Dysregulation of Neurogenic Calcium Signaling and Autism

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Introduction

The calcium ion is one of the most versatile biological signaling molecules, known to regulate physiological systems at every level from membrane potential and ion transporters to kinases, transcription factors, and even cell morphology. It is also one of the most universal and ancient of cellular signals. A diverse host of diseases, importantly including a number of rare syndromes that include a phenotype on the autism spectrum (ASD), are coming to be recognized to be caused by disruptions of intracellular calcium homeostasis, and an emerging pathophysiological mechanism of disease, a *calciumopathy*, is at its formative stage (Stutzmann et al. 2006; Bezprozvanny and Gargus 2008).

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 calcium pumps and carriers at the expense of metabolic energy. It is eliminated back out across the plasma membrane but is also, importantly, sequestered for subsequent rapid release within intracellular storage sites which include the endoplasmic reticulum (ER) and the mitochondria.

Cytosolic *calcium signals* originate either by the rapid release of intracellular stores through intracellular ion channels or by extracellular calcium entering

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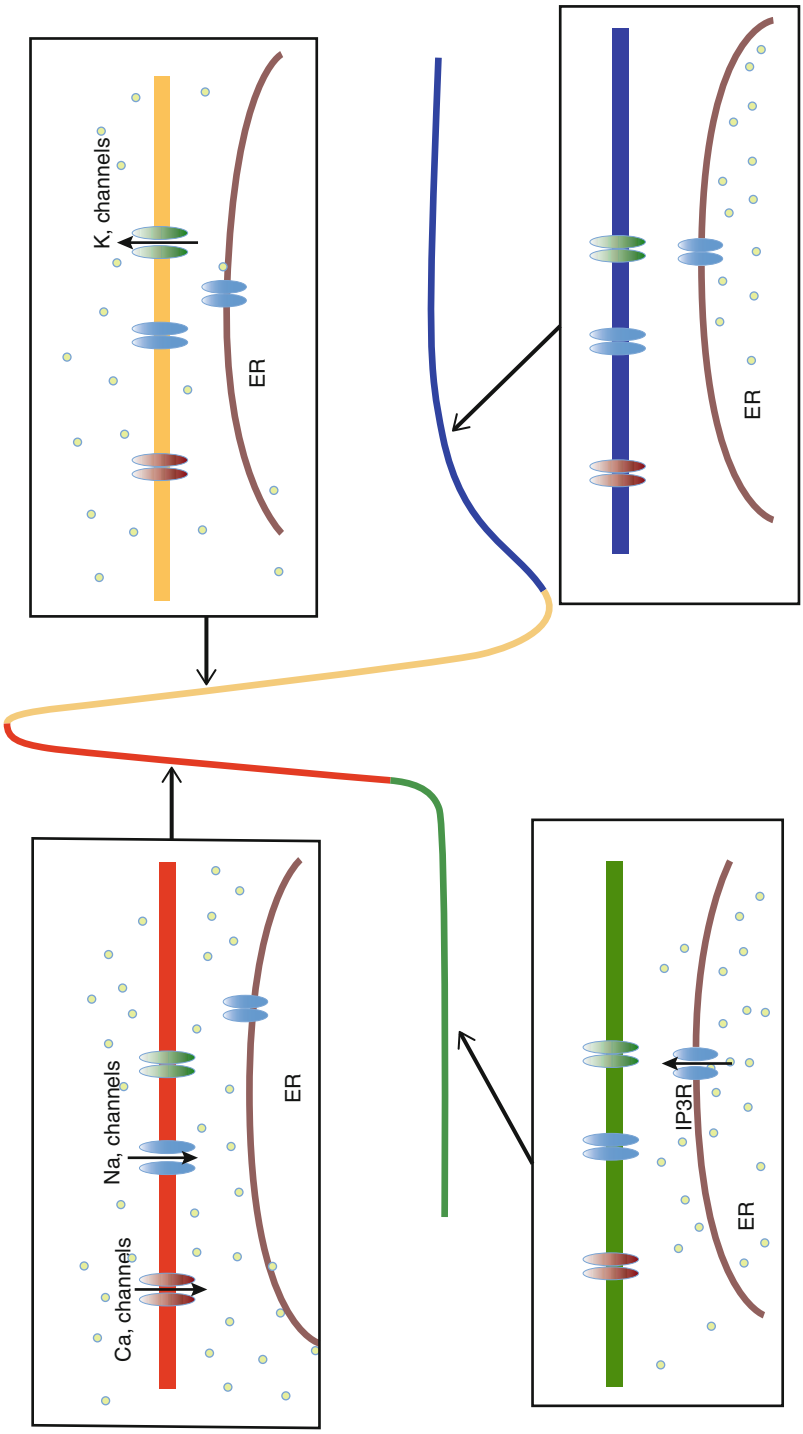

Fig. 1 Initiation of action potential in response to cell activation by a transmembrane receptor

through ion channels across the plasma membrane (Fig. 1). Therefore, to a large extent, calciumopathies represent a subset of the ion channel diseases, the channelopathies (Gargus 2003, 2008, 2009).

Ion channels are a large family of related transmembrane proteins that provide ions a passive pathway through which they can rapidly diffuse down their electrochemical gradient across the hydrophobic barrier of the plasma membrane (Fig. 2). The standing electrochemical gradients that drive ion movements though channels are established by ion pumps – such as the Ca ATPases, and ion carriers – such as the Na/Ca antiporters. While kinetic studies portray channels, pumps, and carriers as radically different transport mechanisms, with channels conducting ions four orders of magnitude faster and seemingly quite differently than the one-at-a-time binding and transmembrane turnover of the pumps and carriers, they are closely related molecular mechanisms (Gargus 2008). In many ways channels act like highly selective water-filled pores that can be opened and closed in a controlled fashion to allow a specific ion species to flow. This causes a miniscule chemical flux but an appreciable electrical current sufficient to change the membrane potential towards the Nernst potential of the conducted ion, which is interior negative for K^+, the predominating permeability at rest, and interior positive for Na^+ and Ca^{2+}. The channel's predominant permeant ion species is dictated by the nature of the channel's selectivity filter, and therefore, an interior-positive depolarization is created by opening Na^+ and Ca^{2+} channels, an increasingly negative hyperpolarization created by opening K^+ channels, and a stabilization of the membrane potential by opening Cl^- channels, since the Cl^- Nernst potential usually is near the resting potential. The most typical channelopathy lesions in well-understood monogenic diseases of the heart, muscle, and nerve cause membrane *hyperexcitability*; thus, mutations in K^+ and Cl^- channels, which physiologically stabilize excitable tissue, typically have pathological lesions that diminish their current, and Na^+ and Ca^{2+} channels, which physiologically excite a tissue, typically have gain-of-function lesions (Gargus 2008). It is predominantly these electrical consequences that underlie the physiology and pathophysiology of the ion channels, but calcium is an important exception to this rule, since it plays an additional critical role in coupling electrical activity to biochemical pathways.

Finally, ion channel families vary in their mechanism of gating. One large family of channels gates in response to changes in the electrical potential across the membrane, the *voltage-gated ion channels* (Fig. 2). These channels respond to a membrane potential change by undergoing a conformational change from "closed" to "open" – a conducting state where 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) (Fig. 1). 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 and hence driving the membrane potential further towards *their* inside-positive Nernst potential, and hence driving still more adjacent voltage-gated channels to open. Ultimately these channels intrinsically inactivate to cease conducting and voltage-gated K^+ channels open to repolarize the membrane, preparing it for conducting

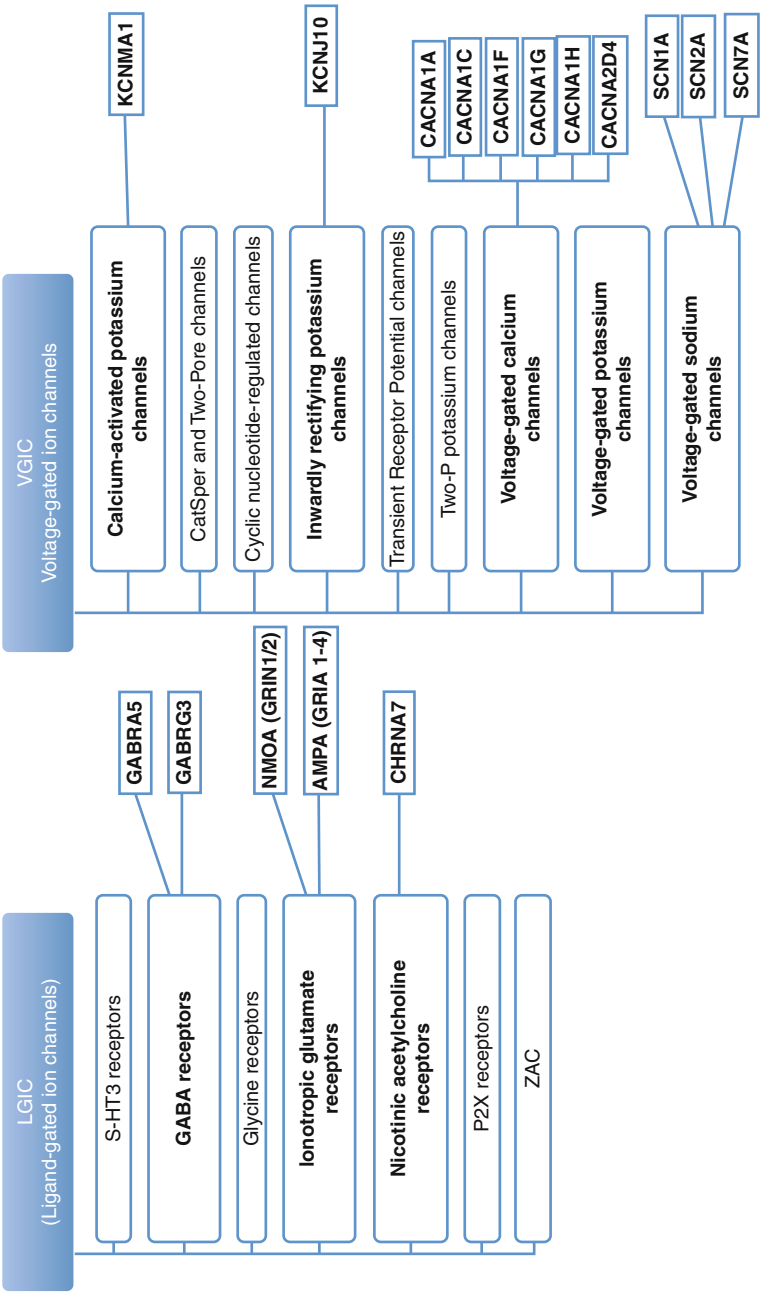

Fig. 2 Classification of ion channels. Channels mentioned in this chapter are shown in bold

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. Intracellular calcium-release channels have complex gating that includes responsiveness to plasma membrane protein 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 resequestered via the ER's calcium ATPase (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) 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 (Hayashi and Su 2007).

Timothy Syndrome: ASD Caused by a Calcium Channelopathy

It is most straightforward to see the importance of calcium channel signaling abnormalities in autism through the lens of *Timothy syndrome* (TS). TS is predominated by the lethal *cardiac arrhythmia syndrome long QT* (*LQT*), so called because of its characteristic EKG findings (Splawski et al. 2004). LQT was one of the founding *channelopathy* diseases, and it has now been shown to be caused by mutations in all of the cardiac ion channels that contribute to the ventricular action potential (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 other LQT mutations, TS (also called LQT8) is a simple *monogenic* dominant channelopathy. Surprisingly, about 80 % of the patients with TS also have a neurodevelopmental phenotype on the autism spectrum (Splawski et al. 2004, 2005). Since so much is understood about the pathogenesis of LQT and the *biophysics* of the ion channels involved, and since

Table 1 Calcium channel genes implicated in ASD

Protein	Description	Normal function	Disease association
CACNA1A	Voltage-regulated P/Q-type calcium channel, alpha 1A subunit	Regulates entry of Ca^{2+} into excitable cells: hormone/ neurotransmitter release, gene expression, cell cycle	Developmental delay, epilepsy, spinocerebellar ataxia
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
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	Associated with 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	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
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

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. It additionally produces other extracardiac symptoms such as *syndactyly*, seizures, immune deficiency, and hypoglycemia. 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. This channel family is well recognized to cause disease, since its close *paralogs CACNA1S* and *CACNA1A* (Table 1) have mutant alleles that cause, respectively, the skeletal muscle diseases *hypokalemic periodic paralysis* and *malignant hyperthermia*, and the neurological diseases hemiplegic migraine, *episodic ataxia*, and *spinocerebellar ataxia* (Gargus 2009). Only rare missense alleles have been recognized at this 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. More recently a novel de novo TS allele was identified in constitutively expressed exon 38, A1473G (Gillis et al. 2012). This caused the full TS, 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 pseudo-monomer domains, and they appear to tightly interact with one another to form the closed state of the channel pore (Depil 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 both of these molecular functions as well.

Since 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 long QT 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 (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 arrhythmia and the channel blocker verapamil can be used to treat TS (Jacobs et al. 2006; Sicouri et al. 2007).

In vitro expression of the mutant and WT versions of the TS channel has been carried out, and kinetic analysis has revealed that the major effect of the TS mutation is to alter the speed with which the opened conducting channel returns to a nonconducting conformation through a process called channel *inactivation* (Barrett and Tsien 2008). The channel inactivation arising from changes in the membrane potential (VDI) is slowed, as would be predicted from the cardiac findings, but a separate mechanism of the inactivation regulated by the calcium signal itself is greatly accelerated. The net result of the mutant is a very *rapid* inactivation of 50 % of the current and then a very slow inactivation of the

remainder (Barrett and Tsien 2008). A major breakthrough in TS research was achieved by creating induced pluripotent stem cells (*iPSCs*) from TS patient fibroblasts (Yazawa et al. 2011). These reprogrammed cells were differentiated first into cardiomyocytes, and they recapitulated in vitro the prolonged APs, irregular electrical activity, and abnormal calcium signals of LQT. Roscovitine, a compound that accelerated VDI, restored calcium and electrical signaling towards control. Next these iPSCs were differentiated into *cortical neurons* (Paşca et al. 2011). They showed wide APs and increased calcium signals, similar to the cardiomyocytes. They also showed altered patterns of calcium-dependent gene expression. Eleven of these genes were previously implicated in ASD. They also showed abnormal expression of *tyrosine hydroxylase* and increased production of norepinephrine and dopamine. Like the cardiomyocytes, all of these phenotypes were reversibly corrected with roscovitine (Paşca et al. 2011). Heterozygous TS transgenic mice carrying a poorly expressed construct with the exon 8 G406R mutation showed behavioral phenotypes suggestive of *ASD* phenotypes. They showed altered social behavior, altered responses to fear conditioning, restricted, repetitive, and perseverative behaviors, as well as altered *ultrasonic vocalizations* (Bader et al. 2011). 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).

Together the biophysical and behavioral findings on expressed TS mutant channels greatly extend the pathophysiology not only of TS but of ASD itself and begin to *render* it a *neurobiological* rather than a strictly behavioral phenotype. As *LQT* is a hyperexcitability syndrome, it suggests that neuronal hyperexcitability is a route to ASD much as it is for epilepsy, a condition long-recognized highly comorbid (a suggestion of shared pathogenesis) with ASD. Since this is such a fundamental and multifaceted signaling mechanism, it holds the potential of representing a core deficit in ASD. Recognition of such a core deficit with tractable pharmacology also brightens the prospect that new *molecular targets* can be discovered against which new generations of drugs can be developed in this disease.

Other Defects in the Calcium Channel Subunits in ASD

Although the TS mutation has proven highly informative, it clearly does not account for even a tiny fraction of the cases with typical ASD. An important difference is that TS is a highly penetrant simple dominant disease, whereas most ASD behaves as a complex *multigenic* disorder such that mutations contributing to typical ASD only incrementally enhance susceptibility to the disease with the phenotype observed only if a sufficient number of such contributing alleles are co-inherited. This leads to the typical inheritance pattern that shows a clustering of ASD in families, with even *identical twins* only ~90 % concordant and *dizygotic fraternal twins*, genetically similar to sibs, ~30 % concordant, but still affected ~20 times the general population risk (Ronald and Hoekstra 2011).

There are however diverse additional suggestions that calcium channel mutations highlighted by the TS mutation are germane to typical ASD. Mutations in calcium channel alpha subunit neuronal *paralogs* of the TS/LQT8 channel behave more like those mutations contributing to a multigenic disease. They do not neatly segregate with ASD but instead appear to contribute susceptibility to autism pathogenesis. The first example is the gene *CACNA1H* (Table 1), where rare alleles have been shown to cluster in cases of familial autism (Splawski et al. 2006). *CACNA1G* (Table 1), another calcium channel alpha subunit *paralog*, is mapped to the chromosome 17q11–q21 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. 2010). More recently deep *resequencing* of functional genomic regions identified potentially causal rare variants contributing to *ASD* in *CACNA1F* (Table 1), 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. Indeed, there is even growing evidence that *CACNA1C* (Table 1) itself contains, in addition to the strong TS alleles, other weak alleles that contribute broadly to cortical dysfunction, such as in schizophrenia, bipolar disease, and major depression (Thimm et al. 2011). An interstitial deletion at chromosome 12p13.33 deleting both the *CACNA1C* major subunit and *CACNA2D4* (Table 1) accessory calcium channel subunit genes caused *developmental delay* in two sibs and their father (Abdelmoity et al. 2011), and in a study of *copy-number* variants (CNVs) in ASD, two affected sibs were found to have a 2p:12p translocation that, again, resulted in the deletion of both genes as well (Smith et al. 2012).

Sodium Channel Defects in ASD

Strong evidence is building for a role of lesions in neuronal voltage-activated sodium channels in typical *polygenic* ASD. Such lesions support a hyperexcitability as well as a calciumopathy mechanism of ASD, since classical calcium-signaling diseases such as *LQT* and *malignant hyperthermia* syndrome (MHS) have well-established dominant pathogenic alleles in cardiac and muscle sodium channel loci, MHS2 and LQT3, respectively. Mutations in neuronal paralogs of the MHS2 and LQT3 sodium channel genes, *SCN1A* and *SCN2A* (Table 2), were long ago found in rare cases of familial autism (Weiss et al. 2003). These neuronal sodium channel genes had previously been shown to contribute pathogenic alleles to the seizure syndrome *GEFS+* and *SCN1A* also to carry null alleles in the severe seizure syndrome *SMEI* (both reviewed in Ma and Gargus 2007) as well as missense alleles in the migraine syndrome *familial hemiplegic*

Table 2 Sodium channel genes 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+, Dravet syndrome, familial autism
SCN2A	Voltage-regulated sodium channel, type 2	Action potential initiation and propagation in excitable cells	Epilepsy, ASD
SCN3A	Voltage-regulated sodium channel, type 3	Action potential initiation and propagation in excitable cells	Epilepsy, ASD
SCN7A	Voltage-regulated sodium channel, type 7	Na+-specific channel, allowing passive flow of ions down their electrochemical gradient	Homozygous deletion in autism
SCN8A	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

migraine (FHM3) (Dichgans et al. 2005; Gargus and Tournay 2007). 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 Tournay 2007). These alleles are found to disrupt cytosolic loop domains at the C-terminus of the protein. This region had originally been identified in the LQT3/SCN5A channel as an *EF-hand*-containing domain key to channel inactivation (Glaaser et al. 2006) and a site of regulatory *calmodulin* binding (Kim et al. 2004). Both *FHM3* and autism alleles of *SCN1A* perturb the same region of the channel protein – those intracellular regions that interact with calmodulin which acts as a bound protein subunit of the channel that confers calcium sensitivity to its regulation (Gargus 2009).

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. 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 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. Another member of the chromosome 2 sodium channel gene cluster is *SCN2A*, first associated with autism using a *candidate gene approach* (Weiss et al. 2003). Recently whole-exome *resequencing* of nearly 1,000 individuals uniquely identified this gene as the sole gene in which two independent probands had nonsense variants that disrupted the same gene, a highly significant result (Sanders et al. 2012), and this finding was again confirmed in a separate large study that found de novo protein-altering mutations in the gene in probands with ASD (O'Roak et al. 2012). Loss-of-function lesions in this region had previously been recognized by *array-CGH* detection in a child with *autistic* features carrying a de novo deletion of chromosome 2q24.2–> q24.3, the region containing *SCN2A* and *SCN3A* (Table 2) (Chen et al. 2010). Similarly, whole-genome resequencing in a small family *quartet* having just one affected *proband*, an unaffected *sib*, and two unaffected parents yielded 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 *paralogs*.

The sum of the evidence on neuronal sodium channels suggests that while hyperexcitability causing gain-of-function lesions that delay inactivation of the channel, much as is seen in *LQT* and MHS to occur, there are also clear cases of deletions causing absence of the channels, as in SCN7A, or *haploinsufficiency*, and these can only be interpreted as loss-of-function lesions that, most simply, reduce or otherwise alter membrane excitability. Again, the fact that bound *calmodulin* acts as a modulatory subunit in this family of channels and that the calciumopathy disease mechanism is caused by pathogenic muscle and cardiac paralogs also serves to implicate abnormal calcium homeostasis in these neuronal lesions.

Potassium Channel Defects in ASD

Early *candidate gene* studies had identified a role for calcium-activated potassium channels – central components in *calcium signaling* and well-established regulators of synaptic activity – in neuropsychiatric phenotypes (Chandy et al. 1998; Grube et al. 2011). The large conductance (BK) family member *KCNMA1* (Table 3), first recognized to carry pathogenic epilepsy alleles [causing the syndrome generalized epilepsy and paroxysmal dyskinesia (Du et al. 2005)], was more recently implicated in ASD. It was shown to be physically disrupted on chromosome 10 by a balanced *reciprocal translocation* in a patient with *ASD*, causing functional haploinsufficiency as assayed by expression studies and electrophysiology. Additionally in the case-control patient cohort of the study, a missense allele was identified that

Table 3 Potassium channel genes implicated in ASD

Protein	Description	Normal function	Disease association
KCNMA1	Calcium-activated large conductance potassium channel, subfamily A	Repolarization of the cell membrane	Epilepsy; implicated in ASD
KCNJ10	ATP-sensitive inward rectifier potassium channel 10	Have a greater tendency to allow potassium to flow into, not outside of, the cell	Seizures and ASD

altered a conserved domain of the channel, with no variants detected in the *control population* (Laumonnier et al. 2006). Subsequently the ATP-dependent inward rectifier potassium channel gene *KCNJ10* (Table 3), also originally identified to carry pathogenic epilepsy alleles [in this case 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. Additionally, while much attention in the *monogenic* ASD *syndrome Fragile X* (*FXS*) has focused upon mGluR5 receptors and enhanced mGluR long-term depression (*LTD*) seen in *fmr1* KO mice (see below), FMRP binds over 400 putative mRNAs (Ashley et al. 1993) and various approaches identify several potassium channel mRNAs as *FMRP* targets (Lee and Jan 2012).

The sum of the evidence however is that while potassium channels remain intriguing mechanistic candidates in ASD (Lee and Jan 2012), to date there have been only limited indications of genetic potassium channel defects in the disorder. They modulate both membrane excitability and calcium signaling so do at least remain important potential downstream targets of therapy (Lee and Jan 2012).

mTOR and ASD Synaptic Defects in Calcium Signaling and Neurosecretion

As discussed in the initial section, as a spreading axonal depolarization wave reaches its terminus at the presynaptic membrane, it stimulate calcium influx and intracellular calcium release, triggering neurotransmitter release into the *synaptic cleft* via the process of excitation-secretion coupling. The calcium signal culminates by initiating the fusion of synaptic vesicles into the presynaptic membrane, and it participates in diverse mechanisms of synaptic modulation that ultimately play a role in synaptic plasticity and learning (Neher and Sakaba 2008). Once in the synaptic cleft, the neurotransmitter binds and activates its receptor, altering excitability of the postsynaptic cell and, through presynaptic receptors that play a feedback regulatory role, modulating further presynaptic vesicle fusion. Calcium not only plays its role in *neuronal plasticity* at this posttranslational regulatory role in modulating

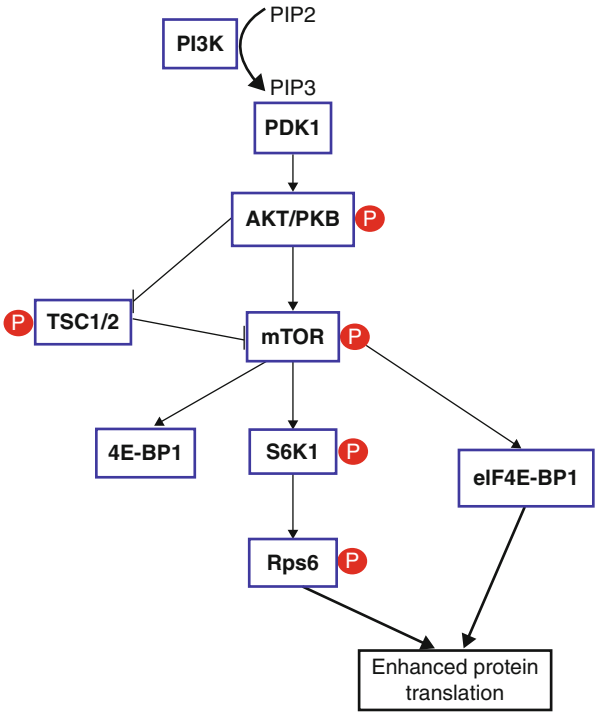

Fig. 3 mTOR pathway

neurosecretion in neuronal networks, but it additionally shapes the composition of the neuronal membranes themselves through the role it plays in the *mTOR* signaling pathway of upstream regulators and downstream effectors, many directly implicated in model monogenic *ASD* syndromes briefly discussed below (Fig. 3).

Fragile X Syndrome

Perhaps the best developed monogenic model of ASD is Fragile X syndrome (FXS) (Iossifov et al. 2012). Carriers of one *FMR1* allele with a "premutation," a modestly expanded trinucleotide repeat (55–200 repeats) at the *FMR1* locus, are at risk for expression of "Fragile X-associated tremor/ataxia syndrome" (*FXTAS*), an aged-onset *monogenic* neurodegenerative disorder associated with decreased FMRP (Table 5) levels (Feng et al. 1995; Jacquemont et al. 2003; Sheridan et al. 2011). *Pluripotent* stem cell-derived neurons from primary fibroblasts of female *premutation* carriers were studied with each *subclone* bearing exclusively either the normal or the expanded (premutation) form of the *FMR1* gene as the active allele on the sole remaining non-lyonized X chromosome. It was shown that neurons harboring the stably active, modestly expanded allele have reduced *neurite* length and functionally abnormal calcium transients of higher amplitude and increased frequency than for neurons harboring the normal active allele

Table 4 Transmembrane receptor genes implicated in ASD

Protein	Description	Normal function	Disease association
CHRNA7	Ligand-gated cation channel	Regulates glutamate release and stimulates inhibitory GABAergic interneuron activity	Epilepsy, schizophrenia, speech and learning problems
GRM5	Metabotropic glutamate receptor mGluR5	Modulates membrane and intracellular ion channels, signaling cascades, including mTOR. Involved in most aspects of normal brain functioning	Disruption leads to various neuropathologic conditions

(Liu et al. 2012). Moreover, a sustained calcium elevation was found in the expanded-allele-expressing neurons after glutamate application. 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 (Liu et al. 2012).

Mouse models of FXS are based upon knockout (KO) mice. One of the many *synaptic proteins* altered in *Fmr1* KO mice is the *metabotropic glutamate receptor* mGluR5 (encoded by *Grm5*) (Table 4). This receptor plays a critical role in neuronal calcium signaling. Phosphorylation and dephosphorylation of a key residue within the C-terminal domain of the activated receptor causes synchronous, oscillatory changes in IP3 and Ca^{2+} levels (Bradley and Challiss 2011). The signaling proteins bind to HOMER scaffold proteins and form a functional complex of mGluR5, *IP3 receptors* (IP3R), and voltage-dependent calcium channels. This signaling complex plays a key role in facilitating intracellular calcium-release and transmembrane calcium currents (Kato et al. 2012). Additionally, the phosphorylation and activity of "mammalian target of rapamycin" (mTOR, see "Tuberous Sclerosis" below), a calcium-*signaling protein* inhibited by rapamycin, are under control of this receptor, as is its upstream kinase, *phosphatidylinositol 3-kinase* (*PI3K*), a predicted FMRP target. Both activities are upregulated in the hippocampus of *Fmr1* KO mice, suggesting that the process impaired in FXS linking overactivation of *mGluRs* and aberrant synaptic plasticity is mTOR signaling (Sharma et al. 2010).

The absence of FMRP in *Fmr1* KO mice produces an upregulation of mGluR5 and an enhancement in the synaptic phenomenon of long-term depression (*LTD*) that relies upon enhanced rapamycin-sensitive protein synthesis triggered by this receptor (Sharma et al. 2010, see below "EIF4E"). This has proven to be a useful target for drug development in FXS, and chronic pharmacological inhibition of this receptor shows a comprehensive phenotypic correction in FXS models from cellular to behavioral features, even when treatment is started *after* full development of the adult phenotype, importantly showing that time-limited early developmental programs of neurogenesis are *not* critical aspects of the lesion (Michalon et al. 2012).

Another component of LTD in excitatory synapses is independent of protein synthesis and is instead endocannabinoid dependent. Postsynaptic mGluR5

Table 5 Cytosolic calcium-signaling protein genes implicated in ASD

Protein	Description	Normal function	Disease association
CYFIP1	Adaptor protein, component of the CYFIP1-eIF4E-FMR1 complex	Mediates translational repression activity of FMR1; role in axon outgrowth	Regulates translational activity through FMRP
FMR1	mRNA-binding protein	Transfers mRNA from nucleus to the cytoplasm, controls wide array of mRNAs encoding multiple proteins	Causes Fragile X syndrome
MECP2	Calcium-dependent transcriptional repressor	Binds to methylated DNA to control gene expression	Rett syndrome
TSC1	Tuberous sclerosis protein 1	Tumor suppressor, growth inhibitory protein. Involved in mTOR regulation	Dominant mutations lead to tuberous sclerosis type 1
TSC2	Tuberin	Tumor suppressor, acts like chaperone for hamartin, stimulates specific GTPases	Dominant mutations lead to tuberous sclerosis type 2
UBE3A	Ubiquitin-protein ligase 3A	Protein ubiquitination	Associated with Angelman syndrome
FKBP	FK506-binding protein	Rapamycin-binding subunit of mTOR complex. Interacts with intracellular calcium-release channels	Deletion is associated with disregulated mTOR signaling and altered synaptic plasticity
PTEN	Phosphatase and tensin homolog	Phosphatase; controls cell cycle. Tumor suppressor	Point mutations in the gene that do not abolish phosphatase activity are implicated in ASD
EIF4E	Eukaryotic translation initiation factor 4E	Component of the eukaryotic translation initiation factor 4F complex. Regulates translation activity. Downstream effector of mTOR signaling and common mediator for various pathways, including the one involving FMRP	Dysregulation leads to ASD-like phenotype

receptors, on HOMER scaffold proteins at the membrane, are linked together into an "eCB signalosome" with the enzyme *diacylglycerol* lipase-α (DGL-α) that produces an endocannabinoid neurotransmitter (eCB). Retrograde eCB signaling to the presynaptic membrane's cannabinoid *GPCR* (encoded by *Cnr1*) inhibits further synaptic glutamate vesicle release by inhibiting adenylate cyclase and thereby the presynaptic calcium channel currents required for *vesicle fusion*, causing this component of LTD (Jung et al. 2007). *FMRP* targets DGL-α to the *signalosome*, and in *Fmr1* KO mice, targeting fails and there is loss of that component of LTD, while restoring eCB signaling corrects the synaptic defects and a set of abnormal behaviors in the *Fmr1* KO mice (Jung et al. 2012b). It is also

of note that eCB signaling through CB1 receptors regulates the activity of forebrain neural circuits involved in the control of systemic energy metabolism and ultimately tissue-level mitochondrial proliferation, with mitochondrial hyperproliferation produced by a defect in this signaling (Jung et al. 2012a). This is a potentially intriguing apparition of the energy-deficient phenotype observed in a subset of *ASD* (Gargus and Imtiaz 2008; Gargus 2010, see below).

Another form of marked presynaptic abnormalities at excitatory hippocampal synapses has been observed in *Fmr1* KO mice – enhanced responses to high-frequency stimulation. These changes were associated with exaggerated calcium influx in presynaptic neurons serving to increase signaling across the synapse (Deng et al. 2011). Long-term potentiation (*LTP*) is another such elemental process in synaptic information processing (Lynch 2004). LTP 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 *AMPA receptors*. The *AMPA* channel is *impermeable* to Ca^{2+}, a feature essential to its function as a nonselective monovalent cation channel (Barbon and Barlati 2011). On the other hand the *NMDA* 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 to remove a luminal Mg^{2+} ion that blocks the receptor's channel (Ogden and Traynelis 2011). This creates a "coincidence detector" in the postsynaptic membrane whereby mild stimuli that cause only small releases of glutamate into the synapse activate some postsynaptic AMPA receptors but leave the NMDA receptors closed. Only when a very strong transmitter release is triggered do the AMPA receptors sufficiently depolarize the membrane to remove the NMDA receptor's Mg^{2+} channel block to provide a calcium conductance pathway, providing a postsynaptic calcium signal. LTP depends upon these changes in postsynaptic calcium. Intracellular injections of the *calcium chelator* EGTA block the development of LTP, strongly suggesting that LTP is caused by a calcium-sensitive modification of the *postsynaptic neuron* (Lynch et al. 1983). NMDA-mediated responses are required to induce LTP, but are not greatly affected by LTP. Conversely AMPA receptors mediate responses that are not essential to elicit LTP (although they *do* play this role physiologically), but it is through their alteration that LTP is manifest. Changes in the *AMPA receptor* components are a key result of the postsynaptic response in LTP (Muller et al. 1988). Additionally, stabilization of LTP depends upon reorganization of the *dendritic spine actin cytoskeleton*, and this involves the participation of the actin-regulatory protein cortactin, a protein also recognized to regulate the potassium channel *KCNA2* and thereby to provide a direct link between actin dynamics, *cell structure*, and membrane excitability (Williams et al. 2007).

The sum of the evidence thus is that pre- and postsynaptic membrane changes recognized in several fundamental processes of synaptic plasticity and learning are calcium sensitive and are altered in *FXS*, some being amenable to pharmacological treatment, rendering this fundamental physiological process a promising target in ASD.

15q11-13 Angelman/Prader-Willi Region

At synapses *FMRP* represses protein synthesis by forming a complex with the "cytoplasmic FMRP interacting protein 1" (CYFIP1) encoded by *CYFIP1* (Table 5) that is mapped to chromosome 15q11 within the locus of the most common chromosomal anomalies observed in ASD. mGluR5 stimulation with selective agonists causes CYFIP1 to dissociate from *eIF4E* at synapses, thereby resulting in synaptic protein synthesis. *TrkB* protein kinase receptor stimulation with *BDNF* (see below "Rett Syndrome") has the same effect. Together this demonstrates that translational repression activity of FMRP is mediated, at least in part, by CYFIP1 (Napoli et al. 2008) and it serves to expand a calcium-sensitive signaling network implicated in the *ASD* phenotype.

Angelman syndrome (AS) puts a focus on another gene at 15q11.2, the maternally expressed E6-AP *ubiquitin ligase* gene *UBE3A* (Table 5). Mouse model knockouts (KO) of maternal *Ube3a* have substantial deficits in *LTP* and learning (Jiang et al. 2010). Calcium signals generated by experience-driven neuronal activity induce *Ube3A* transcription, and Ube3A then regulates *excitatory synapse* development by controlling the degradation of Arc, a synaptic protein that promotes the internalization of *AMPA receptors*. Disruption of Ube3A function in neurons leads to an increase in Arc expression and a decrease in the number of *AMPA* receptors and frequency of miniature excitatory postsynaptic currents at excitatory synapses. Conversely, Ube3A function elevates the surface expression and activity of AMPA receptors by directing the proteasomal degradation of Arc (Greer et al. 2010). TBS-induced actin polymerization within *dendritic spines*, an essential event for stabilizing LTP (see above), is severely impaired in the *Ube3a* KO mice, and this profound defect in activity-driven spine cytoskeletal reorganization is associated with a loss of the synaptic plasticity required for the encoding of long-term memory (Baudry et al. 2012). Long-term memory scores in a fear conditioning paradigm were reduced by 50 % in the KO mice, but positively modulating AMPA receptors with an agonist ampakine CX929 normalized the response, and LTP in hippocampal slices from ampakine-treated KO mice was similar to that in WT controls. Therefore, the spine abnormality, LTP, and learning impairments in the *Ube3a* KO mice were reduced by AMPA-activating drug treatment (Baudry et al. 2012).

More recently four probands were identified with small deletions in this region that included only the *CHRNA7*gene (Table 4). The authors identified another 19 individuals 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 phenotypic features associated with the larger 15q13 microdeletions, suggesting *CHRNA7* was the critical gene responsible for the clinical findings associated with the 15q13 *microdeletion* syndrome (Hoppman-Chaney et al. 2012). The *CHRNA7*/*Chrna7* gene encodes the α7 *nicotinic acetylcholine receptor* (*nAChR*) in man and mice, respectively. It is a homopentameric ligand-gated calcium-conducting cation channel expressed in the hippocampus, where presynaptic α7 nAChRs regulate glutamate release, whereas postsynaptic α7 nAChRs stimulate inhibitory GABAergic *interneuron* activity (Johnstone et al. 2011) and their release

of the major CNS inhibitory neurotransmitter γ-aminobutyric acid (*GABA*) (Adams et al. 2012). GABA is produced from glutamate, the major stimulatory transmitter, and an imbalance in the signaling of these two systems has long been implicated in *ASD* (Gogolla et al. 2009).

It is intriguing that the *combined* modulation of the two different "Cys-loop" family receptors encoded in the AS/PWS region, the α7 nAChR and α5 GABA(A) R, alters hippocampal function in learning paradigms. Transient application of two separate *allosteric modulators*, which individually either inhibit the inhibitory α5 GABA(A)Rs or enhance the activating α7 nAChRs, only jointly causes *LTP* of induced excitatory postsynaptic currents 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 receptors, suggesting the therapeutic utility of this strategy targeting the AS/PWS-encoded receptors (Johnstone et al. 2011).

Finally, the first lesion in the AS/PWS region recognized to be associated with ASD was a *maternally inherited* 15q inverted duplication *marker chromosome* that produced a *tetrasomic* dose of this 20Mb region in the affected patient (Cook et al. 1997). Filipek and coworkers reported that maternal 15q inverted duplication marker chromosomes are associated with a panel of biochemical signs of mild mitochondrial dysfunction, including an elevated serum lactate/pyruvate ratio, ammonia and alanine levels, elevated urinary lactic acid, a secondary carnitine deficiency, and a biopsy-proven partial deficiency of mitochondrial respiratory complex III (Filipek et al. 2003). This same pattern was subsequently recognized as an *endophenotype* in two large chromosomally normal cohorts with typical ASD (Filipek et al. 2004; Oliveira et al. 2007). Interestingly a similar pattern of signs of modest mitochondrial energy deficiency has been observed in another syndromic form of ASD, Rett syndrome.

Rett Syndrome

A pattern of biochemical signs of modest mitochondrial energy deficiency, similar to that seen in ASD patients with 15q inverted duplications, has been observed in another syndromic *monogenic* form of ASD, *Rett syndrome*, caused by mutations in *MECP2* (Table 5). This syndromic form of autism is modeled in *Mecp2* KO mutant mice, and they reveal behavioral changes reminiscent of autism (Moretti et al. 2005). Rett syndrome patients have been shown to have modest *lactic acidosis* and *hyperammonemia* (Eeg-Olofsson et al. 1990) and the mouse model shown to have functional defects in mitochondrial respiratory complex III (Kriaucionis et al. 2006). Together these findings link gene dysregulation within the chromosome 15q11-q13 region in the mammalian brain with *MeCP2* function, and therefore link *Rett syndrome*, *Angelman syndrome*, *Prader-Willi syndrome*, and ASD, and suggest that ASD may be caused by the inability of neurons to generate adaptive responses via calcium-regulated gene expression in response to incoming synaptic activity (Qiu and Cheng 2010).

MeCP2 also binds selectively to the *BDNF* promoter where it functions to repress expression of *BDNF*, the gene encoding "brain-derived neurotrophic factor" (BDNF). Membrane depolarization triggers a calcium-dependent phosphorylation and release of MeCP2 from the promoter, thereby facilitating transcription (Chen et al. 2003). The dendritic BDNF receptor in turn itself evokes calcium transients, and pairing a weak burst of synaptic stimulation with brief dendritic BDNF stimulation causes induction of LTP (see above). This induction is dependent on activation of postsynaptic calcium channels and *NMDA receptors* as well as the postsynaptic calcium transients they mediate (Kovalchuk et al. 2002).

Direct study of neuronal function in the mouse *Mecp2* KO Rett model revealed that excitatory, but not inhibitory, synapses showed less *spontaneous activity* than control. This observation suggests a potential defect in the calcium-dependent processes of excitatory neurosecretion and *synaptic vesicle* trafficking (Nelson et al. 2006). Neurons derived from iPSC clones carrying three different MeCP2 mutations (RTT) showed a reduction in the density of glutamatergic synapses, similar to WT *iPSC* neurons expressing an antisense *knockdown* construct (shMeCP2), while overexpression of MeCP2 increased the synapse number. Together these findings strongly suggest that MeCP2 is a rate-limiting factor regulating glutamatergic synapse number in human neurons (Marchetto et al. 2010). A disturbance in neuronal calcium homeostasis is also observed in *Mecp2* KO mice (Mironov et al. 2009), and in iPSC-derived RTT neurons, spontaneous calcium transients and the frequency of calcium oscillations were decreased (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.

Tuberous Sclerosis

Another important syndromic form of *ASD* that impacts synaptic *calcium signaling* is tuberous sclerosis (*TSC*), caused by dominant mutations in either *TSC1* or *TSC2* (Table 5). The protein products of these two genes heteromultimerize to regulate *mTOR* (Fig. 3) (see "Fragile X Syndrome" above), an 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). While *magnetic resonance spectroscopy* (*MRS*) has been shown to detect elevated CNS lactate in this disease (Yapici et al. 2007), peripheral biochemical markers of mitochondrial energy deficiency have yet to be reported. 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 *Fkbp1A*, is the validated target of rapamycin *immunosuppressants* (Table 5). The protein interacts with intracellular calcium-release channels, and neuronal deletion of *Fkbp1A* is associated with disinhibited mTOR signaling and altered synaptic plasticity and memory (Hoeffer et al. 2008). The *KO mouse* shows increased basal mTOR phosphorylation and enhanced hippocampal *LTP* that is resistant to rapamycin, but not to other blockers of protein translation. This conditional KO displayed enhanced *contextual fear* memory and autistic/obsessive-compulsive-like *perseveration* in several assays, together indicating that FKBP plays a critical role in the regulation of mTOR, LTP, memory, and ASD-like behaviors (Hoeffer et al. 2008). Disrupting *Fkbp1B* with *interfering RNA* (*RNAi*) knockdown also destabilizes Ca^{2+} homeostasis in hippocampal neurons, similar to rapamycin, and is sufficient to induce a characteristic phenotype of Ca^{2+} dysregulation like that seen in very old animals and Alzheimer models, causing impaired *learning and memory* (Gant et al. 2011).

Upstream of TSC/mTOR, negatively regulating the PI3K/AKT signaling pathway, is "phosphatase and *tensin* homolog," a *tumor suppressor gene* encoded by *PTEN* (Table 5). While classically mutations in this gene are associated with the "*PTEN hamartoma* tumor syndrome" (PHTS; includes a family of related classical hereditary tumor syndromes (Hobert and Eng 2009)), individuals with germline mutations that inactivate *PTEN* are prone to develop *neurological disorders*, including *macrocephaly*, epilepsy, developmental delays, and autism. *PTEN* germline mutations are most typically found in a small subset of children with ASD and macrocephaly (Zhou and Parada 2012), and most germline *PTEN* mutations identified in ASD patients are point mutations, not CNVs, but they include nonsense mutations, suggesting a loss-of-function lesion. Functional analysis of *PTEN mutations suggests* that most ASD-associated *PTEN* mutations do not abolish the lipid phosphatase activity of the enzyme and are less severe lesions than PHTS-related alleles (Rodriguez-Escudero et al. 2011), consistent with the absence of correlation of PTEN-associated ASDs and PTEN-associated tumors (Zhou and Parada 2012), although some alleles (R130X) have been identified in both.

Neurofibromatosis

Neurofibromatosis type I (NF1) is a dominant neurocutaneous syndrome that includes *ASD* and is caused by heterozygous loss-of-function mutations in the *NF1 gene* that negatively regulates the activating signaling arm upstream of *mTOR calcium signaling* (Walsh et al. 2013).

EIF4E

In addition to the upstream mTOR regulators TSC1/TSC2, PTEN, and NF1, a downstream effector of calcium-dependent mTOR signaling has also been implicated in ASD. Therefore, it plays a key role in learning and memory through its

control of translation within the synapse and is the final common mediator of *TSC*, NF1, PTEN, and mTOR upstream signaling and is even the final convergent mediator of *FMRP* (Sharma et al. 2010).

Together the mTOR pathway signaling defects, and additional interacting lesions in transsynaptic complexes (Etherton et al. 2011), extend the case that autism pathogenesis involves calcium-signaling defects that culminate in defective excitation-secretion coupling, *synaptic vesicle* trafficking, and neurosecretion (van der Zwaag et al. 2010).

Family of Autism-Related Diseases with Defective Neuronal Calcium Signaling

A number of diseases have long been recognized to be comorbid with autism – seizures, migraine, and bipolar disease (*BPD*) being the most prominent (Gargus 2009, 2010). While there are many competing theories for such familial clustering, an important consideration is that these superficially distinct diseases share some fundamental components of vulnerability, likely arising from a shared subset of susceptibility-conferring loci.

Key Terms

Calciumopathy. Disease caused by abnormal calcium signaling.

Channelopathy. Disease caused by abnormal ion channel function.

Ion channel. A homomeric or heteromeric transmembrane *protein complex* capable of conformational changes that gate opened/closed transitions in a transmembrane pore that generally has a *selectivity filter* that restricts the permeability of specific ionic species.

Synapse. The functional junction between two neurons, where the presynaptic membrane releases neurotransmitter molecules that diffuse across the synaptic gap to activate neuroreceptors on the postsynaptic membrane, altering the excitability of that membrane and potentially triggering a conducted action potential in the postsynaptic cell.

Long (short)-term potentiation (depression). [*LTP*, STP, *LTD*] changes in synaptic function and neuroreceptor vesicle release subsequent to prior activation of the synapse, all serving as elemental components of information processing at a synapse and underlying plasticity and learning.

mTOR. Mammalian target of rapamycin, an 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.

Timothy syndrome. *CACNA1C* mutations that cause *LQT* arrhythmia and ASD. It is a member of a channel family well recognized to cause disease, since its close *paralogs CACNA1S* and *CACNA1A* have mutant alleles that cause, respectively, the skeletal muscle diseases *hypokalemic periodic paralysis* and *malignant*

hyperthermia and the neurological diseases hemiplegic migraine, *episodic ataxia*, and *spinocerebellar ataxia*.

Key Facts

- Cytosolic calcium signals originate either by the rapid release of intracellular stores through intracellular ion channels or by extracellular calcium entering through ion channels across the plasma membrane; therefore, to a large extent, calciumopathies represent a subset of the ion channel diseases, the *channelopathies*.
- The most typical *channelopathy* lesions in well-understood *monogenic* diseases of the heart, muscle, and nerve cause membrane *hyperexcitability*; thus, mutations in K^+ and Cl^-channels, which physiologically stabilize excitable tissue, typically have pathological lesions that diminish their current, and Na^+ and Ca^{2+} channels, which physiologically excite a tissue, typically have gain-of-function lesions.
- It is most straightforward to see the importance of calcium channel signaling abnormalities in autism through the lens of highly penetrant dominant *CACNA1C* mutations that cause *Timothy syndrome* (TS), a syndrome predominated by the biophysically well-understood long QT (LQT) channelopathy syndrome but also with a highly penetrant *ASD* phenotype.
- Mutations in calcium channel alpha subunit neuronal paralogs of the TS channel behave more like those mutations contributing to a *multigenic* disease in that they do not neatly segregate with ASD but instead contribute susceptibility to ASD.
- Strong evidence is building for a role of lesions in neuronal voltage-activated sodium channels in typical *polygenic* ASD and such lesions support a hyperexcitability as well as a calciumopathy mechanism of ASD, since classical *calcium-signaling* diseases such as LQT and malignant hyperthermia syndrome (MHS) have well-established dominant pathogenic alleles in sodium channel loci.
- While potassium channels remain intriguing mechanistic candidates in ASD, to date there have been only limited indications of genetic potassium channel defects in the disorder.
- The calcium signal culminates by initiating the fusion of synaptic vesicles into the presynaptic membrane, and it participates in diverse mechanisms of synaptic modulation that ultimately play a role in synaptic plasticity and learning.
- Pre- and postsynaptic membrane changes recognized in several fundamental processes of synaptic plasticity and learning are calcium sensitive and are altered in *FXS*, some being amenable to pharmacological treatment.
- The ASD-causing AS, *PWS*, RTT, *TSC*, NF1, and *PTEN* lesions converge on the calcium and metabolism sensing *mTOR* signaling pathway suggesting an inability of neurons in ASD to generate adaptive responses via calcium-regulated gene expression.

- A number of diseases comorbid with autism, most prominently seizures, migraine, and bipolar disease, likely reflect a shared subset of susceptibility-conferring loci, many producing constitutionally hyperexcitable neurons.

Summary

- Together the biophysical and behavioral findings on expressed TS mutant channels greatly extend the pathophysiology not only of TS but of ASD itself and begin to *render* it a *neurobiological* rather than strictly behavioral phenotype.
- As *LQT* is a hyperexcitability syndrome, it suggests that neuronal hyperexcitability is a route to ASD much as it is for seizures and epilepsy, a condition long-recognized highly comorbid (a suggestion of shared pathogenesis) with ASD.
- The sum of the evidence on neuronal sodium channels suggests that while hyperexcitability causing gain-of-function lesions that delay inactivation of the channel, much as is seen in LQT and MHS to occur, there are also clear cases of deletions causing absence of the channels, as in SCN7A, or *haploinsufficiency*, and these can only be interpreted as loss-of-function lesions that, most simply, reduce or otherwise alter membrane excitability.
- The sum of the evidence is that pre- and postsynaptic membrane changes recognized in several fundamental processes of synaptic plasticity and learning are calcium sensitive and are altered in models of ASD, some being amenable to pharmacological treatment, rendering this fundamental physiological process a promising target in *ASD*.
- Findings link gene dysregulation within the chromosome 15q11-q13 region in the mammalian brain with *MeCP2* function, and therefore link *Rett syndrome*, *Angelman syndrome*, *Prader-Willi syndrome*, and ASD, and suggest that ASD may be caused by the inability of neurons to generate adaptive responses via calcium-regulated gene expression in response to incoming synaptic activity.
- *eIF4E* plays a key calcium-dependent role in *learning and memory* through its control of translation within the synapse and is the final common mediator of TSC, NF1, PTEN, and mTOR upstream signaling and is even the final convergent mediator of FMRP.
- Together with the mTOR pathway signaling defects, the lesions in the neurexin/neuroligin interacting transsynaptic complexes extend the case that autism pathogenesis involves *calcium-signaling* defects that culminate in defective excitation-secretion coupling, *synaptic vesicle* trafficking, and neurosecretion.

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