

Mitochondrial Membrane Dynamics and Inherited Optic Neuropathies

ELENI BAGLI^{1,2}, ANASTASIA K. ZIKOU³, NIKI AGNANTIS⁴ and GEORGIOS KITSOS²

¹*Institute of Molecular Biology and Biotechnology–FORTH, Division of Biomedical Research, Ioannina, Greece;* Departments of ²*Ophthalmology,* ³*Clinical Radiology,* and ⁴*Pathology, University of Ioannina, Ioannina, Greece*

Abstract. *Inherited optic neuropathies are a genetically diverse group of disorders mainly characterized by visual loss and optic atrophy. Since the first recognition of Leber's hereditary optic neuropathy, several genetic defects altering primary mitochondrial respiration have been proposed to contribute to the development of syndromic and non-syndromic optic neuropathies. Moreover, the genomics and imaging revolution in the past decade has increased diagnostic efficiency and accuracy, allowing recognition of a link between mitochondrial dynamics machinery and a broad range of inherited neurodegenerative diseases involving the optic nerve. Mutations of novel genes modifying mainly the balance between mitochondrial fusion and fission have been shown to lead to overlapping clinical phenotypes ranging from isolated optic atrophy to severe, sometimes lethal multisystem disorders, and are reviewed herein. Given the particular vulnerability of retinal ganglion cells to mitochondrial dysfunction, the accessibility of the eye as a part of the central nervous system and improvements in technical imaging concerning assessment of the retinal nerve fiber layer, optic nerve evaluation becomes critical – even in asymptomatic patients – for correct diagnosis, understanding and early treatment of these complex and enigmatic clinical entities.*

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Correspondence to: Niki Agnantis, Department of Pathology, University of Ioannina, Stavros Niarchos Avenue, 45500 Ioannina, Greece. Tel +30 2651007792, Fax: +302651007792, e-mail: agnantin@gmail.com and Georgios Kitsos, Department of Ophthalmology, University of Ioannina, Stavros Niarchos Avenue, 45500 Ioannina, Greece. Tel: +30 2651099657, Fax: +30 2651007077, e-mail: gkitsos@cc.uoi.gr

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Mitochondria represent a tubular and branched membrane system playing a fundamental role in several cellular processes required for the development and maintenance of an organism, such as metabolism, apoptosis, ion buffering and autophagy (1-3). They reveal a high degree of interconnectivity and plasticity, mainly dictated by metabolic status and developmental stage (4) and, therefore, a constant state of mitochondrial network flux is fundamental. This dynamic state is achieved through mitochondrial dynamics, a complex machinery of highly conserved mechanisms, including mitochondrial fusion, fission, transport, interorganellar communication and mitochondrial quality control (*i.e.* mitophagy), tuned to a variety of signals and stimuli (5-7), and well-orchestrated by specific intracellular proteins.

The morphology and intracellular distribution of mitochondria vary significantly between tissues and cell types, being enriched in areas of increased metabolic demand, such as neurons, especially the presynaptic and postsynaptic terminals (8). Accordingly, it is not surprising that the pathogenic mechanism of various neurodegenerative diseases is established through an underlying deficiency of mitochondrial energy metabolism (9). However, in recent years it has been shown that impairment of mitochondrial dynamics also leads to synaptic dysfunction, dendritic and axonal degeneration and consequently to neurodegeneration (10, 11). In this respect, restoration of mitochondrial function has become, for some time now, the priority target of novel neuroprotective strategies (12).

Mitochondrial membrane dynamics, and more specifically fission and fusion, are indispensable for mitochondrial distribution and homeostasis. Fusion, the physical merger of two neighboring mitochondria, is necessary for the functional complementation between individual mitochondria *via* intermixing and exchanging proteins, respiratory complexes and mitochondrial DNA (mtDNA) nucleoids (13, 14). It is continuously counterbalanced by fission, a process necessary for the appropriate distribution of mitochondria in dividing cells, as well as their transportation and distribution throughout

the length of neurons (15). Furthermore, fission is essential for mitophagy, a quality-control mechanism that enables the elimination of damaged mitochondria by autophagosomes, and for the distribution of mtDNA nucleoids (16).

The association and balance between mitochondrial fusion and fission, and selective mitophagy, are intricate and tightly coordinated (17, 18). By altering the delicate balance between opposing fusional and fissional forces and modifying the architecture of the organelle itself, in particular the cristae, which house the protein complexes of the oxidative phosphorylation system, the cell has been shown to regulate cell proliferation (19) and energy metabolism (4, 20, 21).

Four large guanosine-5'-triphosphate hydrolases (GTPases), members of the dynamin superfamily located in the cytosol or inner (IMM) and outer (OMM) mitochondrial membranes, mediate mitochondrial fusion and fission. Specifically, optic atrophy 1 (OPA1), and mitofusins 1 and 2 (MFN1 and -2) mediate mitochondrial fusion (22), while dynamin-related protein 1 (DRP1) is essential for fission (23).

Mitochondrial fusion is necessarily a multistep process, since at least two distinct membrane fusion events occur. The OMM and IMM, which delineate a mitochondrion, merge with the corresponding membranes on another mitochondrion. These events result in mixing of the membranes, the intermembrane space (IMS), and the matrix. MFN1 and MFN2 are highly conserved and have been identified as mediators of OMM fusion (24). OPA1 on the other hand is involved in IMM fusion and morphology of mitochondrial cristae (25-27). It is synthesized within the cytoplasm as a pre-protein and once transported into the mitochondria undergoes proteolytic cleavage to generate long (L-OPA1) and short (S-OPA1) isoforms of OPA1 (28). This OPA1 processing is reported to be regulated by several mitochondrial proteases including presenilin-associated rhomboid-like (PARL) (25), mitochondrial ATPases associated with diverse cellular activities (m-AAA) proteases (paraplegin and ATPase family gene 3-like protein 2-AFG3L2) (29), the mitochondrial inner membrane AAA (i-AAA) protease yeast mitochondrial escape (YME1)-like 1 ATPase (YME1L) and the membrane-bound metallopeptidase with activities overlapping with the m-AAA protease (OMA1) (30). Mutations in *MFN2* and *OPA1* gene were initially reported as being responsible for the rare neurodegenerative diseases Charcot-Marie-Tooth subtype 2A (CMT2A) and autosomal dominant optic atrophy (DOA), respectively (31, 32).

DRP1, the key player in fission, localizes primarily to the cytosol, but upon activation of mitochondrial fission, it is oligomerized and recruited by OMM-localized receptors, mainly mitochondrial fission factor (MFF) (18, 33, 34), mitochondrial division 49/51 (MiD49/51) (33), and less apparently mitochondrial fission 1 (FIS1) (18, 33), to the OMM, where it forms high-molecular-weight protein complexes marking active or prospective fission sites (23). Mitochondrial recruitment, assembly, activity and stability of DRP1 are

regulated by several post-translational modifications (35, 36).

The recognition of all the implicated molecular players and their exact role in fission/fusion has not been elucidated yet and remains an area of intense investigation. Accumulative evidence suggests that the role of mitochondrial dynamic proteins regarding fusion and fission machinery is not that distinguishable. Mitochondrial fusion proteins, such as OPA1, can regulate mitochondrial fission (30), and DRP1-dependent changes in mitochondrial morphology, on the other hand, might control MFNs and OPA1 (37). Furthermore, recent studies have pinpointed the importance of epigenetic and post-translational modifications of the known key players DRP1, MFNs and OPA1 in the regulation of their function (25, 35, 36, 38, 39). Remarkably, mutations in several of these regulatory proteins (usually involved in protein processing) have been identified lately as causal gene products of syndromic or nonsyndromic inherited optic neuropathies.

Inherited optic neuropathies are a clinically and genetically heterogeneous group of disorders, characterized by typically bilateral, symmetrical, irreversible reduced visual acuity, color vision deficits, visual field defects and the clinical appearance of optic atrophy (40). The clinical spectrum usually varies even between the members of the same family and, in some cases, individuals develop additional neurological complications indicating a greater vulnerability of the central nervous system (CNS) in susceptible mutation carriers (41). Therefore, besides isolated optic neuropathies, optic atrophy is recognized as a prominent feature in many neurodegenerative diseases caused by primary mitochondrial dysfunction. However, because of the heterogeneity and the highly variable phenotypes of these disorders, their diagnosis becomes a challenging task. Moreover genotype-phenotype correlations are usually highly speculative, probably because of tissue-specific expression of different isoforms of the affected protein, secondary unrecognized genetic factors and insufficient knowledge concerning the multi-factorial process of mitochondrial dynamics. However, during the last few years there has been an expansion of data concerning the phenotypic and genotypic spectrum of these disorders because of improved diagnostic imaging [magnetic resonance imaging (MRI), optical coherence tomography (OCT)] and molecular technology (DNA sequencing, transgenic animal models *etc.*). As a result, a greater understanding of the complex molecular mechanisms underpinning this broad range of neurodegenerative diseases is expected in the near future.

In light of this new evidence, the role of the main molecular players of mitochondrial fusion/fission machinery in the pathogenesis of inherited optic neuropathies is discussed in this review. Particular emphasis is placed on novel mitochondrial dynamic proteins, whose mutations were recently identified as accounting for the development of clinical entities involving the optic nerve only or in association with extraocular manifestations.

Mitochondrial Dynamic Proteins Related to the Pathogenesis of Optic Neuropathy

OPA1. The *OPA1* gene, encodes a 960-amino-acid, dynamin-like mitochondrial GTPase. *OPA1* open reading frame (ORF) comprises of 30 exons and 8 transcript variants (42), whose expression is tissue-specific in humans (43-45), and which arise from alternative splicing of three exons. *OPA1* is ubiquitously expressed, localized to the IMS and is firmly anchored to the IMM *via* an *N*-terminal transmembrane segment containing a mitochondrial targeting sequence (MTS) (38, 46). It also displays a GTPase domain, a dynamin central region and a coiled-coil C-terminal domain that correspond to the dynamin GTPase effector domain (GED), involved in oligomerization of this protein into cylindrical tubular structures and catalytic activation (44, 47). The *OPA1* protein is synthesized as a precursor that undergoes complex proteolytic processing within the mitochondria (38). Matrix metalloproteases first remove the IMS during mitochondrial import to give rise to IM-anchored L-*OPA1*, (38, 46), which can undergo further proteolytic cleavage leading to the production of S-*OPA1* (28) (46). S-*OPA1* interacts with uncleaved forms of L-*OPA1* at crista junctions and with subunits of the mitochondrial contact site and cristae organizing system (MICOS), which is involved in the maintenance of crista structure (48-50).

L-*OPA1* is responsible for mitochondrial fusion, while S-*OPA1* was shown to be involved in IMM fission, when accumulated (30, 51), indicating a highly regulated, dual role of *OPA1* in fusion and fission (30). The levels of L- and S-*OPA1* can vary dramatically across tissues (30) and due to various stress conditions. A drop in mitochondrial membrane potential and induction of apoptosis, for instance, may induce massive processing of L-*OPA1* to S-*OPA1* (28, 51). The balance between the different isoforms of *OPA1*, dictated by the post-translational maturational steps of *OPA1*, plays a fundamental role in mitochondrial morphology, the regulation of mitochondrial fusion and fission (25, 28, 51), the organization of cristae, the maintenance of membrane potential, calcium clearance and the assembly of respiratory chain supercomplexes for achieving maximal respiratory efficiency (26, 27, 52, 53). It was recently shown that *OPA1* senses energy substrates in order to regulate crista structure, in a manner dependent on solute carrier 25A (SLC25A) protein (54), while proteolytic activation of *OPA1* is sufficient to stimulate IMM fusion in a process that is sensitive to oxidative phosphorylation (55). Moreover, a direct role of *OPA1* in mtDNA maintenance has been attributed to a small IM-anchored *OPA1*-derived peptide (52) and loss of mtDNA was recently observed upon loss or down-regulation of *OPA1* (52). Finally, *OPA1* was reported to have independent anti-apoptotic activity mediated by particular *OPA1* splice variants (44), oligomerized to complexes that control apoptotic crista remodeling (48).

OPA1 mutations are the most frequently found in patients with non-syndromic DOA, and to date, more than 250 mutations of this gene have been identified (32). Isolated DOA is characterized by a homogeneous phenotype including progressive, bilateral visual impairment occurring during the first two decades of life, temporal optic disc pallor, loss of the central visual field and color vision defect (56, 57). This disease affects primarily the retinal ganglion cells (RGCs), whose axons form the optic nerve and the estimated prevalence is 1:10,000 to 1:50,000. *OPA1* mutations were found in about 60-80% of patients with DOA, with incomplete penetrance in 43-100% of the cases (56). Cases with *de novo* mutation, sporadic cases, and cases with unknown familial history, account together for 50% of all patients (56). The visual impairment is irreversible, usually moderate, but highly variable between and within families, ranging from asymptomatic state to blindness. Up to 20% of *OPA1*-related disorders are syndromic (58) (called DOA-plus syndrome). The extraocular involvement includes sensorineural deafness, ataxia, myopathy, chronic progressive external ophthalmoplegia and peripheral neuropathy, usually presented after optic neuropathy during young adulthood. Visual acuity and optic nerve damage is typically worse in DOA-plus patients compared to ones with isolated optic neuropathy (59) and deafness seems to be the most common extraocular symptom just after optic neuropathy. Brain MRI also discloses various cerebral abnormalities, including lactate peak, as well as cerebellar and cortical atrophy, present even in non-syndromic cases (60). Finally, DOA-plus form presented in young adulthood has been distinguished further into typical DOA-plus and to severe early-onset Behr-like syndromes (61).

Since the spectrum of *OPA1*-related disorders is highly variable regarding the age of onset, the severity of visual loss, and the number, type and severity of extraocular symptoms, it is still unclear if these various DOA phenotypes represent different aspects of one clinical condition or constitute distinct clinical entities (61). Interestingly, a recent report suggests that *OPA1* mutations may also be implicated in systemic conditions, including spastic paraplegia, multiple sclerosis, Parkinsonism or dementia (61). However, despite this large clinical heterogeneity, most dysfunctions concern the central, peripheral and autonomous nervous system.

OPA1 mutations are spread along the coding sequence of the gene but most cluster in the GTPase domain and in the dynamin central region. Single base-pair substitutions represent the most common mutational subtype, followed by deletions, and insertions (47, 62). In some cases, there are two (different) missense mutations on different alleles which, although recessive, lead to a stronger phenotype compared to either of the single mutations (61, 63). Among the few recurrent variants, some have been frequently reported, such as a deletion in the GED at the C-terminus (61). Since *OPA1* oligomerizes (48), mutations in the GED domain of one allele can complement mutations in the GTPase domain of another

allele (64). Genomic rearrangements in *OPA1* gene, as well as deletion of the entire one copy or part of the *OPA1* gene have been reported (63, 65, 66), indicating haploinsufficiency as the main pathogenic mechanism. Overall, the majority of *OPA1* mutations result in premature termination codons and unstable truncated mRNAs, which are degraded by protective surveillance mechanisms operating *via* nonsense-mediated mRNA decay (63, 67, 68), providing further evidence for haploinsufficiency. Missense *OPA1* mutations affecting the catalytic GTPase domain in patients with DOA, on the other hand, are more likely to exert a dominant-negative effect (47, 58, 69, 70) and these patients have a two- to three-fold increased risk of developing syndromic DOA compared to those with truncating mutation (58, 61, 71). Although these syndromic DOA variants are characterized by significant phenotypic variability, a worse visual prognosis as well as thinner retinal nerve fiber layer evaluated by OCT (72) suggest a deleterious gain-of-function effect of these mutations. The different mutations in *OPA1* are usually not correlated with the severity of the disease (73). In this respect, mtDNA instability, due to defective mitochondrial dynamics caused by a dysfunction of the *OPA1* gene, has been identified in DOA-plus patients (70, 74) and could be responsible for the multisystem phenotype through direct functional consequence on the respiratory chain capacity (61, 70, 74, 75). It could also explain the clinical overlap between syndromic DOA and other related to mtDNA mutation-associated disorders. However, mtDNA deletions are also present in patients with isolated DOA (76). Secondary nuclear genes are also suspected to control the severity of the disease in non-syndromic patients (61, 77).

Impairments of mitochondrial morphology (78) and functions, including increased autophagy or apoptosis (44), generation of reactive oxygen species (75) and impaired oxidative phosphorylation (4, 68, 74, 75), are evident in cells derived from affected patients. Although defects in the respiratory chain are less consistent in reports (75, 79), the widespread deleterious consequences of *OPA1* mutations on RGCs, as well as other neuronal cell types and skeletal muscle (61), are well documented.

m-AAA proteases: spastic paraplegia 7 (*SPG7*) and (*AFG3L2*). *SPG7* (paraplegin) and its close homolog *AFG3L2* contain a M41 metallopeptidase domain and an ATPase domain characteristic of the AAA family of ATPases (*m*-AAA proteases) (80). They form a hetero-oligomeric proteolytic complex at the IMM, responsible for the proteolytic cleavage of mitochondrial ribosomal protein L32 (MRLP32) and *OPA1* (29). As a result, they regulate the equilibrium between pro-fusion and pro-fission isoforms of *OPA1* (81). Moreover, *AFG3L2* and *SPG7* homo and hetero-polymeric hexameric functional complexes (82) play essential roles in protein quality control within mitochondria and have broad functional targets

including control of ribosome and respiratory chain subunits assembly (83, 84) and degradation of misfolded proteins (85).

SPG7. Paraplegin is the product of the *SPG7* gene. Recent literature suggests that *SPG7* also plays an essential role in the regulation of the mitochondrial permeability transition pore (86). Impaired axonal transport, ultrastructural mitochondrial abnormalities, including aberrant cristae, as well as hyperfragmentation of the mitochondrial network, have been demonstrated in paraplegin-deficient mice (87), features similar to those of cells isolated from *OPA1*-deficient patients.

Mutations of the *SPG7* gene are responsible for both the autosomal recessive form of hereditary spastic paraplegia (HSP) (88) involving the optic nerve and autosomal dominant non-syndromic optic neuropathy (89).

HSPs are a group of similar neurodegenerative disorders with a clinical presentation of weakness and spasticity in the lower limbs (90). Mutations in *SPG7* were initially reported in three families, two with isolated spasticity and one with a complex phenotype (91), characterized by age of onset ranging from 10 to 45 years (92-95), cerebellar involvement with/without mild cerebellar atrophy observed on MRI scans (91, 93, 94, 96, 97), optic neuropathy (91, 92), ptosis (94, 95) and supranuclear palsy (94). Optic neuropathy usually presents as mild or subclinical visual impairment, revealed only by OCT. *SPG7*-related DOA, on the other hand, is characterized by progressive visual loss starting the first decade of life, with final visual acuity of 1/10-3/10 in the second decade (89).

The mutational spectrum responsible for HSP ranges from missense and nonsense mutations to large intragenic *SPG7* deletions (90). Among them, strong evidence has been provided regarding the pathogenic role of p.Ala510Val variant, which leads to disturbed proteolytic function of the hetero-oligomeric m-AAA protease (92, 93, 97-99). On the other hand, Asp411Ala mutation, the first mutation segregating with isolated autosomal dominant optic neuropathy, is located in the AAA domain of the protein, downstream of the Walker B motif (which with the upstream Walker A motif are implicated in the fixation and hydrolysis of ATP) and causes impaired proteolytic activity (89).

Because paraplegin is closely related to *AFG3L2*, is incapable of self-assembling into homo-oligomers and requires *AFG3L2* for its function, it has been suggested that variants in *AFG3L2* might also act as genetic modifiers contributing to the clinical heterogeneity and the variable severity of *SPG7*-related diseases.

AFG3L2. Heterozygous missense mutations in the *AFG3L2* gene have been shown to be responsible for autosomal-dominant spinocerebellar ataxia type 28, characterized by onset in young adulthood, slowly progressive gait and limb ataxia, increased reflexes, dysarthria and ophthalmoparesis (89, 100, 101). Patients harbouring homozygous *AFG3L2*

mutations present with a more severe phenotype including dystonia, oculomotor apraxia, and progressive myoclonic epilepsy (102), but not optic neuropathy.

However, a novel mutation p.Arg468Cysc in a highly conserved arginine-finger motif in the AAA domain of AFG3L2 was reported recently to account for the development of DOA presenting as bilateral, symmetrical visual loss starting in infancy accompanied by mild mental retardation without signs of cerebellar impairment (103).

i-AAA protease *YME1L*. YME1L1 belongs to the AAA family of ATPases and it is a nuclear genome-encoded ATP-dependent metalloprotease. YME1L1 is embedded in the IMM, while its protease domain faces the IMS (*i*-AAA protease) (104-106). YME1L1 contains a MTS, which is cleaved-off by mitochondrial processing peptidase once it is imported into mitochondria (107). The mature protein then assembles into a homo-oligomeric complex within the IM (104, 108). YME1L1 degrades both IMS and IM proteins, such as lipid transfer proteins (109), components of protein translocases of the IM (110, 111), and OPA1 at S2 site (30, 111, 112). Depletion of YME1L1 was shown to cause increased mitochondrial fission and mitochondrial network fragmentation through acceleration of OMA1-dependent L-OPA1 cleavage, which leads to S-OPA1 accumulation (30, 113), or through a mechanism involving DRP1 and MFF (114). Finally, loss of YME1L1 perturbs crista morphogenesis, and renders cells susceptible to apoptosis (115).

YME1L1 mutations were recently recognized as accounting for infantile-onset developmental delay, muscle weakness, ataxia, and optic nerve atrophy. Homozygous missense mutation (c.616C>T) located in a highly conserved region in the mitochondrial pre-sequence of YME1L1 leads to inhibition of its cleavage by the mitochondrial processing peptidase and the subsequent rapid degradation of YME1L1 precursor protein. The abnormal processing of OPA1, due to impaired YME1L1 function, was shown to cause proliferation defect and mitochondrial network fragmentation (116) and seems to be the main molecular mechanism underlying the pathogenesis of this *YME1L1* mutation.

MFN1 and 2. MFN 1 and -2 are two homolog GTP-binding proteins of the dynamin-superfamily involved in OMM fusion (117) and expressed ubiquitously but differently in various tissues (117, 118). They share an *N*-terminal GTPase domain followed by a first coiled-coiled heptad repeat region (HR1), two adjacent small transmembrane domains and a *C*-terminal second coiled-coiled heptad repeat region (HR2). MFN2, but not MFN1 also possesses an *N*-terminal RAS-binding domain (119). The *N*-terminal and *C*-terminal domains extend perpendicularly into the cytosol, while the hydrophobic domain spans the OMM (117), resembling an unfolded mitofusin conformation optimal for mitochondrial tethering and therefore

permissive for fusion (120, 121). However, it seems that conformational plasticity exists and mitofusins adopt either a fusion-constrained or fusion-permissive molecular conformation directed by specific intramolecular binding interactions through HR1 and HR2 domains (120, 122). MFN1/2 form homo- and hetero-oligomers on the OMM and are required for an elongated mitochondrial network (24). MFN2 plays a fundamental role in mitochondrial dynamics, including mitochondrial transport, mitophagy and communication with other organelles (11, 123), as well as in cellular bioenergetics. Specifically, it coordinates mitochondrial fusion by working in close tandem with its fellow fusogenic protein, OPA1 (124), and it is involved in mitochondrial biogenesis (21, 125) by regulating the expression of nuclear-encoded respiratory chain subunits (126). It is also important for tethering mitochondria to the endoplasmic reticulum (ER) (127) and modulating some functions of the ER (128, 129). Furthermore, it is implicated in apoptotic cell death and OMM permeability, oxidative phosphorylation (126) and microtubule-related mitochondrial transport in axons *via* interaction with Miro-proteins (130, 131). Therefore, it is not a surprise that deletion of *MFN2* is embryonically lethal and alterations of its functions have been associated with a number of different pathological conditions, ranging from neurodegeneration to impaired glucose homeostasis (11, 129). Interestingly, no disease has been associated with mutations of *MFN1*.

MFN2 mutations account for 20% of Charcot-Marie-Tooth disease type 2 (CMT2) cases, making this the most prevalent axonal form of CMT (31, 132). CMT2A is a neurological disorder characterized by complex phenotypes, including not only neuropathy-related features but also systemic impairment of the CNS (11). Both autosomal dominant and autosomal recessive inheritance of these mutations, as well as sporadic new mutations have been described (133). Furthermore, truncation mutations in *MFN2* have been shown to be responsible for hereditary motor sensory neuropathy type VI (HSMN VI), a rare early-onset axonal type of CMT associated with bilateral sub-acute, sometimes improved spontaneously, or acute optic neuropathy (11, 31, 134-136). Although symptoms and disease severity are heterogeneous, the frequency of *MFN2* mutations was shown to be significantly higher among severely affected patients with CMT2A (137, 138). *MFN2* mutations might also be found in all subtypes of CMT (139). Moreover, *MFN2* mutations are linked to a spectrum of clinical manifestations wider than CMT2 neuropathy alone. Indeed, a phenotype resembling the DOA-plus phenotype, characterized by early-onset optic atrophy, axonal neuropathy and mitochondrial myopathy, has been associated with a novel *MFN2* missense mutation (c.629A>T, p.D210V) accompanied by multiple mtDNA deletions (140). Furthermore *MFN2*-related disorders may even mimic multiple sclerosis, when presenting with optic atrophy, brain lesions on MRI and mild or unrecognized neuropathy (141).

Most *MFN2* mutations in CMT2A are missense and cluster within the GTPase and in the HR1 domains (31, 137, 138, 142, 143). A mutational hotspot region located immediately upstream of the GTPase domain has been recognized (31). Previous reports suggested that patients with compound heterozygosity have more severe neuropathy, and their phenotypes are consistent with an additive dominant negative effect of *MFN2* mutations (144). Some mutations lead to a gain of function, since the mutated protein tends to aggregate in mitochondria, while other mutants result in loss of function. Since mitochondrial fusion is impaired (31, 145), the subsequent disequilibrium between fusion and fission processes may negatively affect cell function and serve as a potential mechanism of neurodegeneration (11). However, the pathogenicity of *MFN2* mutations is suggested to rely also on other functions of the MFN2 protein, such as the interaction between mitochondria and the organelle transport machinery. Indeed recently, several studies using *in vitro* and *in vivo* models of CMT2A highlighted the relationship between impaired mitochondrial trafficking due to *MFN2* mutations and axonal degeneration (11).

Particular *MFN2* mutations have been associated with specific clinical features. In this way, visual impairment has been associated with missense or nonsense mutations and incomplete penetrance of visual loss has been linked to Q276R mutation (11). However it seems that a genotype–phenotype correlation cannot be established. The same mutation can be associated with both early- and late-onset disease, as well as different signs and symptoms, even among members of the same family (135), which is probably due to environmental or other factors that modulate genetics (11).

OPA3. OPA3 is composed of at least three exons that are alternatively spliced to produce two major transcripts: OPA3A and OPA3B, (146). Defects in OPA3 have been associated with both recessive and dominant optic neuropathy (147). Interestingly, mutations of *OPA3*, *OPA1* and *SPG7* have all been shown to be responsible for non-syndromic DOA. 3-Methylglutaconic aciduria type III (3-MGCA type III), also known as Costeff optic atrophy syndrome, is a neuro-ophthalmological syndrome caused by recessive mutations in the *OPA3* gene, most prevalent amongst individuals of Iraqi-Jewish origin and typically characterized by early-onset bilateral optic atrophy, late development of a movement disorder (ataxia or extrapyramidal dysfunction) of variable severity beginning in the first or second decade of life, and increased urinary excretion of 3-methylglutaconic acid (148–152). Autosomal dominant OPA3-related disease on the other hand is less common than the recessive form, with only a few families identified to date (147), but highly penetrant (153). It is characterized by optic neuropathy either isolated or more often associated with congenital/infantile lenticular opacity, hearing loss and neurological symptoms (147). It is a

clinically heterogeneous disorder, indicating a complex molecular pathology. Some patients present with poor visual acuity and nystagmus from birth, while others experience a slowly progressive, symmetrical decrease in vision starting in the first two decades of life, or remain asymptomatic until later in life. The optic neuropathy is similar to that caused by *OPA1* mutations characterized by primary involvement of the papillomacular bundle (147). The phenotypic spectrum of autosomal dominant mutations in *OPA3* was further expanded recently with the identification of a *de novo* mutation in exon 2 encoding OPA3 Leu79Val, which leads to an unusually severe phenotype including optic atrophy, cataracts, ataxia, and peripheral and autonomic neuropathy (154).

The functional and biological role of OPA3 is still unclear. The presence of a putative *N*-terminal MTS indicates its import into mitochondria (146). However the studies concerning OPA3 localization in the IMM or OMM are controversial (146, 155, 156). The *N*- and *C*-terminal regions are reported to be exposed to the IMS and cytoplasm, respectively. OPA3 seems to play a regulatory role in mitochondrial dynamics and more specifically in fission, since overexpression of *OPA3* significantly induced mitochondrial fragmentation. It is also referred to as sensitizing cells to apoptosis (156).

The three known 3-MGCA type III-related mutations are all assigned to exon 2 of *OPA3A* (157) and its adjacent splice site (146, 149), while G93S and Q105E mutations lead to DOA associated with cataracts and extrapyramidal signs (158). Overexpression of a familial *OPA3* mutant (G93S) induced mitochondrial fragmentation and spontaneous apoptosis, suggesting that OPA3 mutations may cause optic atrophy *via* a gain-of-function mechanism (156). Furthermore, a novel insertion, c.10_11insCGCCCG/p.V3_G4insAP, which is located in the mitochondrial presequence, results in decreased steady-state levels of the mutant protein compared with the native one and leads to DOA and hearing loss (153). A fragmented mitochondrial network and reduced mitochondrial mass was observed in cells isolated from individuals harboring this novel mutation (153).

SLC25A46. SLC25A46 belongs to the SLC25 family of mitochondrial carrier proteins responsible for the transfer of diverse substrates across the IMM (159). SLC25A46 is an ortholog of UGO1, a yeast outer membrane protein essential for mitochondrial membrane fusion (160–163). It is an integral OMM protein which has been shown to interact with the IMM remodeling protein mitofilin (164), OPA1 and MFN2 (165, 166). Moreover, the oligomerization of OPA1 and the regulation of OPA1-dependent crista modulation, a cellular adaptation to metabolic demand, were shown to be dependent on SLC25A46–OPA1 interaction (54). SLC25A46 also interacts with components of the MICOS complex, which is indispensable for the maintenance of mitochondrial crista

junctions (166-171). However, unlike UGO1, SLC25A46 acts as a pro-fission component in the regulation of mitochondrial dynamics (160, 165). Loss of SLC25A46 results in a hyperfused mitochondrial phenotype in human cells (164, 166, 172) due to increased stability and oligomerization of MFN1/2 on mitochondria (166). A recent study showed that SLC25A46 plays an important role in the maintenance of architecture of mitochondrial cristae and mitochondrial lipid homeostasis by functioning upstream of the MICOS complex and by interacting with the ER/mitochondrial contact site complex, respectively (173, 174). However, further investigation is needed, since this mechanism of action was questioned by another study using a different cell system (166). Since SLC25A46 is involved in mitochondrial ultrastructure *via* interaction with a large number of proteins and formation of transient complexes, it probably acts as a coordinator of rapid changes needed in mitochondrial dynamics and crista morphology in response to changes of the microenvironment.

Recessive *SLC25A46* mutations were recently identified in a syndrome named optic atrophy spectrum disorder that has since been designated hereditary motor and sensory neuropathy type VIB (HMSN_{VIB}), which covers a broad clinical spectrum including early-onset optic atrophy, peripheral neuropathy and cerebellar degeneration, with variable age of onset and severity (164). In particular, *SLC25A46* was identified as a new locus accounting for congenital pontocerebellar hypoplasia (PCH) a lethal condition considered as one extreme of HMSN_{VIB}. The impact of *SLC25A46* mutations on the variable phenotype of HMSN_{VIB} seems to be dictated by the relative stability of the encoded protein (164). In agreement with that, destabilized and nonfunctional mutant *SLC25A46* L341P correlates with the phenotype of lethal congenital PCH syndrome and a homozygous missense mutation in *SLC25A46* (c.425C >T), also causing destabilization and loss of *SLC25A46* function, was shown to account for the development of Leigh syndrome, an early-onset and fatal neurodegenerative disease associated with bilaterally symmetric lesions in the brainstem, basal ganglia, and spinal cord. Therefore, the regulation of *SLC25A46* level seems to be fundamental for its function. In accordance with this, ubiquitin ligases were reported to contribute to the selective and rapid degradation of *SLC25A46* L341p by the proteasome (166).

ATPase family AAA-domain containing protein 3A (ATAD3A). *ATAD3A* is a nuclear-encoded protein, ubiquitously expressed in multicellular organisms. *ATAD3A* interacts simultaneously with both the IMM and OMM and it seems to be devoid of the function of mitochondrial AAA⁺ protease, since it lacks a proteolytic domain. Monoallelic and biallelic variation involving both single-nucleotide variants and copy-number variants at the *ATAD3A* locus was recently recognized in seven families as leading to a primarily neurological disease

characterized by global developmental delay, hypotonia, optic atrophy and axonal neuropathy (175).

The *ATAD3* gene family in humans includes three paralogs (*ATAD3A*, *ATAD3B*, *ATAD3C*) (176, 177). *ATAD3A* has an *N*-terminal domain including two coiled-coil domains with high oligomerization probability that interacts with the OMM and a central transmembrane segment, which anchors the protein in the IMM and positions the *C*-terminal AAA(+) ATPase domain (Walker A and Walker B) in the matrix. *ATAD3A* interacts simultaneously with both mitochondrial membranes and therefore, it regulates mitochondrial dynamics at the interface between the IMM and OMM and is involved in several cell responses, such as cell growth, enhanced channeling of cholesterol for hormone-dependent steroidogenesis (178-180) and mitochondrial fission (178). It was also shown to play a role in mtDNA maintenance and replication (181), as well as cancer cell growth and metastasis (182). Loss of function of *ATAD3A* at contact sites between the OMM and IMM was shown to induce cell fission machinery (178). Since *ATAD3A* can co-immunoprecipitate with MFN2, OPA1 and DRP1, a possible role of *ATAD3A* in mitochondrial dynamics could be maintained through these interactions (178).

Defective ATP-binding *ATAD3A* mutations were shown to interfere with normal oligomer functions leading to fragmentation of mitochondria (178). Furthermore, the p.Arg528Trp variant was shown to act through a dominant-negative mechanism in fibroblasts isolated from affected individuals by generating small mitochondria that trigger mitophagy (175). On the other hand, the recessive copy-number variants lead to infantile lethality through loss of *ATAD3A* function.

DRP1. DRP1 is a dynamin-related GTPase essential for mitochondrial fission in mammalian cells. It is recruited to mitochondria *via* receptors, reversibly associate with the OMM and further assembles around mitochondrial tubules to form an oligomeric ring that constricts and divides the mitochondrion in a GTP-dependent process (23, 183, 184). It contains four distinct domains: GTP-binding, middle, insert B, and GED (185). Mutation of *DRP1* blocks mitochondrial fission, resulting in elongated mitochondrial networks due to unopposed mitochondrial fusion (23). This cellular defect causes developmental lethality in mice (186). Only a single case study of a patient with a *DRP1* mutation has been described (187). This patient was a newborn girl, who died at 37 days due to multisystem abnormalities, including small head circumference, hypotonia, few spontaneous movements, optic atrophy and poor feeding. The heterozygous mutation (A395D) in this patient located in the middle domain, important for self-assembly, suggests that this mutant dramatically disrupts higher-order assembly of DRP1 and thereby reduces assembly-induced GTP hydrolysis in a dominant-negative mode of action

(185). Analysis of the isolated fibroblasts showed elongated mitochondria and peroxisomes.

MFF. MFF is essential for fission of mitochondria and peroxisomes (34, 188). It is considered the major membrane DRP1 receptor and important regulator of organelle fission (33, 184, 189). Interestingly, MFF was shown to efficiently stimulate the GTPase activity of the brain-specific DRP1 isoform compared to ubiquitously expressed isoform and to mediate DRP1 recruitment to synaptic vesicles (189). As a result, when it is knocked-down, mitochondria become elongated (34) and the amount of DRP1 recruited to them is reduced. MFF is anchored in the OMM through its C-terminal transmembrane segment. The sequence of MFF indicates that it also interacts with other proteins and participate in protein complexes that mediate the fission pathway (33, 34).

A truncating mutation in MFF was first identified in a Saudi Arabian boy with delayed psychomotor development, spasticity, optic atrophy, and bilateral, increased signal intensities of the basal ganglia (190). Furthermore, recently three boys with similar overlapping phenotype were found to carry truncating mutations in MFF (191). The disease onset was in the first year of life, characterized by seizures, developmental delay and acquired microcephaly, followed in subsequent years by dysphagia, spasticity, optic neuropathy and peripheral neuropathy (191). Brain MRI showed Leigh-like patterns, with bilateral changes of the basal ganglia and subthalamic nucleus, indicating impaired mitochondrial energy metabolism (191). However, the activity of mitochondrial respiratory chain complexes were found to be normal at least in skeletal muscle. These biallelic loss-of-function variants of MFF were recognized as causing extremely elongated, interconnected mitochondria and peroxisomes, and loss of DRP1 recruitment to the fission nodes in cells isolated from the affected individuals (191).

Conclusion

Inherited optic nerve degeneration due to dysfunction of mitochondrial metabolism and predominantly respiratory chain defects has been identified in patients with Leber's hereditary optic neuropathy harboring mtDNA mutations, as well as individuals carrying mutations in novel genes, such as transmembrane protein 126A (*TMEM126A*), aconitase 2 (*ACO2*), reticulon 4-interacting protein 1 (*RTN4IP1*), chromosome 12 ORF 65 (*C12orf65*), NADH: ubiquinone oxidoreductase core subunit (*S2NDUFS2*), mitochondrial tRNA translation optimization 1 (*MTO1*), and deafness dystonia protein 1 (*DDP1*) (41). However, the pathogenic mechanism underlying RGC loss is much more complex and impairment of mitochondrial network dynamics due to genetic defects has also been shown to account, alone or in combination with bioenergetic crisis, for syndromic or isolated optic neuropathies.

Several inter-related molecular, tissue-specific factors seem to create a complex interacting network not fully understood yet which dictates mitochondrial function and thereby cellular and tissue integrity. This complexity may be responsible for genotype-phenotype disparity and this translates into phenotypic heterogeneity, a hallmark for the need for early and correct diagnosis of these disorders. The application of more advanced techniques regarding CNS (MRI) and nerve fiber layer (OCT) imaging in recent years offers an accurate evaluation of CNS/optic nerve involvement, recognition of asymptomatic patients and subsequent expansion of the phenotypic spectrum. It is obvious that a comprehensive phenotypic profile combined with knowledge gained from detailed genotypic characterization, molecular studies on cells isolated from affected patients and transgenic animal disease models is the only promising approach in order to dissect the relative role of the involved molecules and elucidate the signal pathways underlying these monogenic, as well as complex, neurodegenerative disorders. To that end, generation of induced pluripotent stem cells by reprogramming fibroblasts isolated from affected individuals and their subsequent differentiation into various neural cell populations will expand further the knowledge regarding the susceptibility of particular cell types, such as RGCs, to specific genetic alterations.

Although treatment options concerning disorders of mitochondrial dynamics are currently limited, the future is open for the development of novel neuroprotective strategies and innovative gene-therapy approaches. Novel therapeutic strategies include genetic engineering to correct or silence particular gene mutations and pharmacological approaches targeting the structure of the affected protein (120). Finally, understanding the aspect of mitochondrial function and subsequent cellular process that is affected by a particular genetic defects might offer an alternative more global therapeutic option by enabling the development of efficient treatment targeting the process rather than the gene itself.

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