DEFECTIVE CHANNEL FUNCTION IN AUTISM

Ion channels are a large family of transmembrane proteins that provide ions a passive pathway through which they can rapidly diffuse down their electrochemical gradient across the hydrophobic membrane. Objective quantifiable biochemical markers of this disease have been very hard to come by. However, the high heritability (h²) of ASD, which while still controversial has been calculated at up to 90%, makes it one of the most highly heritable behavioral disorders (Hallmayer et al., 2011; Devlin and Scherer, 2012). This provides powerful assurance that genes and the biochemical pathways they subserve are involved in the disorder. Identifying such alterations in affected individuals would provide an added dimension to the phenotype, perhaps refining more coherent subgroups of ASD (endophenotypes). Environmental impacts, also clearly implicated by monozygotic twins who are discordant for ASD (Hallmayer et al., 2011), may be best understood to perturb these same pathways. Those genes additionally lead to personalization of medicine, potentially serving as new molecular diagnostics of the disease and as targets for the development of new classes of highly selective medications, much as has been the case for cancer. Evidence suggests a complex etiology of autism, and it is not surprising that channelopathies have profound effects on brain functions. In the present work, we summarize existing evidence for the role of ion channel gene defects in the pathogenesis of autism with a focus on calcium signaling and its downstream effects.

Keywords: calcium, mTOR, Fragile X syndrome, tuberous sclerosis, Rett syndrome, Prader-Willi syndrome, Angelman syndrome

AUTISM AND AUTISM SPECTRUM DISORDERS

Autism is a disease that dramatically affects brain function early in development. Its societal consequence and costs are enormous, currently costing over $130 billion per year in the USA alone (Mandell and Knapp, 2012). Worse, its prevalence has been increasing over the last decade with current Center for Disease Control estimates suggesting that nearly 2% of children are affected (Blumberg et al., 2013). Symptoms of autism typically start between the second and third year of life and cause problems of a wide range of severity in various areas of development. It is a neurodevelopmental disorder with three core behavioral features: (1) qualitative impairment in social skills, (2) delayed or disordered language and communicative skills, and (3) restricted and repetitive behaviors. The autistic spectrum disorders (ASD), the preferred term for this broad constellation of pervasive developmental disorders, all share the same three characteristic core deficits. The clinical diagnosis of autism is made by specially trained physicians and psychologists who perform evaluations focused on detailed histories and behavioral observations. ASD diagnosis for research studies is stricter, more time consuming and quantitative, but even at this most refined level ASD remains a group of developmental disorders that are only behaviorally, not yet pathophysiologically, defined (Filipek, 2003). With the May 2013 publication of the new American Psychiatric Association Diagnostic and Statistical Manual (DSM-5), all autism subtypes will be merged into one umbrella diagnosis of ASD.
The standing electrochemical gradients that drive passive ion movements through channels are established by energy-dependent active transport mechanisms such as ion pumps and ion carriers (Gargus, 2008). Ion channels conduct ions four orders of magnitude faster than pumps and carriers, so in many ways channels act like highly selective water-filled pores that can be opened and closed in a controlled fashion (gated) to allow a specific ion species to flow. This causes a minuscule chemical flux but an appreciable electrical current sufficient to change the membrane potential toward the Nernst potential of the conducted ion. Since the sodium-potassium ATPase ion pump maintains a cytoplasm high in $K^+$ and low in $Na^+$ opposite to the cell exterior, the Nernst potential is interior negative for $K^+$ and interior positive for $Na^+$ as it is for $Ca^{2+}$. The channel's predominant permeant ion species is dictated by the nature of the channel's selectivity filter. At rest the predominating membrane permeability is for $K^+$. This means that an interior positive depolarization is created by opening $Na^+$ and $Ca^{2+}$ channels, an increasingly negative hyperpolarization created by opening $K^+$ channels, and, since the $Cl^-$ Nernst potential usually is near the resting potential, a stabilization of the membrane potential is created by opening $Cl^-$-channels.

Ion channel families vary in their mechanism of gating (Figure 1). One large family of channels gate by sensing changes in the electrical potential across the membrane – the voltage-gated ion channels. These channels respond to a membrane potential change by undergoing a conformational change from “closed” to “open.” In the “open” conducting state the channel’s own ionic current flows and thereby further alters the membrane potential. This behavior is critical to their function in perpetuating a propagating action potential (AP). As a patch of membrane begins to depolarize, voltage-gated $Na^+$ and $Ca^{2+}$ channels begin to open, increasing the membrane permeability to sodium and calcium, driving the membrane potential further toward their inside-positive Nernst potential, and hence explosively driving still more adjacent voltage-gated channels to open. Ultimately these channels intrinsically “inactivate” to cease conducting. Finally, voltage-gated $K^+$ channels open to repolarize the membrane, converting the dormant “inactive” sodium and calcium channels into a “closed” (but operable) state again, in preparation for conducting another AP.

A second large class of channels play a role in initiating an AP by inducing the triggering depolarization – the ligand-gated ion channels. They gate in response to the channel protein, typically located at the synaptic junction between cells, binding a ligand released into the synapse. The binding of a wide range of extracellular and intracellular diffusible ligands is able to directly gate ion channels, and many of these ligands are classical synaptic neurotransmitters such as acetylcholine or dopamine. In addition, a large family of ion channels is indirectly gated by ligands, many by the same neurotransmitters mentioned above, but in this case neurotransmitter binding occurs to a heptahelical G-protein coupled receptor (GPCR) and the channel is activated by a second messenger ligand, such as cyclic AMP, or a covalent modification, such as protein phosphorylation.

Like sodium, calcium passively enters the cytoplasm across the plasma membrane and is cleared from the cytoplasm to a level far below extracellular levels by a host of ion pumps and carriers at the expense of metabolic energy. For most ion channels...
it is predominantly the electrical consequences of channel activation that underlie their physiology and pathophysiology, but calcium is an important exception to this rule, since it plays an additional critical role in coupling electrical activity to biochemical pathways. Similar to sodium, calcium is eliminated back across the plasma membrane, but it is also uniquely sequestered for subsequent rapid release within intracellular calcium storage sites (Brom and Carafoli, 2011). Cytosolic calcium signals thus originate by either the rapid release of the intracellular store through intracellular ion channels or by extracellular calcium entering through ion channels across the plasma membrane. The intracellular calcium release channels have complex gating that includes responsiveness to plasma membrane ion channel protein voltage-sensitive conformational changes, changes in levels of cytosolic signaling intermediates, such as inositol 1,4,5-triphosphate (IP3) and changes in cytosolic calcium levels. Until recently the endoplasmic reticulum (ER) had been thought to contain the only dynamic intracellular pool of ionized calcium to participate in cellular signaling. This intracellular store could be rapidly released via intrinsic ER channels, the inositol 1,4,5-triphosphate receptors (IP3R) and the ryanodine receptors (RyR). Once released, this calcium would activate a host of kinases, ion channels and transcription factors, and then be resequencted via the ER’s calcium ATPase pump (SERCA). While mitochondria have long been known to sequester the vast majority of intracellular calcium, only relatively recently has the dynamic nature of this mitochondrial calcium pool been recognized (Spät et al., 2008; Subakalai and Duchen, 2008) and shown to communicate with the ER in the generation of rapid calcium signals, forming a bidirectional link between energy metabolism and cellular signals transmitted via changes in the cytosolic free calcium ion concentration (Danial et al., 2003; Patterson et al., 2004; Hayashi and Su, 2007).

Calcium signals are one of the most universal and ancient of cellular signals (Berridge et al., 2000). It is a versatile biological signal, known to regulate membrane potential, ion transporters, kinases, transcription factors and even cell morphology. It is therefore not surprising that a diverse host of diseases are coming to be recognized to be caused by disruptions of intracellular calcium homeostasis. This is an emerging pathophysiological mechanism of disease, a channelopathy (Stutzm et al., 2006; Berrozpe- vannya and Gargus, 2008; Betenhausen and Marks, 2010; Feske, 2010; Cain and Snutch, 2011), a special subset of the ion channel channelopathy diseases (Gargus, 2003, 2008, 2009).

ASD IN TIMOTHY SYNDROME HAS A CALCIUM CHANNELOPATHY PATHOGENESIS

The phenotype of Timothy syndrome (TS) involves multiple systems and specifically heart, brain, immune and skin cells. It includes mild dysmorphology of the face and syndactyly of fingers and toes, suggesting a perturbation of developmental signals. It is a simple autosomal dominant syndrome with high penetrance, implying that one defective copy of a single specific gene is sufficient to produce the full spectrum of disease. Clinically TS is predominated by prolonged ventricular repolarization and the lethal cardiac arrhythmia syndrome long QT (LQT), so called because of its characteristic EKG finding of a rate-corrected QT (QTc) interval of between 480 and 700 ms (Splawski et al., 2004). In addition to the dysmorphology, other variable extra-cardiac symptoms include seizures, hypotonia, immune deficiency and hypoglycemia. Remarkably, over 80% also have ASD (Splawski et al., 2004, 2005, 2011). The same rare specific allele of CACNA1C, a gene that encodes the “cardiac-expressed” voltage-gated calcium channel, was found to cause TS in all 12 original de novo unrelated cases (Splawski et al., 2004), suggesting that there must be only a very limited range of changes to channel function that create the diverse tissue phenotypes.

Long QT helped establish our understanding of channelopathy pathogenesis and it has now been shown to be caused by mutations in all of the cardiac ion channels that contribute to the ventricular AP (Bokil et al., 2010). The pathogenic alleles in these eight ion channel loci and four loci encoding channel-interacting proteins (LQT 1–12) all prolong the repolarization of the working myocardium, prolonging the QT interval and setting the stage for a fatal arrhythmia. Like most of the LQT mutations, TS (also called LQT8) is a simple monogenic dominant channelopathy, but unlike the others is also highly penetrant for a neurodevelopmental phenotype on the autism spectrum (Splawski et al., 2004, 2005, 2011).

Since so much is understood about the pathogenesis of LQT and the biophysics of the ion channels involved, and since TS makes it so clear that a specific mutation in this calcium channel causes both LQT and autism, TS holds an incomparable potential to reveal the pathophysiology of autism. The TS mutant channel expressed in the heart is also expressed in the neurons of the brain, and it must cause the symptoms in both organs since TS is a simple monogenic disease causing both phenotypes.

Only rare missense alleles have been recognized at the CACNA1C locus, and the specific recurrent de novo TS mutation, G406R, is located in the minor alternatively spliced exon 8A of the gene. Two other alleles in this locus cause a very similar syndrome, but without the syndactyly. These are found in exon 8, not 8A, suggesting cutaneous expression of only the minor transcript (Splawski et al., 2005). The two exons are mutually exclusive, with the vast majority of the mRNA containing exon 8, and both exons encoding the same protein domain. The splicing is developmentally regulated and is mediated by the polypyrimidine tract binding protein PTB (Tang et al., 2011). One of the exon 8 alleles produces exactly the same G406R missense as the classic TS mutation, but causes a severe early lethal disease, likely because of the higher abundance of this transcript isoform. The other allele in this exon, G402S, was only found in a mosaic individual, suggesting that most mutations in this gene are not compatible with viability. Indeed, even for the classical TS alleles somatic mosaicism seems to play a significant role in the variability of this syndrome (Etheridge et al., 2011). More recently, a novel de novo TS allele was identified in constitutively expressed exon 38, A1473G (Gillas et al., 2012). This caused the full TS syndrome, including the minor transcript phenotype of syndactyly, but it was severe like other major transcript alleles, and also caused stroke. The position of this mutation in the channel protein is very similar to the position of the G402S mutation, only in a different “pseudo-monomer” domain of the pseudo-tetrameric structure of this large channel protein. It suggests a special function for the end of transmembrane segment 6.
since this novel lesion is three amino acids away from the end of segment 6 in Domain IV whereas G402S is in the same position in Domain I and G406R is nearby. A conserved structural motif containing these mutated amino acids is found in all four pseudomonomer domains and they appear to tightly interact with one another to form the closed state of the channel pore (Déplé et al., 2011). There are additional suggestions that this domain plays a role in the oligomerization of these channels into synchronized channel clusters capable of enhanced calcium signaling (Dixon et al., 2012), potentially through interaction with anchoring proteins, such as AKAP150 (Cheng et al., 2011) since a TS mutation alters this molecular function as well. Although highly suggestive clinical findings have been observed with the A1473G mutant allele, functional studies of this novel allele have not yet been performed (Gillis et al., 2012).

The TS channel conducts a major component of the inward calcium current underlying the depolarized QT interval. A lengthening of the QT to produce the LQT characteristic of the syndrome suggests that excess current is conducted by the mutant channel. This is supported by the finding that the two missense alleles at this locus that cause the short QT Brugada syndrome, A39V and G490R, are loss-of-function lesions (Antzelevitch et al., 2007; Brugada et al., 2012). It is also supported by the pharmacology of the channel, since the channel opener Bay K 8644 can mimic the TS arhythmia and the channel blocker verapamil can be used to treat TS (Jacobs et al., 2006; Sicouri et al., 2007).

Kinetic analysis of in vitro-expressed mutant and WT versions of the TS channel reveal that the major effect of the TS mutation is to alter the speed with which the open conducting channel returns to a non-conducting conformation through a process called channel inactivation (Antzelevitch et al., 2007; Barrett and Tüsen, 2008). As would be predicted from the cardiac findings in this disorder, channel inactivation caused by changes in the membrane potential (referred to as voltage-dependent inactivation, VDI) are slowed, but there is additionally a different mechanism of inactivation regulated directly by calcium itself, and this process is greatly accelerated by the mutation. The net result of the mutant is a very rapid inactivation of half the current, and then a very slow inactivation of the remainder (Barrett and Tüsen, 2008).

Induced pluripotent stem cells (iPSC) from TS patient fibroblasts (Yazawa et al., 2011) were produced and these reprogrammed cells were differentiated first into cardiomyocytes. These cells recapitulated in vitro the prolonged APs, irregular electrical activity and abnormal calcium signals of LQT, and ros covitine, a compound that accelerated VDI restored calcium and electrical signaling toward control. These iPSC were also differentiated into cortical neurons (Pasač et al., 2011) and they showed wide APs and increased calcium signals, similar to the cardiomyocytes. In the neurons altered patterns of calcium-dependent gene expression were observed, and some of these loci had previously been implicated in ASD. In addition, abnormal levels of the neurotransmitters norepinephrine and dopamine were observed. Like the cardiomyocytes, all of these phenotypes were reversibly corrected with ros covitine (Pasač et al., 2011). However, a different neuronal phenotype, dendritic retraction, also altered in the TS cells, was not associated with calcium permeation, but instead with ectopic activation of RhoA (Krey et al., 2013). Together these iPSC results suggest that there may be cellular phenotypes of TS of potential use in high-throughput screens for novel molecules to treat the clinical syndrome.

Whole animal phenotypes of ASD were also recapitulated with TS alleles. Heterozygous TS transgenic mice carrying a poorly expressed construct with the exon 8 G406R mutation showed abnormal behavioral phenotypes thought to model ASD. They showed altered ultrasonic vocalizations and social behavior, restricted, repetitive and perseverative behaviors, and altered responses to fear conditioning (Baden et al., 2011).

Since LQT is a hyper-excitability syndrome, it suggests that neuronal hyper-excitability is a route to ASD much as it is for seizures and epilepsy, a condition long-recognized highly co-morbid with ASD. As is discussed later, co-morbidity can most simply be seen to arise from a shared genetic architecture of these diseases, with a set of alleles and loci contributing increased susceptibility to both disorders. Since hyper-excitability is such a multifaceted perturbation of fundamental signaling mechanisms, it holds the potential of representing a core deficit in ASD, rendering it a neurobiological, rather than strictly behavioral, disorder. The recognition of such a core deficit brightens the prospect that new molecular targets can be discovered in ASD against which new generations of drugs can be developed for this disease.

THE IMPACT OF MUTATIONS IN OTHER CALCIUM CHANNEL SUBUNITS IN ASD

While it is most straightforward to see the importance of calcium channel signaling abnormalities in autism through the lens of TS, the TS mutations clearly do not account for even a tiny fraction of cases with typical ASD. The key difference is that TS is a highly penetrant simple dominant disease with known causative single gene mutation, whereas most ASD behave as a complex multi-genic disorder (Persico and Napolioli, 2013). This means that mutations contributing to typical ASD cannot be said to “cause ASD” but only to incrementally enhance susceptibility to the disease – the phenotype only being observed if a sufficient number of such contributing alleles are co-inherited, likely together with exposure to additional environmental stressors of the system. This leads to the typical inheritance pattern of a complex multigenic disorder that shows only a clustering of ASD in families, no simple segregation of the trait (Splawski et al., 2006). Another signature that shows the interaction of multiple risk alleles in developing the phenotype of ASD is that while identical (monozygotic) twins, who share 100% of their alleles, are reported up to 80–90% concordant for the disorder, dizygotic fraternal twins – genetically similar to sibs in sharing 50% of their alleles – are only about 30% concordant, but are still affected at about 20 times the general population risk (Ronald and Hoekstra, 2011). That there even is such a thing as an “environmental stressor” in the pathophysiology of ASD is demonstrated by the existence of discordant monozygotic twins who arise from the same fertilized egg and who share their entire genome in common yet have vastly different phenotypes. This discordance is a hopeful sign that therapeutics could be found to mitigate dysfunction in a susceptible genetic background. However, it will likely require a detailed understanding of the genetic architecture of this complex disease before these non-genetic stressors can be identified.
The calcium channel family is well-recognized to cause channelopathy diseases. The close paralog of the TS locus, CACNA1S and CACNA1A, have long been recognized to have highly penetrant, simple dominant mutant alleles that cause, respectively, the skeletal muscle diseases hypokalemic periodic paralysis and malignant hyperthermia (MacManimen and Zvaritch, 2011), and the neurological diseases hemiplegic migraine, episodic ataxia and spinocerebellar ataxia (Gargus, 2009; Pietrobon, 2010). Furthermore, simple dominant pathogenic mutations have also been identified in many of the accessory subunits of these channels as well. There are, in addition, further diverse suggestions that “weak,” poorly penetrant ASD-susceptibility alleles at other calcium channel loci are germane to typical ASD (Table 1). First, mutations in several other calcium channel alpha subunit neuronal paralogs of the TS/LQT8 channel have been found in subjects with ASD, where they behave more like those mutations contributing to a multi-gene disease described above. They do not neatly segregate with ASD in a family, but instead appear to contribute susceptibility to autism pathogenesis (Splawski et al., 2006).

The first example of such a paralog is the gene CACNA1H. In the families segregating mutations in this gene several cases of ASD are observed, all carrying the mutant allele, however, not all with the allele manifest diagnosable ASD. The “risk allele” simply is shown to cluster in such cases of familial autism (Splawski et al., 2006). More recently deep resequencing of functional genomic regions identified potentially causal rare variants contributing to ASD in CACNA1F, an X-linked gene. This gene was first recognized to be a locus of Stationary Night Blindness (Strom et al., 1998) but this resequencing study observed that, in addition to the eye findings, epilepsy and ASD occurred in individuals carrying gain-of-function mutations, whereas loss-of-function lesions caused only the classic Stationary Night Blindness phenotype (Myers et al., 2011). This again suggests that ASD pathogenesis arises from excess calcium signaling, but as was the case for the complex gating changes seen in TS, perhaps perturbed calcium homeostasis is more broadly responsible. CACNA1C, another calcium channel alpha subunit paralog, is mapped to the chromosome 17q11-21 ASD-susceptibility region. It was found to contain single nucleotide polymorphisms (SNPs) associated with ASD in male multiplex families in an AGRE cohort (Strom et al., 2011). The same locus was again found to contain two ASD-associated SNPs in a subsequent larger study that also identified ASD-associated SNPs in CACNA1H and the TS locus CACNA1C (Lu et al., 2012). Indeed, there is even growing evidence that CACNA1C itself also contains other weak alleles that contribute broadly to cortical dysfunction, such as in schizophrenia, bipolar disease (BDP) and major depression (Sklar et al., 2008; Green et al., 2010; Thimm et al., 2011). In some cases mutations of this CACNA1C alpha subunit also alter an adjacent calcium channel accessory subunit, as in the case of an intristitial deletion at chromosome 12p13.33 that deleted both the CACNA1C major subunit and the CACNA2D4 accessory calcium channel subunit genes, causing ASD-like developmental delays in two sibs and their father (Abdelmoity et al., 2011). In a subsequent study of copy number variants (CNVs) in ASD, two affected sibs were found to have a 2p12p translocation that, again, resulted in the deletion of both genes as well (Smith et al., 2012). Eichler and Meyer (2008) also identified exon-disrupting CNV deletions not found in healthy controls in the paralag calcium channel accessory subunit CACNA1D3 in their analysis of recurrent CNV hotspots in ASD (Girirajan et al., 2013). Eichler’s group further identified de novo rare alleles in alpha subunit loci CACNA1D and CACNA1E as “top de novo risk mutations” for autism in a whole exome sequencing study (Y’Roak et al., 2012b), and, using a high density custom microarray in a different cohort of ASD, implicated a completely different type of calcium channel, a calcium-permeable cation channel called a transient receptor potential (TRP) channel, encoded by TRPM1. This gene had previously been shown to carry pathogenic alleles for Complete Congenital Stationary Night Blindness (Audo et al., 2009) and subsequently to participate in the metabolic glutamate receptor signaling cascade (Devi et al., 2013). They observed that the CNV deletion at the 1q13.3 locus that is enriched in ASD, was found in 3 out of 2,588 cases, and that although CHRNA7 had been previously implicated as contributing to neurological defects in this lesion (see below), in their cohort they found no deletions of this gene, but instead found that all five cases had deletions of TRPM1 in this locus, including one homozygous deletion never observed in a control.

In a very powerful genome-wide association study (GWAS) of SNPs in a huge European cohort of over 30,000 cases and a similar number of matched controls, specific variants under-lying genetic effects shared between the five disorders: ASD, attention deficit-hyperactivity disorder, bipolar disorder, major depressive disorder, and schizophrenia revealed that only 4 of the 25,000 human loci were associated with neuropsychiatric disease at “genome-wide significance”—the probability of chance false positive association being less than 5 in 100 million (p < 5 × 10−8). Two of these associated genes encoded calcium channel subunits, CACNA1C, the TS locus, and the accessory calcium channel subunit CACNB2 (Psychiatric Genomics Consortium et al., 2013). Interestingly, although no strong allele of CACNB2 has yet been found to cause a TS-like syndrome, dominant pathogenic loss-of-function missense alleles of the locus cause the short QT syndrome (Antzelevitch et al., 2007). This is the same syndrome caused by dominant loss-of-function alleles at CACNA1C, where a gain-of-function allele causes TS. This finding powerfully implicates the function of this multimeric calcium channel and TS-like pathophysiology in this wide spectrum of neuropsychopathology. It is also consistent with the observation that genes encoding plasma membrane calcium pumps, responsible for creating the calcium gradients dissipated by the channels, have been repeatedly associated with ASD. The calcium ATPase gene ATP2B2 encodes the plasma membrane calcium-transporting pump which extrudes Ca2+ from cytosol into extracellular space, and three studies from different populations have reported association between this locus and ASD (Carayol et al., 2011; Prandini et al., 2012; Yang et al., 2013). It is further intriguing to note that paralogs of the other Brugada syndrome loci encoding sodium and potassium channels, also feature prominently in the architecture of ASD, as discussed below. The typical channelopathy lesions in well-understood monogenic diseases of heart, muscle and nerve cause membrane hyper-excitability. Hence, mutations in Ca2+ and Na+ channels, which physiologically excite a tissue, typically have...
gain-of-function lesions, while mutations in K+ and Ca2+ channels, which physiologically stabilize excitable tissue, typically have pathological lesions that diminish their current (Gargus, 2008).

**SODIUM CHANNEL DEFECTS IN ASD**

As mentioned above, mutations in three voltage-gated sodium channel subunits cause Brugada syndrome, the cardiac alpha subunit SCN5A, and accessory subunits SCN1B and SCN2B. Mutations in neuronal paralogs of the alpha subunit, SCN1A and SCN2A, had long ago been observed in rare cases of familial autism (Weiss et al., 2003) and prior to that had been shown to contribute dominant pathogenic alleles to the seizure syndrome GEFH+− SCNA had also been shown to carry haploinsufficient dominant null alleles in the severe seizure syndrome SMEI/Dravet syndrome (reviewed in Ma and Gargus, 2007), as well as missense alleles in the migraine syndrome familial hemiplegic migraine (FHM3; Dichgans et al., 2009; Gargus and Touray, 2007). Furthermore, SCN1A alleles have also been recognized to cause autism and epilepsy phenotypes together with biopsy-proven mitochondrial disease (Craig et al., 2012). It is particularly intriguing that the autism-associated SCN1A alleles are quite different from the seizure alleles, which produce a more severe lesion in the channel protein, but that they are very similar to the mutations found in the FHM3 families (Gargus and Touray, 2007). These alleles are found to disrupt cysteic loop domains at the C-terminus of the protein, a region originally identified in the Brugada SCN5A channel as an EF-hand-containing domain key to channel inactivation (Gässer et al., 2006) and a site of regulatory calmodulin binding (Kim et al., 2004). Both FHM3 and autism alleles of SCN1A perturb calmodulin-interacting intracellular regions of the channel protein. These regions connect this sodium channel into the calcium signaling pathways of the neuron since they interact with calmodulin that serves as an actual bound protein subunit of the channel (Gargus, 2009).

More recently strong evidence has been building for a role of lesions in neuronal voltage-activated sodium channel alpha subunits in typical polygenic ASD (Table 2). Whole-exome resequencing of nearly 1000 individuals uniquely identified SCN1A as the sole gene in which two independent probands had non-sense variants that disrupted the same gene, a highly significant result (Sanders et al., 2012) and this finding was again confirmed in a separate large resequencing study that found de novo protein altering mutations in the gene in probands with ASD (O’Roak et al., 2012a). Loss of function lesions in this region had previously been recognized by array-comparative genome hybridization (aCGH) detection in a child with autistic features carrying a de novo deletion of chromosome 2q42.4-q23.3, the region containing SCN1A and SCN2A (Chen et al., 2010).

Because of the fact that mutations in SCN1A are capable of causing seizure syndromes and seizures are frequently comorbid with ASD, the question arises whether seizure activity causes the impairments observed in ASD (Cattaneo et al., 2013), or if the ion channel dysfunction itself, independent of seizures, contributes to the ASD pathophysiology. This question has been elegantly approached in heterozygous KO mouse models of SMEI/Dravet syndrome. Scn1a+− heterozygous KO mice develop multiple behavioral phenotypes, including increased anxiety, hyperactivity and stereotyped behaviors, in addition to seizures and ataxia (Han et al., 2012). However, using inhibitory RNA (RNAi) expression of SCN1A could be reduced in focal regions of the brain without producing clinical or EEG-detectable seizures (Bender et al., 2013).

In this case focal loss of expression of this channel was found to cause spatial memory impairment, without an effect on response to a novel object or in more general measures of exploration. This spatial performance was significantly related to hippocampal theta frequency (an inducer of LTP, see below) in the control group, but this relationship was abolished after RNAi knockdown, consistent with a role of this channel in learning paradigms dependent upon hippocampal theta oscillations and independent of a role in seizures. This is consistent with a critical role for SCN1A in the network oscillations contributing to cognitive function (Bender et al., 2013).

Another sodium channel alpha subunit gene came to be implicated in ASD by whole-genome resequencing in a small family quartet having just one affected proband, an unaffected sib and two unaffected parents. The study uncovered yet another neuronal sodium channel paralog underlying the phenotype of autism with epilepsy. They discovered a de novo heterozygous missense mutation in SCN8A in the proband that alters an evolutionarily conserved residue in one of the most abundant sodium channels in the brain. Further, they carried out biophysical measurements of the properties of the mutant channel and demonstrated a dramatic increase in persistent sodium current and incomplete channel inactivation (Veeramah et al., 2012), demonstrating a gain-of-function lesion similar to that seen in the pathogenic cardiac and muscle sodium channel paralogs. A different set of mechanistically unbiased approaches to ASD allele discovery have also pointed to neuronal sodium channel paralogs. The first study involved a large survey of consanguineous Middle Eastern families with autism and the technique of microarray homozygosity mapping. This technique will detect rare variants that, through common descent from a shared parental ancestor, cause recessive disease. The study identified one family that segregated a homozygous deletion of SCN7A (Morrow et al., 2008). This gene lies adjacent to SCN1A within the sodium channel gene cluster at the autism-5 locus (AUTS5) on chromosome 2. While its mRNA is neuronally expressed, no function has yet been observed for the putative ion channel it encodes (Saleh et al., 2005). It is rapidly evolving, having arisen from SCN1A by endoduplication (Plummer and Meisler, 1999), and such rapidly evolving genes are a signature of genes potentially playing human-specific roles, intriguing candidates in neuropsychiatric diseases (Berglund et al., 2009).

While the Brugada syndrome associated sodium channel beta subunit locus SCN1B is well recognized to carry alleles that cause the GEFH+− seizure phenotype (Wallace et al., 1998) and can cause ASD together with this syndrome (Dixon-Salazar et al., 2004), no mutations in these accessory sodium channel subunits have yet been clearly associated with the ASD phenotype independent of seizures. The sum of the evidence on neuronal sodium channels suggests that hyper-excitability-causing gain-of-function lesions that delay inactivation of the channel predominate, much as is seen in LQT and MHS. However, there are also clear cases of deletions causing...
### Table 1 | Calcium channels and calcium channel subunits implicated in ASD.

| Protein   | Description                                  | Normal function                                                                 | Disease association                                      |
|-----------|----------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------|
| CACNA1C   | Voltage-regulated L-type calcium channel, alpha 1C subunit | Regulates entry of Ca$^{2+}$ into excitable cells, muscle contraction, hormone/neurotransmitter release, gene expression, cell cycle | Timothy syndrome, ASD, psychiatric diseases               |
| CACNA1D   | Voltage-regulated calcium channel, alpha 1D subunit | High-voltage activated, long-lasting calcium activity                          | Sinoatrial node dysfunction and deafness, ASD, psychiatric diseases |
| CACNA1E   | Voltage-regulated R-type calcium channel, alpha 1E subunit | Regulates entry of Ca$^{2+}$ into excitable cells, muscle contraction, hormone/neurotransmitter release, gene expression, cell cycle | ASD, psychiatric diseases                                 |
| CACNA1F   | Voltage-regulated L-type calcium channel, alpha 1F subunit | Regulates entry of Ca$^{2+}$ into excitable cells, muscle contraction, hormone/neurotransmitter release, gene expression, cell cycle | ASD and X-linked congenital stationary night blindness     |
| CACNA1G   | Voltage-regulated T-type calcium channel, alpha 1G subunit | Regulates entry of Ca$^{2+}$ into excitable cells, muscle contraction, hormone/neurotransmitter release, gene expression, cell cycle | ASD, intellectual disability, juvenile myoclonic epilepsy  |
| CACNA1H   | Voltage-regulated T-type calcium channel, alpha 1H subunit | Regulates neuronal and cardiac pacemaker activity                             | Familial autism; childhood absence epilepsy               |
| CACNA1I   | Voltage-regulated T-type calcium channel, alpha 1I subunit | Characterized by a slower activation and inactivation compared to other T-channels | Possibly implicated ASD                                    |
| CACNA2D3  | Voltage-regulated calcium channel, alpha 2/delta 3 subunit | Accessory calcium channel subunit; regulates entry of Ca$^{2+}$ into excitable cells | ASD                                                      |
| CACNA2D4  | Voltage-regulated calcium channel, alpha 2/delta 4 subunit | Accessory calcium channel subunit; regulates entry of Ca$^{2+}$ into excitable cells | Gene deletion along with CACNA1C leads to ASD            |
| CACNB2    | Accessory calcium channel beta 2 subunit       | Contributes to the function of calcium channels. Modulates voltage dependence of activation and inactivation and controls trafficking of the calcium channel family | ASD, psychiatric diseases                                 |

### Table 2 | Sodium channels implicated in ASD.

| Protein   | Description                                  | Normal function                                                                 | Disease association                                      |
|-----------|----------------------------------------------|---------------------------------------------------------------------------------|----------------------------------------------------------|
| SCN1A     | Voltage-regulated sodium channel, type 1     | Expressed in brain and muscles; involved in generation/propagation of action potentials | Familial hemiplegic migraine type 3, GEFS$^\ddagger$, SMEI/Dravet syndrome, familial autism |
| SCN1A     | Voltage-regulated sodium channel, type 2     | Action potential initiation and propagation in excitable cells                  | Epilepsy, ASD                                            |
| SCN1A     | Voltage-regulated sodium channel, type 3     | Action potential initiation and propagation in excitable cells                  | Epilepsy, ASD                                            |
| SCN1A     | Voltage-regulated sodium channel, type 7     | Na$^+$-specific channel, allowing passive flow of ions down their electrochemical gradient | Neurotypical deletion in autism                            |
| SCN1A     | Voltage-regulated sodium channel, type 8     | Essential for the rapid membrane depolarization that occurs during the formation of the action potential in excitable neurons | Heterozygous missense mutation was linked to epilepsy and autism |
Potassium-Activated Potassium Channels are Central Components of Neuronal Calcium Signaling and Neurosecretory Pathways and Critical Regulators of Pacemaker-Like Rhythmic, Bursting Synaptic Activity, Particularly Involving Amnestic Transmitters Such as Dopamine. As Such They Are Frequently Identified Candidate Genes Associate with Neuropsychiatric Phenotypes (Chandy et al., 1998). Calcium-Activated Potassium Channels Are Central Components of Neuronal Calcium Homeostasis in These Neuronal Lesions. The Calcium Conductance in the BK Channel Is Regulated by Its Regulatory Subunit (Deng et al., 2013). This Channel’s Regulatory Subunit (Deng et al., 2013) Was Identified to Carry Missense Mutations Causing SESAME Syndrome with Ataxia, Sensorineural Hearing Loss and Tubulopathy; Bockenhauer et al., 2009, Was Identified to Carry Missense Mutations That Altered Highly Conserved Residues in Two Unrelated Families with Seizures and ASD (Sicca et al., 2011). The Effects of Mutations in a Heterologous Expression System Revealed an Increase in Channel Current Suggesting a Gain-of-Function Defect (Sicca et al., 2011). The M. Current Potassium Channel Subunit Gene KCNQ3 (Paralog of the First LQT Gene and Long-Proven Causal Allele of Benign Familial Infantile Seizures; Wang et al., 1998) and Its Paralog Encoding One of Its Heteromultimeric Partners, KCNQ5, Have Also Been Recently Implicated in ASD. A De Novo Translocation Truncating KCNQ3 Was Identified in a Boy with Autism, and Three Patients With ASD Were Recognized to Share the Same Rare Variant of KCNQ5 Caused by a SNP That Creates a Missense Mutation That Could Be Proven to Be Loss-of-Function by Patch Clamp Recording of Oocytes Co-Expressing Both Subunits (Gilling et al., 2013). Potassium Channels Implicated in ASD Are Summarized in Table 3.
indel with the short allele associated with lower transcriptional
posed of repeat elements and the polymorphism is a 44-bp
located 1 kb upstream of the transcription initiation site is com-
principal site of action of tricyclic antidepressants and serotonin
transporter (5-HTT) localized in brain presynaptic
hotspots in ASD (Girirajan et al., 2013). A common polymor-
distributed in the brain, were observed in a large survey of CNV
potassium dependent EAA T glutamate transporter found widely
released into the cleft during synaptic transmission, and mutations
are associated with the disease (Palmieri et al., 2010). Furthermore, all of
study of post-mortem brain tissue showed significantly increased
transport activity by the SLC25A12 transporter in subjects with
ASD. However, no mutations or polymorphisms were found asso-
ciated with other SNPs within the locus, although none
appeared to alter function (Segurado et al., 2005). Subsequently a
study of post-mortem brain tissue showed significantly increased
transport activity by the SLC25A12 transporter in subjects with
ASD. However, no mutations or polymorphisms were found associ-
ciated with the disease (Palmieri et al., 2010). Furthermore, all of
the excess enzyme activity found in brain samples from patients
with ASD was calcium-dependent and was found to be associated
with elevated cytosolic calcium levels in these subjects (Palmieri
et al., 2010). They found that controlling for the calcium levels,
transport activity was identical in isolated mitochondria from
patients and controls. They therefore concluded that the criti-
cal link to this altered mitochondrial metabolism observed in the
brains of patients with autism was in fact caused by altered calcium
homeostasis, although it was never directly studied. Subsequent
studies have found overexpression of SLC25A12 in post-mortem
brain from subjects with ASD (Leygonie-Costello et al., 2008). While
the fundamental abnormality of this transporter in ASD remains
to be unambiguously defined, it is a tantalizing link between
cytosolic calcium homeostasis and mitochondrial energetics, an
often independent important mechanistic theme in ASD etiology
(Gargus, 2010).
### Table 3 | Potassium channels and potassium channel subunits implicated in ASD.

| Protein | Normal function | Disease association |
|---------|----------------|--------------------|
| KCNMA1  | Both voltage- and calcium-sensing channel, controls smooth muscle tone and neuronal excitability | Epilepsy (GEPD); implicated in ASD |
| KCNMB4  | Predominantly expressed in neuronal tissue. Changes voltage dependence and activation kinetics of KCNMA1 | ASD |
| KCNJ10 | Have a greater tendency to allow potassium to flow into, not outside of the cell | Seizures, ataxia, and ASD |
| KCNJ10 | Slower activating and deactivating channel, plays a role in the regulation of neuronal excitability | Seizures and ASD |
| KCNJ5 | Activates slowly with depolarization and expressed in subregions of the brain and skeletal muscle. Can multimerize with KCNQ3 | Implicated in ASD |
| KCND2  | Mediates a rapidly inactivating outward K⁺ current in neurons and the heart | Implicated in ASD |

### Table 4 | Transmembrane receptor genes implicated in ASD.

| Protein | Normal function | Disease association |
|---------|----------------|--------------------|
| ATP2B2 | Pumps Ca out of the cell into the extracellular space | Hearing loss, ASD |
| CADPS2 | Calcium-binding protein, regulates exocytosis of synaptic vesicles | ASD |
| CHRNA7 | GABAergic interneuron activity postsynaptically | Epilepsy, schizophrenia, speech and learning problems |
| GABRQ1 | Conducts chloride ions upon activation, leading to hyperpolarization. Causes inhibitory effect on neurotransmission | SNPs associated with ASD |
| SLC1A1 | Postsynaptic protein, help terminating action of the neurotransmitter glutamate | Schizophrenia, ASD |
| SLC6A4 | Carries neurotransmitter sarcosine from synaptic space, carrying it into presynaptic neurons | Bipolar disorder, depression, obsessive-compulsive disorder, ASD |
| SLC12A2 | Drives chloride into the cell. Ubiquitously expressed in many cell types, including cardiac neurons | ASD |
| SLC25A12 | Transports aspartate from mitochondria to cytosol in exchange for glutamate | ASD |
| TRPM1 | Ca²⁺-permeable cation channel | Congenital stationary night blindness, ASD |
SYNAPSES AND DOWNSTREAM CALCIUM SIGNALING DEFECTS IN ASD

The axonal AP-induced calcium signal culminates by initiating the fusion of synaptic vesicles into the pre-synaptic membrane, and in this fashion participates in diverse mechanisms of synaptic modulation that produce synaptic plasticity and learning (Nebel and Sakaba, 2008). Crossing the synaptic cleft, the neurotransmitter binds and activates its receptor, altering excitability of the post-synaptic cell. It also often feeds back through pre-synaptic receptors to modulate pre-synaptic vesicle fusion. Ultimately the signal is terminated by re-uptake of the neurotransmitter from the synaptic cleft. Calcium not only plays a role in neuronal plasticity in modulating neurosecretion in neuronal networks, but it additionally shapes the composition of the synaptic membranes themselves through the role it plays in the calcium-sensitive mammalian target of rapamycin (mTOR) signaling pathway of upstream regulators and downstream effectors, many directly implicated in model monogenic ASD syndromes.

GLUTAMATE RECEPTORS

Glutamate, the major excitatory neurotransmitter in the central nervous system, activates two major classes of synaptic receptors: ionotropic receptors, which are themselves ligand-gated cation channels, and metabotropic receptors, that are heptahelical GPCRcoupled to a variety of signaling pathways through trimeric G proteins. mGluR5, GRM5-encoded receptors, are coupled via Gq to activate phospholipase C and in turn an IP3 and the calcium signaling second messenger system. Phosphorylation and dephosphorylation of a key residue within the C-terminal domain of the activated receptor cause synchronous, oscillatory changes in IP3 and Ca2+ levels (Collins et al., 2010). The metabotropic glutamate receptor mGluR5 is one of the many synaptic proteins altered in Fmr1 KO mice. FXS is an important monogenic model of ASD (Iossifov et al., 2012), and mouse models of FXS are based upon such knock-out (KO) mice. In the absence of FMRP, it is widely recognized that performance in learning paradigms is impaired in these mice and that this recaptures features observed in FXS patients where defects in learning are directly observed (De Rubens et al., 2012). The absence of FMRP in Fmr1 KO mice produces an up-regulation of mGluR5 and an enhancement in the synaptic phenomenon of LTD that relies upon enhanced mTOR-mediated rapamycin-sensitive protein synthesis triggered by this receptor. This culminates in a reduction of synaptic AMPA glutamate receptors (see below) and chronically decreased synaptic efficiency (Sharma et al., 2010).

Frangible X syndrome is a loss-of-function syndrome, overwhelmingly caused by an extremely large expansion of the trinucleotide CGG repeats (>200) in the 5’ non-coding portion of the human locus, but is also caused by deletion of the locus or rare missense mutations (Collins et al., 2010). Males are predominantly affected, and this lesion typically results in the absence of FMRP, a multi-functional mRNA binding protein (Ashley et al., 1993) and ion channel regulatory subunit (Deng et al., 2013).

Carriers of one FMR1 allele with a “premutation,” a modestly expanded trinucleotide repeat (55–200) at the FMR1 locus, are at risk for expression of “Frangible X-associated tremor/ataxia syndrome” (FTXAS), an aged-onset monogenic neurodegenerative disorder associated with decreased FMRP levels (Feng et al., 1995; Jacqueyont et al., 2003; Sheridan et al., 2011; Cao et al., 2013). iPSC-derived neurons harboring a stably active, modestly expanded allele have reduced neurite length and high amplitude, high frequency functionally abnormal calcium transients compared to neurons harboring the normal active allele (Liu et al., 2012). Moreover, a sustained calcium elevation was found in the expanded allele-expressing neurons after glutamate application (Liu et al., 2012). Subsequently astrocytes derived from heterozygous mice with the FMRP premutation also demonstrated increased Ca2+ oscillations as well as increased sensitivity to glutamate, despite having levels of mGluR5 receptors similar to control (Cao et al., 2013). Comparable studies were not reported with classical FXS alleles, however, these observations suggest a fundamental role of FMRP in synaptic calcium signaling that is sensitive to disruption by pathogenic alleles at the locus and is potentially consistent with the effects of FMRP observed on pre-synaptic BK channels discussed above (Deng et al., 2013).

The postsynaptic membrane mGluR5 receptors are bound to HOMER1 scaffold proteins, together with plasma membrane GRM1-encoded mGluR1 receptors, ER-associated IP3 and RyR, encoded by ITPR1, RYR1 and RYR2, and plasma membrane voltage-dependent calcium channels, to form a functional signaling complex. Together with SHANK1 and SHANK3 scaffolds it plays a key role in facilitating intracellular calcium release and transmembrane calcium currents (Kato et al., 2012). Interestingly, in Fmr1 KO mice disrupted Homer1 complex formation is observed (Ronesi et al., 2012). There are two isoforms of Homer1, long and short, that have opposite actions in signaling. The long form has a coiled-coil domain through which they multimerize to act as a scaffold. The N-terminal domain of the long form binds to the intracellular C-terminal tail of the mGluR5 receptor and has an ability to link them together with the IP3 receptor and other scaffolded signaling membrane proteins and kinases (Ronesi et al., 2012). Homer1 has several splice variants, and extracellular stimuli promote 3’-end processing of Homer1 pre-mRNA, leading to the switch of poly(A) site selection. The short form of Homer1, an immediate early gene transcript, lacks this coiled-coil domain, and thus cannot multimerize or act as a scaffold. It therefore serves to physically dissociate the receptor from the IP3 receptor and other scaffolded proteins. This renders the mGluR5 receptor constitutively active and agonist-independent, enhancing its signaling. In Fmr1 KO mice mGluR5 is preferentially associated with the short form of Homer1. This leads to an increased signaling frequency and prolonged spontaneous persistent activity. Genetic deletion of short-form Homer1 corrects several phenotypes in Fmr1 KO mice, while a short mimetic peptide containing the proline-rich motif of mGluR5 that binds to Homer and disrupts the mGluR5-Homer scaffold, in wild type mice produces several phenotypes of Fmr1 KO mice. Together it suggests an important role of mGluR5-long Homer signaling complexes in normal brain function and of the short form complexes in the disease pathophysiology. In fact, pharmacological inhibition of mGluR5 activity was proven beneficial in correction of autistic phenotypes in both animal models and human patients, probably because of its effect on the constitutively active mGluR5 (Michalos et al., 2012; Ronesi et al., 2012).

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Calcium signaling through metabotropic mGlur5 receptors additionally plays a key role in modulating the function and subunit composition of ionotropic glutamate receptors at the synapse. N-methyl-D-aspartate (NMDA) ionotropic glutamate receptors are a heterotetramer of subunits encoded by GRIN1 and GRIN2A, B, C or D. The switch of subunits in the NMDA receptor from GRIN2B to GRIN2A is an important example of synaptic modulation and experience-dependent regulation of receptor subunit composition in vivo. This long-lasting alteration of the synapse is driven acutely by activity of mGlur5 and involves its downstream phospholipase C, calcium release from IP3-dependent stores, and protein kinase C activity. In mGlur5 KO mice the developmental switch is deficient and therefore the GRIN2B to GRIN2A switch evoked in vivo by visual experience is absent and such learning fails (Matta et al., 2011). The subunit switch causes important changes to NMDA receptor function, altering the amount of calcium influx through the ionotropic receptor channel pore and the types of proteins interacting with the intracellular domain of the receptor. These features regulate the type of long-term synaptic plasticity (LTP or LTD, see below) that NMDA receptor activation can induce (Li et al., 2004; Bartlett et al., 2007).

Long-term potentiation (LTP), the opposite of LTD, can be induced by rapid theta burst stimulation (TBS) in hippocampal slices and it relies upon two classes of ionotropic glutamate receptors, the NMDA receptors and the alpha-amo-nio-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors, a heterotetramer with subunits encoded by GRIN1–4 that function cooperatively as ligand-gated cation channels in the post-synaptic membrane. The AMPA subunit encoded by GRIN2 is subject to RNA editing (CAG→CUG; changing a single amino acid within the second transmembrane domain from glutamine to arginine) which renders the channel impermeable to Ca2+ (α, a feature essential to its function as a non-selective monovalent cation channel (Barbon and Baintan, 2011). On the other hand the NMDA receptor channel is highly calcium permeable. However, it has a complex double-gated mechanism that requires both binding the activating glutamate neurotransmitter and a strong membrane depolarization, usually driven by the AMPA receptors, to remove a laminal Mg2+ ion that blocks the receptor’s channel (Ogden and Traynelis, 2011). Only a very large synaptic glutamate release activates sufficient AMPA channels to remove the Mg2+ ion block to provide an NMDA conductance pathway and hence provide a post-synaptic calcium signal. LTP depends upon this change in post-synaptic calcium since intracellular injection of the calcium chelator EGTA blocks the development of LTP (Lynch et al., 1983). NMDA-mediated responses are required to induce LTP but it is through calcium-dependent modification of the post-synaptic neuron AMPA receptor components that LTP is manifest (Muller et al., 1988).

Loss of FMRP also leads to changes in the synaptic phenomenon of short term potentiation (STP) whereby prior activity of a synapse enhances the probability of synaptic vesicle release with a subsequent stimulation a short while later. This is largely a result of residual elevated calcium levels from the pre-synaptic priming stimulus (Fioravante and Regehr, 2011). Like LTP, STP is considered to be one of the elemental components of information processing at a synapse underlying plasticity and learning as it precedes the development of LTP. Loss of FMRP leads to enhanced responses to high-frequency stimulation and to abnormal exensive enhancement of synaptic processing of natural stimulus trains. These changes are associated with exaggerated calcium influx in pre-synaptic neurons during high-frequency stimulation, enhanced synaptic vesicle recycling, and enlarged readily releasable and reserved vesicle pools, all serving to increase signaling across the synapse (Deng et al., 2011).

Hence results of the loss of FMRP are changes in pre-synaptic and post-synaptic channel function, neurosecretory dysfunction and changes in the expression of a large set of post-synaptic neuronal mRNAs, many themselves changing the composition of the post-synaptic membrane. Therefore, while FXS is a simple mono- genic disease it produces complex polygenic dysregulation of the synapse (De Rubeis et al., 2012). However, the sum of the evidence is that the complex pre- and post-synaptic membrane changes recognized in FXS participate in core fundamental processes of synaptic plasticity and learning, are calcium sensitive, and as such this fundamental physiological process may be a promising target in ASD amenable to pharmacological treatment.
mutations of UBE3A cause AS, implicating it as the critical 15q locus for this syndrome. In contrast to AS, PWS is caused by the absence of the paternally active gene in this critical region of 15q11-13, and while it is uncertain which gene is critical, it is clear that its maternal allele is virtually inactive through imprinting (Chamberlain and Lalonde, 2010). Importantly more than 99% of individuals with PWS have a diagnostic abnormality in the parent-specific methylation imprint within the Prader-Willi critical region (Cassidy et al., 2012) and there is clear evidence that such imprinted genes in this region play a role in ASD-like phenotypes (Chamberlain and Lalonde, 2010; Reikovic et al., 2010; Ingason et al., 2011).

Interestingly deletion of just the small Prader-Willi imprinting center (PWS-IC) within 15q11-13 disrupts long-range imprinted gene expression resulting in PWS (Yasu et al., 2011), and the two PWS-IC sites flank CHRNA7. Recently, four probands were identified with small deletions in 15q13 that included only the CHRNA7 gene, and this was followed by the identification of others also with isolated heterozygous CHRNA7 gene deletions, including the first de novo deletion and one patient homozygous for the deletion. These patients demonstrated the similar wide range of ASD phenotypic features associated with the larger 15q11-13 microdeletions, suggesting CHRNA7 was the critical gene responsible for the clinical findings associated with the 15q13 microdeletion syndrome (Hoppman-Chaney et al., 2013).

A physical interaction between the DNA cis PWS-IC regulatory elements that flank CHRNA7 and the protein trans regulatory element methyl CpG binding protein 2, encoded by MeCP2, is required for optimal expression of AS/PWS region genes implicated in the ASD phenotype (Yasu et al., 2011). MeCP2 acts as a calcium-dependent transcriptional repressor for methylated genes, a global regulator of histone function (Cohen et al., 2011; Li et al., 2011) and plays a key role in the control of neuronal activity-dependent gene regulation. Rett syndrome is an X-linked dominant disorder caused by MeCP2 mutations that are lethal in hemizygous males and cause an ASD-like syndrome in heterozygous females. Patients with Rett syndrome or even those with typical ASD, revealed significantly reduced expression of MeCP2, using a lentiviral vector, increased abundance of the glutamatergic marker in WT and RTT neurons, together strongly suggesting that MeCP2 is a rate-limiting factor regulating glutamatergic synapse number in human neurons (Marchetto et al., 2010).

A disturbance in neuronal calcium homestasis is also observed in MeCP2 KO mice (Mironov et al., 2009), and this phenotype too is recapitulated in iPS-derived Rett neurons. While both WT and Rett neurons showed similar AMPA and sodium and potassium currents in response to depolarizations in this model (but not in other RTT models; Farra et al., 2012), demonstrating that RTT cells are not altered in maturation toward normal electrophysiological activity, spontaneous calcium transients were decreased in the RTT neurons and the frequency of calcium oscillations in Rett neurons and in WT neurons expressing shMeCP2 was abnormally decreased compared to controls (Marchetto et al., 2010). These spontaneous calcium transients could be blocked with the sodium channel blocker tetrodotoxin (TTX) or with AMPA and NMDA glutamate receptor antagonists, and were increased by GABA-A receptor antagonists, demonstrating the sensitivity of this calcium signal to synaptic activity and the presence of glutamatergic and GABAergic synapses in the system. Such neuronal activity-induced calcium influx can trigger the calcium/calmodulin-dependent protein kinase (CaMK), an inducer of MeCP2 phosphorylation.

The importance of GABAergic transmission in these ASD phenotypes is revealed by the study of KO mice lacking MeCP2 selectively only from GABA-releasing neurons. They recapitulate numerous Rett syndrome and ASD phenotypic features, including repetitive behaviors. Furthermore, loss of MeCP2 from just a subset of forebrain GABAergic inhibitory neurons also recapitulates many features of Rett syndrome (Chao et al., 2010). MeCP2-deficient GABAergic neurons show reduced inhibitory quantal size, suggesting less neurotransmitter per synaptic vesicle. This finding is consistent with the observed presynaptic reduction in the GABA-synthesizing enzymes glutamic acid decarboxylase 1 (Gad1) and glutamic acid decarboxylase 2 (Gad2), and decreased GABA immunoreactivity. The pattern is similar to what is observed in the Chrna7 KO mouse. Together this suggests that MeCP2 and CHRNA7 expression is critical for normal function of GABA-releasing inhibitory neurons and that this may contribute to the neuropsychiatric phenotypes in
Rett, other monogenic models of ASD, and perhaps more typical ASD (Chao et al., 2010; Yasui et al., 2011). Together these findings link gene dysregulation in the mammalian brain within the chromosome 15q11-q13 region with MeCP2 function, and therefore link Rett syndrome, AS, PWS and ASD, and suggest that ASD may be caused by the inability of neurons to generate adaptive responses via the neurotransmitters involved in generating synaptic calcium signals and calcium-regulated gene expression (Qiu and Cheng, 2010).

Finally, it is intriguing that the combined modulation of the two different "Cys-loop" superfamily receptors encoded in the AS/PWS region, the α7 nAChR and α5 GABA-AR, alter hippocampal function in learning paradigms. Transient application of two separate allosteric modulators, which individually inhibit either the inhibitory α5 GABA-ARs or enhance the activating α7 nAChRs, only jointly causes LTP of induced excitatory postsynaptic currents (EPSCs) in pyramidal neurons of rat hippocampal slices (Johnstone et al., 2011). Remarkably this effect is replicated by a single compound that was designed to simultaneously carry out both activities specifically on these two related receptors, suggesting the therapeutic utility of this strategy targeting the AS/PWS encoded receptors (Johnstone et al., 2011).

mTOR SIGNALING

Mammalian target of rapamycin is a key cytosolic integrative regulator of calcium signaling and mitochondrial function created by a large multidomain protein kinase that regulates cell growth and metabolism in response to environmental signals (Ramanathan and Schreiber, 2009) and several forms of synaptic plasticity (Hoeffer and Klann, 2010; Figure 2). Upstream signals originate from plasma membrane growth factor receptors that signal through phosphatidylinositol 3-kinase (PI3K) to Akt, or through Ras to ERK, to the TSC1/TSC2 heteromultimer that sits at the center of this growth factor receptor stimulated calcium signaling pathway. The genes that encode these two subunits, TSC2, carry dominant mutations that produce tuberous sclerosis (TSC), another important syndromic form of ASD that impacts synaptic calcium signaling. The protein products of these two TSC genes heteromultimerize to negatively regulate downstream signaling by acting as a GTPase-activating protein (GAP) for the small GTPase RHEB, a direct activator of the protein kinase activity of mTOR. mTOR itself phosphorylates 36 kinase 1 (S6K1) and eukaryotic translation initiation factor 4E binding protein 1 (eIF4E-BP1), leading to enhanced protein translation (Ehninger and Silva, 2011).

Tuberous sclerosis is a neurocutaneous syndrome that produces ASD-like behaviors, seizures, intellectual disability, tumorous growths in the brain and characteristic skin lesions. In a TSC mouse model created by an in vivo postnatal Tsc1 conditional KO in only the CA-1 hippocampal neurons, hippocampal mTOR-LTD was abolished, whereas a protein synthesis-independent form of NMDA receptor-dependent LTD (see above) was preserved (Bateup et al., 2011). Additionally, AMPA and NMDA receptor-mediated EPSCs and miniature spontaneous EPSC frequency were enhanced in Tsc1 KO neurons. These changes in synaptic function occurred in the absence of alterations in presynaptic transmitter release probability, indicating that signaling through Tsc1/2 is required for the expression of specific forms of hippocampal synaptic plasticity and normal excitatory synaptic function (Bateup et al., 2011).

In a different mouse model of TSC, both heterozygous and homozygous loss of Tsc1 was limited to principal cells (PC) in the cerebellum, a region of the brain only recently implicated in the ASD phenotype (Tsai et al., 2012). These KO lesions decrease PC excitability and also result in autistic-like behaviors, including abnormal social interaction, repetitive behavior and vocalizations. Importantly, treatment of these mutant mice with the mTOR inhibitor rapamycin prevented the pathological and behavioral deficits, defining a molecular basis for a cerebellar contribution to ASD (Tsai et al., 2012).

The FKBP rapamycin-binding subunit of mTOR (encoded by Fkbp12; often called Fkbp12) is the validated target of rapamycin and other immunosuppressant and anticancer drugs. The protein is a cis-trans prolyl isomerase that interacts with intracellular calcium release channels. Neuronal deletion of Fkbp12 is associated with disinhibited mTOR signaling and altered synaptic plasticity, and memory (Hoeffer et al., 2008). The KO mouse shows increased basal mTOR and S6K1 phosphorylation as well as an enhancement
A number of diseases have long been recognized to be co-morbid with autism, ADHD, BPD, depression and schizophrenia. It was shown that these superficially distinct diseases share some fundamental component of vulnerability, likely arising from a shared subset of susceptibility-loci. Recently this was explicitly shown to be true for the neuropsychiatric phenotypes of autism, ADHD, BPD, depression and schizophrenia. It was shown that these phenotypically distinct disorders, both childhood- and adult-onset, share common genetic alterations and hence, pathway (Psychiatric Genomics Consortium et al., 2013). In that study, autism-diagnosed calcium signaling was shown to be a joint common susceptibility factor for all various psychopathological conditions.

While these complex neurological diseases are caused overwhelmingly by heterogeneous genetic and environmental factors, the autism and migraine etiological neuronal phenotypes are overwhelmingly by heterogeneous genetic and environmental factors, and possibly useful as a cellular diagnostic in routine clinical care, complementing current complex neurobehavioral testing batteries.

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