

# Networking in Autism: Leveraging Genetic, Biomarker and Model System Findings in the Search for New Treatments

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Autism Spectrum Disorder (ASD) is a common neurodevelopmental disorder affecting approximately 1% of children. ASD is defined by core symptoms in two domains: negative symptoms of impairment in social and communication function, and positive symptoms of restricted and repetitive behaviors. Available treatments are inadequate for treating both core symptoms and associated conditions. Twin studies indicate that ASD susceptibility has a large heritable component. Genetic studies have identified promising leads, with converging insights emerging from single-gene disorders that bear ASD features, with particular interest in mammalian target of rapamycin (mTOR)-linked synaptic plasticity mechanisms. Mouse models of these disorders are revealing not only opportunities to model behavioral perturbations across species, but also evidence of postnatal rescue of brain and behavioral phenotypes. An intense search for ASD biomarkers has consistently pointed to elevated platelet serotonin (5-HT) levels and a surge in brain growth in the first 2 years of life. Following a review of the diversity of ASD phenotypes and its genetic origins and biomarkers, we discuss opportunities for translation of these findings into novel ASD treatments, focusing on mTor- and 5-HT-signaling pathways, and their possible intersection. Paralleling the progress made in understanding the root causes of rare genetic syndromes that affect cognitive development, we anticipate progress in models systems using bona fide ASD-associated molecular changes that have the potential to accelerate the development of ASD diagnostics and therapeutics.

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## ASD HISTORY AND DIAGNOSIS

Leo Kanner initially described ‘early infantile autism’ in a case series in 1943, naming the disorder on the basis of the ‘autistic aloneness’ that he observed in his patients (Kanner, 1943). This social impairment, often considered the defining feature of autism, is intertwined with communication impairment. Nonetheless, these two domains are currently separated in the Diagnostic and Statistical Manual-IV (DSM-IV) criteria (American Psychiatric Association, 2000). Truly, however, social function requires verbal or non-verbal

communication, and, reciprocally, communication necessarily includes social interaction. Restricted, repetitive behaviors, which might be considered as the positive symptoms of autism, are also required to make a diagnosis. Symptoms of autism are commonly recognized as a gradual divergence from the expected pattern of development, but some children appear to show a regression, or loss of, previously acquired skills, most often in the second year of life (Werner and Dawson, 2005). Recent work suggests that a decline in social interaction over the first 12–24 months of age may be common in children with autism (Ozonoff *et al*, 2010), whereas an explicit loss of communication or other skills is less common (Shumway *et al*, 2011).

Autism and related disorders, now termed pervasive developmental disorders (PDDs), have been diagnosed in a steadily increasing number of children over the past twenty years, with current prevalence estimates nearing 1% of

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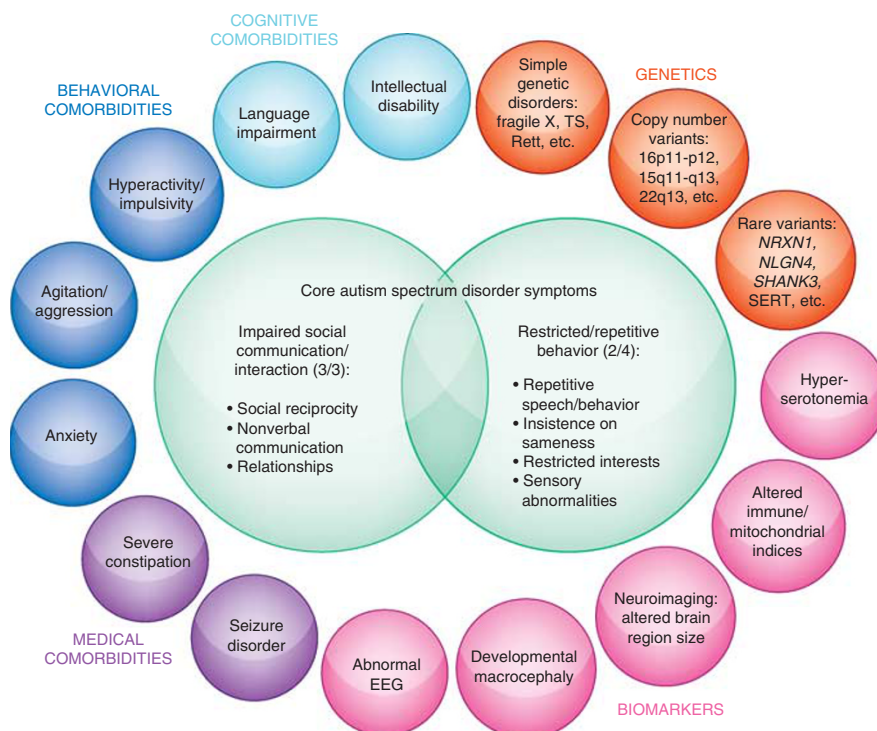
children (Autism and Developmental Disabilities Monitoring Network Surveillance Year 2002 Principal Investigators; Centers for Disease Control and Prevention, 2007). It is not clear how much of this increase is due to broadened criteria, improved diagnostic tools, or other unknown factors. Reflecting the difficulty in finding clear boundaries between the PDDs, the upcoming revision of the DSM (DSM-V at <http://DSM5.org>) will likely include only one diagnosis, Autism Spectrum Disorder (ASD), instead of separating out Asperger's Disorder and Pervasive Developmental Disorder Not Otherwise Specified (PDD-NOS). This spectrum diagnosis reflects the broad heterogeneity observed across individuals with social communication dysfunction and repetitive behavior.

Each of the three current symptom domains can be quite variable from individual to individual sharing a PDD diagnosis. For example, Lorna Wing and others have noted disparate patterns of social motivation, including children who are socially uninterested or 'aloof,' but also children who are socially motivated but qualitatively impaired in their interactions, whom Wing describes as 'active but odd' (Waterhouse *et al*, 1996). Impairment also varies widely in the communication domain, with a substantial number of children who never achieve verbal speech but others whose speech may be most notable for odd prosody or idiosyncratic phrasing. Finally, the repetitive behavior domain is

quite diverse, including intense restricted interests, simple repetitive motor mannerisms, inflexible rituals or routines, or preoccupation with parts of objects, with each child only required to show two of these positive symptoms (American Psychiatric Association, 2000; Richler *et al*, 2007, 2010). Recent work in sibling pairs affected with autism suggests that individual symptom domains or sub-domains may be separately inherited, particularly in the case of repetitive behavior (Georgiades *et al*, 2007; Lam *et al*, 2008; Smith *et al*, 2009; Tadevosyan-Leyfer *et al*, 2003).

## PERSPECTIVE

The planned incorporation of a 'spectrum' term in the description of 'ASD' in the DSM-V reflects an extremely complex and heterogeneous syndrome as notable for its variability as for its core features (Figure 1). Surely, the broadening of phenotype is not unique to ASD, as we now recognize that many brain disorders represent clusters of similar but not identical symptoms. No two individuals present with exactly the same features, attesting to the complexity of our genetic heritage, environmental exposure, and brain development and plasticity. With respect to the complexity of autism, we will argue for an integrative approach that seeks to follow systems that are implicated across multiple research modalities. In the space provided,



**Figure 1.** ASD symptoms, comorbidities, and biomarkers. The core symptoms of ASD are represented in the center and represent the common features required to receive a diagnosis. All three social communication/social interaction symptoms are required to receive a diagnosis in the DSM-V draft criteria. This domain represents the 'negative symptoms' of ASD, that is, absence of appropriate social communication. Two of the four restricted/repetitive behavior symptoms are required to receive a diagnosis in the DSM-V draft criteria. This domain represents the 'positive symptoms' of ASD, that is, the presence of unusual restricted, repetitive, or sensory behaviors. Around the periphery of the figure are symptoms or biomarkers that are not required for an ASD diagnosis but are more common in ASD than in the general populations. Quite a number of comorbid disorders or symptoms are seen in a substantial minority or even a majority of individuals with ASD, spanning cognitive, behavioral/psychiatric, and medical domains. As might be expected from the range of comorbid symptoms, biomarkers and genetic findings also reveal significant heterogeneity across individuals with ASD.

we cannot enumerate all findings across the relevant research modalities. Instead, after a review of comorbidities that illustrate the complexities of the medical problems of ASD and the limited treatments available, we discuss the genetic and environmental factors reported to impact ASD risk and available ASD biomarkers, particularly those that could drive the search for potential therapeutics. Lastly, we discuss findings within networks that converge on the mammalian target of rapamycin (mTOR) pathway and the serotonin (5-hydroxytryptamine, 5-HT) transporter (SERT) as two specific examples of how further preclinical research may provide new opportunities for improved diagnosis and treatment. We refer the reader to other networks and paradigms gaining traction (Bill and Geschwind, 2009; Ehninger *et al*, 2008b; Levitt and Campbell, 2009; Ramocki and Zoghbi, 2008; Sudhof, 2008), as we expect that multiple avenues will need to be pursued given the heterogeneity of ASD.

## COMORBIDITY IN ASD

Children with ASD frequently show comorbid symptoms that are not part of the diagnostic criteria. These include both general and specific cognitive impairment (Munson *et al*, 2008). Approximately 1/3 of children with ASD (although with a decreasing ratio as diagnosis increases) show intellectual disability, or IQ two standard deviations below the mean (Chakrabarti and Fombonne, 2005). Specific cognitive deficits have been described in groups of children with ASD, prominently including impairment in executive function (Hill, 2004). Others have posited 'weak central coherence' as a central deficit in ASD, with patients failing to see or understand group relationships (Happé and Frith, 2006).

Changes in sensory function are common in ASD. Some children with ASD show marked hypersensitivity to specific stimuli, whereas others show apparent insensitivity to the environment, including painful stimuli (Baranek *et al*, 2007; Boyd *et al*, 2010). A few studies show empirical support for altered sensory processing; although more research is needed in this area (Cascio *et al*, 2008; Coskun *et al*, 2009; Kwakye *et al*, 2011; Tommerdahl *et al*, 2007).

Epilepsy is common in ASD, with up to 25% of children affected. The emergence of epilepsy shows two peaks, with some children developing seizures during the first few years of life and a second group developing seizures during adolescence. Beyond those children who have seizures, up to 50% of children with ASD have an abnormal electroencephalogram (Chez *et al*, 2006; Hrdlicka *et al*, 2004; Kim *et al*, 2006). This observation has led some to hypothesize that there is an imbalance favoring excitatory over inhibitory neurotransmission in ASD (Rubenstein and Merzenich, 2003).

Gastrointestinal symptoms have also received considerable attention in ASD (Buie *et al*, 2010; Erickson *et al*, 2005). Despite considerable anecdotal evidence of gastrointestinal dysfunction, available research primarily points to excess

constipation and accompanying encopresis (leakage of stool) in ASD (Ibrahim *et al*, 2009; Wang *et al*, 2011).

A wide variety of behavioral symptoms are also more commonly seen in ASD than in the general population. Hyperactivity and impulsivity are particularly common and frequently lead to impairment (Simonoff *et al*, 2008; Volkmar *et al*, 1999). Aggression and self-injury are also common, particularly in individuals with comorbid intellectual disability (Parikh *et al*, 2008; Volkmar *et al*, 1999). Anxiety appears to be more common (Mattila *et al*, 2010; Simonoff *et al*, 2008); although current measures have difficulty capturing anxiety symptoms in the ASD population (Wood *et al*, 2009). Obsessive-compulsive symptoms are also frequent, but these are difficult to separate from the wide range of repetitive behaviors that can be seen in ASD (Jacob *et al*, 2009; Leyfer *et al*, 2006).

Overall, the pattern of comorbid symptoms in ASD is broad and matches the heterogeneity in core symptoms. Competing approaches can be taken to the patterns of core and comorbid symptoms. We could, as a field, approach each set of symptoms separately, making each diagnosis that is justified by symptoms (ie, describing a child as having ASD with comorbid epilepsy, constipation-encopresis, sensory aversion, and hyperactivity). Alternatively, we could hypothesize that all of the symptoms most likely reflect a single underlying etiology. Neither approach has clearly won out at this point, but comorbid physical and behavioral symptoms are likely to prove important in separating different causes of ASD.

## CURRENT TREATMENT OF ASD

Unfortunately, available treatments for ASDs are inadequate (McPheeters *et al*, 2011; Warren *et al*, 2011). Many children receive treatments based upon applied behavior analysis (ABA), an approach that incrementally reinforces components of core social and communication skills while working to minimize interference from repetitive behavior. Early intensive behavioral interventions that incorporate 30+ hours per week of therapy using ABA principles may improve symptoms for some children but do not often lead to complete remission of symptoms (Dawson *et al*, 2010; Smith *et al*, 2000). Other, less intensive, behavioral interventions targeting social skills have less data to support their use (Beaumont and Sofronoff, 2008; Warren *et al*, 2011). Some treatment strategies seek to work around deficits by adapting communication systems or educational settings to individual needs (Panerai *et al*, 2009; Yoder and Lieberman, 2010). Overall, these behavioral interventions are the mainstay of current treatment and do lead to improved function in some children, but they do not cure ASD symptoms in most children (Warren *et al*, 2011).

Current medications primarily show benefit for comorbid symptoms in ASD, rather than leading to improvement in core symptom domains (McPheeters *et al*, 2011). Some treatments, such as stimulant medications, may be effective

in reducing hyperactivity and impulsivity symptoms (Network, 2005). Other treatments, including the atypical antipsychotics risperidone and aripiprazole, can effectively target comorbid irritability, agitation, and aggression (Marcus *et al*, 2009; McCracken *et al*, 2002). Some evidence shows that atypical antipsychotics may also benefit repetitive behavior symptoms (McDougle *et al*, 2005). The 5-HT reuptake inhibitors (SRIs) may also provide benefit for distressing repetitive behaviors in some people with ASD, but the data regarding population efficacy are inconsistent (Hollander *et al*, 2005; King *et al*, 2009a; McDougle *et al*, 1996). To date, none of these treatments are based upon a specific understanding of the causes of ASD.

## ASD SUSCEPTIBILITY FACTORS

### Genetic Factors

The path to improved diagnosis and treatment of ASD requires a more sophisticated understanding of biological and environmental susceptibilities. Twin and family studies have demonstrated that heritability has a large role in autism. Beginning with work by Susan Folstein and Michael Rutter in the late 1970s, higher ASD concordance rates have been seen in monozygotic *vs* dizygotic twins, supporting a heritability of up to 90% (Bailey *et al*, 1995; Folstein and Rutter, 1977). More recent twin studies have calculated a lower contribution of heritability (Lichtenstein *et al*, 2010; Rosenberg *et al*, 2009), perhaps reflecting changes in diagnostic criteria and population base rate, although heritability estimates remain as high or higher than any other behaviorally defined disorder.

Family studies also support a significant contribution of heritability to autism (Bolton *et al*, 1994). Again, as diagnosis rates have increased in the general population, recurrence rates within families have increased as well, with

estimates ranging from 3 to 11% in large samples (Bolton *et al*, 1994; Constantino *et al*, 2010b; Jorde *et al*, 1991; Ritvo *et al*, 1989). Some studies find a much higher rate of subsequent children with ASD in families with two or more affected children, approaching 50% affected (Zhao *et al*, 2007). The different patterns of recurrence risk suggest that there are multiple different patterns of inheritance contained within the population of ASD. These patterns may favor different genetic approaches in different subsets of ASD.

Several comprehensive reviews of molecular genetic findings in ASD have been published recently (Bill and Geschwind, 2009; Bourgeron, 2009; Levitt and Campbell, 2009; State, 2011; Weiss, 2009). We will therefore not detail the overall genetic findings in ASD but instead will focus on mTor and 5-HT networks where the biological contexts for therapeutic development are most highly developed. In Table 1, we summarize other findings, including the identification of rare copy-number variants (CNVs) and single-gene variants that represent highly penetrant susceptibility factors, establishing, essentially, new genetic syndromes. Interestingly, some of these findings reveal ASD risk associated with both increased and decreased gene dosage. As might be expected from a gene-to-phenotype approach, the impact of a number of these susceptibility variants does not appear to respect the diagnostic boundaries of ASD and includes risk of intellectual disability, ADHD, or schizophrenia. As tabulated, a number of common polymorphisms have also been associated with ASD; although findings in this area have been slower to emerge.

In addition to specific genetic risk factors, an XY karyotype confers an increased risk of ASD, with rates at least four times higher in males than females (Fombonne, 2003). This ratio may be further accentuated in children with higher IQ. Reciprocally, the male-to-female ratio appears lower for children with greater degrees of

TABLE 1 Examples of Molecular Genetic Findings in ASD

Copy-number variants	Uncommon single-gene disruptions/mutations	Associations with common polymorphisms
Maternal duplication of chromosome 15q11-q13 (Christian <i>et al</i> , 2008; Cook <i>et al</i> , 1997)	<i>NLGN4X</i> (Jamain <i>et al</i> , 2003) <i>NLGN3</i> (Jamain <i>et al</i> , 2003) <i>SHANK3</i> (Durand <i>et al</i> , 2007)	<i>SEMA5A</i> (Weiss <i>et al</i> , 2009) <i>CDH9/CDH10</i> (Wang <i>et al</i> , 2009) <i>MET</i> (Campbell <i>et al</i> , 2009, 2006)
Deletions or duplications of chromosome 16p11 (Kumar <i>et al</i> , 2008; Weiss <i>et al</i> , 2008)	<i>SHANK2</i> (Berkel <i>et al</i> , 2010) <i>NRXN1</i> (Bucan <i>et al</i> , 2009; Glessner <i>et al</i> , 2009; Kim <i>et al</i> , 2008; Wisniewicka-Kowalnik <i>et al</i> , 2010) <i>PTCHD1</i> (Noor <i>et al</i> , 2010) <i>IL1RAPL1</i> (Piton <i>et al</i> , 2008) <i>SYNGAP1</i> (Hamdan <i>et al</i> , 2011, 2009; Pinto <i>et al</i> , 2010) <i>SLC6A4</i> (Sutcliffe <i>et al</i> , 2005)	<i>CNTNAP2</i> (Alarcon <i>et al</i> , 2008; Arking <i>et al</i> , 2008; Bakkaloglu <i>et al</i> , 2008) <i>EN2</i> (Benayed <i>et al</i> , 2005; Brune <i>et al</i> , 2008; Gharani <i>et al</i> , 2004)



intellectual disability. Various hypotheses have been raised regarding the increased risk in males. In the general population, average social communication function is lower in males in comparison with females (Constantino and Todd, 2003; Skuse *et al*, 2005). Simon Baron-Cohen and co-workers have hypothesized that fetal testosterone exposure may influence social development, with some support from direct and indirect measures of fetal testosterone (Baron-Cohen, 2010). Skuse *et al* (1997) explored an alternative hypothesis in Turner Syndrome, where girls have a single X chromosome. Girls who received their single X chromosome from their mothers, as is true for all boys, had significantly worse social function than girls who received their X chromosome from their fathers, suggesting that genetic imprinting effects may account for part of the difference in social function. As tabulated, a few genes on the X chromosome have also been examined for a potential role in ASD. Further analysis of sex-specific risk factors could yield distinct treatments for male and female subjects with ASD, as has been suggested recently for PTSD (Kingwood, 2011; Ressler *et al*, 2011).

### Parental and Prenatal Factors

Parental and pregnancy factors appear to affect the risk of ASD. Increasing paternal or maternal age increases the risk of ASD, which may relate to a role for *de novo* genetic variation, including either mutations or CNVs (Durkin *et al*, 2008; Hultman *et al*, 2010; King *et al*, 2009b). Increased loading of pregnancy complications has been reported in children with ASD in comparison with controls; although to date no single complication has been clearly associated with ASD (Gardener *et al*, 2009). Very low birth-weight or extreme preterm birth also increases the risk of ASD. Evidence suggests that these children may have a different pattern of comorbidities, including intellectual or motor disability, in comparison with the general population of children with ASD (Buchmayer *et al*, 2009; Limperopoulos *et al*, 2008; Schendel and Bhasin, 2008; Schieve *et al*, 2010). One recent study reported an increased risk of ASD following a short inter-pregnancy interval, suggesting a potential role for maternal nutritional status or other gestational factors (Cheslack-Postava *et al*, 2011).

A couple of rare environmental exposures during pregnancy also increase the risk of ASD (Chess, 1971). Rubella exposure during the first trimester clearly increases the risk of ASD; although congenital rubella syndrome also includes sensorineural deafness, eye abnormalities, intellectual disability, and cardiac malformations (Duszak, 2009). First-trimester exposure to thalidomide also increases risk; although this is also associated with limb, eye, and cardiac malformations (Stromland *et al*, 1994). Likewise, *in utero* exposure to valproic acid appears to increase risk; although this is also associated with a broader syndrome including neural tube defects (Williams *et al*, 2001). One recent study reported an increased risk of ASD following prenatal exposure to SRI medications (fluoxetine, sertraline, etc.),

especially during the first trimester (Croen *et al*, 2011). Further work will be necessary to understand whether this is related to the drugs themselves, or to target symptoms or conditions that are more common in mothers of children with ASD. As a group, these prenatal risk factors are rare causes of ASD, but they suggest a window of vulnerability to environmental agents that increase ASD risk in the first trimester of pregnancy. Additionally, these causes provide opportunities to establish preclinical models whose investigation may uncover key lessons for idiopathic ASD. In this regard, rodent models of *in utero* viral or valproic acid exposure have been studied and are reviewed elsewhere (Patterson, 2011).

### BIOMARKERS IN ASD

There is a long tradition of biomarker research in ASD. Biomarkers may point toward ASD susceptibility factors in different ways. In theory, a biomarker could contribute directly to susceptibility, but a biomarker also may represent an endophenotype, or a heritable trait resulting from an underlying factor that is the prime contributor to ASD susceptibility (Gottesman and Gould, 2003). Finally, a biomarker may be a secondary result of ASD itself or of ASD treatment. Deciding among these possibilities has therapeutic relevance in narrowing down potential targets and/or using the particular measure as a diagnostic or treatment aid.

The first biomarker described in ASD was elevated whole-blood 5-HT, or hyper-serotonemia, first identified 50 years ago (Schain and Freedman, 1961), and unique to autism among developmental disorders (Hanley *et al*, 1977; Mulder *et al*, 2004). Remarkably, gut enterochromaffin cells that synthesize peripheral 5-HT have never been directly assessed in ASD. In the blood, 5-HT is contained almost exclusively in platelets, which acquire 5-HT through the SERT as they pass through the gut circulation. Not surprisingly, whole-blood 5-HT levels are correlated with SERT-mediated 5-HT uptake (Cook *et al*, 1993; Cross *et al*, 2008). Additional abnormalities have been found in the platelet in ASD, including decreased radioligand binding to the 5-HT<sub>2A</sub> receptor (Cook *et al*, 1993; McBride *et al*, 1989). Importantly, whole-blood 5-HT levels have been shown to be highly heritable, more heritable than ASD itself (Abney *et al*, 2001). Postnatal platelet 5-HT is unlikely to directly affect brain function, given its lack of penetration of the blood-brain barrier, but the protein networks regulating peripheral 5-HT homeostasis are largely conserved in the brain. Indeed, the SERT gene (*SLC6A4*) and the 5-HT receptor 5-HT<sub>2A</sub> gene (*HTR2A*) encode the same protein in the platelets and brain (Cook *et al*, 1994; Lesch *et al*, 1993). Abnormalities in brain 5-HT systems have also been described in ASD, including an altered developmental trajectory of 5-HT turnover (Chugani *et al*, 1999) and decreased radioligand binding to both 5-HT<sub>2A</sub> (Goldberg *et al*, 2009; Murphy *et al*, 2006) and SERT (Nakamura *et al*, 2010).

The known role of the 5-HT system in brain development raises the possibility that changes in 5-HT homeostasis during development result in altered neuronal migration or neurite outgrowth. As one example, 5-HT modulates thalamocortical axon projections to the sensory cortex, with excessive extracellular 5-HT leading to disruption of the somatosensory map in rodents (Bonnin *et al*, 2007; Salichon *et al*, 2001). Recently, Bonnin and colleagues discovered that the placenta synthesizes and releases 5-HT during embryonic brain development at a point before serotonergic raphe axons innervate the forebrain. Moreover, placental release of 5-HT appears to account for the forebrain 5-HT levels at this time, independent of the brain's synthetic capacity (Bonnin *et al*, 2011). These studies add another dimension to the complex role of 5-HT in brain development. Interestingly, the placenta is also a site of high-level expression of SERT proteins (Prasad *et al*, 1996). Indeed, human SERT was first cloned from placenta (Ramamoorthy *et al*, 1993), presaging these most recent studies. The recent finding that prenatal exposure to SRIs may increase ASD risk could therefore point to their activity in the maternal circulation, the placenta, or the developing fetal brain (Croen *et al*, 2011).

Developmental studies of both head circumference and MRI-derived whole-brain size have identified an abnormal rate of brain growth over the first few years in ASD. Using a combination of cross-sectional and longitudinal data, Eric Courchesne and others have identified a surge in brain growth in the first 2 years of life in children with ASD (Courchesne *et al*, 2003, 2001; Hazlett *et al*, 2005; Redcay and Courchesne, 2005; Schumann *et al*, 2010). The cause of this increase in brain growth is unknown, but it appears to be largely symmetric, with increases in both gray matter and white matter (Hazlett *et al*, 2005; Schumann *et al*, 2010). A small number of individuals with ASD do have macrocephaly in adulthood, which can be thought of as a separate biomarker (Courchesne *et al*, 1999), but the majority do not, suggesting a decline in the rate of further brain growth in middle childhood (Courchesne *et al*, 2011). An increased rate of head growth early in life is also seen in some siblings of children with ASD who do not share the diagnosis, so this trait may also be an endophenotype for ASD (Constantino *et al*, 2010a; Elder *et al*, 2008). Interestingly, Joe Piven's group identified an association between an *SLC6A4* promoter polymorphism and cortical gray matter overgrowth in children with autism (Wassink *et al*, 2007), potentially linking compromised 5-HT signaling during development to this well-recognized ASD biomarker.

Structural neuroimaging studies have also sought to identify biomarkers of autism. Regional findings have been inconsistent, but a few patterns may be emerging. First, there appears to be an initial increase in amygdala size in early childhood, followed by a possible decrease in size over time (Kim *et al*, 2010; Mosconi *et al*, 2009; Nacewicz *et al*, 2006; Schumann *et al*, 2009; Stanfield *et al*, 2008). Second, there appears to be a general decrease in long-range

connectivity (Barnea-Goraly *et al*, 2010; Frazier and Hardan, 2009; Shukla *et al*, 2010). These findings are not perfectly consistent, but they have led to a hypothesis of increased local connectivity and decreased distant connectivity (Courchesne and Pierce, 2005). Third, striatal size is increased in multiple studies, with size correlating positively with indices of repetitive behavior (Haznedar *et al*, 2006; Langen *et al*, 2007, 2009; Sears *et al*, 1999). Recent reviews of the structural neuroimaging literature highlight other findings that await consistent replication (Anagnostou and Taylor, 2011; Chen *et al*, 2011; Stanfield *et al*, 2008). Ultimately, these structural findings may connect to post-mortem neuropathological findings in autism, but sample sizes have been difficult to accrue and data are only just beginning to emerge, initial findings included decreased cortical minicolumn width (Casanova *et al*, 2002) and decreased GABA receptor binding (Fatemi *et al*, 2009; Oblak *et al*, 2011) in ASD subjects.

Functional neuroimaging studies in ASD present significant challenges. First, individuals with more severe impairments are unlikely to tolerate the scanner. Second, the inherent behavioral and cognitive differences in ASD may drive changes in brain response. For example, Richie Davidson's group used eye tracking to show that lower fusiform face area activation corresponded to ASD subjects not looking at the face stimulus while in the scanner (Dalton *et al*, 2005). Similarly, tasks that probe cognitive deficits in ASD may show differences in brain activation that arise from the deficit itself, or reflect a compensation for the deficit. Basic sensory processing could also be altered in ASD (Foss-Feig *et al*, 2010; Kwakye *et al*, 2011; Marco *et al*, 2011), resulting in abnormal response to almost any stimulus and without specificity for any particular task. Even with these caveats, quite a number of interesting functional neuroimaging findings have been reported that may relate to structural imaging finding, such as altered amygdala response to social stimuli (Kleinmans *et al*, 2009; Weng *et al*, 2011). The interested reader is referred to several recent reviews on this topic (Anagnostou and Taylor, 2011; Di Martino *et al*, 2009).

Additional peripheral biomarkers are beginning to emerge in ASD. For example, much interest has centered on possible mitochondrial dysfunction (Frye and Rossignol, 2011; Giulivi *et al*, 2010). Although data are accumulating to support the existence of a subgroup of ASD children with such changes, the size of this subgroup and the specificity of these findings remain unclear (Oliveira *et al*, 2005). Peripheral biomarkers related to the immune system have also generated considerable interest. Whereas evidence suggests that there may be altered immune system function in some children with ASD, the specific alterations appear to vary across studies and will require further analysis to reach consensus (Careaga *et al*, 2010). Interestingly, SERT proteins are expressed by B-lymphocytes (Faraj *et al*, 1994; Meredith *et al*, 2005) and 5-HT signaling contributes to immune system function (Aune *et al*, 1994; Hofstetter *et al*, 2005; Young *et al*, 1993).

## GENETIC SYNDROMES THAT SHOW ASD FEATURES

Multiple genetic syndromes include ASD symptoms as part of a broader pattern of dysmorphology and medical morbidity. Space does not permit a review of all such syndromes, but here we highlight a few examples (Table 2) that each have a substantial rate of ASD, include macrocephaly as a prominent feature, and affect signaling through the mTor pathway. A comprehensive review by Betancur (2011) provides extensive detail on many other genetic or genomic disorders that may lead to ASD susceptibility.

The most common, inherited cause of ASD is Fragile X syndrome (FraX), an X-linked recessive disorder that is found in 2–3% of individuals with ASD and is also the most common inherited cause of intellectual disability (Hagerman *et al*, 2009; Loesch *et al*, 2007). An expanding trinucleotide repeat in the 5' untranslated region of the *FMR1* gene causes Fragile X in males (and in some females) with more than 200 copies of the repeat (Kremer *et al*, 1991). Intellectual disability is a defining feature of FraX, along with a characteristic facial appearance, risk of seizures, and macroorchidism. Behavioral features frequently include hyperactivity, anxiety, sensory sensitivity, and avoidance of eye contact (Hagerman *et al*, 2009). Approximately 60% of individuals with FraX meet the criteria for ASD; although patterns of symptoms differ from the general ASD population (Hall *et al*, 2010; Harris *et al*, 2008). As reviewed below and elsewhere (Bhogal and Jongens, 2010; Heulens and Kooy, 2011), significant progress has been made in understanding the molecular changes that connect loss of *FMR1* expression to the observed neurological, cognitive, and behavioral abnormalities in the mouse model. Prominent among the networks disturbed by loss of *FMR1* is the mTor pathway and

associated translational control mechanisms (Penagarikano *et al*, 2007; Sharma *et al*, 2010).

Three additional single-gene disruption disorders cause excessive signaling through the mTor pathway and lead to increased risk of ASD, as reviewed below (Figure 2). *PTEN* mutations lead to a hamartoma tumor syndrome as well as macrocephaly, and often ASD (Butler *et al*, 2005; Goffin *et al*, 2001). Mice lacking *PTEN* expression in neurons show deficient sociability (Kwon *et al*, 2006; Zhou *et al*, 2009), as do mice with hemizygous constitutive *PTEN* knockout (KO) (Page *et al*, 2009). Interestingly, and with relevance to our subsequent section on potential 5-HT mechanisms in ASD, *PTEN* and *SLC6A4* cooperatively exacerbate brain size and sociability phenotypes (Page *et al*, 2009). Tuberous sclerosis is caused by mutations in either hamartin (*TSC1*) or tuberlin (*TSC2*) (Consortium, 1993; van Slegtenhorst *et al*, 1997). Each of these syndromes, as well as related syndromes further upstream or downstream from mTor signaling (Table 1 and Figure 2), result in macrocephaly, a potential connection with this transient biomarker in ASD. As reviewed below, exciting research suggests that targeting control mechanisms for the mTor pathway may benefit individuals with these syndromes, and perhaps idiopathic ASD as well.

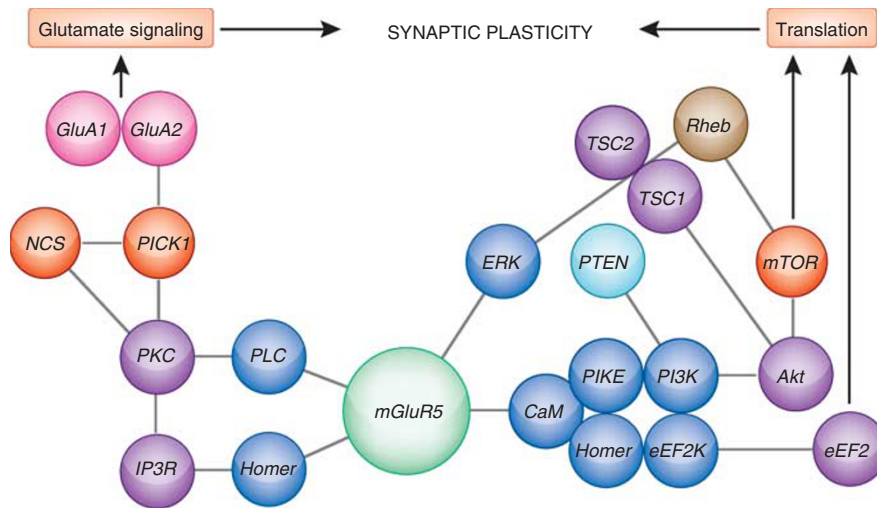
## PATH FROM RARE GENETIC SYNDROMES TO NEW TREATMENTS IN ASD

### mTor and Regulation of Protein Synthesis

A major goal of preclinical studies of ASD susceptibility genes is the translation of disrupted signaling pathways to medications that can relieve physiological and behavioral deficits. In the past, such a challenge seemed naive, as

**TABLE 2** Genetic Syndromes that Affect mTor Signaling and Increase Risk of ASD

Syndrome	Gene	Common symptoms	Effect on mTor
<i>PTEN</i> hamartoma tumor syndrome	<i>PTEN</i>	Macrocephaly Hamartomas in multiple organs Lipomas Cancer risk	Disinhibition
Tuberous sclerosis	<i>TSC1</i> , <i>TSC2</i>	Macrocephaly Hamartomas in multiple organs Renal cysts and angiomyolipomas Facial angiofibromas	Disinhibition
Neurofibromatosis, type-I	<i>NF1</i>	Macrocephaly Fibromatous skin tumors CNS tumors	Disinhibition
Fragile X syndrome	<i>FMR1</i>	Macrocephaly Macroorchidism Risk of seizure disorder	Disinhibition of downstream gene regulation



**Figure 2.** Network of gene products connecting glutamate signaling to translation and synaptic plasticity. The network shown represents physical and signaling interactions between the gene products noted. mGluR5 is centrally located in this illustration to note its capacity for the regulation of both glutamate signaling through ionotropic glutamate receptors and mRNA translation through mTOR-linked signaling pathways. Convergence of FMR1 and TSC1/TSC2 gene products altered that signal through mTOR-linked translation control pathways, independent of mGluRs, are also shown.

lifelong alterations in cognitive function and social reciprocity were believed to derive from irreversible modifications of neuronal wiring established during early brain development. Initial findings suggest that, although the micro-circuitry of the brain in ASD subjects may be altered, deficits in synaptic signaling and plasticity may be of greater significance. The implications of this perspective are not insignificant as they raise the prospect that ASD traits could be ameliorated if the mechanisms compromised can be identified. Adding optimism to this idea, studies with transgenic mouse models of single-gene disorders that bear ASD features have demonstrated remarkable reductions, and in some cases elimination of behavioral deficits following either genetic and/or pharmacological manipulations (Ehninger *et al*, 2008a; Guy *et al*, 2007; Tropea *et al*, 2009; van Woerden *et al*, 2007; Zhou *et al*, 2009). A comprehensive review of these findings has been published recently (Ehninger *et al*, 2008b). As we do not cover them here, the interested reader should pay particular attention to mouse models of neurofibromatosis and Rett syndrome, where striking reversals of behavioral deficits have been achieved in mature animals (Ehninger *et al*, 2008a; Li *et al*, 2005). Here, we outline other instances of such success to highlight the promise that basic research offers for the treatment of ASDs, with reference to pathways linked to mTOR and 5-HT signaling.

As noted above, tuberous sclerosis, derived from heterozygous inactivation of *TSC1* or *TSC2*, presents with ASD features. The diagnostic features of this disorder are epithelial growths ('tubers') visible on MRI scans. However, studies fail to correlate the abundance of tubers with cognitive deficits in humans and in mouse models (Numis *et al*, 2011). Most recently these deficits have been found to derive from neuronal signaling alterations associated with disrupted mTOR signaling. *TSC1* and *TSC2* form a hetero-

dimeric complex that negatively regulates mTOR-signaling pathways that control protein translation. Protein synthesis within synaptic spines is a key feature of the long-term plasticity of neurons and is now known to be required for proper cognitive function, as well as the actions of chronically administered antidepressant medications (Li *et al*, 2010). Ehninger *et al* reasoned that constitutively elevated mTOR signaling could be offset by rapamycin, an mTOR inhibitor. Remarkably, they achieved reversal of deficits in spatial learning and context discrimination with brief (5 day) postnatal administration of rapamycin (Ehninger *et al*, 2008a). These studies nicely converge with studies involving manipulation of the lipid phosphatase PTEN, an upstream regulator of the *TSC1/TSC2*-mTOR pathway, which also can produce amelioration of the structural and neurological features of ASD in mouse models (Zhou *et al*, 2009).

The paradigmatic disorder with ASD features that links to protein translation is FraX (Kremer *et al*, 1991). The FMR1 protein (FMRP) binds to mRNAs and regulates their transport and translation (Ashley *et al*, 1993; Eberhart *et al*, 1996). Huber *et al* (2002) first identified changes in long-term depression, a form of synaptic plasticity, in *Fmr1*-KO mice that is mediated by hyperactivity of metabotropic glutamate receptor type-5 (mGluR5) signaling and linked to the translation and trafficking of AMPA subtype glutamate receptors. These studies led Bear and colleagues (2004) to propose the 'mGluR theory' of FraX, positing that, in the absence of FMRP, mGluR1/5 stimulation results in excessive protein synthesis, thereby leading to excessive trafficking of AMPA receptors away from the cell surface. These investigators tested their hypothesis by decreasing the normal expression of mGluR5 (Dolen *et al*, 2007), demonstrating a remarkable reversal of protein synthesis, dendritic spine alterations, and multiple



behavioral phenotypes. These studies provide robust that manipulation of mGluR5-signaling pathways could provide relief for multiple aspects of FraX. They also provide a cogent example of how context can dramatically impact the penetrance of otherwise devastating genetic changes.

Consistent with the ability of a partial loss of mGluR5 to reverse cognitive deficits of *Fmr1*-deficient mice, the mGluR1/5 antagonist MPEP reverses multiple behavioral abnormalities in *Fmr1*-null mice (de Vrij *et al*, 2008; Levenga *et al*, 2011; Yan *et al*, 2005). Remarkably, Su *et al* (2011) recently reported that early, continuous, treatment with MPEP can even reverse dendritic spine abnormalities in *Fmr1*-KO mice. One preliminary clinical study of an mGluR5 antagonist in adult FraX subjects showed some promise, but only in the subgroup that had complete *FMR1* methylation (Jacquemont *et al*, 2011). Additional trials of mGluR5 antagonists in FraX are ongoing. The use of MPEP to treat features of ASDs may well extend beyond genetic disruptions in *Fmr1*. Thus, Jacki Crawley's group has observed that MPEP can reverse repetitive grooming behavior in the BTBR inbred strain of mice (Silverman *et al*, 2010). Interestingly, loss of the *Drosophila Fmr1* homolog leads to elevated dopamine and 5-HT synthesis (Zhang *et al*, 2005). Gruss and Braun (2001) reported only modest correlates of these changes in monoamines in the *Fmr1*-null mouse; although we must remember that tissue levels provide a limited window on monoamine signaling *in vivo*. Further, the behavioral actions of 5-HT-elevating antidepressants have been linked to mTOR activation (Li *et al*, 2010) and alleles of 5HTTLPR have been found to associate with elevated aggression and destructive behavior in FraX patients (Hessl *et al*, 2008). These findings suggest that features of altered 5-HT signaling, either with respect to early developmental signaling or enduring functional modulation, may derive in part from 5-HT signaling through the mTOR pathway. In this regard, Cleary *et al* (2008) found that the mTOR inhibitor rapamycin exerts activity typical of an SRI in animal models in suppressing struggling in both the forced-swim and tail suspension tests.

In summary, multiple single-gene disorders with ASD features derive from compromised regulation of mTOR-linked signaling pathways. Whether deficits associated with idiopathic ASD derive from these pathways remains to be clarified. The heterogeneity of ASD should at the outset suggest that subgroups, behaviorally or molecularly defined, will likely need to be considered in therapeutic development. The search for synaptic signaling networks that control or are altered by mTOR signaling has revealed mGluR5 as a target whose manipulation can be projected to produce less side effects than targeting mTOR more directly. MPEP itself is likely a 'proof-of-concept' drug, most useful for preclinical animal studies. Additionally, only a limited number of behavioral features and synaptic alterations of ASD models have been queried for responsiveness to mGluR5 manipulation. With respect to the latter point, Suvrathan *et al* (2010) have recently extended the

utility of MPEP for mGluR5 manipulation in reversing pre-synaptic deficits in the amygdala, work that could have relevance for changes in amygdala function in ASD. Finally, mGluR5 is likely but one 'druggable' target, including other surface receptors (eg, IL-1Rs), whose signaling can impact mTOR networks and thus be targeted for potential therapeutics. Interestingly, IL-1Rs have also emerged with respect to control of 5-HT signaling, as discussed below. We suspect that in the coming years, the study of this and other targets will contribute further momentum to an already active area of mTOR-targeted research.

## FROM A BIOMARKER TO GENETIC VARIATION TO NEW TREATMENTS FOR ASD?

### SERT-Mediated 5-HT Homeostasis

Based on the strong heritability of whole-blood 5-HT levels, Ed Cook and Carole Ober's labs mapped quantitative trait loci (QTLs) in a large founder population, revealing association with *ITGB3*, which encodes the SERT-associated protein integrin- $\beta 3$  (Weiss *et al*, 2004). A follow-up scan revealed association of both *ITGB3* and *SLC6A4* with whole-blood 5-HT specifically in males (Weiss *et al*, 2005). Multiple linkage scans in ASD have shown significant evidence for linkage in the chromosome 17q region containing *SLC6A4* (International Molecular Genetic Study of Autism Consortium, 2001; McCauley *et al*, 2004; Stone *et al*, 2004; Sutcliffe *et al*, 2005). Sutcliffe *et al* (2005) showed that this linkage evidence is confined to families containing two or more affected males and no affected females, paralleling the QTL data for hyper-serotonemia noted above. As common *SLC6A4* variants did not explain the strong linkage signal on 17q, the Sutcliffe and Blakely labs searched for rare variants (Sutcliffe *et al*, 2005) and identified multiple, rare non-synonymous variants, with each demonstrating increased 5-HT uptake (Prasad *et al*, 2009, 2005). Interestingly, Dennis Murphy's group identified an SERT variant, Ile425Val, that segregates with obsessive-compulsive disorder (OCD) (as well as other neuropsychiatric disorders including Asperger's syndrome) in two unrelated pedigrees (Ozaki *et al*, 2003). Gary Rudnick's group established that Ile425Val shows constitutively elevated 5-HT transport activity (Kilic *et al*, 2003).

### Targeting Altered 5-HT Signaling in ASD

Following a model of diminished synaptic 5-HT availability, one would predict that agents that reduce 5-HT availability would negatively impact ASD subjects, whereas SRIs should be beneficial. Consistent with this idea, short-term tryptophan depletion worsens ASD symptoms (McDougle *et al*, 1993). Three small, randomized, placebo-controlled trials have found beneficial effects of the SRIs clomipramine, fluvoxamine, and fluoxetine for OCD-like behavior in ASD (Gordon *et al*, 1993; Hollander *et al*, 2005; McDougle *et al*,

TABLE 3 Serotonin Metabolism and Transport Genes

Gene	Protein	Action
<i>TPH1</i>	Tryptophan hydroxylase-1	Rate-limiting enzyme in 5-HT synthesis in the periphery, converts tryptophan to 5-hydroxy-tryptophan
<i>TPH2</i>	Tryptophan hydroxylase-2	Rate-limiting enzyme in 5-HT synthesis in serotonergic neurons, converts tryptophan to 5-hydroxy-tryptophan
<i>DDC</i>	Aromatic L-amino acid decarboxylase	Enzyme in 5-HT and dopamine synthesis, converts 5-hydroxy-tryptophan to 5-HT
<i>VMAT2</i>	Vesicular monoamine transporter, type-2	Transports 5-HT into pre-synaptic vesicles in a proton-dependent manner
<i>SLC6A4</i>	Serotonin transporter (SERT)	Transports 5-HT into the pre-synaptic neuron in a sodium-dependent manner
<i>MAOA</i>	Monoamine oxidase	Converts 5-HT to 5-hydroxyindoleacetic acid (5-HIAA), its primary metabolite

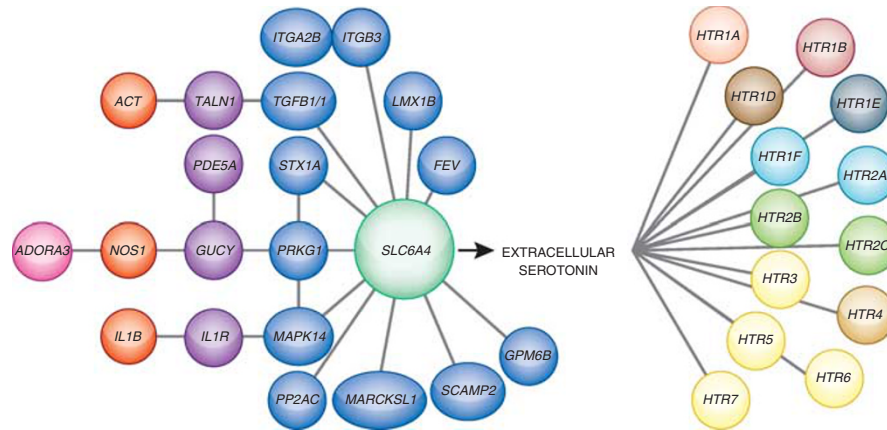
1996). By contrast, one large, randomized trial reported no efficacy for the SRI citalopram for repetitive behavior in ASD subjects (King *et al*, 2009a). Additional studies are needed that use selection criteria based on biomarkers, genotypes, or more specific impairing symptoms. The hyper-functional SERT variants were found in individuals with high scores on measures of rigid-compulsive traits and sensory aversion (Sutcliffe *et al*, 2005), suggesting that SERT blockade might be most beneficial for these individuals. Despite the appeal of SRIs as available drugs, however, complete blockade of SERT is likely to overshoot a modulated restoration of 5-HT homeostasis (Table 3). Below we discuss the network of gene products that regulate SERT where novel therapeutics could be developed that conditionally diminish SERT activity, permitting SERT to maintain a basal level of control over synaptic 5-HT.

Prominent pathways that regulate SERT trafficking and catalytic function include those linked to PKC, PKG, and p38 MAPK signaling (Samuvel *et al*, 2005; Steiner *et al*, 2009; Zhu *et al*, 2005) (Figure 3). Importantly, many of the rare SERT coding variants show altered sensitivity to these signals (Prasad *et al*, 2009, 2005). Certainly these networks, like mTOR pathways, are widespread and have many targets, making their therapeutic manipulation problematic. As with mTOR, however, there are ways to enter these pathways in a more cell-specific manner. For example, brain SERT activity can be enhanced by adenosine-A3 and IL-1 $\beta$  receptors (A3AR and IL-1R), with loss of regulation evident in A3AR and IL-1R-KO mice, respectively (Zhu *et al*, 2010, 2007). A3ARs signal to SERT through PKG and p38 MAPK, whereas IL-1Rs bypass PKG to activate p38 MAPK directly.

Given the capacity of A3AR and IL-1R activation to mimic the elevated 5-HT transport activity observed

constitutively in ASD-associated SERT coding variants, it appears reasonable to consider whether pharmacological modulation of A3AR- or IL-1R-signaling pathways could lead to novel ASD therapeutics. Although a connection between IL-1Rs and 5-HT signaling is growing (Capuron and Miller, 2011; Zhu *et al*, 2011), we focus our discussion here on A3ARs. Among the CNS adenosine receptor subtypes, A3ARs are the least studied, receiving more attention in the control of peripheral immune function and inflammation (Hasko *et al*, 2008). Indeed, early investigators questioned whether A3ARs exist in the brain at all (Rivkees *et al*, 2000). However, more recent efforts have established clear actions of A3AR agonists in protection of the CNS against hypoxia/ischemic and inflammatory insults (Borea *et al*, 2009; Haas and Selbach, 2000; Taliani *et al*, 2010), as well as support for synaptic signaling and plasticity (Brand *et al*, 2001; Costenia *et al*, 2001; Dunwiddle *et al*, 1997; Macek *et al*, 1998).

Recently, Zhu *et al* (2011) demonstrated that SERT and A3ARs colocalize in raphe neurons and can form an A3AR agonist-sensitive physical complex, providing further rationale to pursue pharmacological manipulation of A3AR-linked signaling for 5-HT-associated disorders. Trials of the A3AR-selective agonist CF 101 (IB-MECA) have been initiated and the agent has been found to be well-tolerated and capable of anti-inflammatory effects. Future studies will hopefully explore the action of this agent on behaviors supported by CNS 5-HT signaling. Additional proteins that coordinate A3AR signaling to SERT (Figure 3) should also be considered as potential therapeutic targets. SERTs exist in physical complexes with other signaling, scaffolding, and cytoskeletal proteins. Among these are NOS1 that produces nitric oxide to activate guanyl cyclase, producing cGMP and thereby activating the cGMP-responsive kinase PKG1 $\alpha$  (Carneiro and Blakely, 2006; Carneiro *et al*, 2008; Chanrion



**Figure 3.** Network of gene products regulating the expression, trafficking, and activity of the antidepressant-sensitive 5-HT transporters (SERT, SLC6A4). SERT controls the extracellular availability of 5-HT in the brain and periphery, and also recycles serotonin for further rounds of release. SERT-mediated clearance of 5-HT limits the amplitude and duration of signaling of more than a dozen 5-HT receptors. We hypothesize that just as risk for ASD can derive from genetic variation in SERT that impacts 5-HT signaling during brain development, risk for ASD can also be generated through environmental or genetic perturbations of SERT-regulatory control mechanisms.

*et al*, 2007; Quick, 2003; Steiner *et al*, 2009). Phosphodiesterases, such as PDE2, 5, and 9, hydrolyze cGMP, thereby reducing PKG stimulation of SERT. Agents that manipulate one or more of these steps in the A3AR-signaling pathway to SERT may provide novel approaches to normalize 5-HT-associated behavioral deficits.

Paralleling efforts to model disruptions in mTor-regulatory pathways using mouse models, we have produced SERT Ala56-knock-in mice, introducing the most common of the hyper-functional ASD SERT variants into the native SERT gene locus (Veenstra-VanderWeele *et al.*, unpublished data (submitted)). Importantly, SERT Ala56 mice show hyper-serotonemia and enhanced rates of 5-HT clearance *in vivo*, as predicted from findings of elevated 5-HT transport *in vitro*. As seen with the variant in transfected cells, mid-brain SERT Ala56 protein shows p38 MAPK-dependent hyper-phosphorylation, further linking physiological alterations to the SERT-regulatory network discussed above. SERT Ala56 mice show hypersensitivity at 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors that likely arises as compensation for decreased synaptic 5-HT availability. Finally, we observe abnormalities in social, communication, and repetitive behavior in the SERT Ala56 mice, opening the door to detailed mechanistic studies in a model of autism with both face and construct validity. Studies are underway to understand the developmental *vs* dynamic effects of the SERT Ala56 variant in these animals, and to explore whether the physiological and behavioral alterations seen can be reversed. 5-HT<sub>1A</sub>- and 5-HT<sub>2A</sub>-based agents should be considered given the altered receptor sensitivities noted in the SERT Ala56 mice. Both receptors are currently targeted by pharmacologically complex agents such as risperidone (D<sub>2</sub> and 5-HT<sub>2A/2C</sub> antagonist, etc.), which is effective for treatment of irritability in autism (McCracken *et al*, 2002; McPheeters *et al*, 2011), and buspirone (5-HT<sub>1A</sub> partial agonist; D<sub>2</sub>,  $\alpha_1$ ,  $\alpha_2$  antagonist, etc.), which is under

study in a large, randomized trial in autism (Edwards *et al*, 2006).

## SUMMARY

ASD is a heterogeneous and multi-factorial condition, and identifying subgroups of individuals will be necessary to gain traction into its pathophysiology and novel opportunities for treatment. Possible subgroups can be identified based on biomarkers, such as macrocephaly or indicators of mitochondrial dysfunction, or genetic findings, such as the neurexin–neuroligin system. Two of the established ASD subgroups are based on abnormal mTOR and 5-HT signaling in some individuals with ASD. These two networks represent opportunities to move from genetic and biomarker findings to model systems that allow studies of mechanisms and potential novel treatments for ASD. Multiple single-gene disorders with ASD features converge on altered control of mTOR signaling. Elegant work on the connection of mTOR signaling to glutamate-supported synaptic plasticity has led to considerations of regulatory glutamate receptors, particularly mGluR5, as a potential target for novel ASD medications. Hyper-serotonemia has now reached its 50th anniversary as a biomarker in ASD, compelling consideration of recent genetic studies that point to dysregulation of SERT and/or its regulatory networks. ASD-associated SERT coding variants provide a framework to better understand the contribution of altered 5-HT homeostasis to brain development and ASD-related behavior. We suggest future consideration of agents that engage proteins mediating SERT stimulation by A3ARs (eg, A3AR, NOS1, PKG1, p38 MAPK) but remind ourselves that, as with targeting mTOR pathways, the heterogeneity of ASD will likely require multiple approaches that are tailored to individuals with specific symptoms, genetics, and biomarker profiles.



## FUTURE RESEARCH DIRECTIONS

It is clear that new therapies are desperately needed for ASD. We believe that the soundest way forward is to follow the biology and decode its messages, whether they are established by genetics, the environment, or the continual dialog between the two. From single-gene disorders with ASD features comes a realization that the mTOR-signaling network may have many nodes where ASD risk is embedded. From biomarker, developmental, and genetic studies comes a reminder of how much, despite decades of study, we still have to learn about 5-HT, such as whether its signaling regulators might themselves represent one such node of the mTOR pathway and vice versa. Should this prove to be the case, agents that target specific 5-HT receptors and their signaling partners may prove beneficial in ASD treatment. As with mGluR5 and mTOR, the biology of 5-HT signaling can teach us how to target widespread signaling pathways in nuanced ways that can offer more help than harm. In that light, we suggest that targeting of SERT-regulatory pathways, as opposed to the pharmacological bludgeoning of the transporter itself, as with SRIs, may offer options for ASD treatments. Clearly, new animal models are needed, particularly those that result from environmental or genetic perturbations that lead to ASD traits in some individuals, as in the use of rare, penetrant coding mutations in genes expressed in brain areas impacted by the disorder, early in development. The era of trying to use animals with odd behaviors as models for neuropsychiatric disease is drawing to a close. Just because there is evidence of mitochondrial dysfunction or changes in the immune system, does not mean that we can accept any model that generates such changes. We can do better. Our models must be increasingly linked to the explicit mechanisms responsible for these changes, at least in some ASD subjects. Finally, we have noted above the intersections emerging between mTOR and 5-HT networks in the brain. There are observations outside the CNS that suggest similar convergences (eg, Soll *et al*, 2010). The study of 5-HT has long captured the interest of translational researchers. The elucidation of translational mechanisms influenced by a convergence of 5-HT/mTOR-signaling pathways may further fuel this interest.

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