Signalling Dysregulation of the PI3K/AKT/mTOR Pathway and Its Links to Autophagy and Mitochondrial Function in Autism Spectrum Disorder

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Groningen, Netherlands

November 2025

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Abstract

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition defined by atypical communication and social interaction, along with restricted and repetitive behaviours, the aetiology of which involves variation in brain connectivity and synaptic signalling. The mechanistic target of rapamycin (mTOR) is a serine/threonine kinase crucial for regulating neuronal growth, circuit formation, and synaptic plasticity. Aberrant mTOR signalling is associated with multiple neurological disorders, including ASD. This essay aims to delineate the mechanistic role of the PI3K/AKT/mTOR pathway in ASD neuropathology, specifically focusing on metabolic regulation. Preclinically, aberrant mTOR hyperactivation is established as a shared molecular feature in multiple syndromic ASD models (such as TSC, PHTS and FXS), consistently correlating with defective neuronal development and atypical behavioural phenotypes. As a consequence of this hyperactivation, impaired autophagy is linked to suppressed and ineffective synaptic pruning during early brain development, which alters synaptic plasticity. Furthermore, neurons are rendered metabolically vulnerable by depriving the energy supply necessary for synaptic function while simultaneously inducing mitochondrial oxidative damage and suppressing quality control mechanisms like mitophagy. By focusing on these metabolic mechanisms, this essay aims to assess molecular processes underlying ASD and highlight potential targets for therapeutic intervention.

Introduction

Autism Spectrum Disorder (ASD) is a neurodevelopmental condition defined by atypical communication and social interaction, as well as restricted and repetitive patterns of behaviour (Lord et al., 2020). These features manifest in a broad spectrum of severity, thus termed a "spectrum" disorder. Clinically, it is distinguished by the characteristic challenges in social-emotional reciprocity, non-verbal communication, building and understanding relationships, along with stereotyped behaviours, insistence on sameness, fixed interests and unusual sensory input tolerances (American Psychiatric Association, 2022). Common comorbidities include intellectual disability, speech delay, motor challenges, epilepsy, attention deficit hyperactivity disorder (ADHD), anxiety and sleep disorders, each of which further complicates diagnosis and management (Lord et al., 2020). The global prevalence is estimated to be around 1-2% of the population and is diagnosed considerably more often in males, at an estimated 4:1 ratio (Lord et al., 2020). The sex differences in ASD diagnosis emerge from mechanisms attributed to genetic, hormonal and neurodevelopmental factors that influence behavioural and cognitive phenotypes as well as diagnostic criteria shaped by overt male behavioural traits, reflecting the broader challenge of accurately representing the full spectrum of ASD phenotypes (Ferri et al., 2018).

The aetiology of ASD arises from both genetic and environmental factors that drive neurodevelopmental variation and contribute to changes in brain connectivity and synaptic signalling (Lord et al., 2020). Several regions of the brain are implicated, including the prefrontal cortex, which supports executive functions, the striatum, which is associated with habit formation and social reward, the amygdala, which links emotional processing and social cognition and the cerebellum, which governs motor behaviour, balance and coordination (Wang et al., 2023). The genetic background plays a dominant role, reaching up to 80% heritability, and several genes involved in synaptic homeostasis have been associated with ASD pathogenesis (Moyses-Oliveira et al., 2020). Furthermore, different environmental triggers are considered to increase the vulnerability of developing ASD in genetically predisposed individuals (Sealey et al., 2016). Despite considerable insight into the involvement of aberrant synaptic plasticity, the molecular mechanisms linking these genetic and environmental factors to the behavioural phenotype remain incompletely understood (Delorme et al., 2013).

The mechanistic target of rapamycin (mTOR) is a serine/threonine kinase known to integrate growth signals with nutrient and energy status in order to regulate cellular growth and proliferation by promoting anabolic metabolism and suppressing catabolic activity (Saxton & Sabatini, 2017). It is part of the PI3K-related kinase family and consists of two complexes, mTOR Complex 1 (mTORC1), which interacts with regulatory-associated protein of mTOR (Raptor) and promotes protein, nucleotide and lipid synthesis while suppressing autophagy, and mTORC2, which associates with rapamycin-insensitive companion of mTOR (Rictor) and mediates cytoskeletal remodelling (Saxton & Sabatini, 2017). The signalling cascade of mTORC1 depends on upstream regulators, including the PI3K/AKT, while mTORC2 acts as a negative regulator via AKT phosphorylation (Saxton & Sabatini, 2017). Aberrant mTOR signalling is associated with multiple neurological disorders, developmental and degenerative, such as ASD, epileptic encephalopathy and Alzheimer's disease, among others (Lipton & Sahin, 2014). During neurodevelopment, the mTOR pathway is involved in processes of neuronal growth, circuit formation and importantly, synaptic plasticity (Lipton & Sahin, 2014). Aberrant mTOR activity is also a link between several forms of syndromic ASD, indicating the presence of a shared molecular dysfunction despite the different genetic drivers (Thomas et al., 2023; Winden et al., 2018). Currently, animal models are the primary means of research to uncover how mTOR pathway dysregulation contributes to the development of ASD (Dana et al., 2020; Drehmer et al., 2024).

Autophagy is a conserved intracellular recycling pathway that removes unnecessary or damaged cellular components by promoting the encapsulation of aberrant material within double-membrane vesicles called autophagosomes, which subsequently fuse with lysosomes for cargo degradation (Klionsky et al., 2021). Autophagy begins after the activation of the ULK kinase complex, marking the site of autophagosome formation and the recruitment of additional machinery for the nucleation of the phagophore structure. Multiple ATG proteins are involved in the process of elongation, including the LC3 (ATG8) conjugation system, where LC3-II is formed via lipidation and gets incorporated into the autophagosomal membrane. During selective autophagy, cargo recognition is mediated by autophagy receptors (e.g., p62) that bind ubiquitinated targets. The mature autophagosome detaches from its formation site and fuses with a lysosome, generating an autolysosome, inside of which lysosomal enzymes degrade the cargo and inner membrane, releasing the material back to the cytoplasm for reuse (Lahiri et al., 2019). As a major regulator of anabolic metabolism, mTORC1 has an inhibitory effect on autophagy by

phosphorylating the ULK1 complex, thus preventing autophagosome formation at its earliest stage, to counteract processes of catabolic metabolism and biomolecule degradation (Deleyto-Seldas & Efeyan, 2021). Disruption of autophagy is involved in pathogenic mechanisms of a wide range of neurological disorders, including ASD (Klionsky et al., 2021).

Given the brain's increased metabolic demands, a disproportionate rate of energy production is required to sustain the homeostasis of neurotransmission and support synaptic plasticity. During neurodevelopment, these energy demands are amplified and functional mitochondria are crucial not only for ATP synthesis but also to control synaptic calcium levels, apoptotic signalling and redox balance (Rojas-Charry et al., 2021). Mitochondrial activity is tightly controlled by signalling pathways, including the mTOR, which enable cells to sense and integrate nutrient and energy availability to regulate mitochondrial biogenesis, turnover and metabolic flux (Khaliulin et al., 2025). Such regulation is especially important for neurodevelopment, during which neurons are still adapting their independent metabolic control to the fluctuating nutrient environment (Deleyto-Seldas & Efeyan, 2021). Mitochondrial dysfunction can therefore impair neuronal maturation and connectivity by inhibiting the energy supply that fuels the processes of neuronal proliferation, differentiation, quality control and circuit formation (Rojas-Charry et al., 2021). Recognising this, mitochondrial dysfunction has been implicated in neurodevelopmental pathologies, and since the late 1990s, ASD has been hypothesised to be, at least in part, a mitochondrial disease (Lombard, 1998).

In this essay, I examine how disruptions in neuronal metabolic regulation, particularly through PI3K/AKT/mTOR signalling, autophagy and mitochondrial function, contribute to the aetiology of ASD. Building on evidence from syndromic ASD, the essay first considers clinical data from syndromic ASD and preclinical data linking mTOR dysregulation to characteristic behavioural phenotypes in mouse models. It then explores autophagy as a direct metabolic process controlled by mTOR, before addressing mitochondrial dysfunction as an alternative pathway influencing neurodevelopment. By focusing on these metabolic mechanisms, this essay aims to assess molecular processes underlying ASD and highlight potential targets for therapeutic intervention.

Dysregulated mTOR Signalling Linked to Syndromic ASD

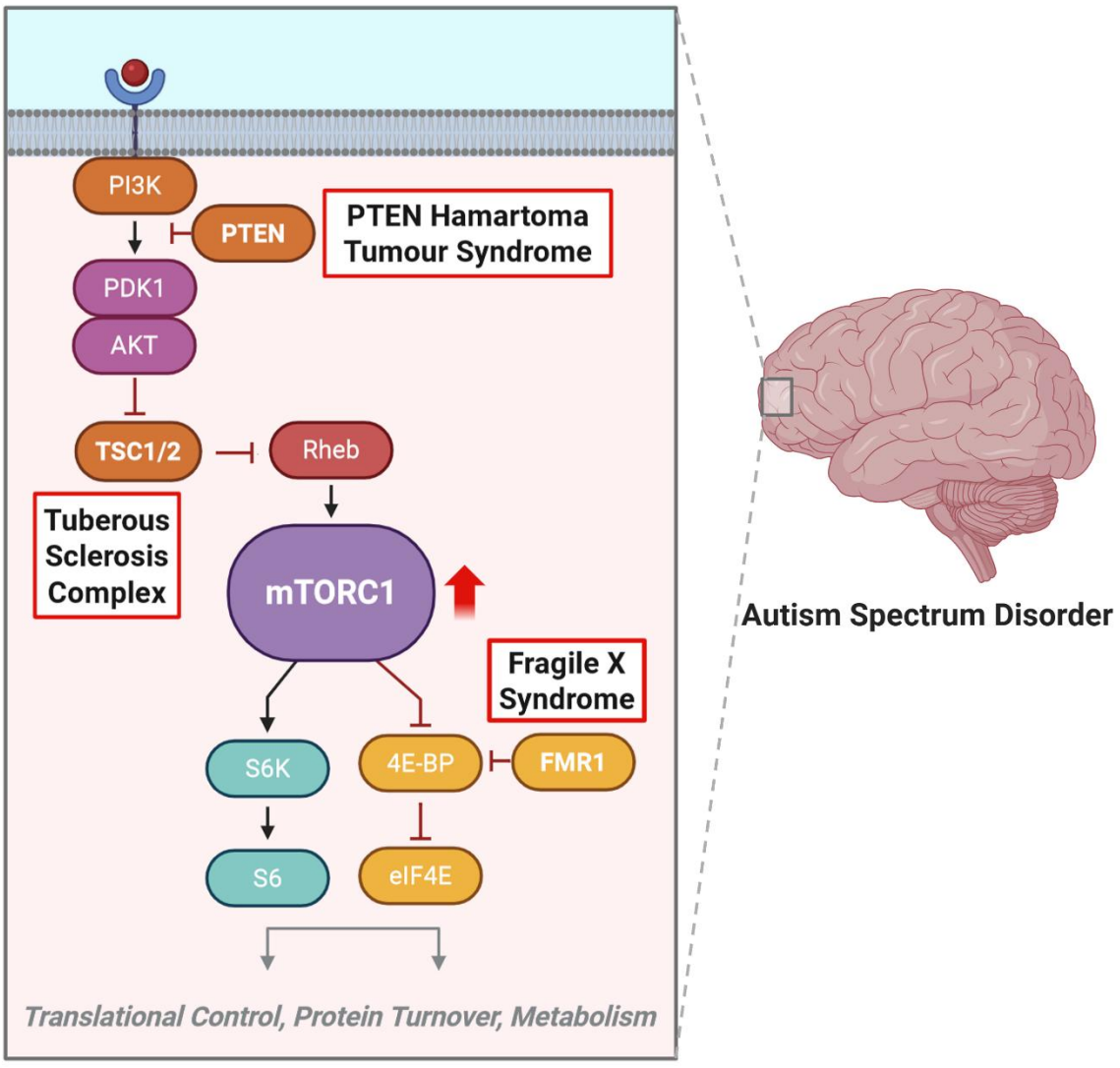

Figure 1 Overview of PI3K/AKT/mTOR pathway and associated syndromic ASD.
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Tuberous Sclerosis Complex

TSC is a genetic neurocutaneous disorder, diagnosed in approximately 1 per 6.000–10.000 infants. Genetic alterations in either the *TSC1* or *TSC2* gene drive the growth of benign tumours or lesions that can affect multiple organs such as the brain, lungs, heart, skin and kidneys. Patients with TSC often manifest neurological symptoms like epileptic seizures, as well as symptoms of cognitive and neuropsychiatric disorders (Henske et al., 2016). Furthermore, TSC is a leading genetic cause of syndromic ASD, with an estimated 40–60% of TSC patients having an ASD

diagnosis (Winden et al., 2018). As discussed, the TSC complex operates upstream of the mTORC1 and functions as an inhibitor of Rheb, which subsequently signals mTORC1 inhibition. Haploinsufficiency of *TSC1/2* disrupts this negative feedback, mTORC1 remains unrestrained and protein synthesis gets dysregulated (Thomas et al., 2023). Even though the signalling function of the TSC complex is not limited to the mTOR pathway, it is widely considered that the core neurobehavioral features of ASD are driven by the hyperactive signalling of mTORC1 (Winden et al., 2018).

Both clinical and preclinical studies have consistently linked mTOR pathway hyperactivation to these ASD neurobehavioral features. In a study by Tang et al., evidence from the postmortem brains of ASD patients implicates the overactivation of mTOR in the development of ASD. Analysis of pyramidal neurons of the temporal cortex revealed higher density of dendritic spines in ASD patients, a phenomenon attributed to deteriorated developmental pruning. The elevated levels of p-mTOR suggest that mTOR hyperactivation regulates neuronal connectivity via deficient synaptic pruning (Tang et al., 2014). In another study by Prohl et al., the brains of infants with TSC were longitudinally studied during the first two years of life to capture the differences in brain connectivity between the individuals who developed ASD and those who did not (Prohl et al., 2019). In addition to more severe developmental delay and epilepsy, individuals with ASD lacked typical organisation in multiple white matter tracts, including the arcuate fasciculus, which is involved in language and social communication. During infancy, the observed decline in white matter structure and organisation was progressive(Prohl et al., 2019), which sets a specific timeframe for when the changes in the developing brain of ASD individuals may give rise to symptoms of verbal communication.

Moreover, preclinical studies have established that loss of TSC complex function leads to ASD behavioural traits in transgenic mice. Both partial and complete conditional knockout (cKO) of *Tsc1* in cerebellar Purkinje cells caused mice to exhibit ASD behavioural phenotypes, characterised by decreased interest in social interaction, repetitive grooming and abnormal communication via ultrasonic vocalisations, as well as cognitive inflexibility and deficits in motor learning and novelty exploration (Tsai et al., 2012). The Purkinje cells of these mice showed upregulated mTOR activity, were structurally bigger and had increased spine density, while their excitability was reduced, likely due to cellular stress (Tsai et al., 2012). Similarly, heterozygous deletion of *Tsc2* increased neuronal spine density and synaptic coupling due to mTOR

hyperactivity, causing hyperconnectivity and ASD phenotypes in mice (Pagani et al., 2021). While the structural integrity of the white matter remained intact, increased long-range functional connectivity was observed among the insular, striatal, and prefrontal regions in *Tsc2* haploinsufficient mice. Similar observations were reported in the anterior insula, basal ganglia, and prefrontal regions of children with idiopathic ASD, which regulates the integration of executive processes, social and emotional control and habit formation (Pagani et al., 2021). Together, both the clinical and preclinical observations are of particular significance since TSC provides the most direct mechanistic link between mTOR hyperactivation, developmental synaptic pruning dysfunction, early life abnormal white matter organisation and ASD phenotypes that include key characteristics like impaired sociability and communication.

PTEN Hamartoma Tumour Syndrome

PTEN hamartoma tumour syndromes (PHTS) refer to the spectrum of disorders caused by heterozygous germline pathogenic variants in the *PTEN* gene, a known tumour suppressor, which predispose carriers to benign and/or cancerous tumours in thyroid, renal, breast and endometrial tissues (Rademacher & Eickholt, 2019). PHTS is commonly comorbid with various neurodevelopmental conditions like intellectual disability, epileptic seizures, macrocephaly and ASD, with the latter reaching up to 5% of patients (Thomas et al., 2023). PTEN is a lipid phosphatase, an antagonist of the PI3K/AKT/mTOR pathway that converts phosphatidylinositol-trisphosphate to phosphatidylinositol-bisphosphate via dephosphorylation, and is known to impact lipid and mitochondrial metabolic processes, among others (Rademacher & Eickholt, 2019).

There is mounting evidence that involves *PTEN* mutations with the PI3K/AKT/mTOR pathway in ASD phenotypes. Whole-exome sequencing data from blood and oral samples in a cohort of patients with macrocephaly, developmental delay and ASD suggest a causative association between mutations in the *PTEN* gene, as well as other genetic alterations in genes that encode components of the PI3K/AKT/mTOR pathway and its regulators, and a progressive increase in symptom severity (Yeung et al., 2017). However, in patients with *PTEN* mutations who were also diagnosed with ASD, the decreased expression of *PTEN* observed was accompanied by levels of phosphorylated downstream targets of mTOR comparable to those of patients without an ASD diagnosis (Frazier et al., 2015), which implies that PTEN may not drive the social and behavioural impairments but rather they emerge as a consequence of clinical severity. Furthermore,

the ASD patients were characterised by higher head circumference, higher brain and callosal volume, decreased total and frontal cortical thickness and abnormal development and maturation of white matter. Neurocognitive deficits were also pronounced, with lower IQ, slower processing speed, impaired working memory and adaptive functioning. Such phenotypes were also observed in *Pten* mutant mice, which exhibited higher corpus callosum thickness and upregulation of genes involved in myelination (Frazier et al., 2015).

Regarding genetically induced ASD behavioural phenotypes, *Pten* haploinsufficient mice presented reduced social interest and social novelty preferences, along with brain enlargement. Specifically, male transgenic mice exhibited increased repetitive behaviour (more marble burying), depression-like behaviour (greater immobility in tail suspension and forced swim tests), reduced anxiety-like behaviour (more exploration of open, brightly lit areas) and impaired social recognition. On the other hand, female mice exhibited atypical circadian activity patterns and impaired emotional learning/memory (less freezing in trace fear conditioning) (Clipperton-Allen & Page, 2014). Another study found that neuronal *Pten* cKO results in fewer social interactions and repetitive behaviour deficits, but no changes in ultrasonic communication (Lugo et al., 2014). The brains of mice lacking Pten showed hyperactive PI3K/AKT/mTOR signalling, based on the increased phosphorylation markers of AKT, S6 and S6K, and disrupted synaptic signalling and plasticity (Lugo et al., 2014). Importantly, mTOR inhibition by either rapamycin or deletion of the gene encoding for Raptor reversed the induced dendritic enlargement and increased spine density in mice with *Pten* KO dentate gyrus granule neurons, while at the same time promoting excitatory synaptic transmission (Tariq et al., 2022). Since *PTEN* mutations affect both cellular metabolic processes and cognition, and despite the fact that their influence on social and behavioural deficits may be indirect, they highlight the importance of investigating hyperactive mTOR signalling in Pten-deficient models as a potential mechanistic explanation of ASD phenotypes.

Fragile X Syndrome

Fragile X syndrome (FXS) is a neurodevelopmental disorder that most typically rises from a CGG repeat expansion in the promoter of the *FMR1* gene, which results in reduced levels or complete absence of the signalling protein (Hagerman et al., 2017). FXS affects approximately 1 male per 5.000 and 1 female per 4.000–8.000 individuals, with intellectual disability, epilepsy and ASD as major neurodevelopmental manifestations, in addition to anxiety, hyperactivity and

impulsive behaviours (Hagerman et al., 2017). The prevalence of ASD symptoms in FXS patients reaches approximately one out of every two males and one out of every four females during early development (Hagerman et al., 2017). Molecularly, the role of fragile X messenger ribonucleoprotein 1 (*FMR1*) is associated with suppressed initiation of synaptic protein translation, since FMR1 is an mRNA-binding protein that competitively inhibits the function of eIF4E, thereby preventing protein synthesis (Thomas et al., 2023). Although still unclear how, it is widely accepted that FMR1 is capable of both regulating and being regulated by mTOR signalling.

In ASD, loss of *FMR1* expression is considered to be involved in mTORC1 pathway hyperactivity. Translational profiles and protein phosphorylation patterns of blood (lymphocytes) and post-mortem brain tissue samples from FXS patients revealed that, despite the absence of increased expression of mTOR-related genes, phosphorylation levels were found higher in the S6, S6K, AKT, eIF4E signalling proteins in both types of tissue samples (Hoeffer et al., 2012), which are the direct functional consequences of overactive mTOR signalling and are also linked to inhibition of autophagy and upregulation of mitochondrial metabolism. In *Fmr1* KO mice, hyperactivation of mTOR was associated with pronounced dysregulation of synaptic plasticity (Sharma et al., 2010). Within the hippocampus, both mTOR phosphorylation and enzymatic activity were elevated, accompanied by increased activation of its downstream effectors, S6K, 4E-BP, and eIF4F. Notably, mGluR-dependent long-term depression (LTD) was increased, yet this abnormal LTD persisted even upon pharmacological inhibition of mTOR, indicating that sustained mTOR hyperactivity uncouples synaptic protein synthesis and LTD. The study indicated that the dysregulation of mTOR is driven by upstream PI3K/AKT signalling due to the increased levels of phosphorylated AKT and increased expression of PI3K catalytic subunits. Inhibition of PI3K normalised both AKT phosphorylation and mTOR activity, highlighting a critical role of PI3K/AKT signalling in mTOR-mediated synaptic dysfunction (Sharma et al., 2010). Therefore, these models are essential for translational research to study how hyperactive mTOR signalling is involved in developmental protein synthesis at synapses and leads to ASD behaviours.

Indirectly Dysregulated mTOR Signalling Linked to ASD

While several ASD syndromes involve direct mutations in mTOR pathway components and regulators, others exhibit indirect mTOR dysregulation in which mutations in genes that are not part of the canonical mTOR cascade lead to its aberrant activation or suppression. Among

these, the Angelman syndrome is a rare and severe neurodevelopmental disorder, affecting approximately 1 per 20.000 individuals (Buiting et al., 2016). Clinical symptoms include seizures, microcephaly and motor deficits such as ataxia and dystonia/hyperreflexia (Buiting et al., 2016), along with hyperactivity, severe intellectual disability, speech impairment and ASD (Winden et al., 2018). Angelman syndrome has a genetic origin caused by mutations in the ubiquitin–protein ligase E3A (*UBE3A*) gene, resulting in the loss of enzyme function involved in substrate ubiquitination for proteasomal degradation (Buiting et al., 2016). Preclinical *Ube3A*-deficient models provide insight into the role of mTOR hyperactivation in motor deficits. It has been established that deletion of *Ube3A* alters neuromotor function and reduces spine density in Purkinje neurons due to overactive mTORC1 signalling, which in turn upregulates S6K activity in order to phosphorylate Rictor and inhibit the negative feedback loop of mTORC2/AKT, further amplifying pathway hyperactivation (Sun et al., 2015).

Given the sex-biased prevalence of ASD, exploring X-linked mutations is particularly relevant. Notably, pathogenic variants of the *RAB39B* gene have been associated with the molecular regulation of vesicular trafficking and synaptic function during neuronal development, and cause clinical manifestations of macrocephaly, intellectual disability, language and motor delay, as well as ASD with increased severity in male carriers (Woodbury-Smith et al., 2017). In mouse models, *RAB39b* deletion hyperactivates the PI3K/AKT/mTOR pathway and induces atypical behaviours (Zhang et al., 2020). Lastly, the Phelan-McDermid syndrome (PMS)-SHANK3 related is a cluster of rare neurodevelopmental disorders which clinical features include primarily intellectual disability, epilepsy, loss of balance and coordination, and ASD due to genetic alterations that inhibit the function of the postsynaptic scaffolding protein *SHANK3* (Phelan et al., 2024). Contrary to the previously discussed syndromic ASD cases, PMS models derived from *Shank3* knockdown mice reported downregulation of mTORC1 signalling due to the abnormal regulation of AKT dephosphorylation, despite the observed preclinical hallmarks of decreased spine density, synaptic transmission, and behavioural deficits, including sociability and repetitive grooming associated with ASD (Bidinosti et al., 2016). Collectively, the syndromic ASD models establish that aberrant mTOR signalling links specific but diverse genetic alterations with consistent synaptic and behavioural deficits. We will now focus on autophagy and mitochondrial homeostasis which directly reflect mTOR's impact on neuronal metabolic function during development.

Impaired Autophagy in ASD

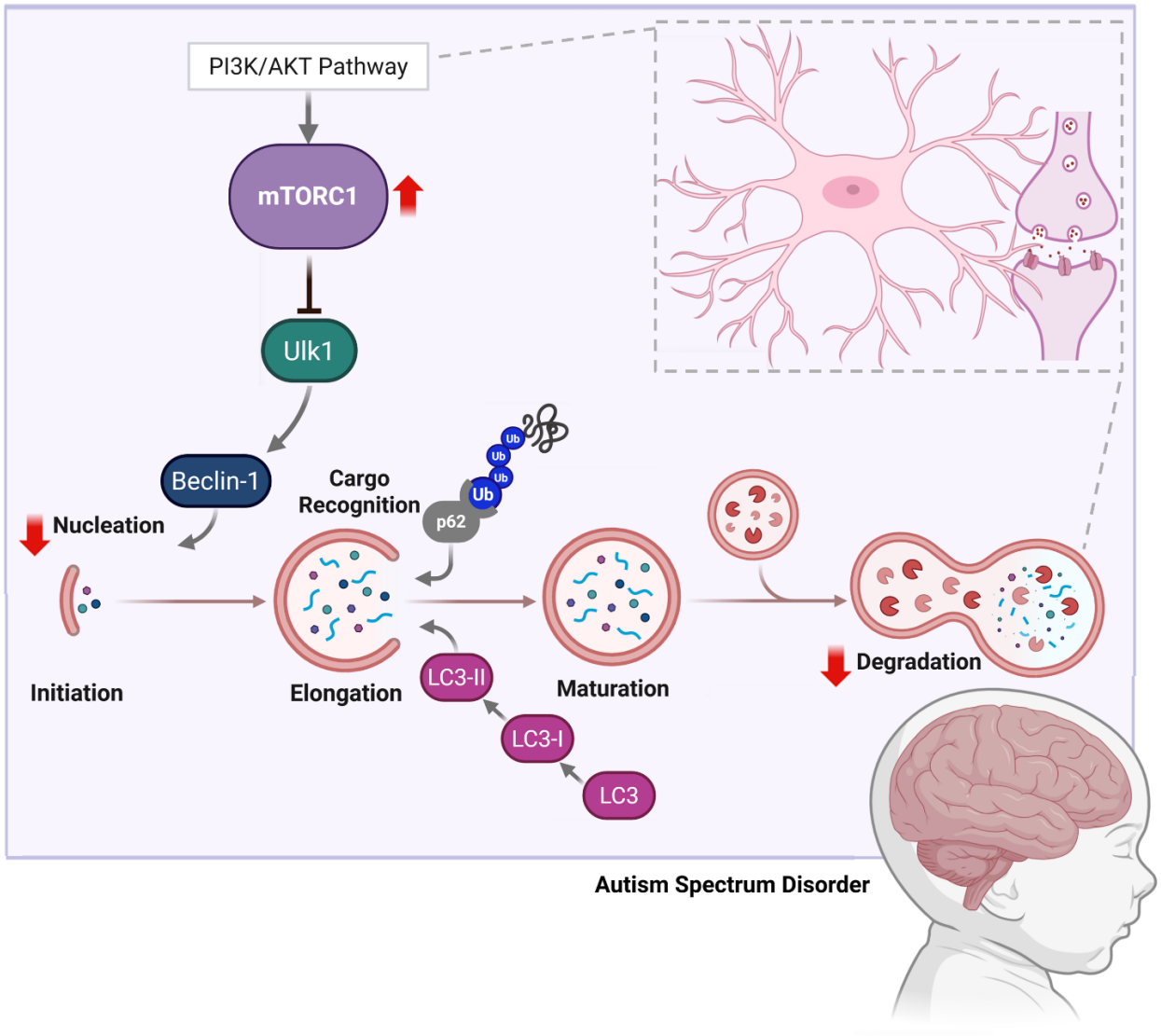

Figure 2 Overview of impaired autophagy in ASD.
Created in Biorender.

There is now considerable evidence supporting the involvement of defective neuronal autophagy via hyperactivation of mTORC1 signalling as the mechanism responsible for the irregular synaptic function observed in ASD. Accordingly, the study by Tang et al. demonstrated that mTORC1 hyperactivity prevents normal synaptic pruning during development, which was corroborated with data from postmortem brain analyses of ASD patients, *in vivo Tsc1/2* mutant

murine models and *in vitro* hippocampal neuron cultures (Tang et al., 2014). The analysis of human postmortem brains revealed the hallmark of defective pruning. Typically, spine density decreases from childhood to adolescence but the developmental pruning was found to be reduced in ASD patient samples. Morphological analyses of pyramidal neurons in the temporal cortex showed that non-ASD individuals exhibited around 45% reduction in dendritic spine density from childhood to adolescence, while ASD patients showed 16% reduction. The cortical samples revealed that the postsynaptic density marker PSD95 declined with age typically, but remained stable in ASD individuals. Additionally, increased p-mTOR and p-S6 were observed, associating the morphological differences with mTORC1 pathway hyperactivation. At the same time, autophagy markers, such as decreased LC3-II and increased p62, indicated that autophagy is halted during initiation, meaning that nucleation and autophagosome formation cannot functionally occur and cargo proteins accumulate instead of being degraded. Causality was investigated through the use of *Tsc1/2* mutant mice. In these preclinical ASD models, neuronal mTORC1 signalling was found overactive, leading to impaired spine pruning and reduced autophagy. Furthermore, loss of autophagy was directly linked to ASD phenotypes. Neuronal autophagy was genetically inhibited and *Atg7* cKO mice displayed dysfunctional pruning and ASD-like social behaviours. Importantly, pharmacological inhibition of mTORC1 via rapamycin confirmed that the hyperactive signalling suppresses autophagy, leading to failure in synaptic pruning, which manifests as ASD behaviours using different murine models. They demonstrated that both pruning and social behaviour are improved upon rapamycin treatment in *Tsc2*+/- mice, however, neither pruning nor sociability was affected in mice that functionally lacked neuronal autophagy regardless of mTORC1 hyperactivity (Tang et al., 2014). Dysfunctional autophagy has been further implicated as the mechanistic link between developmental synaptic pruning and ASD behaviours. Specifically in microglia, *Atg7* deletion was able to induce behavioural changes in mice attributed to ineffective pruning (Kim et al., 2017). Given their role during brain development, mutant microglia that lacked the ability to perform autophagy were capable of engulfing synaptic material but not of degrading it, resulting in increased synaptic density. Key social behaviours were found impaired in these conditionally mutant mice, as indicated by the three-chamber, marble-burying and nest-building assays, highlighting deficient autophagy and irregular synaptic pruning as potential molecular bases for ASD phenotypes (Kim et al., 2017).

Studies employing alternative ASD mouse models have reported similar findings. Yan et al. used *Fmr1*-KO mice to study how the pathological triad of mTORC1 hyperactivation, impaired autophagy and irregular synaptic function influences cognitive skills (Yan et al., 2018). In the hippocampus, autophagosome formation and cargo degradation were found reduced, evident by the decreased LC3-II and increased p62 levels. mTORC1 signalling was confirmed to be overactive and was linked to the aberrant phosphorylation of Ulk1 and Beclin-1 proteins, inhibiting the autophagic process. At the synaptic level, impaired autophagy led to abnormal accumulation of ubiquitinated synaptic proteins. Suppression of mTORC1 reversed these effects and improved dendritic spine density and morphology of hippocampal pyramidal neurons in an autophagy-dependent manner. By restoring the autophagic flux, *Fmr1*-KO mice demonstrated normalised synaptic plasticity and improved cognitive performance in novel object recognition tests (Yan et al., 2018), proving that autophagy disruption causally drives the impairments. In addition, Lieberman et al. investigated the role of autophagy in the deep brain during development and employed environmental models, implicating mTORC1 signalling dysfunction as the contributing factor of ASD phenotypes (Lieberman et al., 2020). This study highlighted the involvement of mTORC1 in the regulation of autophagy during the first 30 days post birth, a neurodevelopmental process that, when disrupted, is able to induce behavioural impairments in mice. In fact, the striatum of prenatally valproic acid (VPA)-treated mice exhibited aberrant mTORC1 activity, leading to suppression of Beclin-1 and reduced autophagic capacity. Much like the genetically induced ASD models, VPA mice displayed the hallmarks of impaired autophagy, i.e. decreased LC3-II and increased p62 markers, synaptic transmission and ASD behavioural phenotypes, with the latter showing improvement upon pharmacological mTOR inhibition (Lieberman et al., 2020). Seeking to integrate and contextualise relevant insights across diverse ASD models, a recent systematic review and meta-analysis examined shared molecular alterations that have been reported in literature involving mTOR signalling. The findings provide compelling support for mTOR-regulated events that are characterised by heterogeneous responses based on differences in animal species, brain regions, developmental stages, sex and genetic versus environmentally-induced models. Nonetheless, mTORC1 hyperactivation was found consistent across models, reflected by the upregulation of downstream targets and effector proteins of the pathway, as well as the negative regulation of Beclin-1 and LC3-II (Abromeit et al., 2025). Collectively, the literature points out the shared state of autophagic inefficiency due to aberrant mTORC1 signalling

in the brains of ASD model mice as the underlying molecular mechanism responsible for ASD pathogenesis during neurodevelopment, strengthening the potential translational value of these preclinical findings.

Insights from independent investigations using mice to model intellectual disability, macrocephaly and ASD have described alternative mechanisms by which loss of *RAB39b* leads to ASD phenotypes via mTORC1. In the study by Zhang et al., absence of cerebellar *RAB39b* was found to contribute to the hyperactivation of PI3K/AKT/mTORC1 signalling, which was correlated with recognised ASD behaviours (Zhang et al., 2020). However, autophagy involvement was excluded based on the levels of autophagic markers and the lack of regulation of class III PI3K components. Instead, the researchers proposed that loss of *RAB39b* results in hyperactive mTORC1 signalling during early brain development through dysregulation of class I PI3K components and downstream activity, thereby promoting abnormal proliferation and differentiation of cortical progenitor cells with consequent structural abnormalities of macrocephaly and atypical behaviours of ASD (Zhang et al., 2020). On the contrary, Niu et al. demonstrated that deletion of *RAB39b* triggers mTORC1 hyperactivation, impairing synaptic homeostasis and plasticity, and rendering neurotransmission dysfunctional in hippocampal neurons via an autophagy-dependent mechanism (Niu et al., 2020). Moreover, disruption of autophagy gave rise to specific defects in learning, memory and social behaviours, linked to the role of hippocampal function. In detail, *Rab39b* mutant mice performed poorly in tasks regarding motor skill learning, object recognition, short-term working and spatial memory, and social novelty recognition, all of which are common ASD manifestations (Niu et al., 2020). Therefore, mTORC1 hyperactivation might be implicated in more than one hallmark of ASD through distinct molecular mechanisms beyond autophagy regulation, according to the developmental stage and the brain regions that are mostly affected.

Despite the general consensus that hyperactive mTORC1 signalling suppresses autophagy during the early stages of nucleation and autophagosome formation, consequently inducing ASD phenotypes, research has also implicated variable findings and interpretations. In a study that *Shank3*-mutant mice were used as PMS models to investigate autophagy in ASD, distinct expression profiles were reported that reflected hallmarks of synaptic dysfunction (Deri et al., 2025). However, both LC3-II and p62 were found elevated, which substantiated the hypothesis that there is an underlying mechanism capable of initiating autophagy upregulation, yet cargo

degradation does not occur. They proposed that in *Shank3*-mutant mice, decreased expression of *Tsc2* suppresses the inhibitory signal of mTORC1, which blocks the initial stages of autophagy but this threshold is surpassed by oxidative species that promote autophagosome formation. In addition, mTORC1 signalling influences lysosomal membrane integrity by lysosomal-associated membrane protein 1 (LAMP1) destabilisation, blocking the fusion of autophagosomes and lysosomes, thus reducing autophagy degradation (Deri et al., 2025). It should be acknowledged again that in PMS models, mTOR has been found downregulated (Bidinosti et al., 2016), suggesting variability across studies. Mutations in the ASD-risk related gene *Arrb2*, involved in the neuronal immune functions of microglia, have been also linked to hyperactive AKT/mTOR signalling and reduced autophagic capacity (Peng et al., 2023). In the hippocampus of *Arrb2* KO mice, decreased LC3-II and increased p62 autophagy markers indicated impaired autophagosome formation and flux, AKT and mTOR phosphorylation were confirmed to be elevated and evidence of dysregulated mitochondrial metabolism was observed with decreased membrane potential and subsequent reduction of adenosine triphosphate (ATP) synthesis and increased oxidative burden. Nonetheless, deletion of *Arrb2* was not sufficient to produce ASD behavioural phenotypes and mice exhibited typical performance in assays, including three-chamber test, open field test, elevated plus maze and Y-maze test for repetitive behaviour (Peng et al., 2023). Therefore, the core pathology of hyperactive mTORC1 signalling in ASD may not be attributed to the universal suppression of autophagy, but rather to a complex and context-dependent dysregulation that can be influenced by the genetic background. Taken together, while the evidence sets autophagy as a central metabolic process influenced by mTOR signalling in ASD models, a closer look at the way mitochondrial activity is influenced may help integrate the current knowledge on neuronal metabolic dysfunction in ASD.

Mitochondrial Abnormalities & Metabolic Dysfunction in ASD

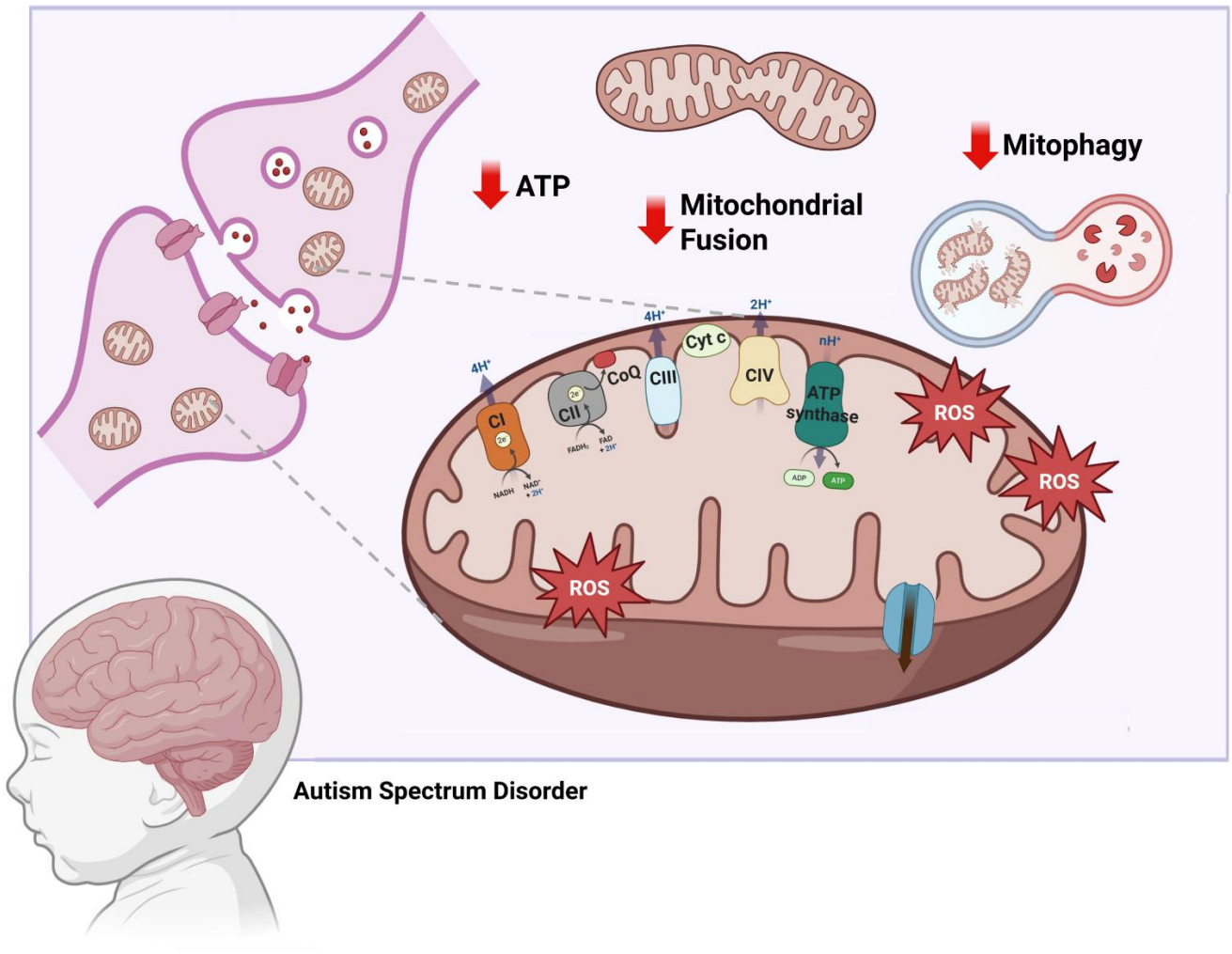

Figure 3 Overview of synaptic mitochondrial dysfunction in ASD.
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Converging data consistently support the involvement of mTORC1 signalling in molecular hallmarks of ASD, including mitochondrial abnormalities and metabolic dysfunction. As shown by Ebrahimi-Fakhari et al., mTORC1 hyperactivation contributes to impaired mitochondrial turnover and metabolic support at axonal and presynaptic sites in neurons of *Tsc1/2* transgenic mice (Ebrahimi-Fakhari et al., 2016). Notably, loss of *Tsc2* induced accumulation of dysfunctional mitochondria, without evident upregulation of mitochondrial biogenesis. Instead, mTORC1 hyperactivation was involved in deficient turnover of neuronal mitochondria with aberrant morphology, depleted respiration and consequently, decreased membrane potential. The process

of mitophagy was halted at the stages of autophagosome formation and lysosomal fusion, evident by the accumulation of the mitophagy/autophagy markers PINK1, Parkin, p62 and LC3-II. Specifically in hippocampal axons, loss of *Tsc2* was found to influence the local recruitment of mitochondria, which appeared motile but unstable, and preferentially transported towards the soma and away from the axon. At the presynaptic level, mitochondria failed to be transported and maintained in sufficient numbers on site, likely contributing to dysregulated synaptic transmission. Similar findings were corroborated in *Tsc1*-mutant mice as well as TSC patient-derived iPSCs differentiated into cortical neurons (Ebrahimi-Fakhari et al., 2016). Thus, absence of negative mTORC1 regulation by the TSC complex disrupts the mitochondrial quality control capacity of neurons and causes metabolic challenges by decreased energy supply at presynaptic sites.

mTORC1 hyperactivity caused by the loss of negative regulation via PTEN has been implicated in the impaired metabolic support of developing dendrites. Murine models with *Pten* cKO in the Purkinje cells of the cerebellum provided insight into how aberrant mTOR signalling contributes to the mitochondrial imbalance that affects dendritic connectivity during early-life development (Walsh et al., 2025). During the first two weeks of development, the *Pten* cKO cerebellar dendrites exhibited a spike of elongation and branching, which then was reversed. By the first month, cerebellar Purkinje cells were found abnormal in structural integrity and synaptic connectivity, resulting from decreased spine density, reduced dendrite thickening and excessive neurofilament proteins. Markedly, mTOR hyperactivation was observed after the first two weeks of development and so was the increased phosphorylation of downstream signalling proteins. Regarding the mitochondria, *Pten* cKO triggered reduced activity, degradation and irregular transport from soma to dendrite, rendering the synapses unable to maintain energy homeostasis. These molecular findings were supplemented by ASD-linked traits in motor and social behaviour, with sex-specific exemptions, that include early motor deficits in females and more hyperactive behaviours, while males exhibited deteriorated fine motor skills and social traits (Walsh et al., 2025). Additionally, a study by Feng et al. has proposed an alternative PTEN-controlled signalling pathway that is involved in neuronal bioenergetics and mitochondrial biogenesis (Feng et al., 2021). *Pten*-deficient primary neurons were characterised by increased mitochondria paired with increased activity of ETC I. They demonstrated that PTEN inhibition stimulates the generation of mitochondria via the AKT/GSK3β/PGC−1α pathway. When PTEN is absent, the activity of AKT is elevated, which induces the phosphorylation of glycogen synthase kinase 3 beta (GSK3β) and

its subsequent deactivation. With GSK3β inactive, peroxisome proliferator-activated receptor-gamma coactivator 1-alpha (PGC−1α) signalling can recruit the transcription of nuclear respiratory factor 1 (NRF−1) and mitochondrial transcription factor A (TFAM) to regulate the expression of nuclear and mitochondrial genes, respectively, to produce new mitochondria. In VPA-treated mice with confirmed ASD behavioural phenotypes, PTEN abundance was reduced, contrary to the increased levels of both PGC−1α and cytochrome c oxidase IV (COX IV), which are indicative of increased mitochondrial content (Feng et al., 2021). Whether the upregulation of mitochondrial biogenesis via AKT/GSK3β/PGC−1α results in functional organelles that can participate in the synaptic metabolic demands remains unknown. Nevertheless, recognising that there is an alternative pathway capable of regulating mitochondrial biogenesis suggests additional translational opportunities that could target the energy imbalance in neurodevelopmental disorders.

In a study by Shen et al., *FMR1* loss is implicated in insufficient mitochondrial respiration and defective synaptic connectivity in the forebrain, which is tightly linked to its role in protein synthesis (Shen et al., 2019). *Fmr1*-deficient mice exhibited obstructed maturation of dentate gyrus neurons, less morphologically complex dendrites with shorter and fewer branches and reduced excitatory signalling. These immature neurons had distinct transcriptional profiles of differential gene expression involved in neuronal maturation, synaptic function, organelle organisation, intracellular trafficking, metabolism and were characterised by increased oxidative burden. Dysfunctional mitochondria displayed fragmented structures. Supporting this, *Mfn1* and *Mfn2* genes were expressed at lower levels, indicating reduced mitochondrial fusion. Importantly, impaired neuronal function was attributed to deficient mitochondrial fusion, as both pharmacological and genetic upregulation of *Mfn2* rescued mitochondrial morphology and restored dendritic growth and branching. ASD behavioural phenotypes were also rescued by the pharmacological treatment (Shen et al., 2019). During the developmental peak of synapse formation, postnatal mice with loss of *Fmr1* were found to have dysfunctional mitochondria due to increased electron leak through open cyclosporine A-sensitive mitochondrial pores (mPTP), which induce ROS (Griffiths et al., 2020). This, coupled with the observed increase in glucose fermentation and hyperactive mitochondrial enzymes, indicated the inefficiency of the mitochondria to maintain the proton gradient and ATP levels, even though they require a high rate of substrate for oxidation. Nevertheless, treatment with coenzyme Q10 (CoQ) to promote electron shuttle in the inner membrane ameliorated the effects of the inefficient proton pumping, rescued

the synaptic density in the dendrites, but only partially improved the ASD-phenotypes, not including social behaviour (Griffiths et al., 2020). Similarly, the neuronal mitochondria of older *Fmr1*-deficient mice, although active, failed to maintain the bioenergetic capacity of the cell and displayed decreased levels of ATP (D'Antoni et al., 2020). However, this was not accompanied by a glycolytic flux and subsequent production of lactate to replenish ATP levels, suggesting a different metabolic profile that the one observed during neurodevelopment. Instead, the observed hyperactive state of all mitochondrial respiratory chain complexes suggests that oxidative phosphorylation remains as an active route for energy production. Furthermore, research found that the mitochondria of *Fmr1*-deficient neurons are in a state of metabolic inflexibility, evident by the increased mitochondrial expression of glycerol-3-phosphate dehydrogenase (G3PDH), which indicates that glycerol-phosphate is used as the primary shuttle to fuel the electron transport chain (ETC), resulting in lower ATP yield and increased reactive oxygen species (ROS) production. Indeed, higher levels of ROS were observed in older adult mice, but not in younger ones, suggesting that long-term mitochondrial hyperactivation leads to the accumulation of oxidative damage, especially as antioxidant mechanisms are suppressed with age (D'Antoni et al., 2020). In sum, *Fmr1* loss disrupts the efficacy of mitochondrial metabolism by blocking mitochondrial fusion and energy production that manifests with age as metabolic inflexibility and increased oxidative stress.

Building on the preceding literature that links mitochondrial dysfunction to ASD, Angelman syndrome provides another example in which disrupted metabolism and synaptic signalling contribute to ASD phenotypes. Murine models that lacked *Ube3a* expression were reported to exhibit certain metabolic vulnerabilities, especially affecting the CA1 region of the hippocampus, which is known to have increased energy demands to maintain synaptic activity. Hailing Su et al demonstrated that mutations in the *Ube3a* gene result in irregular mitochondrial morphology and structure in CA1 neurons, with evident oxidative damage, condensed organelle size and damaged cristae while mitochondrial abundance remained stable (Su et al., 2011). As a consequence, neuronal communication and synaptic transmission deteriorated, evident by the reduced presynaptic vesicle turnover. These events were coupled with insufficient ETC complex III function, suggesting that the structural mitochondrial abnormalities and respiratory dysfunction contribute to the synaptic impairment (Su et al., 2011). As an approach to establish the relationship between oxidative stress and ASD behavioural traits, Santini et al. focused on mitochondrial

metabolism in *Ube3a*-deficient mice (Santini et al., 2015). The study corroborated that CA1 neurons display impaired synaptic plasticity due to the increase in mitochondrial ROS production. Interestingly, the *Ube3a*-mutant mice performed poorly on both cognitive and motor assays, indicating that the mitochondrial oxidative stress could influence skills that are not specific to the function of the hippocampus and can extend to other regions like the cerebellum. However, by targeting mitochondrial ROS production to diminish the oxidative burden, the memory deficits were reversed, but the motor coordination deficits persisted, implying that excess hippocampal mitochondrial ROS is selectively responsible for the cognitive performance (Santini et al., 2015). In conclusion, hyperactive mTORC1 signalling is a central mechanism that links many distinct genetic causes of ASD to disruption of mitochondrial function and synaptic energy support that manifest as atypical ASD-like behavioural traits.

Discussion

In this essay, signalling dysregulation of the mTOR pathway emerges as a key factor in ASD pathogenesis. As a central hub of metabolic regulation, mTOR activity has been implicated in critical neurodevelopmental processes that require precise fine-tuning between anabolic growth and catabolic activity during periods of nutrient fluctuations and intercellular quality control (Deleyto-Seldas & Efeyan, 2021). Preclinically, the aberrant activation of mTOR signalling has been established as a shared molecular feature in multiple models of syndromic forms of ASD, including TSC, PHTS, FXS, Angelman syndrome and PMS, and despite the functional diversity of the genetically altered genes, mTOR dysregulation consistently correlates with defective neuronal development, disrupted connectivity and atypical behavioural phenotypes. Nonetheless, due to the complexity of mTOR signalling, claims of its involvement in ASD pathogenesis are insufficient without specifying the mechanisms affected by its regulation. As a consequence of mTOR hyperactivation, impaired autophagy is linked to suppressed and ineffective synaptic pruning during early brain development, altering neuronal connectivity and the way synaptic signalling is processed. Furthermore, neurons are rendered metabolically vulnerable by depriving energy supply for synaptic function while simultaneously inducing mitochondrial oxidative damage and suppressing quality control mechanisms like mitophagy. Collectively, evidence from patient-derived data and behavioural mouse models, induced by either environmental or genetic factors, provides strong support that these metabolic events are implicated in the aetiology of ASD and constitute the underlying molecular mechanisms that contribute to its neurodevelopmental pathogenesis.

Since mTOR dysregulation links neuronal pathology to behavioural deficits, it is rational to consider mTOR as a potential target for therapeutic intervention. Everolimus is an FDA-approved anti-epileptic compound that acts as an mTOR inhibitor and has been proven effective for the treatment of seizures in TSC patients (French et al., 2016). However, clinical trials of everolimus in TSC-associated ASD have yielded no therapeutic benefit (Krueger et al., 2017; Overwater et al., 2019). TSC patients aged 4–17 years without comorbid epileptic seizures reported no measurable improvement in symptoms related to ASD, social deficits, communication, executive functioning skills, sleep and sensory regulation after 12 months of everolimus administration (Overwater et al., 2019). As a consequence, preclinical findings examining

alternative targets may be particularly informative. The reviewed literature in this essay indicates that targeting pathways that maintain intracellular quality control or metabolic homeostasis rescues ASD deficits in mouse models. When autophagy is restored by silencing the expression of Raptor in neurons through lentiviral transduction, both synaptic and cognitive impairments are normalised (Yan et al., 2018). Improving mitochondrial fusion has also shown therapeutic potential since it restores late neuron maturation and thus synaptic function, as well as both cognitive and behavioural performance (Shen et al., 2019). In addition, interventions that ameliorate defects of oxidative phosphorylation with antioxidants have been proposed. Notably, administration of CoQ during synaptic development improves ASD traits like repetitive behaviours and hyperactivity but does not affect sensory processing, anxiety and importantly, social behaviour (Griffiths et al., 2020). Lastly, administration of MitoQ, a CoQ derivative designed to accumulate in the mitochondria to neutralise ROS, leads to cognitive improvements in memory tasks specifically (Santini et al., 2015). Since oxidative stress in developing neurons is often a secondary phenomenon, due to the higher antioxidant capacity of younger cells, the efficacy of antioxidants during early life might be limited, while targeting autophagy or mitochondrial fusion shows broader improvements that include social and cognitive behaviours. Although these findings are preclinical they provide a strong rationale for considering them as candidate therapeutic targets and they highlight the need for further research to examine their translational potential as a monotherapy or in combination.

The lack of translational success in behavioural and cognitive improvement may reflect the limitations of current preclinical models, though useful for mechanistic insight. The variability across species, brain regions, developmental stages, sex and experimental parameters in preclinical ASD models, and the clinical heterogeneity of ASD patients may explain this gap between outcomes. Specifically for neurodevelopmental disorders, limitations often stem from the challenge of replicating human behaviours in rodents, since rodents lack complex social cognition and language, as well as from the differences in the neurodevelopmental period, including duration, complex cortical development and neuronal heterogeneity (Zhao & Bhattacharyya, 2018). To address some of the limitations of rodent studies, patient-derived models give the ability to study molecular pathways in a context that better reflects human biology and patient-specific genetic variation which may increase translational value (Zhao & Bhattacharyya, 2018). Another factor that may explain the lack of efficacy is that the events underlying ASD pathogenesis occur

during early neurodevelopment and may only be partially reversible by the time of intervention, limiting the potential for behavioural improvement. To understand when interventions are most effective, it is crucial to study both behavioural and molecular changes from early development to adulthood. For example, mTOR inhibition improves social behaviour in adult *Tsc1* mutant mice when administered during the third postnatal week (Amegandjin et al., 2021). Furthermore, the underrepresentation of females in existing literature limits the applicability of conclusions and highlights the necessity of examining both sexes to represent the whole spectrum of phenotype variation in ASD individuals (Ferri et al., 2018).

Ultimately, the discussed findings support that the aetiology of ASD involves dysregulation of mTOR signalling, impaired autophagy and mitochondrial dysfunction, and while they provide the potential for therapeutic intervention, translating them into meaningful clinical targets relies on targeting the specific pathways driving dysfunction in each individual, i.e., personalised strategies. Nevertheless, future research to understand the genetic and neurodevelopmental basis of ASD is justified, as a means to reduce disease burden and improve patient quality of life, as a means to uncover the fundamental mechanisms of human social behaviour and communication, and as a means to challenge the stigma and recognise neurodiversity as a form of biological variation.

List of Abbreviations

4EBP	eIF4E-binding Protein
ADHD	Attention deficit hyperactivity disorder
AKT	Protein kinase B, PKB
ASD	Autism spectrum disorder
ATG	"Autophagy-related" gene or protein
ATP	Adenosine triphosphate
CA1	Cornu ammonis 1
cKO	Conditional knockout
CoQ	Coenzyme Q10
COX IV	Cytochrome c oxidase IV
eIF4E	Eukaryotic initiation factor 4E
eIF4F	Eukaryotic initiation factor 4F
ETC	Electron transport chain
FDA	Food and drug administration
FMR1	Fragile X messenger ribonucleoprotein 1
FXS	Fragile X syndrome
G3PDH	Glycerol-3-phosphate dehydrogenase
GSK3β	Glycogen synthase kinase 3 beta
iPSC	Induced pluripotent stem cell
KO	Knockout
LAMP1	Lysosomal-associated membrane protein 1
LC3	Microtubule-associated protein 1 light chain 3
LTD	Long-term depression
MFN1	Mitofusin 1
mGluR	Metabotropic glutamate receptor
mPTP	Cyclosporine A-sensitive mitochondrial pores
mTORC1	Mechanistic target of rapamycin complex 1
mTORC2	Mechanistic target of rapamycin complex 2
NRF-1	Nuclear respiratory factor 1
p62	Sequestosome 1
PGC−1α	Peroxisome proliferator-activated receptor-gamma coactivator 1-alpha
PHTS	PTEN hamartoma tumour syndromes
PI3K	Phosphatidylinositol-3 kinase
PINK1	PTEN-induced kinase 1
PMS	Phelan-McDermid syndrome
PSD95	Postsynaptic density protein 95

PTEN	Phosphatase and tensin element on chromosome 10
RAB39B	Ras analogue in brain 39b
ROS	Reactive oxygen species
S6	Ribosomal Protein S6
S6K	Ribosomal protein S6 kinase
SHANK3	SH3 and multiple ankyrin repeat domains 3
TFAM	Mitochondrial transcription factor A
TSC	Tuberous sclerosis complex
UBE3A	Ubiquitin–protein ligase E3A
ULK	Unc-51-like kinase
VPA	Valproic Acid

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