Identification of Novel Functional Brain Proteins for Treatment-Resistant Schizophrenia: Based on a Proteome-wide Association Study

Wenming Wei[*1], Huijie Zhang[*1], Bolun Cheng[1], Xiaoyue Qin[1], Dan He[1], Na Zhang[1], Yijing Zhao[1], Qingqing Cai[1], Sirong Shi[1], Xiaoge Chu[1], Yan Wen[1], Huan Liu[1], Yumeng Jia[1], Feng Zhang[#1].

[1] Key Laboratory of Trace Elements and Endemic Diseases, Collaborative Innovation Center of Endemic Disease and Health Promotion for Silk Road Region, School of Public Health, Health Science Center, Xi'an Jiaotong University, 710061, P. R. China

*The two authors contributed equally to this work

Corresponding author:

Feng Zhang

School of Public Health, Xi'an Jiaotong University Health Science Center

Email: fzhxjtu@mail.xjtu.edu.cn

Xi'an, P. R. China 710061

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Abstract

Objective: Genetic approaches are increasingly advantageous in characterizing treatment-resistant schizophrenia (TRS). Our aim is to identify TRS-associated functional brain proteins, providing a potential pathway for improving psychiatric classification and developing better-tailored therapeutic targets.

Methods: TRS-related proteome-wide association studies (PWAS) were conducted on genome-wide association studies (GWAS) from CLOZUK and the Psychiatric Genomics Consortium (PGC), which provided TRS individuals (n = 10,501) and non-TRS individuals (n = 20,325), respectively. The reference datasets for the human brain proteome were obtained from ROS/MAP and Banner, with 8,356 and 11,518 proteins collected respectively. We then performed colocalization analysis and functional enrichment analysis to further explore the biological functions of the proteins identified by PWAS.

Results: In PWAS, 2 statistically significant proteins were identified using the ROS/MAP and then replicated using the Banner reference dataset, including CPT2 ($P_{PWAS\text{-}ROS/MAP} = 4.15\times10^{-2}$, $P_{PWAS\text{-}Banner} = 3.38\times10^{-3}$) and APOL2 ($P_{PWAS\text{-}ROS/MAP} = 4.49\times10^{-3}$, $P_{PWAS\text{-}Banner} = 8.26\times10^{-3}$). Colocalization analysis identified 3 variants that were causally related to protein expression in the human brain, including *CCDC91* (PP4 = 0.981), *PRDX1* (PP4 = 0.894), and *WARS2* (PP4 = 0.757). We extended PWAS results from gene-based analysis to pathway-based analysis, identifying 14 gene ontology (GO) terms and the only candidate pathway for TRS, metabolic pathways (*all* $P < 0.05$).

Conclusions: Our results identified two protein biomarkers, and cautiously support that the pathological mechanism of TRS is linked to lipid oxidation and inflammation, where mitochondria-related functions may play a role.

Keywords: treatment-resistant schizophrenia; proteome-wide association study; human brain proteins; lipid oxidation; inflammation; mitochondria

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Introduction

Treatment-resistant schizophrenia (TRS) refers to approximately one-third of schizophrenia patients who do not adequately alleviate their psychotic symptoms despite standard antipsychotic treatment (*1*). Patients with TRS have higher rates of unemployment, poorer quality of life and poorer social and occupational functioning than individuals who respond to treatment(*2*). Clozapine is the only antipsychotic recommended for TRS, which is effective in about 60% of cases (*3*) and improves indicators of morbidity and mortality(*1*). Nevertheless, studies have shown that identification difficulties with TRS led to delays in clozapine prescription, which in turn was associated with reduced responsiveness of patients to clozapine (*4*). This makes early identification of TRS critical and the ascertainment of biomarkers of TRS a priority for the field of schizophrenia research.

Evidence suggests that earlier age of schizophrenia onset is a robust predictor of TRS, and that male gender, autumn/winter birth, poor premorbid functioning and rural upbringing may also contribute(*5*). However, a gap in the literature exists in the genetics of TRS, particularly biomarkers. To date, there is considerable heterogeneity in the genetic findings associated with TRS. A family history of schizophrenia is likely linked to developing TRS(*6*). Candidate gene research investigating the involvement of specific targets in TRS mostly clustered around the serotoninergic and dopaminergic systems, as well as on systems involved in oxidative stress and inflammation(*7*). Conversely, a small sample study of TRS, defined by American Psychiatric Association criteria, did not find any significant association among the 384 candidate loci(*8*). The collective interpretation of these results is made difficult by the slightly different recruitment TRS criteria(*9*). And the underrepresentation of individuals with treatment-resistant psychiatric symptoms in studies similarly reduced statistical efficiency(*10*). Another critical question in the TRS field is whether TRS represents a more severe form of schizophrenia, or if it represents a distinct subtype of schizophrenia with a different symptom profile and pathophysiology. Some clinical, imaging, biological and genetic evidence supports the latter(*11, 12*). Given the

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

potentially complex genetic architecture of this trait and the discrepancies in the clinical definition of TRS, there remains disagreement on the best approach to maximize the informativity and power of genetic studies on TRS. Our work was designed as a proteomics analysis to identify functional brain proteins that distinguish TRS from schizophrenia, in an attempt to provide a new perspective on this issue by exploring the expression products.

Proteomic techniques are increasingly being used to screen for biomarkers of schizophrenia, while no studies have yet applied proteomics to TRS. Proteome-wide association study (PWAS) captures any variant affecting the coding regions of genes, and then assigns each protein-coding gene functional affecting scores, and is widely utilized to robustly prioritize candidate genes(*13*). PWAS is a high-throughput approach where proteomic studies detect fewer expressed proteins than expressed genes detected by the transcriptome, but protein expression provides an accurate functional profile and reflects the complex interactions between genes and the environment, presenting an unbiased picture of the current physiological state. The importance of those interactions has been increasing in the research of neurological diseases(*10*). We performed two independent PWAS to validate each other, and functional enrichment and annotation analysis was conducted based on the results. Colocalization analysis was performed to identify variant loci that were causally related to the expressed proteins. These methods may identify biomarkers and discern the specific mechanisms underlying TRS, thereby offering proof of its classification as a subtype of schizophrenia and facilitating the early detection of TRS.

Materials and Methods

GWAS summary data

The GWAS summary data of treatment-resistant schizophrenia (TRS) and non-treatment resistant schizophrenia (non-TRS) were derived from a recently published study(*12*). TRS patients were derived from the CLOZUK1 and CLOZUK2 cohorts, with a total sample size of 10,501 individuals(*14*). All TRS patients were prescribed clozapine following at least two failed trials of antipsychotics and in

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

accordance with National Institute for Health and Care Excellence guidelines for TRS. Non-TRS patients were derived from 34 studies that were included in the meta-analysis by the Schizophrenia Working Group of the Psychiatric Genomics Consortium (PGC), with a total sample size of 20,325 schizophrenia patients(*15*). Fourteen studies with clinical records identified and excluded individuals with TRS, and cases from the remaining twenty studies without clinical records were conservatively included in our analysis as non-TRS individuals. Due to the number of different datasets and genotyping arrays involved in this analysis, the processing of TRS and non-TRS GWAS samples was performed separately on the data generated by the original study(*16, 17*). Both of these imputations used the SHAPEIT/IMPUTE2 pipeline. SNPs were restricted to minor allele frequencies (MAF) of 5% or higher and called in at least 20,000 combined samples, and any strand-ambiguous markers (A/T, G/C) with MAF≥40% were discarded in both datasets.

Human brain proteome reference weights for PWAS

Two human brain proteome reference weight datasets were obtained from recent publicly available studies. The discovery dataset was derived from Religious Orders Study and Rush Memory and Aging Project (ROS/MAP) cohorts, recruiting 391 individuals from two longitudinal clinical-pathologic cohort studies of aging and Alzheimer's disease(*18*). After quality control, 8,356 proteins from 376 subjects were included in our analysis. Among these, 262 were female and the average age at death was 89 years. The final clinical diagnosis included no cognitive impairment, mild cognitive impairment (MCI), Alzheimer's disease (AD) dementia, or other causes of dementia. The confirmation dataset was derived from the Banner Sun Health Research Institute (Banner) containing 198 individuals(*19*). After quality control, 11,518 proteins from 152 subjects were quantified. Of these, 87 were female and the average age at death was 85 years. Only individuals with a final diagnosis of AD or normal cognition were included. Both of the proteomic reference datasets utilized the same quality control procedures to identify and control the effects of clinical covariates (that is, age, gender, and final clinical diagnosis of cognitive status) before estimating protein weights. Details can be found in the Supplementary Material.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Proteome-wide association study analysis

Proteome-wide association study (PWAS) analysis was performed by integrating the TRS GWAS data with two brain proteomes using the FUSION pipeline (http://gusevlab.org/projects/fusion/). Briefly, we utilized FUSION to calculate protein weights in both the discovery and confirmation datasets individually. Subsequently, we combined the genetic effect of TRS (TRS GWAS z-score) with pre-computed protein weights by calculating the linear sum of z-score × weight of independent SNPs to perform the PWAS of TRS. Only proteins identified in the discovery dataset and replicated in the confirmation dataset were considered TRS-associated proteins. The linkage disequilibrium reference panel routinely utilized 1,190,321 HapMap SNPs from 489 individuals of European descent from the 1000 Genomes Project in FUSION. We implemented 2,000 permutations to control the potential impact of multiple testing on PWAS results. The proteins with permutated P value < 0.05 were considered significant. The design is presented in the Supplementary Material (Figure S1).

Colocalization Analysis

Our research performed a colocalization analysis of all genes identified by the two-stage PWAS using the coloc R package. We evaluated the colocalization status of a gene by calculating the PP that the genetic and functional associations derived from a shared causal SNP (PP4). Genes with PP4 > 0.75 are considered to be colocalized.

Functional enrichment and annotation analysis

The GO annotation and KEGG pathway enrichment analyses of the genes identified by PWAS were performed by the DAVID tool (https://david.ncifcrf.gov/). GO enrichment analysis includes Biological Process (BP), Cellular Component (CC), and Molecular Function (MF) analysis. KEGG database is a bioinformatics resource for mining significantly metabolic pathways enriched in the gene list. The purpose was to extract important GO terms and KEGG pathways, which can depict the characteristics of TRS. The results were considered statistically significant if $P < 0.05$.

Results

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

PWAS results of TRS

In PWAS, 2 significant proteins were identified in the discovery reference dataset and replicated in the confirmation reference dataset, including CPT2 ($P_{PWAS\text{-}ROS/MAP}$ = 4.15×10^{-2}, $P_{PWAS\text{-}Banner}$ = 3.38×10^{-3}) and APOL2 ($P_{PWAS\text{-}ROS/MAP}$ = 4.49×10^{-3}, $P_{PWAS\text{-}Banner}$ = 8.26×10^{-3}). Specifically, in the discovery stage, a total of 24 TRS-related candidate proteins were identified using the ROS/MAP reference dataset, such as PRDX1 ($P_{PWAS\text{-}ROS/MAP}$ = 1.00×10^{-3}), APOL2 ($P_{PWAS\text{-}ROS/MAP}$ = 4.49×10^{-3}), and WARS2 ($P_{PWAS\text{-}ROS/MAP}$ = 6.41×10^{-3}). In the confirmation stage, a total of 19 TRS-related candidate proteins were identified using the Banner reference dataset, such as CPT2 ($P_{PWAS\text{-}Banner}$ = 3.38×10^{-3}) and ABCC1 ($P_{PWAS\text{-}Banner}$ = 4.25×10^{-3}). Statistically significant genes identified in PWAS analysis are shown in Table 1 and Figure 1.

Colocalization Analysis

Of all 41 proteins identified by PWAS, colocalization analysis identified 3 genes that are causal for TRS and encoded functional proteins, including *CCDC91* (PP4 = 0.981), *PRDX1* (PP4 = 0.894), and *WARS2* (PP4 = 0.757). The results of the colocalization analysis of the genes identified by PWAS are presented in Table 1.

Functional enrichment and annotation analysis

GO enrichment analysis of all 41 genes identified by PWAS results were shown in Table 2. DAVID detected 14 GO terms, such as mitochondrion (GO:0005739, *P* value < 0.0001), Golgi apparatus (GO:0005794, *P* value = 0.0145), oxidoreductase activity (GO:0016620, *P* value = 0.0338), and protein domain specific binding (GO:0019904, *P* value = 0.0117). For pathway enrichment analysis of the genes identified by PWAS, DAVID detected only one candidate pathway for TRS, metabolic pathways (hsa01100, *P* value = 0.0312).

Discussion

In this work, we performed TRS-associated PWAS and found a total of 41 proteins that were differentially expressed, 2 of which were duplicated in the PWAS analysis of the discovery and confirmation dataset, suggesting a prominent role in the

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

biological processes of TRS, including CPT2 and APOL2. Due to polygenic inheritance, a complex trait is often influenced by multiple genes with similar functions as annotated in gene pathways. We extended PWAS results from gene-based analysis to pathway-based analysis, identifying 14 GO terms and 1 candidate pathway for TRS. Aiming to understand the mechanisms driving GWAS risk loci, we performed a colocalization analysis of PWAS-identified genes and identified 3 variants that were causally related to expression in the human dorsolateral prefrontal cortex.

TRS may be associated with abnormalities in the β-oxidative metabolic pathway of long-chain fatty acids in mitochondria. We discovered a novel TRS-related protein CPT2. Carnitine palmitoyl transferases 2 (CPT2) is the core protein of a catalytically active multiprotein complex localized in the inner mitochondrial membrane, assisting long-chain fatty acids to enter the mitochondrial matrix for oxidation and energy. The role of the lipid regulatory system on TRS has been supported by studies. One work found that clozapine altered the activity of the AMPK-ACC-CPT1 pathway, a central pathway of lipid metabolism, to affect the lipid compositions of the neuronal membranes in the rat frontal cortex(*20*). Abnormalities in membrane lipid composition have also been reported in the frontal cortex of patients with schizophrenia(*21*). Beta-oxidation may be potentially linked to the pathogenesis of TRS. The metabolite of clozapine is capable of interacting with a wide range of neurotransmitter receptors, suggesting that TRS may have a neurobiological etiology(*22*). One study found that deletion of CPT2 in the nervous system leads to elevated expression of β-oxidation enzymes(*23*). Individuals with genetic disorders in mitochondrial fatty acid β-oxidation may suffer from neurological disorders, including seizures, encephalopathies, and cortical atrophy(*24-26*). CPT activity is present in almost all brain regions especially the brainstem(*27*), and carnitine shuttle and β-oxidation genes are expressed primarily in astrocytes and neural stem cells(*28*), suggesting that CTP2 deficiency may involve the central nervous system. Our GO and pathway enrichment analysis also support the CTP2-centred bio-metabolic processes.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

TRS may be linked to cholesterol transport and homeostasis. The other brain protein we identified was apolipoprotein L2 (APOL2). The apolipoprotein family of proteins facilitates the tightly regulated delivery of lipids and lipophilic substrates to specific cells in the brain, as well as regulating signal transduction pathways(*29*). APOL2, mainly localized at the endoplasmic reticulum, is implicated in cholesterol biosynthesis and trafficking and is thought to mediate cell death induced by interferon-gamma or viral infection, indicating a role in inflammatory processes(*30*). Dysregulation of the inflammatory response system has been associated with the pathophysiology of schizophrenia(*31*). Prior works have found the gene *APOL2* was upregulated in the brains of schizophrenic patients, and polymorphisms in this gene were linked to schizophrenia risk(*32*). Differential expression of *APOL2* has also been observed in individuals with substance use disorders, including cocaine, cannabis, and phencyclidine(*33*). *APOL2* is highly expressed in some brain regions, including the hippocampus, intralobular white matter and the medulla(*34*). However, the biological function of APOL2 in the brain remains unclear.

Three gene variants identified by colocalization analysis implied a potential association with TRS pathogenesis, including *CCDC91*, *PRDX1*, and *WARS2*. *CCDC91* is highly expressed in the central nervous system, located in the nucleoplasm and trans-Golgi networks and is involved in subcellular transport and protein localization in the Golgi complex. One study showed an increased incidence of copy number variation in *CCDC91* in bipolar disorder patients(*35*). Its interacting partners, GGA1 and GGA2, have been implicated in the pathophysiology of Alzheimer's disease through interactions with β-amyloid precursors(*36*). Also, our work identified one GO annotation associated with *CCDC91*: identical protein binding. PRDX is a protein family with antioxidant enzyme activity that reduces hydrogen peroxide and alkyl hydroperoxides in cells. Numerous studies have demonstrated the anti-inflammatory and anti-apoptotic effects of PRDX1(*37*). It appears to have neuroprotective activities in neuronal cells, which reduce reactive oxygen species-mediated cell death in schizophrenia(*38*). Antipsychotic drugs affect *PRDX1* expression. *PRDX1* expression was increased in haloperidol-treated C6 cells

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

but decreased in C6 cells treated with risperidone and clozapine(*39*). *WARS2* encodes mitochondrial tryptophan-tRNA synthetase, a homologous class Ic enzyme. The clinical spectrum associated with *WARS2* defects appears to be quite broad, including clinical features (cardiomyopathy, movement disorders, retinitis pigmentosa, optic atrophy, hypoglycemia, etc.) as well as the age of onset and clinical course(*40*). Also, *WARS2* mutations cause dopa-responsive early-onset parkinsonism and progressive myoclonus ataxia(*41*). These works support the contention that the biparental loss-of-function *WARS2* variants cause mitochondrial dysfunction and disease. The effect of variants in these three genes on TRS needs further study.

Functional enrichment and annotation analysis revealed several mitochondrial-related results implicating mitochondrial function in the pathogenesis of TRS. Some evidence has confirmed that mitochondrial dysfunction is an important pathological factor in schizophrenia, including decreased mitochondrial respiration due to altered complex I activity(*42*), motor deficits, altered mitochondrial dynamics(*43*), increased levels of mitochondrial DNA mutations(*44*) and decreased cognitive abilities in mitochondrial diseases(*45*). One study identified a strong correlation between a TRS susceptibility gene and mitochondrial dysfunction, which correlates with the dysregulation of NRG-1/mTOR/miR143-3p signaling(*46*). More attention should be paid to the role of mitochondria in TRS.

Our results revolve more around lipid oxidation and inflammation, which tends to be, but is not fully explained by the "inflammation and oxidative stress" hypothesis of the neurobiological mechanisms of TRS. More evidence is being mined to support this hypothesis. A study showed elevated lipid peroxidation in patients with TRS compared to treatment-responsive schizophrenia patients and healthy controls. This exacerbated peroxidation process in TRS may reflect a deeper abnormality in the fatty acid content of synaptic membranes, leading to the dysfunction of neurons as well as its microenvironment(*47*). Early neuroinflammation and chronic hyperactivation are thought to contribute to schizophrenia; high levels of inflammation may also play a role in treatment resistance(*48*). There is no universally accepted or defined mechanism for TRS, limited by different criteria for TRS or small study sample sizes.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Other hypotheses have been proposed regarding the neurobiological mechanisms of TRS, including differences in dopaminergic function, glutamate dysregulation and serotonin dysregulation. Previous studies have provided genetic evidence for different hypotheses, indicating that TRS may develop through multiple pathways or change in potential mechanisms at specific times (*49*). These theories are not mutually exclusive, and combining several pathways may contribute to the neurobiology of TRS(*50*). These results are insufficient to highlight the distinctiveness of TRS from schizophrenia, and more characteristic markers are needed to understand the biological processes and prove the heterogeneity of TRS.

Our work is the first TRS-related study with proteomic analysis based on a large sample of GWAS summary data, providing an accurate functional profile that presents an unbiased picture of current physiological status. Despite this, the study has certain limitations. TRS is an underreported diagnosis, and although our definition of the phenotype is in line with international criteria, we acknowledge that there may still be individuals with treatment-resistant symptoms in non-TRS datasets. This could result in imperfect phenotypes and misclassifications that weaken our findings and reduce the exploratory power of the brain proteins analyzed. And, the study sample is mainly of European descent and the conclusions may not pertain to non-European countries. Furthermore, we performed a cross-sectional proteomics study, so no exploration of longitudinal changes in biomarkers was available. More research is needed to test our results in different patient populations and different phases of illness. Additionally, it is worth noting that the age and brain tissue preparation technique of the individuals included in the proteome reference datasets may have a slight impact on the final protein expression results. Finally, some proteins identified in the discovery PWAS failed to be replicated in the confirmation PWAS, which is attributed to the limited sample size and the stochastic nature of high-throughput proteomic sequencing.

In conclusion, we performed PWAS analysis and identified two TRS-associated brain proteins, CPT2 and APOL2. Colocalization analysis based on PWAS results identified three variants that were causally related to protein expression, including *CCDC91*, *PRDX1*, and *WARS2.* Our results cautiously support that the pathological

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

316 mechanism of TRS is linked to lipid oxidation and inflammation, where
317 mitochondria-related functions may play a role.
318
319 **Ethics Approval**
320 This study is based on publicly available summarized data. The protocol and data
321 collection were approved by the ethics committee of each genome-wide association
322 study.
323
324 **Author contributions**
325 Material preparation, data collection and analysis were performed by Wenming Wei
326 and Huijie Zhang. The first draft of the manuscript was written by Wenming Wei. The
327 figures and tables were made by Bolun Cheng, Xiaoyue Qin and Dan He. The
328 literature searches were performed by Na Zhang, Yijing Zhao, Qingqing Cai, Xiaoge
329 Chu, Sirong Shi, Liu Huan, Yan Wen and Yumeng Jia. The study design was
330 performed by Feng Zhang.
331
332 **Data Availability**
333 The datasets can be downloaded from the Psychiatric Genomics Consortium website
334 (http://pgc.unc.edu).
335
336 **Funding**
337 This work was supported by the National Natural Scientific Foundation of China
338 [81922059] and the Natural Science Basic Research Plan in Shaanxi Province of
339 China [2021JCW-08].
340
341 **Consent to Participate**
342 Written informed consent was obtained from each participant of previously published
343 GWASs before data collection.
344
345 **Consent for Publication**

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

All the authors have read and approved the final version of the manuscript.

Competing interests

The authors report no financial interests or potential conflicts of interest.

Acknowledgements

Not applicable.

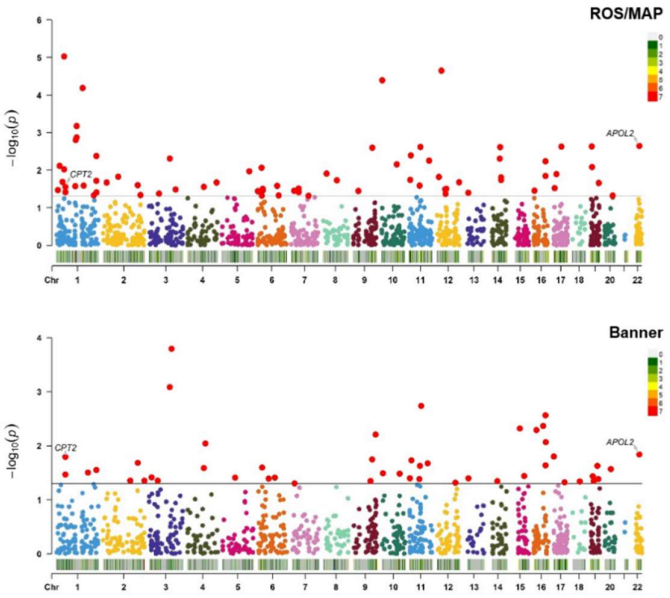

Figure 1. Manhattan Plots of significant human brain proteins identified in PWAS

Note: PWAS, proteome-wide association study. Each point corresponds to a single test of association between a gene and phenotype, plotted according to genomic position on the x-axis and the strength of association ($-\log_{10}$ *P*-value) on the y-axis. Two common statistically significant proteins resulting from the analysis were mapped out.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Table 1. Significant proteins identified by PWAS

Brain Proteins		Chromosome	Permutation P value		COLOC.PP4
Symbol	Name		ROS/MAP	Banner	
CPT2	Carnitine Palmitoyltransferase 2	1	4.15×10^{-2}	3.38×10^{-2}	0.056
APOL2	Apolipoprotein L2	22	4.49×10^{-3}	8.26×10^{-3}	0.017
CCDC91	Coiled-Coil Domain Containing 91	12	2.27×10^{-2}	-	0.981
PRDX1	Peroxiredoxin 1	1	1.00×10^{-3}	-	0.894
WARS2	Tryptophanyl TRNA Synthetase 2	1	6.41×10^{-3}	-	0.757
FLAD1	Flavin Adenine Dinucleotide Synthetase 1	1	2.94×10^{-2}	-	0.065
NIT1	Nitrilase 1	1	1.26×10^{-2}	-	0.055
ICA1L	Islet Cell Autoantigen 1 Like	2	3.11×10^{-2}	-	0.042
RABEP1	Rabaptin, RAB GTPase Binding Effector Protein 1	17	4.03×10^{-2}	-	0.036
1-Mar	Mitochondrial Amidoxime Reducing Component 1	1	4.21×10^{-2}	-	0.025
GANAB	Glucosidase II Alpha Subunit	11	1.07×10^{-2}	-	0.023
CORO7	Coronin 7	16	< 0.0001	-	0.022
TMEM25	Transmembrane Protein 25	11	< 0.0001	-	0.016
FUT8	Fucosyltransferase 8	14	< 0.0001	-	0.014
ALDH4A1	Aldehyde Dehydrogenase 4 Family Member A1	1	3.79×10^{-2}	-	0.012
DBNL	Drebrin Like	7	2.09×10^{-2}	-	0.01
LETMD1	LETM1 Domain Containing 1	12	1.17×10^{-2}	-	0.005
TTC19	Tetratricopeptide Repeat Domain 19	17	4.92×10^{-2}	-	0.002
RASA4B	RAS P21 Protein Activator 4B	7	4.35×10^{-2}	-	0.001
ALAD	Aminolevulinate Dehydratase	9	1.20×10^{-3}	-	0.001
TPP1	Tripeptidyl Peptidase 1	11	9.24×10^{-3}	-	0.001

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

TUBA4A	Tubulin Alpha 4a	2	3.23×10^{-2}	-	< 0.001
NPM2	Nucleophosmin/Nucleoplasmin 2	8	4.80×10^{-2}	-	< 0.001
COQ10A	Coenzyme Q10A	12	4.34×10^{-3}	-	< 0.001
ABCC1	ATP Binding Cassette Subfamily C Member 1	16	-	4.25×10^{-3}	0.06
DHODH	Dihydroorotate Dehydrogenase	16	-	3.01×10^{-2}	0.051
MRVI1	Murine Retrovirus Integration Site 1 Homolog	11	-	2.81×10^{-2}	0.049
ALDH5A1	Aldehyde Dehydrogenase 5 Family Member A1	6	-	1.63×10^{-2}	0.04
COA7	Cytochrome C Oxidase Assembly Factor 7	1	-	2.48×10^{-2}	0.032
ANKMY2	Ankyrin Repeat And MYND Domain Containing 2	7	-	3.80×10^{-2}	0.026
TGM2	Transglutaminase 2	20	-	4.11×10^{-2}	0.008
C1orf27	Chromosome 1 Open Reading Frame 27	1	-	2.32×10^{-2}	0.007
GRIA4	Glutamate Ionotropic Receptor AMPA Type Subunit 4	11	-	2.31×10^{-2}	0.006
GALK2	Galactokinase 2	15	-	2.67×10^{-2}	0.003
TMEM245	Transmembrane Protein 245	9	-	< 0.0001	0.002
KHDRBS2	KH RNA Binding Domain Containing	6	-	2.03×10^{-2}	0.001
TMEM109	Transmembrane Protein 109	11	-	2.00×10^{-4}	0.001
NUDT16	Nudix Hydrolase 16	3	-	< 0.0001	< 0.001
TMEM43	Transmembrane Protein 43	3	-	< 0.0001	< 0.001
PPA2	Inorganic Pyrophosphatase 2	4	-	2.54×10^{-2}	< 0.001
CRAT	Carnitine O-Acetyltransferase	9	-	2.77×10^{-2}	< 0.001

Note: PWAS, proteome-wide association study. Only significant permutation P values are presented.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

Table 2. GO enrichment analysis results of TRS-associated genes identified by PWAS

Name	*P* value	Fold enrichment
GO:0006807~nitrogen compound metabolic process	0.0244	78.5959
GO:0006491~N-glycan processing	0.0330	57.9128
GO:0005739~mitochondrion	0.0000	4.8738
GO:0005743~mitochondrial inner membrane	0.0014	6.9664
GO:0005759~mitochondrial matrix	0.0052	6.9069
GO:0005794~Golgi apparatus	0.0145	3.3611
GO:0042470~melanosome	0.0149	15.6914
GO:0070062~extracellular exosome	0.0151	2.4289
GO:0008458~carnitine O-octanoyltransferase activity	0.0054	358.3048
GO:0042802~identical protein binding	0.0098	2.8254
GO:0019904~protein domain specific binding	0.0117	8.1743
GO:0048039~ubiquinone binding	0.0126	153.5592
GO:0003824~catalytic activity	0.0237	12.2149
GO:0016620~oxidoreductase activity, acting on the aldehyde or oxo group of donors, NAD or NADP as acceptor	0.0338	56.5744

Note: PWAS, proteome-wide association study.

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

References

1. R. R. Conley, D. L. Kelly, Management of treatment resistance in schizophrenia. *Biological Psychiatry* **50**, 898-911 (2001).
2. F. Iasevoli *et al.*, Treatment resistant schizophrenia is associated with the worst community functioning among severely-ill highly-disabling psychiatric conditions and is the most relevant predictor of poorer achievements in functional milestones. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **65**, (2016).
3. S. Kumra *et al.*, Clozapine and "high-dose" olanzapine in refractory early-onset schizophrenia: A 12-week randomized and double-blind comparison. *Biological Psychiatry* **63**, 524-529 (2008).
4. P. R. Shah *et al.*, Clozapine response trajectories and predictors of non-response in treatment-resistant schizophrenia: a chart review study. *Eur Arch Psy Clin N* **270**, 11-22 (2020).
5. S. E. Smart, A. P. Kepinska, R. M. Murray, J. H. MacCabe, Predictors of treatment resistant schizophrenia: a systematic review of prospective observational studies. *Psychol Med* **51**, 44-53 (2021).
6. K. Kowalec *et al.*, Increased schizophrenia family history burden and reduced premorbid IQ in treatment-resistant schizophrenia: a Swedish National Register and Genomic Study. *Mol Psychiatr* **26**, 4487-4495 (2021).
7. C. Pisanu, A. Squassina, Treatment-Resistant Schizophrenia: Insights From Genetic Studies and Machine Learning Approaches. *Front Pharmacol* **10**, (2019).
8. C. Teo *et al.*, Analysis of treatment-resistant schizophrenia and 384 markers from candidate genes. *Pharmacogenet Genom* **22**, 807-811 (2012).
9. K. Hodgson, P. McGuffin, C. M. Lewis, Advancing psychiatric genetics through dissecting heterogeneity. *Hum Mol Genet* **26**, R160-R165 (2017).
10. A. Hofer *et al.*, Why do indiviuals with schizophrenia drop out of observational clinical trials? *Psychiatry Research* **256**, 1-5 (2017).
11. F. C. Nucifora, E. Woznica, B. J. Lee, N. Cascella, A. Sawa, Treatment resistant schizophrenia: Clinical, biological, and therapeutic perspectives. *Neurobiology of Disease* **131**, 104257 (2019).
12. A. F. Pardiñas *et al.*, Interaction Testing and Polygenic Risk Scoring to Estimate the Association of Common Genetic Variants With Treatment Resistance in Schizophrenia. *JAMA Psychiatry* **79**, 260 (2022).
13. T. S. Wingo *et al.*, Integrating human brain proteomes with genome-wide association data implicates novel proteins in post-traumatic stress disorder. *Mol Psychiatr* **27**, 3075-3084 (2022).
14. A. F. Pardinas *et al.*, Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. *Nat Genet* **50**, 381-+ (2018).
15. C. Schizophrenia Working Group of the Psychiatric Genomics, Biological insights from 108 schizophrenia-associated genetic loci. *Nature* **511**, 421-427 (2014).
16. S. W. Choi, P. F. O'Reilly, PRSice-2: Polygenic Risk Score software for biobank-scale data. *Gigascience* **8**, (2019).
17. J. Tiihonen *et al.*, Real-World Effectiveness of Antipsychotic Treatments in a Nationwide Cohort of 29 823 Patients With Schizophrenia. *Jama Psychiatry* **74**, 686-693 (2017).

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

18. A. P. Wingo *et al.*, Shared proteomic effects of cerebral atherosclerosis and Alzheimer's disease on the human brain. *Nat Neurosci* **23**, 696-+ (2020).
19. A. P. Wingo *et al.*, Integrating human brain proteomes with genome-wide association data implicates new proteins in Alzheimer's disease pathogenesis. *Nat Genet* **53**, 143-+ (2021).
20. M. K. Kim *et al.*, The effect of clozapine on the AMPK-ACC-CPT1 pathway in the rat frontal cortex. *Int J Neuropsychoph* **15**, 907-917 (2012).
21. S. Narayan, S. R. Head, T. J. Gilmartin, B. Dean, E. A. Thomas, Evidence for Disruption of Sphingolipid Metabolism in Schizophrenia. *J Neurosci Res* **87**, 278-288 (2009).
22. A. F. Pardinas, M. J. Owen, J. T. R. Walters, Pharmacogenomics: A road ahead for precision medicine in psychiatry. *Neuron* **109**, 3914-3929 (2021).
23. C. J. White *et al.*, Determining the Bioenergetic Capacity for Fatty Acid Oxidation in the Mammalian Nervous System. *Mol Cell Biol* **40**, (2020).
24. I. Tein, J. Vajsar, L. MacMillan, W. G. Sherwood, Long-chain L-3-hydroxyacyl-coenzyme A dehydrogenase deficiency neuropathy: Response to cod liver oil. *Neurology* **52**, 640-643 (1999).
25. T. Tyni *et al.*, Long-chain 3-hydroxyacyl-coenzyme A dehydrogenase deficiency with the G1528C mutation: Clinical presentation of thirteen patients. *J Pediatr-Us* **130**, 67-76 (1997).
26. J. L. Merritt, M. Norris, S. Kanungo, Fatty acid oxidation disorders. *Ann Transl Med* **6**, (2018).
27. A. Virmani *et al.*, The Carnitine Palmitoyl Transferase (CPT) System and Possible Relevance for Neuropsychiatric and Neurological Conditions. *Molecular Neurobiology* **52**, 826-836 (2015).
28. Z. G. Xie, A. Jones, J. T. Deeney, S. K. Hur, V. A. Bankaitis, Inborn Errors of Long-Chain Fatty Acid beta-Oxidation Link Neural Stem Cell Self-Renewal to Autism. *Cell Rep* **14**, 991-999 (2016).
29. Y. H. Li, J. Cam, G. J. Bu, Low-density lipoprotein receptor family. *Molecular Neurobiology* **23**, 53-67 (2001).
30. J. Galindo-Moreno *et al.*, Apolipoprotein L2 contains a BH3-like domain but it does not behave as a BH3-only protein. *Cell Death Dis* **5**, (2014).
31. N. Muller, M. Riedel, M. Ackenheil, M. J. Schwarz, The role of immune function in schizophrenia: an overview. *Eur Arch Psy Clin N* **249**, 62-68 (1999).
32. S. Takahashi *et al.*, Association of SNPs and haplotypes in APOL1, 2 and 4 with schizophrenia. *Schizophrenia Research* **104**, 153-164 (2008).
33. E. Lehrmann *et al.*, Transcriptional changes common to human cocaine, cannabis and phencyclidine abuse. *Plos One* **1**, e114 (2006).
34. A. Luo *et al.*, Epigenetic aging is accelerated in alcohol use disorder and regulated by genetic variation in APOL2. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology* **45**, 327-336 (2020).
35. X. F. Chen *et al.*, Novel Association Strategy with Copy Number Variation for Identifying New Risk Loci of Human Diseases. *Plos One* **5**, (2010).
36. J. H. Herskowitz *et al.*, GGA1-mediated endocytic traffic of LR11/SorLA alters APP intracellular distribution and amyloid-beta production. *Mol Biol Cell* **23**, 2645-2657 (2012).

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press

37. G.-Q. Yang *et al.*, Prdx1 Reduces Intracerebral Hemorrhage-Induced Brain Injury via Targeting Inflammation- and Apoptosis-Related mRNA Stability. *Frontiers in Neuroscience* **14**, 181 (2020).
38. Y. M. Lee *et al.*, Oxidative modification of peroxiredoxin is associated with drug-induced apoptotic signaling in experimental models of Parkinson disease. *J Biol Chem* **283**, 9986-9998 (2008).
39. M.-L. Chen, Two-dimensional gel electrophoresis revealed antipsychotic drugs induced protein expression modulations in C6 glioma cells. *Progress in Neuro-Psychopharmacology & Biological Psychiatry* **40**, 1-11 (2013).
40. S. B. Wortmann *et al.*, Biallelic variants in WARS2 encoding mitochondrial tryptophanyl-tRNA synthase in six individuals with mitochondrial encephalopathy. *Human Mutation* **38**, 1786-1795 (2017).
41. M. Skorvanek *et al.*, WARS2 mutations cause dopa-responsive early-onset parkinsonism and progressive myoclonus ataxia. *Parkinsonism & Related Disorders* **94**, 54-61 (2022).
42. D. Ben-Shachar, Mitochondrial multifaceted dysfunction in schizophrenia; complex I as a possible pathological target. *Schizophrenia Research* **187**, 3-10 (2017).
43. K. H. Flippo, S. Strack, An emerging role for mitochondrial dynamics in schizophrenia. *Schizophrenia Research* **187**, 26-32 (2017).
44. V. F. Goncalves *et al.*, A Comprehensive Analysis of Nuclear-Encoded Mitochondrial Genes in Schizophrenia. *Biological Psychiatry* **83**, 780-789 (2018).
45. J. Geng *et al.*, Andrographolide sulfonate improves Alzheimer-associated phenotypes and mitochondrial dysfunction in APP/PS1 transgenic mice. *Biomed Pharmacother* **97**, 1032-1039 (2018).
46. J. Sun *et al.*, miR143-3p-Mediated NRG-1-Dependent Mitochondrial Dysfunction Contributes to Olanzapine Resistance in Refractory Schizophrenia. *Biological Psychiatry* **92**, 419-433 (2022).
47. V. Medina-Hernandez *et al.*, Increased lipid peroxidation and neuron specific enolase in treatment refractory schizophrenics. *J Psychiatr Res* **41**, 652-658 (2007).
48. O. D. Howes, R. McCutcheon, Inflammation and the neural diathesis-stress hypothesis of schizophrenia: a reconceptualization. *Transl Psychiat* **7**, (2017).
49. D. M. Ruderfer *et al.*, Polygenic overlap between schizophrenia risk and antipsychotic response: a genomic medicine approach. *Lancet Psychiat* **3**, 350-357 (2016).
50. O. D. Howes, S. Kapur, A neurobiological hypothesis for the classification of schizophrenia: type A (hyperdopaminergic) and type B (normodopaminergic). *Br J Psychiatry* **205**, 1-3 (2014).

https://doi.org/10.1192/j.eurpsy.2023.20 Published online by Cambridge University Press