Molecular Psychiatry (2022) 27:95–112
https://doi.org/10.1038/s41380-021-01037-w

PERSPECTIVE

An alternative splicing hypothesis for neuropathology of schizophrenia: evidence from studies on historical candidate genes and multi-omics data

Chu-Yi Zhang [1,2] · Xiao Xiao [1,3] · Zhuohua Zhang [4,5] · Zhonghua Hu [4,5,6,7,8,9,10] · Ming Li [1,2,3]

Received: 4 October 2020 / Revised: 8 January 2021 / Accepted: 22 January 2021 / Published online: 8 March 2021

Abstract

Alternative splicing of schizophrenia risk genes, such as *DRD2*, *GRM3*, and *DISC1*, has been extensively described. Nevertheless, the alternative splicing characteristics of the growing number of schizophrenia risk genes identified through genetic analyses remain relatively opaque. Recently, transcriptomic analyses in human brains based on short-read RNA-sequencing have discovered many "local splicing" events (e.g., exon skipping junctions) associated with genetic risk of schizophrenia, and further molecular characterizations have identified novel spliced isoforms, such as $AS3MT^{d2d3}$ and $ZNF804A^{E3E4}$. In addition, long-read sequencing analyses of schizophrenia risk genes (e.g., *CACNA1C* and *NRXN1*) have revealed multiple previously unannotated brain-abundant isoforms with therapeutic potentials, and functional analyses of *KCNH2*-3.1 and *Ube3a1* have provided examples for investigating such spliced isoforms in vitro and in vivo. These findings suggest that alternative splicing may be an essential molecular mechanism underlying genetic risk of schizophrenia, however, the incomplete annotations of human brain transcriptomes might have limited our understanding of schizophrenia pathogenesis, and further efforts to elucidate these transcriptional characteristics are urgently needed to gain insights into the illness-correlated brain physiology and pathology as well as to translate genetic discoveries into novel therapeutic targets.

Alternative splicing is an essential molecular mechanism underlying risk of schizophrenia

Schizophrenia is a severe disabling mental illness with a global lifetime prevalence of ~1% [1]. Accumulating studies have indicated a strong genetic component in schizophrenia pathogenesis [2], and genetic analyses including genome-wide association studies (GWASs) have reported multiple single-nucleotide polymorphisms (SNPs) associated with this illness [3–5]. There is a growing consensus

✉ Zhonghua Hu
huzhonghua@csu.edu.cn

✉ Ming Li
limingkiz@mail.kiz.ac.cn

1 Key Laboratory of Animal Models and Human Disease Mechanisms of the Chinese Academy of Sciences and Yunnan Province, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

2 Kunming College of Life Science, University of Chinese Academy of Sciences, Kunming, Yunnan, China

3 KIZ-CUHK Joint Laboratory of Bioresources and Molecular Research in Common Diseases, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, China

4 Institute of Molecular Precision Medicine and Hunan Key Laboratory of Molecular Precision Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China

5 Center for Medical Genetics and Hunan Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan, China

6 Department of Critical Care Medicine, Xiangya Hospital, Central South University, Changsha, Hunan, China

7 National Clinical Research Center for Geriatric Disorders, Xiangya Hospital, Central South University, Changsha, Hunan, China

8 Hunan Key Laboratory of Animal Models for Human Diseases, School of Life Sciences, Central South University, Changsha, Hunan, China

9 Eye Center of Xiangya Hospital and Hunan Key Laboratory of Ophthalmology, Central South University, Changsha, Hunan, China

10 National Clinical Research Center on Mental Disorders, Changsha, Hunan, China

that genetic risk of schizophrenia tends to affect mRNA expression in human brains [6–10]. During the past few years, mRNA expression analyses using multiple approaches (such as real-time quantitative PCR, microarray or RNA-sequencing (RNA-seq)) have identified many dysregulated genes associated with schizophrenia. While the functional outcomes of altered mRNA expression related to schizophrenia pathology are being investigated, the impact of the much more complicated regulatory network of RNA processing during transcription has drawn growing attention, and the role of alternative splicing in schizophrenia has been explored [11–13]. In the human genome, majority of the multi-exon genes are alternatively spliced during transcription [14], resulting in cassette exons, microexons, intron retention, alternative 5′ and 3′ splice sites, alternative promoters, and alternative untranslated regions (UTRs) (Fig. 1) [15], and thereby producing diverse transcriptomes, proteomes, and phenomes [16]. In this perspective, we mainly discuss the contributions of alternative splicing to neuropathology of schizophrenia, highlighting potential mechanisms by which miss-splicing events related to schizophrenia genetic risk facilitate its pathogenesis.

Alternative splicing in brain is highly complex [17], suggesting that essential roles of appropriate RNA splicing in myriad neuronal development and functions [14], and aberrant splicing of particular genes may underlie the pathogenesis of brain disorders [18]. For example, Gomafu, a long noncoding RNA whose expression is decreased in postmortem brains of schizophrenia patients, acts as a scaffold for splicing factors such as serine/arginine-rich SF 1, and knockdown of Gomafu increases the expression of schizophrenia-associated isoforms of *ErbB4* and *DISC1* [19]. Similarly, compared with longer exons, alternative microexons (i.e., a class of exons comprising 3–27 nucleotides, are strongly conserved and usually frame-preserving [20]) are preferentially brain-enriched and regulate neuronal differentiation [21, 22], and Ganda et al. found significant enrichment of switched isoforms with microexons in schizophrenia patients [23]. In addition, cumulative studies have also reported dysregulated mRNA levels of alternatively spliced isoforms of schizophrenia risk genes in brains of patients, which will be described in the following sections.

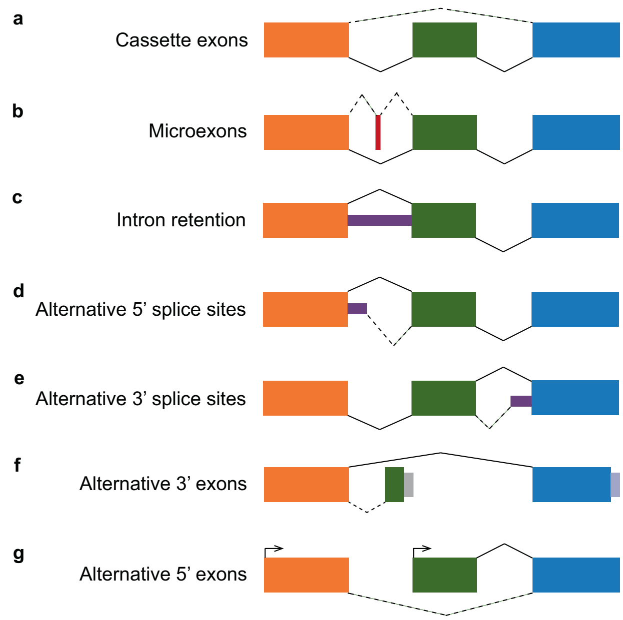


Fig. 1 Alternative splicing patterns. The solid and dash V-shaped lines represent two distinct splicing options, respectively. **a** Cassette exons: the most common pattern of alternative splicing is the inclusion or exclusion of a cassette exon in the mRNA. **b** Microexons: a special type of cassette exon with 3–27 nucleotides shows enrichment in neuron-specific transcripts. **c** Intron retention: the omission of intron exclusion leaves the retained intronic sequence (shown as purple block) in mature mRNA transcript. **d, e** Alternative 5′/3′ splice sites: through selecting different combinations of the 5′ (donor)/3′ (acceptor) splice sites, exons can be extended or shortened in length. **f, g** Alternative 5′/3′ exons: different transcriptional initiation or termination sites generate alternative 5′-terminal exons or 3′-terminal exons with alternative polyadenylation sites (shown as gray blocks).

Alternative splicing promotes understanding of schizophrenia pathogenesis

Neurotransmitter dysfunction (including dopamine, glutamate, and γ-aminobutyric acid (GABA) systems) and neurodevelopmental disturbances are central hypotheses of schizophrenia pathology [24]. However, many questions still remain regarding how genetic risks contribute to these hypothesized models. Intriguingly, several essential genes involving in these schizophrenia hypotheses are alternatively spliced (Table 1), producing different isoforms with distinct functions, which may in part explain the disease pathogenesis. We herein briefly discuss the alternative splicing patterns of several genes in human brains, including *DRD2* (encoding dopamine 2 receptor), *GRM3* (encoding metabotropic glutamate receptor 3 (mGluR3)), *GAD1* (encoding glutamic acid decarboxylase), *DISC1* (encoding the disrupted in schizophrenia 1 scaffold protein), *NRG1* (encoding neuregulin 1), and *ErbB4* (encoding erb-b2 receptor tyrosine kinase 4).

Dopamine hypothesis of schizophrenia

The dopamine hypothesis of schizophrenia is originated from the observations that neuroleptic drugs (e.g., chlorpromazine) could block brain dopamine receptor dates back to the ~1960s [25–27]. Until now, many major antipsychotic drugs are still designed to antagonize dopamine 2 receptor and are proven effective in alleviating positive

Table 1 Alternative spliced isoforms in the schizophrenia risk genes.

Gene	Splicing type	Diagnostic association			Genotypic association					
		Primary isoforms	Brain region	Direction (in SZ)	Primary isoforms	Brain region	Risk SNP	Location	Allele[a]	Direction (with risk allele)
DRD2	Full length	D2LR	DLPFC	↓ [32]	D2LR	–				
						–				
	Exon 6 skipping	D2SR	DLPFC	↑ [32]	D2SR	DLPFC	rs1076560	Intron 6	G/**T**	↓ [33]
			Hippocampus	↓ [32]		Striatum	rs1076560	Intron 6	G/**T**	↓ [33]
GAD1	Full length	GAD67	DLPFC	↓ [63]	GAD67	DLPFC	rs3749034	5′ UTR	A/**G**	↓ [151]
			Hippocampus	↓ [63]		Hippocampus	rs3749034	5′ UTR	A/**G**	↓ [151]
	Alternative 3′ exon	GAD25	–		GAD25	DLPFC	rs3749034	5′ UTR	A/**G**	↑ [63]
			–			Hippocampus	rs3749034	5′ UTR	A/**G**	↑ [64]
GRM3	Full length	mGluR3	DLPFC	↑ [50]	mGluR3	–				
	Exon 4 skipping	mGluR3Δ4	–		mGluR3Δ4	DLPFC	rs2228595	Exon 3	C/**T**	↑ [50]
ZDHHC8	Full length	ZDHHC8	–		ZDHHC8	–				
	Intron 4 retention	ZDHHC8-trucated	–		ZDHHC8-trucated	Fetal brain	rs175174	Intron 4	G/**A**	↑ [152]
NRXN1	Alternative promoter	NRXN1-α	–		NRXN1-α	–				
		NRXN1-β	DLPFC	↑ [129]	NRXN1-β	–				
NRG1	Alternative 5′ exon Alternative 3′ exon Exon skipping	NRG1-I type	DLPFC	↑ [89]	NRG1-I type	Hippocampus	SNP8NRG221132	Promoter	G/**A**	↑ [90]
			Hippocampus	↑ [90]						
		NRG1-IV type	–		NRG1-IV type	Hippocampus	rs6994992	Promoter	C/**T**	↑ [90]
						DLPFC	rs6994992	Promoter	C/**T**	↑ [90]
		NRG1-III type	–		NRG1-III type	Hippocampus	rs7014762	Promoter	A/**T**	↓ [92]
						DLPFC	SNP8NRG221533	Intergenic	T/**C**	↑ [93]
							SNP8NRG241930	Intergenic	G/**T**	↑ [93]
		NRG1-II/V/ VI type	–		NRG1-II/V/ VI type	–				
NRG3	Alternative 5′ exon Exon skipping	NRG3-I/IV type	DLPFC	↑ [153]	NRG3-I/IV type	–				
		NRG3-II/III type	–		NRG3-II/III type	DLPFC	rs10748842	Intron 1	C/**T**	↑ [153, 154]
ErbB4	Exon 16 inclusion	JM-a	DLPFC	↑ [99, 100]	JM-a	DLPFC	rs4673628	Intron 12	G/**A**	↑ [100]
			PFC layer 4	↑ [99]		Hippocampus	rs4673628	Intron 12	G/**A**	↑ [100]
	Exon 15b inclusion	JM-b	DLPFC	↓ [99]	JM-b	–				
			PFC layer 4	↓ [99]						
	Exon 26 inclusion	CYT-1	DLPFC	↑ [100, 155]	CYT-1	Hippocampus	rs7598440	Intron 3	G/**A**	↑ [100]
			PFC layer 4	↑ [99]		DLPFC	rs7598440	Intron 3	G/**A**	↑ [100]
	Exon 26 skipping	CYT-2	DLPFC	↓ [99]	CYT-2	–				

Table 1 (continued)

Gene	Splicing type	Diagnostic association			Genotypic association					
		Primary isoforms	Brain region	Direction (in SZ)	Primary isoforms	Brain region	Risk SNP	Location	Allele[a]	Direction (with risk allele)
DISC1	Exon 3 skipping	DISC1-Δ3	Hippocampus	↑ [76]	DISC1-Δ3	Hippocampus	rs821597	Intron 10	G/**A**	↑ [76]
	Exon 7,8 skipping	DISC1-Δ7Δ8	Hippocampus	↑ [76]	DISC1-Δ7Δ8	Hippocampus	rs6675281	Exon 9	C/**T**	↑ [76]
							rs821616	Exon 11	A/**T**	↑ [76]
	Alternative 3′ exon	DISC1-Esv1	Hippocampus	↑ [76]	DISC1-Esv1	–				
TrkB	Full length	TrkB-TK+	DLPFC	↓ [156, 157]	TrkB-TK+	–				
			Hippocampus	↓ [158]		–				
	Alternative 3′ exon	TrkB-TK−	DLPFC	↑ [156]	TrkB-TK−	–				
	Alternative 3′ exon	TrkB-Shc	DLPFC	↑ [156]	TrkB-Shc	–				
BDNF	Alternative 5′ exon	BDNF I–IX	Hippocampus	↓ [159]	BDNF I–IX	–				
		BDNF II–IX	DLPFC	↓ [159, 160]	BDNF II–IX	–				
			Parietal cortex	↓ [160]		–				
			Hippocampus	↓ [159]		–				
		BDNF IV–IX	Parietal cortex	↓ [160]	BDNF IV–IX	–				
		BDNF VI–IX	Hippocampus	↓ [159, 160]	BDNF VI–IX	–				
PPP1R1B	Full length	DARPP-32	–		DARPP-32	–				
	Alternative 5′ exon	t-DARPP-32	DLPFC	↑ [161]	t-DARPP-32	DLPFC	rs907094	Intron 5	A/**G**	↑ [161]
							rs879606	Intergenic	G/**A**	↑ [161]

SZ schizophrenia, *SNP* single-nucleotide polymorphism, *DLPFC* dorsolateral prefrontal cortex.

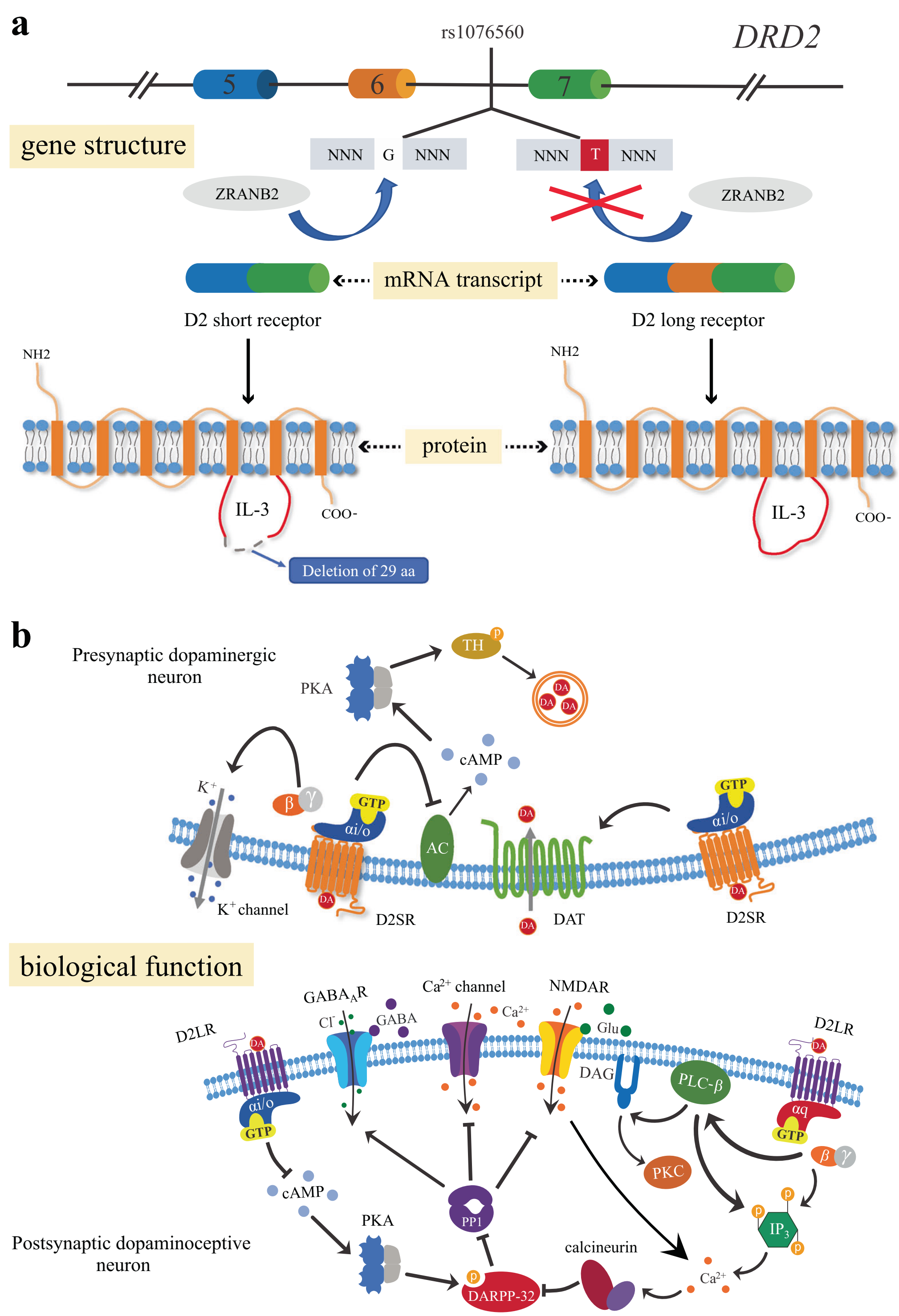
[a]The reported schizophrenia risk alleles in previous studies were marked in bold.

symptoms among schizophrenia patients. The dopamine 2 receptor is a central molecule of dopamine signaling involved in schizophrenia and a major antipsychotic drug target [28, 29], and SNPs spanning *DRD2* showed genome-wide associations with the illness [3]. The *DRD2* gene is transcribed primarily into two isoforms, D2-short receptor (D2SR, skipping exon 6) and D2-long receptor (D2LR, inclusion of exon 6) [30]. D2SR encodes a presynaptic receptor of dopamine, whereas the protein encoded by D2LR mainly mediates postsynaptic dopamine signaling [30, 31], and multiple studies have examined changes of these *DRD2* isoforms in schizophrenia. A recent study found elevated mRNA expression of D2SR and simultaneously reduced expression of D2LR in the dorsolateral prefrontal cortex (DLPFC) of schizophrenia patients compared with controls [32], although inconsistent results have also been reported [33]. In addition, studies have consistently found significant associations between these isoforms and schizophrenia genetic risk. For example, the mRNA expression of D2SR has been found to be significantly associated with a schizophrenia risk SNP rs1076560 in the intron 6 of *DRD2* [33, 34]. Furthermore, Cohen et al. showed that rs1076560 affected binding affinity for a splicing regulator ZRANB2 in an in vitro oligonucleotide assay, and this SNP was correlated with changes of D2SR/D2LR ratio in a minigene assay when ZRANB2 was co-expressed [34]. Intriguingly, rs1076560 was significantly associated with activity and functional connectivity of striatum/DLPFC during working memory tasks [33, 35], as well as that of amygdala/DLPFC during emotion processing [36], providing hints for the physiological impact of D2SR. Therefore, schizophrenia genetic risk (e.g., rs1076560) likely modulates the balance between D2SR and D2LR, and thereby affects D2 receptor-mediated signaling and physiological consequences (Fig. 2). However, the transcription of *DRD2* is likely also affected by other schizophrenia risk factors in addition to this SNP, as its disease risk allele is unlikely the causal factor for the increased *DRD2* expression in schizophrenia [12].

Glutamate and GABA hypothesis of schizophrenia

Apart from the dopamine hypothesis for schizophrenia pathogenesis, it has also been known for ~30 years that antagonists of N-methyl-D-aspartate receptor (NMDAR), such as phencyclidine and ketamine, could induce schizophrenia-like negative symptoms and subtle cognitive impairments [37, 38], promoting the formation of glutamate hypofunction hypothesis [39, 40]. Indeed, the lost dendritic spines in postmortem brains of schizophrenia patients were primarily characterized as excitatory glutamatergic synapses [41]. The mGluR3 is a type of G-protein-coupled receptor (GPCR) modulating glutamate neurotransmission and synaptic plasticity through enhancing glutamate uptake [42], and previous studies have reported significant associations between SNPs in *GRM3* (e.g., rs2228595) and risk of schizophrenia, cognition, prefrontal, and hippocampal physiology, as well as glutamate neurotransmission [3, 43]. Several lines of evidence have also demonstrated the effects of mGluR2/3 agonists, such as LY354740 and LY379268, on ameliorating NMDAR antagonist-induced behavioral defects in animals [44–47]. Corti et al. reported decreased dimeric form of mGluR3 in schizophrenia patients [48]. Notably, abundant presence of a spliced isoform lacking exon 4 in mGluR3 (mGluR3Δ4) was identified in human brain. This isoform was predicted to translate into a truncated protein that lacked the transmembrane domain while contained a novel intracellular C-terminal and a partially truncated extracellular ligand binding domain [49]. Intriguingly, mRNA expression of mGluR3Δ4 in human brain, rather than mGluR3, was associated with the schizophrenia risk SNP rs2228595 [50]. The mGluR3Δ4 also possesses altered functions compared with mGluR3. Despite lacking the transmembrane region, mGluR3Δ4 still locates at the plasma membrane in cultured hippocampal neurons [49] probably due to its heterodimerization with mGluR3. As dimerization between some full-length GPCRs and their truncated isoforms has been commonly seen, the resultant complexes generally show unique subcellular localizations, ligand binding properties, and pharmacological potentials compared with the canonical homodimers formed by full-length GPCRs [51–53]. Likewise, a recent study showed physical interactions between mGluR3Δ4 and mGluR3, and transfection of mGluR3Δ4 could cause deficits in ligand binding availability and decreased membrane mGluR3 abundance [54]. Together, these results suggest a possible mechanism underlying schizophrenia risk through negative modulation of mGluR3 function by mGluR3Δ4 [50].

In addition, dysfunction of GABAergic neurotransmission has been implicated in schizophrenia through studies of human postmortem brain tissues and in vivo levels of GABA [55–57], and alternative splicing of *GAD1* gene likely contributes to GABA dysfunction in the brains of schizophrenics [58]. There are two primary isoforms transcribed by *GAD1*, a full-length isoform encoding the active protein glutamic acid decarboxylase 67 (GAD67, 67 kDa) and a shorter isoform GAD25 (25 kDa) encoding a protein lacking the enzymatic domain [59]. As the most abundant *GAD1* transcript in human brain, GAD67 regulates the synthesis of GABA [60], and the mRNA and protein levels of this isoform were both decreased in the postmortem brains of schizophrenia patients compared with normal controls [58, 61–64]. Further analyses by Hyde et al. and Tao et al. respectively demonstrated an increased ratio of GAD25 to GAD67 in schizophrenics compared with healthy controls [63, 64]. Intriguingly, the altered expression



ratio of *GAD1* isoforms could be predicted by a schizophrenia risk SNP (rs3749034) within this gene. Given that transcription of GAD25 might have jeopardized appropriate expression of GAD67, the molecule necessary for mature GABA signaling, this mis-splicing-mediated developmental deficit might underlie pathogenesis of schizophrenia [64]. Remarkably, Tao et al. also discovered ten novel *GAD1* transcripts in human brains, among which four of them showed a life span trajectory expression pattern that is anticorrelated with the expression of GAD67 [63]. These results provided novel hints into schizophrenia pathogenesis, which not only highlighted potential effects of the reduced mature isoform (GAD67), but also emphasized influences of the truncated transcripts (e.g., GAD25).

◀ **Fig. 2 The association between schizophrenia genetic risk and alternative splicing of *DRD2*. a** The schizophrenia risk allele at rs1076560 (T-allele) facilitates the inclusion of exon 6 and is also associated with higher mRNA level of D2LR by abolishing the binding site of ZRANB2, while G allele has opposite effect. D2SR mRNA lacks exon 6 comparing with D2LR mRNA, which results in a deletion of 29 amino acids (represented by gray dash lines) at IL-3 (represented by red lines) of D2SR. **b** Different IL-3 structures of D2SR and D2LR affect their binding properties with G-protein subunits and the downstream signaling pathways activated. Upper figure: D2SR predominantly locates at presynaptic dopaminergic neurons and acts as auto-receptor to provide a negative feedback for modulation of neuron firing and dopamine neurotransmission. Activation of D2SR reduces dopamine synthesis via regulation of TH activity, facilitates dopamine reuptake partially through increasing the surface expression of DAT, and represses neuron excitability. Bottom figure: D2LR mainly locates at postsynaptic dopaminoceptive neurons. Activation of D2LR inhibits cAMP production, thereby mediating the phosphorylation state of DARPP-32, which is a major target for dopamine in striatum and also a potent inhibitor of a multifunctional PP1. The D2LR-triggered DARPP-32/PP1 cascade shows impacts on a wide range of downstream effectors including neurotransmitter receptors and ion channels in dopaminoceptive neurons, e.g., striatal medium spiny neurons. Moreover, D2LR signaling regulates intracellular calcium levels involving $G_{\beta\gamma}$ activation of PLCβ-IP3-calcineurin cascade. It is noteworthy that activation of D1-class and D2 heterodimer receptors modulates calcium signaling mediated by Gαq and PLCβ/IP3 pathway. AC adenylyl cyclase, β, γ, α/io, αq, subunits of G-protein complex, cAMP 3′,5′-cyclic adenosine monophosphate, DA dopamine, DAG diacylglycerol, DARPP-32 dopamine- and cAMP-regulated phosphoprotein 32 kDa, DAT dopamine transporter, GTP guanosine triphosphate, IL-3 third intracellular loop, IP3 inositol 1,4,5-trisphosphate, p phosphorylated, PKA protein kinase A, PKC protein kinase C, PLC-β phospholipase C-β, PP1 protein phosphatase 1, TH tyrosine hydroxylase.

Neurodevelopmental hypothesis of schizophrenia

Neurodevelopmental hypothesis of schizophrenia suggests that perturbation of early central nervous system development may be the key etiology for later onset of schizophrenia symptoms. This theory has been supported by early epidemiological and circumstantial data as well as more recent brain-specific molecular and genetic findings [65–67]. It is noteworthy that some schizophrenia risk genes related to dysregulation of neurotransmitter systems (e.g., *GAD1*) also play essential roles in regulation of neurodevelopment [68]. In addition, there are multiple schizophrenia risk genes (like *DISC1* and *NRG1*) exerting pivotal functions in embryonic and postnatal neurodevelopmental processes. *DISC1* gene is genetically associated with schizophrenia [69, 70], and encodes a scaffold protein regulating neuron proliferation, migration, neurite outgrowth, synaptogenesis, and integration of newborn neurons in multidimensional pathways [71–75]. Intriguingly, Nakata et al. reported that human *DISC1* mRNA underwent extensive alternative splicing, and expression of several *DISC1* isoforms were changed during brain development and in schizophrenia patients [76]. Among these *DISC1* transcripts, the one that missing exon 3 (Δ3), or exons 7 and 8 (Δ7Δ8), as well as the one with an insertion variant in exon 3 (extra short variant-1, Esv1) exhibited higher mRNA levels in the brain of schizophrenics than healthy controls [76]. Moreover, expressions of *DISC1* Δ3 and Δ7Δ8 isoforms were associated with schizophrenia risk SNPs (rs821616, rs6675281, and rs821597). The full-length DISC1 protein has been found to exert functions in early neuronal development and synapse maturation via interacting with other proteins [71], whereas Newburn et al. found that the truncated DISC1 proteins respectively encoded by these spliced isoforms (Δ3, Δ7Δ8, and Esv1) showed altered binding abilities with DISC1-interacting proteins, e.g., reduced binding for NDEL1 and PDE4B, and intact binding for FEZ1 and GSK3B [77]. Moreover, all the truncated DISC1 proteins could be co-immunoprecipitated with full-length DISC1, suggesting that they likely formed a complex and modulate the biological function of DISC1 through affecting its protein interaction network [77].

NRG1-ErbB4 signaling plays essential roles in axon/dendrite development and synapse formation/plasticity [78, 79], and both *NRG1* and its receptor *ErbB4* were perturbed in schizophrenia [80–83]. *NRG1* gene is primarily transcribed into six types of isoforms (I–VI) with distinct amino-terminal regions via alternative 5′ flanking regulatory elements usage, and these isoforms exhibit diverse expression patterns, properties, and features in neurodevelopment [82, 84]. For example, the most mature *NRG1* isoforms are soluble and act as chemoattractants, but the membrane-bound *NRG1*-III acts in a contact-dependent manner [82]. *NRG1* has been confirmed as a schizophrenia susceptibility gene via linkage analyses and early candidate studies [85–88]. Subsequently, several risk SNPs (SNP8NRG221132, rs6994992, rs7014762, SNP8NRG221533, and SNP8NRG241930) have been reported to affect expression of *NRG1* isoforms (*NRG1*-I, *NRG1*-IV, and *NRG1*-III), suggesting their potential roles in schizophrenia pathogenesis [89–93]. Consistently, schizophrenia-like behavioral (e.g., deficits in social interaction) and endophenotypic deficits (e.g., decreased prepulse inhibition) shown in mice with modified expression of *NRG1*-I/IV/III isoforms corroborated their involvement in schizophrenia, whereas mice overexpressing different isoforms exhibited subtly different deficits, suggesting possible isoform-specific biological mechanisms [94–97]. Meanwhile, four primary isoforms of *ErbB4* have been characterized. They are generated by alternative splicing at the juxtamembrane (JM) and cytoplasmic (CYT) locus, and are therefore named JM-a, JM-b, CYT-1, and CYT-2 [98]. A splicing shift from major JM-b/CYT-2 isoforms to minor JM-a/CYT-1 isoforms in schizophrenia patients was associated with a risk haplotype containing three SNPs (rs7598440, rs707284, and rs839523) in *ErbB4* [99, 100]. This shift, which leads to suppressed ErbB4 kinase activity and a subsequent reduction of excitatory synapses on parvalbumin-containing GABAergic interneurons, likely contributes to defects in synapse pruning and cognitive deficits in schizophrenia [101, 102].

Alternative splicing analyses are urgently needed in the current omics era post GWAS

While earlier candidate risk gene studies have significantly strengthened the understanding of schizophrenia pathogenesis, the panorama of alternative splicing in schizophrenia remains less clear. With the advancements in high-throughput genetic analyses of schizophrenia, the number of potential disease risk loci has also rapidly grown. Unlike the previously defined genes with well-characterized functionality and clinical applications, many of these newly-identified disease risk loci have been less-studied, let alone their transcription and splicing patterns that are potentially essential in schizophrenia pathogenesis. Therefore, further endeavors defining schizophrenia risk transcripts within these loci are urgently needed to interpret the current massive genetic data at the post GWAS era.

To reveal additional schizophrenia risk genes and their disease-associated isoforms, multiple methods have been developed to uncover disease relevant transcriptomic characteristics on gene-, isoform-, exon-, junction- to single base-levels using the high-throughput RNA-seq approach [103]. Gandal et al. recently conducted gene-level and annotation-guided isoform-level analyses using the RNA-seq data from DLPFC tissues of schizophrenia patients and normal controls [23]. Both methods highlighted generally similar pathways and cell-type enrichment in schizophrenics compared with controls, despite that isoform-level analyses identified multiple differentially expressed transcripts that were not significant in gene-level analyses. Intriguingly, these transcripts exhibited significant overlap with excitatory neuron clusters and enrichment for neuron projection development, mRNA metabolism, and synaptic pathways. In addition, networks built by the differentially expressed isoforms exhibited increased resolution in the disease-specific biological insights [23], and isoform-level changes likely provided extra clues for the neuronal and synaptic characteristics of schizophrenia. Therefore, they concluded that changes at the isoform level, compared with the gene level, showed larger effect sizes and genetic enrichment and a greater disease specificity. Another advantage of isoform-level analyses compared with gene-level analyses is the consideration of potentially distinct impact of certain isoforms in different illnesses. Although GWAS has identified multiple shared genetic risk genes across psychiatric disorders, different alternatively spliced isoforms of a single risk gene might exert unique functions in each illness, probably resulting from their specific expression pattern in distinct developmental stages and brain regions/cell types [104]. This speculation is concordant with the isoform-level analyses in Gandal et al. study [23], which emphasized the importance of splicing and isoform-level gene regulatory mechanisms in defining cell type and disease specificity. A study by Yang et al. further implied that isoforms of a single gene could function like different genes through interacting with extremely different protein networks [105]. Adding more complexity to the functional impact of alternative splicing in schizophrenia, developmental stage-specific expression patterns of isoforms have been implicated by several studies as well. For example, Jaffe et al. compared the human cortex transcriptome differences across developmental stages, and found that genes with developmental stage-related isoforms shifts were more likely to locate at schizophrenia GWAS risk loci than those without isoforms shifts [106]. In addition, Walker et al. conducted splicing QTL analyses using mid-gestational human brain and found that schizophrenia-associated SNPs were significantly enriched for prenatal splicing QTL loci [107]. Therefore, future endeavors are called to reveal potential effects of schizophrenia genetic risk on splicing events in developing brains, as current studies are mainly based on adult postmortem brain tissues.

Local splicing analysis of RNA-seq data

To gain more comprehensive insights into the expression patterns of known and unknown isoforms in schizophrenia, the “local splicing” analysis, which circumvents the limitations of imputation and assembling from short reads guided by existing transcriptomic annotations (e.g., inaccurate quantifications, incomplete annotations [108], loss of sequencing coverage, statistical analysis bias, etc.), is also adopted for RNA-seq analysis. For example, Gandal et al. investigated “local splicing” events using de novo aligned RNA-seq reads of human DLPFC [23], and they observed multiple types of “local splicing” changes in schizophrenia, such as exon skipping, alternative 5′ exon inclusion, and alternative 3′ splice-site usage. They found that genes with altered “local splicing” in schizophrenia showed significant enrichment for cell communication, actin cytoskeleton, synapse and neuronal development, as well as guanosine triphosphatase receptor activity [23]. In addition, Jaffe et al. detected numerous previously unannotated splice junctions tagging potential transcripts with alternative exonic boundaries or exon skipping using brain DLPFC RNA-seq samples, suggesting the incomplete annotations of human brain transcriptomes in existing databases [109]. They also identified numerous schizophrenia GWAS risk SNPs associated with these novel junctions (or unannotated transcribed sequences) [106]. Takata et al. analyzed RNA-seq data of DLPFC tissues [110], and identified many alternative splicing events including exon skipping, alternative usage of splice sites, and intron retentions. Their further analyses of these splicing events revealed that the splicing QTL SNPs were significantly enriched at schizophrenia GWAS risk loci [110]. Similarly, using RNA-seq data of postmortem samples across 13 brain regions, Ma et al. investigated the exon–exon splice junctions (for exon skipping events), and found that some schizophrenia GWAS risk SNPs were significantly associated with the expression of exon skipping junctions in several genes including *CYP2D6* and *SNX19* [111]. Notably, the exon skipping junction in *SNX19* has also been detected in independent brain RNA-seq samples [112]. We herein also briefly summarize several representative "local splicing" events associated with schizophrenia GWAS risk SNPs that were retrieved from two DLPFC RNA-seq studies (Table 2) [106, 110].

Despite identifying "local splicing" events associated with risk of schizophrenia through RNA-seq analyses, experimental validations using molecular approaches are necessary to verify such "local splicing" events in organisms. For example, Li et al. conducted RNA-seq analysis followed by experimental validations and identified a human-unique isoform within the *AS3MT* gene, which lacks exon 2 and 3 (named $AS3MT^{d2d3}$) compared with the full-length *AS3MT* transcript ($AS3MT^{full}$) [113]. Briefly, to explore the potential mechanisms underlying the schizophrenia GWAS locus at 10q24.32, which contains numerous genome-wide significant risk SNPs spanning multiple genes, Li et al. performed junction analysis of RNA-seq data in human DLPFC tissues. They showed that the junction skipping exon 2 and 3 of *AS3MT* (referred as $AS3MT^{d2d3}$) was significantly associated with the schizophrenia risk SNP rs7085104 [113]. This eQTL association was further confirmed in independent studies [114, 115]. The mRNA level of $AS3MT^{d2d3}$ isoform was elevated in the DLPFC of schizophrenia patients compared with normal controls [113], and overexpression of $AS3MT^{d2d3}$ in neurons resulted in a significant reduction of mushroom dendritic spine density [114], mimicking the endophenotypes observed in the brains of schizophrenia patients [116–119]. Cai et al. also found that the variable number of tandem repeat in high linkage disequilibrium with rs7085104 regulated the mRNA expression of $AS3MT^{d2d3}$ using an in vitro minigene splicing assay [114]. Altogether, these studies have identified an alternatively spliced isoform $AS3MT^{d2d3}$, which likely accounts for (at least part of) the molecular mechanisms underlying genetic risk of schizophrenia in the 10q24.32 GWAS locus.

Besides exon skipping, other types of alternative splicing events, such as alternative 5′ exon inclusion, have generated transcripts associated with schizophrenia risk in human brains. For example, Tao et al. discovered a novel spliced transcript in the schizophrenia risk gene *ZNF804A* in human brains [120]. This novel isoform arises from a new 5′ UTR in the intron 2 of this gene and lacks the first 2 exons compared with the wild-type $ZNF804A^{Full}$ transcript (named $ZNF804A^{E3E4}$). Predictive analysis suggested that $ZNF804A^{E3E4}$ might encode a protein lacking the zinc finger domain, the vital functional element in $ZNF804A^{Full}$ [121]. Intriguingly, the schizophrenia risk SNP rs1344706 was significantly associated with lower mRNA expression of $ZNF804A^{E3E4}$ rather than $ZNF804A^{Full}$ in human fetal brains, corroborating the reduced $ZNF804A^{E3E4}$ mRNA in brains of schizophrenia patients relative to healthy controls [120]. It has been reported that ZNF804A protein is present in dendrites and synapses, and knockdown of the aggregated isoforms reduces dendritic spine density and inhibits neurite formation [122, 123]. Surprisingly, overexpression of $ZNF804A^{E3E4}$ in cultured neurons provokes more mushroom dendritic spines than overexpression of $ZNF804A^{Full}$, suggesting a pronounced and specific functional effect of this isoform on mature spines compared to wild-type transcript [124]. Therefore, decreased $ZNF804A^{E3E4}$ expression during early brain development likely explains the molecular mechanism underlying genetic risk of schizophrenia in this GWAS locus.

Long-read sequencing analysis

Despite the recent enlightening discoveries of alternative splicing in schizophrenia using RNA-seq data, short-read sequencing has also brought challenges to accurate assembling and quantification of isoforms since certain sequences are likely shared by multiple transcripts of a single gene [103]. Meanwhile, although RNA-seq supplemented with molecular characterizations have identified novel schizophrenia risk isoforms, these analyses usually require extensive efforts. Therefore, feasible strategies for efficiently characterizing more target genes and isoforms, e.g., long-read sequencing, are needed.

Long-read sequencing allows increased sequencing resolution and transcript assembling accuracy that are required for deciphering the alternative splicing profiles. A recent nanopore long-read sequencing analysis characterized the alternative splicing of *CACNA1C* in human brains [125], a psychiatric risk gene encoding the $Ca_V1.2$ voltage-gated calcium channel alpha1 subunit [3]. The authors applied two complementary approaches, exon-level analysis and splice-site-level analysis, which incorporated novel exons, novel junctions between annotated exons, and new combination of known junctions, and they identified 241 novel transcripts within the *CACNA1C* gene. Notably, many of the novel transcripts were abundant in human brain and were predicted to have functional impact on $Ca_V1.2$. These

Table 2 Local splicing events associated with schizophrenia risk SNPs.

RNA-seq resource	Gene	Local splicing type	Local splicing events in the referenced transcript	Local splicing position (hg19)	Index SNP	SNP position	SNP annotation	SNP splicing QTL p value (FDR) [reference]	SNP schizophrenia GWAS p value [reference]
RNA-seq data in Jaffe et al. study [106]. The DLPFC sample was from Lieber Institute for Brain Development. A total of 495 individuals with ages across the life span were analyzed, including 175 patients with schizophrenia.	*AKT3*	Alternative usage of splice sites at the acceptor site	"Exon 12-Intron 12" in ENST00000366539	chr1:243706262-243708811	rs14403	chr1:243663893	Intergenic variant	1.23E−05 [106]	1.71E−10 [4]
	AS3MT	Exon skipping	"Exon 1-Exon 4" in ENST00000369880	chr10:104629351-104632204	rs11191419	chr10:104612335	Intergenic variant	7.57E−28 [106]	4.11E−21 [5]
	CYP2D6	Exon skipping	"Exon 5-Exon 7" in ENST00000360608	chr22:42523637-42524175	rs1023500	chr22:42340844	Intergenic variant	3.48E−07 [106]	3.08E−11 [4]
	DOC2A	Alternative usage of splice sites at the donor site	"Exon 6-Exon 7" in ENST00000350119	chr16:30018412-30018499	rs12691307	chr16:29939877	Intergenic variant	1.69E−10 [106]	1.34E−10 [4]
	FANCL	Exon skipping	"Exon 3-Exon 5" in ENST00000233741	chr2:58449178-58456948	rs75575209	chr2:58138192	Intron variant	3.04E−33 [106]	4.60E−09 [4]
	FAM57B	Alternative usage of splice sites at the acceptor site	"Exon 3-Exon 4" in ENST00000380495	chr16:30037091-30037929	rs12691307	chr16:29939877	Intergenic variant	1.15E−03 [106]	1.34E−10 [4]
	GNL3	Alternative usage of splice sites at the donor site	"Exon 1-Exon 2" in ENST00000418458	chr3:52720072-52720784	rs2535627	chr3:52845105	Intergenic variant	1.19E−08 [106]	8.55E−12 [5]
	MAD1L1	Alternative usage of splice sites at the donor site	"Intron 13-Exon 14" in ENST00000399654	chr7:2041757-2048106	rs10650434	chr7:2025096	Intron variant	5.82E−06 [106]	1.10E−18 [4]
	NOSIP	Alternative usage of splice sites at the donor site	"Intron 1-Exon 2" in ENST00000596358	chr19:50063950-50067411	rs56873913	chr19:50091199	Intron variant	1.20E−03 [106]	2.58E−09 [4]
	NRGN	Alternative usage of splice sites at the donor site	"Intron 1-Exon 2" in ENST00000284292	chr11:124612650-124615398	rs55661361	chr11:124613957	Intron variant	3.57E−06 [106]	1.74E−15 [4]
	NRGN	Alternative usage of splice sites at the donor site	"5′flanking-Exon 2" in ENST00000284292	chr11:124589765-124615398	rs55661361	chr11:124613957	Intron variant	1.78E−04 [106]	1.74E−15 [4]

Table 2 (continued)

RNA-seq resource	Gene	Local splicing type	Local splicing events in the referenced transcript	Local splicing position (hg19)	Index SNP	SNP position	SNP annotation	SNP splicing QTL *p* value (FDR) [reference]	SNP schizophrenia GWAS *p* value [reference]
	NRGN	Alternative usage of splice sites at the donor site	"5′flanking-Exon 2" in ENST00000284292	chr11:124589528-124615398	rs55661361	chr11:124613957	Intron variant	1.90E–03 [106]	1.74E–15 [4]
	SNX19	Exon skipping	"Exon 8-Exon 10" in ENST00000265909	chr11:130749607-130773149	rs10791097	chr11:130718630	Intron variant	1.13E–25 [106]	3.29E–11 [4]
	TSNARE1	Alternative usage of splice sites at the acceptor site	"Exon 12-Intron 12" in ENST00000524325	chr8:143354949-143356141	rs4129585	chr8:143312933	Intron variant	8.20E–03 [106]	9.26E–18 [4]
RNA-seq data in Takata et al. study [110]. The DLPFC sample was from CommonMind Consortium. A total of 285 individuals without neuropsychiatric diseases or neurological insults were included.	*DPYD*	Exon skipping	"Exon 1-Exon 3" in ENST00000370192	chr1:98293753-98386439	rs1801265	chr1:98348885	Missense variant	8.70E–03 [110]	1.22E–10 [4]
	FLOT1	Exon skipping	"Exon 4-Exon 6" in ENST00000376389	chr6:30708576-30709390	rs1059612	chr6:30708955	Intron variant	4.16E–39 [110]	4.79E–28 [4]
	NEK4	Exon skipping	"Exon 3-Exon 5" in ENST00000233027	chr3:52797641-52800193	rs2230535	chr3:52800284	Synonymous variant	9.15E–10 [110]	2.79E–11 [5]
	NEK4	Exon skipping	"Exon 4-Exon 6" in ENST00000233027	chr3:52794953-52799902	rs2276817	chr3:52860936	Synonymous variant	1.73E–03 [110]	5.77E–10 [5]
	SNAP91	Intron retention	"Intron 13" in ENST00000369694	chr6:84315523-84317417	rs217291	chr6:84393942	Intron variant	5.45E–23 [110]	1.30E–13 [5]
	SRA1	Exon skipping	"Exon 2-Exon 4" in ENST00000336283	chr5:139,930,755-139936731	rs778591	chr5:140025484	Intron variant	2.50E–07 [110]	4.14E–08 [5]

transcripts might reveal novel mechanisms of schizophrenia pathogenesis that were previously hidden by the incomplete annotation of *CACNA1C* transcripts [125].

The schizophrenia risk gene *NRXN1* can be transcribed into *NRXN1*-α and *NRXN1*-β depending on the usage of two alternative promoters [126–128], and Jenkins et al. previously found that mRNA levels of *NRXN1*-β were significantly higher in the DLPFC of schizophrenia patients compared with controls, whereas *NRXN1*-α levels were consistent between diagnostic groups [129]. Intriguingly, a recent PacBio long-read Iso-seq analysis has described alternatively spliced *NRXN1* isoform patterns in the induced pluripotent stem cell (iPSC)-derived neurons from psychiatric patients with non-recurrent *NRXN1*3′/5′ heterozygous deletions [130]. Apart from the altered mRNA levels of some known *NRXN1*-α isoforms, there were dozens of novel isoforms identified in the iPSC-derived neurons carrying the mutant allele in 3′-*NRXN1*+/−. The authors then performed comparative analysis of the novel and conventional isoforms, and found generally similar exon inclusion patterns except for exons encompassed by the 3′-deletion. As a result, the *NRXN1* deletion perturbs *NRXN1*-α isoform repertoire, which might alter the protein expression profile of this gene and thereby participate in psychiatric illnesses [130].

Functional analyses of alternatively spliced isoforms in brain and in schizophrenia

So far, transcriptomic analyses have identified numerous schizophrenia-relevant alternative splicing events, majority of which occur in genes playing key roles in neurodevelopment, synaptic plasticity, and cognition. Therefore, it is of great importance to uncover the functional complexity of these diverse alternative splicing events and relevant isoforms in schizophrenia. Although many of these isoforms are yet to be functionally characterized, several groups have reported inspiring findings highlighting specific roles of disease-associated isoforms (distinct from the full-length transcripts) in brain development aberrations linked with schizophrenia. We herein briefly summarize the functional analyses of two alternatively spliced isoforms with critical roles in brain development and potentially schizophrenia pathogenesis.

Alternatively spliced isoforms encoding proteins with unique functional characteristics

KCNH2 encodes the human ether-a-go-go-related voltage-gated potassium channel controlling neuronal firing patterns with characteristic electrophysiological properties: slow activation, fast inactivation, and slow and voltage-dependent deactivation [131, 132]. Huffaker et al. reported that SNPs in the *KCNH2* gene were significantly associated with risk of schizophrenia, cognition, as well as brain structure and physiology [133], but the risk SNPs did not affect expression of *KCNH2*-1A (full-length transcript) or *KCNH2*-1B (a minor isoform). Notably, they identified a primate-specific and brain-enriched isoform (*KCNH2*-3.1) lacking the first two exons but containing a previously undescribed 5′ extension from exon 3 compared with *KCNH2*-1A. Moreover, *KCNH2*-3.1 mRNA expression was associated with schizophrenia risk alleles and was significantly increased in patients, suggesting that its elevated expression likely confers risk of the illness [133]. Intriguingly, individuals carrying schizophrenia risk alleles associated with increased *KCNH2*-3.1 expression showed better response to antipsychotics [134, 135], indicating the potential of this isoform in clinical applications.

Accordingly, a transgenic mouse model that mimics the increased expression of *KCNH2*-3.1 in schizophrenia patients was used to interrogate the functional impact of KCNH2-3.1 on cortical and hippocampal circuit [136, 137]. Electrophysiological recordings revealed faster ERG channel deactivation kinetics and increased firing rate in neurons of prefrontal cortex slices prepared from *KCNH2*-3.1 overexpressed mice compared with wild-type mice [136]. Interestingly, in *KCNH2*-3.1 transgenic mice, long-term potentiation induced in hippocampal CA1 synapses by theta burst stimulation is impaired, which is in accordance with the hippocampal-dependent memory deficits measured in object location task [136]. These data suggest that *KCNH2*-3.1 isoform might regulate information processing in prefrontal cortex and hippocampal microcircuit. Moreover, *KCNH2*-3.1 transgenic mice display impaired synaptic connectivity and transmission in ventral hippocampus-medial PFC long-range projection [137].

Alternatively spliced isoforms exerting noncoding function

While many spliced isoforms encode proteins with significant physiological impact, some are also found to exert functions in a coding-independent manner, such as *Ube3a1*. *Ube3a1* refers to an alternatively spliced transcript of the *UBE3A* gene in the chromosome 15q11.2 locus, and duplications of this genomic region confer risk of schizophrenia in diverse populations [138–142]. *Ube3a* originally encodes a ubiquitin E3 ligase that plays an important role in dendrite and spine development, synaptic plasticity, and excitatory/inhibitory imbalance [143–145]. There are three isoforms identified for *Ube3a*. Both *Ube3a2/3* transcripts are translated into the full-length protein, while *Ube3a1* contains a unique 3′UTR producing a truncated isoform due to the alternative polyadenylation [146]. Interestingly, Valluy et al. found that *Ube3a1* and *Ube3a2/3* transcripts have opposite effects on dendrite complexity. Knockdown of *Ube3a1* with shRNA specifically targeting its 3′UTR in cultured neurons increased dendritic complexity, whereas knockdown of *Ube3a2/3* RNA reduced dendritic complexity. In addition to increasing dendrites complexity, *Ube3a1* RNA knockdown reduced sizes of dendritic spines and amplitude of miniature excitatory postsynaptic currents, suggesting that Ube3a1 could promote spine maturation but prevent the overgrowth of dendrites [146]. Surprisingly, only the mRNA form of *Ube3a1* has been detected in neurons, and the unique 3′UTR of *Ube3a1* RNA might contain signals for localization at the dendritic compartments. Intriguingly, further investigations indicated that *Ube3a1* RNA acted as a competing endogenous RNA and sequestered miR-134 from its natural target dendritic genes, resulting in abnormal translation and function of these genes [146]. Therefore, the coding-independent functionality of spliced isoforms are also of potentially great significance in schizophrenia.

Conclusions and perspectives

So far, as the importance of alternative splicing in pathogenesis of multiple diseases, such as autism, amyotrophic lateral sclerosis, and Parkinson's disease, has been acknowledged, and therapeutic strategies based on correcting splicing defects are being investigated. For example, the clinical usage of antisense oligonucleotides or CRISPR/Cas9 that could recognize specific RNA splicing regulatory elements has been extensively studied [147]. However, such therapies are likely more effective for Mendelian diseases than for complex disorders such as schizophrenia. As the polygenic nature of schizophrenia requires intervention of hundreds or thousands of genes involved in its pathogenesis, treatment with oligonucleotides or CRISPR/Cas9-based methods is not feasible currently. Hence, novel phenomics strategy might offer new insights into the complex interaction network between risk genes and clinical features of schizophrenia patients, and thereby revealing potential hub pathways underlying the endophenotypes in schizophrenia pathology. Although intervention of altered alternative splicing in schizophrenia is impractical, targeting the spliced mRNAs or proteins of schizophrenia-associated isoforms might be possible with a handful of new techniques. For example, Liu et al. recently developed a genetic method called "isoTarget" for in vivo isoform functionality characterization, which could knock out or tag an isoform in a cell-type-specific manner through inserting a cassette sequence into an exon [148]. It is of great interest to explore the possibility of applying this technique to agonize or antagonize GPCRs (e.g., DRD2, mGluR3, etc.) to ameliorate schizophrenia symptoms while reduce side effects and declined effects during chronic treatment [39, 149]. Considering the splicing diversity of these genes, better understanding of the structure and expression patterns of their isoforms in vivo could benefit the discovery of compounds specifically targeting the pharmacologically effective sites without activation elsewhere [150]. Overall, greater attentions into altered alternative splicing of schizophrenia risk genes are necessary for efficient translation of genetic discoveries into the understanding of controlling of the illness.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (81722019 and 81971259 to ML, 31872778 to ZH). ZH was also supported by Xiangya Hospital Start-up Research Grants, The innovative team program 2019RS1010 from Department of Science & Technology of Hunan Province, The innovation-driven team project 2020CX016 from Central South University, 111 Grant (B13036), and Hunan 100 Talents Program.

Compliance with ethical standards

Conflict of interest The authors declare no competing interests.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Marder SR, Cannon TD. Schizophrenia. N Engl J Med. 2019;381:1753–61.
2. Sullivan PF, Kendler KS, Neale MC. Schizophrenia as a complex trait: evidence from a meta-analysis of twin studies. Arch Gen Psychiatry. 2003;60:1187–92.
3. Schizophrenia Working Group of the Psychiatric Genomics Consortium. Biological insights from 108 schizophrenia-associated genetic loci. Nature. 2014;511:421–7.
4. Pardinas AF, Holmans P, Pocklington AJ, Escott-Price V, Ripke S, Carrera N, et al. Common schizophrenia alleles are enriched in mutation-intolerant genes and in regions under strong background selection. Nat Genet. 2018;50:381–9.
5. Lam M, Chen CY, Li Z, Martin AR, Bryois J, Ma X, et al. Comparative genetic architectures of schizophrenia in East Asian and European populations. Nat Genet. 2019;51:1670–8.
6. Edwards SL, Beesley J, French JD, Dunning AM. Beyond GWASs: illuminating the dark road from association to function. Am J Hum Genet. 2013;93:779–97.
7. French JD, Edwards SL. The role of noncoding variants in heritable disease. Trends Genet. 2020;36:880–91.
8. Yang Z, Zhou D, Li H, Cai X, Liu W, Wang L, et al. The genome-wide risk alleles for psychiatric disorders at 3p21.1 show convergent effects on mRNA expression, cognitive function and mushroom dendritic spine. Mol Psychiatry. 2020;25:48–66.
9. Chang H, Cai X, Li HJ, Liu WP, Zhao LJ, Zhang CY, et al. Functional genomics identify a regulatory risk variation rs4420550 in the 16p11.2 schizophrenia-associated locus. Biol Psychiatry. 2021;89:246–55.
10. Yang Z, Cai X, Qu N, Zhao L, Zhong BL, Zhang SF, et al. Identification of a functional 339-bp Alu polymorphism in the schizophrenia-associated locus at 10q24.32. Zool Res. 2020;41:84–9.

11. Reble E, Dineen A, Barr CL. The contribution of alternative splicing to genetic risk for psychiatric disorders. Genes Brain Behav. 2018;17:e12430.
12. Kleinman JE, Law AJ, Lipska BK, Hyde TM, Ellis JK, Harrison PJ, et al. Genetic neuropathology of schizophrenia: new approaches to an old question and new uses for postmortem human brains. Biol Psychiatry. 2011;69:140–5.
13. Glatt SJ, Cohen OS, Faraone SV, Tsuang MT. Dysfunctional gene splicing as a potential contributor to neuropsychiatric disorders. Am J Med Genet B Neuropsychiatr Genet. 2011;156B:382–92.
14. Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, et al. Alternative isoform regulation in human tissue transcriptomes. Nature. 2008;456:470–6.
15. Park E, Pan Z, Zhang Z, Lin L, Xing Y. The expanding landscape of alternative splicing variation in human populations. Am J Hum Genet. 2018;102:11–26.
16. Nilsen TW, Graveley BR. Expansion of the eukaryotic proteome by alternative splicing. Nature. 2010;463:457–63.
17. Yeo G, Holste D, Kreiman G, Burge CB. Variation in alternative splicing across human tissues. Genome Biol. 2004;5:R74.
18. Li YI, van de Geijn B, Raj A, Knowles DA, Petti AA, Golan D, et al. RNA splicing is a primary link between genetic variation and disease. Science. 2016;352:600–4.
19. Barry G, Briggs JA, Vanichkina DP, Poth EM, Beveridge NJ, Ratnu VS, et al. The long non-coding RNA Gomafu is acutely regulated in response to neuronal activation and involved in schizophrenia-associated alternative splicing. Mol Psychiatry. 2014;19:486–94.
20. Scheckel C, Darnell RB. Microexons—tiny but mighty. EMBO J. 2015;34:273–4.
21. Irimia M, Weatheritt RJ, Ellis JD, Parikshak NN, Gonatopoulos-Pournatzis T, Babor M, et al. A highly conserved program of neuronal microexons is misregulated in autistic brains. Cell. 2014;159:1511–23.
22. Laurent B, Ruitu L, Murn J, Hempel K, Ferrao R, Xiang Y, et al. A specific LSD1/KDM1A isoform regulates neuronal differentiation through H3K9 demethylation. Mol Cell. 2015;57:957–70.
23. Gandal MJ, Zhang P, Hadjimichael E, Walker RL, Chen C, Liu S, et al. Transcriptome-wide isoform-level dysregulation in ASD, schizophrenia, and bipolar disorder. Science. 2018;362:eaat8127.
24. McCutcheon RA, Krystal JH, Howes OD. Dopamine and glutamate in schizophrenia: biology, symptoms and treatment. World Psychiatry. 2020;19:15–33.
25. Carlsson A, Lindqvist M. Effect of chlorpromazine or haloperidol on formation of 3-methoxytyramine and normetanephrine in mouse brain. Acta Pharm Toxicol. 1963;20:140–4.
26. van Rossum JM. The significance of dopamine-receptor blockade for the mechanism of action of neuroleptic drugs. Arch Int Pharmacodyn Ther. 1966;160:492–4.
27. Seeman P, Lee T, Chau-Wong M, Wong K. Antipsychotic drug doses and neuroleptic/dopamine receptors. Nature. 1976; 261:717–9.
28. Creese I, Burt DR, Snyder SH. Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs. Science. 1976;192:481–3.
29. Hietala J, Syvalahti E, Vuorio K, Rakkolainen V, Bergman J, Haaparanta M, et al. Presynaptic dopamine function in striatum of neuroleptic-naive schizophrenic patients. Lancet. 1995; 346:1130–1.
30. Usiello A, Baik JH, Rouge-Pont F, Picetti R, Dierich A, LeMeur M, et al. Distinct functions of the two isoforms of dopamine D2 receptors. Nature. 2000;408:199–203.
31. Lindgren N, Usiello A, Goiny M, Haycock J, Erbs E, Greengard P, et al. Distinct roles of dopamine D2L and D2S receptor isoforms in the regulation of protein phosphorylation at presynaptic and postsynaptic sites. Proc Natl Acad Sci U S A. 2003;100:4305–9.
32. Kaalund SS, Newburn EN, Ye T, Tao R, Li C, Deep-Soboslay A, et al. Contrasting changes in DRD1 and DRD2 splice variant expression in schizophrenia and affective disorders, and associations with SNPs in postmortem brain. Mol Psychiatry. 2014;19:1258–66.
33. Zhang Y, Bertolino A, Fazio L, Blasi G, Rampino A, Romano R, et al. Polymorphisms in human dopamine D2 receptor gene affect gene expression, splicing, and neuronal activity during working memory. Proc Natl Acad Sci U S A. 2007;104:20552–7.
34. Cohen OS, Weickert TW, Hess JL, Paish LM, McCoy SY, Rothmond DA, et al. A splicing-regulatory polymorphism in DRD2 disrupts ZRANB2 binding, impairs cognitive functioning and increases risk for schizophrenia in six Han Chinese samples. Mol Psychiatry. 2016;21:975–82.
35. Bertolino A, Fazio L, Caforio G, Blasi G, Rampino A, Romano R, et al. Functional variants of the dopamine receptor D2 gene modulate prefronto-striatal phenotypes in schizophrenia. Brain. 2009;132:417–25.
36. Blasi G, Lo Bianco L, Taurisano P, Gelao B, Romano R, Fazio L, et al. Functional variation of the dopamine D2 receptor gene is associated with emotional control as well as brain activity and connectivity during emotion processing in humans. J Neurosci. 2009;29:14812–9.
37. Krystal JH, Karper LP, Seibyl JP, Freeman GK, Delaney R, Bremner JD, et al. Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses. Arch Gen Psychiatry. 1994;51:199–214.
38. Javitt DC. Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia. Hillside J Clin Psychiatry. 1987;9:12–35.
39. Moghaddam B, Javitt D. From revolution to evolution: the glutamate hypothesis of schizophrenia and its implication for treatment. Neuropsychopharmacology. 2012;37:4–15.
40. Coyle JT, Ruzicka WB, Balu DT. Fifty years of research on schizophrenia: the ascendance of the glutamatergic synapse. Am J Psychiatry. 2020;177:1119–28.
41. Sweet RA, Henteleff RA, Zhang W, Sampson AR, Lewis DA. Reduced dendritic spine density in auditory cortex of subjects with schizophrenia. Neuropsychopharmacology. 2009; 34:374–89.
42. Harrison PJ, Lyon L, Sartorius LJ, Burnet PW, Lane TA. The group II metabotropic glutamate receptor 3 (mGluR3, mGlu3, GRM3): expression, function and involvement in schizophrenia. J Psychopharmacol. 2008;22:308–22.
43. Egan MF, Straub RE, Goldberg TE, Yakub I, Callicott JH, Hariri AR, et al. Variation in GRM3 affects cognition, prefrontal glutamate, and risk for schizophrenia. Proc Natl Acad Sci U S A. 2004;101:12604–9.
44. Engel M, Snikeris P, Matosin N, Newell KA, Huang XF, Frank E. mGluR2/3 agonist LY379268 rescues NMDA and GABAA receptor level deficits induced in a two-hit mouse model of schizophrenia. Psychopharmacology. 2016;233:1349–59.
45. Profaci CP, Krolikowski KA, Olszewski RT, Neale JH. Group II mGluR agonist LY354740 and NAAG peptidase inhibitor effects on prepulse inhibition in PCP and D-amphetamine models of schizophrenia. Psychopharmacology. 2011;216:235–43.
46. Cartmell J, Monn JA, Schoepp DD. Attenuation of specific PCP-evoked behaviors by the potent mGlu2/3 receptor agonist, LY379268 and comparison with the atypical antipsychotic, clozapine. Psychopharmacology. 2000;148:423–9.
47. Cartmell J, Monn JA, Schoepp DD. The metabotropic glutamate 2/3 receptor agonists LY354740 and LY379268 selectively

attenuate phencyclidine versus d-amphetamine motor behaviors in rats. J Pharm Exp Ther. 1999;291:161–70.
48. Corti C, Crepaldi L, Mion S, Roth AL, Xuereb JH, Ferraguti F. Altered dimerization of metabotropic glutamate receptor 3 in schizophrenia. Biol Psychiatry. 2007;62:747–55.
49. Sartorius LJ, Nagappan G, Lipska BK, Lu B, Sei Y, Ren-Patterson R, et al. Alternative splicing of human metabotropic glutamate receptor 3. J Neurochem. 2006;96:1139–48.
50. Sartorius LJ, Weinberger DR, Hyde TM, Harrison PJ, Kleinman JE, Lipska BK. Expression of a GRM3 splice variant is increased in the dorsolateral prefrontal cortex of individuals carrying a schizophrenia risk SNP. Neuropsychopharmacology. 2008;33:2626–34.
51. Milligan G. G protein-coupled receptor dimerization: function and ligand pharmacology. Mol Pharm. 2004;66:1–7.
52. Chow KB, Sun J, Chu KM, Tai Cheung W, Cheng CH, Wise H. The truncated ghrelin receptor polypeptide (GHS-R1b) is localized in the endoplasmic reticulum where it forms heterodimers with ghrelin receptors (GHS-R1a) to attenuate their cell surface expression. Mol Cell Endocrinol. 2012;348:247–54.
53. Wise H. The roles played by highly truncated splice variants of G protein-coupled receptors. J Mol Signal. 2012;7:13.
54. Garcia-Bea A, Bermudez I, Harrison PJ, Lane TA. A group II metabotropic glutamate receptor 3 (mGlu3, GRM3) isoform implicated in schizophrenia interacts with canonical mGlu3 and reduces ligand binding. J Psychopharmacol. 2017;31:1519–26.
55. Yoon JH, Maddock RJ, Rokem A, Silver MA, Minzenberg MJ, Ragland JD, et al. GABA concentration is reduced in visual cortex in schizophrenia and correlates with orientation-specific surround suppression. J Neurosci. 2010;30:3777–81.
56. Schmidt MJ, Mirnics K. Neurodevelopment, GABA system dysfunction, and schizophrenia. Neuropsychopharmacology. 2015;40:190–206.
57. de Jonge JC, Vinkers CH, Hulshoff Pol HE, Marsman A. GABAergic mechanisms in schizophrenia: linking postmortem and in vivo studies. Front Psychiatry. 2017;8:118.
58. Kilonzo VW, Sweet RA, Glausier JR, Pitts MW. Deficits in glutamic acid decarboxylase 67 immunoreactivity, parvalbumin interneurons, and perineuronal nets in the inferior colliculus of subjects with schizophrenia. Schizophr Bull. 2020;46:1053–9.
59. Szabo G, Katarova Z, Greenspan R. Distinct protein forms are produced from alternatively spliced bicistronic glutamic acid decarboxylase mRNAs during development. Mol Cell Biol. 1994;14:7535–45.
60. Erlander MG, Tillakaratne NJ, Feldblum S, Patel N, Tobin AJ. Two genes encode distinct glutamate decarboxylases. Neuron. 1991;7:91–100.
61. Curley AA, Arion D, Volk DW, Asafu-Adjei JK, Sampson AR, Fish KN, et al. Cortical deficits of glutamic acid decarboxylase 67 expression in schizophrenia: clinical, protein, and cell type-specific features. Am J Psychiatry. 2011;168:921–9.
62. Akbarian S, Kim JJ, Potkin SG, Hagman JO, Tafazzoli A, Bunney WE Jr., et al. Gene expression for glutamic acid decarboxylase is reduced without loss of neurons in prefrontal cortex of schizophrenics. Arch Gen Psychiatry. 1995;52:258–66.
63. Tao R, Davis KN, Li C, Shin JH, Gao Y, Jaffe AE, et al. GAD1 alternative transcripts and DNA methylation in human prefrontal cortex and hippocampus in brain development, schizophrenia. Mol Psychiatry. 2018;23:1496–505.
64. Hyde TM, Lipska BK, Ali T, Mathew SV, Law AJ, Metitiri OE, et al. Expression of GABA signaling molecules KCC2, NKCC1, and GAD1 in cortical development and schizophrenia. J Neurosci. 2011;31:11088–95.
65. Birnbaum R, Weinberger DR. Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci. 2017;18:727–40.
66. Weinberger DR. The neurodevelopmental origins of schizophrenia in the penumbra of genomic medicine. World Psychiatry. 2017;16:225–6.
67. Weinberger DR. Implications of normal brain development for the pathogenesis of schizophrenia. Arch Gen Psychiatry. 1987;44:660–9.
68. Represa A, Ben-Ari Y. Trophic actions of GABA on neuronal development. Trends Neurosci. 2005;28:278–83.
69. Millar JK, Wilson-Annan JC, Anderson S, Christie S, Taylor MS, Semple CAM, et al. Disruption of two novel genes by a translocation co-segregating with schizophrenia. Hum Mol Genet. 2000;9:1415–23.
70. Chubb JE, Bradshaw NJ, Soares DC, Porteous DJ, Millar JK. The DISC locus in psychiatric illness. Mol Psychiatry. 2008;13:36–64.
71. Brandon NJ, Sawa A. Linking neurodevelopmental and synaptic theories of mental illness through DISC1. Nat Rev Neurosci. 2011;12:707–22.
72. Clapcote SJ, Lipina TV, Millar JK, Mackie S, Christie S, Ogawa F, et al. Behavioral phenotypes of Disc1 missense mutations in mice. Neuron. 2007;54:387–402.
73. Hikida T, Jaaro-Peled H, Seshadri S, Oishi K, Hookway C, Kong S, et al. Dominant-negative DISC1 transgenic mice display schizophrenia-associated phenotypes detected by measures translatable to humans. Proc Natl Acad Sci U S A. 2007;104:14501–6.
74. Mao Y, Ge X, Frank CL, Madison JM, Koehler AN, Doud MK, et al. Disrupted in schizophrenia 1 regulates neuronal progenitor proliferation via modulation of GSK3beta/beta-catenin signaling. Cell. 2009;136:1017–31.
75. Kim JY, Liu CY, Zhang F, Duan X, Wen Z, Song J, et al. Interplay between DISC1 and GABA signaling regulates neurogenesis in mice and risk for schizophrenia. Cell. 2012;148:1051–64.
76. Nakata K, Lipska BK, Hyde TM, Ye T, Newburn EN, Morita Y, et al. DISC1 splice variants are upregulated in schizophrenia and associated with risk polymorphisms. Proc Natl Acad Sci U S A. 2009;106:15873–8.
77. Newburn EN, Hyde TM, Ye T, Morita Y, Weinberger DR, Kleinman JE, et al. Interactions of human truncated DISC1 proteins: implications for schizophrenia. Transl Psychiatry. 2011;1:e30.
78. Fazzari P, Paternain AV, Valiente M, Pla R, Lujan R, Lloyd K, et al. Control of cortical GABA circuitry development by Nrg1 and ErbB4 signalling. Nature. 2010;464:1376–80.
79. Ting AK, Chen Y, Wen L, Yin DM, Shen C, Tao Y, et al. Neuregulin 1 promotes excitatory synapse development and function in GABAergic interneurons. J Neurosci. 2011;31:15–25.
80. Harrison PJ, Law AJ. Neuregulin 1 and schizophrenia: genetics, gene expression, and neurobiology. Biol Psychiatry. 2006;60:132–40.
81. Mei L, Nave KA. Neuregulin-ERBB signaling in the nervous system and neuropsychiatric diseases. Neuron. 2014;83:27–49.
82. Mei L, Xiong WC. Neuregulin 1 in neural development, synaptic plasticity and schizophrenia. Nat Rev Neurosci. 2008;9:437–52.
83. Del Pino I, Garcia-Frigola C, Dehorter N, Brotons-Mas JR, Alvarez-Salvado E, Martinez de Lagran M, et al. Erbb4 deletion from fast-spiking interneurons causes schizophrenia-like phenotypes. Neuron. 2013;79:1152–68.
84. Tan W, Wang Y, Gold B, Chen J, Dean M, Harrison PJ, et al. Molecular cloning of a brain-specific, developmentally regulated neuregulin 1 (NRG1) isoform and identification of a functional promoter variant associated with schizophrenia. J Biol Chem. 2007;282:24343–51.

85. Hall J, Whalley HC, Job DE, Baig BJ, McIntosh AM, Evans KL, et al. A neuregulin 1 variant associated with abnormal cortical function and psychotic symptoms. Nat Neurosci. 2006;9:1477–8.
86. Yang JZ, Si TM, Ruan Y, Ling YS, Han YH, Wang XL, et al. Association study of neuregulin 1 gene with schizophrenia. Mol Psychiatry. 2003;8:706–9.
87. Alaerts M, Ceulemans S, Forero D, Moens LN, De Zutter S, Heyrman L, et al. Support for NRG1 as a susceptibility factor for schizophrenia in a northern Swedish isolated population. Arch Gen Psychiatry. 2009;66:828–37.
88. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, et al. Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet. 2002;71:877–92.
89. Hashimoto R, Straub RE, Weickert CS, Hyde TM, Kleinman JE, Weinberger DR. Expression analysis of neuregulin-1 in the dorsolateral prefrontal cortex in schizophrenia. Mol Psychiatry. 2004;9:299–307.
90. Law AJ, Lipska BK, Weickert CS, Hyde TM, Straub RE, Hashimoto R, et al. Neuregulin 1 transcripts are differentially expressed in schizophrenia and regulated by 5’ SNPs associated with the disease. Proc Natl Acad Sci U S A. 2006;103:6747–52.
91. Moon E, Rollins B, Mesen A, Sequeira A, Myers RM, Akil H, et al. Lack of association to a NRG1 missense polymorphism in schizophrenia or bipolar disorder in a Costa Rican population. Schizophr Res. 2011;131:52–7.
92. Nicodemus KK, Law AJ, Luna A, Vakkalanka R, Straub RE, Kleinman JE, et al. A 5’ promoter region SNP in NRG1 is associated with schizophrenia risk and type III isoform expression. Mol Psychiatry. 2009;14:741–3.
93. Weickert CS, Tiwari Y, Schofield PR, Mowry BJ, Fullerton JM. Schizophrenia-associated HapICE haplotype is associated with increased NRG1 type III expression and high nucleotide diversity. Transl Psychiatry. 2012;2:e104.
94. Papaleo F, Yang F, Paterson C, Palumbo S, Carr GV, Wang Y, et al. Behavioral, neurophysiological, and synaptic impairment in a transgenic neuregulin1 (NRG1-IV) murine schizophrenia model. J Neurosci. 2016;36:4859–75.
95. Olaya JC, Heusner CL, Matsumoto M, Sinclair D, Kondo MA, Karl T, et al. Overexpression of neuregulin 1 type III confers hippocampal mRNA alterations and schizophrenia-like behaviors in mice. Schizophr Bull. 2018;44:865–75.
96. Chen YJ, Johnson MA, Lieberman MD, Goodchild RE, Schobel S, Lewandowski N, et al. Type III neuregulin-1 is required for normal sensorimotor gating, memory-related behaviors, and corticostriatal circuit components. J Neurosci. 2008;28:6872–83.
97. Yin DM, Chen YJ, Lu YS, Bean JC, Sathyamurthy A, Shen C, et al. Reversal of behavioral deficits and synaptic dysfunction in mice overexpressing neuregulin 1. Neuron. 2013;78:644–57.
98. Veikkolainen V, Vaparanta K, Halkilahti K, Iljin K, Sundvall M, Elenius K. Function of ERBB4 is determined by alternative splicing. Cell Cycle. 2011;10:2647–57.
99. Chung DW, Volk DW, Arion D, Zhang Y, Sampson AR, Lewis DA. Dysregulated ErbB4 splicing in schizophrenia: selective effects on parvalbumin expression. Am J Psychiatry. 2016;173:60–8.
100. Law AJ, Kleinman JE, Weinberger DR, Weickert CS. Disease-associated intronic variants in the ErbB4 gene are related to altered ErbB4 splice-variant expression in the brain in schizophrenia. Hum Mol Genet. 2007;16:129–41.
101. Chung DW, Wills ZP, Fish KN, Lewis DA. Developmental pruning of excitatory synaptic inputs to parvalbumin interneurons in monkey prefrontal cortex. Proc Natl Acad Sci U S A. 2017;114:E629–E37.
102. Chung DW, Chung Y, Bazmi HH, Lewis DA. Altered ErbB4 splicing and cortical parvalbumin interneuron dysfunction in schizophrenia and mood disorders. Neuropsychopharmacology. 2018;43:2478–86.
103. Frazee AC, Sabunciyan S, Hansen KD, Irizarry RA, Leek JT. Differential expression analysis of RNA-seq data at single-base resolution. Biostatistics. 2014;15:413–26.
104. Araki Y, Hong I, Gamache TR, Ju S, Collado-Torres L, Shin JH, et al. SynGAP isoforms differentially regulate synaptic plasticity and dendritic development. Elife. 2020;9:e56273.
105. Yang X, Coulombe-Huntington J, Kang S, Sheynkman GM, Hao T, Richardson A, et al. Widespread expansion of protein interaction capabilities by alternative splicing. Cell. 2016;164:805–17.
106. Jaffe AE, Straub RE, Shin JH, Tao R, Gao Y, Collado-Torres L, et al. Developmental and genetic regulation of the human cortex transcriptome illuminate schizophrenia pathogenesis. Nat Neurosci. 2018;21:1117–25.
107. Walker RL, Ramaswami G, Hartl C, Mancuso N, Gandal MJ, de la Torre-Ubieta L, et al. Genetic control of expression and splicing in developing human brain informs disease mechanisms. Cell. 2019;179:750–71.e22.
108. Zhang D, Guelfi S, Garcia-Ruiz S, Costa B, Reynolds RH, D’Sa K, et al. Incomplete annotation has a disproportionate impact on our understanding of Mendelian and complex neurogenetic disorders. Sci Adv. 2020;6:eaay8299.
109. Jaffe AE, Shin J, Collado-Torres L, Leek JT, Tao R, Li C, et al. Developmental regulation of human cortex transcription and its clinical relevance at single base resolution. Nat Neurosci. 2015;18:154–61.
110. Takata A, Matsumoto N, Kato T. Genome-wide identification of splicing QTLs in the human brain and their enrichment among schizophrenia-associated loci. Nat Commun. 2017;8:14519.
111. Ma L, Shcherbina A, Chetty S. Variations and expression features of CYP2D6 contribute to schizophrenia risk. Mol Psychiatry. 2020. https://doi.org/10.1038/s41380-020-0675-y.
112. Ma L, Semick SA, Chen Q, Li C, Tao R, Price AJ, et al. Schizophrenia risk variants influence multiple classes of transcripts of sorting nexin 19 (SNX19). Mol Psychiatry. 2020;25:831–43.
113. Li M, Jaffe AE, Straub RE, Tao R, Shin JH, Wang Y, et al. A human-specific AS3MT isoform and BORCS7 are molecular risk factors in the 10q24.32 schizophrenia-associated locus. Nat Med. 2016;22:649–56.
114. Cai X, Yang ZH, Li HJ, Xiao X, Li M, Chang H. A human-specific schizophrenia risk tandem repeat affects alternative splicing of a human-unique isoform $AS3MT^{d2d3}$ and mushroom dendritic spine density. Schizophr Bull. 2021;47:219–27.
115. Aygün N, Elwell AL, Liang D, Lafferty MJ, Cheek KE, Courtney KP, et al. Genetic influences on cell type specific gene expression and splicing during neurogenesis elucidate regulatory mechanisms of brain traits. bioRxiv. 2020:349019.
116. Glantz LA, Lewis DA. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. Arch Gen Psychiatry. 2000;57:65–73.
117. Osimo EF, Beck K, Reis Marques T, Howes OD. Synaptic loss in schizophrenia: a meta-analysis and systematic review of synaptic protein and mRNA measures. Mol Psychiatry. 2019;24:549–61.
118. Penzes P, Cahill ME, Jones KA, VanLeeuwen JE, Woolfrey KM. Dendritic spine pathology in neuropsychiatric disorders. Nat Neurosci. 2011;14:285–93.
119. Forrest MP, Parnell E, Penzes P. Dendritic structural plasticity and neuropsychiatric disease. Nat Rev Neurosci. 2018;19:215–34.
120. Tao R, Cousijn H, Jaffe AE, Burnet PW, Edwards F, Eastwood SL, et al. Expression of ZNF804A in human brain and alterations in schizophrenia, bipolar disorder, and major depressive disorder: a novel transcript fetally regulated by the psychosis risk variant rs1344706. JAMA Psychiatry. 2014;71:1112–20.

121. Chang H, Xiao X, Li M. The schizophrenia risk gene ZNF804A: clinical associations, biological mechanisms and neuronal functions. Mol Psychiatry. 2017;22:944–53.
122. Deans PJM, Raval P, Sellers KJ, Gatford NJF, Halai S, Duarte RRR, et al. Psychosis risk candidate ZNF804A localizes to synapses and regulates neurite formation and dendritic spine structure. Biol Psychiatry. 2017;82:49–61.
123. Huang Y, Huang J, Zhou QX, Yang CX, Yang CP, Mei WY, et al. ZFP804A mutant mice display sex-dependent schizophrenia-like behaviors. Mol Psychiatry. 2020. https://doi.org/10.1038/s41380-020-00972-4.
124. Zhou D, Xiao X, Li M. The schizophrenia risk isoform ZNF804A^{E3E4} affects dendritic spine. Schizophr Res. 2020;218:324–5.
125. Clark MB, Wrzesinski T, Garcia AB, Hall NAL, Kleinman JE, Hyde T, et al. Long-read sequencing reveals the complex splicing profile of the psychiatric risk gene CACNA1C in human brain. Mol Psychiatry. 2020;25:37–47.
126. Missler M, Sudhof TC. Neurexins: three genes and 1001 products. Trends Genet. 1998;14:20–6.
127. Hu Z, Xiao X, Zhang Z, Li M. Genetic insights and neurobiological implications from NRXN1 in neuropsychiatric disorders. Mol Psychiatry. 2019;24:1400–14.
128. Ullrich B, Ushkaryov YA, Sudhof TC. Cartography of neurexins: more than 1000 isoforms generated by alternative splicing and expressed in distinct subsets of neurons. Neuron. 1995;14:497–507.
129. Jenkins AK, Paterson C, Wang Y, Hyde TM, Kleinman JE, Law AJ. Neurexin 1 (NRXN1) splice isoform expression during human neocortical development and aging. Mol Psychiatry. 2016;21:701–6.
130. Flaherty E, Zhu S, Barretto N, Cheng E, Deans PJM, Fernando MB, et al. Neuronal impact of patient-specific aberrant NRXN1alpha splicing. Nat Genet. 2019;51:1679–90.
131. Shepard PD, Canavier CC, Levitan ES. Ether-a-go-go-related gene potassium channels: what's all the buzz about? Schizophr Bull. 2007;33:1263–9.
132. Morais Cabral JH, Lee A, Cohen SL, Chait BT, Li M, Mackinnon R. Crystal structure and functional analysis of the HERG potassium channel N terminus: a eukaryotic PAS domain. Cell. 1998;95:649–55.
133. Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, et al. A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. Nat Med. 2009;15:509–18.
134. Apud JA, Zhang F, Decot H, Bigos KL, Weinberger DR. Genetic variation in KCNH2 associated with expression in the brain of a unique hERG isoform modulates treatment response in patients with schizophrenia. Am J Psychiatry. 2012;169:725–34.
135. Heide J, Zhang F, Bigos KL, Mann SA, Carr VJ, Shannon Weickert C, et al. Differential response to Risperidone in schizophrenia patients by KCNH2 genotype and drug metabolizer status. Am J Psychiatry. 2016;173:53–9.
136. Carr GV, Chen J, Yang F, Ren M, Yuan P, Tian Q, et al. KCNH2-3.1 expression impairs cognition and alters neuronal function in a model of molecular pathology associated with schizophrenia. Mol Psychiatry. 2016;21:1517–26.
137. Ren M, Hu Z, Chen Q, Jaffe A, Li Y, Sadashivaiah V, et al. KCNH2-3.1 mediates aberrant complement activation and impaired hippocampal-medial prefrontal circuitry associated with working memory deficits. Mol Psychiatry. 2020;25:206–29.
138. Liao HM, Chao YL, Huang AL, Cheng MC, Chen YJ, Lee KF, et al. Identification and characterization of three inherited genomic copy number variations associated with familial schizophrenia. Schizophr Res. 2012;139:229–36.
139. Boot E, Kant SG, Otter M, Cohen D, Nabanizadeh A, Baas RW. Overexpression of chromosome 15q11-q13 gene products: a risk factor for schizophrenia and associated psychoses? Am J Psychiatry. 2012;169:96–7.
140. Noor A, Dupuis L, Mittal K, Lionel AC, Marshall CR, Scherer SW, et al. 15q11.2 duplication encompassing only the UBE3A gene is associated with developmental delay and neuropsychiatric phenotypes. Hum Mutat. 2015;36:689–93.
141. Ingason A, Kirov G, Giegling I, Hansen T, Isles AR, Jakobsen KD, et al. Maternally derived microduplications at 15q11-q13: implication of imprinted genes in psychotic illness. Am J Psychiatry. 2011;168:408–17.
142. Li Z, Chen J, Xu Y, Yi Q, Ji W, Wang P, et al. Genome-wide analysis of the role of copy number variation in schizophrenia risk in Chinese. Biol Psychiatry. 2016;80:331–7.
143. Greer PL, Hanayama R, Bloodgood BL, Mardinly AR, Lipton DM, Flavell SW, et al. The Angelman syndrome protein Ube3A regulates synapse development by ubiquitinating arc. Cell. 2010;140:704–16.
144. Sato M, Stryker MP. Genomic imprinting of experience-dependent cortical plasticity by the ubiquitin ligase gene Ube3a. Proc Natl Acad Sci U S A. 2010;107:5611–6.
145. Ebert DH, Greenberg ME. Activity-dependent neuronal signalling and autism spectrum disorder. Nature. 2013;493:327–37.
146. Valluy J, Bicker S, Aksoy-Aksel A, Lackinger M, Sumer S, Fiore R, et al. A coding-independent function of an alternative Ube3a transcript during neuronal development. Nat Neurosci. 2015;18:666–73.
147. Siva K, Covello G, Denti MA. Exon-skipping antisense oligonucleotides to correct missplicing in neurogenetic diseases. Nucleic Acid Ther. 2014;24:69–86.
148. Liu H, Pizzano S, Li R, Zhao W, Veling MW, Hu Y, et al. isoTarget: a genetic method for analyzing the functional diversity of splicing isoforms in vivo. Cell Rep. 2020;33:108361.
149. Patil ST, Zhang L, Martenyi F, Lowe SL, Jackson KA, Andreev BV, et al. Activation of mGlu2/3 receptors as a new approach to treat schizophrenia: a randomized Phase 2 clinical trial. Nat Med. 2007;13:1102–7.
150. Marti-Solano M, Crilly SE, Malinverni D, Munk C, Harris M, Pearce A, et al. Combinatorial expression of GPCR isoforms affects signalling and drug responses. Nature. 2020;587:650–6.
151. Straub RE, Lipska BK, Egan MF, Goldberg TE, Callicott JH, Mayhew MB, et al. Allelic variation in GAD1 (GAD67) is associated with schizophrenia and influences cortical function and gene expression. Mol Psychiatry. 2007;12:854–69.
152. Mukai J, Liu H, Burt RA, Swor DE, Lai WS, Karayiorgou M, et al. Evidence that the gene encoding ZDHHC8 contributes to the risk of schizophrenia. Nat Genet. 2004;36:725–31.
153. Kao WT, Wang Y, Kleinman JE, Lipska BK, Hyde TM, Weinberger DR, et al. Common genetic variation in Neuregulin 3 (NRG3) influences risk for schizophrenia and impacts NRG3 expression in human brain. Proc Natl Acad Sci U S A. 2010;107:15619–24.
154. Paterson C, Wang Y, Hyde TM, Weinberger DR, Kleinman JE, Law AJ. Temporal, diagnostic, and tissue-specific regulation of NRG3 isoform expression in human brain development and affective disorders. Am J Psychiatry. 2017;174:256–65.
155. Silberberg G, Darvasi A, Pinkas-Kramarski R, Navon R. The involvement of ErbB4 with schizophrenia: association and expression studies. Am J Med Genet B Neuropsychiatr Genet. 2006;141B:142–8.
156. Wong J, Rothmond DA, Webster MJ, Weickert CS. Increases in two truncated TrkB isoforms in the prefrontal cortex of people with schizophrenia. Schizophr Bull. 2013;39:130–40.

157. Weickert CS, Ligons DL, Romanczyk T, Ungaro G, Hyde TM, Herman MM, et al. Reductions in neurotrophin receptor mRNAs in the prefrontal cortex of patients with schizophrenia. Mol Psychiatry. 2005;10:637–50.
158. Thompson Ray M, Weickert CS, Wyatt E, Webster MJ. Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. J Psychiatry Neurosci. 2011;36:195–203.
159. Reinhart V, Bove SE, Volfson D, Lewis DA, Kleiman RJ, Lanz TA. Evaluation of TrkB and BDNF transcripts in prefrontal cortex, hippocampus, and striatum from subjects with schizophrenia, bipolar disorder, and major depressive disorder. Neurobiol Dis. 2015;77:220–7.
160. Wong J, Hyde TM, Cassano HL, Deep-Soboslay A, Kleinman JE, Weickert CS. Promoter specific alterations of brain-derived neurotrophic factor mRNA in schizophrenia. Neuroscience. 2010;169:1071–84.
161. Kunii Y, Hyde TM, Ye T, Li C, Kolachana B, Dickinson D, et al. Revisiting DARPP-32 in postmortem human brain: changes in schizophrenia and bipolar disorder and genetic associations with t-DARPP-32 expression. Mol Psychiatry. 2014;19:192–9.