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Mitochondria in complex psychiatric disorders: lessons from mouse models of 22q11.2 deletion syndrome

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Summary

Mitochondrial ATP synthesis, calcium buffering, and trafficking affect neuronal function and survival. Several genes implicated in mitochondrial functions map within the genomic region associated with 22q11.2 deletion syndrome (22q11DS), which is a key genetic cause of neuropsychiatric diseases. Although neuropsychiatric diseases impose a serious health and economic burden, their etiology and pathogenesis remain largely unknown because of the dearth of valid animal models and challenges in investigating the pathophysiology in neuronal circuits. Mouse models of 22q11DS are becoming valid tools for studying human psychiatric diseases, because they have hemizygous deletions of the genes that are deleted in patients and exhibit neuronal and behavioral abnormalities consistent with neuropsychiatric disease. The deletion of some 22q11DS genes implicated in mitochondrial function leads to abnormal neuronal and synaptic function. Herein, we summarize recent findings on mitochondrial dysfunction in 22q11DS and extend those findings to the larger context of schizophrenia and other neuropsychiatric diseases.

Keywords

22q11.2 deletion syndrome; schizophrenia; mitochondria; synapse; synaptic plasticity; calcium; ATP

Introduction

Serious mental illness affects an estimated 9.8 million adults in the United States, which represents 4.2% of the nation's adult population [1]. Globally, 7.4% of all disability-adjusted life years, a measure of time lost due to health problems, disability, or early death, is caused by mental and behavioral disorders [2]. In the United States, mental health disorders are associated with the highest economic burden, accounting for $201 billion in annual healthcare spending [3]. Despite the severity of these disabilities and the associated economic burden, progress in the field of neuropsychiatry has been hampered by the

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Conflict of Interest

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complexity of higher-order neurobiological functions and the dearth of valid animal or cellular models of psychiatric diseases [4].

Mouse models of 22q11.2 deletion syndrome (22q11DS) hold promise to increase our understanding of the complex biology of mental illnesses [5–7]. 22q11DS (also known as velocardiofacial or DiGeorge syndrome) is the most common microdeletion syndrome in humans [8–10], with a prevalence of 1 in 4000 live births. The syndrome is caused by a hemizygous microdeletion (1.5–3 Mb) on the long arm of chromosome 22 [11]. It is one of the strongest genetic risk factors for schizophrenia [12,13]. Schizophrenia develops in 23% to 43% of individuals with 22q11DS [14–18], most of whom experience psychosis [19,20]. Furthermore, 30% to 50% of nonschizophrenic individuals with 22q11DS exhibit subthreshold symptoms of psychosis [21]. Nonpsychotic behavioral abnormalities are present from early adulthood in patients with 22q11DS [22,23], but psychotic symptoms and schizophrenia appear only in adulthood [24–26]. In addition to schizophrenia, 25% to 50% of individuals with 22q11DS exhibit comorbid illnesses such as attention-deficit hyperactive disorder, autism spectrum disorder, anxiety, and mood disorders [27,28]. The varied outcomes of patients with this syndrome suggest deficits in multiple neural circuits.

All genes within the human 22q11.2 genomic region, except for one, are present on mouse chromosome 16 but in a slightly shuffled order. Mouse models with deletions spanning different syntenic regions have been developed over the past several years (Fig. 1) [29]. The availability of these mouse models and the overlap of different neuropsychiatric phenotypes with 22q11DS provide a valuable experimental tool to dissect the genetic causes and investigate the molecular and cellular mechanisms of these phenotypes [29,30]. Using mouse models of 22q11DS (22q11DS mice), our group investigated the genetic causes of neural circuit deficits that give rise to abnormalities that resemble the cognitive and positive symptoms of schizophrenia [31–33]. Several groups have reported working-memory deficits in patients with 22q11DS and in 22q11DS mice [34–37]. We recently identified that haploinsufficiency of the 22q11DS gene *Mrpl40* contributes to working-memory deficits. *Mrpl40* encodes one of the mitochondrial proteins in the large ribosomal subunit [34]. *Zdhhc8,* another putative mitochondrial gene within the 22q11 genomic region, has also been implicated in a working-memory disorder [38]. *Prodh*, a gene encoding mitochondrial proline dehydrogenase, has been implicated in deficient sensorimotor gating [39]. Altogether, six 22q11DS genes—*Prodh, Slc25a1, Mrpl40, Zdhhc8, T10*, and *Txnrd2*— encode mitochondrial proteins (Fig. 1B). Computational predictions suggest that three other 22q11DS genes—*Gnbl1*, *Rtn4r,* and *Ufd1l*— also encode proteins that are important for mitochondria [40]. On the basis of these findings, we propose that the mitochondrion is most likely a key organelle in the pathophysiology of 22q11DS and schizophrenia.

An analysis of several published studies on genomic, transcriptomic, and proteomic factors associated with schizophrenia revealed 295 genes that mediate mitochondrial structure or function [41]. Moreover, 57 of those genes have been implicated in more than one study [41]. Twenty-two genes encoding mitochondrial proteins have been mapped within the 108 genetic risk loci (encompassing more than 300 genes) and were identified by the largest schizophrenia genome-wide association study (GWAS) to date [41–43]. This preponderant association of mitochondrial genes with schizophrenia and 22q11DS justifies further testing

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of the hypothesis that complex psychiatric disorders, including schizophrenia, have a mitochondrial origin. This hypothesis has been strengthened by a recent proteomics and metabolomics study that identified multiple mitochondrial proteins that are deregulated in 22q11DS mice [44]. The intertwining of mitochondrial function with multiple cellular processes is also considered a common denominator in various psychiatric disorders [45,46]. The comorbidity of mitochondrial disorders and autism, depression, anxiety, or bipolar disorder is well established [46]. Although these disorders may not qualify as classic mitochondrial diseases, many abnormal proteins encoded by nuclear genes can affect mitochondrial function, thereby compromising the functioning of neural circuits [45].

In this essay, we summarize recent findings on the interdependence of mitochondrial and neural functions. Furthermore, we discuss how defects in 22q11DS nuclear-encoded genes may affect mitochondrial function in neural circuits and lead to the neuropathophysiology associated with 22q11DS symptoms. We also touch upon how mitochondrial functions vary across cell types in the central nervous system (CNS).

Mitochondrial and neuronal functions are interdependent

Synaptic transmission and plasticity in neural circuits are highly energy-dependent processes. Mitochondria, the primary cellular organelles that provide adenosine triphosphate (ATP), also partake in other neuronal functions at the synapse, such as calcium buffering, lipid biogenesis, and redox regulation (Fig. 2).

Mitochondrial ATP supports synaptic function

The electrochemical nature of synaptic signaling makes the process a big ATP consumer [47]. ATP is required primarily by the sodium–potassium ATPases, which maintain the resting membrane potential in neurons; various calcium pumps [e.g., plasma membrane and (sarco)endoplasmic reticulum calcium ATPase 2 (SERCA2)], which maintain low cytosolic calcium concentration; vesicular ATPases (vATPases), which fill synaptic vesicles with neurotransmitters; and synaptic vesicle exocytosis and endocytosis [47]. A recent study showed that under conditions of ATP depletion synaptic vesicle endocytosis is the most affected event [48]. In that study, activity-dependent synaptic ATP consumption was measured in individual presynaptic terminals by using the synaptically targeted luciferase reporter Syn-ATP [48]. Neurotransmitters are loaded into vesicles by vesicular transporters, which are aided by vacuolar ATPases. These molecular complexes have the same architecture as mitochondrial ATP synthase (also an ATPase) but are oriented in the opposite direction. This converse orientation enables the complexes to use the energy derived from ATP hydrolysis to drive a motor that generates a proton and pH gradient to help load the neurotransmitters into vesicles [49]. Single-vesicle imaging has shown that glutamate and gamma-aminobutyric acid (GABA), two major neurotransmitters in the mammalian brain, are loaded into vesicles by different classes of vesicular transporters. Compared with glutamate transporters, GABA vesicular transporters rely more on the proton gradient generated by vATPases for loading, which suggests that excitatory neurons and inhibitory neurons have different needs for vATPases [50]. Apart from its role as an energy currency, ATP also acts as a neurotransmitter and activates purinergic receptors [51]. Moreover, ATP

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degradation leads to the production of adenosine, another important neurotransmitter, which acts through various adenosine-specific receptors [51]. In turn, adenosine is released from neurons and glial cells via different mechanisms and has different effects on glutamate- and GABA-dependent synaptic transmission [52]. The multifaceted roles of ATP in the CNS support the notion that energy demand and synaptic function are intertwined. Therefore, any decline in mitochondrial ATP production can substantially and non-uniformly affect neuronal circuits.

Mitochondrial calcium buffering modulates synaptic calcium levels

Mitochondria possess an intricate molecular machinery that regulates calcium uptake and release. The molecular identification of the high-affinity (K_d < 2 nM) mitochondrial calcium uniporter (MCU) channel in 2011 [53–55] spurred research on mitochondrial calcium regulation. The MCU channel is inhibited at rest by regulatory proteins MICU1 and MICU2, and this inhibition is lifted under conditions of excess cytosolic calcium [56]. In humans, MICU1 mutations result in myopathy, learning deficits, and movement disorders [57]. MICU3, a less well-characterized regulatory protein of MCU, is exclusively expressed in the CNS [58], which suggests a specialized role for mitochondrial calcium in the nervous system. The calcium-buffering capacity of the mitochondrial matrix sink is maintained by releasing excess matrix calcium through the mitochondrial sodium– and proton–calcium exchangers and the low-conductance mode of the mitochondrial permeability transition pore (mPTP) [56,59]. An increase in mitochondrial calcium concentration is also closely tied to the tricarboxylic acid cycle and ATP synthesis [60]. This association can, therefore, indirectly affect synaptic calcium levels, because the calcium pumps localized on the endoplasmic reticulum and plasma membrane are ATP dependent. The 22q11DS mice have deficits in hippocampal short-term and long-term synaptic plasticities, which are the cellular correlates of learning and memory, and these deficits depend on mitochondrial calcium level [34] and Serca2 activity [30,32]. In a comprehensive GWAS, the *ATP2A2* gene, which encodes SERCA2, was associated with schizophrenia [43], suggesting an interplay between the calcium-handling machinery in the mitochondria and endoplasmic reticulum in the pathophysiology of the disease.

Mitochondrial redox regulation may contribute to the age dependence of 22q11DS phenotypes

Oxidative phosphorylation in mitochondria entails a series of electron-transfer reactions in the respiratory-chain complexes that eventually lead to the reduction of molecular oxygen to water [61]. These reactions are not foolproof and can result in electron leak, especially at complexes I and III, which gives rise to reactive oxygen species (ROS) [61]. Mitochondria have various antioxidants, such as superoxide dismutase-2, catalase, glutathione peroxidase, peroxiredoxins, and thioredoxins, to counteract ROS-induced damage [61,62]. Although multiple data sources have suggested that ROS are major contributors to the process of ageing, recent re-evaluation suggests that ROS are merely the mediators of age-dependent cellular damage [63,64]. Strikingly, some of the phenotypes associated with 22q11DS mice are also age dependent [31,33]. MicroRNA biogenesis may be the determinant of age dependence in these studies. Given the critical involvement of mitochondrial oxidative

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phosphorylation in many synaptic functions, oxidation/reduction (redox) imbalance can also be a factor in age dependence of the 22q11DS phenotypes.

Mitochondrial lipid metabolism in synaptic vesicle recycling

Signal transduction at chemical synapses is mainly achieved by the release of synaptic vesicles containing neurotransmitters. Most attention has been focused on synaptic proteins and the need of ATP for synaptic vesicle recycling, but the rapid turnover of lipid membranes in the process has been underappreciated [65,66]. Although most phospholipids are synthesized in the endoplasmic reticulum, mitochondria-associated membranes (i.e., the contact sites between mitochondria and the endoplasmic reticulum) harbor enzymes involved in lipid biosynthesis [67–69]. Thus, further investigations are warranted to elucidate the role of mitochondria-associated membranes in the recycling of synaptic vesicles.

Mitochondrial trafficking may underlie the pathogenesis of neuropsychiatric diseases

Because of their polarity and varied architecture, neuronal cells face the challenge of distributing mitochondria throughout their dendritic arbor and the thin axonal branches over very long distances [70,71]. The relevance of mitochondrial trafficking to neuropsychiatric diseases is exemplified by the *DISC1 (Disrupted in schizophrenia 1)* gene. Mutations in *DISC1* have been associated with several neuropsychiatric illnesses [72,73], and the putative underlying cause is alterations in the mitochondria–microtubule complex involved in intracellular trafficking [74,75]. *DISC1* is also involved in dendritic morphogenesis [28]. The mitochondrial receptor Miro1, which mediates mitochondrial trafficking, has calcium-sensing EF-hand domains, suggesting that calcium signals that are crucial for synaptic plasticity also affect mitochondrial trafficking [70]. Neuronal mitochondrial trafficking is an active field of research, and we refer readers to other recent, more comprehensive reviews on this topic [70,71]. Other relevant mitochondrial events, such as mitochondrial fission and fusion, are also reviewed elsewhere [76,77]. The mitochondrial functions discussed thus far in this review apply to all cell types. However, some cell types (e.g., oligodendrocytes, interneurons, and astrocytes) have unique mitochondrial functions. This specialization indicates that these cell types in the CNS could be more vulnerable to some, but not other, mitochondrial deficits.

Mitochondria play a role in myelination by oligodendrocytes

The myelin sheath formed by oligodendrocytes is composed mainly of lipids. Mitochondria in oligodendrocytes are primarily located within the cytoplasmic ridges along the sheath and are characterized by reduced surface area of cristae, which suggests that ATP synthesis is not the primary function of these organelles in these cells [78]. Oligodendrocytes express metabotropic glutamatergic and purinergic receptors, which mediate calcium transient responses to the vesicular release of glutamate and ATP [79,80]. This form of extrasynaptic communication has been implicated in activity-dependent myelination [79,80]. An important ultrastructural alteration seen post-mortem in the brains of patients with schizophrenia is the paucity of mitochondria in oligodendrocytes. This finding suggests impaired connectivity in long-range neuronal connections [81,82]. Defective myelination is a common feature of

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many primary and secondary mitochondrial diseases [83], a fact that further supports the importance of mitochondria in myelination.

Mitochondrial function is crucial for the higher metabolic needs of interneurons

Multiple types of interneurons contribute to the complexity of neuronal circuit organization and function in the brain [84]. Parvalbumin-positive (PV) interneurons are particularly relevant to the this review, because these cells have higher numbers of mitochondria than other neuronal cell types and are enriched with cytochrome c, which is essential for oxidative phosphorylation [85]. PV interneurons are essential for setting the gamma frequencies observed in hippocampal–cortical networks [85]. The supercritical density of sodium channels in PV interneurons, which provides excellent conduction velocities in the specialized axonal arbor, comes at the expense of excess energy demand [85]. The *Cox10* gene encodes cytochrome oxidase, which is the terminal enzyme in the electron transport chain. In PV neurons, deletion of *Cox10* leads to abnormal gamma- and theta-frequency oscillations in the prefrontal cortex and hippocampus of anaesthetized mice, thereby further supporting the notion that PV interneurons have a higher metabolic need than do most neurons [86]. An integrative analysis of various measures in post-mortem hippocampal samples from the Stanley Neuropathology Consortium found a decrease in PV interneuron density in the CA2 region [87]. Reduced feed-forward inhibition arising from decreased density of PV interneurons in the CA2 area underlies social-cognition deficits in the *Df(16)A*$^{+/-}$ mouse model of 22q11DS [88]. These findings highlight the pathophysiologic intersection between the higher metabolic needs of PV interneurons and the greater representation of mitochondrial genes in 22q11DS.

Mitochondrial ATP production may regulate glutamate uptake by astrocytes

A major function of astrocytes is to recycle the excitatory neurotransmitter glutamate as inactive glutamine [89]. The astrocyte-localized, ATP-dependent enzyme glutamine synthetase serves this function [90,91] and demonstrates the unique need for mitochondrial ATP synthesis in astrocytes. Astrocytic uptake of glutamate is a secondary energy-dependent process [47,91]. The electrochemical gradient established by the sodium–potassium ATPase is used by glutamate transporters in astrocytes, hence glutamate transport indirectly depends on mitochondrial ATP production [47]. Glutamate uptake by astrocytes after neuronal activity immobilizes mitochondria in the perisynaptic processes, underlining the homeostatic need for mitochondrial localization in astrocytes [92].

Nuclear-encoded mitochondrial genes are implicated in the pathophysiology of 22q11DS

As mentioned earlier, six 22q11DS genes—*Prodh, Slc25a1, Mrpl40, Zdhhc8, T10*, and *Txnrd2*—are colocalized with mitochondria or implicated in mitochondrial function. Here we review recent data on these genes and their roles within mitochondria (Fig. 3).

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Prodh

PRODH was one of the earliest genes implicated in 22q11DS pathophysiology [39]. The gene encodes proline dehydrogenase (also known as proline oxidase), which is involved in the catabolic conversion of proline to pyrroline-5 carboxylate (P5C) [93]. The P5C intermediary can be recycled to proline by P5C reductase or can be converted to glutamate by P5C dehydrogenase [93]. Extracellular proline at physiological concentrations can potentiate hippocampal NMDA (N-methyl-D-aspartate) glutamate receptors [94]. A recent report suggests that proline is a GABA mimetic that competes with intracellular GABA synthesis [95]. The metabolic relations among proline, glutamate, and GABA; the possibility of proline acting as a neurotransmitter; the high incidence of hyperprolinemia in patients with 22q11DS; and evidence of sensorimotor-gating deficits in *Prodh*-deficient mice all point to the importance of P5C in some aspects of 22q11DS pathophysiology [39,96,97]. However, the role of *PRODH* in schizophrenia is debatable [97].

Slc25a1

The *Slc25a1* gene encodes a citrate transporter that catalyzes the efflux of citrate/isocitrate from the mitochondrial matrix in exchange for cytosolic malate [98]. Exported citrate molecules are converted to acetyl-coenzyme A, which is required for fatty acid synthesis [98]. Apart from serving as a source of citrate, which is needed for fatty acid synthesis, mitochondria provide the ATP required for the synthetic process [98]. Many Krebs' cycle intermediates are altered in patients carrying mutations in *SLC25A1*, with symptoms manifesting as developmental delay, hypotonia, and seizures [99]. Imbalanced citrate metabolism often leads to the accumulation of 2-hydroxyglutarate. This can interfere with alpha-ketoglutarate uptake by astrocytes and lead to deficits in anaplerotic neurotransmitter synthesis. The importance of this gene in lipid biogenesis and the ATP dependence of the conversion might underlie the agenesis of the corpus callosum observed in a patient with *SLC25A1* mutations [100].

Mrpl40

Mrpl40 encodes one of more than 50 proteins in the large ribosomal subunit of mitochondria [101]. Two elegant studies reporting the crystal structure of mammalian mitoribosomes identified the encoded protein mL40 at the P-finger of the large ribosomal subunit [102,103]. The function of this P-finger, which is unique to mammals, is yet to be determined [102,103]. Because of its ribosomal location, mL40 might be involved in some aspects of translation or posttranslational modification of nascent proteins. In a study of the association between nonsynonymous single-nucleotide polymorphisms (SNP) and schizophrenia, *MRPL40* was one of the top 25 candidates and was the only 22q11DS gene identified [104].

We identified *Mrpl40* in our recent screening of genes that mediate short-term plasticity deficits in 22q11DS mice [34]. By using the ultrasensitive genetically encoded calcium indicator GCaMP6 [105] imaged in either the cytoplasm of presynaptic terminals or the presynaptic mitochondria (mitGCaMP6) (Fig. 4), we showed that haploinsufficiency of *Mrpl40* affects the mPTP, resulting in abnormal mitochondrial calcium handling in presynaptic terminals of hippocampal neurons (Fig. 5). This in turn leads to abnormal short-term synaptic plasticity and working-memory deficit. To our knowledge, this is the first

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study that connects a nuclear-encoded mitochondrial protein, mitochondrial calcium, synaptic plasticity, and working memory. The GCaMP6 indicator enabled us to measure mitochondrial calcium uptake during ongoing synaptic activity and replicate mitochondrial calcium abnormalities observed in *Mrpl40*$^{+/-}$ mice by inhibiting adenine nucleotide translocases, which regulate the mPTP [34,106]. We also showed that increased activity of adenine nucleotide translocase 1 in presynaptic neurons rescues the synaptic plasticity deficit caused by *Mrpl40* haploinsufficiency, and decreased activity of the enzyme mimics the deficit.

Unlike most studies that have implicated the mPTP in cell death, our experiments indicated that the mPTP has a more subtle role, such as affecting calcium levels in the presynaptic cytoplasm. This notion is consistent with a recent report suggesting that the mPTP acts in a physiologic low-conductance mode [59,107]. It remains to be determined how Mrpl40, a part of the mitochondrial ribosome, affects the mPTP. Progress in the field of mitochondrial translation and the development of novel tools to study post-translational modifications in mitochondria will help elucidate the molecular link between *Mrpl40* and the mPTP. In contrast to the mPTP-dependent calcium-extrusion defect, which increases mitochondrial and cytosolic calcium levels, a defect in the MCU should increase cytosolic calcium levels but decrease mitochondrial calcium levels. Defects in either the MCU complex or the mPTP alter mitochondria–cytoplasm calcium exchange and eventually lead to increased neurotransmitter release. Two recent studies reporting mitochondrial calcium defects used different approaches to calculate the number of mitochondria localized to presynaptic terminals [34,108]. The first study addressing the role of *Mrpl40* in short-term plasticity used three-dimensional electron microscopy in hippocampal sections [34], whereas the second study examining LKB1 kinase regulation of the MCU used a mitochondria-targeted fluorescent protein in primary neuronal cultures [108]. Both methods revealed that approximately 50% of presynaptic terminals have mitochondria, suggesting that presynaptic terminals are heterogeneous in energy demand and calcium regulation by mitochondria.

Zdhhc8

The *Zdhhc8* gene encodes one of many palmitoyl transferases involved in S-palmitoylation, a process by which palmitoyl groups are added to certain proteins [109]. The palmitoyl code is used to sort proteins to specific subcellular compartments, and defects in that code can lead to various neuropsychiatric conditions [109]. *ZDHHC8* was identified as a risk gene for schizophrenia in a high-density SNP analysis within the 22q11DS region [110], although this association was not found in studies of different populations [109]. A recent study showed that *Zdhhc8* affects terminal axonal arborization, thereby affecting hippocampal–prefrontal connections and working memory [38]. Three pieces of complementary evidence support the localization of Zdhhc8 to the mitochondria: bioinformatics analysis to determine the mitochondrial localization signal, localization of the GFP-fusion protein, and colocalization with a mitochondrial marker [40]. However, an earlier study reported colocalization of Zdhhc8 with Golgi markers [110], raising some uncertainties about the specific subcellular localization of this protein.

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T10

T10 is the mouse ortholog of the human *TANGO2* (Transport and Golgi Organization 2) gene [40]. Mitochondrial localization of the encoded protein in mouse tissues has been confirmed using the same approaches used to identify Zdhhc8 localization [40]. However, studies in flies suggest that T10 is localized in the Golgi complex and cytosol, with a putative function in redistributing Golgi membranes to the endoplasmic reticulum [111] and raising questions about differences across species. Three unrelated individuals with biallelic truncating mutations in *TANGO2* showed an infancy-onset metabolic disorder accompanied by encephalopathy and multiple organ disorder [112]. The metabolic signatures in these patients suggested defective mitochondrial fatty acid oxidation [112]. Fibroblast cell lines derived from the affected individuals revealed defective palmitate-dependent mitochondrial oxygen consumption and no gross difference in Golgi organization [112]. This result further supports a role for *TANGO2* in mitochondrial beta-oxidation of fatty acids. However, the precise molecular mechanisms of *TANGO2* and *T10* remain unknown.

Txnrd2

The *Txnrd2* gene encodes thioredoxin reductase 2 and plays a key role in redox signaling by maintaining thioredoxin in a reduced state [113]. Reduced thioredoxin is needed to scavenge the free radicals generated in excess from the mitochondrial electron transport chain [113]. *Txnrd2* has been implicated in ageing and some heart diseases [114]. Of the several antioxidant systems (e.g., thioredoxin-2, glutathione, and catalase), the thioredoxin-2 system catalyzes approximately 60% ± 20% of the ROS detoxification in rat brain mitochondria [115]. Inhibition of thioredoxin-2 by 1-chloro-2,4-dinitrobenzene in hippocampal slice cultures revealed that interneurons and microglial cells are the most prone to ROS toxicity [115].

Other 22q11DS genes

In addition to the genes that encode mitochondrial proteins, other genes in the 22q11 region might indirectly affect mitochondrial function. For example, *Dgcr8,* which is involved in microRNA processing, may affect mitochondrial calcium buffering, because *miR-25* overexpression reduces MCU activity [116]. *Dgcr8*-dependent depletion of *miR-25* and *miR-185* also affects SERCA2 [32,33], which depends on mitochondrial ATP for its function.

Mitochondrial heteroplasmy can lead to incomplete penetrance of neuropsychiatric phenotypes

The number of copies of mitochondrial DNA (mtDNA) in a single cell varies from 100 to 1000, depending on the cell type [117]. The mtDNA is degraded and replicated, even in postmitotic cells [117]. This feature gives rise to a situation wherein a proportion of mtDNA carries mutations that cause heteroplasmy [117]. Inherited mtDNA heteroplasmy manifests as pathology when it reaches a critical threshold, usually during the prenatal stage of development [118,119]. Even in healthy individuals, the proportion of mutant mtDNA accumulation increases with age [117]. Although these mutations might not reach a

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pathogenic threshold by themselves, they can be pathogenic when combined with nuclear DNA mutations. The additive effect of nuclear DNA and mtDNA mutations, in turn, may depend on the level of heteroplasmy achieved and the criticality of the gene product for mitochondrial function. The mtDNA heteroplasmy acquired by postmitotic cells over their lifespan most likely contributes to the incomplete penetrance and late onset of neuropsychiatric conditions in patients with 22q11DS. This hypothesis is supported by the fact that the penetrance of all 22q11DS symptoms is incomplete, and even monozygotic twins with 22q11DS exhibit phenotypic discordance [120].

Conclusions and outlook

In this review, we have discussed several roles of mitochondria that impinge on the integrity of synaptic function and how different cell types vary in their mitochondrial needs. We highlighted how the 22q11DS mitochondrial genes can affect different aspects of mitochondrial function and took a glimpse at some recent evidence supporting the involvement of these genes in neuropsychiatric etiopathogenesis. We have also speculated on how mitochondrial defects contribute to the age dependence and incomplete penetrance of neuropsychiatric phenotypes. We propose that defects in nuclear-encoded mitochondrial proteins do not uniformly affect all neural circuits, because the circuits have heterogeneous demands. For example, compared with some unmyelinated circuits, myelinated long-range connections will be more vulnerable to defective mitochondrial fatty acid metabolism.

The idea that mitochondrial deficits give rise to complex psychiatric diseases instead of whole organismal pathology is implicit. The challenge in delineating these subtleties lies in integrating the various *in vitro* tools for studying mitochondria into the context of neural circuits, which are usually studied in rodent brain slices or *in vivo.* An amenable approach is to transition to relevant cellular models after the neural circuit underpinnings have been identified. Once a firm mechanistic link is established using cellular and brain slice models, unique features of the mitochondrial subcellular compartment can be used to target therapeutics with subcellular precision. Meanwhile, mouse models of 22q11DS, with their well-established relevance to neuropsychiatric diseases, serve as a springboard to connect various dots, such as nuclear genes encoding mitochondrial proteins, the mitochondrial functional deficit caused by their mutation, neural circuit dysfunction, and the spectrum of behavioral alterations.

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Abbreviation list

22q11DS	22q11.2 deletion syndrome
ATP	Adenosine triphosphate
CNS	Central nervous system

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GABA	Gamma-amino butyric acid
GCaMP6	Genetically encoded ultrasensitive calcium indicator
GWAS	Genome-wide association study
HCX	H^+-Ca^{2+} exchanger
MCU	Mitochondrial calcium uniporter
mitGCaMP6	GCaMP6 targeted to mitochondria
mPTP	Mitochondrial permeability transition pore
mtDNA	Mitochondrial DNA
NMDA	N-methyl-D-aspartate
NADPH and NADP	Nicotinamide adenine dinucleotide phosphate
NCLX	Na^+-Ca^{2+} exchanger
P5C	Pyrroline-5 carboxylate
PV	Parvalbumin
redox	Oxidation/reduction
ROS	Reactive oxygen species
SERCA2	(Sarco)endoplasmic reticulum calcium ATPase 2
SNP	Single nucleotide polymorphism
vATPase	Vesicular ATPase

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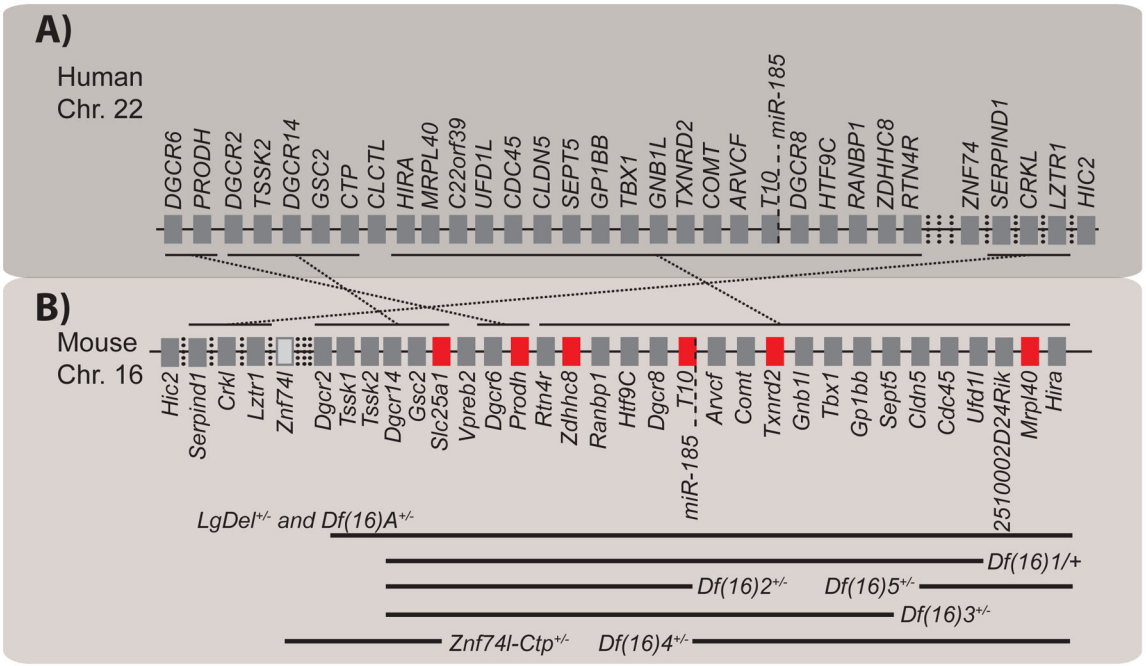

Figure 1. Mouse models of 22q11DS and mitochondrial genes

(**A**, **B**) Genes hemizygously deleted on chromosome (Chr.) 22 in human patients (**A**) and on chromosome 16 in mouse models of 22q11DS (**B**). The genes important for mitochondrial function are labeled in red (**B**). Hemizygous deletions spanning different subregions in mouse models are indicated by solid black lines.

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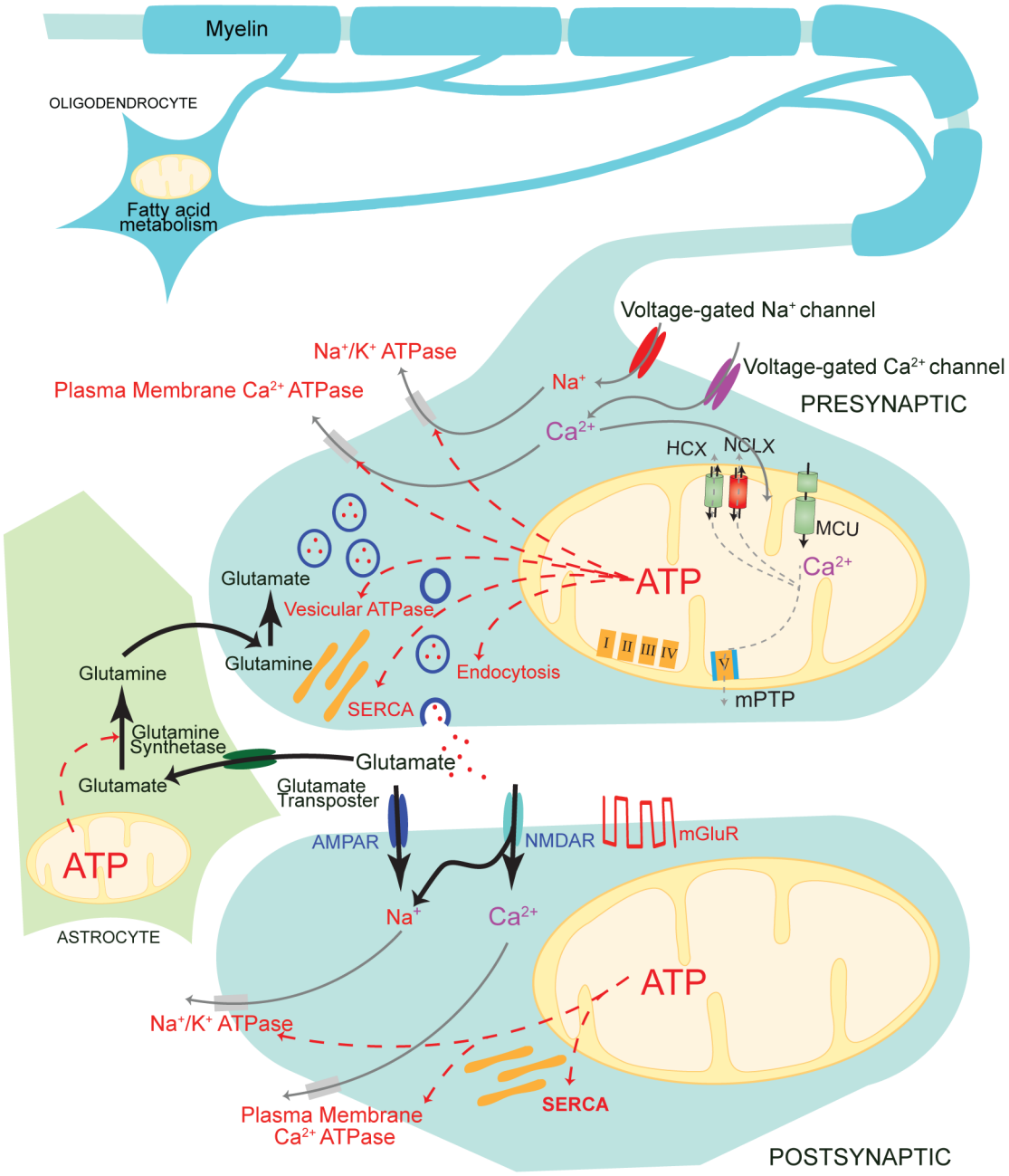

Figure 2. Interdependence of synaptic and mitochondrial functions

Presynaptically, adenosine triphosphate (ATP) derived from mitochondrial oxidative phosphorylation is used by the sodium–potassium ATPase for maintaining the resting membrane potential, various ATPases that restore cytosolic calcium levels after action potentials, vesicular ATPases (vATPases) that load neurotransmitters into vesicles, and vesicular endocytosis. Postsynaptically, ATP is consumed primarily for pumping out the ions that mediate synaptic currents. Presynaptic mitochondria also play an important role in the calcium buffering mediated by the mitochondrial calcium uniporter (MCU) complex, the sodium–calcium exchanger (NCLX), the proton–calcium exchanger (HCLX), and the

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mitochondrial permeability transition pore (mPTP). In astrocytes, an important consumer of ATP is glutamine synthetase, which is needed to maintain the glutamate–glutamine cycle. In oligodendrocytes, mitochondrial lipid metabolism plays a role in myelination. AMPAR; α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; mGluR, metabotropic glutamate receptor; NMDAR, N-methyl-D-aspartate receptor;SERCA, (sacro)endoplasmic reticulum calcium ATPase.

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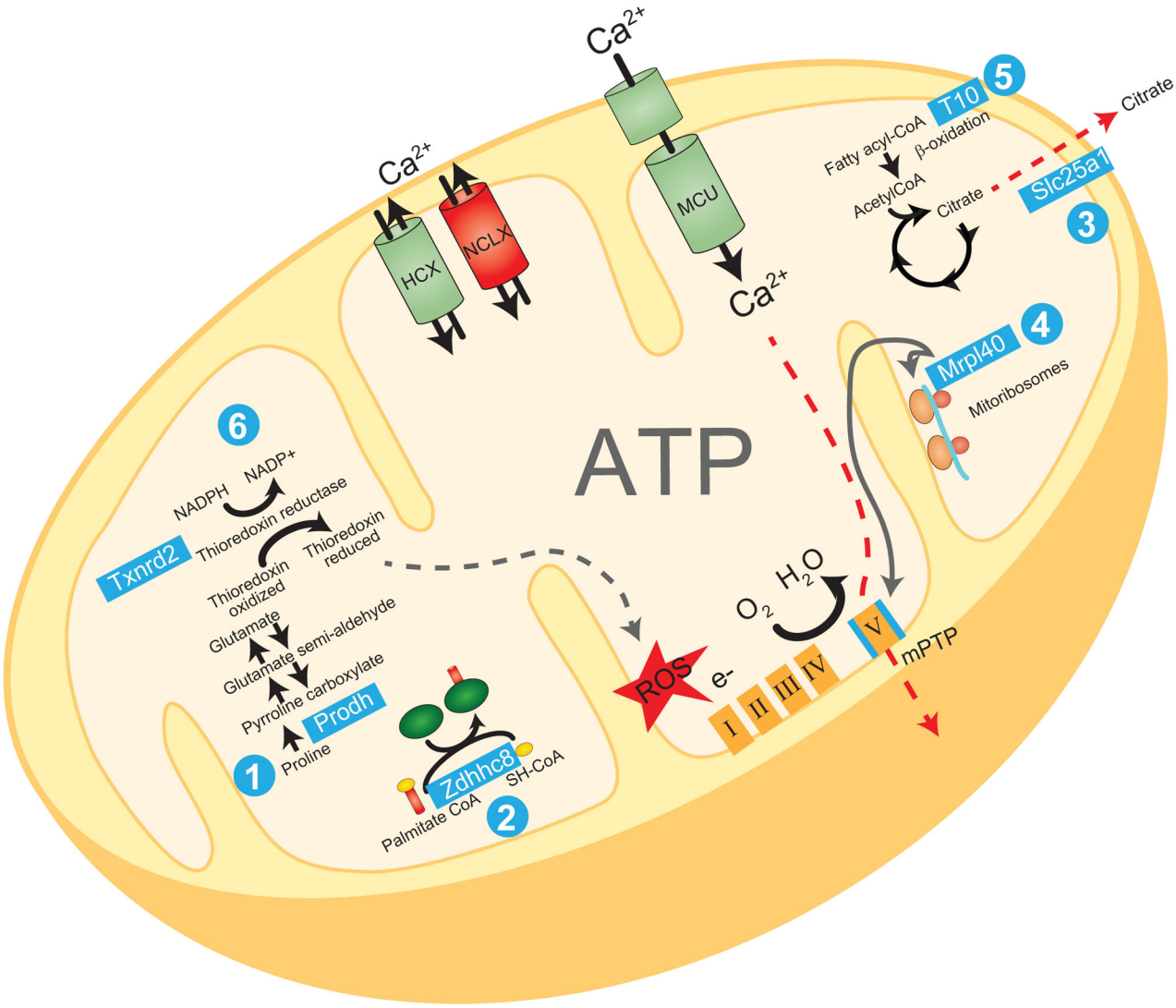

Figure 3. 22q11DS genes encoding mitochondrial proteins and their functions

(**1**) *Prodh* encodes mitochondrial proline dehydrogenase, which is involved in proline metabolism and is interconnected to glutamate synthesis. (**2**) *Zdhhc8* encodes a protein that is involved in palmitoylation of proteins. (**3**) *Slc25a1* encodes the mitochondrial citrate transporter, which mediates fatty acid metabolism. (**4**) *Mrpl40* encodes one of the proteins in the mitochondrial large ribosomal subunit, which affects calcium extrusion via the permeability transition pore (mPTP). (**5**) *T10* encodes a protein that is involved in fatty acid oxidation. (**6**) *Txnrd2* encodes thioredoxin reductase 2, which maintains thioredoxin in a reduced state and contributes to oxidation/reduction (redox) regulation. HCX, hydrogen–calcium exchanger; MCU, mitochondrial calcium uniporter; NADPH and NADP+, nicotinamide adenine dinucleotide phosphate; NCLX, sodium–calcium exchanger; ROS, reactive oxygen species.

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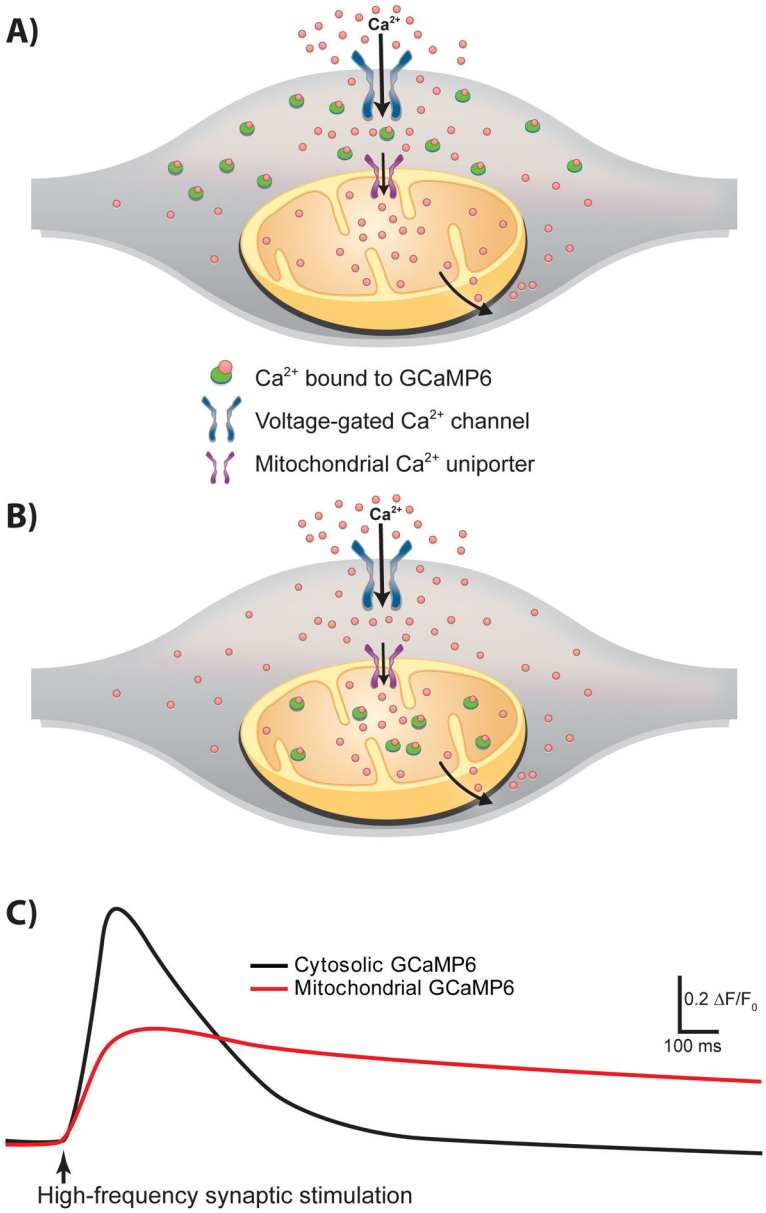

Figure 4. Measurements of activity-dependent calcium transients by using the ultrasensitive genetically encoded calcium indicator GCaMP6

(**A**) Expression of GCaMP6 under the synapsin promoter enables the measurement of calcium levels in the neuronal cytosol/presynaptic terminal. (**B**) GCaMP6 targeted to mitochondria enables the measurement of mitochondrial calcium transients in isolation. (**C**) Mitochondrial calcium transients have a slower rise and much slower decay kinetics than do cytosolic transients in response to a train of stimulations delivered to axonal projections.

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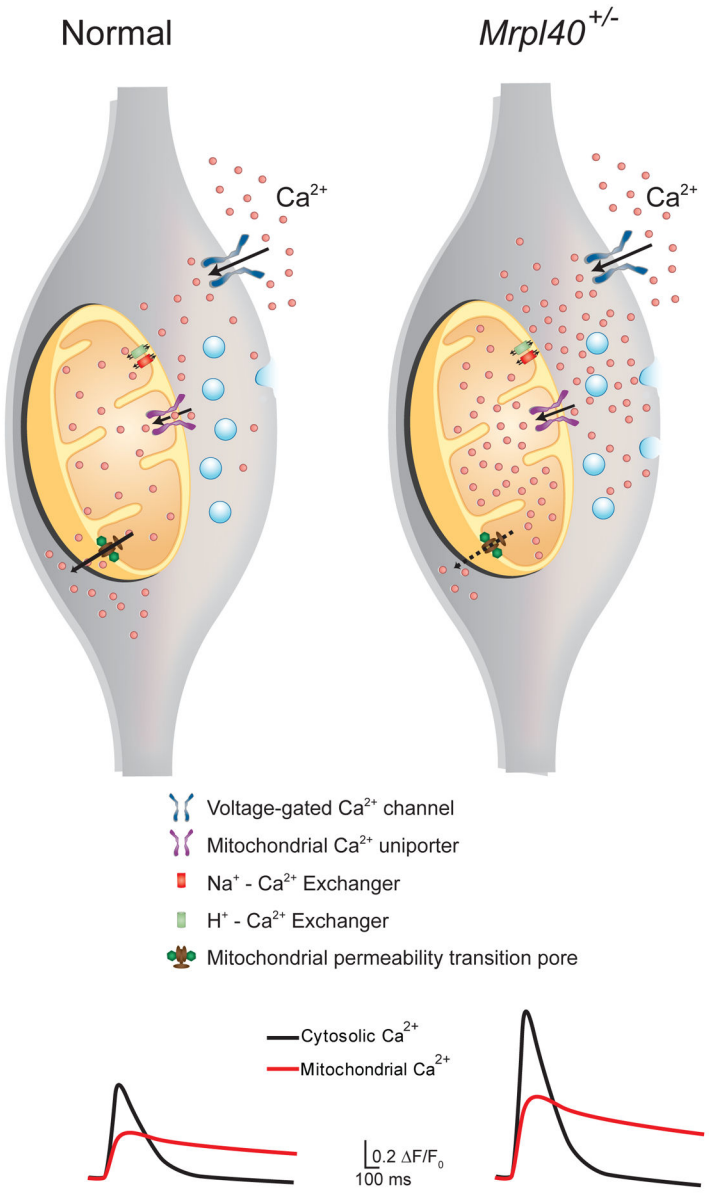

Figure 5. *Mrpl40* haploinsufficiency leads to mPTP-dependent alterations in mitochondrial and presynaptic calcium handling

(**Left**) In normal wild-type presynaptic terminals, the mitochondrial calcium-buffering capacity is aided by controlled influx through the mitochondrial uniporter complex (MUC), bidirectional fluxes through the sodium–calcium and proton–calcium exchangers (NCLX and HCLX, respectively), and slow extrusion of calcium through the low-conductance mode of the mitochondrial permeability transition pore (mPTP). (**Right**) In *Mrpl40*-haploinsufficient terminals, slow extrusion of calcium via the mPTP is hindered, leading to calcium accumulation in the mitochondrial matrix. The resulting reduction in mitochondrial calcium-buffering capacity causes calcium build-up in the presynaptic cytosol, leading to increased release of synaptic vesicles. Traces at the bottom represent mitochondrial and

cytosolic calcium transient currents in wild-type and *Mrpl40*$^{+/-}$ axon terminals measured using GCaMP6.

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