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Orchestration of Neurodevelopmental Programs by RBFOX1: Implications for Autism Spectrum Disorder

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Abstract

Neurodevelopmental and neuropsychiatric disorders result from complex interactions between critical genetic factors and as-yet-unknown environmental components. To gain clinical insight, it is critical to develop a comprehensive understanding of these genetic components. *RBFOX1*, an RNA splicing factor, regulates expression of large genetic networks during early neuronal development and haploinsufficiency causes severe neurodevelopmental phenotypes including autism spectrum disorder (ASD), intellectual disability, and epilepsy. Genomic testing in individuals and large patient cohorts has identified phenotypically similar cases possessing copy number variations in *RBFOX1*, implicating the gene as an important cause of neurodevelopmental disease. However, a significant proportion of the observed structural variation is inherited from phenotypically normal individuals, raising questions regarding overall pathogenicity of variation at the *RBFOX1* locus. In this article, we discuss the molecular, cellular, and clinical evidence supporting the role of *RBFOX1* in neurodevelopment and present a comprehensive model for the contribution of structural variation in *RBFOX1* to ASD.

Keywords

autism; autism spectrum disorder; RNA splicing; RBFOX1; copy number variation; neurodevelopment; A2BP1; FOX1

Neurodevelopment and the *RBFOX1* RNA Splicing Factor

During development, a series of intricate programs of gene regulation must specifically occur within neurons, resulting in both temporal and spatial patterns of distinct gene expression. This results in an organized program of molecular and cellular actions and interactions that translate into the connectivity that underlies the function of the neurotypical human brain. Not surprisingly, disruption of these regulatory programs has been shown to

cause a broad range of neurodevelopmental disorders including autism spectrum disorder, schizophrenia, and many others (Bill & Geschwind, 2009; B. L. Fogel et al., 2012; Pescosolido, Yang, Sabbagh, & Morrow, 2012). Over the past decade, we have come to better understand the workings of various aspects of this complex system through the study of key regulatory factors that guide these neurodevelopmental cascades. Many of these factors directly influence gene expression through transcription and/or pre-mRNA alternative splicing, both fundamental processes to the development of tissue-specific genetic programs.

The *RBFOX1* gene (also referred to as *A2BP1* or *FOX1*) encodes a splicing regulatory factor, specifically expressed in neurons and muscle, responsible for widespread effects, both enhancing and inhibiting the alternative splicing of many cellular pre-mRNAs (B. L. Fogel, et al., 2012; Underwood, Boutz, Dougherty, Stoilov, & Black, 2005; E. T. Wang et al., 2008). Several lines of evidence indicate that *RBFOX1* regulates the alternative splicing of large tissue-specific gene networks including multi-species comparative genomics of splicing regulatory elements (Yeo, Nostrand, & Liang, 2007), bioinformatic analysis of genes alternatively spliced during embryonic cell differentiation (Yeo et al., 2007), genome-wide target site prediction strategies (Zhang et al., 2008), genome-wide transcriptome assessment (E. T. Wang, et al., 2008), multi-species RNA-binding protein motif analysis (Ray et al., 2013), and gene-specific knockdown in differentiated human neural progenitor cell lines (B. L. Fogel, et al., 2012). In addition to mediating alternative splicing, functional roles for *RBFOX1* have been identified in the transcriptional regulation of additional gene networks by directly mediating RNA stability (Ray, et al., 2013), influencing transcription (Usha & Shashidhara, 2010), or indirectly through effects on other regulators of gene expression (B. L. Fogel, et al., 2012).

***RBFOX1* Genetic Variation and Autism Spectrum Disorder**

The available molecular and cellular evidence described above supports *RBFOX1* as a high-level regulatory factor in early brain development, so it is not surprising that a growing number of patients with neurodevelopmental phenotypes have been identified with mutations disrupting *RBFOX1*. These phenotypes, some of which are quite severe, include syndromes of autism spectrum disorder (ASD), intellectual disability, and epilepsy as well as other neuropsychiatric phenotypes.

The *RBFOX1* gene is quite large, spanning 2.4 MB on chromosome 16p13.3, making it one of the largest genes in the human genome. It must be noted that the nomenclature used to describe the *RBFOX1* genetic architecture over the years has not been consistent across the literature, and it can often be difficult to compare the functional significance of reported variants. This is due, in part, to the fact that the *RBFOX1* gene utilizes multiple promoters and undergoes a wide variety of alternative splicing in a tissue-specific fashion, with many of the functional cellular transcripts likely not yet characterized or annotated (B. L. Fogel, et al., 2012; Underwood, et al., 2005) (Figure 1). Furthermore, the major neuronal transcripts initially reported in the literature for humans have been shown to actually be muscle-specific transcripts, the same as originally reported in the mouse (B. L. Fogel, et al., 2012). We advocate the use of the naming scheme first published in Underwood et al. (B. L. Fogel, et

al., 2012; Underwood, et al., 2005) for describing variants found in patients (Figure 1A; Supplemental Table 1). Standardized nomenclature is particularly important for determining the pathogenicity of an individual sequence or structural change likely to affect neuron-specific transcripts or isoforms.

One of the earliest clinical descriptions of a neurodevelopmental phenotype associated with *RBFOX1* was of two patients with likely ASD, mental retardation, and epilepsy associated with haploinsufficiency caused by *de novo* translocations involving chromosome 16 (Bhalla et al., 2004). This was followed by the description of another patient with a similar syndrome, also caused by a haploinsufficiency due to a *de novo* translocation, which incorporated validated rating scales to clinically diagnose the patient with autism (Martin et al., 2007). These two initial case reports provided strong confirmatory evidence of causality between *RBFOX1* mutation and human disease, resulting in its classification as an ASD candidate gene (Simons Foundation Autism Research Initiative gene database; <https://sfari.org/>).

Other structural variations in *RBFOX1*, most notably copy number variations (CNVs; arbitrarily defined as deletions or duplications greater than 1 kilobase in size), have also been associated with human neurodevelopmental disease. CNVs occur commonly in the population and represent a significant source of human genetic variation (Brent L. Fogel & Geschwind, 2012) (Database of Genomic Variants, DGV; <http://dgv.tcag.ca/dgv/app/home>) (Iafate et al., 2004). The link between *RBFOX1* CNVs and autism has been suggested by the presence of *de novo* CNVs within ASD cohorts (Sebat et al., 2007) and in samples from isolated autistic patients (Wintle et al., 2011).

More recently, a number of case reports have highlighted specific *RBFOX1* CNVs linked to ASD. Mikhail et al. reported a 3-year-old microcephalic boy with developmental delay, language delay, and an intragenic deletion involving noncoding exons 6 and 1D in *RBFOX1* of unknown inheritance status as his parents could not be tested (Mikhail et al., 2011). Davis et al. reported the case of a 12-year-old boy with autism determined through validated rating scales, global hypotonia, a mild developmental left hemiparesis, and deficits in motor planning and coordination with the dominant right hand (Davis et al., 2012). The patient was found to have a deletion involving noncoding exon 5 of *RBFOX1* inherited through the father who was not clinically evaluated (Davis, et al., 2012). Most recently, Zhao reported 13 patients with deletions (1 maternally inherited involving noncoding exon 6, 1 *de novo* involving multiple coding exons between 1D and 10, and the inheritance of the rest could not be determined) and 1 patient with a duplication (of undetermined inheritance) within the *RBFOX1* locus (Zhao, 2013). Two patients in this report were excluded from phenotypic analysis due to 1) the finding of an alternate Mendelian genetic diagnosis in addition to a deletion involving *RBFOX1* exon 5, and 2) the presence of multiple non-neurological congenital anomalies of unclear etiology in a patient with an *RBFOX1* duplication involving exons 1D and 7 (Zhao, 2013). The major clinical findings in the other patients (who all possessed intronic deletions except for one that involved noncoding exon 6) included global developmental delay (58%), epilepsy (50%), macro- or microcephaly (50%), and renal problems (33%) (Zhao, 2013). Of note, 50% of the patients with epilepsy also had developmental delay, one patient with epilepsy but without developmental delay was noted

as intellectually disabled, and one patient with developmental delay but without epilepsy was given a clinical diagnosis of autism (Zhao, 2013). The extent of the clinical evaluation for ASD each patient received in this cohort was unfortunately not reported, so it cannot be assumed the other patients lacked such phenotypes.

In general, it has been presumed that the above CNVs lead to reduced *RBFOX1* expression. If true, then it would be consistent with the observations that *RBFOX1* haploinsufficiency results in a syndrome characterized primarily by neurodevelopmental and neurological phenotypes including ASD, intellectual disability, and epilepsy (Bhalla, et al., 2004; Martin, et al., 2007). Data from knockdown studies in human neural progenitor cell lines modeling haploinsufficiency during neuronal differentiation demonstrate widespread changes in RNA splicing and gene expression (B. L. Fogel, et al., 2012), and studies of the *Rbfox1* neural-specific knockout mouse show alterations in synaptic transmission, increased membrane excitability, and a predisposition to seizures (Gehman et al., 2011). Interestingly, whole transcriptome analysis in the brains of autistic patients reveals decreased levels of *RBFOX1* and dysregulation of *RBFOX1*-dependent alternative splicing (Voineagu et al., 2011), similar to the effects seen in haploinsufficient neuronal cell lines (B. L. Fogel, et al., 2012). However, in the majority of cases, the impact of the identified CNV to *RBFOX1* expression or function is unclear, as evidenced by the presence of identical CNVs in controls and unaffected family members (Figure 1B and 1C).

To better understand the contribution of structural variation in *RBFOX1* to the development of autism and related disorders, we compiled all published CNVs including those from 2 of the largest ASD cohorts, the Autism Genetic Resource Exchange (AGRE), a primarily multiplex cohort of families with multiple affected siblings, and the Simons Simplex Cohort (SSC), which contains families with only a single affected child (Figure 1B and 1C). In the AGRE cohort, we found that 2.2% of patients carried a CNV in the *RBFOX1* locus compared to 0.7% of unaffected siblings (OR=3.19, p=0.006, 95% CI [1.27, 10.28]). In contrast, the SSC did not show a significantly increased odds ratio, with 2.6% of probands and 2.4% of their normal siblings having a CNV (OR=1.11, p=0.77, 95% CI [0.59, 2.12]). While we cannot rule out ascertainment bias or differences in population structure, this data demonstrates a significant enrichment of *RBFOX1* CNVs in a multiplex but not in a simplex cohort. This analysis reveals two additional striking features. The first is that, irrespective of cohort, CNVs in *RBFOX1* tend to be inherited from an unaffected parent. Second, we observe a locational bias of CNVs toward the 5' untranslated exons 1–7 (Figure 1B) compared to the constitutively translated exons 8–21 (Figure 1C) with a preponderance of ASD-related deletions occurring in the intron prior to exon 7, which contains a potential translational start site (Figure 1B). Although it is tempting to speculate that these CNVs lead to a correlative alteration of the expression level of *RBFOX1*, qualitatively, we also observe a large number of CNVs in this intron within unaffected individuals in the DGV (Iafate, et al., 2004) (Figure 1B and C). Although the CNVs in the DGV appear to be somewhat smaller overall than those in those identified in ASD patients, we unfortunately cannot conclude that larger CNVs have a higher impact on phenotype, primarily due to differences in acquisition between the groups. Therefore, taking all these observations together, we conclude that while *RBFOX1* CNVs confer a heritable risk of developing ASD, the majority

of *RBFOX1* CNVs do not cause haploinsufficiency in isolation and other factors, genetic or environmental, likely contribute.

Further complicating this clinical landscape, the phenotypic spectrum of disease associated with disruption of *RBFOX1* regulation and/or function appears to extend into a cacophony of other phenotypes, some commonly found in ASD patients, such as epilepsy and developmental delay. *RBFOX1* CNVs have been reported in patients with idiopathic generalized epilepsy, including one patient with epilepsy and ASD that removes exon 7 (Lal et al., 2013). Additionally, the intron prior to exon 7 has a high number of CNVs identified within the International Standard for Cytogenomic Arrays (ISCA) database (<https://www.iscaconsortium.org/>) that are associated with phenotypes such as global developmental, intellectual, and speech delay. It is possible these reports could reflect populations that are on the ASD spectrum, but incompletely characterized. *RBFOX1* CNVs have also been identified in populations of individuals with schizophrenia (International Schizophrenia Consortium, 2008; Melhem et al., 2011; Priebe et al., 2013; Xu et al., 2008), however at a very low rate. The strongest evidence for this association is an increased risk for male-specific schizophrenia (OR=8.2, 95% CI, 0.8–84.4) due to duplications occurring just prior to exon 6, predicted to be inherited from a single founder event (Melhem, et al., 2011). Lastly, various cancer cells also show structural variation in *RBFOX1* (Andersen et al., 2011; Bass et al., 2011; Linnebacher et al., 2013) suggesting either a potential post-developmental role in cellular growth and/or differentiation in other tissues or disease consequences of aberrant expression/regulation of *RBFOX1* in non-specific tissues.

Finally, although unlikely to be directly causative, *RBFOX1* single nucleotide polymorphism (SNP) variants have also been implicated in autism (K. Wang et al., 2009) as well as a diverse array of other human conditions including Alzheimer disease (Kohannim et al., 2012), bipolar disorder (Le-Niculescu et al., 2009), attention-deficit hyperactivity disorder (Elia et al., 2010), schizoaffective disorder (Hamshere et al., 2009), obesity (Ma et al., 2010), and refractive error (Stambolian et al., 2013). It is unclear whether these SNPs may, in some cases, co-segregate with other rare sequence or structural variants directly influencing *RBFOX1* expression or regulation.

The Contributions of *RBFOX1* Model Systems to Molecular Pathogenesis

The first member of the *RBFOX1* family of alternative splicing factors was initially reported in a search for modifiers of sexual differentiation in *C. elegans* (Hodgkin, Zellan, & Albertson, 1994). Feminization on X (*fox-1*) was identified as a dominant factor that feminizes XO males, and causes high levels of male lethality due to its ability to splice the *xol-1* [XO (male) lethality] gene (Kuroyanagi, 2009). The *Drosophila melanogaster* homolog (*dA2bp1*, also known as *CG3206*) and zebrafish homologs (*rbfox1* and *rbfox1l*) were subsequently identified and shown upon constitutive knockdown to be embryonic lethal, suggesting an early role in embryogenesis (Bajpai, Sambrani, Stadelmayer, & Shashidhara, 2004; Gallagher et al., 2011; Hodgkin, et al., 1994). Although the mechanism underlying this early lethality has not been explained, it provides one potential explanation for the high sequence conservation in *RBFOX1* observed from worms to humans and the lack of CNVs found within the coding region of patient and control samples (Figure 1C).

Human RBFOX1 was originally identified through its interaction with ataxin-2, the protein causing the neurodegenerative disease spinocerebellar ataxia type 2 (SCA2) (Shibata, Huynh, & Pulst, 2000). Although the functional significance of this interaction is not yet fully understood, it likely contributes to ataxin-2's established role in RNA processing and translation (C. Lim & Allada, 2013). Interestingly, RBFOX1 is present in other protein-protein and gene interaction networks related to cerebellar ataxia (B.L. Fogel & Perlman, 2011; J. Lim et al., 2006), and it affects the splicing and transcription of other ataxia genes in human neural progenitor cells (B. L. Fogel, et al., 2012), an intriguing observation given that cerebellar dysfunction may contribute to the autistic phenotype (Fatemi et al., 2012).

Jin and coworkers were the first to confirm that zebrafish *rbfox1l* was involved in alternative RNA splicing (Jin et al., 2003). They demonstrated that *rbfox1l* could bind to an intronic GCAUG pentanucleotide and affect splicing by repressing exon inclusion if the binding site was upstream or enhancing inclusion if the site were downstream (Jin, et al., 2003). The mechanism of repressing exon inclusion involves the hindrance of pre-spliceosomal complexes (Kuroyanagi, 2009), and can be overcome by overexpression of spliceosomal components (Zhou & Lou, 2008). By extension, reduction of RBFOX1 due to haploinsufficiency in patients could allow spliceosome factors to outcompete RBFOX1 for binding, thereby altering splicing for some targets. The mechanism for enhancing exon inclusion is less well defined. Sun and coworkers demonstrated that the c-terminus of RBFOX1 is required for this process, implicating protein-protein interactions as key to enhancing exon usage (Sun, Zhang, Fregoso, & Krainer, 2012). Our recent studies of splicing in human neural progenitor cell lines demonstrated globally that downstream sequences tend to function as enhancers while upstream sequences can mediate both repression and enhancement of specific exons (B. L. Fogel, et al., 2012), suggesting a role for local context in determining the function of RBFOX1. Furthermore, only 56% of the splicing events colocalized with the canonical RBFOX1 binding site, suggesting an interplay of both direct and indirect regulatory mechanisms, or possibly non-canonical binding sites that have yet to be discovered (B. L. Fogel, et al., 2012).

A Model for the Dysregulation of *RBFOX1* in Human Neurodevelopmental Disease

As discussed above, a great deal of scientific evidence supports a role for *RBFOX1* in the regulation of gene expression during human neurodevelopment. Clinical evidence further supports an association with neurodevelopmental disease in humans when haploinsufficient. The observation of noncoding *de novo* structural variants in *RBFOX1* in patients with ASD would be consistent with the hypothesis that such variation leads to haploinsufficiency. However, the finding that the majority of structural variation in these patients is inherited, including variants similar to those seen *de novo* (Figure 1), appears to counteract that hypothesis. An imprinting effect seems unlikely as there does not appear to be a sex preference to CNV inheritance and disease association. How then does one reconcile this data with the scientific evidence? One possible conclusion is that much of the noncoding structural variation seen is in fact non-pathogenic and the observation of multiple CNVs in ASD patients is the product of the large size of the *RBFOX1* gene, the commonality of

structural variation in the human genome (Brent L. Fogel & Geschwind, 2012), and an increased rate in the occurrence of such variation at that locus (Bass, et al., 2011; Yi & Li, 2005). However, if this were the case, it would be expected that CNVs in *RBFOX1* would occur at equal frequency in the population as a whole, without enrichment in ASD cohorts. Therefore, the finding of an enrichment in inherited *RBFOX1* CNVs using stringent criteria in a large multiplex ASD cohort, but not in a large simplex cohort as described above, points to a different interpretation (see Figure 2). In this model, *RBFOX1* CNVs confer an increased risk of developing ASD, dependent upon unknown genetic or environmental factors that influence *RBFOX1*-regulated cellular programs.

Regulation of splicing factors can have profound consequences with regard to neurodevelopmental outcome. There are two examples of *Rbfox1* transcriptional regulation. In zebrafish, the homeobox-transcription factor *Otp* in complex with phospho-Creb binds the *rbfox1* promoter in response to physical or osmotic stress (Amir-Zilberstein et al., 2012). Subsequently, *Rbfox1* regulates splicing of the *Pacap*-receptor, *pac1*, that in turn modulates corticotropin-releasing hormone (*Crh*) levels, stimulating recovery from stress (Amir-Zilberstein, et al., 2012). Increases in *Crh* can manifest as increased anxiety or disturbances of sleep (Holsboer & Ising, 2008), both of which are common in ASD (Mazzone, Ruta, & Reale, 2012; Richdale & Schreck, 2009). A second candidate transcriptional regulator is *Myt1l*, which can bind four distinct binding sites in the *Rbfox1* promoter (Hu et al., 2013). In an analysis of human and mouse expression data, consistent coexpression is seen between *RBFOX1* and *MYT1L* in a dataset from patients with frontotemporal dementia (chosen due to an observed 2 fold reduction of *MYT1L*) and in two large databases that collate expression data; the UCLA Gene expression tool (UGET) and COXPRESdb (Bill, unpublished observation) (Chen-Plotkin et al., 2008) (Day et al., 2009) (Day, Carlson, Dong, O'Connor, & Nelson, 2007; Obayashi & Kinoshita, 2011). Since *MYT1L* has been shown to be critical for the transdifferentiation of iPSCs (Takahashi & Yamanaka, 2006), and has been associated with ASD (Meyer, Axelsen, Sheffield, Patil, & Wassink, 2012), intellectual delay and obesity (Stevens et al., 2011), and schizophrenia (Y. Lee et al., 2012; Van Den Bossche et al., 2013; Vrijenhoek et al., 2008), this is a finding of particular clinical relevance. As the regulation of *RBFOX1* transcription is not yet fully understood, it is possible that CNVs in the 5' UTR may interfere with this process, either constitutively or in response to certain stressors requiring modifications in *RBFOX1* expression (Figure 2).

It is known that many genes, including *RBFOX1*, undergo extensive alternative splicing (B. L. Fogel, et al., 2012; Underwood, et al., 2005), and that such splicing patterns can be altered by changes to the cellular environment (J. A. Lee, et al., 2009). *Rbfox1* RNA has been shown to be regulated via effects on RNA stability (Pistoni et al., 2013) and alternative splicing (Damianov & Black, 2010; Lee, et al., 2009). *Rbfox1* can autoregulate the splicing of its own RNA binding domain creating a functional dominant negative, and thus regulate its own activity (Damianov & Black, 2010). Furthermore, Lee et al. have demonstrated that cellular depolarization, known to widely affect RNA splicing, causes alternative splicing of murine *Rbfox1* in neurons, resulting in a change in subcellular localization of the protein and subsequent reversal of the effects of depolarization on the splicing of other *Rbfox1* targets, which they interpret as a novel adaptive mechanism to chronic stimuli (J. A. Lee, et al.,

2009). Regulation of *RBFOX1* alternative splicing in response to cellular stressors could therefore play a meaningful role in neurodevelopment, and provide another possible regulatory mechanism CNVs may affect (Figure 2).

Given the extensive networks of genes regulated both directly and indirectly by *RBFOX1* (B. L. Fogel, et al., 2012; Ray, et al., 2013), it is likely that various stimuli could occur throughout the course of neuronal development which require an adaptive response from *RBFOX1*, alone or in conjunction with other factors, to maintain normal expression profiles. Therefore, it may be supposed that mutations in *RBFOX1* could exist which impair these adaptive responses while having no, or minimal, effect on routine function. Applying this scenario to human neurodevelopment, noncoding variation that directly disrupts *RBFOX1* expression would act to cause haploinsufficiency and therefore disease, whereas variation altering accessory regulatory responses (e.g., various target binding sequences) would only lead to disease under cellular conditions requiring function of those elements. If such conditions are rare, or only transient, then the ability to mount a limited adaptive response may be tolerated, but if the condition is such that the adaptive response is essential for normal brain development, this inability to respond could result in disease. This synergizes well with the suspected contribution of environmental factors to ASD if exposure occurs during critical points in brain development (Herbert, 2010), and suggests a mechanism whereby variation in the *RBFOX1* gene could modify ASD risk under certain conditions, but not others. Families tend to stay in the same environment for long periods of time and, therefore, children are often exposed to the same environmental stressors as their siblings. If *RBFOX1* CNVs confer ASD risk in a particular environmental context, the pathogenicity of such variants would be most apparent in families with multiple affected children. This model could explain why enrichment of *RBFOX1* CNVs is seen in a multiplex, but not a simplex, cohort, as shown above. Further work will be essential to confirm this hypothesis as well as to identify and characterize such regulatory elements and determine what cellular or environmental signals trigger their use. If validated, therapies stimulating such factors or inhibiting the triggering signals could be useful to minimize ASD risk in families with known pathogenic CNVs in *RBFOX1* and possibly other genes as well. Until such evidence is available however, we must continue to observe caution in the interpretation of inherited *RBFOX1* CNVs as pathogenic when passed on from unaffected individuals.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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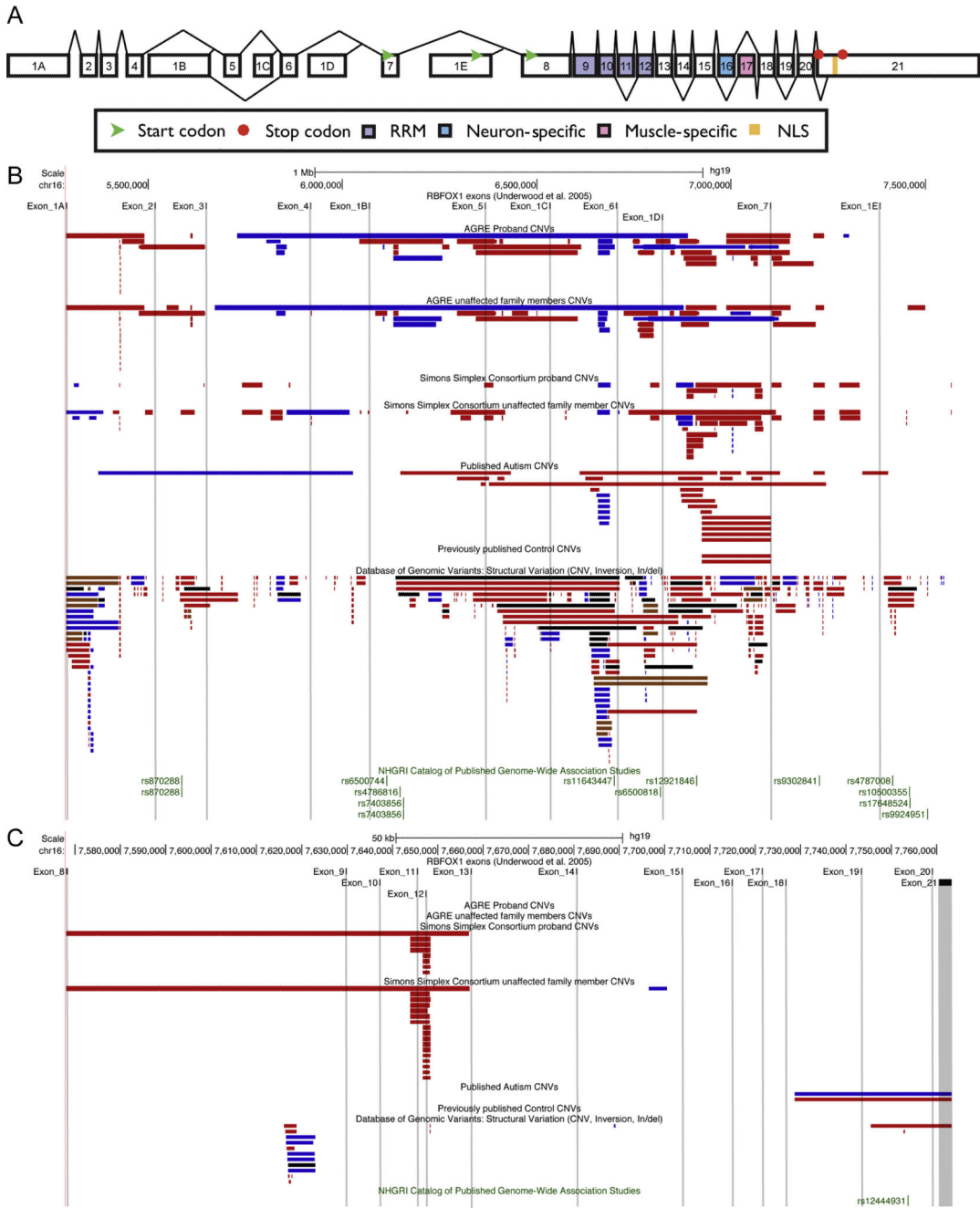


Figure 1. *RBFOX1* Genomic Architecture and Copy Number Variation associated with Autism Spectrum Disorder

A) *RBFOX1* gene structure is complex with multiple transcriptional-start sites, translational-start sites, multiple isoforms, and alternate endings. The distribution of CNVs associated with autism spectrum disorder in the *RBFOX1* locus [HG19-chr16:5289804–7763340] are shown and compared to the database of genomic variants. B) Exons 1A through 1E. C) Exons 8 through 21. Observed structural variation is shown clustered at the 5' untranslated region between exons 1 through 7. Single nucleotide polymorphisms related to the locus and showing genome-wide association in ASD also follow a similar distribution pattern (green).

Red bars = deletions; Blue bars = duplications; Brown bars = insertions and deletions; Black bars = unknown feature.

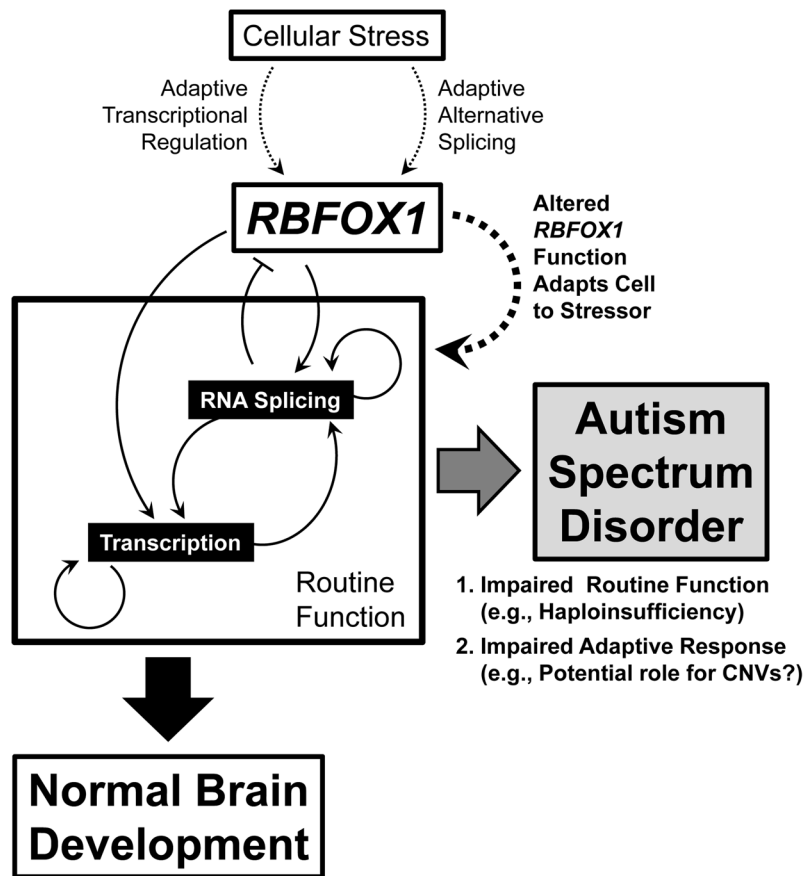


Figure 2. Proposed Model for *RBFOX1* Dysregulation in Autism Spectrum Disorder
 During neurodevelopment, *RBFOX1* regulates large genetic networks via direct effects on RNA splicing, mRNA stability, and transcriptional regulation as well as indirect effects on gene transcription leading to normal neuronal development. If this function is disrupted (e.g., by haploinsufficiency of *RBFOX1*), development is sufficiently perturbed resulting in autism spectrum disorder (ASD). As discussed in the text, *RBFOX1* may play a further role in the adaptive response to cellular stress by regulating RNA splicing or transcriptional effects in response to environmental stimuli to maintain normal function. If this process is perturbed, this may also result in ASD. Inherited structural variation (CNVs) may damage *RBFOX1* RNA processing or transcriptional signals and thus impair this adaptive response, thereby increasing the risk of ASD occurring upon exposure to certain environmental stressors during critical stages in neurodevelopment.