Heinzer *et al. J Transl Genet Genom* 2024;8:1-12
DOI: 10.20517/jtgg.2023.39

Journal of Translational Genetics and Genomics

Review

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What have genetic studies of rare sequence variants taught us about the aetiology of schizophrenia?

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How to cite this article: Heinzer L, Curtis D. What have genetic studies of rare sequence variants taught us about the aetiology of schizophrenia? *J Transl Genet Genom* 2024;8:1-12. https://dx.doi.org/10.20517/jtgg.2023.39

Received: 6 Sep 2023 **First Decision:** 5 Dec 2023 **Revised:** 18 Dec 2023 **Accepted:** 2 Jan 2024 **Published:** 12 Jan 2024

Academic Editor: Richard E. Frye **Copy Editor:** Fangyuan Liu **Production Editor:** Fangyuan Liu

Abstract

With a population prevalence of 1%, schizophrenia is widespread, yet the aetiology of this psychiatric disorder remains elusive. There is an evident genetic component of schizophrenia, with heritability estimates lying at 60%-80%. While genome-wide association studies have identified 120 gene loci associated with schizophrenia risk, these involved common variants that confer only small effects on individual risk (median odds ratio < 1.2). The recent emergence of whole exome sequencing (WES) technologies has facilitated the identification of rare sequence variants, including some protein-truncating variants that have significant effects on risk. Three key large-scale WES studies have demonstrated that rare sequence variants in the genes *SETD1A*, *CACNA1G*, *CUL1*, *GRIA3*, *GRIN2A*, *HERC1*, *RB1CC1*, *SP4*, *TRIO*, *XPO7*, and *AKAP11* confer substantial risk for schizophrenia. These genes are highly expressed in central nervous system neurons and their products participate in diverse molecular functions including synaptic transmission, transcriptional regulation, and ubiquitin ligation. The understanding of these functional roles illuminates putative molecular mechanisms which may lead to schizophrenia-like phenotypes. It will also be possible to develop model systems in which the effects of impaired function of these genes can be further explored. Genetic studies of rare variants to date suggest that glutamatergic system dysregulation, chromatin modification, and the ubiquitin-proteasome system play key roles in schizophrenia aetiology.

Keywords: Schizophrenia, exome sequencing, rare sequence variants, protein-truncating variants, glutamatergic dysfunction, ubiquitin-proteasome system

INTRODUCTION

Schizophrenia is a severe and chronic psychiatric disorder with a population prevalence in the order of 1%,

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whose aetiology remains elusive[1,2]. It has long been clear that genetic factors can contribute to risk, and twin, family, and adoption studies estimate the heritability of schizophrenia to lie between 60%-80%[3,4]. The most comprehensive genome-wide association study (GWAS) to date implicated 287 significant risk variants and these signals were fine-mapped to 120 genes[4]. As is expected for a trait likely to be under strong negative selection, the common variants identified by GWAS are associated with a relatively small risk for schizophrenia (median OR < 1.2)[5]. There can also be a lack of clarity as to which genes are implicated and the nature of the change in gene function that leads to increased risk. A number of extremely rare copy number variants (CNVs) have been found to have large effects on schizophrenia risk, and for these, the risk is clearly associated with either a decrease (for deletions) or an increase (for duplications) in gene dosage[6]. However, in general, with the exception of the *NRXN1* deletion, these CNVs implicate large regions of the genome, so it is unclear which specific genes are implicated.

Whole exome sequencing (WES) offers advantages over GWAS and other previously employed approaches for a number of reasons. It is able to detect extremely rare inherited and *de novo* variants, some of which may have large effects on risk. Of these rare variants, some can be characterised as having an effect on the gene such that it cannot produce a useful product. These may be termed loss of function (LOF) variants, although some authors use the term protein-truncating variants (PTVs). If such variants are found to be convincingly associated with disease, then it is reasonable to make the claim that impaired functioning of the gene, with a reduction in its product, increases the risk of disease. Overall, this may assist in developing insights into pathogenesis. Another advantage of WES is that, when such an effect is identified, it becomes possible to study animal models in which gene function is impaired, allowing a comprehensive investigation of impacts ranging from molecular mechanisms up to behavioural phenotypes.

This focussed review explores the 11 genes recently implicated in schizophrenia risk by the first large-scale WES studies, carried out on thousands of cases and controls as well as trios consisting of cases and their parents[7-9]. For all of these genes, PTVs were shown to confer a substantial effect on risk and reached exome-wide levels of statistical significance, although in the case of one gene, *AKAP11*, this was achieved only in a joint analysis of schizophrenia and bipolar disorder. A more recent custom sequencing study confirmed *AKAP11* as a risk gene for schizophrenia and supported the involvement of most of the other genes, as well as identifying a novel gene, *SRRM2*, as achieving exome-wide significance[10]. However, both this study and a study of exome-sequenced participants in the UK Biobank found PTVs in one of the genes from the original set of WES genes, *CACNA1G*, to be relatively common in controls, casting doubt on whether it is, in fact, a schizophrenia risk gene[11]. Through the assimilation of information from WES studies, gene profiles, and relevant model systems, this review investigates what research into rare sequence variants can tell us about the aetiology of schizophrenia.

INDIVIDUAL RISK GENES IMPLICATED IN SCHIZOPHRENIA THROUGH WES

A number of sequencing studies have suggested several genes to be possibly involved in schizophrenia pathophysiology[12]. To focus this investigation, we restrict attention to the 11 genes implicated at exome-wide significance by the first three large WES studies referred to above. These implicated genes that have been found to confer schizophrenia risk are *SETD1A, CACNA1G, CUL1, GRIA3, GRIN2A, HERC1, RB1CC1, SP4, TRIO*, *XPO7*, and *AKAP11*. Having identified these genes as being well evidenced through systematic, large-scale studies, we went on to carry out an exhaustive review of the available literature for each of them in order to gain insights into their functions and their possible relevance to schizophrenia pathogenesis. The genes are listed in Table 1 and a detailed account of their functions related to schizophrenia is provided below.

Table 1. Summary of genes implicated in schizophrenia pathogenesis through large-scale exome sequencing studies

Gene symbol	Gene name	Putative role in schizophrenia pathogenesis
SETD1A	SET domain containing 1A, histone lysine methyltransferase	Regulates gene expression
CACNA1G	Calcium voltage-gated channel subunit alpha1 G	Involved in neuronal excitation
CUL1	Cullin 1	Encodes component of an E3 ubiquitin ligase complex
GRIA3	Glutamate ionotropic receptor AMPA type subunit 3	Involved in ionotropic glutamatergic transmission
GRIN2A	Glutamate ionotropic receptor NMDA type subunit 2A	Codes for NMDA receptor subunit
HERC1	HECT and RLD domain containing E3 ubiquitin protein ligase family member 1	Encodes an E3 ubiquitin ligase
RB1CC1	RB1 inducible coiled-coil 1	Involved in neurodevelopment
SP4	Sp4 transcription factor	Modulates expression of NMDA receptor subunit genes
TRIO	Trio Rho guanine nucleotide exchange factor	Has a role in glutamatergic transmission
XPO7	Exportin 7	Role unclear
AKAP11	A-kinase anchoring protein 11	Possibly involved in neuronal plasticity, target of E3 ubiquitin ligases

SETD1A

SETD1A (SET Domain Containing 1A), previously referred to as *KMT2F*, encodes a histone lysine-methyltransferase, which is a catalytic subunit of the highly conserved mammalian Set/COMPASS complex[13]. This complex mediates mono-, di- and trimethylation of the lysine 4 residue on the histone H3 protein (H3K4)[9]. These histone marks are associated with gene activation and implicate SETD1A as a transcription regulator[13,14]. SETD1A is particularly responsible for H3K4 trimethylation (H3K4me3), which is a chromatin modification that has been reported to be found at the transcription start sites of active genes[15,16]. Downstream effects of this transcriptional regulation include modulation of cortical synaptic dynamics and axonal branching[9,13]. In addition to being implicated in schizophrenia risk, *SETD1A* variants are also associated with other neurodevelopmental disorders and early-onset epilepsy[13,17]. Given these results and the fact that *SETD1A* is expressed in the developing brain, it seems likely that *SETD1A* plays a significant role in the development and maintenance of healthy brain function[17].

CACNA1G

CACNA1G (Calcium Voltage-Gated Channel Subunit Alpha-1G Subunit) encodes the $Ca_V3.1$ subunit of the low-voltage-activated T-type calcium channel[18]. These voltage-sensitive calcium channels facilitate calcium ion entry into excitable neuronal cells, as well as being involved in calcium-dependent processes, including cell division, cell death, gene expression, and neurotransmitter release[19]. These T-type calcium channels are highly expressed in deep cerebellar nuclei and Purkinje neurons. These neurons are specific to the cerebellar cortex and appear to be involved in cognition and emotion[20,21]. Along with being a schizophrenia risk gene, *CACNA1G* is also associated with the risk of severe intellectual or developmental disability. To date, most research into *CACNA1G* has been conducted on spinocerebellar ataxia-42, a disorder characterised by cerebellar atrophies and cognitive developmental defects[20].

CUL1

CUL1 (Cullin 1) encodes a scaffolding protein, which is a core component of the SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligase complex[22]. The SCF complex is invariably composed of scaffolding protein CUL1, RING finger protein RBX1, and adaptor protein SKP1, as well as a variable F-box component[23]. This complex mediates the ubiquitination and subsequent degradation of proteins regulating cell-cycle progression, signal transduction, and cell proliferation[23,24]. CUL1 is the most extensively characterised member of the cullin (CUL) protein family, and aberrant expression of this protein has been

shown to induce SCF E3 ligase dysfunction[23,25]. The existing literature investigating CUL1 is largely focussed on its implication in various cancers, including breast cancer metastasis, colorectal cancer, ovarian cancer, and melanoma[22-24].

E3 ubiquitin ligases are vital components of the ubiquitin-proteasome system (UPS), which is fundamental for protein degradation in neurons[26]. Furthermore, there is strong evidence that the UPS fulfils a key role in synaptic transmission[26]. The UPS fulfils its autophagy functions via a cascade of reactions depicted in Figure 1, primarily involving E1 ubiquitin-activating enzymes and E2 ubiquitin-conjugating enzymes in addition to E3 ubiquitin-protein ligase enzymes[26]. Three major classes of E3 ligases exist, the HECT domain-containing E3s, RING domain-containing E3s, and RBR family E3s, whereby the SCF complex implicating CUL1 belongs to the RING E3 ligases[27]. Overall, E3 ligases determine ubiquitination specificity and transfer ubiquitin to the target proteins[27].

GRIA3

The protein product encoded by *GRIA3* (Glutamate Ionotropic Receptor AMPA Type Subunit 3) is the GluA3 subunit of AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionate)-type ionotropic glutamate receptors (AMPARs)[28]. Overall, AMPARs are the predominant excitatory neurotransmitter receptors in mammalian brains and play a key role in hippocampal synaptic long-term potentiation and transmission[29]. Specifically, the GluA3 AMPAR subunit has been suggested to shape the synaptic transmission properties and activity-dependent plasticity of synapse end bulbs, the presynaptic region of neurons that releases neurotransmitters into the synaptic cleft[30]. Located on chromosome Xq25, alternative splicing at the *GRIA3* locus results in different isoforms of GluA3, which may cause their signal transduction properties to vary[31]. X-linked Intellectual Developmental Disorder is also known to be associated with GRIA3[32].

GRIN2A

GRIN2A (Glutamate Ionotropic Receptor NMDA Type Subunit 2A) is a particularly compelling schizophrenia risk gene as it has also been a significant GWAS hit for the disorder, as well as being implicated in eliciting clinical effects relating to the *N*-methyl-D-aspartate receptor (NMDAR)[12]. This gene encodes the 2A subunit of the NMDAR, which is a member of the glutamate-gated ion channel protein family alongside AMPARs and kainite receptors[12,33]. NMDARs are heterotetramers consisting of two glycine-binding GRIN1 and two glutamate-binding GRIN2 subunits, of which there are four variants (GRIN2A-GRIN2D)[33]. Mutations in each GRIN subunit gene have been shown to cause encephalopathies that tend to be initially diagnosed as epilepsy, autism, or schizophrenia[34]. Otherwise known as GluN2A, GRIN2A is the most abundant GRIN2 subunit in the central nervous system (CNS) and is thought to play a crucial role during postnatal brain development[35]. Functionally, NMDARs are characterised by calcium permeability and they mediate a component of excitatory synaptic transmission in the CNS[35]. *GRIN2A* mutations and polymorphisms have previously been implicated in a variety of developmental brain disorders, including epilepsy and mental retardation[35].

HERC1

The *HERC1* (HECT And RLD Domain Containing E3 Ubiquitin Protein Ligase Family Member 1) gene encodes another component of the UPS, specifically an E3 ubiquitin ligase. This member of the HERC protein family belongs to the HECT domain-containing class of E3s. In addition to its role in autophagy via the UPS, HERC1 has also been shown to be involved in membrane transport processes via guanine nucleotide exchange factor (GEF) activity and its ability to bind to clathrin[36]. Moreover, the HERC1 ubiquitin ligase regulates cellular proliferation and migration via the RAF/MEK/ERK signalling pathway

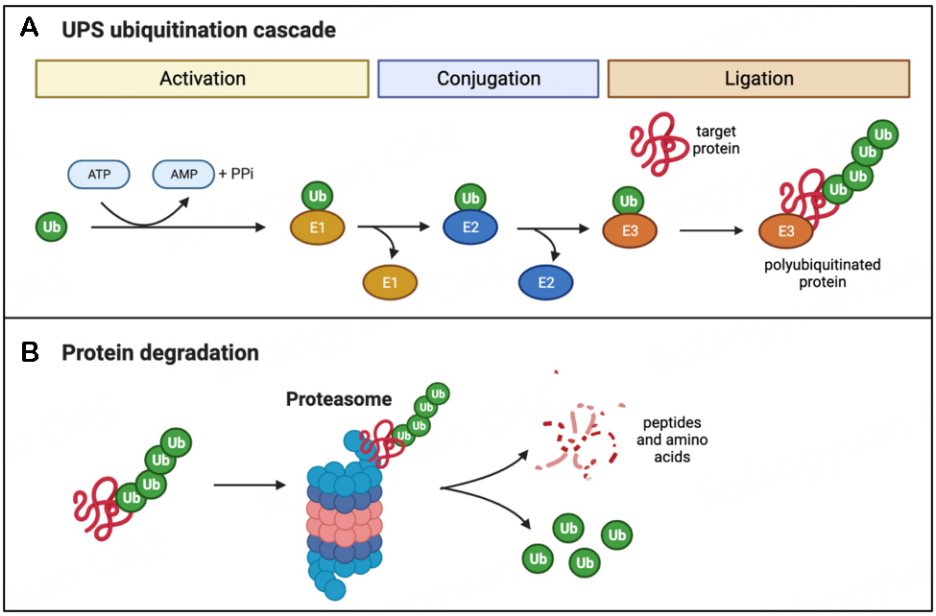

Figure 1. The cascade of reactions involved in the UPS. (A) The ubiquitination cascade involves the E1, E2, and E3 ubiquitin ligases and produces a polyubiquitinated end-product. (B) The polyubiquitinated protein is then located by the proteasome which degrades and deubiquitinates the protein. Created with BioRender.com.

and the RAF-dependent MKK3/p38 signalling pathways, respectively[37]. In humans, *HERC1* mutations are known to cause a variety of intellectual disability syndromes with or without cerebellar abnormalities[38]. Affected individuals are often diagnosed with macrocephaly, motor development delay, and epilepsy, in addition to intellectual disability[39].

RB1CC1

The protein encoded by *RB1CC1* (RB1 Inducible Coiled-Coil 1) is a DNA-binding transcription factor (TF), commonly referred to as FIP200[40]. It is responsible for activating the expression of the retinoblastoma 1 (*RB1*) gene by binding to the GC-rich region found upstream of the promoter[41]. Additionally, it interacts with various signalling pathways to regulate cellular processes in a coordinated fashion, including cell-cycle progression, differentiation, and senescence, as well as neural migration and neurodegeneration[42]. Overall, there is strong evidence supporting the role of RB1CC1 as a tumour suppressor as it enhances the expression of RB1, a gene in which mutational inactivation can lead to cancer[41].

SP4

SP4 (Specificity Protein 4 Transcription Factor) encodes a TF which is a member of the Sp/KLF family of TFs[43]. Similarly to RB1CC1, it recognises GC-rich sequences around the promoters of various genes and activates transcription; however, SP4 TFs are restrictively expressed in neuronal cells[43]. Common and rare variants in *SP4* have been elucidated as risk factors for schizophrenia, possibly through distinct pathological molecular mechanisms[44]. The *SP4* gene has been proposed to play a crucial role in hippocampal development and function and has also been associated with bipolar disorder in humans[45].

TRIO

TRIO (Trio Rho Guanine Nucleotide Exchange Factor) encodes a guanine nucleotide exchange factor (GEF), which facilitates the activation of Rho GTPases[46]. The TRIO RhoGEF protein regulates GEF-

dependent and Rho GTPase-powered cytoskeletal rearrangements and membrane trafficking, the two processes required for neurite growth and development[47]. Neurites are specific functional structures, which form when post-mitotic neurons differentiate into mature neurons in response to developmental signals[47]. Furthermore, TRIO serves a role in glutamatergic neurotransmission and long-term potentiation (LTP) alongside its paralog Kalirin[48]. As a form of synaptic plasticity, LTP strengthens glutamatergic synapses, which is postulated to be a mechanism underlying memory and learning[49]. *TRIO* LoF mutants have been shown to affect dendritic branching, axon guidance, and synaptic transmission[46,50]. A number of studies have implicated *TRIO* in autism spectrum disorder and intellectual disability[46].

XPO7

To date, *XPO7* (Exportin-7) is one of the least characterised genes of the exportin family genes, with relatively little being known about its function[51]. Like other exportins, XPO7 mediates the nuclear export of proteins with broad specificity and does so via GTP-dependent binding of cargo inside the nucleus[52]. The encoded protein is a RAN GTPase and thus is also referred to as RAN Binding Protein 16. In addition to facilitating cargo export, XPO7 also acts as a nuclear import receptor for proteins with nuclear localisation signals[52]. Recent studies have elucidated *XPO7* as a novel senescence regulator[53]. Given that senescence is a stress response that limits the replication capacity of old, damaged and preneoplastic cells, this gene's role in cancer and neoplastic formation is being increasingly researched[51].

AKAP11

AKAP11 (A-Kinase Anchoring Protein 11) encodes the AKAP11 protein, also known as AKAP220, which acts as a scaffolding protein[7]. This scaffolding protein binds to the regulatory subunits of the protein kinase A (PKA) holoenzyme, with the purpose of restricting PKA to discrete intracellular compartments[7,54]. This provides spatiotemporal regulation of PKA activity, as well as enabling substrate-specific targeting for phosphorylation and dephosphorylation[7]. Due to this characteristic, AKAP11 is also classified as an autophagy receptor, mediating PKA activation, which in turn regulates cellular metabolism[54]. Overall, the PKA protein is involved in a wide range of biological processes, one of which is neuronal plasticity, which has relevance for the investigation into neurodevelopmental disorders[12]. Nonetheless, the role of AKAP11 in the brain and its biological role in psychiatric diseases remain largely uncharacterized[12].

EXPLORING THE PUTATIVE ROLES OF IMPLICATED GENES IN MODEL SYSTEMS

Investigations of these 11 genes and the functional roles of the gene products throw light on a variety of molecular mechanisms that may contribute to the schizophrenia pathophysiology. Since the association with schizophrenia is due to variants that cause loss of function of these genes, relevant *in vitro* and *in vivo* models can be established involving either heterozygous or homozygous knockouts[12]. By exploring these newly established models and revisiting existing ones, further inferences about potentially implicated molecular pathways can be made. In particular, to date such investigations implicate chromatin modification, glutamatergic system dysregulation and ubiquitin-proteasome system (UPS) impairment as putative molecular mechanisms involved in the aetiology of schizophrenia.

GLUTAMATERGIC SYSTEM DYSREGULATION

The identification of *GRIN2A* and *SP4*, both of which encode protein products implicated in NMDAR formation and functionality, adds further weight to the evidence from previous studies suggesting that impairment of NMDAR functioning is associated with symptoms observed in schizophrenia. Acute administration of the NMDAR antagonists phencyclidine (PCP) or methamphetamine (METH) can induce schizophrenia-like symptoms in human controls and regressive symptoms in schizophrenia patients[12,43,55].

In wild-type rodents, repeated METH administration induces NMDAR dysfunction and leads to a reduction in *Grin2a* expression[56]. Likewise, anti-NMDAR autoantibody encephalitis can produce symptoms resembling those observed in schizophrenia[34]. These autoantibodies cause receptor internalisation from the plasma membrane of neurons, resulting in NMDAR hypofunction[34]. In experimental mouse models, both PCP and anti-NMDAR autoantibodies induce NMDAR hypofunction in excitatory neurons and elicit schizophrenia-like symptoms, including memory deficits[34].

The *Sp4* hypomorphic mouse is a further valuable model that aids our understanding of the putative NMDAR-specific hypoglutamatergic function underlying schizophrenia[43]. The mouse *Sp4* gene is expressed abundantly in the hippocampal CA1 region. Relative to wild-type mice, *Sp4* hypomorphic mice display impaired LTP in the hippocampal CA1 and impaired spatial learning and memory, which mimic cognitive deficits seen in schizophrenia[43]. Following the publication of the SCHEMA results and confirmation of *SP4* as a schizophrenia risk gene, further investigations into *Sp4* hypomorphic mice were conducted[44]. The hippocampal vacuolisation observed in these mice with reduced *Sp4* expression was found to be associated with the deficits in LTP as well as the observed sensorimotor gating, learning, and memory deficits[44,57].

GRIA3 codes for GluA3, an AMPAR subunit, and dysfunction of AMPARs has also been implicated in schizophrenia aetiology, although this has been less extensively investigated. Knockout models of this gene in mice have been established and studied, yielding results that show impairment in excitatory neurotransmission in the medial prefrontal cortex (mPFC) and reduced neuronal activity[58]. In mice, heterozygous deletion of *Gria3* was associated with aggressive behaviour, which could be alleviated by the injection of an adeno-associated virus vector expressing GluA3 into the mPFC[58].

CHROMATIN MODIFICATION

With the discovery of rare LOF variants in *SETD1A* conferring a large increase in risk for schizophrenia, epigenetic dysregulation gained attention as an important mechanism in the pathogenesis of the disorder[8]. The *Setd1a* haploinsufficiency mouse model (*Setd1a*$^{+/-}$) has been investigated extensively in order to throw light on the epigenetic mechanisms presumed to be involved in schizophrenia pathophysiology[59].

Setd1a$^{+/-}$ mice have been shown to have approximately a 50% decrease in Setd1a protein in frontal brain regions[60]. The effects of this haploinsufficiency in the mPFC and striatum of the *Setd1a*$^{+/-}$ mice were also investigated, yielding valuable results[59]. Single-cell RNA sequencing revealed that the H3K4me3-dependent transcriptional function of Setd1a differed between cell types and neuronal subtypes in these brain regions[59]. In the mPFC, the decrease in H3K4me3 correlated with a decrease in gene expression in excitatory neurons, particularly in *Foxp*$^{2+}$ neurons[59]. In these mPFC-localised neurons, morphological defects including dendrite morphology defects and exocytosis impairment have been observed[59-61]. In addition, schizophrenia-like behavioural phenotypes, including impaired sociality, sensorimotor gating defects, and deficits in working memory, were observed in these *Setd1a*$^{+/-}$ mice[59-61]. However, there are some discrepancies between the behavioural phenotypes observed in *Setd1a*$^{+/-}$ mice as part of the different studies that have been conducted, which may be attributable to differing genetic backgrounds or behaviour-testing protocols[59].

Investigations into the *Setd1a* haploinsufficiency mouse model have also revealed an involvement of Setd1a in excitatory synaptic plasticity. In the mPFC of *Setd1a*$^{+/-}$ mice, excitatory synaptic transmission was observed to be attenuated in layer 2 and 3 (L2/3) pyramidal neurons (PNs)[61]. While postsynaptic SETD1A is crucial for the functionality of excitatory synapses, the reduction of Setd1a in L2/3 PNs further caused a

reduction in the probability of glutamate release from presynaptic terminals[61]. Changes in the short-term plasticity of *Setd1a*$^{+/-}$ mice have been elucidated through observation of increases in short-term depression[17]. These observations suggest that the observed alteration of signalling processes and synaptic plasticity in these haploinsufficiency mouse models may be contributing factors to cognitive symptoms[17]. Overall, these findings relating to *SETD1A* suggest that schizophrenia risk may be mediated by abnormal synaptic development and function, arising as a result of aberrant epigenetic modifications[57].

UBIQUITIN-PROTEASOME SYSTEM IMPAIRMENT

The ubiquitin-proteasome system (UPS) plays a fundamental role in neuronal homeostasis via protein degradation[26]. Localised at synapses, the UPS has been implicated in synaptic development, maintenance, and plasticity. Given these implications, dysfunction of this degradation system has been related to various neurological disorders[26]. Components of the UPS are found to be mis-regulated in numerous diseases and E3 ligases may be of particular interest given their role in specifying ubiquitination targets for degradation[62]. The schizophrenia risk genes *HERC1, CUL1*, and *AKAP11* all have a role in the UPS.

The *tambaleante (tbl)* mutant mouse is an extensively characterised model organism that carries a spontaneous mutation in the HERC1 E3 ubiquitin ligase[26,63]. The *tbl* mouse was first described as a model of adult cerebellar ataxia, which is caused by Purkinje neuron death in the cerebellum[38]. NMDARs and GABA neurotransmitters are essential in Purkinje neuron development[21]. Cerebellum-related spatial learning alterations are observed in *tbl* mutant mice, as well as impairments in hippocampal learning and memory[38]. In addition to having a profound impact on the growth and maintenance of the cerebellar and hippocampal structures, *Herc1* has also been implicated in the amygdala through *tbl* mouse studies[63]. The amygdala plays a crucial role in associative learning, which is impaired in *tbl* mice[63]. These learning deficits are associated with a lower density of glutamatergic synaptic vesicles in the lateral amygdala and hippocampus of *tbl* mice[39,63]. These glutamatergic synapses are a component of dendritic spines. E3 ligases of the UPS have previously been implicated in the maintenance of the size and density of such spines on neurons of the lateral amygdala[63]. These results collectively suggest that HERC1 E3 ubiquitin ligase may play a role in the regulation of postsynaptic dendritic spinogenesis and the maintenance of homeostasis within presynaptic terminals[63].

As noted previously, CUL1 is an invariable component of the SCF E3 ubiquitin ligases. Few genetic models exist that have specifically investigated *CUL1*, but models exploring overall SCF complex alterations are available. It has been shown in mice that engineered ubiquitin variants that target the *Cul1* binding site work to inhibit SCF ubiquitin ligases[62]. However, no investigation into behavioural phenotypes or brain-related morphological and functional defects was undertaken in this study. Total knockout of F-box protein *Fbxw7* in mice induces alterations of the overall SCF complex, manifesting in vascular development abnormalities in the brain and ultimately causing embryonic lethality[25]. FBXW7 is a well-characterised variant of the F-box protein, the variable component of the SCF complex, and has been implicated in neurodevelopment and neurodegeneration[27]. Despite the current lack of brain-specific investigations focussed on *CUL1*, results from the existing studies suggest that alterations in the SCF complex may have implications for neurodevelopment and neurological functions.

A recent study investigating FBXW7 implicated AKAP11 as a target of SCF complex-mediated degradation. The FBXW7 component of the SCF complex binds to target substrates via a phosphodegron consensus motif and is found on proteins targeted by the UPS for proteasomal degradation[25]. Such FBXW7-binding phosphodegrons were located on AKAP11 proteins, suggesting that the SCF complex E3 ligase may be involved in maintaining levels of this neuronal synapse plasticity regulator[12,64].

DISCUSSION

The use of WES has led to the initial identification of 11 genes in which very rare PTVs confer substantially increased risk of schizophrenia: *SETD1A, CACNA1G, CUL1, GRIA3, GRIN2A, HERC1, RB1CC1, SP4, TRIO, XPO7*, and *AKAP11*[7-9]. The exploration of model systems targeting some of these genes suggests putative convergent neurobiological and molecular mechanisms that seem to be impaired in schizophrenia[12]. A role for glutamatergic system dysregulation is supported by the association with schizophrenia of PTVs in *GRIA3, GRIN2A*, and *SP4* and is augmented by studies of NMDAR antagonists, NMDAR autoantibodies, *Sp4* hypomorphic mouse models, and *Gria3* knockout models. While considerable research in this area of schizophrenia pathophysiology has been carried out, the majority of animal studies investigating NMDAR antagonists have specifically focussed on effects in adulthood[56]. Further studies could be conducted that allow for the examination of behavioural changes and neural circuit impacts throughout early developmental stages[56]. Disruption of chromatin modification by *Setd1a* dysfunction has effects on neuronal structure and function, again including glutamatergic neurons, among others. The involvement of *HERC1*, *CUL1*, and *AKAP11* suggests that impairment of the UPS could also be a component of schizophrenia pathogenesis, and in particular, the impairment of E3 ligases encoded by *HERC1* and *CUL1* seems to elicit dysfunction in this degradation mechanism[62,63]. Interestingly, predating the identification of these genes, a number of studies had suggested that there might be abnormalities of the UPS associated with schizophrenia, and these findings were recently reviewed, although the authors concluded that it was difficult to determine whether such abnormalities were a cause or a consequence of the illness[65]. Previous GWAS results have highlighted glutamatergic genes and the UPS but not particularly chromatin modification[4].

Our current knowledge of the biological mechanisms that are affected by these schizophrenia risk genes has been largely limited to animal models and the translational applicability of these to humans may be limited[17]. Future studies could be conducted as multi-scale studies, which maintain investigations into animal models while incorporating disease modelling based on human induced pluripotent stem cells (iPSCs)[66]. Such multi-scale studies have the potential to facilitate clarification of the molecular pathology, enable cross-species verification, and initiate the development of clinically valid biomarkers with translational potential to patients[66].

For a number of the implicated genes, little is understood about their role to date. This situation is certain to change as further functional studies are carried out, focussing on phenotypes that seem especially relevant to schizophrenia. As we gain more knowledge, it seems possible that we may be able to develop a more coherent understanding of the mechanisms that can be involved in schizophrenia pathogenesis, hopefully condensing around a relatively small number of key processes.

CONCLUSION

WES studies have proven to be of significant value in revealing schizophrenia risk genes, with future studies likely to contribute further to our understanding of the molecular mechanisms that may be involved in schizophrenia pathophysiology. Elucidating key processes that are disordered in schizophrenia holds the potential to lead to improvements in treatment and a shift towards targeting underlying biological causes.

DECLARATIONS

Authors' contributions

Carried out the literature searches and drafted the main manuscript: Heinzer L
Assisted in the writing process: Curtis D

Availability of data and materials
Not applicable.

Financial support and sponsorship
None.

Conflicts of interest
All authors declared that there are no conflicts of interest.

Ethical approval
Ethical approval was obtained by the researchers carrying out the cited studies.

Consent for publication and copyright
Not applicable.

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REFERENCES
1. Patel KR, Cherian J, Gohil K, Atkinson D. Schizophrenia: overview and treatment options. *Pharm Ther* 2014;39:638-45. PMC
2. McCutcheon RA, Reis Marques T, Howes OD. Schizophrenia-an overview. *JAMA Psychiatry* 2020;77:201-10. DOI PubMed
3. Kanwal A, Pardo JV, Naz S. RGS3 and IL1RAPL1 missense variants implicate defective neurotransmission in early-onset inherited schizophrenias. *J Psychiatry Neurosci* 2022;47:E379-90. DOI PubMed PMC
4. Trubetskoy V, Pardiñas AF, Qi T, et al. Mapping genomic loci implicates genes and synaptic biology in schizophrenia. *Nature* 2022;604:502-8. DOI
5. Henriksen MG, Nordgaard J, Jansson LB. Genetics of Schizophrenia: overview of methods, findings and limitations. *Front Hum Neurosci* 2017;11:322. DOI PubMed PMC
6. Marshall CR, Howrigan DP, Merico D, et al. Contribution of copy number variants to schizophrenia from a genome-wide study of 41,321 subjects. *Nat Genet* 2017;49:27-35. DOI
7. Palmer DS, Howrigan DP, Chapman SB, et al. Exome sequencing in bipolar disorder identifies AKAP11 as a risk gene shared with schizophrenia. *Nat Genet* 2022;54:541-7. DOI
8. Singh T, Kurki MI, Curtis D, et al; Swedish Schizophrenia Study; INTERVAL Study; DDD Study; UK10 K Consortium. Rare loss-of-function variants in SETD1A are associated with schizophrenia and developmental disorders. *Nat Neurosci* 2016;19:571-7. DOI PubMed PMC
9. Singh T, Poterba T, Curtis D, et al. Rare coding variants in ten genes confer substantial risk for schizophrenia. *Nature* 2022;604:509-16. DOI PubMed PMC
10. Liu D, Meyer D, Fennessy B, et al; Psychiatric Genomics Consortium Phase 3 Targeted Sequencing of Schizophrenia Study Team. Schizophrenia risk conferred by rare protein-truncating variants is conserved across diverse human populations. *Nat Genet* 2023;55:369-76. DOI
11. Curtis D. Clinical features of UK Biobank subjects carrying protein-truncating variants in genes implicated in schizophrenia pathogenesis. *Psychiatr Genet* 2022;32:156-61. DOI PubMed
12. Herzog LE, Wang L, Yu E, et al. Mouse mutants in schizophrenia risk genes GRIN2A and AKAP11 show EEG abnormalities in common with schizophrenia patients. *Transl Psychiatry* 2023;13:92. DOI PubMed PMC
13. Mukai J, Cannavò E, Crabtree GW, et al. Recapitulation and reversal of schizophrenia-related phenotypes in setd1a-deficient mice. *Neuron* 2019;104:471-87.e12. DOI PubMed PMC
14. Chong ZS, Khong ZJ, Tay SH, Ng SY. Metabolic contributions to neuronal deficits caused by genomic disruption of schizophrenia risk gene SETD1A. *Schizophrenia* 2022;8:115. DOI PubMed PMC
15. Kusch T. Histone H3 lysine 4 methylation revisited. *Transcription* 2012;3:310-4. DOI PubMed PMC
16. Lee JH, Skalnik DG. Wdr82 is a C-terminal domain-binding protein that recruits the Setd1A Histone H3-Lys4 methyltransferase complex to transcription start sites of transcribed human genes. *Mol Cell Biol* 2008;28:609-18. DOI PubMed PMC
17. Wang S, Bleeck A, Nadif Kasri N, Kleefstra T, van Rhijn JR, Schubert D. SETD1A mediated H3K4 methylation and its role in neurodevelopmental and neuropsychiatric disorders. *Front Mol Neurosci* 2021;14:772000. DOI PubMed PMC
18. Barresi S, Dentici ML, Manzoni F, et al. Infantile-onset syndromic cerebellar ataxia and CACNA1G mutations. *Pediatr Neurol* 2020;104:40-5. DOI
19. Calcium channel, voltage-dependent, T type, alpha-1G subunit; CACNA1G. Available from: https://www.omim.org/entry/604065

[Last accessed on 4 Jan 2024].

20. Chemin J, Siquier-Pernet K, Nicouleau M, et al. De novo mutation screening in childhood-onset cerebellar atrophy identifies gain-of-function mutations in the CACNA1G calcium channel gene. *Brain* 2018;141:1998-2013. DOI
21. Paul MS, Limaiem F. Histology, purkinje cells. Available from: https://www.ncbi.nlm.nih.gov/books/NBK545154/ [Last accessed on 4 Jan 2024].
22. Huang YF, Zhang Z, Zhang M, et al. CUL1 promotes breast cancer metastasis through regulating EZH2-induced the autocrine expression of the cytokines CXCL8 and IL11. *Cell Death Dis* 2018;10:2. DOI PubMed PMC
23. Chen G, Li G. Increased Cul1 expression promotes melanoma cell proliferation through regulating p27 expression. *Int J Oncol* 2010;37:1339-44. DOI
24. Michail O, Moris D, Theocharis S, Griniatsos J. Cullin-1 and -2 protein expression in colorectal cancer: correlation with clinicopathological variables. *In Vivo* 2018;32:391-6. DOI PubMed PMC
25. Zhou W, Wei W, Sun Y. Genetically engineered mouse models for functional studies of SKP1-CUL1-F-box-protein (SCF) E3 ubiquitin ligases. *Cell Res* 2013;23:599-619. DOI PubMed PMC
26. Bachiller S, Rybkina T, Porras-García E, et al. The HERC1 E3 ubiquitin ligase is essential for normal development and for neurotransmission at the mouse neuromuscular junction. *Cell Mol Life Sci* 2015;72:2961-71. DOI
27. Yang Y, Zhou X, Liu X, Song R, Gao Y, Wang S. Implications of FBXW7 in neurodevelopment and neurodegeneration: molecular mechanisms and therapeutic potential. *Front Cell Neurosci* 2021;15:736008. DOI PubMed PMC
28. Davies B, Brown LA, Cais O, et al; WGS500 Consortium. A point mutation in the ion conduction pore of AMPA receptor GRIA3 causes dramatically perturbed sleep patterns as well as intellectual disability. *Hum Mol Genet* 2017;26:3869-82. DOI PubMed PMC
29. Sumi T, Harada K. Mechanism underlying hippocampal long-term potentiation and depression based on competition between endocytosis and exocytosis of AMPA receptors. *Sci Rep* 2020;10:14711. DOI PubMed PMC
30. Antunes FM, Rubio ME, Kandler K. Role of GluA3 AMPA receptor subunits in the presynaptic and postsynaptic maturation of synaptic transmission and plasticity of endbulb-bushy cell synapses in the cochlear nucleus. *J Neurosci* 2020;40:2471. DOI PubMed PMC
31. Park YH, Broyles HV, He S, McGrady NR, Li L, Yorio T. Involvement of AMPA receptor and its flip and flop isoforms in retinal ganglion cell death following oxygen/glucose deprivation. *Invest Ophthalmol Vis Sci* 2016;57:508-26. DOI PubMed
32. Trivisano M, Santarone ME, Micalizzi A, et al. GRIA3 missense mutation is cause of an X-linked developmental and epileptic encephalopathy. *Seizure* 2020;82:1-6. DOI
33. Myers SJ, Yuan H, Kang JQ, Tan FCK, Traynelis SF, Low CM. Distinct roles of GRIN2A and GRIN2B variants in neurological conditions. *F1000Res* 2019;8:1940. DOI PubMed PMC
34. Nakazawa K, Sapkota K. The origin of NMDA receptor hypofunction in schizophrenia. *Pharmacol Ther* 2020;205:107426. DOI PubMed PMC
35. Poltavskaya EG, Fedorenko OY, Kornetova EG, et al. Study of early onset schizophrenia: associations of GRIN2A and GRIN2B polymorphisms. *Life* 2021;11:997. DOI PubMed PMC
36. Garcia-Gonzalo FR, Bartrons R, Ventura F, Rosa JL. Requirement of phosphatidylinositol-4,5-bisphosphate for HERC1-mediated guanine nucleotide release from ARF proteins. *FEBS Lett* 2005;579:343-8. DOI PubMed
37. Pedrazza L, Schneider T, Bartrons R, Ventura F, Rosa JL. The ubiquitin ligase HERC1 regulates cell migration via RAF-dependent regulation of MKK3/p38 signaling. *Sci Rep* 2020;10:824. DOI PubMed PMC
38. Pérez-Villegas EM, Pérez-Rodríguez M, Negrete-Díaz JV, et al. HERC1 ubiquitin ligase is required for hippocampal learning and memory. *Front Neuroanat* 2020;14:592797. DOI PubMed PMC
39. Lalonde R, Strazielle C. The Herc1 gene in neurobiology. *Gene* 2022;814:146144. DOI PubMed
40. Wang C, Liang CC, Bian ZC, Zhu Y, Guan JL. FIP200 is required for maintenance and differentiation of postnatal neural stem cells. *Nat Neurosci* 2013;16:532-42. DOI PubMed PMC
41. Ikebuchi K, Chano T, Ochi Y, et al. RB1CC1 activates the promoter and expression of RB1 in human cancer. *Int J Cancer* 2009;125:861-7. DOI
42. Guo A, Lun P, Chen J, et al. Association analysis of risk genes identified by SCHEMA with schizophrenia in the Chinese Han population. *Psychiatr Genet* 2022;32:188-93. DOI
43. Zhou X, Nie Z, Roberts A, et al. Reduced NMDAR1 expression in the Sp4 hypomorphic mouse may contribute to endophenotypes of human psychiatric disorders. *Hum Mol Genet* 2010;19:3797-805. DOI PubMed PMC
44. Zhang Q, Huo JH, Guo L, Wang L, Wang C, Li M. Common and rare variants within SP4 exert distinct molecular mechanisms contributing to the risk of schizophrenia. *Psychiatry Res* 2022;318:114948. DOI
45. Zhou X, Tang W, Greenwood TA, et al. Transcription factor SP4 is a susceptibility gene for bipolar disorder. *PLoS One* 2009;4:e5196. DOI PubMed PMC
46. Wei C, Sun M, Sun X, et al. RhoGEF trio regulates radial migration of projection neurons via its distinct domains. *Neurosci Bull* 2022;38:249-62. DOI PubMed PMC
47. Tao T, Sun J, Peng Y, et al. Golgi-resident TRIO regulates membrane trafficking during neurite outgrowth. *J Biol Chem* 2019;294:10954-68. DOI PubMed PMC
48. Sadybekov A, Tian C, Arnesano C, Katritch V, Herring BE. An autism spectrum disorder-related de novo mutation hotspot discovered in the GEF1 domain of Trio. *Nat Commun* 2017;8:601. DOI PubMed PMC

49. Herring BE, Nicoll RA. Kalirin and Trio proteins serve critical roles in excitatory synaptic transmission and LTP. *Proc Natl Acad Sci USA* 2016;113:2264-9. DOI PubMed PMC
50. Ba W, Yan Y, Reijnders MR, et al. TRIO loss of function is associated with mild intellectual disability and affects dendritic branching and synapse function. *Hum Mol Genet* 2016;25:892-902. DOI PubMed PMC
51. Modepalli S, Martinez-Morilla S, Venkatesan S, et al. An in vivo model for elucidating the role of an erythroid-specific isoform of nuclear export protein exportin 7 (Xpo7) in murine erythropoiesis. *Exp Hematol* 2022;114:22-32. DOI PubMed PMC
52. Aksu M, Pleiner T, Karaca S, et al. Xpo7 is a broad-spectrum exportin and a nuclear import receptor. *J Cell Biol* 2018;217:2329-40. DOI PubMed PMC
53. Innes AJ, Sun B, Wagner V, et al. XPO7 is a tumor suppressor regulating p21(CIP1)-dependent senescence. *Genes Dev* 2021;35:379-91. DOI PubMed PMC
54. Deng Z, Li X, Blanca Ramirez M, et al. Selective autophagy of AKAP11 activates cAMP/PKA to fuel mitochondrial metabolism and tumor cell growth. *Proc Natl Acad Sci USA* 2021;118:e2020215118. DOI PubMed PMC
55. Veleanu M, Urrieta-Chávez B, Sigoillot SM, et al. Modified climbing fiber/Purkinje cell synaptic connectivity in the cerebellum of the neonatal phencyclidine model of schizophrenia. *Proc Natl Acad Sci USA* 2022;119:e2122544119. DOI PubMed PMC
56. Oka M, Ito K, Koga M, Kusumi I. Changes in subunit composition of NMDA receptors in animal models of schizophrenia by repeated administration of methamphetamine. *Prog Neuropsychopharmacol Biol Psychiatry* 2020;103:109984. DOI PubMed
57. Farsi Z, Sheng M. Molecular mechanisms of schizophrenia: insights from human genetics. *Curr Opin Neurobiol* 2023;81:102731. DOI PubMed
58. Peng SX, Pei J, Rinaldi B, et al. Dysfunction of AMPA receptor GluA3 is associated with aggressive behavior in human. *Mol Psychiatry* 2022;27:4092-102. DOI
59. Chen R, Liu Y, Djekidel MN, et al. Cell type-specific mechanism of Setd1a heterozygosity in schizophrenia pathogenesis. *Sci Adv* 2022;8:1077. DOI PubMed PMC
60. Clifton NE, Bosworth ML, Haan N, et al. Developmental disruption to the cortical transcriptome and synaptosome in a model of SETD1A loss-of-function. *Hum Mol Genet* 2022;31:3095-106. DOI PubMed PMC
61. Nagahama K, Sakoori K, Watanabe T, et al. Setd1a insufficiency in mice attenuates excitatory synaptic function and recapitulates schizophrenia-related behavioral abnormalities. *Cell Rep* 2020;32:108126. DOI
62. Gorelik M, Orlicky S, Sartori MA, et al. Inhibition of SCF ubiquitin ligases by engineered ubiquitin variants that target the Cul1 binding site on the Skp1-F-box interface. *Proc Natl Acad Sci USA* 2016;113:3527-32. DOI PubMed PMC
63. Pérez-Villegas EM, Negrete-Díaz JV, Porras-García ME, et al. Mutation of the HERC 1 ubiquitin ligase impairs associative learning in the lateral amygdala. *Mol Neurobiol* 2018;55:1157-68. DOI
64. Singh N, Zeke A, Reményi A. Systematic discovery of FBXW7-binding phosphodegrons highlights mitogen-activated protein kinases as important regulators of intracellular protein levels. *Int J Mol Sci* 2022;23:3320. DOI PubMed PMC
65. Luza S, Opazo CM, Bousman CA, Pantelis C, Bush AI, Everall IP. The ubiquitin proteasome system and schizophrenia. *Lancet Psychiatry* 2020;7:528-37. DOI PubMed
66. Nakazawa T. Modeling schizophrenia with iPS cell technology and disease mouse models. *Neurosci Res* 2022;175:46-52. DOI PubMed