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PTEN mutations in autism spectrum disorder and congenital hydrocephalus: developmental pleiotropy and therapeutic targets

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Abstract

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Declaration of interests

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Search strategy and selection criteria.

We searched PubMed for articles in all year ranges with multiple combinations of search terms including, “autism spectrum disorder”, “congenital hydrocephalus”, “ventriculomegaly”, “PTEN”, “mTORC1”. There were no language exclusions and articles chosen were based on relevance to topics covered in this review.

The lack of effective treatments for autism spectrum disorder (ASD) and congenital hydrocephalus (CH) reflects the limited understanding of the biology underlying these common neurodevelopmental disorders. Although ASD and CH have been extensively studied as independent entities, recent human genomic and pre-clinical animal studies have uncovered shared molecular pathophysiology. Here, we review and discuss phenotypic, genomic, and molecular similarities between ASD and CH, and identify the PTEN-PI3K-mTOR (phosphatase and tensin homolog-phosphoinositide 3-kinase-mammalian target of rapamycin) pathway as a common underlying mechanism that holds diagnostic, prognostic, and therapeutic promise for individuals with ASD and CH.

Keywords

neurodevelopment disorders; ventriculomegaly; macrocephaly; mTOR; rapamycin

Common biological pathways in autism spectrum disorders and congenital hydrocephalus

Neurodevelopmental disorders (NDDs) include a wide range of neurological, physiological, and genetic conditions that impair normal brain development and function. While the clinical heterogeneity of NDDs reflects their multifactorial pathogenesis, the extensive symptomatic and genetic overlap among NDDs [1] suggests common underlying biological pathways. Within the intricate landscape of NDDs, autism spectrum disorders (ASDs) are a significant but poorly understood subgroup with a steadily increasing prevalence. ASDs affect one in 54 children in the United States, with a male-to-female ratio of 4.3:1 [2]. ASD is characterized by communication difficulties, restricted interests, and repetitive behaviors that often manifest before age 3 [3]. Individuals with ASD typically display deficits in socioemotional reciprocity, non-verbal communication, and social adaptation. Characteristic behaviors include motor stereotypies, repetitive speech (e.g. echolalia), and rigid adherence to behavioral, social, and environmental routine. ASD is also frequently associated with altered sensitivity to sensory stimuli, sleep dysfunction, gastrointestinal abnormalities, and motor deficits [3]. These and other social and behavioral deficits among affected individuals can vary widely in severity.

Enlargement of the brain's cerebrospinal fluid (CSF)-filled ventricles (**ventriculomegaly**, see Glossary) is frequently observed in individuals with ASD [4–6], and CSF-related structural brain abnormalities such as congenital hydrocephalus (CH) are associated with ASD [7–10]. Approximately 20% of children with CH are also diagnosed with ASD – a rate 10-fold higher than the 1.9% rate in the general population [2] [11, 12]. CH has been classically attributed to decreased CSF clearance and resultant increased intracranial pressure [13]. Based on this paradigm, the mainstay of CH treatment has been neurosurgical CSF diversion [14], with high rates of complication, morbidity, and treatment failure [14, 15]. Interestingly, some CH cases, including those accompanied by comorbidities such as ASD or other NDDs [16], can be characterized by ventriculomegaly with normal or low intracerebral pressure, a critical distinction with implications for treatment [17, 18]. These observations suggest that impaired CSF dynamics alone might not drive development of

ventriculomegaly in these patients. Moreover, the ventriculomegaly, **macrocephaly** [19, 20], and other CSF-related structural abnormalities frequently accompanying ASD suggest a degree of shared etiology between CH and ASD.

The mammalian target of rapamycin (mTOR) pathway (Figure 1) is an intracellular signaling pathway that regulates protein synthesis, cell growth, and metabolism. Emerging evidence suggests that mTOR signaling may play a central mechanistic role during neurodevelopment in both ASDs and CH [21, 22]. Although the mTOR pathway has been previously implicated in ASDs [23], recent genetic evidence supports its additional critical function in sporadic, neurosurgically treated CH [24]. Through its two major complexes, mTORC1 and mTORC2, mTOR controls cellular metabolism, proliferation, differentiation and survival [25]. *Phosphatase and Tensin Homolog* (*PTEN*) encodes a lipid and protein phosphatase that negatively regulates mTOR through PIP3 hydrolysis, preventing AKT-mediated activation of mTORC1 [25]. Importantly, ***de novo*** loss-of-function mutations in *PTEN* are strongly associated with both ASDs and CH [24, 26]. This intriguing link between seemingly disparate NDDs suggests the hypothesis that ASD – a disorder defined mainly by behavioral manifestations – and CH – a structural brain abnormality classically attributed to impaired CSF “plumbing” – may share, at least in part, a common targetable molecular origin.

The current lack of pharmacological interventions targeting core features of ASD or CH reflects, in part, a limited understanding of the biology underlying these disorders, traditionally studied as independent entities. However, overlapping neurodevelopmental pathways and shared pathogenic elements of ASD and CH are beginning to emerge. Here, we discuss the phenotypic, genetic, and molecular similarities between ASD and CH, and identify the PTEN-PI3K (phosphoinositide 3-kinase)-mTOR pathway as a common underlying mechanism for subsets of patients with these disorders.

Phenotypic similarities between ASD and CH

Although ASD currently lacks **pathognomonic** neuroradiographic findings, the prevalence of structural brain abnormalities is higher in ASD compared to healthy controls, typically developing children, and children with developmental delay [27, 28]. Abnormal head sizes often described in ASD may be associated with specific genetic etiologies. Macrocephaly occurs in nearly 20% of individuals with ASD [20], and the altered ASD brain growth trajectory is characterized by overgrowth in the first few years of life, followed by a period of slowed growth [29–31]. The severity of brain structural abnormalities in children also correlates with likelihood of an ASD diagnosis. Computed tomography imaging demonstrates major structural brain abnormalities in 64% of hydrocephalic patients with ASD and 28% of those without ASD [12]. Moreover, increased extra-axial CSF spaces in neonates detected by magnetic resonance imaging (MRI) can predict future ASD diagnosis [7, 8, 10]. Such MRI detection of extra-axial CSF collections (or “benign external hydrocephalus”) analyzed by a machine learning algorithm exhibits 84% sensitivity and 60% specificity in ASD diagnosis, suggesting its use as a biomarker for a subtype of ASD [9] and reinforcing the potential of MRI as part of the diagnostic approach to ASD [32].

Gene discovery and integrative genomics implicate common pathways in ASD and CH

The complex, highly heritable but polygenic character of NDDs [33–35] has contributed to the difficulties historically encountered in their investigation. However, recent advances in human genetics are revealing new insights into the pathophysiology of these disorders. Genomic technologies such as genome-wide association studies, whole exome sequencing (WES), and whole genome sequencing have highlighted numerous disease-risk loci and afforded novel insights into ASD pathophysiology [26, 36, 37]. Recent WES analyses of families with affected offspring and unaffected parents (termed “case-parent trio design”) and of families with multiple affected individuals have similarly identified *de novo* and rare inherited mutations that impart large effects on CH risk [24, 38].

ASD is highly heritable, with concordance rates higher in monozygotic (50–90%) than in dizygotic twins (up to 30%), and sibling recurrence rates up to 18.7% [39–45]. The first ASD risk genes were identified in rare monogenic disorders such as Fragile X syndrome (*FMR1*), tuberous sclerosis complex (*TSC1*, *TSC2*), neurofibromatosis type 1 (NF1), and *PTEN*-associated macrocephaly [46] [47], syndromes directly or indirectly associated with dysregulated mTOR signaling (reviewed in [48]). While most inherited ASD risk is associated with common variants [49–51], recent progress in ASD gene discovery reflects identification of rare ASD risk variants. Large-scale WES and genotyping studies have revealed significant association of rare *de novo* variants, gene-disrupting single-nucleotide variants, and copy number variants with ASD risk [52–54, 58], leading to the identification of growing numbers of ‘high-confidence’ ASD risk genes [55, 56]. A recent analysis [26] examined rare *de novo* and case-control variants from almost 12,000 affected individuals, and identified 102 risk genes (FDR≤0.1) [26]. The negative regulator of mTOR signaling, *PTEN*, was among the top risk genes identified both by this study [26] and other WES studies [56–58].

Discovery of “high confidence” risk genes has implicated convergent pathways contributing to ASD biology, including the regulation of gene expression and neuronal communication [26, 55, 56]. Integrative genomic approaches leveraging coexpression networks to construct a spatiotemporal map of ASD risk gene expression have identified mid-fetal glutamatergic projection neurons as a point of convergence with potential disease implications [59, 60]. Moreover, ASD genes are most strongly enriched in early excitatory neurons and striatal interneurons, implicating maturing and mature neurons of both excitatory and inhibitory lineages [26]. These studies highlight the power of **integrative genomics** to elucidate the spatiotemporal dynamics of ASD risk genes and reveal central roles for these genes in early brain development and excitatory-inhibitory signaling.

WES has also recently illuminated the pathogenic mechanisms underlying CH. Although nearly 40% of CH cases are predicted to have genetic etiology [16], mutations in CH genes identified prior to availability of WES account for fewer than 5% of primary cases [61]. The multiple biological processes affected by these *bona fide* genes initially failed to suggest a unified paradigm of CH pathogenesis [62]. However, recent WES of the largest cohort of sporadic CH patients to date have revealed additional genes with significant burden of

rare damaging mutations [24]. Remarkably, all of these genes are implicated in regulation of neural stem cells (NSCs), suggesting many CH cases may arise from impaired prenatal neurogliogenesis [24]. Perhaps unexpectedly, CH and ASD exhibited significant overlap of genes with rare, damaging *de novo* mutations, including *PTEN* and *MTOR* [24].

As with ASD genetic studies, CH-associated variants were further studied through an integrative genomics approach, using a bulk RNA-seq transcriptome characterized by WGCNA modules [63] and a single-cell [single-cell RNA sequencing (scRNA-seq)] transcriptome characterized by cell types [64] of the midgestational human brain. Of note, CH risk genes converged in a module implicated in ASD and developmental disorders, yet these genes were also found in cell types critical to fetal neurogenesis at earlier stages of differentiation than the postmitotic stages typically thought to be pertinent to autism and other developmental disorders [24]. These findings suggest that CH and ASD may arise from intersecting neurodevelopmental pathways, with disruption of early NSCs effecting a more severe structural phenotype as seen in CH. Taken together, WES and associated integrative genomics studies of ASD and CH reveal a surprising degree of convergence, and highlight PTEN-PI3K-mTOR signaling as one potentially shared pathway.

PTEN mutations and ASD

Prior to its association with ASD, *PTEN* was first identified as a tumor suppressor gene based on mutations present in cancer specimens [65, 66] and inherited cancer syndromes [67–69]. *PTEN* mutations cause Cowden syndrome, an autosomal dominant disorder characterized by macrocephaly, benign **hamartomas**, and an increased risk of breast, thyroid, and other cancers in adults. *PTEN* mutations also cause Bannayan-Riley-Ruvalcaba syndrome, a childhood-onset autosomal dominant disorder similarly characterized by macrocephaly and hamartomas, along with developmental delay, **lipomas**, and penile freckling [67, 68]. These disorders are collectively considered PTEN hamartoma tumor syndromes (PHTSs), reflecting their shared genetic etiology and similar clinical features [69]. Interestingly, while recent WES studies have confirmed *PTEN* as a high-confidence ASD risk gene [26], the association of *PTEN* with ASD was first established by earlier targeted sequencing of the *PTEN* gene in individuals with ASD and macrocephaly [70–73]. It was hypothesized [70] that *PTEN* mutations might be associated with co-occurrence of ASD and macrocephaly, based on reports of ASD-like behaviors in individuals with PHTS [74–76] in whom macrocephaly is common. Multiple studies have since documented *PTEN* mutations in at least 5–10% (up to 22%) of individuals with ASD accompanied by macrocephaly [70–73, 77–79].

Macrocephaly is the strongest clinical feature associated with *PTEN* mutations, and individuals with ASD and *PTEN* mutations typically have “extreme” macrocephaly (>2.5–3 standard deviations above the mean) [70, 73, 77, 78]. For this reason, *PTEN* mutation screening in children with ASD and severe macrocephaly (>2.5 standard deviations) is recommended as part of clinical genetic diagnostic evaluation [80]. While brain imaging in children with ASD, macrocephaly, and *PTEN* mutations may not show major findings, abnormalities on brain MRI have included ventriculomegaly, polymicrogyria, dilation of perivascular spaces, and periventricular and other white matter abnormalities [70, 72–74, 79,

81]. *PTEN* mutations have also been associated with seizures [82]. In addition, a WES study of 21 children with ASD, developmental delay, and macrocephaly identified in 10 of the 21 children (largely *de novo*) mutations in *PTEN* and other mTOR pathway-associated genes (*MTOR, PIK3CA*, and *PPP2R5D*) [81]. Nearly 47.6% of this cohort carried mutations in the PI3K-mTOR-AKT pathway and 19% had mutations in *PTEN* [81]. All patients with these mutations exhibited **megalencephaly**, half demonstrated **polymicrogyria** and white matter abnormalities, and one-third developed ventriculomegaly [81].

Pleiotropy of PTEN mutations

The spectrum of neurodevelopmental outcomes associated with *PTEN* mutations (Figure 2, Table 1, and Table S1 in the supplemental information online) poses a challenge to establishing genotype-phenotype correlations for *PTEN*. Children with ASD, macrocephaly, and *PTEN* mutations often lack hamartomas or other features of PHTS, and the association of these mutations with long-term cancer risk is unclear, although clinical surveillance for cancer in these individuals and family members carrying *PTEN* mutations is recommended [70, 71, 73]. However, reports have identified families in which a *PTEN* variant associated with Cowden syndrome or Bannayan-Riley-Ruvalcaba syndrome in a parent is identified in a child with ASD [71, 73, 75, 76, 83]. Conversely, the same variants initially identified in individuals with ASD and macrocephaly have also been found in Cowden syndrome and Bannayan-Riley-Ruvalcaba syndrome [73]. This suggests that, with the exception of macrocephaly, *PTEN* variant expressivity can be influenced by other factors such as genetic background.

Evidence indeed suggests that *PTEN* variant function might correlate with clinical presentation. For example, over 50% of cancer-associated *PTEN* variants result in protein truncation, and most cancer-associated missense variants lead to complete loss of phosphatase activity [84]. These missense variants might have **dominant negative** effects, resulting in more severe cancer phenotypes in animal models [85, 86]. By contrast, the majority of ASD-associated variants are missense [83]. Functional assessment in a yeast-based assay found that ASD-associated variants result in partial **loss of function (LoF)** compared to complete LoF of PHTS-associated variants [87]. Another study found that ASD-associated *PTEN* variants, though unstable when transduced in glioblastoma cells and in mouse hippocampal neurons, retained catalytic activity, whereas PHTS variants led to complete LoF in glioblastoma cells [88]. Altered ubiquitination and defective nuclear-cytoplasmic shuttling may also cause PTEN LoF [89–92]; reviewed in [25]. For example, some ASD-associated *PTEN* variants result in nuclear exclusion of PTEN and increased soma size when introduced into mouse dentate gyrus *in vivo* [91]. Restoring nuclear PTEN expression and lipid phosphatase activity rescued soma size and phospho S6 (p-S6) levels [91]. These findings suggest that mutations may affect PTEN function by altering its subcellular localization as well as its stability and activity [91].

Recent studies have developed computational approaches to assess genotype-phenotype correlation for *PTEN* variants [93]. One study found 106 missense and nonsense variants disrupted PTEN function in five different model systems via multiple mechanisms [94], including induced instability, loss of catalytic or substrate-binding activity, and dominant

negative effects. Whereas unstable variants were highly correlated across different assays, other functional effects were less consistent, and genotype-phenotype correlations could not be assigned, reflecting clinical overlap among these variants [94]. ***PTEN* pleiotropy** might represent an avenue for exploring common mechanisms across disorders, despite this complexity. For example, missense mutations at *PTEN* R130 have been associated with ASD, CH, PHTS, and somatic cancers [24, 94] (Figure 2), suggesting common biology.

While integrative genomics implicates overlap in CH- and ASD-associated pathways (Figure 3), the number of CH versus ASD-associated *PTEN* variants identified to date is more limited (Figure 2, Table S2), complicating genotype-phenotype correlations. Identifying additional CH-associated *PTEN* variants is likely to provide greater leverage for differentiating variant function and revealing unique and shared downstream pathways as potential targets. As WES studies of CH and ASD have implicated altered neural proliferation and differentiation during early and mid-fetal development, both processes directly affected by PTEN and downstream mTOR signaling, these pathways may represent a convergent “hub” predisposing to a range of NDDs.

Animal models for studying PTEN function

Animal models have provided important insights into the role of the *PTEN-PI3K-AKT-mTOR* pathway in the pathogenesis of ASD and CH. The *PTEN* gene is evolutionarily conserved across several species, including zebrafish, mice, and humans, and *Pten* LoF animal models have been instrumental in elucidating the effects of LoF mutations on basic neurodevelopmental mechanisms (see Table S2 in the supplemental information online).

Pten mouse models exhibit macrocephaly and ASD-like behavioral phenotypes

Because homozygous Pten LoF causes embryonic lethality in mice at about embryonic day 9.5 [95, 96], conditional *Pten* LoF mouse models have been developed to investigate spatial and temporal functions of PTEN in the brain. The first autism-associated Pten LoF mouse model was generated via homozygous *Pten* knockout in postmitotic, mature neurons (Nse-Cre × Pten$^{flx/flx}$; *Nse-Pten*). *Nse-Pten* mice exhibit macrocephaly and brain overgrowth due to neuronal hypertrophy and progressive enlargement of the cerebral cortex and dentate gyrus, as well as increased dendritic arborizations [97]. Hypertrophic neurons in the dentate gyrus have increased levels of p-Akt, p-S6, and p-GSK3ß, indicating activation of the AKT and mTOR pathways [97]. *Nse-Pten* mice also develop “ASD-like” behavioral phenotypes, including deficits in social interaction, impaired learning, and altered sensory processing [97]. Morphological and behavioral phenotypes in *Pten* mouse models have been recently reviewed [25, 99, 100].

Additional mouse models have recapitulated macrocephaly, regional brain size alterations and/or neuronal hypertrophy, as well as behavioral deficits resulting from haploinsufficient or cell-type specific loss of Pten [98, 101–106]. Interestingly, these studies highlight the complex interplay of signaling pathways downstream of PTEN. For example, haploinsufficient *Pten*$^{+/-}$ mice show brain overgrowth at birth due to neuronal hyperplasia,

as well as an increase in markers of ß-catenin activity in the cortex, while adult *Pten*$^{+/-}$ mice show an increase in glia [103]. Genetic suppression of ß-catenin, not mTOR, reversed overgrowth in adult cortex, suggesting that Wnt/ß-catenin signaling downstream of PTEN might modulate brain overgrowth [103]. By contrast, genetic suppression of mTOR rescued social deficits in *Pten*$^{+/-}$ female mice as well as hypertrophy of cortical layer V neurons, implicating mTOR signaling in controlling these phenotypes [104]. *PTEN* LoF has also been shown to lead to decreased apoptosis and abnormal neuronal migration, and may not lead to increased proliferation of some progenitor populations [102, 107, 108], suggesting that mechanisms other than increased proliferation might also contribute to increased brain size.

Pten mouse models display seizures, excitatory-inhibitory imbalance, and synaptic alterations

Several *Pten* mouse models develop seizures [97, 98, 101, 102], analogous to the comorbidity of epilepsy in humans with ASD [97], and possibly reflecting a shared imbalance between cortical excitation and inhibition in ASD and epilepsy [109]. For example, epilepsy was observed in mice lacking *Pten* expression in astrocytes, 50–80% of cortical neurons, and in 80–90% of hippocampal pyramidal and granule neurons (*Gfap-Cre* × *Pten*$^{flx/flx}$; *Gfap-Pten*) [110]. Mouse models have also revealed a central role of PTEN in synaptic structure and function. For example, *Nse-Pten* mutants display thickened and ectopic dendrites, increased spine density, and hypertrophic axon tracts [97]. *Gfap-Pten* mice exhibit altered synaptic structures in both the cortex and cerebellum [101], including increased numbers of immature dendritic spines and synapses and increased pre-synaptic terminal size [101]. Synaptic plasticity is also disrupted in these mice [101].

Imbalance between excitatory and inhibitory neurons and altered synaptic transmission has also been characterized in mouse Pten LoF studies of excitatory neurons (*Nse-Cre, CaMKIIα-Cre*), cortical interneuron progenitors (*Nkx2.1-Cre, DlxI12b-Cre and SST-IRES-Cre*), and astrocytes (*Gfap-Cre*) [97, 101, 102, 104, 111, 112]. For example, *Pten* LoF in inhibitory neurons and precursors (*Nkx2.1-Cre, DlxI12b-Cre*) resulted in an increased ratio of parvalbumin/somatostatin (PV/SST) interneurons, likely reflecting increased apoptosis of SST neurons. Increased inhibitory postsynaptic current (IPSC) frequency in layer II/III excitatory neurons was also observed in P30 *Nkx2.1-Cre+;Pten*$^{flx/flx}$ mice [102]. Screening of five ASD-associated PTEN variants using an *in vivo* complementation assay revealed these alleles as hypomorphic in their inability to rescue either increased soma size or PV-to-SST ratio, without dominant negative effects [102].

Myelination deficits

Several studies in *Pten* mouse models have identified myelination defects which may contribute to circuit and behavioral dysfunction [101, 113–115]. For example, a nuclear-excluded Pten mouse model (*Pten*$^{m3m4/m3m4}$) displayed abnormalities in oligodendrocyte development and morphology with disrupted myelination [114]. Brain MRIs of adult Pten haploinsufficient mice identified abnormal scaling affecting multiple brain regions, with prominent white matter enlargement [106]. Cortical cultures from these mice showed increased glial cell proliferation [106]. Interestingly, gene expression profiles of three ASD

mouse models, including *PTEN (Pten*$^{m3m4/m3m4}$), *MECP2 (Rett Syndrome)*, and *TCF4* (Pitt-Hopkins syndrome), found enrichment of myelination-related genes among the shared differentially expressed genes [115], suggesting altered myelination as another point of convergence among ASD-associated genes.

Pten mouse models display congenital ventriculomegaly

Pten plays a major regulatory role in the cellular and molecular processes governing NSC proliferation and differentiation into mature neurons, ependymal cells, and oligodendrocytes. This suggests the hypothesis that *Pten* LoF in NSCs may not only produce structural and functional deficits in the development of the cortical circuits underlying ASDs and epilepsy, but also underpin structural and functional deficits during development of the proximal ciliated ventricular epithelium. Consistent with this, *Pten* mutant mice lacking *Pten* expression in all cortical layers, cerebellum, and hippocampus, without diminished astrocyte expression (*NGfap*-Pten), develop not only epilepsy, but also progressive ventriculomegaly resulting in early fatality [98, 116]. In addition, homozygous *Pten* knockout in NSCs (*Nestin*-Pten) results in macrocephaly and ventriculomegaly due to increased proliferation, decreased apoptosis, and increased NSC size [108, 117].

While few studies examined CSF dynamics directly in *Pten* mutant mice, an intriguing hypothesis to explain both ventriculomegaly and megalencephaly is the overaccumulation of CSF, as CSF and its hydrostatic pressure promote neural progenitor proliferation and brain enlargement [118, 119]. Interestingly, *Pten* expression has been localized to the apical membrane of the choroid plexus [120], within close proximity to ion transporters and water channels, which regulate the production of CSF (recently reviewed in [121]). Disrupted *Pten* protein phosphatase activity led to increased levels of phosphorylated Dishevelled, a key component of WNT signaling, which prevented the formation of both primary and multicilia in ependymal cells, which are thought to be responsible for the circulation of CSF [122]. Thus, developmental pleiotropy of *Pten* LoF mouse models in ASD, epilepsy, and CH may be related to abnormal development of ependymal cells, choroid plexus epithelial cells, and neural progenitors, all deriving from a common progenitor pool within prenatal germinal niches [123, 124], leading to functional deficits in both assembly of cortical circuits and in the proximal ventricular zone/subventricular zone.

Zebrafish models of PTEN

The transparency and rapid embryonic neurodevelopment of zebrafish offer unparalleled ability to visualize early developmental phenotypes. The duplicated teleost genome has given rise to two zebrafish *PTEN* orthologs, *ptena* and *ptenb*, encoding Ptena and Ptenb proteins (which are 88% and 86% identical to the human protein, respectively) [125]. Both paralogs are broadly expressed during early somitogenesis and in the central nervous system by 24 h post fertilization (hpf) [125]. Homozygous LoF of either allele is viable and exhibits no obvious morphological phenotype, but LoF of both *ptena* and *ptenb* results in embryonic lethality at 5 days post fertilization (dpf) [126]. Homozygous double mutants display an apparent increase in head size at 4 dpf [126]. While their development is grossly normal until 48 hpf, double mutants display severe developmental abnormalities by 4 dpf, partially

reversible by administration of a PI3K inhibitor from 2 dpf, implicating PI3K pathway hyperactivation [126]. Homozygous double mutants also exhibit increased cell proliferation and decreased apoptosis. At 7 months, *ptenb* null mutants exhibit eye tumors with increased p-Akt expression [126]. These studies demonstrate overlapping cellular and molecular phenotypes in zebrafish and mouse models, consistent with evolutionary conservation of PTEN-PI3K-AKT signaling.

Pharmacological rescue in PTEN mouse models

There is evidence from preclinical models that pharmacological inhibition of mTOR signaling can reverse abnormal phenotypes. For example, treatment of *Nse-Pten* mice with the mTORC1 inhibitor, rapamycin, reversed social deficits, seizures, and structural abnormalities, including macrocephaly, neuronal hypertrophy, and spine density [127]. Similarly, rapamycin decreased seizures and rescued neuronal hypertrophy in mice lacking *Pten* in all cortical layers, cerebellum, and hippocampus using a modified *Gfap-Cre* (*Gfap-cre x Pten*$^{flx/flx}$*; NGfap-Pten*) [128]. In addition, early treatment of *Pten*$^{+/-}$ mice with a pharmacological inhibitor of the mTOR component, S6K1, reversed social deficits present in female mice as well as neuronal hypertrophy in cortical layer V [104]. Further, this treatment rescued increased activity and connectivity in the medial prefrontal cortex and basolateral amygdala when administered during development, but not in adulthood, suggesting there are critical periods during which mTORC1 modulation is important for the establishment of these circuits [104]. Preclinical investigations in mice have also demonstrated rapamycin efficacy in preventing ventriculomegaly [129].

Targeting the mTOR pathway in ASD and CH

No FDA-approved drugs are currently available to treat core symptoms of ASD or to attenuate ventriculomegaly in CH, but active drug discovery efforts are ongoing. At present, the only FDA-approved pharmacological treatments for ASD are atypical antipsychotics, risperidone and aripiprazole, indicated for aggression and irritability, but not for the core deficits [130]. Given evidence from preclinical studies, a number of clinical studies have investigated targeting the mTOR pathway in NDDs. Most progress to date has been in TSC, where studies have demonstrated benefit for mTOR inhibitors in treating TSC-associated seizures (reviewed in [131]). Specifically, everolimus is FDA-approved for adjunctive treatment of complex partial seizures in children (>2 years old) and adults with TSC [132]. Sirolimus is also effective in treating seizures in children with TSC [133].

At present, clinicaltrials.gov includes studies investigating pharmacological treatment targeting the mTOR pathway for ASD-associated behaviors in TSC and neurocognition in PHTS. NCT02991807[I] is testing mTOR inhibitor everolimus in individuals with *PTEN* mutations to assess safety and possible improvements in neurocognition and behavior. The completed study NCT01730209[II] tested everolimus in children and adolescents aged 4–17 years with TSC, yet found no improvement in intelligence quotient or ASD behaviors [134]. Another double-blind randomized, placebo-controlled trial (NCT01289912[III]) also found no significant improvement in neurocognition or behavior in children and adolescents with TSC following 6 months of everolimus treatment [135]. The lack of mTOR inhibitor efficacy in

treating ASD-associated behaviors might reflect the need for earlier treatment, in view of the importance of critical periods of plasticity during neurodevelopment [131, 134, 135]. In addition, early seizure treatment in children with TSC may be important for improving neurocognitive and behavioral outcomes [135], a finding supported by a recent preclinical study showing that everolimus improves social behaviors in *Tsc2*$^{+/-}$ rats but not after early status epilepticus [136]. Interestingly, a recent retrospective study showed that both everolimus and sirolimus attenuated ventriculomegaly in six of 13 TSC patients diagnosed with obstructive hydrocephalus secondary to subependymal giant cell astrocytoma [137]. Because TSC1/2 is downstream of PTEN, it is possible that targeting different components of the PI3K-AKT-mTOR pathway will be important for treating specific clinical features of these disorders.

Concluding Remarks and Future Perspectives

Emerging evidence from genetics and integrative genomics strongly implicates PTEN and mTOR signaling in both ASD and CH, suggesting these disorders might share similar pathophysiological mechanisms (Figure 3). While CH has been classically attributed to abnormal CSF “plumbing,” and ASD is primarily characterized by behavioral symptoms without pathognomonic structural brain abnormalities, convergence on a common pathway has the potential to shape our conceptualization of the two disorders. For example, this reconceptualization supports characterizing CH as an NDD, similar to ASD, and suggests that children with CH may benefit from early behavioral screening and intervention, while structural imaging might be relevant for a subset of children with ASD at risk for ventriculomegaly. Moreover, the identification of PTEN-PI3K-mTOR as a central pathway has the potential not only to shed light on novel mechanisms underlying both disorders but also to present new avenues for developing novel therapeutics.

Understanding the precise mechanisms by which *PTEN* LoF leads to a range of neurodevelopmental phenotypes represents a critical next step (see **Outstanding Questions**). For example, it will be important to investigate in animal models the neural cell types, developmental time points, and molecular components downstream of PTEN that are most relevant for the development of specific phenotypes. The considerable pleiotropy of *PTEN* also represents a central challenge. Additional preclinical studies aimed at differentiating mechanisms by which specific *PTEN* variants cause diverse phenotypes are needed to elucidate how *PTEN* LoF predisposes to a range of clinical disorders and identify improved therapeutic targets. At the same time, gaining insight into a common pathway underlying ASD and CH holds important scientific and clinical implications for diagnosis and treatment of both disorders and identifies a targetable pathway that might lead to new avenues for pharmacological development.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Glossary

De novo mutation
a mutation that is not inherited, but arose in the gametes or during early embryonic development

Dominant negative mutation
a mutation that causes the gene product to suppress the normal function of the wild-type gene product

Hamartoma
a benign tumor that can occur in different parts of the body (e.g. brain, skin), often associated with a genetic condition

Integrative genomics
An analysis approach that integrates data from large-scale genetic studies with biological data sets, such as RNA-seq and protein-protein interaction networks

Lipoma
benign tumor consisting of fatty tissue

Loss of function (LoF) mutation
a mutation resulting in a loss of the gene product's function

Macrocephaly
increased head circumference, defined as over 2 standard deviations above average

Megalencephaly
abnormal enlargement of the brain, defined as brain weight over 2.5 standard deviations above average

Pathognomonic
a characteristic feature of a disorder that defines the diagnosis

Pleiotropy

the production of two apparently unrelated effects by the same gene mutation, or in the clinical context, the association of mutations in a particular gene with multiple clinical outcomes. For example, *PTEN* is highly pleiotropic because mutations in this gene have been associated with ASD, CH, and cancer

Polymicrogyria
abnormal brain development characterized by an increased number of small surface folds

Ventriculomegaly
enlargement of the CSF-filled brain ventricles

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Resources

I. This study is registered with ClinicalTrials.gov (NCT0299180). https://clinicaltrials.gov/ct2/show/NCT02991807

II. This study is registered with ClinicalTrials.gov (NCT01730209). https://clinicaltrials.gov/ct2/show/NCT01730209

III. This study is registered with ClinicalTrials.gov (NCT01289912). https://clinicaltrials.gov/ct2/show/NCT01289912

Outstanding Questions Box

How does PTEN disruption in specific neural cell types and brain regions predispose to the circuit and behavioral deficits seen in ASD?

Which period of development is most critical for PTEN function and does this period differ in distinct NDDs?

What is the mechanism underlying the pleiotropy of PTEN mutations? To what extent is the type of mutation (i.e., missense, nonsense, splice site) or its function (i.e., LoF, dominant negative) associated with specific clinical features?

How can the pleiotropy of PTEN mutations be leveraged to identify specific therapeutic targets for PTEN-related disorders?

There is evidence that PI3K-Akt-mTOR might represent a common pathway across NDDs. What are the mechanisms by which disruption of this common pathway leads to seemingly disparate clinical presentations of CH and/or ASD?

How can cellular and animal model systems be leveraged for screening the functionality of PTEN variants?

How can animal models of PTEN mutation help uncover translationally relevant therapeutic targets for future investigation in NDDs?

Highlights

- Structural brain abnormalities, such as enlargement of the brain's ventricles, occur at an increased frequency in individuals with autism spectrum disorder (ASD). Conversely, rates of ASD are increased in congenital hydrocephalus (CH) compared to the general population. These observations raise the question of whether the two disorders might share common neurodevelopmental mechanisms.
- Mutations in *PTEN* are associated with a range of neurodevelopmental disorders, including ASD and CH.
- Integrative genomics highlights PTEN-PI3K-mTOR as one potentially shared pathway between ASD and CH.
- Mouse models of *PTEN* loss of function exhibit macrocephaly, social deficits, seizures, synaptic alterations, myelination defects, and enlarged brain ventricles.
- Preclinical studies in mouse models suggest that targeting the mTOR pathway can reverse structural and behavioral abnormalities associated with *PTEN* loss of function.
- The PTEN-PI3K-mTOR pathway may represent a potential pharmacological target for further investigation in ASD and CH.

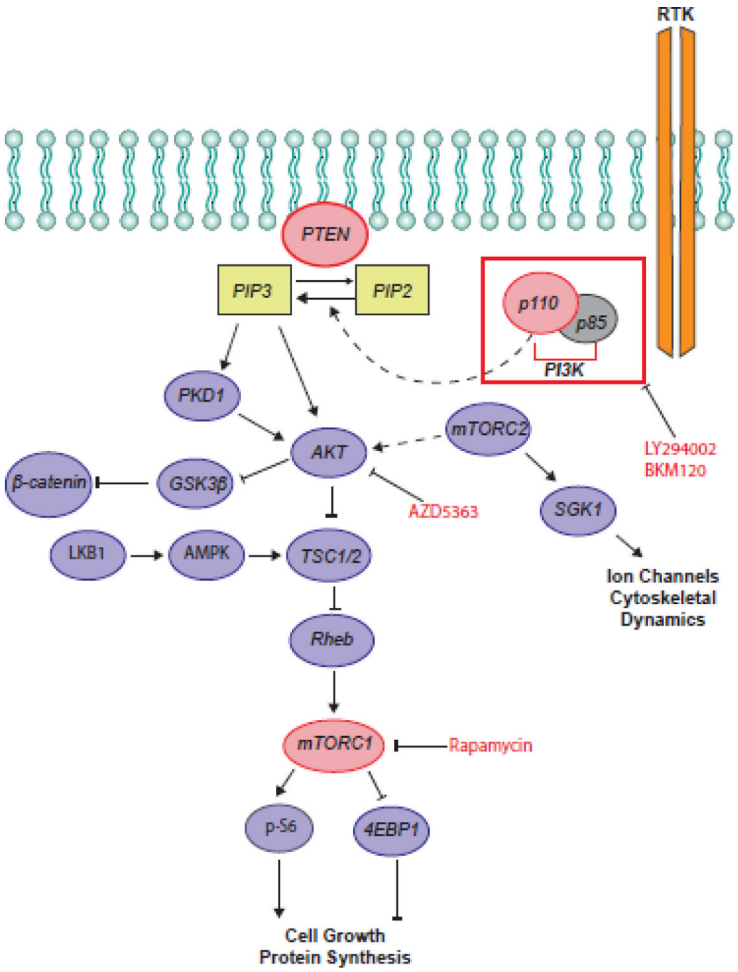

Figure 1. The PTEN-PI3K-AKT-mTOR pathway and therapeutic targets.

Activation of PI3K-AKT signaling can occur by binding of growth factors, hormones, or cytokines to receptor tyrosine kinases (RTK), leading to activation of phosphatidylinositol 3-phosphate kinase (PI3K). PI3K phosphorylates phosphatidylinositol-4,5-bisphosphate (PIP2), generating phosphatidylinositol 3,4,5-trisphosphate (PIP3), leading to the activation of AKT by phosphorylation at Thr^{308} (p-AKT). PTEN opposes the effect of PI3K by hydrolyzing PIP3 to PIP2. p-AKT deactivates glycogen synthase kinase-3 β (GSK3β), which is a negative regulator of β-catenin, and suppresses the tuberous sclerosis complex (TSC1/TSC2), a negative regulator of mTORC1. mTORC1 suppresses eukaryotic translation initiation factor 4E-binding protein (4EBP1) and activates p-S6, leading to increased protein translation. mTORC2 modulates cytoskeletal remodeling and ion channel activation and leads to phosphorylation of AKT at Ser^{473}. Pharmacological treatment with LY294002 and BKM120 inhibits PI3K activity. AZD5363 inhibits AKT. Rapamycin inhibits mTORC1 activity. Abbreviations: AKT, protein kinase B; AMPK, AMP-activated protein kinase; mTOR, mammalian target of rapamycin; mTORC1, mammalian target of rapamycin complex 1; mTORC2, mammalian target of rapamycin complex 2; PTEN, phosphatase and tensin homolog; PKD1, polycystin 1; LKB1, liver kinase B1; SGK1, serum/glucocorticoid regulated kinase 1.

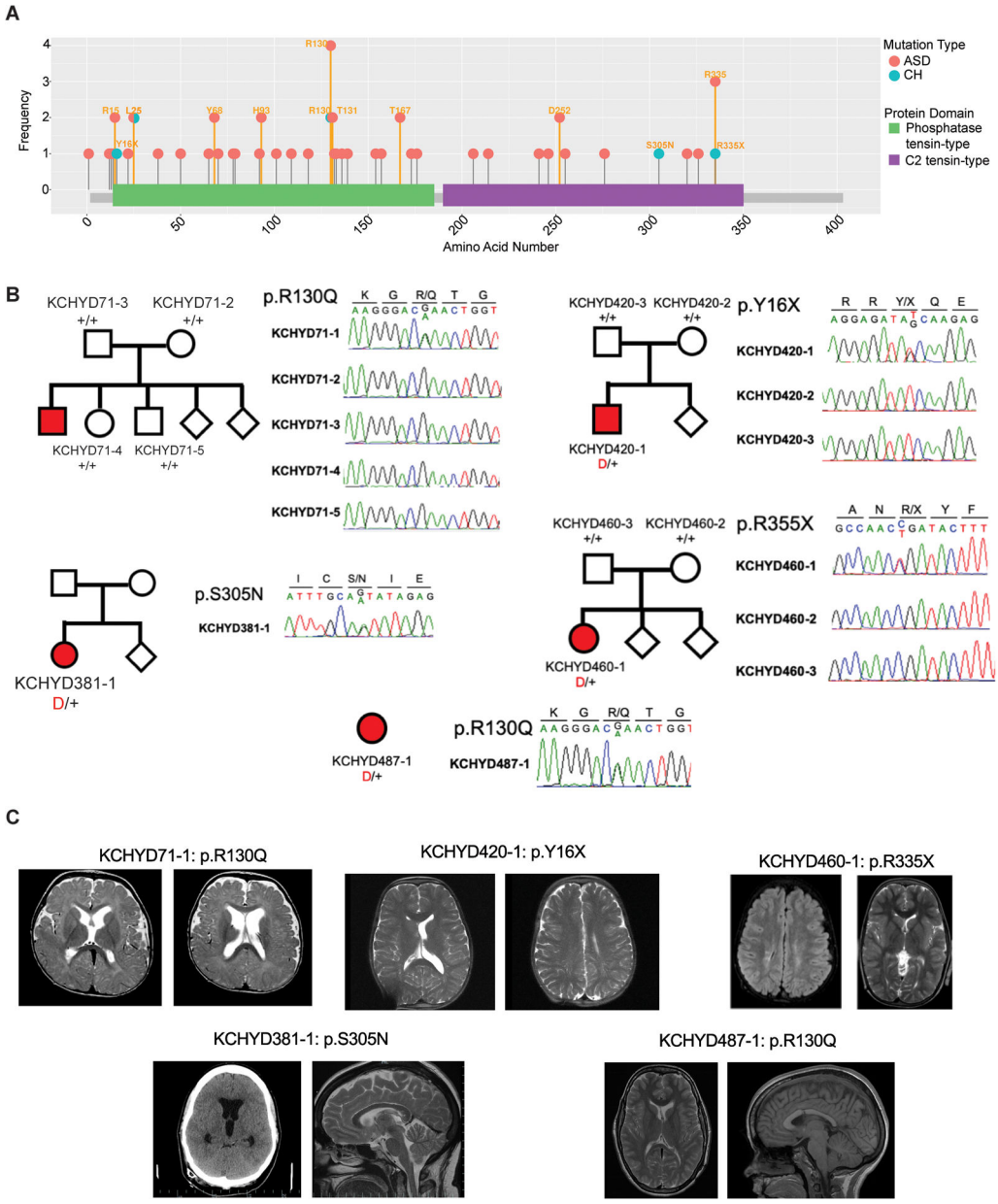

Figure 2. *PTEN* mutations in autism spectrum disorder (ASD) and congenital hydrocephalus (CH).

(A) *PTEN* mutations identified in individuals with ASD (pink) and CH (blue) are shown in relation to PTEN functional domains. This figure was generated using the MutPlot program [138] to map the missense and truncating mutations identified in our literature search (Table S1 in the supplemental information online) onto the human PTEN protein domains reported in UniProt [139]. Splice site mutations are not shown, but are listed in Table S1 in the supplemental information online. Mutation frequency at each residue (based on Table S1 in the supplemental information online) is shown on the y axis. Additional information about clinical features associated with mutations can be found in Table S1 in the supplemental information online. **(B)** Family pedigrees and Sanger sequencing electropherograms of individuals who underwent neurosurgical treatment for sporadic CH and harbor *PTEN*

mutations, corresponding to reported mutations from Table 1. **(B)** Adapted, with permission, from [24]. Abbreviation: PTEN, phosphatase and tensin homolog. **(C)** Representative T1 or T2-weighted axial and sagittal brain MRIs or head CT images of neurosurgically treated CH patients harboring indicated PTEN mutations, adapted, with permission, from [24].

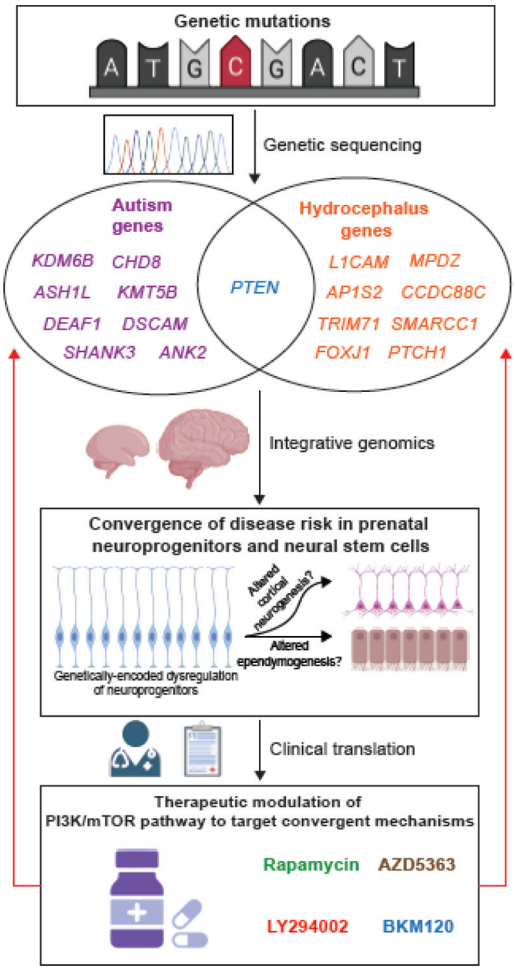

Figure 3. Integrative genomics leads to a convergent neuroscience approach to autism spectrum disorder (ASD) and congenital hydrocephalus (CH).

A. Mutation detection in ASD and CH patients using “case-parent trio design” whole exome sequencing approaches have identified *de novo* mutations that carry a high risk burden. The autism genes listed in the figure, selected from [26], were identified as ASD predominant with family-wise error rate of 0.05 or less, and the hydrocephalus genes are high-confidence genes selected from [24], with *PTEN* being the only gene shared between the two groups. **B**. Integrative genomic analyses reveal that spatial and temporal co-expression networks shared between ASD and CH candidate genes converge upon neuroprogenitors and neural stem cells in the embryonic brain. Ongoing basic science studies are examining altered cortical neurogenesis and altered ependymogenesis, as well as ASD and CH pathogenesis at the molecular, cellular, and circuit levels. **C**. Therapeutic modulation of the PI3K/mTOR pathway can inform the development of future potential treatments of ASD and CH in individuals carrying mutations in key genes within this pathway. Figure created using Biorender.com. Abbreviations: mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homolog.

Table 1.

Neurosurgical treatments, cortical malformations, and developmental sequalae in PTEN-CH patients (modified from [24]) with permission.

Subject	Treatment	Aqueductal stenosis	Cavum septum pellucidum	Cerebellar tonsillar ectopia	Corpus callosum abnormalities	Intracranialcyst	Megalencephaly	Polymicrogyria	Septal agenesis	White matter signal abnormality	White matter volume loss	Craniofacial abnormality	Developmental delay	Epilepsy	Hernia	Macrocephaly	Skeletal abnormalities
KCHYD71-1	ETV	+	−	+	+	−	−	+	−	+	+	−	−	−	−	+	−
KCHYD420-1	Shunted	−	−	+	−	−	−	+	−	−	+	−	+	−	+	+	−
KCHYD460-1	Shunted>ETV> Shunted	−	+	+	−	−	−	−	−	+	+	+	+	−	+	+	+
KCHYD381-1	Shunted	+	−	+	−	−	−	−	−	+	−	−	+	−	−	+	−
KCHYD487-1	Shunted	+	−	+	+	−	−	−	−	+	+	−	+	−	−	+	−

ETV: Endoscopic third ventriculostomy

KCHYD71-1 also exhibits mildly rotated thalami, prominence of the subarachnoid spaces overlying the cerebral convexities, small ill-defined enhancing focus in the left centrum semiovale adjacent to a medullary vein may represent a capillary telangiectasia or developmental venous anomaly

KCHYD420-1 also exhibits dilated perivascular spaces and pineal cyst

KCHYD460-1 also exhibits asymmetric caudate nuclei, signal abnormality in the globus pallidus, prominence of the extra-axial CSF spaces overlying the left cerebral convexity most pronounced at the parietal region near the vertex

KCHYD381-1 also exhibits pineal cyst

KCHYD487-1 also exhibits right lateral intraventricular cyst