of drug efficacy, or patients will continue to suffer unnecessarily.

It is generally believed that understanding the genetic determinants of drug response will help to guide therapeutic strategies toward a better efficacy profile [8]. Pharmacogenetics is the field of research and clinical practice that focuses on the influence of genetics on drug response. Primary pharmacogenetic studies based on prior knowledge have investigated several candidate genes that are suggested to be considered when prescribing medications used in psychiatry. Commonly used candidates in pharmacogenetics include genes involved in the absorption, distribution, metabolism or excretion of drugs (e.g., CYP2D6, CYP2C19, CYP2C9, ABCB1, SLC6A4, COMT) or genes involved in the immune system (e.g., HLA-A, and HLA-B) [8]. However, candidate gene approaches mostly focus on potential

1Bio-X Institutes, Key Laboratory for the Genetics of Developmental and Neuropsychiatric Disorders (Ministry of Education), Shanghai Jiao Tong University, Shanghai 200030, China. 2School of Life Sciences and Biotechnology, Shanghai Jiao Tong University, Shanghai 200240, China. 3School of Engineering, Westlake University, 18 Shilongshan Road, Hangzhou 310024 Zhejiang Province, China. 4Institute of Advanced Technology, Westlake Institute for Advanced Study, 18 Shilongshan Road, Hangzhou 310024 Zhejiang Province, China. 5The Fourth People’s Hospital of Wuhu, No.1 East Wuxia Road, Wuhu 241003, China. 6The Second People’s Hospital of Lishui, No.69 Beihu Road, Lishui 323020, China. 7The Third People’s Hospital of Shangrao, No.1 Fenghuang East Avenue, Taokan Road, Shangrao 334000, China. 8Shanghai Yangpu district mental health center, No.585 Jungong Road, Yangpu District, Shanghai 900093, China. 9These authors contributed equally: Mingzhe Zhao, Jingsong Ma, Mo Li, Wenli Zhu. ✉ email: huaic@sjtu.edu.cn; chinsir@sjtu.edu.cn

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genes with functional relevance, and there can still be unknown genes or unidentified genetic variants that may help determine the drug response. The whole-genome application of pharmacogenetics termed pharmacogenomics enables contributions from novel and less obvious genes to be detected, especially in the area of drug-target genetics, which is more complex and less well understood than the pharmacogenetics of drug metabolism [9]. Yu et al. conducted a large pharmacogenomic genome-wide association study (GWAS) using the Chinese Antipsychotics Pharmacogenomics Consortium (CAPOC) and the Chinese Antipsychotics Pharmacogenetics Consortium (CAPEC) samples and found that single-nucleotide polymorphisms (SNPs) in MEGF10, SLC1A1, and PCDH7 were associated with antipsychotic treatment response [10]. Overall, 20.8% of the total variation in response to antipsychotics was attributed to common SNPs across the genome [10]. However, almost without exception, even this well-powered GWAS identified loci that could explain only a small proportion of the genetic variance for antipsychotic treatment response, revealing the so-called missing heritability problem [11, 12]. Incomplete linkage disequilibrium between the causal variants and common SNP markers may explain a small part of the heritability underestimation. More importantly, a large number of rare variants with large effects were missed by current GWAS [13, 14]. Whole-exome sequencing (WES) offers a cost-effective strategy for investigating rare variants in drug-response studies [15]. Wang et al. conducted a pharmacogenomic WES study which found a greater burden of rare damaging variants in the reduced NMDA(N-methyl-D-aspartate)-mediated synaptic currents and reduced AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid)-mediated synaptic current gene sets curated in patients with poor response to antipsychotic medications, but no SNPs and single gene achieved genome-wide significance [16]. It is worth noting that most of the large sample GWAS and WES studies mainly used samples treated with different antipsychotics that have varying pharmacokinetic properties and mechanisms of action. Mixed antipsychotics for pharmacogenomic study may lead to spurious associations that often cannot be replicated due to heterogeneity [17].

The common variants are thought to impart subtle effects on gene function, often through changes to gene regulation [18]. The rare variants may have larger effects on gene function, such as nonsynonymous variants that alter the amino acid sequence of the resulting protein, and as a result lead to large changes in drug response. Multiple variants contributing to drug response are segregating in the population at a wide allele frequency spectrum, and a combination of common and rare variants may be required to obtain a more complete picture of the genetic architecture of drug response [19].

To investigate the genetic mechanisms underlying the difference in treatment response, we performed a discovery GWAS and WES to analyze the role of both common and rare variants of response to eight weeks of acute-phase treatment with risperidone and contribute to the development of personalized antipsychotic prescriptions.

**MATERIALS AND METHODS**

**Study participants**

This study was conducted in accordance with the Strengthening the Reporting Of Pharmacogenetic Studies (STROPS) reporting guideline [20]. Subjects were recruited from the inpatient departments of psychiatric hospitals in Shanghai, Wuhu, Lishui, and Shangrao, China. The inclusion criteria for participants were as follows: Han Chinese ancestry, diagnosis of schizophrenia by two psychiatric physicians without divergence based on the Structured Clinical Interview of DSM-IV, total Positive and Negative Syndrome Scale (PANSS) scores of more than 60, physically healthy with all laboratory parameters within normal limits, taken oral medication and written informed consent. Participants were excluded from the study if they had severe or unstable physical disease; were pregnant or breastfeeding; were diagnosed with schizoaffective disorder, schizophreniform disorder, delusional disorder, or other cognitive disorders; did not have a guardian. This study was approved by the Ethical Committee of Bio-X Institutes of Shanghai Jiao Tong University. All participants were asked to appoint a legal guardian to provide written informed consent and help patients with decision making before enrolling in this study.

**Phenotyping**

The treatment response was evaluated by percentage change on PANSS to antipsychotic medication. During an eight weeks of risperidone treatment, drug efficacy was evaluated four times (baseline, week two, week four, week eight) based on the change in PANSS scores from baseline. The PANSS reduction rate for each participant at week eight was calculated by the following formula. The 50% was set as threshold to define responders (PANSS reduction rate ≥ 50%) and nonresponders (PANSS reduction rate < 50%).

\[
\text{PANSS percentage change} = \frac{\text{PANSS week eight scores} - \text{PANSS baseline scores}}{\text{PANSS baseline scores}} \times 100
\]

**Chip genotyping**

Genomic DNA was extracted from peripheral blood with the QiAamp DNA Blood Mini Kit (QIAGEN) and then was genotyped with the Illumina Global Screening Array-24 v1.0 Beadchip (Illumina, San Diego, CA, USA). Samples and markers underwent QC before the association analysis using PLINK software (version 1.9, http://www.cog- genomics.org/plink/) [21, 22], and the reference haplotypes were derived from phase I of the 1000 Genomes Project (release version 3). SNPs with imputation quality scores less than 0.8 were removed from further analyses.

**Whole exome DNA sequencing**

There were 227 individuals being designed for the WES. Whole-exome capture libraries were constructed with Agilent SureSelect Human All Exon V6 (Agilent Technologies, Santa Clara, CA, USA) following the manufacturer's protocols and then were assessed with Agilent 2100 Bioanalyzer High Sensitivity DNA chip (Agilent Technologies, Santa Clara, CA, USA). Trusted high-level libraries were sequenced on Illumina X10 (Illumina, San Diego, CA, USA).

Raw sequencing reads with FASTQ format were mapped to the human reference genome (hg19) using the Burrows-Wheeler Aligner tool (BWA, version 0.7.17, http://bio-bwa.sourceforge.net/bwa.shtml). Polymerase chain reaction (PCR) duplicates were detected with the Picard tool (version 2.15.0, http://broadinstitute.github.io/picard/) using the mapped reads. Then, the Genome Analysis Toolkit (GATK, version 3.8, https://software. broadinstitute.org/gatk/) was applied for local realignment and quality score recalibration (BSQR), which can realign insertion-deletions (indels) and correct for the base quality scores from BAM files. The HaplotypeCaller tool embedded in GATK was applied to call single nucleotide variants (SNVs). Every called variant file with VCF formats was merged using the GenotypeGVCFs tool and then filtered using the VariantRecalibrator tool embedded in GATK.

**Common SNP association analysis**

Association analyses between genetic variants, age, sex, and the first five principal components of population structure and PANSS percentage change values were assessed with linear regression under an additive genetic model implemented in PLINK (version 1.9 beta). Given differences in sample size, phenotypic characterization, and effect size between pharmacogenomic GWAS and complex-disease GWAS [23], an accepted genome-wide significance threshold on the order of \( P < 1 \times 10^{-5} \) was used for our pharmacogenomic GWAS [24]. A P value less than \( 5 \times 10^{-8} \) was
We investigated individual differences in treatment response could be moderately significant.

Several secondary analyses were conducted based on the results of GWAS. The tissue-specific expression patterns of genes in human tissues in genotype-tissue expression Portal (GTEx, http://www.gtexportal.org/home/) were investigated. The GTEx database collected approximately 17382 RNA-seq samples across 54 tissues from 948 postmortem donors [25]. We explored expression quantitative trait loci (eQTL) data in the brain eQTL database, using three algorithms (CMC, PRICE, SKAT-O) in RVTESTS [27, 28].

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RESULTS

GWAS

We totally recruited 226 patients with schizophrenia in multiple centers of China (Shanghai:102, Wuhu:39, Lishui:32, Shangrao:53) and divided them into two groups (responder group/nonresponder group, Shanghai:28/74, Wuhu:8/31, Lishui:8/24, Shangrao:22/31) based on the percentage change in PANSS after an eight weeks of risperidone treatment. The total response rate is 29.2% under the treatment of risperidone in our samples. Significant differences were found between the two groups in baseline PANSS total scores, endpoint PANSS total scores, and PANSS percentage changes but not on age and sex (Table 1). Among 196 genotyped patients, 189 of them remained after quality control. All patients included in the GWAS were unrelated Han Chinese without population stratification after principal component analysis. There are 700,078 genotyped SNPs in raw chip data. After imputation and quality control, a linear regression analysis with 446,504 common SNPs on the PANSS percentage change values was conducted.

Figure 1 illustrates the Manhattan plot of the GWAS common SNP association results. As the Fig. 1 shows that 312 SNPs were weakly associated with risperidone treatment response (i.e., $P < 5 \times 10^{-5}$), approximately half of which were intergenic variants that could not be annotated with gene symbols, and the nonintergenic variants with gene symbols were listed in Supplementary Table S1. Nevertheless, 185 SNPs in raw chip data. After imputation and quality control, a linear regression analysis with 446,504 common SNPs on the PANSS percentage change values was conducted.

Fig. 1 Manhattan plots of $P$ values against their respective chromosomal positions for the genome-wide association study. The blue line corresponds to the $P < 1 \times 10^{-5}$, while the red line represents the genome-wide significance level ($P < 5 \times 10^{-8}$).

Table 1. Demographic and clinical characteristics of samples.

| Variables               | Responders Group | Nonresponders Group | t/χ² | P value |
|-------------------------|-------------------|---------------------|------|---------|
| Age, mean (SD)          | 36.40 (10.621)    | 38.48 (12.205)      | −1.197 | 0.233   |
| Sex                     |                   |                     |      |         |
| Male, No. (%)           | 38 (57.6)         | 84 (52.5)           | 0.485 | 0.486   |
| Female, No. (%)         | 28 (42.4)         | 76 (47.5)           |      |         |
| Clinical assessment, mean (SD) |         |                     |      |         |
| Baseline PANSS total scores | 80.70 (16.423) | 85.74 (14.466)   | −2.295 | 0.023   |
| Endpoint PANSS total scores | 35.73 (4.056) | 51.43 (9.856)       | −12.509 | 0.000   |
| PANSS percentage changes | 69.749 (6.322) | 61.81 (12.050)      | 17.853 | 0.000   |

PANSS positive and negative syndrome scale.

REPORTED AS A FINDING OF INTEREST BECAUSE MARKERS ASSOCIATED WITH IMPORTANT INDIVIDUAL DIFFERENCES IN TREATMENT RESPONSE COULD BE MODERATELY SIGNIFICANT.

Several secondary analyses were conducted based on the results of GWAS. The tissue-specific expression patterns of genes in human tissues in genotype-tissue expression Portal (GTEx, http://www.gtexportal.org/home/) were investigated. The GTEx database collected approximately 17382 RNA-seq samples across 54 tissues from 948 postmortem donors [25]. We explored expression quantitative trait loci (eQTL) data in the brain eQTL database, using three algorithms (CMC, PRICE, SKAT-O) in RVTESTS [27, 28].

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The other SNPs were not associated with gene expression (Supplementary Table S3).

WES

The WES data were generated on the 226 patients and 222 of them remained after quality control. The data had an average per-target depth of coverage of × 74.57, with 97.5% of all targeted bases covered at × 10 or greater (94.1% at ≥20×). After taking a series of quality controls, 108,813 SNVs were identified in the protein-coding regions. Figure 2 depicts the Manhattan plots of the common SNP-based association analysis. Four SNPs showed a genome-wide significant association with the treatment response to risperidone, in PACC1, SPTBN1, and HMGXB3 (i.e., \( P < 1 \times 10^{-5} \)), and seven SNPs were weakly associated with the risperidone treatment response (i.e., \( P < 5 \times 10^{-5} \)) (Table 2). For rare SNVs, we identified ten genes that reached the significance \( P < 0.05 \) of all three algorithms (CMC, PRICE, SKAT-O) in RVTESTS in gene-based association analyses (Table 3).

Convergent pathways between GWAS and WES

The results of the GWAS were correlated with those of the WES, which were represented in commonalities between genetic pathways identified through GWAS and WES. GRM7 in the GWAS is the G-protein coupled receptor (GPCR) for glutamate, which is one of the most important neurotransmitters in the central nervous system. GPR12 in the WES belongs to the GPCR1 family and it can promote neurite outgrowth and block myelin inhibition in neurons. GRM7 and GPR12 are all in the peptide ligand-binding receptor pathway that are related with GPCR signaling pathway. Both of them play an important role in cell metabolism and the expression of genes in brain. SPTBN1 in the WES is an actin crosslinking and molecular scaffold protein that interacts with calmodulin in a calcium-

Table 2. Genomic regions of common variants in the whole exome sequencing analyses.

| CHR | SNP      | Position      | A1 | A2 | Gene   | variant location | BETA  | P         |
|-----|----------|---------------|----|----|--------|-----------------|-------|-----------|
| 2   | rs2271326| 54871519      | T  | C  | SPTBN1 | synonymous variant | −34.24 | 3.18E-06 |
| 2   | rs2271326| 54864964      | G  | A  | SPTBN1 | intron variant    | −34.24 | 3.18E-06 |
| 5   | rs186497515| 149410319     | A  | T  | HMGBX3 | missense variant  | −30.33 | 5.05E-06 |
| 15  | rs181987 | 99679655      | A  | C  | TTC23  | intron variant    | −21.06 | 2.98E-05 |
| 15  | rs260089 | 99678145      | A  | G  | TTC23  | 3 prime UTR variant| −21.06 | 2.98E-05 |
| 15  | rs7167599| 99672599      | C  | G  | TTC23  | downstream gene variant | −21.06 | 2.98E-05 |
| 19  | rs11673029| 58491627      | C  | T  | ZNF606 | missense variant  | −10.01 | 3.24E-05 |
| 19  | rs9304808| 58514183      | C  | T  | ZNF606 | 5 prime UTR variant| −10.01 | 3.24E-05 |
| 19  | rs9304809| 58514475      | T  | G  | ZNF606 | 5 prime UTR variant| −10.01 | 3.24E-05 |
| 10  | rs149024826| 23578843      | C  | T  | C10orf67 | missense variant | −22.08 | 3.52E-05 |

CHR: chromosome, SNP: single-nucleotide polymorphism, A1: minor allele, A2: major allele.

Table 3. Gene-based association results in the whole exome sequencing analyses.

| Gene    | \( P \) (CMC) | \( P \) (PRICE) | \( P \) (SKAT-O) |
|---------|---------------|----------------|-----------------|
| MAP2K3  | 3.00E-06      | 0.000          | 2.67E-06        |
| C20orf194| 2.53E-05     | 0.0002         | 0.0002          |
| CCDC188 | 4.02E-05      | 0.0001         | 7.18E-05        |
| FAM1828 | 0.0001        | 0.0003         | 0.0002          |
| FOXP1   | 0.0003        | 0.002          | 0.0003          |
| NHLRC2  | 0.0003        | 0.002          | 0.0003          |
| CDK11B  | 0.0007        | 0.0007         | 0.0006          |
| GPR12   | 0.0003        | 0.002          | 0.0003          |
| ATCH8   | 0.003         | 0.003          | 0.003           |
| CNTLN   | 0.002         | 0.004          | 0.002           |

CMC: combined multivariate and collapsing, SKAT-O: optimized sequence kernel association test.
dependent manner and is thus candidate for the calcium-dependent movement of the cytoskeleton at the membrane. (9) SPTBN1 and GRM7 are all involved in the signaling by the GPCR pathway. MAP2K3 in the WES is a dual specificity protein kinase that participates in the MAP kinase-mediated signaling cascade. Importantly, MAP2K3 and GRM7 are both involved in three signal transduction pathways (Neuropathic pain-signaling/G-Beta Gamma signaling/CREB pathway) in the brain. The three pathways are all related to the GPCR signaling pathway. On the whole, findings from the GWAS and the WES can build up a pathway network where the GRM7 is the central hub gene and the GPCR signaling pathway may be the main signal transduction pathway in pathophysiology of schizophrenia and response to risperidone.

Significantly, what we combined the findings of GWAS and WES with targets (DRD2 and HTR2A) of risperidone, an extended pathway network was found. The GRM7 and MAP2K3 are involved in the G-Beta Gamma signaling with DRD2 and HTR2A. The GRM7 and GPR12 participate in the peptide ligand-binding receptor pathway with these two targets. Furthermore, one of the target pathways of risperidone termed the neuroactive ligand-receptor interaction is part of the peptide ligand-binding receptor pathway.

**DISCUSSION**

To the best of our knowledge, this is the first study so far to identify both common variants with GWAS and rare variants with WES of treatment response to antipsychotics in patients with schizophrenia. We identified several SNPs associated with the treatment response to risperidone in the GWAS. The WES revealed four SNPs located in three genes showing genome-wide significant association with treatment response to risperidone and ten genes were significantly associated with response status in the gene-based association analyses.

Our results supported the hypothesis that the genetic architecture of drug response was likely to be similar to those of common diseases that were determined by a combination of multiple common and rare variants [29]. Several explanations for the genetic architecture of drug response are plausible. First, multiple variants contributing to drug response are segregating in the population at a wide allele frequency spectrum. Second, rare variants may impart larger effects on gene function, in addition to the variants with major impact, common variants with subtle effects also contribute to variation in drug response.

To identify the association between common SNPs and treatment response, we performed SNP-based association analysis through GWAS. First, we identified three novel GRM7 SNPs (rs141134664, rs57521140, and rs73809055), with genome-wide significance. GRM7 belongs to the G protein-coupled receptor family, a major excitatory neurotransmitter in the central nervous system that is involved in many signal transduction pathways in pathophysiology of schizophrenia and response to risperidone.

We identified several SNPs associated with the treatment response to risperidone in the GWAS. First, we identified three novel GRM7 SNPs (rs141134664, rs57521140, and rs73809055), with genome-wide significance. GRM7 belongs to the G protein-coupled receptor family, a major excitatory neurotransmitter in the central nervous system that is involved in many signal transduction pathways in pathophysiology of schizophrenia and response to risperidone.

HTATIP2 is an oxidoreductase required for tumor suppression and may act as a redox sensor linked to transcription through regulation of nuclear import [43]. Our eQTL results indicated that the identified SNPs in this gene might affect the treatment response through a similar process in which PRMT3 is involved. PALMA2AKAP2 belongs to the paralemmin down gene family which may have evolved continguously with the paralemmin genes and are associated with other paralemmin paralogs in humans and several other taxa [44]. A SNP (rs4978848) in this gene was reported to be associated with cognition [45]. Therefore, although the SNP in this gene did not have eQTL effects in our study, further studies of its effects on PALMA2AKAP2-related signals are justified.

For common SNPs tested in WES, we found four SNPs achieved genome-wide significance. Two SNPs (rs2271323 and rs2271326) were located in SPTBN1, which is a member of a family of betaspectrin genes with functions in the determination of cell shape, arrangement of transmembrane proteins, and organization of organelles [46]. It was reported that SPTBN1 interacted with calmodulin in a calcium-dependent manner, which was believed to have close link with the pathophysiological process of schizophrenia [47]. Another SNP (rs117462017) belongs to PACC1 that can mediate import of chloride ion in response to extracellular acidic pH and is involved in acidosis-induced cell death by mediating chloride influx and subsequent cell swelling [48, 49]. The last SNP was a missense variant located in HMGB3, which is one of the noncanonical high mobility group (HMG) genes. The encoded protein of this gene contains an HMG-box domain found in DNA binding proteins such as transcription factors and chromosomal proteins [50]. Although no study has reported the association between HMGB3 and the treatment response of antipsychotics or schizophrenia, this missense variant can affect the function of transcription factors which play an important role in transcription. Further studies of its effect on the treatment response of antipsychotics or schizophrenia are needed.

To identify the association between rare SNVs and the treatment response of antipsychotics, we performed gene-based association analysis using WES. We found GPR12 with significance in our study. GPR12 belongs to the GPCR family and can promote neurite outgrowth and block myelin inhibition in neurons [51]. It has been reported that GPR12 was associated with response to antipsychotic drug [52]. We were aware of another GPCR family gene (GRM7) that was also significant in the GWAS results, which indicated that the signal transduction pathway mediated by GPCRs may play an important role in the pharmacodynamics and pharmacokinetics of antipsychotic drugs. Given remarkably similar results from the GWAS and WES, we are more convinced that the variance of treatment
response among patients with schizophrenia emerged as the result of the interaction between common variants and rare variants. Another significant gene that we found was MAP2K3, which is a dual specificity protein kinase and belongs to the MAP kinase kinase family [53]. This kinase participates in the MAP kinase-mediated signaling cascade which is the downstream signal of the signal transduction pathway mediated by GPCR. Active MAPK is transferred into the nucleus and then phosphorylates some transcription factors [54]. The whole signal transduction pathway is believed to be associated with the pathophysiological process of schizophrenia and the treatment response of antipsychotics [42]. In addition, we also found eight other genes that passed the three tests in the gene-based association analysis. Although, we did not annotate any available evidence that can support the potential role of antipsyhtics or schizophrenia in the treatment response, future studies may need to validate them.

We noticed a lack of shared identified loci between GWAS and WES, for which there are two possible explanations. First, although some pharmacogenomic effects tend to be larger and involve fewer genes than those detected in GWAS and WES studies for complex diseases, small sample sizes may still have insufficient power to detect ideal convergence. Second, the minor allele frequency distribution of variants for pharmacogenomics showed an obvious excess of low allele frequency variants and a significant number of drug response variants with high allele frequency in the population compared with common diseases and other complex traits. Multiple variants with a wide allele frequency spectrum contribute to drug response thus GWAS and WES may detect different signals.

Although a shared locus was not found between GWAS and WES, association signals tend to cluster into key pathways that drive treatment response etiology. We noticed that GRM7 was associated with SPTBN1, MAP2K3, and GPR12 in different signaling pathways. Our findings were supported by the core gene omnigenic model which assumes that most traits can be directly affected by a modest number of genes or gene pathways with specific roles in disease etiology, as well as their direct regulators [18]. These genes are “core genes”, such as GRM7, that tend to have biologically interpretable roles in treatment response. There are also a large number of genes without direct effects called “peripheral genes”, such as SPTBN1, MAP2K3, and GPR12, that are propagated through regulatory networks to a much smaller number of core genes [55]. The signaling pathways we identified were all associated with DRD2 and HTR2A which are targets of risperidone. Drug pharmacokinetics and pharmacodynamics operate within networks of proteins that are responsible for drug metabolism, transport, and drug target. Genetic influences on drug response are frequently identified within these networks. Network analysis showed that GWAS- or WES-reported genes were close to drug target genes in a biological network [56, 57], and distributions showed that distances from a GWAS- or WES-reported gene to the closest drug targets were on average much shorter than those of a random gene to a closest drug target [58], which is consistent with our findings.

There are several limitations of the present study. First, although a large proportion of the current pharmacogenomic GWAS or WES findings have been reported with small sample sizes, such as ours, which might inflate the type I error rate and reduce power for detecting solidly associated genetic markers. Replication and extension of these findings are needed in studies with much larger samples. Second, nongenetic factors, such as environmental factors, duration of illness, and previous antipsychotics, which might affect interindividual differences in antipsychotic drug response, were not considered in our analyses. Third, although a significance threshold on the order of P < 1 x 10^{-5} is acceptable in pharmacogenomic studies, the risk of false positive results may exist when statistical power is low. Fourth, we provided a new research paradigm for identifying susceptibility loci for treatment response to antipsychotic drugs, but other omics technologies are also necessary to better characterize the joint contribution of variants and decipher the molecular pathways they affect.

In summary, we explored a GWAS and WES combined approach to comprehensively analyze the role of common and rare variants in the efficacy of risperidone in schizophrenic patients. We have identified several important loci and genes that are involved in the efficacy of risperidone. Our findings provide an illustration of the future potential of this approach in guiding the treatment of schizophrenia. The preliminary nature of our findings precludes our abilities to translate the findings into the prediction of clinical response. However, these findings warrant regulations and should be extended with larger samples to confirm their use in the development of personalized medicine.

REFERENCES

1. Thaker GK, Carpenter WT Jr. Advances in schizophrenia. Nat Med. 2001;7:667–71.
2. McGrath J, Saha S, Chant D, Welham J. Schizophrenia: a concise overview of incidence, prevalence, and mortality. Epidemiol Rev. 2008;30:67–76.
3. Walker ER, McGee RE, Druss BG. Mortality in mental disorders and global disease burden implications: a systematic review and meta-analysis. JAMA Psychiatry. 2015;72:334–41.
4. Haddad PM, Correll CU. The acute efficacy of antipsychotics in schizophrenia: a review. Recent Prrt Adv Pharmacol. 2018;303–18.
5. Lieberman JA, Stroup TS, McEvoy JP, Swartz MS, Rosenheck RA, Perkins DO, et al. Effectiveness of antipsychotic drugs in patients with chronic schizophrenia. N Engl J Med. 2005;353:1209–23.
6. Cloutier M, Agbogbu MS, Guerin A, Nitsulescu R, Ramanakumar AV, Kamat SA, et al. The Economic Burden of Schizophrenia in the United States in 2013. J Clin Psychiatry. 2016;77:764–71.
7. Pennington M, McCreone P. The Cost of Relapse in Schizophrenia. Pharmaco- economics. 2017;35:921–36.
8. Bousman CA, Bengesser SA, Atchison KJ, Amare AT, Aschauer H, Baune BT, et al. Review and consensus on pharmacogenomic testing in psychiatry. Pharma- copsychoi 2021;5:45–17.
9. McCutcheon RA, Krystal JH, Howes OD. Dopamine and glutamate in schizo- phrenia: biology, symptoms and treatment. World Psychiatry. 2020;19:15–33.
10. Yu H, Yan H, Wang L, Li J, Tan L, Deng W, et al. Five novel loci associated with antipsychotic treatment response in patients with schizophrenia: a genome-wide association study. Lancet Psychiatry. 2018;5:327–38.
11. van Calker D, Serchov T. The “missing heritability”-Problem in psychiatry: Is the interaction of genetics, epigenetics and transposable elements a potential solution? Neurosci Biobehav Rev. 2021;126:22–42.
12. Manolio TA, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, et al. Finding the missing heritability of complex diseases. Nature 2009;461:747–53.
13. Park JH, Wacholder S, Gail MH, Peters JU, Jacobs KB, Chanock SJ, et al. Estimation of effect size distribution from genome-wide association studies and implications for future discoveries. Nat Genet. 2010;42:570–5.
14. Hirschhorn JN. Genowide association studies—illuminating biologic pathways. N Engl J Med. 2009;360:1699–701.
15. Fukunaga K, Momozawa Y, Mushiroda T. Update on next generation sequencing of pharmacokinetics-related genes: development of the Pkseq panel, a platform for amplicon sequencing of drug-metabolizing enzyme and drug transporter genes. Drug Metab Pharmacokinet. 2021;37:100370.
16. Wang Q, Man Wu H, Yue W, Yan H, Zhang Y, Tan L, et al. Effect of damaging mutations in minisix-pain related genes sets on response to short-term antipsychotic medication in chinese patients with schizophrenia: a randomized clinical trial. JAMA Psychiatry. 2018;75:1261–9.
17. Islam F, Men X, Yoshida K, Zai CC, Müller DJ. Pharmacogenetics-guided advances in antipsychotic treatment. Clin Pharm Ther. 2021;110:582–8.
18. Boyle EA, Li VI, Pritchard JK. An expanded view of complex traits: from polygenic to omniogenic. Cell 2017;169:1177–86.
19. Fabbri C, Kasper S, Kautzky A, Zohar J, Souery D, Montgomery S, et al. A polygenic predictor of treatment-resistant depression using whole exome sequencing and genome-wide genotyping. Transl Psychiatry. 2020;10:50.
20. Chaplin M, Kirkham JJ, Dwan K, Sloan DJ, Davies G, Jorgensen AL. Strengthening the reporting of pharmacogenetic studies: development of the STROPS guideline. PLoS Med. 2020;17:e1003244.
21. Delaneau O, Zagury JF, Marchini J. Improved whole-chromosome phasing for disease and population genetic studies. Nat Methods. 2013;10:5–6.
22. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. PLoS Genet. 2009;5:e1000529.
23. Daly AK. Genome-wide association studies in pharmacogenomics. Nat Rev Genet. 2010;11:241–6.
24. Mu G, Xiang Q, Zhang Z, Liu C, Zhang H, Liu Z, et al. PNPT1 and PCGF3 variants associated with angiotensin-converting enzyme inhibitor-induced cough: a nested case-control genome-wide study. Pharmacogenomics 2020;21:501–14.
25. The GTEx Consortium atlas of genetic regulatory effects across human tissues. Science 2015;350:629–33.
26. Ramasamy A, Trabzuni D, Guelfi S, Varghese V, Smith C, Walker R, et al. Genetic variability in the regulation of gene expression in ten regions of the human brain. Nat Neurosci. 2014;17:1418–28.
27. Asimit J, Zeggini E. Rare variant association analysis methods for complex traits. Annu Rev Genet. 2010;44:293–308.
28. Zhan X, Hu Y, Li B, Abeasis GR, Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics 2016;32:1423–6.
29. Roden DM, Wilke RA, Kroemer HK, Stein CM. Pharmacogenomics: the genetics of variable drug responses. Circulation 2011;123:1661–70.
30. Song JM, Kang M, Park DH, Park S, Lee S, Suh YH. Pathogenic GRM7 mutations associated with neurodevelopmental disorders impair axon outgrowth and presynaptic terminal development. J Neurosci. 2021;41:3344–59.
31. Sacchetti E, Magri C, Minelli A, Valsecchi P, Traversa M, Calza S, et al. The GRM7 gene, early response to risperidone, and schizophrenia: a genome-wide association study and a confirmatory pharmacogenetic analysis. Pharmacogenomics J. 2017;17:146–54.
32. Stevenson JM, Reilly JL, Harris MS, Patel SR, Weiden PJ, Prasad KM, et al. Anti-psychotic pharmacogenomics in first episode psychosis: a role for glutamate genes. Transl Psychiatry. 2016;6:739.
33. Li Q, Wineinger NE, Fu DJ, Libiger O, Alphs L, Savitz A, et al. Genome-wide association studies in pharmacogenomics. Nat Rev Genet. 2012;13:308–19.
34. Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Patterson N, et al. LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet. 2015;47:291–5.
35. Guccione E, Richard S. The regulation, functions and clinical relevance of arginine vasopressin. Curr Hypertens Rep. 2018;20:40.
36. Bassani S, van Beelen E, Rossel M, Voisin N, Morgan A, Arribat Y, et al. Variants in the sarcoglycan complex, is reduced in muscular dystrophy. Hum Mol Genet. 2002;11:2147–56.
37. Wheeler MT, Zarnegar S, McNally EM. Zeta-sarcoglycan, a novel component of the sarcoglycan complex, is reduced in muscular dystrophy. Hum Mol Genet. 2002;11:2147–56.
38. Fabbri C, Kasper S, Kautzky A, Bartova L, Dold M, Zohar J, et al. Genome-wide association study of treatment-resistance in depression and meta-analysis of three independent samples. Br J Psychiatry. 2019;214:36–41.
39. Lam M, Hill WD, Trampush JW, Yu J, Knowles E, Davies G, et al. Pleiotropic meta-analysis of cognition, education, and schizophrenia differentiates roles of early neurodevelopmental and adult synaptic pathways. Am J Hum Genet. 2019;105:334–50.
40. Li Q, Wineinger NE, Fu DJ, Libiger O, Alphs L, Savitz A, et al. Variants in USP48 encoding ubiquitin hydrolase are associated with autosomal dominant non-syndromic hereditary hearing loss. Hum Mol Genet. 2021;30:1785–96.
41. Sinha S, Varghese V, Smith C, Walker R, et al. Genetic meta-analysis of p38 MAP kinase activation in vivo. Genes Dev. 2003;17:1969–78.
42. Grimesy NJ, Lin Y, Narala R, Rada CC, Mejia-Pena H, Trejo J. G protein-coupled receptors activate p38 MAPK via a non-canonical TAB1-TAB2-TAB3-dependent pathway in endothelial cells. J Biol Chem. 2019;294:5867–78.
43. Bray NR, Wijmenga C, Sullivan PF, Yang J, Visscher PM. Common disease is more complex than implied by the core gene omnigenic model. Cell 2018;173:1573–80.
44. Yeh SH, Yeh HY, Soo VW. A network flow approach to predict drug targets from microarray data, disease genes and interactome network - case study on prostate cancer. J Clin Bioinforma. 2012;2:1.
45. Jia P, Wang L, Fanous AH, Fato CN, Edwards TL, Zhao Z. Network-assisted investigation of combined causal signals from genome-wide association studies in schizophrenia. PLoS Comput Biol. 2018;14:e1006515.
46. Rossin EJ, Lage K, Raychaudhuri S, Xavier RJ, Tataar D, Benita Y, et al. Proteins encoded in genomic regions associated with immune-mediated disease physically interact and suggest underlying biology. PLoS Genet. 2011;7:e1001273.

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AUTHOR CONTRIBUTIONS
SYQ, CH, LH, contributed to the study design, and critically revised the manuscript; MZZ, JSM, ML, WZ designed the study, participated in data extraction, analyzed the data and drafted the manuscript; WZ, LS, HW, NZ conducted the experiment and revised the manuscript; SCW, CFP, XXL, collected the data; KY, TCT, RXS revised the manuscript.

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COMPETING INTERESTS
The authors declare no competing interests.

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