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Defective Phosphoinositide Metabolism in Autism

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Abstract

Phosphoinositides are essential components of lipid membranes and crucial regulators of many cellular functions, including signal transduction, vesicle trafficking, membrane receptor localization and activity, and the determination of membrane identity. These functions depend on the dynamic and highly regulated metabolism of phosphoinositides and require finely balanced activity of specific phosphoinositide kinases and phosphatases. There is increasing evidence from genetic and functional studies that these enzymes are often dysregulated or mutated in autism spectrum disorders; in particular, phosphoinositide 3-kinases and their regulatory subunits appear to be affected frequently. Examples of autism spectrum disorders with defective phosphoinositide metabolism are Fragile X syndrome and autism disorders associated with mutations in the phosphoinositide 3-phosphatase PTEN, but recent genetic analyses also suggest that select non-syndromic, idiopathic forms of autism may have altered activity of phosphoinositide kinases and phosphatases. Isoform-specific inhibitors for some of the phosphoinositide kinases have already been developed for cancer research and treatment, and a few are being evaluated for the use in humans. Taken together, this offers exciting opportunities to explore altered phosphoinositide metabolism as therapeutic target in individuals with certain forms of autism. This review summarizes genetic and functional studies identifying defects in phosphoinositide metabolism in autism and related disorders, describes published preclinical work targeting phosphoinositide 3-kinases in neurological disorders, and discusses the opportunities and challenges ahead to translate these findings from animal models and human cells into clinical application in humans.

Graphical Abstract

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CONFLICT OF INTEREST

The author declares no conflict of interest.

Regulated phosphoinositide metabolism is crucial for cellular function. Several phosphoinositide kinases and phosphatases are mutated or dysfunctional in autism spectrum disorders and other neurodevelopmental diseases, suggesting a shared molecular defect with treatment potential.

Keywords

phosphoinositide 3-kinase; PTEN; signal transduction; schizophrenia; autism; biomarker; therapeutic strategy; Fragile X syndrome

INTRODUCTION

Autism disorders are a spectrum of diseases including individuals who are nonverbal and have IQs below 40 on the one end of the spectrum, and high-functioning individuals who can have above-average intellectual capabilities in certain domains, but are impaired in social interaction and communication skills (Lord and Bishop 2015). The underlying neurobiology of autism spectrum disorders seems to reflect this phenotypic variety: many genetic and functional studies suggest that the molecular mechanisms underlying autism spectrum disorders are complex and as heterogeneous as the disease phenotype (De Rubeis and Buxbaum 2015). Nonetheless, evidence is emerging that defects in certain cellular pathways are overrepresented and might be shared among different forms of autism (Cusco et al. 2009; Pinto et al. 2014; Sanders et al. 2015). Such pathways may be potential therapeutic targets with broader applicability, in particular if specific drugs for components of these pathways already exist. Recent studies suggest that defects in lipid kinases and phosphatases regulating phosphoinositides and their phosphorylated derivatives are such a shared pathological mechanism in autism spectrum disorders and other neurodevelopmental diseases. This review describes and critically assesses recent findings of altered phosphoinositide metabolism in autism and related disorders, outlines the implications for the development of novel therapeutic strategies, and discusses the challenges the field is facing to successfully translate these findings into treatment options.

PHOSPHOINOSITIDE FUNCTION AND METABOLISM

Phosphoinositides control a variety of cellular mechanisms

Lipid membranes, the physical barriers for organelles and cells, are not static. In fact, components of the lipid bilayers, which constitute all cellular membranes, are powerful regulators of a wide array of functions in the cell (Laganowsky et al. 2014). They can activate intracellular signal transduction, serve as a source for the generation of second messengers within the cell, control protein content of membranes, and regulate the flow of molecules in and out of the cell. Particularly phosphoinositides, the phosphorylated derivatives of phosphatidylinositol, which make up only a small fraction of the lipid membrane, are involved in all processes of membrane dynamics in virtually every cell of the human body. They define organelle identity, regulate localization and function of transmembrane proteins, and control activity of proteins involved in intracellular signal transduction (Balla 2013). Phosphoinositides are particularly crucial in defining membrane identity, with phosphatidylinositol 3-phosphate (PI3P) marking endosomes, phosphatidylinositol 4-phosphate (PI4P) residing predominantly in the membranes of the Golgi apparatus, and phosphatidylinositol 4,5-bisphosphate (PI(4,5)P₂) denoting plasma membranes (Di Paolo and De Camilli 2006). PI(4,5)P₂ is furthermore essential for vesicle trafficking at the membrane (Downes et al. 2005) and an important precursor of phosphatidylinositol 3,4,5-triphosphate (PI(3,4,5)P₃).

Apart from these direct roles at the cell membrane, phosphoinositides have indirect effects on cellular function via activating downstream signal transduction pathways. PI(4,5)P₂, for example, is the precursor for two second messengers, diacylglycerol (DAG) and inositol triphosphate (IP₃), which activate PKC as well as intracellular calcium release (Berridge 2009; Bishop et al. 1992). PI(3,4,5)P₃, in turn, activates intracellular signaling cascades through interaction with the pleckstrin homology domains of, for example, Akt and phosphoinositide-dependent protein kinase 1 (PDK1) (Downward 1998). This leads to activation of downstream signaling molecules, including the major signaling hub mechanistic target of rapamycin (mTOR). mTOR regulates a variety of central cellular functions, such as protein synthesis (Ma and Blenis 2009), mRNA transcription (Laplane and Sabatini 2013), as well as cell proliferation and apoptosis (Laplane and Sabatini 2012). mTOR is also an important regulator of autophagy, a cellular mechanism that removes damaged organelles and macromolecules (Kim and Guan 2015); moreover, mTOR activity controls the cytoskeleton, in particular microtubule and actin dynamics through two separate mTOR-containing complexes, mTORC1 and mTORC2 (Malik et al. 2013). This brief overview of mTOR-dependent processes shows that mTOR is involved in virtually every aspect of cellular function and emphasizes the widespread effects phosphoinositide-mediated regulation of downstream signaling can have on cells. Notably, another important regulator of mTOR, tuberous sclerosis complex 1/2 (TSC1/2), which is also downstream of PI(3,4,5)P₃, is mutated in Tuberous Sclerosis, a genetic disease associated with a high occurrence of epilepsy and autism (Davis et al. 2015a; Saxena and Sampson 2015).

The large variety of phosphoinositide-dependent processes in the cell is even further expanded by studies showing that phosphoinositides do not only reside and function in the

cytosol, but are also present and metabolized in the nucleus (Divecha et al. 1991; Manzoli et al. 1982), where they regulate mRNA biogenesis (Didichenko and Thelen 2001; Resnick et al. 2005) and mRNA export to the cytosol (Okada et al. 2008), as well as DNA repair and cell cycle entry (Silió et al. 2012). The role of phosphoinositide signaling and metabolism in the nucleus is reviewed in detail in Davis et al. 2015b.

It is well established that in neurons, phosphoinositides are involved in fundamental processes including, but not limited to, signal transduction regulating synapse structure and synaptic protein synthesis, synaptic vesicle endocytosis and exocytosis, and the recruitment of ion channels and neurotransmitter receptors to synapses (Viaud et al. 2015). To add to the complexity of cellular processes regulated by phosphoinositides in neurons, PI(4,5)P₂ has been shown to control ion channel function (Furst et al. 2014; Suh and Hille 2005), and recent work demonstrates that activity-regulated PI(3,5)P₂ synthesis is important for AMPA receptor trafficking mediating synaptic plasticity (McCartney et al. 2014).

Regulated phosphoinositide metabolism is crucial for cellular function

Phosphoinositides are phosphorylated at the 3rd, 4th and/or 5th carbon molecule within the inositol ring, leading to a total of seven different phosphorylated phosphatidylinositol derivatives (Fig. 1). As described above, different phosphoinositides vary widely in their function and subcellular localization and can regulate a plethora of cellular processes by recruiting enzymes, signaling molecules and ion channels or neurotransmitter receptors to cellular membranes. It is noteworthy that although many phosphoinositide kinases and phosphatases are present in both the nucleus and the cytoplasm, the exact signaling pathways and regulatory subunits involved in phosphoinositide metabolism can differ in these cellular compartments (see for example (Ahn et al. 2004)), suggesting unique phosphoinositide regulation depending on the subcellular localization (reviewed in Davis et al. 2015b).

Each of the phosphoinositides serves as a precursor for other phosphoinositides, so that perturbations in their metabolism can have cascading, widespread effects in the cell. Specific phospholipid kinases and phosphatases that convert phosphoinositides from one form to the other thus play important roles in regulating membrane receptor trafficking and intracellular signaling (Dyson et al. 2015; Vanhaesebroeck et al. 2010). A recent study, for instance, showed that PI3P hydrolysis to phosphatidylinositol (PI) by the lipid phosphatase MTM1 (Myotubularin 1), which is mutated in the X-linked disease centronuclear myopathy, is essential for surface delivery of endosomes and subsequent exocytosis (Ketel et al. 2016). It is thus not surprising, that deficiencies in phospholipid metabolism have been associated with impaired neuronal function and linked to neurological diseases, such as Alzheimer's disease and amyloid lateral sclerosis (Wen et al. 2012), but also to neurodevelopmental disorders including autism and schizophrenia (Gross and Bassell 2014). The enzymes involved in phosphoinositide synthesis and hydrolysis, lipid kinases and phosphatases, are therefore of central interest to understand underlying disease mechanisms and to identify targeted treatment options.

ENZYMES INVOLVED IN PHOSPHOINOSITIDE METABOLISM ARE AFFECTED IN AUTISM SPECTRUM DISORDERS

Several studies have suggested that mutations in genes coding for proteins involved in phosphoinositide metabolism, phosphoinositide kinases, phosphoinositide phosphatases and their regulators are associated with autism (Butler et al. 2005; Conti et al. 2012; Cusco et al. 2009; Pinto et al. 2014) (Fig. 1). These mutations lead to loss of expression in some cases, but may also affect enzyme function, membrane localization, or stability. Genetic studies provide important information about potential underlying disease mechanisms, but it is not always clear what the effect of a certain mutation on the enzyme's function in the cell is. To evaluate these mutations as disease mechanisms and therapeutic targets, it is thus mandatory to carefully analyze their functional consequences on phosphoinositide composition of neuronal membranes and phosphoinositide-mediated downstream signaling and function. Recent work also connects alterations in phosphoinositide kinase function that are secondary to the disease-causing genetic defect with autism or related disorders (Gross et al. 2010; Papaleo et al. 2016; Poopal et al. 2016; Sharma et al. 2010). Together with the genetic studies, these observations are of particular interest from a therapeutic point of view, because the same class of enzymes is frequently affected in cancer, and has thus been a target of drug discovery and development since many years. As outlined below, recent work in patient cell lines and in animal models has assessed how mutations or changes in expression of phosphoinositide kinases and phosphatases may alter neuronal function, providing initial insight into their role as disease-mediating molecular defect and potential therapeutic target.

PHOSPHOINOSITIDE KINASES AND THEIR REGULATORS IN AUTISM

Phosphoinositide kinases are distinguished by the carbon atom on the inositol ring whose hydroxyl group they phosphorylate. Of all phosphoinositide kinases, to date the most prominent role in autism is taken by the catalytic and regulatory isoforms of the phosphoinositide 3-kinase (PI3K) family, which phosphorylate the hydroxyl group on the third C atom of the inositol ring, giving rise to PI(3)P, PI(3,4)P₂, PI(3,5)P₂, and PI(3,4,5)P₃ (Hawkins et al. 2006). A reason for this apparent bias might lie in their biological function and in particular their central role in regulating mTOR and mTOR-dependent protein synthesis. Defects in protein synthesis and mTOR signaling are frequently associated with autism spectrum disorders (Kelleher and Bear 2008; Richter et al. 2015), which often may involve defective signaling through PI3K isoforms. This section will mainly focus on dysregulated PI3K signaling in autism and potential therapeutic strategies arising from these defects, but will also cover more recent evidence for other, less well-studied phosphoinositide kinases involved in autism and related disorders.

Defects in phosphoinositide-3 kinases and their regulatory subunits – shared molecular mechanisms in autism with treatment potential

PI3K-dependent signaling regulates cell growth and proliferation. Consequently, mutations in PI3K catalytic subunits have been found in different forms of cancer and malformations throughout the body, including the brain (Hevner 2015; Martini et al. 2014), and a lot of effort has been put towards the development of selective drugs targeting PI3K catalytic

subunits (Vanhaesebroeck et al. 2016). Altered brain growth and development has also been observed in autism (Courchesne et al. 2003), suggesting that molecular pathways involved in regulating cell growth are dysregulated in autism. Therefore, these drugs are becoming more and more attractive to assess the importance of defective PI3K signaling in autism and related disorders. The following sections will describe recent progress in analyzing altered PI3K signaling in autism, and highlight a few studies that used subunit-specific inhibitors to assess their therapeutic potential in preclinical studies with mouse or human cell models of autism.

There are three classes of PI3K catalytic subunits, of which the class I family is of particular interest in autism research, because all four class I catalytic subunits, p110 α , β , γ and δ have been shown to be mutated or dysregulated in autism. P110 α , β and δ belong to the class IA family, whereas p110 γ is the only member of the class IB family. They are distinguished by the regulatory subunits they are associated with (p50–55/p85 for class IA and p101/p84 for class IB) (Hawkins et al. 2006). Previously, it was thought that class IA PI3K catalytic subunits mainly signal downstream of receptor tyrosine kinases, whereas p110 γ is the major isoform downstream of G protein-coupled receptors (GPCRs); however, this view has been challenged by more recent work showing that p110 β is predominantly associated with GPCRs in macrophages and in neurons (Gross et al. 2015c; Guillermet-Guibert et al. 2008), corroborating the notion that a lot is still to be learned about the specific, non-redundant functions of PI3K catalytic subunits (Vanhaesebroeck et al. 2010). Genetic rescue studies and pharmacological studies with the recently developed selective inhibitors for all four of the class I PI3K catalytic subunits will be essential for understanding these distinct functions.

It is noteworthy that class I PI3K subunits, apart from their function as lipid kinases, were also shown to possess protein kinase activity (Vanhaesebroeck et al. 1999). The specific substrates, cellular functions and thus relevance for autism are still rather unclear, and will thus not be further discussed in this review.

Dysregulated p110 β could be a therapeutic target in Fragile X syndrome and other autism spectrum disorders—Two of the class IA catalytic subunits, p110 β and p110 δ , have been shown to be important for brain disease-associated behavior and molecular mechanisms using pharmacological or genetic approaches. There are several strong indications for a role of overexpressed p110 β , encoded by the *PIK3CB* gene, in autism, by both genetic and functional studies. A duplication in the chromosomal region covering *PIK3CB* was associated with autism in at least two separate studies (Cusco et al. 2009; Pinto et al. 2014). Moreover, increased levels of p110 β in Fragile X syndrome (FXS) may contribute to phenotypes associated with this inherited intellectual disability and autism spectrum disorder (Gross and Bassell 2012; Gross et al. 2010; Gross et al. 2015c; Kumari et al. 2014; Miyashiro et al. 2003; Sharma et al. 2010). FXS is caused by loss of expression of the Fragile X Mental Retardation Protein (FMRP), an mRNA-binding protein that usually suppresses the translation of its mRNA targets, among many other functions (Gross et al. 2015b; Santoro et al. 2012). The p110 β mRNA is associated with FMRP (Ascano et al. 2012; Gross et al. 2010; Miyashiro et al. 2003) leading to increased expression and function of p110 β in *Fmr1* knockout (KO) mice, a mouse model for FXS (Gross et al. 2010; Sharma

et al. 2010), as well as in FXS patient cell lines (Gross and Bassell 2012; Kumari et al. 2014).

Several lines of evidence from preclinical studies suggest that p110 β is a promising therapeutic target in FXS. TGX-221, a selective p110 β inhibitor (Jackson et al. 2005), rescues increased PI3K signaling in lymphoblastoid cells from patients with FXS, and excessive protein synthesis in both lymphoblastoid cells and fibroblasts from patients (Gross and Bassell 2012; Kumari et al. 2014). In the FXS mouse model, genetic reduction of p110 β expression by half using mice heterozygous for *Pik3cb* rescues molecular defects in signaling and protein synthesis, reduces increased dendritic spine density, and improves autistic-like behavior, but did not improve spontaneous neocortical activity (Gross et al. 2015c). Nevertheless, the therapeutic promise of p110 β in FXS is supported by the observation that local reduction of p110 β in the prefrontal cortex of *Fmr1* KO mice improves goal-directed behavior, a form of higher-order cognition that is particularly impaired in individuals with FXS (Gross et al. 2015c).

The next step to further explore p110 β as a therapeutic target in preclinical studies will be to test if brain-permeable p110 β -selective inhibitors improve core FXS phenotypes in developing or adult *Fmr1* KO mice. Results of these studies will be of broader interest for autism research, because, as mentioned above, duplications in the gene locus of *PIK3CB* have been associated with autism, suggesting overactive p110 β as a shared molecular mechanism. Of particular interest and further discussed below, p110 β activity plays an important role in overactive PI3K signaling caused by mutations in phosphatase and tensin homologue deleted on chromosome 10 (PTEN) (Jia et al. 2008; Wee et al. 2008), a hotspot for autism-associated mutations (Tilot et al. 2015).

Altered expression and function of p110 δ may be a shared mechanism in autism and schizophrenia—The class I PI3K catalytic subunit p110 δ is mainly known for its role in lymphocytes, and p110 δ -selective inhibitors have been proven as effective treatment for lymphomas and other hematological cancers (Furman et al. 2014). Selective inhibition of p110 δ may even have much broader applicability for a variety of cancers by reversing tumor-induced immune tolerance (Ali et al. 2014). Apart from this core role in the immune system, an increasing number of studies show that p110 δ is important for neuronal function and dysregulated in brain diseases. P110 δ enzymatic activity is needed for axonal outgrowth and regeneration in sensory neurons (Eickholt et al. 2007). On the other hand, inhibiting p110 δ activity improves recovery from stroke in a mouse model by reducing tissue-damaging neuroinflammation (Low et al. 2014).

Two recent studies have used human lymphoblastoid cells and discovered overactive and dysregulated p110 δ in schizophrenia and autism (Law et al. 2012; Poopal et al. 2016). Law et al. (2012) showed that increased p110 δ mRNA expression in patients with a risk polymorphism in the upstream ErbB4 receptor is associated with dysregulated neuregulin 1-mediated PI(3,4,5)P₃ production in lymphoblastoid cell lines. In a pharmacologically induced psychosis mouse model and in a rat model of schizophrenia induced by lesions in the ventral hippocampus, the p110 δ -selective inhibitor IC87114 rescued behavioral abnormalities and normalized Akt signaling (Law et al. 2012). More recently, elevated

p110 δ levels were shown in a novel transgenic mouse model of schizophrenia that overexpresses a specific isoform of Neuregulin 1 (Papaleo et al. 2016). Interestingly, a p110 δ -selective inhibitor reversed aberrant sensorimotor gating and cognitive deficits in these mice (Papaleo et al. 2016). Increased p110 δ expression and downstream signaling were also detected in lymphoblastoid cells from autistic members of a multiplex family with autism, but not in the healthy sibling or parents, and the p110 δ -selective inhibitor IC87114 rescued increased protein synthesis rates in one of the affected cell lines (Poopal et al. 2016).

Further studies in patient cells and in mouse models are needed to assess the relevance of dysregulated p110 δ for autism and to explore the therapeutic applicability of p110 δ -selective inhibitors for this disease. P110 δ negatively regulates PTEN (Papakonstanti et al. 2007), making it, similarly as p110 β , an interesting target in PTEN-associated autism. Notably, p110 δ -selective inhibitors are already approved for human use in leukemia and lymphoma patients (Yang et al. 2015).

Defects in p110 α and p110 γ are weakly linked to autism—The link between the other class I PI3K catalytic subunits, p110 α and p110 γ , and autism is weaker, and mainly based on genetic analyses, while functional studies are rare. Very recent work has identified a de novo missense mutation in *PIK3CA*, the gene coding for p110 α in a patient with autism and macrocephaly (Turner et al. 2016). Given the important role for p110 α in brain growth and the frequently observed mutations in *PIK3CA* in individuals with brain anomalies (Rivière et al. 2012), the mutation is likely to be disease-relevant. Interestingly, p110 α mRNA was also identified as an mRNA target of FMRP in a large-scale screen (Ascano et al. 2012), but so far it is unknown if loss of FMRP leads to increased p110 α expression and function, as observed for the FMRP-target p110 β . In the context of the above described studies using p110 β -specific inhibitors or gene knockdown in patient cell lines and mouse models of FXS, it will be interesting if a p110 α -selective inhibitor is similarly effective in ameliorating molecular, cellular and behavioral phenotypes in FXS, or if a combined application of p110 α - and p110 β -selective drugs even has synergistic effects.

P110 γ (*PIK3CG*) is well known for its role in immune and cardiovascular systems (Costa et al. 2011; Kerfant et al. 2006), but more recent work also demonstrates p110 γ 's importance for neuronal function. In the first study analyzing the functional role of p110 γ in the brain, Kim et al. (2011) used *Pik3cg* knockout mice and a p110 γ -selective inhibitor (AS-605240) to show that p110 γ is necessary for NMDA-dependent LTD and that its functional loss impairs behavioral flexibility and contextual fear memory in mice (Kim et al. 2011). While these results strongly suggest an important role of p110 γ for cognition and memory, it is less clear if defects in p110 γ contribute to autism in humans. A study analyzing individuals with autism for small nucleotide polymorphisms (SNPs) in genes involved in PI3K signaling discovered SNPs in *PIK3CG* (Serajee et al. 2003). Yet, this mutation does not affect the amino acid sequence, questioning the relevance of the SNP for the disease phenotype. To evaluate the role of this SNP for autistic phenotypes, it will be essential to analyze if stability or transcription and translation efficiency of the mRNA are altered by this mutation. The chromosomal location 7q22, which includes *PIK3CG*, has been identified as a potential autism susceptibility locus in case studies and gene linkage analyses (Cukier et al. 2009; Trikalinos et al. 2006), although the importance of this locus for autism could not be

confirmed in a more recent, comprehensive study (Sanders et al. 2015). More detailed and functional studies are needed to further evaluate p110 γ in autism.

Alterations in regulatory subunits of PI3K support an important role of PI3K signaling in autism—Not only class I PI3K catalytic subunits, but also their associated regulatory subunits have been shown to be altered in autism spectrum disorders. Mutations in the regulatory subunit p85 β (*PIK3R2*) were found to be associated with megalencephaly and autism (Rivière et al. 2012). Screens for mRNA binding partners of FMRP, the protein lost in FXS, identified mRNAs for the PI3K regulatory subunits p85 β (*PIK3R2*) and PIKE (PI3K enhancer, *CENTG1*) (Brown et al. 2001; Darnell et al. 2011). So far, it is unclear if loss of FMRP leads to dysregulated p85 β expression; however, two independent studies confirmed that protein levels of PIKE are increased in *Fmr1* KO mice (Gross et al. 2010; Sharma et al. 2010). The importance of the PI3K activator PIKE for FXS phenotypes was corroborated by showing that genetic reduction of PIKE in either mouse or fly models rescues FXS-associated phenotypes on the molecular, cellular, behavioral and cognitive level (Gross et al. 2015a). PIKE appears to be a promising target in FXS and potentially other forms of autism, firstly, because it links metabotropic glutamate receptors to the intracellular PI3K machinery, which is defective in FXS (Ronesi et al. 2012; Ronesi and Huber 2008), and secondly, because it is important for dendritic spine morphology (Chan et al. 2011). So far, PIKE has been targeted for inhibition using small peptides interfering with its association with the scaffolding protein Homer or the PI3K catalytic subunits (Rong et al. 2003), but there are no selective drugs available yet. Thus, to date, the therapeutic applicability of PIKE is limited due to lack of specific antagonists that are suitable for the use in humans.

Class II and III PI3K – a less studied group of phosphoinositide kinases with weak links to autism—In contrast to class I PI3K, members of the class II and class III PI3K families, which are mainly involved in the production of PI(3)P and PI(3,5)P₂, respectively, so far have not been shown to be altered in autism. Single nucleotide polymorphisms in the class II isoforms PIK3C2A and PIK3C2G are associated with schizophrenia (Jungerius et al. 2007; Ruderfer et al. 2014); however, functional studies are needed to assess the relevance of these genetic findings for schizophrenic phenotypes or possibly other brain diseases, such as autism.

OTHER PHOSPHOINOSITIDE KINASES AND THEIR REGULATORS IN AUTISM AND SCHIZOPHRENIA

Apart from PI3K, a few other lipid kinases involved in phosphoinositide metabolism have been suggested to be altered in autism and related disorders. Although the association is generally weaker and needs to be confirmed in larger scale genetic screens or in functional studies, some of these results are interesting and warrant further work. A recent study, for example, has identified rare deleterious mutations in the gene *Eighty-five Requiring 3A* (*EFR3A*), a regulator of phosphoinositide 4-kinases (PI4K), in individuals with autism, suggesting a role for phosphatidylinositol 4-phosphate (PI(4)P) synthesis in autism (Gupta et al. 2014). In this study, Gupta et al. (2014) performed deep sequencing of the *EFR3A* gene

in 2,196 autism cases and 3,389 controls, and detected twice as many mutations in this gene in autism compared to control. EFR3A is a palmitoylated membrane protein that is essential for membrane localization and thus activation of PI4K (Bojjireddy et al. 2015; Nakatsu et al. 2012). While its exact role in neurons is unclear, it is known that PI(4)P is a precursor of PI(4,5)P₂ and thus important for vesicle trafficking and the determination of membrane identity (Nakatsu et al. 2012). The observed mutations appeared to be pathological, as crystal structure analyses suggested that they resided in regions critical for EFR3A function. However, so far, it is unknown if these identified *EFR3A* mutations lead to neuronal dysfunction and autistic phenotypes.

Of note, a role of the PI4K/EFR3A signaling complex in neurological disorders was also supported by a study identifying SNPs in *PIK4CA*, the gene coding for PI4K α , in patients with schizophrenia (Jungerius et al. 2007). This is of particular interest, because *PIK4CA* is located within the chromosomal region 22q11, which is often affected by pathogenic copy number variations (CNVs). 22q11 deletion syndrome (a.k.a. velocardiofacial syndrome (VCFS)) is characterized by varying degrees of congenital heart disease, craniofacial abnormalities and immunodeficiency, and is often associated with neurological disorders, including schizophrenia, neurodevelopmental delay and autism (Swillen and McDonald-McGinn 2015). 22q11 has also recently been identified as one out of six risk loci for autism-associated CNVs (Sanders et al. 2015). Again, the physiological consequences of these SNPs or changes in gene dosage for PI4K function are unknown, and a role in autistic-like behavior is not established yet. Preclinical studies in animal models will be critical before exploring EFR3A/PI4K as potential drug targets in autism.

Other genetic studies have suggested that phosphoinositide-phosphate 5-kinases (PIP5Ks), in particular PI(4)P5K, are affected in autism spectrum disorders. PI(4)P5Ks use PI(4)P as substrates and catalyze the rate-limiting step in the biosynthesis of PI(4,5)P₂. PI(4,5)P₂ is a crucial regulator of synaptic vesicle trafficking (Di Paolo et al. 2004), ion channel function (Suh and Hille 2005), and intracellular signaling (Gericke et al. 2013). PI(4)P5K isoform 3 is encoded on chromosomal region 2q33.3, which was found to be duplicated in a few individuals with developmental delay and autism (Cusco et al. 2009; Lukusa et al. 1999; Usui et al. 2013). Deletion of this region was also associated with autism and epilepsy in another case (Brandau et al. 2008). A role for PI(4)P5K in brain disorders is further supported by studies suggesting that mutations in PI(4)P5K are associated with schizophrenia (Bakker et al. 2007; He et al. 2007), and by work showing that PI(4)P5K is necessary for NMDA-induced AMPA receptor endocytosis during synaptic long-term depression (Unoki et al. 2012). PI(4)P5K thus seems to play an important role in enduring forms of synaptic plasticity necessary for learning and memory and other brain functions. Yet, similarly as for the PI4K regulator EFR3A, the link between PI(4)P5K and autism (or any other brain disease) is weak and will have to be further assessed in animal models.

Components of the other family of phosphoinositide 5-kinases, PI(3)P5Ks, which use PI(3)P as substrates to generate PI(3,5)P₂ have also been shown to be important for neuronal function. The PI(3)P5K PIKfyve, for instance, regulates AMPA receptor trafficking and synaptic plasticity (McCartney et al. 2014). The PIKfyve complex, which also includes the PIKfyve activator and phosphoinositide 5-phosphatase FIG4, is regulated by amyloid

precursor protein (APP) and has been associated with Alzheimer's disease (Currinn et al. 2015; Currinn and Wassmer 2016). However, so far no association of PIKfyve dysregulation with neurodevelopmental brain disorders such as autism has been reported.

Taken together, PI3K isoforms seem to be much further developed and thus currently more promising as treatment target in autism than other phosphoinositide kinases, not only because of the availability of selective and potent inhibitors. Nonetheless, as knowledge about these other phosphoinositide kinases is increasing, so may the opportunities to explore them as therapeutic targets in autism spectrum disorders.

PTEN AND OTHER PHOSPHOINOSITIDE PHOSPHATASES IN AUTISM

Although there are many different phosphoinositide phosphatases that counteract the effects of phosphoinositide kinases, one stands out for its involvement in autism and cancer, the phosphatase and tensin homologue deleted on chromosome 10 (PTEN). Disorders associated with *PTEN* mutations are characterized by the frequent occurrence of tumors, developmental delay, autism and epilepsy (Conti et al. 2012; Elia et al. 2012; Leslie and Longy 2016). Moreover, the relevance of impaired PTEN function for increased seizure susceptibility and autistic-like phenotypes has been corroborated multiple times using mouse models, in which PTEN was deleted in select cell populations of the brain (Backman et al. 2001; Kwon et al. 2006; Kwon et al. 2001; Pun et al. 2012).

PTEN catalyzes the reaction from $PI(3,4,5)P_3$ to $PI(4,5)P_2$. In most cases, the *PTEN* mutations observed in human diseases lead to complete or partial inactivation of the phospholipid phosphatase activity (Leslie and Longy 2016). This causes subsequent accumulation of $PI(3,4,5)P_3$ and activation of downstream signaling cascades, which include mTOR (Domanskyi et al. 2011; Pun et al. 2012). Both, the direct effects of PTEN on $PI(4,5)P_2/PI(3,4,5)P_3$ ratios at the plasma membrane and the indirect effects on the activity of mTOR-dependent downstream signaling may contribute to alterations in synaptic and neuronal connectivity associated with *PTEN* mutations (Tilot et al. 2015). Defects in neuronal connectivity are believed to underlie autism spectrum disorders (Maximo et al. 2014), suggesting that strategies reversing these alterations mediated by impaired PTEN function may ameliorate the autistic phenotype.

MTOR inhibitors are already approved for the use in humans, and are thus attractive drug candidates for PTEN-associated brain disorders. A preclinical study in a PTEN deletion mouse model that exhibits autistic-like behavior has shown that the mTOR-selective inhibitor rapamycin reduces anxiety, improves social behavior and decreases seizure frequency (Zhou et al. 2009). Notably, in this study, rapamycin also prevented or reversed macrocephaly in mice treated pre- or post-symptomatically, respectively. This is in contrast to aberrant migration of PTEN-deficient neurons in the dentate gyrus, as shown in a recent study (Getz et al. 2016): Neuronal migration deficits were prevented by pre-symptomatic rapamycin treatment, but were not reversed in older mice when the deficits had already been established. These latter findings suggest that treatment with mTOR inhibitors in PTEN-associated autism spectrum disorders most likely will have to start very early before the manifestation of symptoms, reducing feasibility.

Another strategy to offset the loss of PTEN activity is to directly counteract the upstream defect by inhibiting the production of PI(3,4,5)P₃ using PI3K antagonists. Such a strategy is expected to not only normalize PI(3,4,5)P₃-dependent downstream signaling, but also correct direct effects of altered PI(3,4,5)P₃ levels on membrane protein and vesicle trafficking. However, a general inhibition of PI3K might not be desirable because of the expected profound side effects on basic cellular function, such as cell growth and proliferation. To overcome this problem, specific PI3K catalytic subunits could be targeted, which would leave the other PI3K subunits functional to preserve these basic cellular functions. Again, the autism field can take advantage of the advances that have been made by cancer scientists who face a similar problem. Accumulating research shows that many tumors caused by mutations in *PTEN* require p110 β activity, making p110 β a valid therapeutic target (Wee et al. 2008; Yuzugullu et al. 2015). If a similar relationship could be shown between *PTEN* mutations and p110 β in autism, preclinical studies testing p110 β -selective inhibitors in *PTEN* autism mouse models would be the next logical step towards developing targeted treatments for these subtypes of autism.

It is important to acknowledge that PTEN also has phosphoinositide-unrelated functions, for example as protein phosphatase (Myers et al. 1997) or, independent of its enzymatic activities, through protein-protein interactions (Tang and Eng 2006). So far, the relative contributions of the different functions of PTEN in *PTEN*-associated diseases are unknown. Early evidence suggested that PTEN's phospholipid phosphatase activity is crucial for its tumor-suppressing function (Myers et al. 1998), which was confirmed in later studies (Johnston and Raines 2015; Leslie and Longy 2016; Spinelli et al. 2015). Mutations that lead to only partial loss of function of PTEN enzymatic activity, however, seem to be preferentially associated with autism (Leslie and Longy 2016; Spinelli et al. 2015). In addition, some mutations observed in patients seem to affect the nuclear versus cytoplasmic localization of PTEN (Lobo et al. 2009), suggesting that the subcellular localization of PTEN is important for the development of *PTEN*-associated tumors and autism. The role of PTEN's protein phosphatase activity as a tumor suppressor or regulator of neuronal function is less clear. More work is needed to understand how different PTEN-mediated enzymatic or non-enzymatic activities affect the brain, and how their dysregulation may cause autistic-like defects.

To date, there is no strong connection between autism and any of the other phosphoinositide phosphatases. Notably, a few phosphoinositide phosphatases have been shown to play roles in neurodegenerative diseases. FIG4 (a.k.a. Sac3), for instance, which dephosphorylates PI(3,5)P₂ to PI(3)P, but also functions as an activator of the PI(3)P 5-kinase PYKfyve (Bharadwaj et al. 2016), is mutated in a severe form of Charcot-Marie-Tooth disease CMT4J (Chow et al. 2007). Mutations in FIG4 were also found in Yunis-Varon syndrome, a neurological disorder with skeletal defects and severe neuronal loss in the central nervous system (Nakajima et al. 2013), as well as in some forms of amyloid lateral sclerosis, although a causal link is not proven yet (Chow et al. 2009). Last but not least and as discussed above, FIG4 is part of the PIKfyve complex, which has been shown to be regulated by APP and thus may play a role in Alzheimer's disease (Currinn et al. 2015; Currinn and Wassmer 2016). Mutations in a different phosphoinositide phosphatase, myotubularin-related 2 (*MTMR2*), which dephosphorylates both PI(3,5)P₂ and PI(3)P at the

third hydroxyl group, lead to another form of Charcot-Marie-Tooth disease, CMT4B1 (Bolino et al. 2000).

The fact that four different phosphoinositide kinases and phosphatases regulating the biosynthesis or hydrolysis of PI(3,5)P₂ (PI3K, MTMR2, PIKfyve, FIG4, see Fig. 1) are implicated in neuronal disorders, strongly suggests an essential role for this specific phosphoinositide in neuronal function. Yet again, similarly as in the case of PIKfyve, a potential involvement of either of the two phosphatases, FIG4 or MTMR2, in autism or other neurodevelopmental disorders remains elusive.

ALTERED PHOSPHOINOSITIDE METABOLISM AS BIOMARKER IN AUTISM

Most of the above discussed phospholipid kinases and phosphatases are expressed in other organs and cells of the body, apart from the brain. Defects in their function or expression levels are thus expected to alter phospholipid contents in virtually all cells. Indeed, several studies have shown that in peripheral cells, such as lymphoblastoid cells from patients with autism and other neurodevelopmental disorders, the amount, production or activity-regulated turnover of phosphoinositide-phosphates is altered (Gross and Bassell 2012; Law et al. 2012; Poopal et al. 2016; Rivière et al. 2012). In lymphoblastoid cell lines from patients with FXS, for example, PI(3,4,5)P₃ production by p110 β is increased (Gross and Bassell 2012), but it is not known whether this change in p110 β activity leads to increased PI(3,4,5)P₃ levels in lymphoblastoid cells, as observed at synapses from *Fmr1* knockout mice (Gross et al. 2010). Similarly, in lymphoblastoid cells from a patient with autism, p110 δ -mediated PI(3,4,5)P₃ production is increased, but again it is unclear whether this leads to a net increase in PI(3,4,5)P₃ (Poopal et al. 2016). Small nucleotide polymorphisms in ERBB4, a known genetic risk variation in schizophrenia, lead to increased expression of p110 δ and impaired neuregulin 1-mediated PI(3,4,5)P₃ production in patient cells (Law et al. 2012). Lastly, in lymphoblastoid cells from a patient with Cowden syndrome, a disease associated with multiple cancers and autism, which is caused by mutations in the lipid phosphatase PTEN (Leslie and Longy 2016), PI(3,4,5)P₃ levels are increased (Rivière et al. 2012). These perturbations do not seem to lead to detectable symptoms related to lymphocyte dysfunctions in patients, suggesting that neurons and the brain are particularly sensitive to small changes in phosphoinositide content. However, the fact that defects are detectable in easy accessible, peripheral human cells opens up the possibility to use them as quantitative biomarkers to assess the efficiency of drug treatments to correct the underlying molecular defect. Efforts to develop large-scale quantitative assays to detect altered phosphoinositide levels or metabolism in patient cells are needed in order to identify patient cohorts with this shared defect.

CHALLENGES AND PROMISES FOR THERAPEUTIC APPLICATION

So far, there are no clinical trials in autism testing specific inhibitors of phospholipid kinases or phosphatases. As discussed, some critical basic and preclinical studies are needed to advance the exciting findings in patient cell lines and mouse models to the next step, the application in humans. One caveat is that, so far, there are very few basic research studies in animal models assessing if the observed alterations in phosphoinositide kinases or

phosphatases (mutations or altered expression) indeed lead to autistic phenotypes. Even the many studies analyzing various PTEN deletions in animal models do not truly mimic the mutations found in human patients. More recently, efforts have started to develop PTEN mouse models that more closely resemble the mutations in humans. For example, a new mouse model with a mutation altering the subcellular localization of PTEN was reported, which mimics some mutations in human patients with autism (Lobo et al. 2009). These mice show male-specific alterations in social motivation, suggesting that *PTEN* mutations observed in human patients may indeed cause autistic-like phenotypes (Tilot et al. 2014). Further basic research is needed to obtain a clearer picture of how phosphoinositide kinases and phosphatases influence brain development and function in order to assess their contribution to autism and other brain disorders. Once their relevance for the disease phenotype is established, the next step will be to develop biomarker assays to identify patient groups that have defects in phosphoinositide metabolism.

Nonetheless, the transition to the next phase in evaluating phosphoinositide kinases and phosphatases as treatment targets in autism will be accelerated tremendously because of the intriguing overlap between molecular pathways affected in autism and in cancer (Crawley et al. 2016). This offers not only the unique opportunity to repurpose drugs that were originally designed to treat cancers, but also enables the autism research community to learn from results of the clinical trials in cancer. The latter is particularly important, because the targeted PI3K pathways appear to display some plasticity, which leads to drug resistance in cancer cells when treated with high doses of certain inhibitors over longer periods of time (Ramos and Bentires-Alj 2015). Evidence of the underlying mechanisms in cancer (Masui et al. 2015) may educate the design of therapeutic strategies in autism.

It is noteworthy that except for *PTEN*, none of the recent large scale genomic studies (De Rubeis et al. 2014; Dong et al. 2014; Sanders et al. 2015) identified any of the phosphoinositide kinases, phosphatases or regulatory subunits as susceptibility genes. These genes also do not lie within the *bona fide* autism-associated chromosomal copy number variations identified in these studies, altogether suggesting that genetic defects in these enzymes are rather rare. However, so far, these genetic studies have mainly analyzed protein coding sequences, whereas mutations in noncoding sequences, which could affect for example expression levels, are scarce. Given that a few PI3K catalytic and regulatory subunits were shown to be upregulated in certain autism spectrum disorders and that pharmacological or genetic reduction of either of them rescued specific phenotypes, it is also conceivable that altered PI3K activity secondary to the primary, disease-causing mutation is nevertheless relevant for disease development.

Together with the above discussed recent advances suggesting a role of p110 β or p110 δ in regulation of PTEN signaling, a proven hotspot of mutations in autism, and the availability of subunit-selective drugs, there is thus a strong indication to further study phosphoinositide kinases in the etiology of autism and as potential treatment targets. Considering that in many cases the cause of defects in phosphoinositide kinases or phosphoinositide-dependent signaling in autism may not be a mutation in these genes, it will be essential to perform large scale functional studies in idiopathic autism to identify the cohort of patients who will

benefit from the expanding tool box of selective drugs targeting phosphoinositide metabolism.

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References

- Ahn JY, Rong R, Liu X, Ye K. PIKE/nuclear PI 3-kinase signaling mediates the antiapoptotic actions of NGF in the nucleus. *The EMBO Journal*. 2004; 23(20):3995–4006. [PubMed: 15385964]
- Ali K, Soond DR, Pineiro R, Hagemann T, Pearce W, Lim EL, Bouabe H, Scudamore CL, Hancox T, Maecker H, Friedman L, Turner M, Okkenhaug K, Vanhaesebroeck B. Inactivation of the PI3K p110 δ breaks regulatory T cell-mediated immune tolerance to cancer. *Nature*. 2014; 510(7505):407–411. [PubMed: 24919154]
- Ascano M Jr, Mukherjee N, Bandaru P, Miller JB, Nusbaum JD, Corcoran DL, Langlois C, Munschauer M, Dewell S, Hafner M, Williams Z, Ohler U, Tuschl T. FMRP targets distinct mRNA sequence elements to regulate protein expression. *Nature*. 2012; 492(7429):382–386. [PubMed: 23235829]
- Backman SA, Stambolic V, Suzuki A, Haight J, Elia A, Pretorius J, Tsao MS, Shannon P, Bolon B, Ivy GO, Mak TW. Deletion of Pten in mouse brain causes seizures, ataxia and defects in soma size resembling Lhermitte-Duclos disease. *Nat Genet*. 2001; 29(4):396–403. [PubMed: 11726926]
- Bakker SC, Hoogendoorn ML, Hendriks J, Verzijlbergen K, Caron S, Verduijn W, Selten JP, Pearson PL, Kahn RS, Sinke RJ. The PIP5K2A and RGS4 genes are differentially associated with deficit and non-deficit schizophrenia. *Genes Brain Behav*. 2007; 6(2):113–119. [PubMed: 17410640]
- Balla T. Phosphoinositides: Tiny Lipids With Giant Impact on Cell Regulation. *Physiological Reviews*. 2013; 93(3):1019–1137. [PubMed: 23899561]
- Berridge MJ. Inositol trisphosphate and calcium signalling mechanisms. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2009; 1793(6):933–940. [PubMed: 19010359]
- Bharadwaj R, Cunningham KM, Zhang K, Lloyd TE. FIG4 regulates lysosome membrane homeostasis independent of phosphatase function. *Human Molecular Genetics*. 2016; 25(4):681–692. [PubMed: 26662798]
- Bishop WR, Pachter JA, Pai JK. Regulation of phospholipid hydrolysis and second messenger formation by protein kinase C. *Adv Enzyme Regul*. 1992; 32:177–192. [PubMed: 1323204]
- Bojjireddy N, Guzman-Hernandez ML, Reinhard NR, Jovic M, Balla T. EFR3s are palmitoylated plasma membrane proteins that control responsiveness to G-protein-coupled receptors. *Journal of Cell Science*. 2015; 128(1):118–128. [PubMed: 25380825]
- Bolino A, Muglia M, Conforti FL, LeGuern E, Salih MA, Georgiou DM, Christodoulou K, Hausmanowa-Petrusewicz I, Mandich P, Schenone A, Gambardella A, Bono F, Quattrone A, Devoto M, Monaco AP. Charcot-Marie-Tooth type 4B is caused by mutations in the gene encoding myotubularin-related protein-2. *Nat Genet*. 2000; 25(1):17–19. [PubMed: 10802647]
- Brandau DT, Lund M, Cooley LD, Sanger WG, Butler MG. Autistic and dysmorphic features associated with a submicroscopic 2q33.3–q34 interstitial deletion detected by array comparative genomic hybridization. *American Journal of Medical Genetics Part A*. 2008; 146A(4):521–524. [PubMed: 18203195]
- Brown V, Jin P, Ceman S, Darnell JC, O'Donnell WT, Tenenbaum SA, Jin X, Feng Y, Wilkinson KD, Keene JD, Darnell RB, Warren ST. Microarray identification of FMRP-associated brain mRNAs and altered mRNA translational profiles in fragile X syndrome. *Cell*. 2001; 107(4):477–487. [PubMed: 11719188]

- Butler MG, Dasouki MJ, Zhou XP, Talebizadeh Z, Brown M, Takahashi TN, Miles JH, Wang CH, Stratton R, Pilarski R, Eng C. Subset of individuals with autism spectrum disorders and extreme macrocephaly associated with germline PTEN tumour suppressor gene mutations. *J Med Genet.* 2005; 42(4):318–321. [PubMed: 15805158]
- Chan CB, Liu X, Pradoldej S, Hao C, An J, Yepes M, Luo HR, Ye K. Phosphoinositide 3-kinase enhancer regulates neuronal dendritogenesis and survival in neocortex. *J Neurosci.* 2011; 31(22): 8083– 8092. [PubMed: 21632930]
- Chow CY, Landers JE, Bergren SK, Sapp PC, Grant AE, Jones JM, Everett L, Lenk GM, McKenna-Yasek DM, Weisman LS, Figlewicz D, Brown RH, Meisler MH. Deleterious Variants of FIG4, a Phosphoinositide Phosphatase, in Patients with ALS. *The American Journal of Human Genetics.* 2009; 84(1):85–88. [PubMed: 19118816]
- Chow CY, Zhang Y, Dowling JJ, Jin N, Adamska M, Shiga K, Szigeti K, Shy ME, Li J, Zhang X, Lupski JR, Weisman LS, Meisler MH. Mutation of FIG4 causes neurodegeneration in the pale tremor mouse and patients with CMT4J. *Nature.* 2007; 448(7149):68–72. [PubMed: 17572665]
- Conti S, Condo M, Posar A, Mari F, Resta N, Renieri A, Neri I, Patrizi A, Parmeggiani A. Phosphatase and Tensin Homolog (PTEN) Gene Mutations and Autism: Literature Review and a Case Report of a Patient With Cowden Syndrome, Autistic Disorder, and Epilepsy. *Journal of Child Neurology.* 2012; 27(3):392–397. [PubMed: 21960672]
- Costa C, Martin-Conte EL, Hirsch E. Phosphoinositide 3-kinase p110 γ in immunity. *IUBMB Life.* 2011; 63(9):707–713. [PubMed: 21800408]
- Courchesne E, Carper R, Akshoomoff N. Evidence of brain overgrowth in the first year of life in autism. *JAMA.* 2003; 290(3):337–344. [PubMed: 12865374]
- Crawley JN, Heyer W-D, LaSalle JM. Autism and Cancer Share Risk Genes, Pathways, and Drug Targets. *Trends in Genetics.* 2016; 32(3):139–146. [PubMed: 26830258]
- Cukier HN, Skaar DA, Rayner-Evans MY, Konidari I, Whitehead PL, Jaworski JM, Cuccaro ML, Pericak-Vance MA, Gilbert JR. Identification of chromosome 7 inversion breakpoints in an autistic family narrows candidate region for autism susceptibility. *Autism Research.* 2009; 2(5):258–266. [PubMed: 19877165]
- Curinn H, Guscott B, Balklava Z, Rothnie A, Wassmer T. APP controls the formation of PI(3,5)P₂ vesicles through its binding of the PIKfyve complex. *Cellular and Molecular Life Sciences.* 2015; 73(2):393–408. [PubMed: 26216398]
- Curinn H, Wassmer T. The amyloid precursor protein (APP) binds the PIKfyve complex and modulates its function. *Biochemical Society Transactions.* 2016; 44(1):185–190. [PubMed: 26862204]
- Cusco I, Medrano A, Gener B, Vilardell M, Gallastegui F, Villa O, Gonzalez E, Rodriguez-Santiago B, Vilella E, Del Campo M, Perez-Jurado LA. Autism-specific copy number variants further implicate the phosphatidylinositol signaling pathway and the glutamatergic synapse in the etiology of the disorder. *Hum Mol Genet.* 2009; 18(10):1795–1804. [PubMed: 19246517]
- Darnell J, Van Driesche S, Zhang C, Hung K, Mele A, Fraser C, Stone E, Chen C, Fak J, Chi S, Licatalosi D, Richter J, Darnell R. FMRP stalls ribosomal translocation on mRNAs linked to synaptic function and autism. *Cell.* 2011; 146:247– 261. [PubMed: 21784246]
- Davis PE, Peters JM, Krueger DA, Sahin M. Tuberous Sclerosis: A New Frontier in Targeted Treatment of Autism. *Neurotherapeutics.* 2015a; 12(3):572–583. [PubMed: 25986747]
- Davis WJ, Lehmann PZ, Li W. Nuclear PI3K signaling in cell growth and tumorigenesis. *Frontiers in Cell and Developmental Biology.* 2015b; 3:24. [PubMed: 25918701]
- De Rubeis S, Buxbaum JD. Genetics and genomics of autism spectrum disorder: embracing complexity. *Human Molecular Genetics.* 2015; 24(R1):R24–R31. [PubMed: 26188008]
- De Rubeis S, He X, Goldberg AP, Poultney CS, Samocha K, Ercument Cicek A, Kou Y, Liu L, Fromer M, Walker S, Singh T, Klei L, Kosmicki J, Fu S-C, Aleksic B, Biscaldi M, Bolton PF, Brownfeld JM, Cai J, Campbell NG, Carracedo A, Chahrour MH, Chiocchetti AG, Coon H, Crawford EL, Crooks L, Curran SR, Dawson G, Duketis E, Fernandez BA, Gallagher L, Geller E, Guter SJ, Sean Hill R, Ionita-Laza I, Jimenez Gonzalez P, Kilpinen H, Klauck SM, Kolevzon A, Lee I, Lei J, Lehtimäki T, Lin C-F, Ma'ayan A, Marshall CR, McInnes AL, Neale B, Owen MJ, Ozaki N, Parellada M, Parr JR, Purcell S, Puura K, Rajagopalan D, Rehnstrom K, Reichenberg A, Sabo A,

- Sachse M, Sanders SJ, Schafer C, Schulte-Ruther M, Skuse D, Stevens C, Szatmari P, Tammimies K, Valladares O, Voran A, Wang L-S, Weiss LA, Jeremy Willsey A, Yu TW, Yuen RKC, Cook EH, Freitag CM, Gill M, Hultman CM, Lehner T, Palotie A, Schellenberg GD, Sklar P, State MW, Sutcliffe JS, Walsh CA, Scherer SW, Zwick ME, Barrett JC, Cutler DJ, Roeder K, Devlin B, Daly MJ, Buxbaum JD. The DDDS Homozygosity Mapping Collaborative for A Consortium UK, The Autism Sequencing C. Synaptic, transcriptional and chromatin genes disrupted in autism. *Nature*. 2014; 515(7526):209–215. [PubMed: 25363760]
- Di Paolo G, De Camilli P. Phosphoinositides in cell regulation and membrane dynamics. *Nature*. 2006; 443(7112):651–657. [PubMed: 17035995]
- Di Paolo GD, Moskowitz HS, Gipson K, Wenk MR, Voronov S, Obayashi M, Flavell R, Fitzsimonds RM, Ryan TA, Camilli PD. Impaired PtdIns(4,5)P₂ synthesis in nerve terminals produces defects in synaptic vesicle trafficking. *Nature*. 2004; 431(7007):415–422. [PubMed: 15386003]
- Didichenko SA, Thelen M. Phosphatidylinositol 3-kinase C2a contains a nuclear localization sequence and associates with nuclear speckles. *Journal of Biological Chemistry*. 2001; 276(51):48135–42. [PubMed: 11606566]
- Divecha N, Banfi H, Irvine RF. The polyphosphoinositide cycle exists in the nuclei of Swiss 3T3 cells under the control of a receptor (for IGF-I) in the plasma membrane, and stimulation of the cycle increases nuclear diacylglycerol and apparently induces translocation of protein kinase C to the nucleus. *The EMBO Journal*. 1991; 10(11):3207–3214. [PubMed: 1655412]
- Domanskyi A, Geisler C, Vinnikov IA, Alter H, Schober A, Vogt MA, Gass P, Parlato R, Schutz G. Pten ablation in adult dopaminergic neurons is neuroprotective in Parkinson's disease models. *The FASEB Journal*. 2011; 25(9):2898–2910. [PubMed: 21593433]
- Dong S, Walker Michael F, Carriero Nicholas J, DiCola M, Willsey AJ, Ye Adam Y, Waqar Z, Gonzalez Luis E, Overton John D, Frahm S, Keaney John F III, Teran Nicole A, Dea J, Mandell Jeffrey D, Hus Bal V, Sullivan Catherine A, DiLullo Nicholas M, Khalil Rehab O, Gockley J, Yuksel Z, Sertel Sinem M, Ercan-Sencicek AG, Gupta Abha R, Mane Shrikant M, Sheldon M, Brooks Andrew I, Roeder K, Devlin B, State Matthew W, Wei L, Sanders Stephan J. De Novo Insertions and Deletions of Predominantly Paternal Origin Are Associated with Autism Spectrum Disorder. *Cell reports*. 2014; 9(1):16–23. [PubMed: 25284784]
- Downes CP, Gray A, Lucocq JM. Probing phosphoinositide functions in signaling and membrane trafficking. *Trends in Cell Biology*. 2005; 15(5):259–268. [PubMed: 15866030]
- Downward J. Mechanisms and consequences of activation of protein kinase B/Akt. *Current Opinion in Cell Biology*. 1998; 10(2):262–267. [PubMed: 9561851]
- Dyson JM, Fedele CG, Davies EM, Mitchell CA. Phosphoinositide phosphatases: Just as important as the kinases. *Sub-Cellular Biochemistry*. 2015; 58:215–279.
- Eickholt BJ, Ahmed AI, Davies M, Papakonstanti EA, Pearce W, Starkey ML, Bilancio A, Need AC, Smith AJH, Hall SM, Hamers FP, Giese KP, Bradbury EJ, Vanhaesebroeck B. Control of Axonal Growth and Regeneration of Sensory Neurons by the p110 δ PI 3-Kinase. *PLoS One*. 2007; 2(9):e869. [PubMed: 17846664]
- Elia M, Amato C, Bottitta M, Grillo L, Calabrese G, Esposito M, Carotenuto M. An atypical patient with Cowden syndrome and PTEN gene mutation presenting with cortical malformation and focal epilepsy. *Brain and Development*. 2012; 34(10):873–876. [PubMed: 22469695]
- Furman RR, Sharman JP, Coutre SE, Cheson BD, Pagel JM, Hillmen P, Barrientos JC, Zelenetz AD, Kipps TJ, Flinn I, Ghia P, Eradat H, Ervin T, Lamanna N, Coiffier B, Pettitt AR, Ma S, Stilgenbauer S, Cramer P, Aiello M, Johnson DM, Miller LL, Li D, Jahn TM, Dansey RD, Hallek M, O'Brien SM. Idelalisib and Rituximab in Relapsed Chronic Lymphocytic Leukemia. *The New England journal of medicine*. 2014; 370(11):997–1007. [PubMed: 24450857]
- Furst O, Mondou B, D'Avanzo N. Phosphoinositide Regulation of Inward Rectifier Potassium (Kir) Channels. *Frontiers in Physiology*. 2014;4. [PubMed: 24478717]
- Gericke, A., Leslie, NR., Losche, M., Ross, AH. PtdIns(4,5)P₂-Mediated Cell Signaling: Emerging Principles and PTEN as a Paradigm for Regulatory Mechanism. In: Capelluto, GSD., editor. *Lipid-mediated Protein Signaling*. Dordrecht: Springer Netherlands; 2013. p. 85-104.
- Getz SA, DeSpensa T Jr, Li M, Luikart BW. Rapamycin prevents, but does not reverse, aberrant migration in Pten knockout neurons. *Neurobiology of Disease*. 2016

- Gross C, Bassell GJ. Excess Protein Synthesis in FXS Patient Lymphoblastoid Cells Can Be Rescued with a p110beta-Selective Inhibitor. *Mol Med*. 2012; 18(1):336–345. [PubMed: 22207187]
- Gross C, Bassell GJ. Neuron-specific regulation of class I PI3K catalytic subunits and their dysfunction in brain disorders. *Frontiers in molecular neuroscience*. 2014; 7:12. [PubMed: 24592210]
- Gross C, Chang CW, Kelly SM, Bhattacharya A, McBride SM, Danielson SW, Jiang MQ, Chan CB, Ye K, Gibson JR, Klann E, Jongens TA, Moberg KH, Huber KM, Bassell GJ. Increased Expression of the PI3K Enhancer PIKE Mediates Deficits in Synaptic Plasticity and Behavior in Fragile X Syndrome. *Cell reports*. 2015a; 11(5):727–736. [PubMed: 25921541]
- Gross C, Hoffmann A, Bassell GJ, Berry-Kravis EM. Therapeutic Strategies in Fragile X Syndrome: From Bench to Bedside and Back. *Neurotherapeutics*. 2015b; 12(3):584–608. [PubMed: 25986746]
- Gross C, Nakamoto M, Yao X, Chan CB, Yim SY, Ye K, Warren ST, Bassell GJ. Excess phosphoinositide 3-kinase subunit synthesis and activity as a novel therapeutic target in fragile X syndrome. *J Neurosci*. 2010; 30(32):10624–10638. [PubMed: 20702695]
- Gross C, Raj N, Molinaro G, Allen AG, Whyte AJ, Gibson JR, Huber KM, Gourley SL, Bassell GJ. Selective Role of the Catalytic PI3K Subunit p110beta in Impaired Higher Order Cognition in Fragile X Syndrome. *Cell reports*. 2015c; 11(5):681–688. [PubMed: 25921527]
- Guillemet-Guibert J, Bjorklof K, Salpekar A, Gonella C, Ramadani F, Bilancio A, Meek S, Smith AJ, Okkenhaug K, Vanhaesebroeck B. The p110beta isoform of phosphoinositide 3-kinase signals downstream of G protein-coupled receptors and is functionally redundant with p110gamma. *Proc Natl Acad Sci U S A*. 2008; 105(24):8292–8297. [PubMed: 18544649]
- Gupta AR, Pirruccello M, Cheng F, Kang HJ, Fernandez TV, Baskin JM, Choi M, Liu L, Ercan-Sencicek AG, Murdoch JD, Klei L, Neale BM, Franjic D, Daly MJ, Lifton RP, De Camilli P, Zhao H, Šestan N, State MW. Rare deleterious mutations of the gene EFR3A in autism spectrum disorders. *Molecular Autism*. 2014; 5(1):1–14. [PubMed: 24410847]
- Hawkins PT, Anderson KE, Davidson K, Stephens LR. Signalling through Class I PI3Ks in mammalian cells. *Biochem Soc Trans*. 2006; 34(Pt 5):647–662. [PubMed: 17052169]
- He Z, Li Z, Shi Y, Tang W, Huang K, Ma G, Zhou J, Meng J, Li H, Feng G, He L. The PIP5K2A gene and schizophrenia in the Chinese population — A case-control study. *Schizophrenia Research*. 2007; 94(1–3):359–365. [PubMed: 17555944]
- Hevner RF. Brain overgrowth in disorders of RTK–PI3K–AKT signaling: A mosaic of malformations. *Seminars in Perinatology*. 2015; 39(1):36–43. [PubMed: 25432429]
- Jackson SP, Schoenwaelder SM, Goncalves I, Nesbitt WS, Yap CL, Wright CE, Kenche V, Anderson KE, Dopheide SM, Yuan Y, Sturgeon SA, Prabakaran H, Thompson PE, Smith GD, Shepherd PR, Daniele N, Kulkarni S, Abbott B, Saylik D, Jones C, Lu L, Giuliano S, Hughan SC, Angus JA, Robertson AD, Salem HH. PI 3-kinase p110beta: a new target for antithrombotic therapy. *Nat Med*. 2005; 11(5):507–514. [PubMed: 15834429]
- Jia S, Liu Z, Zhang S, Liu P, Zhang L, Lee SH, Zhang J, Signoretti S, Loda M, Roberts TM, Zhao JJ. Essential roles of PI(3)K-p110beta in cell growth, metabolism and tumorigenesis. *Nature*. 2008; 454(7205):776–779. [PubMed: 18594509]
- Johnston SB, Raines RT. Conformational Stability and Catalytic Activity of PTEN Variants Linked to Cancers and Autism Spectrum Disorders. *Biochemistry*. 2015; 54(7):1576–1582. [PubMed: 25647146]
- Jungerius BJ, Hoogendoorn MLC, Bakker SC, Van't Slot R, Bardoel AF, Ophoff RA, Wijmenga C, Kahn RS, Sinke RJ. An association screen of myelin-related genes implicates the chromosome 22q11 PIK4CA gene in schizophrenia. *Mol Psychiatry*. 2007; 13(11):1060–1068. [PubMed: 17893707]
- Kelleher RJ 3rd, Bear MF. The autistic neuron: troubled translation? *Cell*. 2008; 135(3):401–406. [PubMed: 18984149]
- Kerfant B-G, Rose RA, Sun H, Backx PH. Phosphoinositide 3-kinase γ Regulates Cardiac Contractility by Locally Controlling Cyclic Adenosine Monophosphate Levels. *Trends in Cardiovascular Medicine*. 2006; 16(7):250–256. [PubMed: 16980183]

- Ketel K, Krauss M, Nicot A-S, Puchkov D, Wieffer M, Muller R, Subramanian D, Schultz C, Laporte J, Haucke V. A phosphoinositide conversion mechanism for exit from endosomes. *Nature*. 2016; 529(7586):408–412. [PubMed: 26760201]
- Kim JI, Lee HR, Sim SE, Baek J, Yu NK, Choi JH, Ko HG, Lee YS, Park SW, Kwak C, Ahn SJ, Choi SY, Kim H, Kim KH, Backx PH, Bradley CA, Kim E, Jang DJ, Lee K, Kim SJ, Zhuo M, Collingridge GL, Kaang BK. PI3Kgamma is required for NMDA receptor-dependent long-term depression and behavioral flexibility. *Nat Neurosci*. 2011; 14(11):1447–1454. [PubMed: 22019731]
- Kim YC, Guan K-L. mTOR: a pharmacologic target for autophagy regulation. *The Journal of Clinical Investigation*. 2015; 125(1):25–32. [PubMed: 25654547]
- Kumari D, Bhattacharya A, Nadel J, Moulton K, Zeak NM, Glicksman A, Dobkin C, Brick DJ, Schwartz PH, Smith CB, Klann E, Usdin K. Identification of Fragile X Syndrome-Specific Molecular Markers in Human Fibroblasts: A Useful Model to Test the Efficacy of Therapeutic Drugs. *Human mutation*. 2014; 35(12):1485–1494. [PubMed: 25224527]
- Kwon CH, Luikart BW, Powell CM, Zhou J, Matheny SA, Zhang W, Li Y, Baker SJ, Parada LF. Pten regulates neuronal arborization and social interaction in mice. *Neuron*. 2006; 50(3):377–388. [PubMed: 16675393]
- Kwon CH, Zhu X, Zhang J, Knoop LL, Tharp R, Smeyne RJ, Eberhart CG, Burger PC, Baker SJ. Pten regulates neuronal soma size: a mouse model of Lhermitte-Duclos disease. *Nat Genet*. 2001; 29(4):404–411. [PubMed: 11726927]
- Laganowsky A, Reading E, Allison TM, Ulmschneider MB, Degiacomi MT, Baldwin AJ, Robinson CV. Membrane proteins bind lipids selectively to modulate their structure and function. *Nature*. 2014; 510(7503):172–175. [PubMed: 24899312]
- Laplante M, Sabatini DM. mTOR signaling in growth control and disease. *Cell*. 2012; 149(2):274–293. [PubMed: 22500797]
- Laplante M, Sabatini DM. Regulation of mTORC1 and its impact on gene expression at a glance. *Journal of Cell Science*. 2013; 126(8):1713–1719. [PubMed: 23641065]
- Law AJ, Wang Y, Sei Y, O'Donnell P, Piantadosi P, Papaleo F, Straub RE, Huang W, Thomas CJ, Vakkalanka R, Besterman AD, Lipska BK, Hyde TM, Harrison PJ, Kleinman JE, Weinberger DR. Neuregulin 1-ErbB4-PI3K signaling in schizophrenia and phosphoinositide 3-kinase-p110delta inhibition as a potential therapeutic strategy. *Proc Natl Acad Sci U S A*. 2012; 109(30):12165–12170. [PubMed: 22689948]
- Leslie NR, Longy M. Inherited PTEN mutations and the prediction of phenotype. *Seminars in Cell & Developmental Biology*. 2016; 52:30–38. [PubMed: 26827793]
- Lobo GP, Waite KA, Planchon SM, Romigh T, Nassif NT, Eng C. Germline and somatic cancer-associated mutations in the ATP-binding motifs of PTEN influence its subcellular localization and tumor suppressive function. *Human Molecular Genetics*. 2009; 18(15):2851–2862. [PubMed: 19457929]
- Lord C, Bishop SL. Recent advances in autism research as reflected in DSM-5 criteria for autism spectrum disorder. *Annual review of clinical psychology*. 2015; 11:53–70.
- Low PC, Manzanero S, Mohannak N, Narayana VK, Nguyen TH, Kvaskoff D, Brennan FH, Ruitenber MJ, Gelderblom M, Magnus T, Kim HA, Broughton BRS, Sobey CG, Vanhaesebroeck B, Stow JL, Arumugam TV, Meunier FA. PI3Kδ inhibition reduces TNF secretion and neuroinflammation in a mouse cerebral stroke model. *Nature communications*. 2014; 5:3450.
- Lukusa T, Devriendt K, Jaeken J, Fryns JP. Mild dysmorphic signs in two male sibs with partial trisomy 2q32.1-->q35 due to maternal ins(14;2) translocation. *Clinical dysmorphism*. 1999; 8(1):47–51. [PubMed: 10327251]
- Ma XM, Blenis J. Molecular mechanisms of mTOR-mediated translational control. *Nat Rev Mol Cell Biol*. 2009; 10(5):307–318. [PubMed: 19339977]
- Malik AR, Urbanska M, Macias M, Skalecka A, Jaworski J. Beyond control of protein translation: What we have learned about the non-canonical regulation and function of mammalian target of rapamycin (mTOR). *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*. 2013; 1834(7):1434–1448. [PubMed: 23277194]

- Manzoli FA, Capitani S, Mazzotti G, Barnabei O, Maraldi NM. Role of chromatin phospholipids on template availability and ultrastructure of isolated nuclei. *Adv Enzyme Regul.* 1982; 20:247–262. [PubMed: 6180606]
- Martini M, De Santis MC, Braccini L, Gulluni F, Hirsch E. PI3K/AKT signaling pathway and cancer: an updated review. *Annals of medicine.* 2014; 46(6):372–383. [PubMed: 24897931]
- Masui K, Tanaka K, Ikegami S, Villa GR, Yang H, Yong WH, Cloughesy TF, Yamagata K, Arai N, Cavenee WK, Mischel PS. Glucose-dependent acetylation of Rictor promotes targeted cancer therapy resistance. *Proceedings of the National Academy of Sciences.* 2015; 112(30):9406–9411.
- Maximo JO, Cadena EJ, Kana RK. The Implications of Brain Connectivity in the Neuropsychology of Autism. *Neuropsychology Review.* 2014; 24(1):16–31. [PubMed: 24496901]
- McCartney AJ, Zolov SN, Kauffman EJ, Zhang Y, Strunk BS, Weisman LS, Sutton MA. Activity-dependent PI(3,5)P₂ synthesis controls AMPA receptor trafficking during synaptic depression. *Proceedings of the National Academy of Sciences.* 2014; 111(45):E4896–E4905.
- Miyashiro KY, Beckel-Mitchener A, Purk TP, Becker KG, Barret T, Liu L, Carbonetto S, Weiler IJ, Greenough WT, Eberwine J. RNA cargoes associating with FMRP reveal deficits in cellular functioning in *Fmr1* null mice. *Neuron.* 2003; 37(3):417–431. [PubMed: 12575950]
- Myers MP, Pass I, Batty IH, Van der Kaay J, Stolarov JP, Hemmings BA, Wigler MH, Downes CP, Tonks NK. The lipid phosphatase activity of PTEN is critical for its tumor suppressor function. *Proceedings of the National Academy of Sciences.* 1998; 95(23):13513–13518.
- Myers MP, Stolarov JP, Eng C, Li J, Wang SI, Wigler MH, Parsons R, Tonks NK. P-TEN, the tumor suppressor from human chromosome 10q23, is a dual-specificity phosphatase. *Proceedings of the National Academy of Sciences.* 1997; 94(17):9052–9057.
- Nakajima J, Okamoto N, Shiraishi J, Nishimura G, Nakashima M, Tsurusaki Y, Saitsu H, Kawashima H, Matsumoto N, Miyake N. Novel FIG4 mutations in Yunis-Varon syndrome. *Journal of human genetics.* 2013; 58(12):822–824. [PubMed: 24088667]
- Nakatsu F, Baskin JM, Chung J, Tanner LB, Shui G, Lee SY, Pirruccello M, Hao M, Ingolia NT, Wenk MR, De Camilli P. PtdIns4P synthesis by PI4KIII α at the plasma membrane and its impact on plasma membrane identity. *The Journal of Cell Biology.* 2012; 199(6):1003–1016. [PubMed: 23229899]
- Okada M, Jang S-W, Ye K. Akt phosphorylation and nuclear phosphoinositide association mediate mRNA export and cell proliferation activities by ALY. *Proceedings of the National Academy of Sciences.* 2008; 105(25):8649–8654.
- Papakonstanti EA, Ridley AJ, Vanhaesebroeck B. The p110 δ isoform of PI 3-kinase negatively controls RhoA and PTEN. *The EMBO Journal.* 2007; 26(13):3050–3061. [PubMed: 17581634]
- Papaleo F, Yang F, Paterson C, Palumbo S, Carr GV, Wang Y, Floyd K, Huang W, Thomas CJ, Chen J, Weinberger DR, Law AJ. Behavioral, Neurophysiological, and Synaptic Impairment in a Transgenic Neuregulin1 (NRG1-IV) Murine Schizophrenia Model. *J Neurosci.* 2016; 36(17):4859–4875. [PubMed: 27122041]
- Pinto D, Delaby E, Merico D, Barbosa M, Merikangas A, Klei L, Thiruvahindrapuram B, Xu X, Ziman R, Wang Z, Vorstman Jacob AS, Thompson A, Regan R, Pilorge M, Pellecchia G, Pagnamenta Alistair T, Oliveira B, Marshall Christian R, Magalhaes Tiago R, Lowe Jennifer K, Howe Jennifer L, Griswold Anthony J, Gilbert J, Duketis E, Dombroski Beth A, De Jonge Maretha V, Cuccaro M, Crawford Emily L, Correia Catarina T, Conroy J, Conceicao Ines C, Chiacchetti Andreas G, Casey Jillian P, Cai G, Cabrol C, Bolshakova N, Bacchelli E, Anney R, Gallinger S, Cotterchio M, Casey G, Zwaigenbaum L, Wittemeyer K, Wing K, Wallace S, van Engeland H, Tryfon A, Thomson S, Soorya L, Roge B, Roberts W, Poustka F, Moug S, Minshew N, McInnes LA, McGrew Susan G, Lord C, Leboyer M, Le Couteur Ann S, Kolevzon A, Jimenez Gonzalez P, Jacob S, Holt R, Guter S, Green J, Green A, Gillberg C, Fernandez BA, Duque F, Delorme R, Dawson G, Chaste P, Cate C, Brennan S, Bourgeron T, Bolton Patrick F, Bolte S, Bernier R, Baird G, Bailey Anthony J, Anagnostou E, Almeida J, Wijsman Ellen M, Vieland Veronica J, Vicente Astrid M, Schellenberg Gerard D, Pericak-Vance M, Paterson Andrew D, Parr Jeremy R, Oliveira G, Nurnberger John I, Monaco Anthony P, Maestrini E, Klauck Sabine M, Hakonarson H, Haines Jonathan L, Geschwind Daniel H, Freitag Christine M, Folstein Susan E, Ennis S, Coon H, Battaglia A, Szatmari P, Sutcliffe James S, Hallmayer J, Gill M, Cook Edwin H, Buxbaum Joseph D, Devlin B, Gallagher L, Betancur C, Scherer Stephen W. Convergence of Genes and Cellular

- Pathways Dysregulated in Autism Spectrum Disorders. *The American Journal of Human Genetics*. 2014; 94(5):677–694. [PubMed: 24768552]
- Poopal AC, Schroeder LM, Horn PS, Bassell GJ, Gross C. Increased expression of the PI3K catalytic subunit p110 δ underlies elevated S6 phosphorylation and protein synthesis in an individual with autism from a multiplex family. *Molecular Autism*. 2016; 7:3. [PubMed: 26770665]
- Pun RY, Rolle IJ, Lasarge CL, Hosford BE, Rosen JM, Uhl JD, Schmeltzer SN, Faulkner C, Bronson SL, Murphy BL, Richards DA, Holland KD, Danzer SC. Excessive activation of mTOR in postnatally generated granule cells is sufficient to cause epilepsy. *Neuron*. 2012; 75(6):1022–1034. [PubMed: 22998871]
- Ramos P, Bentires-Alj M. Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. *Oncogene*. 2015; 34(28):3617–3626. [PubMed: 25263438]
- Resnick AC, Snowman AM, Kang BN, Hurt KJ, Snyder SH, Saiardi A. Inositol polyphosphate multikinase is a nuclear PI3-kinase with transcriptional regulatory activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2005; 102(36):12783–12788. [PubMed: 16123124]
- Richter JD, Bassell GJ, Klann E. Dysregulation and restoration of translational homeostasis in fragile X syndrome. *Nature reviews Neuroscience*. 2015; 16(10):595–605. [PubMed: 26350240]
- Riviere J-B, Mirzaa GM, O’Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzenuber JA, Gripp KW, Nikkel SM, Worthylake T, Sullivan CT, Ward TR, Butler HE, Kramer NA, Albrecht B, Armour CM, Armstrong L, Caluseriu O, Cytrynbaum C, Drolet BA, Innes AM, Lauzon JL, Lin AE, Mancini GMS, Meschino WS, Reggin JD, Sagar AK, Lerman-Sagie T, Uyanik G, Weksberg R, Zirn B, Beaulieu CL, Majewski J, Bulman DE, O’Driscoll M, Shendure J, Graham JM, Boycott KM, Dobyns WB. Consortium FC. De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. *Nature genetics*. 2012; 44(8):934–940. [PubMed: 22729224]
- Ronesi JA, Collins KA, Hays SA, Tsai NP, Guo W, Birnbaum SG, Hu JH, Worley PF, Gibson JR, Huber KM. Disrupted Homer scaffolds mediate abnormal mGluR5 function in a mouse model of fragile X syndrome. *Nat Neurosci*. 2012; 15(3):431–440. S431. [PubMed: 22267161]
- Ronesi JA, Huber KM. Homer interactions are necessary for metabotropic glutamate receptor-induced long-term depression and translational activation. *J Neurosci*. 2008; 28(2):543–547. [PubMed: 18184796]
- Rong R, Ahn JY, Huang H, Nagata E, Kalman D, Kapp JA, Tu J, Worley PF, Snyder SH, Ye K. PI3 kinase enhancer-Homer complex couples mGluRI to PI3 kinase, preventing neuronal apoptosis. *Nat Neurosci*. 2003; 6(11):1153–1161. [PubMed: 14528310]
- Ruderfer DM, Fanous AH, Ripke S, McQuillin A, Amdur RL, Gejman PV, O’Donovan MC, Andreassen OA, Djurovic S, Hultman CM, Kelsoe JR, Jamain S, Landen M, Leboyer M, Nimgaonkar V, Nurnberger J, Smoller JW, Craddock N, Corvin A, Sullivan PF, Holmans P, Sklar P, Kendler KS. Polygenic dissection of diagnosis and clinical dimensions of bipolar disorder and schizophrenia. *Mol Psychiatry*. 2014; 19(9):1017–1024. [PubMed: 24280982]
- Sanders Stephan J, He X, Willsey AJ, Ercan-Sencicek AG, Samocha Kaitlin E, Cicek AE, Murtha Michael T, Bal Vanessa H, Bishop Somer L, Dong S, Goldberg Arthur P, Jinlu C, Keaney John F III, Klei L, Mandell Jeffrey D, Moreno-De-Luca D, Poultney Christopher S, Robinson Elise B, Smith L, Solli-Nowlan T, Su Mack Y, Teran Nicole A, Walker Michael F, Werling Donna M, Beaudet Arthur L, Cantor Rita M, Fombonne E, Geschwind Daniel H, Grice Dorothy E, Lord C, Lowe Jennifer K, Mane Shrikant M, Martin Donna M, Morrow Eric M, Talkowski Michael E, Sutcliffe James S, Walsh Christopher A, Yu Timothy W, Ledbetter David H, Martin Christa L, Cook Edwin H, Buxbaum Joseph D, Daly Mark J, Devlin B, Roeder K, State Matthew W. Insights into Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci. *Neuron*. 2015; 87(6):1215–1233. [PubMed: 26402605]
- Santoro MR, Bray SM, Warren ST. Molecular mechanisms of fragile X syndrome: a twenty-year perspective. *Annual review of pathology*. 2012; 7:219–245.
- Saxena A, Sampson JR. Epilepsy in Tuberous Sclerosis: Phenotypes, Mechanisms, and Treatments. *Seminars in neurology*. 2015; 35(03):269–276. [PubMed: 26060906]

- Serajee F, Nabi R, Zhong H, Mahbulul H. Association of INPP1, PIK3CG, and TSC2 gene variants with autistic disorder: implications for phosphatidylinositol signalling in autism. *Journal of Medical Genetics*. 2003; 40(11):e119–e119. [PubMed: 14627686]
- Sharma A, Hoeffler CA, Takayasu Y, Miyawaki T, McBride SM, Klann E, Zukin RS. Dysregulation of mTOR Signaling in Fragile X Syndrome. *J Neurosci*. 2010; 30(2):694–702. [PubMed: 20071534]
- Silio V, Redondo-Munoz J, Carrera AC. Phosphoinositide 3-kinase β regulates chromosome segregation in mitosis. *Molecular Biology of the Cell*. 2012; 23(23):4526–4542. [PubMed: 23051731]
- Spinelli L, Black FM, Berg JN, Eickholt BJ, Leslie NR. Functionally distinct groups of inherited PTEN mutations in autism and tumour syndromes. *Journal of Medical Genetics*. 2015; 52(2): 128–134. [PubMed: 25527629]
- Suh B-C, Hille B. Regulation of ion channels by phosphatidylinositol 4,5-bisphosphate. *Current Opinion in Neurobiology*. 2005; 15(3):370–378. [PubMed: 15922587]
- Swillen A, McDonald-McGinn D. Developmental trajectories in 22q11.2 deletion syndrome. *American Journal of Medical Genetics Part C: Seminars in Medical Genetics*. 2015; 169(2):172–181.
- Tang Y, Eng C. PTEN Autoregulates Its Expression by Stabilization of p53 in a Phosphatase-Independent Manner. *Cancer Research*. 2006; 66(2):736–742. [PubMed: 16424003]
- Tilot AK, Frazier TW, Eng C. Balancing Proliferation and Connectivity in PTEN-associated Autism Spectrum Disorder. *Neurotherapeutics*. 2015; 12(3):609–619. [PubMed: 25916396]
- Tilot AK, Gaugler MK, Yu Q, Romigh T, Yu W, Miller RH, Frazier TW, Eng C. Germline disruption of Pten localization causes enhanced sex-dependent social motivation and increased glial production. *Human Molecular Genetics*. 2014; 23(12):3212–3227. [PubMed: 24470394]
- Trikalinos TA, Karvouni A, Zintzaras E, Ylisaukko-oja T, Peltonen L, Jarvela I, Ioannidis JPA. A heterogeneity-based genome search meta-analysis for autism-spectrum disorders. *Mol Psychiatry*. 2006; 11(1):29–36. [PubMed: 16189507]
- Turner Tychele N, Hormozdiari F, Duyzend Michael H, McClymont Sarah A, Hook Paul W, Iossifov I, Raja A, Baker C, Hoekzema K, Stessman Holly A, Zody Michael C, Nelson Bradley J, Huddleston J, Sandstrom R, Smith Joshua D, Hanna D, Swanson James M, Faustman Elaine M, Bamshad Michael J, Stamatoyannopoulos J, Nickerson Deborah A, McCallion Andrew S, Darnell R, Eichler Evan E. Genome Sequencing of Autism-Affected Families Reveals Disruption of Putative Noncoding Regulatory DNA. *The American Journal of Human Genetics*. 2016; 98(1): 58–74. [PubMed: 26749308]
- Unoki T, Matsuda S, Kakegawa W, Van Ngo Thai B, Kohda K, Suzuki A, Funakoshi Y, Hasegawa H, Yuzaki M, Kanaho Y. NMDA Receptor-Mediated PIP5K Activation to Produce PI(4,5)P₂ Is Essential for AMPA Receptor Endocytosis during LTD. *Neuron*. 2012; 73(1):135–148. [PubMed: 22243752]
- Usui D, Shimada S, Shimojima K, Sugawara M, Kawasaki H, Shigematu H, Takahashi Y, Inoue Y, Imai K, Yamamoto T. Interstitial Duplication of 2q32.1–q33.3 in a Patient With Epilepsy, Developmental Delay, and Autistic Behavior. *American Journal of Medical Genetics Part A*. 2013; 161(5):1078–1084.
- Vanhaesebroeck B, Guillemet-Guibert J, Graupera M, Bilanges B. The emerging mechanisms of isoform-specific PI3K signalling. *Nat Rev Mol Cell Biol*. 2010; 11(5):329–341. [PubMed: 20379207]
- Vanhaesebroeck B, Higashi K, Raven C, Welham M, Anderson S, Brennan P, Ward SG, Waterfield MD. Autophosphorylation of p110 δ phosphoinositide 3-kinase: a new paradigm for the regulation of lipid kinases in vitro and in vivo. *The EMBO Journal*. 1999; 18(5):1292–1302. [PubMed: 10064595]
- Vanhaesebroeck B, Whitehead MA, Pineiro R. Molecules in medicine mini-review: isoforms of PI3K in biology and disease. *Journal of molecular medicine (Berlin, Germany)*. 2016; 94(1):5–11.
- Viaud J, Mansour R, Antkowiak A, Mujalli A, Valet C, Chicanne G, Xuereb J-M, Terrisse A-D, Severin S, Gratacap M-P, Gaits-Iacovoni F, Payrastra B. Phosphoinositides: Important lipids in the coordination of cell dynamics. *Biochimie*. 2015

- Wee S, Wiederschain D, Maira S-M, Loo A, Miller C, deBeaumont R, Stegmeier F, Yao Y-M, Lengauer C. PTEN-deficient cancers depend on PIK3CB. *Proceedings of the National Academy of Sciences*. 2008; 105(35):13057–13062.
- Wen, PJ., Osborne, SL., Meunier, FA. Phosphoinositides in Neuroexocytosis and Neuronal Diseases. In: Falasca, M., editor. *Phosphoinositides and Disease*. Dordrecht: Springer Netherlands; 2012. p. 87-98.
- Yang Q, Modi P, Newcomb T, Queva C, Gandhi V. Idelalisib: First-in-Class PI3K Delta Inhibitor for the Treatment of Chronic Lymphocytic Leukemia, Small Lymphocytic Leukemia, and Follicular Lymphoma. *Clinical Cancer Research*. 2015; 21(7):1537–1542. [PubMed: 25670221]
- Yuzugullu H, Baitsch L, Von T, Steiner A, Tong H, Ni J, Clayton LK, Bronson R, Roberts TM, Gritsman K, Zhao JJ. A PI3K p110[beta]-Rac signalling loop mediates Pten-loss-induced perturbation of haematopoiesis and leukaemogenesis. *Nature communications*. 2015; 6:8501.
- Zhou J, Blundell J, Ogawa S, Kwon CH, Zhang W, Sinton C, Powell CM, Parada LF. Pharmacological inhibition of mTORC1 suppresses anatomical, cellular, and behavioral abnormalities in neural-specific Pten knock-out mice. *J Neurosci*. 2009; 29(6):1773–1783. [PubMed: 19211884]

SIGNIFICANCE STATEMENT

Highly regulated and dynamic phosphoinositide metabolism is essential for cellular function. Not surprisingly, mutations and defects in the enzymes involved in this process, phosphoinositide kinases and phosphatases, lead to human disease. In the past, the focus for therapy development targeting these defects has been on cancer. More recently, evidence is emerging that phosphoinositide kinases and phosphatases are also affected in certain forms of autism, opening up opportunities for disease-targeted treatment. This review discusses possibilities and challenges arising from these findings that warrant further studies to test phosphoinositide kinase-specific drugs for the treatment of autism spectrum disorders with defective phosphoinositide metabolism.

Fig. 1. Overview of defects in phosphoinositide metabolism in autism

Shown are phosphatidylinositol and its seven phosphorylated derivatives, as well as phosphoinositide kinases and phosphatases that have been shown or suggested to be dysregulated in autism and related diseases. Indicated in the insets are the disease, the affected subunit (if applicable), and the type of study (genetic or functional). Bold font indicates strong experimental support for a role of the respective enzyme in the disease. MTMR2, FIG4 and PIKfyve, implicated in neurodegenerative diseases such as Charcot Marie-Tooth disease and Alzheimer's disease, are shown as examples for autism-unrelated neurological disorders. Note that phosphorylation and dephosphorylation between PtdIns(4)P and PtdIns(3,4)P₂ is not shown, although enzymes catalyzing the conversion between these phosphoinositides exist.