The importance of fasciculation and elongation protein zeta-1 in neural circuit establishment and neurological disorders

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Abstract

The human brain contains an estimated 100 billion neurons that must be systematically organized into functional neural circuits for it to function properly. These circuits range from short-range local signaling networks between neighboring neurons to long-range networks formed between various brain regions. Compelling converging evidence indicates that alterations in neural circuits arising from abnormalities during early neuronal development or neurodegeneration contribute significantly to the etiology of neurological disorders. Supporting this notion, efforts to identify genetic causes of these disorders have uncovered an over-representation of genes encoding proteins involved in the processes of neuronal differentiation, maturation, synaptogenesis and synaptic function. Fasciculation and elongation protein zeta-1, a Kinesin-1 adapter, has emerged as a key central player involved in many of these processes. Fasciculation and elongation protein zeta-1-dependent transport of synaptic cargoes and mitochondria is essential for neuronal development and synapse establishment. Furthermore, it acts downstream of guidance cue pathways to regulate axo-dendritic development. Significantly, perturbing its function causes abnormalities in neuronal development and synapse formation both in the brain as well as the peripheral nervous system. Mutations and deletions of the fasciculation and elongation protein zeta-1 gene are linked to neurodevelopmental disorders. Moreover, altered phosphorylation of the protein contributes to neurodegenerative disorders. Together, these findings strongly implicate the importance of fasciculation and elongation protein zeta-1 in the establishment of neuronal circuits and its maintenance.

Key Words: fasciculation and elongation protein zeta-1; neurological disorder; neuronal development; neuronal differentiation; neuronal networks; synapse formation; synaptic function

Introduction

The fundamental underpinning of our brain function lies undeniably in its dense and complex networks of interconnected neurons. The estimated 100 billion neurons contained in the normal human brain are systematically and intricately organized into functional units ranging from local networks connecting neighboring neurons to long-range networks that span across different brain regions (Richards and Van Hooser, 2018). Formation of neural circuits is organized into distinct phases stretching over 20 years (Birnbaum and Weinberger, 2017). Neurogenesis and neuronal migration, the earliest phases, occupy only a small fraction of this process (Molnar et al., 2019). In comparison to this, functional wiring (synaptogenesis) and optimization (synaptic pruning) of the neural circuits extend into young adulthood, reflecting the incredible complexity involved in their establishment, wiring and optimization. Indeed, perturbations affecting these two critical phases of brain formation (i.e. initial wiring and subsequent optimization) are believed to be the origins of neuropsychiatric disorders including schizophrenia and autism-spectrum disorders (Birnbaum and Weinberger, 2017).

Functional wiring is a dynamic and highly complex process regulated by a diverse assortment of seemingly disparate signaling pathways to coordinate axon and dendrite specification, elongation, arborization and guidance. These events eventually lead to synaptogenesis when the two processes come into contact. Functional wiring is closely tied to the completion of neuronal migration and begins when differentiating neurons project axons and dendrites to their relevant cellular connections, guided by both extrinsic and intrinsic cues (Ledda and Paratcha, 2017). Upon reaching their cellular targets, additional signaling by trans-synaptic signaling molecules allow contacting axons and their target dendrites to initiate synapse formation (Sudhof, 2018). Strikingly,

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many of these pathways ultimately converge to modulate cytoskeletal dynamics and intracellular transport. This intersection is critical given that the rapid elongation of both types of processes and the subsequent formation of up to ten thousands of synapses in each neuron must be efficiently coordinated and supported by the synthesis and delivery of an incredible amount of biological raw materials to the sites of neurite expansion and synapse development (Rizalar et al., 2021). Indeed, defects in many proteins involved in these pathways are linked to human neurological disorders with severe mental disabilities, mostly involving cognitive and motor dysfunctions (Sleigh et al., 2019).

One protein that has been inextricably linked to many of the abovementioned processes is known as fasciculation and elongation protein zeta-1 (FEZ1). UNC-76, the nematode homolog of FEZ1, was first discovered in *Caenorhabditis elegans* (*C. elegans*) as a protein essential for normal development of its nervous system (Desai et al., 1988; Bloom and Horvitz, 1997). Since then, other studies have highlighted its importance in neuronal development, and the formation and maintenance of synapses in both the central as well as peripheral nervous system. These findings strongly indicate that loss of FEZ1 in neurons can trigger alterations that affect the formation and maintenance of central and peripheral neuronal networks that underlie neurodevelopmental and neurodegenerative disorders. This review highlights recent advances in our knowledge of FEZ1's function and its relevance in causing neurological disorders.

Search Strategy and Selection Criteria

Articles cited in this review published from 1974 to 2021 were searched on the PubMed database using the following keywords: fasciculation and elongation protein zeta 1, Unc-76, neuronal development, neurodegeneration, neurodevelopmental disorders, schizophrenia, Jacobsen syndrome, autophagy. Results were further filtered by title and abstract.

Fasciculation and Elongation Protein Zeta-1 Is Selectively Expressed in Nervous Tissues

Unc-76, the *C. elegans* homolog of the mammalian *FEZ1* gene, was first identified as part of a group of genes associated with locomotory behavior in a series of elegant studies (Brenner, 1974; White et al., 1976). Subsequent studies determined that the gene is essential for axon outgrowth and guidance and further highlighted its restricted expression to nervous tissues at all stages of development (Hedgecock et al., 1985; Desai et al., 1988; Bloom and Horvitz, 1997). In mammals, a similarly restricted pattern of expression to nervous tissues is observed (Fujita et al., 2004; Honda et al., 2004; Sakae et al., 2008). In rodent brains, FEZ1 could already be detected prenatally and increases with development. High levels of FEZ1 expression persist in both rat and human adult brains (Whitehouse et al., 2002). Within the brain, regional differences in expression are observed, with the prefrontal cortex and cerebellum expressing the highest levels of the protein (Sakae et al., 2008; Gunaseelan et al., 2021). FEZ1 is also notably well-expressed in the spinal cord. Both neurons and astrocytes appear to express FEZ1 (He et al., 2009; Butkevich et al., 2016; Gunaseelan et al., 2021). Mammalian genomes contain a separate gene encoding the related *FEZ2* that has a much broader expression but whose function is much less understood (Fujita et al., 2004). A word of caution – it should be noted that *FEZ1*/*Unc-76* is often erroneously confused with another mammalian gene named *LZTS1* (unfortunately also known as *FEZ1*) in the literature as well as public databases. Careful examination shows these genes are distinct from each other and located on different chromosomes. Sequence comparisons of their gene products (i.e., mRNA and protein) do not show any significant alignment. As an example, annotations of the human orthologs of both genes as derived from the NCBI Gene database is shown in **Table 1**.

Table 1 | **Comparison between human *FEZ1* and *LZTS1***

Official gene symbol/ Full name	Synonyms	Chromosomal location	Length (aa)	Major function
FEZ1				
Fasciculation and elongation protein zeta 1	UNC-76	11q24.2	392	Kinesin-1 adapter
LZTS1				
Leucine zipper tumor suppressor 1	F37; FEZ1	8p21.3	596	Transcription factor

Fasciculation and Elongation Protein Zeta-1 as a Kinesin-1 Adapter for Intracellular Transport in Neurons

Possibly the most studied function of FEZ1 relates to its role as a Kinesin-1 adapter. Concomitant binding of FEZ1 and JIP1 to the tail of Kinesin-1 was reported to activate the motor (Blasius et al., 2007). Other studies have confirmed the FEZ1/Kinesin-1 interaction and further identified mitochondria as well as synaptic proteins as major cargoes of the FEZ1/Kinesin-1 motor complex in neurons (Gindhart et al., 2003; Ikuta et al., 2007; Toda et al., 2008; Chua et al., 2012; Butkevich et al., 2016; Sure et al., 2018). In all instances, binding to motor and cargoes require the coiled coil domain of FEZ1.

The function of FEZ1 in axonal transport is regulated by kinases. In particular, phosphorylation of the conserved Serine residue (positions 58 and 143 in mammalian and *Drosophila* FEZ1, respectively) by ATG1/UNC-51 or microtubule affinity regulating kinases is required for axonal transport of synaptic cargoes (Toda et al., 2008; Butkevich et al., 2016; Malikov and Naghavi, 2017). Additionally, PKCζ phosphorylates FEZ1 at its C-terminal region although its relevance to transport remains unknown (Kuroda et al., 1999; Lanza et al., 2009).

Fasciculation and Elongation Protein Zeta-1 Is Required for Axo-Dendrite Development

During brain development, developing neurons send projecting axons and dendrites to their targets. Transport of biomolecules is crucial to support the growth of rapidly extending neuronal processes and, thus, indispensable for the formation of neural circuits (Aiken and Holzbaur, 2021). Early studies on UNC-76 in *C. elegans* first illustrated its importance in axonal outgrowth and guidance. In particular, chemosensory neurons of *unc-76* mutant worms exhibited ectopic branching with enlarged axonal terminals that terminated prematurely (Hedgecock et al., 1985). Moreover, similar defects in axonal outgrowth and bundling were observed in the hermaphrodite-specific neuron motor neurons of *unc-76* mutant worms, where the axons failed to grow in normal fascicles (Bloom and Horvitz, 1997).

The role of mammalian FEZ1 in axo-dendritic development is evolutionarily conserved (**Figure 1**). Using nerve growth factor-treated PC12 cells as a model for neuritogenesis, expression of FEZ1 temporally coincided with the appearance of neurites in a manner dependent on its phosphorylation by PKCζ as well as ubiquitinylation by the U-box-type ubiquitin ligase E4B (Kuroda et al., 1999; Okumura et al., 2004). More importantly, recent studies employing CRISPR-based strategies further demonstrate that both axon and dendrite development in developing hippocampal neurons are significantly impaired when FEZ1 expression is abrogated (Chua et al., 2021). In particular, total axonal length and the length of the longest axon were significantly shortened in FEZ1-deficient

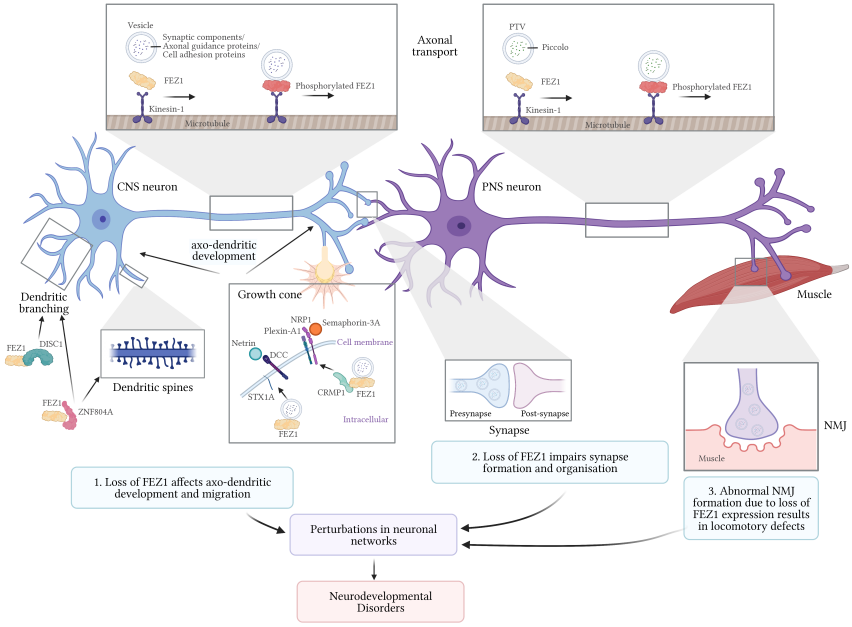

Figure 1 | **FEZ1 is critical for neurodevelopment.** In CNS and PNS neurons, FEZ1 participates in both axo-dendritic development as well as synapse formation and organization. (1) FEZ1 acts downstream effector of guidance cue signaling pathways to regulate axo-dendritic development and migration. FEZ1 also interacts with DISC1 or ZNF804A to modulate dendrite length, branching and short spine density. (2) FEZ1/Kinesin-1 complexes transport synaptic components essential for synapse formation and organization. (3) Likewise, FEZ1 role's in transporting synaptic components during motor neuron development is essential for proper formation of neuromuscular junctions (NMJ). Loss of FEZ1 in these processes eventually leads to neuronal network perturbations and potentially contributes to neurodevelopmental disorders. CNS: Central nervous system; CRMP1: collapsin response mediator protein 1; DCC: deleted in colorectal cancer; DISC1: disrupted in schizophrenia 1; FEZ1: fasciculation and elongation zeta 1; NMJ: neuromuscular junction; NRP1: neuropilin 1; PNS: peripheral nervous system; PTV: piccolo transport vesicle; STX1A: syntaxin-1A; ZNF804A: zinc finger protein 804A. Image was created using Biorender graphic software (https://biorender.com/).

neurons *versus* control neurons. Axonal branching was also dramatically reduced. Dendrites in FEZ1-deficient neurons showed markedly reduced complexity and lesser branching as compared to control neurons. Motor neurons lacking FEZ1 expression also exhibited similar abnormalities in axo-dendritic development (Gunaseelan et al., 2021). Additionally, total dendrite length was shorter in FEZ1-deficient motor neurons versus control motor neurons. Moreover, neurons in *unc-76* mutant worms showed impaired ability to regenerate axons following laser axotomy (Chen et al., 2011). Neurite development defects in cortical neurons knocked down for or expressing zinc finger protein 804A (ZNF804A), a FEZ1-binding protein, appears to be rescued by over-expressing FEZ1 (Dong et al., 2021). Curiously, loss of FEZ1 in an adult hippocampal neurogenesis model reportedly induces over-proliferation of dendrites without impairing axon development (Kang et al., 2011).

Fasciculation and Elongation Protein Zeta-1 Acts Downstream of Guidance Cues to Regulate Axo-Dendrite Development

An outstanding question in intracellular transport pertains to how motors are targeted to deposit their cargoes at sites where they are required. Nowhere is this more relevant than during the wiring of neural circuits during brain development. Migration of developing axons and dendrites to their cellular targets during the formation of neural circuits depends on guidance cue signaling (Ledda and Paratcha, 2017; Yogev and Shen, 2017; Bellon and Mann, 2018; Pinto-Costa and Sousa, 2021). Growth cones located at neurite tips direct migrating axons and dendrites to their synaptic targets through interactions with various guidance cues such as Netrins, Slits, Semaphorins and Ephrins (Seiradake et al., 2016). Through their receptors, these guidance molecules regulate the activity of Rho-family GTPases through guanine nucleotide exchange factors and GTPase activating proteins, which further result in modulating cytoskeletal dynamics (Niftullayev and Lamarche-Vane, 2019; Pasterkamp and Burk, 2021). However, how guidance cue signaling contributes to targeting delivery of cargoes towards or away from navigating neuronal processes remain largely known.

Strikingly, FEZ1 is present in growth cones of cultured rat hippocampal neurons and human motor neurons (Miyoshi et al., 2003; Chua et al., 2012; Gunaseelan et al., 2021). This suggested that it may be involved in guidance cue-mediated delivery of cargoes to direct expansion (or retraction) of migrating neuronal processes. Indeed, FEZ1 forms separate complexes with the Netrin-1 receptor Deleted in Colorectal Cancer and Semaphorin-3A receptor complex (Plexin-A1 and neuropilin-1) (Chua et al., 2021). FEZ1 also binds CRMP1 (a downstream Semaphorin-3A effector) and Syntaxin-1A (required for Netrin-1 mediate exocytosis in axonal growth cones) (Cotrufo et al., 2011; Chua et al., 2012). Importantly, treatment of control neurons with Netrin-1 or Semaphorin-3A increased exuberance of dendritic branches and axonal growth. In marked contrast to this, FEZ1-deficient neurons are unresponsive to either stimulation and axo-dendrite development remains stunted. Collectively, these results indicate that the protein acts as a convergence point downstream of guidance cues signaling pathways to regulate targeted delivery of cargoes to migrating neurites as they navigate to their destinations.

Fasciculation and Elongation Protein Zeta-1 Contributes to Synapse Formation and Organization through the Delivery of Synaptic Proteins

Chemical synapses play predominant roles in most neural circuits. It consists of two juxtaposed components from two adjacent neurons, namely the presynaptic specialization (in axon) and postsynaptic density (in dendrite) (Chua et al., 2010). Presynaptic specializations are regions of cell terminals with neurotransmitters-filled synaptic vesicles, which can be released at the presynaptic active zones upon stimulation. Meanwhile, postsynaptic densities are enriched with neurotransmitter receptors, channels and downstream signaling molecules to sense the released neurotransmitters in the synaptic cleft (Kaizuka and Takumi, 2018). Importantly, synaptic transport is a crucial process that ensures a continuous supply of synaptic proteins and organelles to synapses while preventing build-up of defective synaptic components at the terminals in order to maintain synaptic homeostasis (Guedes-Dias and Holzbaur, 2019; Vasudevan and Koushika, 2020).

As previously mentioned, FEZ1 is an important adapter for Kinesin-1-mediated anterograde transport in axons. Synaptic proteins constitute major cargoes transported by the FEZ1/Kinesin-1 transport complex in neurons. These include

presynaptic active zone scaffold proteins Bassoon, Piccolo and Liprin-α, synaptic vesicle proteins VAMP/Synaptobrevin and postsynaptic proteins, including the postsynaptic density-95 family of membrane-associated guanylate kinases and members of the ionotropic glutamate receptor family (Butkevich et al., 2016).

Confirming its role in synaptic protein transport, axonal cargo aggregates are observed in neurons of *C. elegans unc-76*/*FEZ1* mutants, which are indicative of defective axonal transport (Chua et al., 2012). Similarly, deletion of FEZ1 in developing human motor neurons significantly delayed axonal delivery of the active zone protein Piccolo (Gunaseelan et al., 2021). While Piccolo was already present in both proximal and distal neurites in 86% of control motor neurons as early as DIV9, less than 6% of FEZ1-deficient motor neurons showed proximal presence of the protein at the same timepoint. The defects in synaptic protein transport have a strong negative impact on synapse formation and function. In *C. elegans*, *unc-76* mutants show a dramatic loss and disorganization of presynaptic specializations (Butkevich et al., 2016). In the latter instance, this could arise from disrupted interaction with UNC-69/SCOCO as both proteins are required to maintain normal presynaptic organization (Su et al., 2006). Post-synaptically, FEZ1 over-expression rescued spine density reductions in neurons with abnormal levels of ZNF804A (Dong et al., 2021). Synaptic loss caused by perturbation of FEZ1 function is also recapitulated in the peripheral nervous system. In *Drosophila*, motor neuron specific knockdown of UNC-76 expression resulted in a significant decrease in the number of synaptic boutons in these animals as compared to control flies (Gunaseelan et al., 2021). The abnormalities corroborated strongly with locomotion defects observed in these mutant flies and is accompanied by a significantly shortened lifespan. Importantly, synaptic and locomotion defects (but not lifespan) were rescued with pharmacological activation (using rapamycin and metformin) of ATG1, one of the two known FEZ1/UNC-76 activating kinases. Notably, the locomotion and survival defects observed in motor neuron specific *unc-76* knockdown flies were also observed in *unc-76* null *Drosophila* mutants (Gindhart et al., 2003). Taken together, the recent evidence highlights the critical importance of FEZ1 in the proper development of neurons and their synapses and, thus, in the establishment of functional neural circuits during the development of the brain and peripheral nervous system.

Perturbation of Fasciculation and Elongation Protein Zeta-1 Function as a Contributing Factor in Causing Neurodevelopmental Disorders

Given its importance in the formation of neural circuits, it is not surprising that FEZ1 alterations have been linked to various brain disorders. *FEZ1* gene polymorphisms and reduced mRNA expression have been reported in patients affected by schizophrenia (SCZ) (Yamada et al., 2004; Lipska et al., 2006; Rampino et al., 2014; Vachev et al., 2015; Tang et al., 2017). Despite its complex etiology and pathophysiology, schizophrenia is now widely regarded as a neurodevelopmental disorder with origins rooted in perturbations affecting the establishment and optimization of neural circuits over the course of brain development (Birnbaum and Weinberger, 2017; McCutcheon et al., 2020). In particular, abnormalities in neural circuits involving the dorsolateral prefrontal cortex and hippocampal formation and their associated networks have been implicated. Magnetic resonance imaging studies of brains of schizophrenic patients revealed that significant volume loss, particularly in the temporal and prefrontal cortices, associated with the progressive loss of grey and white matter are already detectable upon the onset of psychosis that further deteriorates with progression of the disorder (Schwarz et al., 2019; Cetin-Karayumak et al., 2020). At the cellular level, observations of reductions in dendritic length, complexity and spine density together with lower presynaptic specializations and defective activity-induced release of synaptic vesicles further support the existence of altered or loss of synaptic connectivity between neurons in the affected areas (Hunt et al., 2017; Kahn, 2020).

As discussed previously, loss of FEZ1 alone is sufficient to disrupt the development of neurons and the networks they form. In addition, FEZ1 is known to either physically interact with SCZ-related risk genes or be involved in SCZ-implicated pathways. For instance, interactions at the protein, as well as, epistatic level have been reported between FEZ1 and disrupted in schizophrenia 1 (DISC1) or ZNF804A. Interestingly, the effect of perturbing these interactions is synergistic and affects neurite development (Miyoshi et al., 2003; Kang et al., 2011; Zhou et al., 2018; Dong et al., 2021). Deletion of histone deacetylase 11 (*HDAC11*), another SCZ-associated gene, leads to decreased expression of *FEZ1* in the hippocampus (Bryant et al., 2017). Moreover, perturbed synaptic function has been identified as common denominator in a broad range of neuropsychiatric disorders (Gandal et al., 2018; Koopmans et al., 2019). Reduced synapse number and disrupted synapses observed in the absence of FEZ1 strongly argues for its involvement not only in SCZ, but also in other neuropsychiatric disorders.

Apart from polymorphisms, the *FEZ1* gene is frequently deleted in patients suffering from Jacobsen syndrome (JS) (Grossfeld et al., 2004). The gene is located at the long arm of chromosome 11 (11q). Deletions ranging from 7 to 16 Mb in size of the 11q terminal region lead to heterozygous loss of a number of genes, of which only some have been functionally characterized. Amongst other clinical symptoms, intellectual disability exists in JS patients and the extent of chromosomal deletion is known to positively correlate with increasing disability. A significant proportion of patients also manifest attention deficit hyperactivity disorder, autism spectrum disorder and, less frequently, schizophrenia or bipolar disorders (Mattina et al., 2009). Psychomotor impairments, including gross and fine motor delays are also commonly identified. Importantly, the locomotion defects were recapitulated in a *Drosophila* motor neuron specific knockdown model, indicating that partial loss of *FEZ1* at least contributes to such impairments (Gunaseelan et al., 2021). Given FEZ1's importance in neuronal development and formation of neuronal networks, it would not be surprising if partial loss of the protein also contributes to the behavioral and cognitive impairments observed in JS patients.

Studies using mouse models have at least partially confirmed that perturbation of FEZ1 function can cause behavioral phenotypes relevant to neuropsychiatric disorders. *FEZ1*-knockout mice exhibited a hyperactive phenotype caused by increased dopaminergic signaling in the nucleus accumbens, which is reminiscent of observations in schizophrenia patients (Sakae et al., 2008). The expression of FEZ1 in inhibitory neurons further suggest that the alternations in dopaminergic transmission could have arisen from impaired GABAergic transmission in FEZ1-deficient inhibitory neurons. This finding is significant given that perturbations of the dopaminergic, glutamatergic and GABAergic neurotransmitter systems have all been implicated in causing schizophrenia (Canitano and Pallagrosi, 2017; Egerton et al., 2020). A follow up study by the same group further reported hyperactivity and impulsivity phenotypes in these animals that could be treated using methylphenidate or guanfacine, which have been used for the treatment of attention deficit hyperactivity disorder (Sumitomo et al., 2018).

Loss of Fasciculation and Elongation Protein Zeta-1 Function at Later in Life Is Associated with Neurodegeneration

Expression of FEZ1 persists late into adulthood (Whitehouse et al., 2002). While perturbing FEZ1 function early in life is linked to neurodevelopmental disorders, loss of its function at later stages contributes to neurodegenerative disorders (**Figure 2**). Axonal transport defects in the form of aggregates and synapse loss are observed in neurons missing FEZ1 (Gindhart et al., 2003; Toda et al., 2008; Chua et al., 2012). Moreover, progressive aggregation of FEZ1 is observed in brains of the triple-transgenic mouse model of AD (3xTg-AD). These features are frequently found in neurodegenerative diseases such as Alzheimer's disease (Bitetto and Di Fonzo, 2020). Importantly, FEZ1 is phosphorylated by microtubule affinity regulating kinases, which also phosphorylate Tau and are implicated in the formation of neurofibrillary tangles and disruption of axonal transport (Butkevich et al., 2016; Combs et al., 2019). Loss of FEZ1 phosphorylation either via substituting the targeted serine with an alanine residue or by using microtubule affinity regulating kinases/PAR-1 mutants also causes axonal transport effects.

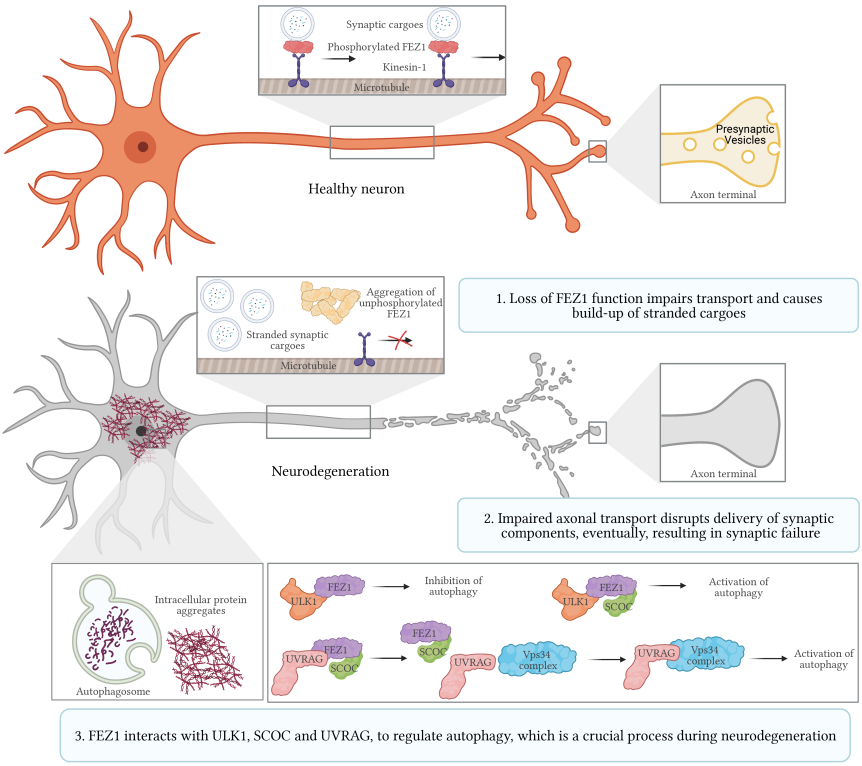

Figure 2 | Loss of FEZ1 function is implicated in neurodegeneration.
(1) The loss of FEZ1 function causes perturbations in axonal transport, leading to the build-up of stranded cargoes along with unphosphorylated FEZ1 aggregates. (2) Disrupted axonal transport blocks synaptic cargo delivery, which in turn, affects synaptic function. (3) Neurodegenerative phenotypes are commonly associated with intracellular protein aggregates. Autophagy is a catabolic process often upregulated under neurodegenerative conditions in order to remove these aggregates. FEZ1 is a regulator of autophagy via its interaction with various proteins such as ULK1, SCOC and UVRAG. FEZ1: Fasciculation and elongation protein zeta-1; SCOC: short coiled coil protein; ULK1: UNC-51-like kinase; UVRAG: ultraviolet radiation resistance associated gene. Image was created using Biorender graphic software (https://biorender.com/).

In addition to stranded cargoes, loss of FEZ1 in neurons causes the abnormal appearance of axonal autophagosomes (Chua et al., 2012). In agreement with these observations, FEZ1 inhibits autophagy but formation of a FEZ1-UNC-51-like kinase-short coiled coil protein (SCOC) complex allows autophagy to proceed (McKnight et al., 2012). Alternatively, release of ultraviolet radiation resistance associated gene (UVRAG) from a complex with FEZ1 and SCOC is thought to allow it to initiate autophagy via associating with the vacuolar protein sorting 34 complex. Loss of FEZ1 could prevent the formation of this complex and allow UVRAG to constitutively activate autophagy. Increased autophagy to remove damaged cellular components has been implicated as a protective mechanism in neurodegeneration (Menzies et al., 2017). This could indicate an attempt by affected neurons to clear up axonal aggregates. As such, it would be interesting to investigate if autophagic abnormalities associated with loss of FEZ1 is linked to susceptibility to neurodegeneration and its onset.

FEZ1 may be implicated in other neurodegenerative disorders as well. Axonal aggregates have not been observed in motor neurons lacking FEZ1 (Gunaseelan et al., 2021). However, given the identification of Kinesin-1 mutations in ALS patients, it will be relevant to determine if FEZ1 malfunctions could also contribute to motor neurone disorders. Moreover, given the involvement of dysfunctional mitochondrial dynamics in neurodegenerative diseases it would also be important to determine if abnormalities in mitochondrial trafficking arising from perturbed FEZ1 function could also contribute to neuronal degeneration. Finally, while FEZ1 also binds Huntingtin, its role in Huntington's disease remains uncharacterized (Goehler et al., 2004).

Conclusion

Accumulating evidence has highlighted the importance of FEZ1 in the development, formation and maintenance of neuronal networks in the brain as well as peripheral nervous system. Notably, altered FEZ1 function causes abnormalities in the development and maintenance of neuronal networks that are associated with neurodevelopmental as well as neurodegenerative disorders involving cognitive and motor deficits. Further elucidation of the protein and its functions will provide important insights into our understanding of how neurons are wired together for the proper functioning of the brain and its associated neuronal networks.

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References

Aiken J, Holzbaur ELF (2021) Cytoskeletal regulation guides neuronal trafficking to effectively supply the synapse. Curr Biol 31:R633-650.

Bellon A, Mann F (2018) Keeping up with advances in axon guidance. Curr Opin Neurobiol 53:183-191.

Birnbaum R, Weinberger DR (2017) Genetic insights into the neurodevelopmental origins of schizophrenia. Nat Rev Neurosci 18:727-740.

Bitetto G, Di Fonzo A (2020) Nucleo-cytoplasmic transport defects and protein aggregates in neurodegeneration. Transl Neurodegener 9:25.

Blasius TL, Cai D, Jih GT, Toret CP, Verhey KJ (2007) Two binding partners cooperate to activate the molecular motor Kinesin-1. J Cell Biol 176:11-17.

Bloom L, Horvitz HR (1997) The Caenorhabditis elegans gene unc-76 and its human homologs define a new gene family involved in axonal outgrowth and fasciculation. Proc Natl Acad Sci U S A 94:3414-3419.

Brenner S (1974) The genetics of caenorhabditis elegans. Genetics 77:71-94.

Bryant DT, Landles C, Papadopoulou AS, Benjamin AC, Duckworth JK, Rosahl T, Benn CL, Bates GP (2017) Disruption to schizophrenia-associated gene Fez1 in the hippocampus of HDAC11 knockout mice. Sci Rep 7:11900.

Butkevich E, Hartig W, Nikolov M, Erck C, Grosche J, Urlaub H, Schmidt CF, Klopfenstein DR, Chua JJ (2016) Phosphorylation of FEZ1 by microtubule affinity regulating kinases regulates its function in presynaptic protein trafficking. Sci Rep 6:26965.

Canitano R, Pallagrosi M (2017) Autism spectrum disorders and schizophrenia spectrum disorders: excitation/inhibition imbalance and developmental trajectories. Front Psychiatry 8:69.

Cetin-Karayumak S, Di Biase MA, Chunga N, Reid B, Somes N, Lyall AE, Kelly S, Solgun B, Pasternak O, Vangel M, Pearlson G, Tamminga C, Sweeney JA, Clementz B, Schretlen D, Viher PV, Stegmayer K, Walther S, Lee J, Crow T, et al. (2020) White matter abnormalities across the lifespan of schizophrenia: a harmonized multi-site diffusion MRI study. Mol Psychiatry 25:3208-3219.

Chen L, Wang Z, Ghosh-Roy A, Hubert T, Yan D, O'Rourke S, Bowerman B, Wu Z, Jin Y, Chisholm AD (2011) Axon regeneration pathways identified by systematic genetic screening in C. elegans. Neuron 71:1043-1057.

Chua JJ, Kindler S, Boyken J, Jahn R (2010) The architecture of an excitatory synapse. J Cell Sci 123:819-823.

Chua JJ, Butkevich E, Worseck JM, Kittelmann M, Gronborg M, Behrmann E, Stelzl U, Pavlos NJ, Lalowski MM, Eimer S, Wanker EE, Klopfenstein DR, Jahn R (2012) Phosphorylation-regulated axonal dependent transport of syntaxin 1 is mediated by a Kinesin-1 adapter. Proc Natl Acad Sci U S A 109:5862-5867.

Chua JY, Ng SJ, Yagensky O, Wanker EE, Chua JJE (2021) FEZ1 forms complexes with CRMP1 and DCC to regulate axon and dendrite development. eNeuro doi: 10.1523/ENEURO.0193-20.2021.

Combs B, Mueller RL, Morfini G, Brady ST, Kanaan NM (2019) Tau and axonal transport misregulation in tauopathies. Adv Exp Med Biol 1184:81-95.

Cotrufo T, Perez-Branguli F, Muhaisen A, Ros O, Andres R, Baeriswyl T, Fuschini G, Tarrago T, Pascual M, Urena J, Blasi J, Giralt E, Stoeckli ET, Soriano E (2011) A signaling mechanism coupling netrin-1/deleted in colorectal cancer chemoattraction to SNARE-mediated exocytosis in axonal growth cones. J Neurosci 31:14463-14480.

Desai C, Garriga G, McIntire SL, Horvitz HR (1988) A genetic pathway for the development of the Caenorhabditis elegans HSN motor neurons. Nature 336:638-646.

Dong F, Mao J, Chen M, Yoon J, Mao Y (2021) Schizophrenia risk ZNF804A interacts with its associated proteins to modulate dendritic morphology and synaptic development. Mol Brain 14:12.

Egerton A, Grace AA, Stone J, Bossong MG, Sand M, McGuire P (2020) Glutamate in schizophrenia: neurodevelopmental perspectives and drug development. Schizophr Res 223:59-70.

Fujita T, Ikuta J, Hamada J, Okajima T, Tatematsu K, Tanizawa K, Kuroda S (2004) Identification of a tissue-non-specific homologue of axonal fasciculation and elongation protein zeta-1. Biochem Biophys Res Commun 313:738-744.

Gandal MJ, Haney JR, Parikshak NN, Leppa V, Ramaswami G, Hartl C, Schork AJ, Appadurai V, Buil A, Werge TM, Liu C, White KP, CommonMind C, Psych EC, i P-BWG, Horvath S, Geschwind DH (2018) Shared molecular neuropathology across major psychiatric disorders parallels polygenic overlap. Science 359:693-697.

Gindhart JG, Chen J, Faulkner M, Gandhi R, Doerner K, Wisniewski T, Nandlestadt A (2003) The kinesin-associated protein UNC-76 is required for axonal transport in the Drosophila nervous system. Mol Biol Cell 14:3356-3365.

Goehler H, Lalowski M, Stelzl U, Waelter S, Stroedicke M, Worm U, Droege A, Lindenberg KS, Knoblich M, Haenig C, Herbst M, Suopanki J, Scherzinger E, Abraham C, Bauer B, Hasenbank R, Fritzsche A, Ludewig AH, Büssow K, Coleman SH, et al. (2004) A protein interaction network links GIT1, an enhancer of huntingtin aggregation, to Huntington's disease. Mol Cell 15:853-865.

Grossfeld PD, Mattina T, Lai Z, Favier R, Jones KL, Cotter F, Jones C (2004) The 11q terminal deletion disorder: a prospective study of 110 cases. Am J Med Genet A 129A:51-61.

Guedes-Dias P, Holzbaur ELF (2019) Axonal transport: driving synaptic function. Science doi: 10.1126/science.aaw9997.

Gunaseelan S, Wang Z, Tong VKJ, Ming SWS, Razar R, Srimasorn S, Ong WY, Lim KL, Chua JJE (2021) Loss of FEZ1, a gene deleted in Jacobsen syndrome, causes locomotion defects and early mortality by impairing motor neuron development. Hum Mol Genet 30:5-20.

He J, Liu J, Zhang Z, Sun M, Zhu T, Xia C (2009) Expression of fasciculation and elongation protein zeta-1 (FEZ1) in cultured rat neonatal astrocytes. Mol Cell Biochem 325:159-167.

Hedgecock EM, Culotti JG, Thomson JN, Perkins LA (1985) Axonal guidance mutants of Caenorhabditis elegans identified by filling sensory neurons with fluorescein dyes. Dev Biol 111:158-170.

Honda A, Miyoshi K, Baba K, Taniguchi M, Koyama Y, Kuroda S, Katayama T, Tohyama M (2004) Expression of fasciculation and elongation protein zeta-1 (FEZ1) in the developing rat brain. Brain Res Mol Brain Res 122:89-92.

Hunt MJ, Kopell NJ, Traub RD, Whittington MA (2017) Aberrant network activity in schizophrenia. Trends Neurosci 40:371-382.

Ikuta J, Maturana A, Fujita T, Okajima T, Tatematsu K, Tanizawa K, Kuroda S (2007) Fasciculation and elongation protein zeta-1 (FEZ1) participates in the polarization of hippocampal neuron by controlling the mitochondrial motility. Biochem Biophys Res Commun 353:127-132.

Kahn RS (2020) On the origins of schizophrenia. Am J Psychiatry 177:291-297.

Kaizuka T, Takumi T (2018) Postsynaptic density proteins and their involvement in neurodevelopmental disorders. J Biochem 163:447-455.

Kang E, Burdick KE, Kim JY, Duan X, Guo JU, Sailor KA, Jung DE, Ganesan S, Choi S, Pradhan D, Lu B, Avramopoulos D, Christian K, Malhotra AK, Song H, Ming GL (2011) Interaction between FEZ1 and DISC1 in regulation of neuronal development and risk for schizophrenia. Neuron 72:559-571.

Koopmans F, van Nierop P, Andres-Alonso M, Byrnes A, Cijsouw T, Coba MP, Cornelisse LN, Farrell RJ, Goldschmidt HL, Howrigan DP, Hussain NK, Imig C, de Jong APH, Jung H, Kohansalnodehi M, Kramarz B, Lipstein N, Lovering RC, MacGillavry H, Mariano V, et al. (2019) SynGO: an evidence-based, expert-curated knowledge base for the synapse. Neuron 103:217-234.

Kuroda S, Nakagawa N, Tokunaga C, Tatematsu K, Tanizawa K (1999) Mammalian homologue of the Caenorhabditis elegans UNC-76 protein involved in axonal outgrowth is a protein kinase C zeta-interacting protein. J Cell Biol 144:403-411.

Lanza DC, Silva JC, Assmann EM, Quaresma AJ, Bressan GC, Torriani IL, Kobarg J (2009) Human FEZ1 has characteristics of a natively unfolded protein and dimerizes in solution. Proteins 74:104-121.

Ledda F, Paratcha G (2017) Mechanisms regulating dendritic arbor patterning. Cell Mol Life Sci 74:4511-4537.

Lipska BK, Peters T, Hyde TM, Halim N, Horowitz C, Mitkus S, Weickert CS, Matsumoto M, Sawa A, Straub RE, Vakkalanka R, Herman MM, Weinberger DR, Kleinman JE (2006) Expression of DISC1 binding partners is reduced in schizophrenia and associated with DISC1 SNPs. Hum Mol Genet 15:1245-1258.

Malikov V, Naghavi MH (2017) Localized phosphorylation of a kinesin-1 adaptor by a capsid-associated kinase regulates HIV-1 motility and uncoating. Cell Rep 20:2792-2799.

Mattina T, Perrotta CS, Grossfeld P (2009) Jacobsen syndrome. Orphanet J Rare Dis 4:9.

McCutcheon RA, Reis Marques T, Howes OD (2020) Schizophrenia-an overview. JAMA Psychiatry 77:201-210.

McKnight NC, Jefferies HB, Alemu EA, Saunders RE, Howell M, Johansen T, Tooze SA (2012) Genome-wide siRNA screen reveals amino acid starvation-induced autophagy requires SCOC and WAC. EMBO J 31:1931-1946.

Menzies FM, Fleming A, Caricasole A, Bento CF, Andrews SP, Ashkenazi A, Füllgrabe J, Jackson A, Jimenez Sanchez M, Karabiyik C, Licitra F, Lopez Ramirez A, Pavel M, Puri C, Renna M, Ricketts T, Schlotawa L, Vicinanza M, Won H, Zhu Y, et al. (2017) Autophagy and neurodegeneration: pathogenic mechanisms and therapeutic opportunities. Neuron 93:1015-1034.

Miyoshi K, Honda A, Baba K, Taniguchi M, Oono K, Fujita T, Kuroda S, Katayama T, Tohyama M (2003) Disrupted-In-Schizophrenia 1, a candidate gene for schizophrenia, participates in neurite outgrowth. Mol Psychiatry 8:685-694.

Molnar Z, Clowry GJ, Sestan N, Alzu'bi A, Bakken T, Hevner RF, Huppi PS, Kostovic I, Rakic P, Anton ES, Edwards D, Garcez P, Hoerder-Suabedissen A, Kriegstein A (2019) New insights into the development of the human cerebral cortex. J Anat 235:432-451.

Niftullayev S, Lamarche-Vane N (2019) Regulators of Rho GTPases in the nervous system: molecular implication in axon guidance and neurological disorders. Int J Mol Sci 20:1497.

Okumura F, Hatakeyama S, Matsumoto M, Kamura T, Nakayama KI (2004) Functional regulation of FEZ1 by the U-box-type ubiquitin ligase E4B contributes to neuritogenesis. J Biol Chem 279:53533-53543.

Pasterkamp RJ, Burk K (2021) Axon guidance receptors: Endocytosis, trafficking and downstream signaling from endosomes. Prog Neurobiol 198:101916.

Pinto-Costa R, Sousa MM (2021) Microtubules, actin and cytolinkers: how to connect cytoskeletons in the neuronal growth cone. Neurosci Lett 747:135693.

Rampino A, Walker RM, Torrance HS, Anderson SM, Fazio L, Di Giorgio A, Taurisano P, Gelao B, Romano R, Masellis R, Ursini G, Caforio G, Blasi G, Millar JK, Porteous DJ, Thomson PA, Bertolino A, Evans KL (2014) Expression of DISC1-interactome members correlates with cognitive phenotypes related to schizophrenia. PLoS One 9:e99892.

Richards SEV, Van Hooser SD (2018) Neural architecture: from cells to circuits. J Neurophysiol 120:854-866.

Rizalar FS, Roosen DA, Haucke V (2021) A presynaptic perspective on transport and assembly mechanisms for synapse formation. Neuron 109:27-41.

Sakae N, Yamasaki N, Kitaichi K, Fukuda T, Yamada M, Yoshikawa H, Hiranita T, Tatsumi Y, Kira JI, Yamamoto T, Miyakawa T, Nakayama KI (2008) Mice lacking the schizophrenia-associated protein FEZ1 manifest hyperactivity and enhanced responsiveness to psychostimulants. Hum Mol Genet 17:3191-3203.

Schwarz E, Doan NT, Pergola G, Westlye LT, Kaufmann T, Wolfers T, Brecheisen R, Quarto T, Ing AJ, Di Carlo P, Gurholt TP, Harms RL, Noirhomme Q, Moberget T, Agartz I, Andreassen OA, Bellani M, Bertolino A, Blasi G, Brambilla P, et al. (2019) Reproducible grey matter patterns index a multivariate, global alteration of brain structure in schizophrenia and bipolar disorder. Transl Psychiatry 9:12.

Seiradake E, Jones EY, Klein R (2016) Structural perspectives on axon guidance. Annu Rev Cell Dev Biol 32:577-608.

Sleigh JN, Rossor AM, Fellows AD, Tosolini AP, Schiavo G (2019) Axonal transport and neurological disease. Nat Rev Neurol 15:691-703.

Su CW, Tharin S, Jin Y, Wightman B, Spector M, Meili D, Tsung N, Rhiner C, Bourikas D, Stoeckli E, Garriga G, Horvitz HR, Hengartner MO (2006) The short coiled-coil domain-containing protein UNC-69 cooperates with UNC-76 to regulate axonal outgrowth and normal presynaptic organization in Caenorhabditis elegans. J Biol 5:9.

Sudhof TC (2018) Towards an understanding of synapse formation. Neuron 100:276-293.

Sumitomo A, Saka A, Ueta K, Horike K, Hirai K, Gamo NJ, Hikida T, Nakayama KI, Sawa A, Sakurai T, Tomoda T (2018) Methylphenidate and guanfacine ameliorate ADHD-like phenotypes in Fez1-deficient mice. Mol Neuropsychiatry 3:223-233.

Sure GR, Chatterjee A, Mishra N, Sabharwal V, Devireddy S, Awasthi A, Mohan S, Koushika SP (2018) UNC-16/JIP3 and UNC-76/FEZ1 limit the density of mitochondria in C. elegans neurons by maintaining the balance of anterograde and retrograde mitochondrial transport. Sci Rep 8:8938.

Tang J, Fan Y, Li H, Xiang Q, Zhang DF, Li Z, He Y, Liao Y, Wang Y, He F, Zhang F, Shugart YY, Liu C, Tang Y, Chan RCK, Wang CY, Yao YG, Chen X (2017) Whole-genome sequencing of monozygotic twins discordant for schizophrenia indicates multiple genetic risk factors for schizophrenia. J Genet Genomics 44:295-306.

Toda H, Mochizuki H, Flores R, 3rd, Josowitz R, Krasieva TB, Lamorte VJ, Suzuki E, Gindhart JG, Furukubo-Tokunaga K, Tomoda T (2008) UNC-51/ATG1 kinase regulates axonal transport by mediating motor-cargo assembly. Genes Dev 22:3292-3307.

Vachev TI, Stoyanova VK, Ivanov HY, Minkov IN, Popov NT (2015) Investigation of fasciculation and elongation protein zeta-1 (FEZ1) in peripheral blood reveals differences in gene expression in patients with schizophrenia. Balkan J Med Genet 18:31-38.

Vasudevan A, Koushika SP (2020) Molecular mechanisms governing axonal transport: a C. elegans perspective. J Neurogenet 34:282-297.

White JG, Southgate E, Thomson JN, Brenner S (1976) The structure of the ventral nerve cord of Caenorhabditis elegans. Philos Trans R Soc Lond B Biol Sci 275:327-348.

Whitehouse C, Chambers J, Howe K, Cobourne M, Sharpe P, Solomon E (2002) NBR1 interacts with fasciculation and elongation protein zeta-1 (FEZ1) and calcium and integrin binding protein (CIB) and shows developmentally restricted expression in the neural tube. Eur J Biochem 269:538-545.

Yamada K, Nakamura K, Minabe Y, Iwayama-Shigeno Y, Takao H, Toyota T, Hattori E, Takei N, Sekine Y, Suzuki K, Iwata Y, Miyoshi K, Honda A, Baba K, Katayama T, Tohyama M, Mori N, Yoshikawa T (2004) Association analysis of FEZ1 variants with schizophrenia in Japanese cohorts. Biol Psychiatry 56:683-690.

Yogev S, Shen K (2017) Establishing neuronal polarity with environmental and intrinsic mechanisms. Neuron 96:638-650.

Zhou Y, Dong F, Lanz TA, Reinhart V, Li M, Liu L, Zou J, Xi HS, Mao Y (2018) Interactome analysis reveals ZNF804A, a schizophrenia risk gene, as a novel component of protein translational machinery critical for embryonic neurodevelopment. Mol Psychiatry 23:952-962.

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