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Regulatory Genes and Pathways Disrupted in Autism Spectrum Disorders

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Abstract

Autism spectrum disorder (ASD) is a highly prevalent and complex genetic disorder. The complex genetic make-up of ASD has been extensively studied and both common and rare genetic variants in up to 1000 genes have been linked to increased ASD risk. While these studies highlight the genetic complexity and begin to provide a window for delineating pathways at risk in ASD, the pathogenicity and specific contribution of many mutations to the disorder are poorly understood. Defining the convergent pathways disrupted by this large number of ASD-associated genetic variants will help to understand disease pathogenesis and direct future therapeutic efforts for the groups of patients with distinct etiologies. Here, we review some of the common regulatory pathways including chromatin remodeling, transcription, and alternative splicing that have emerged as common features from genetic and transcriptomic profiling of ASD. For each category, we focus on one gene (*CHD8, FOXP1*, and *RBFOX1*) that is significantly linked to ASD and functionally characterized in recent years. Finally, we discuss genetic and transcriptomic overlap between ASD and other neurodevelopmental disorders.

Keywords

transcription; FOXP1; splicing; CHD8; RBFOX1; network; autism

Introduction

Autism spectrum disorder (ASD) is a neurodevelopmental condition with a prevalence of 1 in 59 children in the United States per recent estimates[1]. ASD is characterized by impairments in reciprocal social interactions as well as the presence of repetitive and restricted behaviors and interests[2]. The increased prevalence of the disease in siblings of ASD patients and greater ASD concordance rates in monozygotic twins compared with dizygotic twins confirmed that ASD has a major heritable component[3,4,5-7]. Within the last decade, numerous large-scale family-based whole exome and genome sequencing studies

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have identified a rapidly growing number of genes linked to ASD[8-16]. These studies, which include family cohorts with sporadic ASD (simplex) or with more than one affected individual (multiplex), resulted in the discovery of rare or common variants with various inheritance patterns. Interestingly, these studies uncovered the involvement of non-inherited genetic variants such as *de novo* (spontaneous)[8,13,14,17] and somatic mutations[18-20]. *De novo* mutations could arise in the germ cells of one parent or in the fertilized egg during embryogenesis resulting in an affected child with unaffected parents. Somatic mutations can occur at the later stages of development and yield mosaic individuals with distinct genomic content in subsets of cells[21]. Recurrence of genetic variants in independent cohorts as well as overlap of genes with inherited, *de novo*, and somatic mutations substantiates the pathogenicity of these mutations in ASD[22] and rank them to a “high-confidence” category. Taken together, these studies underscore the complexity of the genetic landscape of the disease and begin to illuminate the biological pathways at risk in ASD. This complex genetic architecture also raises the possibility that certain combinations of common genetic variants contribute to ASD by modifying the pathogenic effects of rare inherited, *de novo* or somatic mutations.

Given the progress in gene discovery in large-scale family based studies, the pressing challenge now is to prioritize high-confidence causal genes in ASD for further functional studies validating and defining the pathogenicity. Several approaches have been taken to pinpoint high confidence causative genetic variants. First, recurrent mutations of a given gene found in independent family cohorts and unrelated individuals with ASD can “rank” the gene to a high confidence category[23,24]. Second, predictions of the damaging potential of a mutation to gene structure and function are also taken into account when prioritizing the loss-of-function (LOF) mutations[8]. Third, a network-level approach involving functional annotation, gene lists implicated in brain development, neuronal function or monogenetic syndromes is being used to assess the functional relevance of newly identified genes[25,26]. Fourth, integrating transcriptomic analysis of ASD post-mortem tissue is also providing information on the pathways disrupted in ASD[27-34]. Knowledge acquired from transcriptome studies can be used as a framework to assess the novel candidate genes for their involvement in particular pathways affected in ASD. These approaches have helped predict pathogenicity of the large catalog of ASD-associated genetic variants; however, the functional impact of each mutation on the developing brain still needs to be determined and validated in experimental models including three-dimensional brain organoids[22] and rodent models[36].

The polygenic nature of ASD is also supported by the fact that many high confidence ASD mutations reside in regulatory genes encoding chromatin remodelers, transcription factors (TFs) and RNA binding proteins (RBPs) that can further regulate a multitude of developmental programs rather than a single gene function. Thus, loss-of-function mutations within one of these key master regulators can cause ASD by leading to dysregulation of an entire network of genes that coexpress and function together during critical windows of neurodevelopment.

Here, we will review the recent progress on three gene regulatory pathways implicated in ASD as common mechanisms (chromatin remodeling, transcriptional control and alternative

splicing) with the focus and example of high-confidence and relatively well-studied ASD genes linked to these respective pathways (*CHD8*, *FOXP1, RBFOX1*) **(Figure 1).** We will describe the mechanistic insights that have emerged from cell and animals models for these high-confidence ASD genes. Finally, we will discuss increasing evidence for shared molecular features of ASD with other neurodevelopmental disorders, in particular schizophrenia (SCZ).

Chromatin Remodeling

Regulation of gene expression plays a predominant role in cell fate determination and maintenance during human brain development[37]. The local chromatin state surrounding any given gene is an important determinant for the gene to be “on” or “off” and is regulated by the chromatin remodeling complexes[38]. Proper regulation of chromatin states is critical for ensuring key genetic programs are in place during developmental stages. The role of gene regulation at the chromatin level in human cortical development and function is further supported by the identification of mutations in chromatin remodeling genes linked to neurodevelopmental and neuropsychiatric disorders[39,40].

The gene encoding the chromodomain helicase DNA-binding protein 8, *CHD8*, has emerged as a high-confidence ASD gene. Recurrence of rare, *de novo*, LOF mutations in *CHD8* among unrelated individuals with ASD points to chromatin remodeling as a converging molecular disease mechanism[8,13,14,17,23,41,42]. In addition to typical features of ASD, patients harboring *CHD8* mutations often are co-morbid for macrocephaly, facial dysmorphisms, and intellectual disability[42,43].

CHD8 is an ATP-dependent chromatin remodeling protein, which is a member of the chromodomain-helicase-DNA binding protein family. Regulatory roles for CHD8 in Wnt signaling[44] and apoptosis[45] have been implicated; however, knowledge of the cellular function of CHD8, particularly in brain, is sparse. The strong genetic link of CHD8 in ASD has fuelled mechanistic studies geared towards understanding the role of CHD8 in brain development and function along with the consequences of reduced CHD8 levels in animal and cell models.

Modeling disease-associated haploinsufficieny of CHD8 through knockdown studies in human neural cell models followed by RNA-sequencing (RNA-seq) facilitated the identification of the subset of genes regulated by CHD8[46-48]. While reduced CHD8 levels lead to altered expression of hundreds of genes, other known ASD risk genes are significantly enriched among the downregulated but not upregulated genes upon CHD8 reduction[46-48]. The use of chromatin immunoprecipitation-sequencing (ChIP-seq) in cell models[46,47] and human midfetal brain tissue[47] showed that CHD8 binds to active promoter regions marked with trimethylated histone H3 lysine 4. In agreement with knockdown studies, genes that are identified as direct targets of CHD8 in developing human brain are also enriched for ASD candidate genes[47]. These data highlight the possibility that the majority of ASD risk genes are co-expressed and subject to co- and crossregulation. CHD8 is likely to have a prominent regulatory role in critical co-expression networks and the loss

of CHD8 thereby contributes to ASD pathogenesis by disrupting numerous downstream cellular processes.

Multiple genetically modified mice with decreased expression of CHD8 have been developed to characterize the impact of reduced CHD8 levels on brain development and behavioral outcomes **(Table 1)**. Whereas homozygous deletion of *Chd8* is embryonically lethal in mice[45], haploinsufficient models of *Chd8* have been established through conventional exon targeting[49,50], *in utero* knockdown of CHD8[51], or introducing a gene-disrupting mutation via CRISPR/Cas9 gene editing[52,53]. Consistent with the macrocephaly observed in patients harboring *CHD8* mutations[42], imaging[49,50,53] and histological[52] examination of heterozygous *Chd8* mutant mice show increased brain volume relatively to wild type littermates. CHD8 mutant and *in utero* knockdown models manifest some degree of altered behavior potentially relevant to clinical features of ASD however; these results are highly variable between studies. While two of the heterozygous knockout[49,53] and knockdown models[51] show mild deficits in social interaction, two different knockout models are reported to have normal social behavior[50,52]. Similarly, cognitive deficits are found only in one of the models[52]. The most recent study also reported a motor deficit in mutant mice[50], while former models did not show atypical motor function. Platt *et al.*[53] observed an increased acquired motor learning phenotype in Chd8 knock-in mice. The authors linked this behavior to synaptic dysfunction of spiny projection neurons in the nucleus accumbens (NAc) via a region-specific targeting of Chd8 in adult animals in NAc[53]. These findings implicate region-specific roles for CHD8 and support the role of NAc dysfunction in ASD. Although these studies are able to recapitulate various behavioral aspects of the disease, discrepancies in the behavioral outcomes among studies need to be addressed. While such behavioral assays could be confounded by genetic background, sex, or age of the animals tested as well as the sensitivity of the techniques, these differences might also result from uncharacterized differences in CHD8 dose among different models.

Gene expression studies of these mice captured subtle yet widespread changes in gene expression consistent with the studies in ASD human data. Differentially expressed genes are consistently enriched for functional annotations including chromatin and histone modification, and cell-cycle regulation[49,51,52]. These data suggest involvement of a network of epigenetic modifiers in CHD8-mediated gene regulation. Dysregulation of cell-cycle genes are consistent with the macrocephaly phenotypes. More specifically, mice heterozygous for *Chd8* show elevated expression levels of genes involved in early fetal development and downregulation of genes expressed during mid-fetal stages, indicating a developmental delay[49]. Based on gene expression profiles, each study has identified potential downstream mechanisms of *Chd8* haploinsufficiency including activation of the REST complex[49] or disrupted Wnt signaling[51,53]. Remarkably, Gompers *et al.*[52] identified downregulation of a group of genes responsible for RNA processing and widespread alternative splicing changes in *Chd8* heterozygous mice. Thus, CHD8 can indirectly regulate alternative splicing, another convergent mechanism implicated in ASD discussed below, by controlling the expression of RNA processing genes.

Genetic evidence for the involvement of *CHD8* in ASD is particularly strong. Studies of cell and animal models show that CHD8 is required for neuronal function and regulates a

network of genes critical for early neurodevelopment. Moving forward to therapeutic strategies would require addressing a number of remaining questions including: 1) What is the mechanism CHD8 uses to either repress or activate genes? 2) What is the therapeutic window for reversing phenotypes related to dysfunction of CHD8? 3) Can CHD8-regulated events also be dysregulated due to environmental factors in the absence of a mutation?

Transcriptional Control

Transcription factors play a key role in intricate regulation of the spatial and temporal gene expression patterns important for brain development[37,54]. Work over the past few decades has identified a number of transcription factors that cooperatively and/or hierarchically control proper brain development. Variants in genes encoding transcription factor and dysregulated gene expression have been reported in neurodevelopmental disorders highlighting the need for the identification of gene networks regulated by the transcription factors implicated in both brain development and disease states.

The gene encoding the Forkhead box transcription factor 1, *FOXP1*, has been implicated in neurodevelopmental disorders such as ASD and ID[55]. Numerous studies have identified geneinterrupting variants of *FOXP1* including heterozygous deletions, duplications, and missense and nonsense mutations in both case reports and recent large-scale profiling of patients with ASD and ID, ranking *FOXP1* as one of the high-confidence causal ASD genes[17,56-58,40,59-61]. Moreover, *FOXP2*, a paralog of *FOXP1* is linked to human speech and language development suggesting a prominent role for FOXP proteins in human cognitive function including language[55,62-64]. Collectively, these studies have provided strong evidence for *FOXP1* mutations underlying specific cognitive phenotypes, and have prompted research on FOXP1 function in brain.

Several groups have begun to elucidate a role for FOXP1 in neurodevelopment and cognitive function **(Table 1)**. Mice with brain-specific deletion of *Foxp1* exhibit widespread morphological defects throughout the brain including enlarged lateral ventricles, impaired striatal development, and decreased density of CA1 neurons in hippocampus[65]. These structural alterations are accompanied by an excitatory/inhibitory (E/I) imbalance in hippocampal CA1 neurons. Behavioral deficits in these mice include increased repetitive behaviors, decreased social interest and impairments in spatial memory. Moreover, heterozygous knockout *Foxp1* mice modeling patient-relevant haploinsufficiency show increased excitability of striatal spiny projection neurons (SPNs) and defects in neonatal ultrasonic vocalizations (USVs)[66]. Gene expression and co-expression module preservation analyses of the heterozygous knockout mice with human neuronal data demonstrate that Foxp1 orchestrates gene expression networks important for striatal development and function that are at risk in ASD. These results from the brain-specific and heterozygous knockout mice models support the functional significance of FOXP1 in neurodevelopment, social and cognitive function, and vocal communication; however, these studies are limited in linking behavioral outcomes to region-specific defects caused by the loss of Foxp1. This is relevant because unlike CHD8, FOXP1 is not widely expressed in the brain.

To address a region-specific role of Foxp1, *in utero* knockdown of Foxp1 expression in developing neocortex results in defective neuronal migration and neurite development; however, behavioral outcomes of decreased cortical levels of Foxp1 in this model have yet to be reported[67]. A more complete characterization of the brain region-specific role of Foxp1 comes from studies of conditional knockout mice with loss of Foxp1 in the pyramidal neurons of the neocortex and the hippocampus[68]. These mice exhibit hyperactivity, decreased sociability, impaired hippocampal-based spatial learning and memory highlighting the role of Foxp1 in the hippocampus[68]. Consistent with behavioral deficits indicative of impaired hippocampal function, these mice present with a decreased late-phase long-term potentiation (LTP) response. Pathways disturbed due to loss of Foxp1 in the hippocampus that could potentially contribute to the LTP and spatial learning deficits were examined using genomic approaches. Gene ontology categories of differentially expressed genes downstream of Foxp1 in the hippocampus include abnormal synaptic transmission, and abnormal learning/memory/conditioning in agreement with the behavioral and electrophysiological characterization[68]. In addition, deletion of Foxp1 in pyramidal neurons of the forebrain results in impaired vocal communication in postnatal stages in mice[69]. Structural changes that occurred from loss of cortical Foxp1 include reduced overall neocortical size and mispositioning of neurons in the deep layers of the mouse neocortex[69]. Transcriptional networks regulated by Foxp1 in early development include genes that are responsible for neurogenesis and neuronal migration. Both in hippocampus and neocortex, Foxp1 regulated genes are enriched for other ASD genes[68,69]. Taken together, these studies provide insights into the role of Foxp1 in distinct brain regions and highlights brain-region specific features of a complex disorder.

In summary, Foxp1 regulates distinct sets of transcriptional programs in different brain regions and loss of Foxp1 function yields social and cognitive deficits. Disentangling these diverse functions of Foxp1 in different brain regions and cell-types will be important for understanding region-specific pathophysiology of the disease and guiding future therapeutic efforts. Future studies focused on the role of Foxp1 in striatum will be important for complete understanding of the molecular basis for complex ASD presentation as striatal circuits are affected in ASD and brain-specific Foxp1 knockout mice show striking striatal defects.

Alternative Splicing

There is a growing body of evidence showing the prominent role of alternative post-transcriptional processing events including alternative splicing (AS) and polyadenylation in human brain. Considering the limited number of protein coding genes in the human genome, AS is increasingly recognized as the primary source of transcriptomic and proteomic diversity and complexity driving the species-specific features of humans including brain evolution[70-72]. AS is coor post-transcriptionally regulated by RNA binding proteins (RBPs) and tightly controlled during normal development stages in a tissue-specific manner[73]. Consistent with the presumed role of alternative splicing regulation in human brain, erroneous AS regulation has been implicated in many neurologic diseases including frontotemporal dementia and myotonic dystrophy[74].

Transcriptomic profiling of ASD has increasingly pointed to dysregulation of AS as a convergent mechanism for disease pathogenesis. ASD-linked copy number variations and chromosomal translocation in one particular neuronal RBP with a role in AS, *RBFOX1*, have been highlighted in patient cohorts[11,75,76]. In addition, transcriptomic analyses of ASD postmortem brains have identified dysregulated RBFOX1 function as a common feature of genetically distinct ASD cases, supporting a prominent role for loss and/or dysregulation of RBFOX1 activity in ASD pathogenesis[27,28].

Several studies have begun to characterize the role of RBFOX1 and understand the functional impact of defective RBFOX1 function **(Table 1).** Brain-specific knockout mice show spontaneous and induced seizures and aberrantly increased neuronal activity[77]. The loss of *Rbfox1* resulted in increased excitability in dentate gyrus consistent with the imbalanced E/I activity observed in other ASD models[77]. A separate study also reported decreased inhibitory synaptic transmission in CA1 neurons of this model[78]. Whole-transcriptome profiling by RNA-seq identified gene expression and alternative splicing changes in the knockout mice[77]. *In utero* knockdown of *Rbfox1* caused defects in neuronal migration, neuronal placement, and dendritic arbor formation during corticogenenis; however, behavioral consequences of these defects have yet to be determined[79]. Specific functional consequences of loss of Rbfox proteins were also investigated in motor neurons differentiated from mouse embryonic stem cells (ESCs) lacking all three members of the Rbfox protein family (Rbfox1, Rbfox, and Rbfox3)[80]. These triple knockout neurons show immature electrophysiological activity and defective axon initial segment assembly (AIS). Remarkably, defects in AIS have previously been implicated for ASD as high-confidence variants are found in genes involved in this process including *SCN2A*[81]. Depletion of Rbfox proteins led to missplicing of genes encoding cytoskeletal, cell membrane and synaptic proteins. Moreover, studies in mice delineated differential roles of cytoplasmic and nuclear isoforms of Rbfox1[82]. In addition to canonical splicing regulation by nuclear Rbfox1, the cytoplasmic isoform of Rbfox1 elicits distinct functions including regulation of RNA stability and translation. Vamp1, a vSNARE protein was identified as one of the downstream targets of cytoplasmic Rbfox1 and shown to be downregulated in *Rbfox1* knockout mice as a result of loss of post-transcriptional regulation. Forced expression of Vamp1 using AAV mediated delivery in *Rbfox1* knockout mice rescued inhibitory synaptic transmission defects in those mice[78]. These findings suggest several aspects of RNA metabolism including translation efficiency and stability might be dysregulated in ASD due to the loss of RBFOX1 function.

Studies of human neural progenitor cells suggested that a larger network of RBPs along with RBFOX1 is co-expressed during development, and can potentially function together in post-transcriptional regulation of cortical development[83,84]. Moreover, the overlapping targets in this network are important for neuronal development and are likely disrupted in ASD. For example, the ELAVL2 binding motif was enriched in alternatively spliced exons in human neurons with decreased levels of RBFOX1 suggesting a coordinated combinatorial regulation of RNA processing by RBFOX1 and ELAVL2. Consistent with this hypothesis, transcripts misspliced in postmortem ASD brains are also enriched for cellular targets of several RBPs including SRRM4[29] and PTB1[27].

These data highlight RBP function, including those of RBFOX1, as essential for cortical development and function, and at risk in ASD. Taken together, dysregulation of RNA processing may be a unifying feature of genetically diverse ASD cases, and regulation of these processes might be viable targets for therapeutic strategies.

Overlapping Pathways

Genetic and transcriptomic profiling of ASD has found overlapping molecular underpinning for ASD and other neurodevelopmental disorders, in particular schizophrenia (SCZ). In fact, genetic variants in *CHD8*[85], *FOXP1*[86], and *RBFOX1*[87] have been also reported in patients with SCZ. Additional *de novo* mutations have been identified in overlapping chromatin and synaptic genes in ASD and SCZ patients[8,88]. Moreover, examination of microarray and RNA-seq data from postmortem brain tissue across several neuropsychiatric disorders including ASD and SCZ has revealed similar transcriptome signatures for ASD and SCZ with downregulation of synaptic genes and upregulation of astroglial genes[31]. While these data highlight the possibility of shared pathways at risk in both ASD and SCZ, these results cannot explain how perturbations of similar genes results in strikingly distinct clinical representations. One potential explanation is that distinct combinations of common genetic variants in each individual genome determine the expressivity and penetrance of rare, disease-associated variants. Additionally, the biological impact of different mutations on the same gene might lead to distinct pathogenesis leading to ASD or SCZ. For example, missense variants in the *SCN2A* gene encoding for a neuronal sodium channel have been linked to both ASD[59] and infantile seizures[89]. The majority of *SCN2A* variants associated with infantile seizures are predicted to have gain of function effects leading to a hyperexcitability phenotype[90,91]. In contrast, bioinformatics and electrophysiological characterization of ASD-associated missense *SCN2A* variants have revealed their LoF effects leading to drastic reduction on channel conductance[92]. One recent example of differential pathogenesis caused by distinct mutations of the same gene came from patients harboring mutations in the *PUM1* gene, a gene encoding an RBP[93]. Based on the severity of each LoF mutation in this gene, individuals harboring mutations either present with a severe developmental delay or developed an adult-onset ataxia[93]. A similar dichotomous effect was also reported for genetic variants of the gene RORα, which encodes the RAR-related orphan nuclear receptor alpha[94]. In this case, individuals with LoF variants presented with ID and ASD, whereas individuals with dominant toxic variants showed ID and ataxia. Finally, it is worth noting that these transcriptome data come from bulk brain tissue, and thus the reported gene expression levels represent the average levels across highly heterogeneous cell types and fail to determine cell-specific differences between unaffected, ASD, and SCZ brain tissue. Integrating data from studies profiling single-cell RNA-sequencing (scRNA-seq) of unaffected human brain[95-97] with the list of disease associated genes lists[27,28,32,98,99] has begun to provide insights into the cell-specific biology of these disorders. One such study[100] showed that high confidence ASD-candidate genes and downstream targets of ASD gene including those of CHD8 are enriched in inhibitory neurons. While this finding is in agreement with the E/I imbalance hypothesis, this study is limited to cortical scRNA-seq and lacks data from other brain regions such as the striatum and hippocampus, which are known to be involved in ASD pathology. A similar approach[101] identified the cell types affected in

SCZ by integrating both mouse and human scRNA-seq datasets[35,95,102-106] with genes linked to SCZ[107]. Inclusion of datasets spanning more diverse brain regions in this study led to the identification of SPNs, pyramidal cells in hippocampal CA1, pyramidal cells of the somatosensory cortex and cortical interneurons as cell types connected to SCZ[101]. Ultimately, scRNA-seq of disease-affected brain tissue will be more informative to characterize cell-specific pathways perturbed in ASD.

Conclusion

Genomics has identified altered regulatory processes including chromatin remodeling, transcription and alternative splicing as key contributors to ASD. Functionally relevant disease models have begun to provide insights into the basic function of ASD-associated chromatin remodelers, TFs, and RBPs. Collectively, these data demonstrate the involvement of functionally connected gene regulatory networks in ASD pathogenesis. However, these regulatory networks might be biased or underexplored due to the nature of the genetic studies focusing on LoF mutations and haploinsufficiency as primary disease mechanisms. Future studies should include identifying potential gain-of-function mutations, as neurons are known to be sensitive to overexpression and/or misfolding of certain proteins.

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Abbreviations

AIS	axon initial segment
AS	alternative splicing
ASD	autism spectrum disorder
ChIP-seq	chromatin immunoprecipitation-sequencing
E/I	excitatory/inhibitory
ESCs	embryonic stem cells
ID	intellectual disability
LTP	long-term potentiation
SCZ	schizophrenia
SPN	spiny projection neurons
RBP	RNA-binding protein
scRNA-seq	single-cell RNA-sequencing

TF	transcription factor

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Highlights

- Chromatin remodeling, transcription, and alternative splicing are disrupted in ASD.
- *CHD8, FOXP1*, and *RBFOX1* are high confidence ASD genes related to these functions.
- Cell and animal models have begun to elucidate the molecular function of these genes.
- There are converging molecular pathways between ASD and other neurodevelopmental disorders.

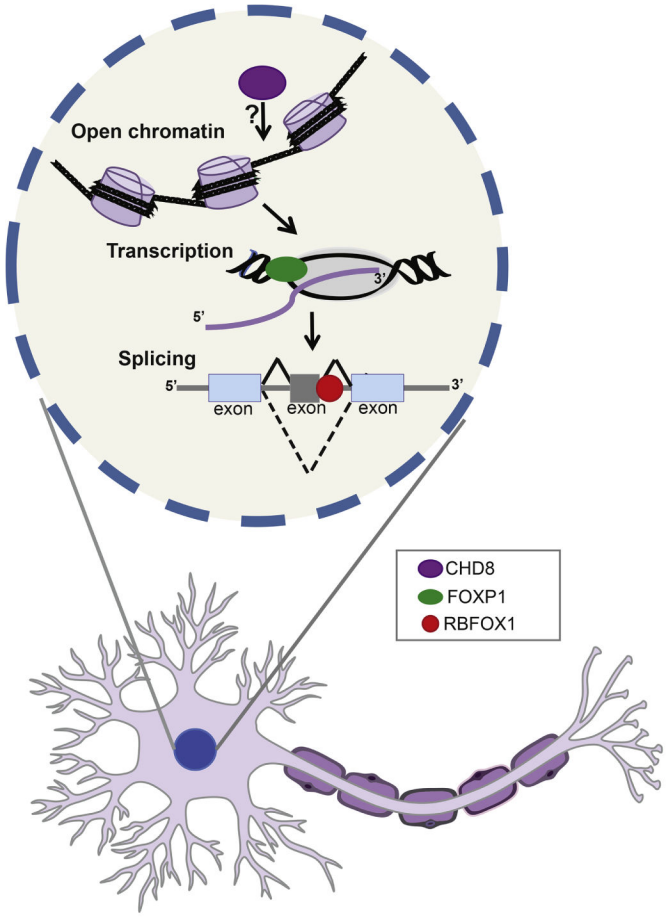

Figure 1: Regulatory genes disrupted in ASD.

High confidence ASD genes regulate multiple levels of gene expression. CHD8 (purple) is a chromatin remodeler and is associated with open chromatin and active promoters. FOXP1 is a transcriptional factor (green) active or repress its transcriptional targets. RBFOX1 (red) is a RNA binding protein and regulates RNA metabolism including splicing.

Table 1:

Mouse models of regulatory ASD genes (*CHD8*, *FOXP1*, *RBFOX1*).

	Reference	Strategy Used	Morphological Phenot	Behavioral Phenotype	Physiological Deficits	Downstream Targets
CHD8	Katayama et al[49], 2016	Heterozygous s knockout (Cre-mediated)	macrocephaly	Increased anxiety, deficits in social behaviour, normal learning	n.d.	neuronal development, REST complex
	Durak et al.[51], 2016	*in utero* knockdown (in developing cortex)	Defective neural progenitor proliferation	deficits in social behaviour, normal learning	n.d.	cell cycle, Wnt signaling
	Platt et al. [53], 2017	heterozygou s knockout (CRISPR-mediated)	macrocephaly	mild social defects, increased anxiety, no repetative behaviour, increased acquired motor learning	decreased inhibitory signaling in SPNs of Nac	chromatin remodelling, mRNA processing, cell cycle, Wnt signaling
	Gompers et al.[52], 2016	heterozygou s knockout (CRISPR-mediated)	macrocephaly	normal social behaviour, no repetative behaviour, learning and memory impairment	n.d.	RNA processing, chromatin remodelling, cell cycle
	Suetterlin et al.[50], 2018	heterozygou s knockout (Cre-mediated)	macrocephaly	normal social behaviour, delayed motor development, hypoactivity in adults	n.d.	CNS development, cell adhesion, axon guidance
FOXP1	Bacon et al.[65], 2015	brain-specific knockout (Nestin.Cre-mediated)	enlarged lateral ventricle, abnormalitie s in striatum, decreased neuronal density in hippocampu s	increased repetitive behavior, decreased social interest and impairment s in patial memory	decreased excitability and increased excitatory synaptic transmission in hippocampal pyramidal neurons.	chromatin, nucleosome, cell cyle
	Araujo et al.[66], 2015	heterozygous knockout	n.d.	defects in neonatal ultrasonic vocalizations	increased excitability of striatal SPNs	striatal development
	Li et al.[67], 2015	in utero knockdown (in developing cortex)	defective neuronal migration, defective Neurite development	n.d.	n.d.	n.d.
	Araujo et al.[68], 2017	conditional knockout in forebrain pyramidal neurons (Emx.Cre-mediated)	decreased hippocampal volume	hyperactivity, decreased sociability, impaired hippocampal-based spatial learning	decreased late-phase long-term potentiatio n (LTP) response	neurogenesis, neural differantiation, synaptic transmission

	Reference	Strategy Used	Morphological Phenot	Behavioral Phenotype	Physiological Deficits	Downstream Targets
	Usui et al.[69], 2017	conditional knockout in forebrain pyramidal neurons (Emx.Cre-mediated)	reduced neocortical size and mispositioning of deep layer neurons	impaired postnatal vocal communicatio n	n.d.	neurogenesi s and neuronal migration
RBFOX1	Gehman et al.[77], 2011	brain-specific knockout (Nestin.Cre-mediated)	normal gross morphology	seizures	hyperexcitbility in hippocampal neurons	SNARE complex, neurotransmitter genes, ion channels
	Hamada et al.[79], 2015	*in utero* knockdown (in developing cortex)	defects in neuronal migration, neuronal placement, and dendritic arbor formation	n.d.	n.d.	n.d.

SPN: spiny projection neuron, Nac: nucleus accumbens, n.d: not determined