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Biochimica et Biophysica Acta 1659 (2004) 107–114

BIOCHIMICA ET BIOPHYSICA ACTA
BBA

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Review

Mitochondrial medicine

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Received 29 June 2004; received in revised form 2 August 2004; accepted 9 August 2004
Available online 19 August 2004

Abstract

After reviewing the history of mitochondrial diseases, I follow a genetic classification to discuss new developments and old conundrums.

In the field of mitochondrial DNA (mtDNA) mutations, I argue that we are not yet scraping the bottom of the barrel because: (i) new mtDNA mutations are still being discovered, especially in protein-coding genes; (ii) the pathogenicity of homoplasmic mutations is being revisited; (iii) some genetic dogmas are chipped but not broken; (iv) mtDNA haplotypes are gaining interest in human pathology; (v) pathogenesis is still largely enigmatic.

In the field of nuclear DNA (nDNA) mutations, there has been good progress in our understanding of disorders due to faulty intergenomic communication. Of the genes responsible for multiple deletions and depletion of mtDNA, mutations in *POLG* have been associated with a great variety of clinical phenotypes in humans and to precocious aging in mice. Novel pathogenetic mechanisms include alterations in the lipid milieu of the inner mitochondrial membrane and mutations in genes controlling mitochondrial motility, fission, and fusion.

While I am very honored to give the opening talk at the Sixth Euromit, I feel like Tonio, the clown in Leoncavallo's *I Pagliacci*, who sings the opening aria "Io sono il Prologo" (I am the Prologue). Just like that introductory clown, I think my contribution is to propose a classification of the mitochondrial diseases that will serve as a prologue, a sort of framework for the far more detailed and scholarly presentations that will follow in the next three and a half days. But, first, a little history.

1. Historical considerations

The concept of mitochondrial disease was introduced in 1962, when a team of investigators at the Karolinska University in Stockholm, including Rolf Luft, an endocrinologist, Lars Ernster, a cell biologist, and Björn Afzelius, a morphologist, described a young Swedish woman with severe hypermetabolism not due to thyroid dysfunction [1]. This example of clinical investigation at its best was based on three sets of data: (i) morphological evidence of abnormal mitochondria in muscle; (ii) biochemical documentation of "loose coupling" of oxidation and phosphorylation in isolated muscle mitochondria; and (iii) good correlation between biochemical defect and clinical features. Curiously, this "prototypical" mitochondrial disease is also the rarest: only one additional case of Luft syndrome has been documented in more than 40 years since the first report [2,3].

During the decade of the 1960s, the attention of clinical scientists was largely directed to clinical myopathies and to muscle morphology: "megaconial" and "pleoconial" congenital myopathies were described by G. Milton Shy and Nicholas Gonatas [4,5]. Interestingly, Gonatas and Shy foretold the importance of mitochondrial DNA (mtDNA), when they wrote :"If mitochondria are self-replicating organelles as recent morphological and chemical evidence has suggested, these two myopathies [megaconial and pleoconial] may be due to a defective gene" [6], implicating a mtDNA gene. In 1963, W. King Engel introduced a modification of the Gomori trichrome histochemical stain

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doi:10.1016/j.bbabio.2004.08.003

that made possible to identify muscle fibers with excessive mitochondrial proliferation, which he dubbed "ragged-red fibers" (RRF) [7].

Biochemical studies were not conducted systematically until the 1970s, when specific biochemical assays led to the description of several defects of mitochondrial metabolism, including pyruvate dehydrogenase (PDH) deficiency [8], carnitine deficiency [9,10], carnitine palmitoyltransferase (CPT) deficiency [11], as well as the first example of cytochrome *c* oxidase (COX) deficiency in Leigh syndrome, which was identified here in Nijmegen [12]. Soon, it became possible to propose a rational biochemical classification of the mitochondrial diseases based on defects in the five major steps of mitochondrial metabolism: substrate transport, substrate utilization, Krebs cycle, electron transport chain, and oxidation/phosphorylation coupling [13]. However, the term "mitochondrial diseases" ended up being restricted to defects of the respiratory chain. This conventional wisdom is justified by the biochemical complexity of the respiratory chain and by its dual genetic control, explaining the extraordinary clinical and genetic heterogeneity of mitochondrial diseases.

The molecular age of mitochondrial diseases began in 1988, with the description of the first pathogenic mutations in mtDNA, large-scale single deletions in patients with "mitochondrial myopathies" [14] and a point mutation in the gene encoding subunit 4 of complex I (ND4) in a family with Leber's hereditary optic neuropathy (LHON) [15]. Within two years of these discoveries, large-scale deletions were associated with various forms of progressive external ophthalmoplegia (PEO), including Kearns–Sayre syndrome (KSS) [16,17], and apparently specific point mutations with maternally inherited multisystemic syndromes, such as MERRF (myoclonic epilepsy with RRF) [18] and MELAS (mitochondrial encephalomyopathy, lactic acidosis, and strokelike episodes) [19]. In the next 14 years, new pathogenic mutations of mtDNA were reported at the rate of about eight per year, such that 118 point mutations were listed in the January, 2004, catalogue of *Neuromuscular Disorders* [20]. To these must be added numerous rearrangements (single deletions, duplications, or both) [21].

In 1989, Zeviani et al. [22] described several Italian families in whom PEO was inherited as an autosomal dominant trait and there were multiple rather than single mtDNA deletions in muscle, the first example of a new group of mitochondrial disorders apparently due to defects of intergenomic signaling. A primary nuclear defect could impair mtDNA integrity, as in the case of multiple mtDNA deletions, or mtDNA copy number, as documented by Moraes et al. [23] in patients with mtDNA depletion, an autosomal recessive trait.

As the "mtDNA fever", i.e., the search for new mtDNA pathogenic mutations, abated towards the end of the century, there was a return to mendelian genetics, searching for nuclear DNA (nDNA) defects directly or indirectly responsible for respiratory chain dysfunction. Perhaps predictably, the first such defect was discovered in the respiratory chain complex entirely encoded by nDNA: the group of Arnold Munnich in Paris described two sisters with Leigh syndrome (LS) and complex II deficiency, who had a homozygous mutation in the flavoprotein-encoding gene [24]. In 1999, the Nijmegen group of Jan Smeitink identified, in rapid succession, several pathogenic mutations in highly conserved genes of complex I [25]: again, the most common phenotype was LS.

The molecular genetics of arguably the most common cause of LS, COX deficiency, proved a harder nut to crack because, as it turned out, all mutations described thus far affect genes encoding COX-assembly proteins, not COX subunits. Mutations were first identified in the SURF1 ancillary protein by the groups of Eric Shoubridge in Montreal [26] and Massimo Zeviani in Milano [27]. Whereas *SURF1* gene mutations cause typical LS, mutations in other COX-assembly genes seem to affect one other target organ besides the brain: cardiopathy is associated with mutations in *SCO2* [28] or *COX15* [29], liver disease with mutations in *SCO1* [30], nephropathy with mutations in *COX10* [31]. The recent description of mutations in the *LRPPRC* gene (which encodes an mRNA-binding protein) in patients with COX-deficient LS, French Canadian type (LSFC) is interesting not just for its results but also for its methodology, as the gene was identified through the novel approach of integrative genomics [32]. Integrative genomics also facilitated the identification of the gene *ETHE1*, responsible for ethylmalonic encephalomyopathy (EE) [33]. Although the encoded matrix protein has unknown function, it must affect COX integrity, since muscle biopsies from children with EE show marked COX deficiency.

Indirect "hits", i.e., mutations in ancillary proteins, are also responsible for the very few mendelian disorders due to defects of complex III and complex V. Mutations in *BCS1L*, a complex III assembly protein, have been associated with LS [34] and with GRACILE (*g*rowth *r*etardation, *a*minoaciduria, *c*holestasis, *i*ron overload, *l*actacidosis, and *e*arly death) a fatal infantile multisystemic disease [35]. Another multisystemic and rapidly fatal disease of infancy has been attributed to mutations in ATP12, a complex V assembly protein [36].

Although this brief historical review has brought us to the present, it is far from complete. The pathogeneses of known mutations remain largely unexplained, at the same time that new pathogenic mechanisms are emerging, such as defects of the inner membrane lipid milieu or defects of mitochondrial fission and fusion. Besides "primary" mitochondrial diseases, there are numerous conditions—including aging and late-onset neurodegenerative diseases—in which mitochondrial involvement may be secondary or indirect or not conclusively documented [37].

Last, but certainly not least, therapy remains woefully inadequate, as we still treat mitochondrial patients with the "white magic" of vitamin and cofactor mixtures.

2. Annotated genetic classification

What follows is a series of brief discussions on unsolved problems and emerging issues in mitochondrial diseases grouped according to a genetic and biochemical classification (Table 1). Hopefully, some answers will be provided by the presentations at this meeting: surely, more questions will be added to the list.

2.1. Mutations in mtDNA

2.1.1. Are we scraping the bottom of the barrel?

I first asked this question four years ago, when "only" 97 pathogenic point mutations peppered the mtDNA [38]. My answer—now as then—is a resounding "no" for several reasons. First, new mtDNA mutations, especially in protein-coding genes, are still been identified at a fairly brisk pace. Second, we are "rediscovering" that homoplasmic mutations can be pathogenic. Third, we have stumbled into some unexpected—indeed "heretical"—findings, such as the occasional paternal inheritance of mtDNA. Fourth, we have to understand the physiopathological significance of mtDNA haplotypes. Fifth, we still do not understand the detailed pathogenic mechanisms of mtDNA mutations, at least of those impairing mitochondrial protein synthesis, which are still the majority. Let us consider these points one by one.

2.1.1.1. New mtDNA mutations: the ND5 story. Because of the syllogistic reasoning prevalent in medicine, I think we had made unwarranted generalizations based on two common disorders due to mutations in protein-coding genes. As both NARP/MILS (neuropathy, ataxia, retinitis pigmentosa/maternally inherited Leigh syndrome) and LHON, the former associated with mutations in the ATPase 6 gene and the latter with mutations in ND genes, are maternally inherited, multisystemic, and not associated with RRF, we concluded that *all* mutations in protein-coding mtDNA genes would have the same characteristics. The first demonstration that we were wrong on all three accounts came from the observation of sporadic patients with isolated myopathy, RRF in their muscle biopsies, specific biochemical defects in one of the respiratory chain complexes, and corresponding mutations in *ND* genes, *COX* genes, or—more often—in the *cyt b* gene [39]. More recently, another protein-coding gene has attracted a lot of attention and attained the status of "hotspot", *ND5*. To be sure, *ND5* mutations are maternally inherited and multisystemic, but usually associated with RRF. In 1997, we were surprised to find the first *ND5* mutation (G13513A) in a patient with MELAS, who had had his first stroke at the relatively late age of 42 [40]. The same mutation, however, was soon reported in another MELAS patient [41], in a patient with LHON/MELAS overlap syndrome [42], and—surprisingly—in patients with typical LS [43,44]. Other mutations in the same gene have now been described in patients with MELAS [45,46], LS/MELAS overlap [47], MELAS/MERRF overlap [48], and even a three-way LHON/MELAS/LS overlap [46]. The cautionary lesson here is that we should not underestimate complex I as a potential cause of mitochondrial diseases (after all, it is by far the largest respiratory chain component), and that the biochemical defect in muscle may or may not be severe.

Table 1
Genetic classification of the mitochondrial diseases

Defects of mtDNA	Defects of nDNA
Mutations in protein synthesis genes	Mutations in respiratory chain subunits
tRNA, rRNA, rearrangements	Complex I, Complex II
Mutations in protein-coding genes	Mutations in ancillary proteins
Multisystemic (LHON, NARP/MILS)	Complex IV, Complex III, Complex V
Tissue-specific	Defects of intergenomic signaling
	PEO with multiple Δ-mtDNA
	mtDNA depletion
	Defects of the lipid milieu
	Barth syndrome
	Defects of motility/fusion/fission
	Autosomal dominant optic atrophy
	CMT type 2A

LHON, Leber's hereditary optic neuropathy; NARP, neuropathy, ataxia, retinitis pigmentosa; MILS, maternally inherited Leigh syndrome; PEO, progressive external ophthalmoplegia; CMT, Charcot–Marie–Tooth disease; Δ denotes large-scale deletion.

2.1.1.2. Homoplasmic, yet pathogenic. We seem to have forgotten that the very first pathogenic point mutation (G11778A in *ND4*) described in a large family with LHON was homoplasmic [15], because most subsequent pathogenic mutations were, in fact, heteroplasmic and the degree of heteroplasmy usually correlates with clinical severity, which is intuitively satisfactory. Thus, heteroplasmy was included among the canonical criteria for pathogenicity. In addition, the mutation in question ought to: (i) be absent in normal subjects of the same ethnic background (i.e., not a neutral polymorphism); (ii) alter an evolutionarily conserved (presumably functionally important) site; (iii) cause a functional alteration, demonstrable as a specific biochemical defect (mutations in protein-coding genes), as a defect of respiration or protein synthesis in cybrid cell lines (deletions or mutations in tRNA genes), or as a spatial correlation between mutations and ragged-red or COX-negative fibers (single fiber PCR [49]). We are now rediscovering the importance of homoplasmic pathogenic mtDNA mutations. Most ND mutations associated with LHON are homoplasmic [50]; so are several mutations causing non-syndromic deafness, including the A1555G mutation in the 12S rRNA gene [51], and two mutations in the $tRNA^{Ser(UCN)}$ gene, A7455G, which was "almost homoplasmic" (>95%) in one large kindred [52], and T7511C [53]. In collaboration with the Newcastle/Tyne group, we

have described two families with maternally inherited hypertrophic cardiomyopathy and a homoplasmic mutation (A4300G) in the $tRNA^{Ile}$ gene [54]. Another sad and puzzling report of pathogenic homoplasmy reported by the Newcastle group regarded a mildly myopathic woman who had seven children from four partners: six died within hours of birth and the only survivor had LS: mother and children were homoplasmic for a mutation (C1624T) in the $tRNA^{Val}$ gene [55]. A third example of homoplasmy from Newcastle is less straightforward, but may be more common than we thought. In a large family with the T14709C mutation in the $tRNA^{Glu}$ gene, which is typically associated with myopathy and diabetes, McFarland et al. [56] found that the mutation had attained homoplasmy in at least three members of the family. Surprisingly, however, one such person, with homoplasmic mutation in blood, was asymptomatic.

The homoplasmy of pathogenic mtDNA mutations raises two important questions, one practical and one scientific. The practical question is how to prove pathogenicity, when most of the canonical criteria listed above are not applicable, at least for tRNA or rRNA mutations. One approach that has been used successfully in at least two cases [54,56] is the determination of steady-state levels of the tRNA in question in tissues or cybrids using high-resolution Northern blots. The scientific question is how a homoplasmic mutation can be pathogenic in some family members but not in others and how can it result in different phenotypes. There are four possible—and not mutually exclusive—factors conditioning the phenotypic expression of a homoplasmic mtDNA mutation: environmental factors, mtDNA haplotype, nDNA background, and tissue-specific expression of interacting genes. The importance of the environment is best exemplified by the effect of aminoglycoside exposure on triggering deafness in some carriers of the A1555G mutation [51,57]. The influence of mtDNA haplotype is illustrated by the different penetrance of the A7455G mutation in a Scottish compared with a New Zealand family. The higher frequency of deafness in the New Zealand pedigree was ascribed to the coexistance of three "secondary" LHON mutations in ND genes, which were absent in the Scottish family [58]. The influence of the nuclear background has been suggested by studies of cell cultures harboring the A1555G deafness mutation or the A3460G LHON mutation. In cultured lymphoblastoid cells, features of mitochondrial dysfunction were more severe in lines derived from symptomatic than from asymptomatic carriers of the A1555G mutation [59]. Cybrids homoplasmic for the A3460G mutation but derived from two different rho^0 cell lines (osteosarcoma or lung) showed different degrees of complex I deficiency, presumably related to the different nuclear backgrounds [60].

2.1.1.3. Breaking dogmas? About two years ago, a surprising report cast doubts on one of the tenets of mitochondrial genetics, the maternal inheritance of mtDNA. A patient with myopathy and a microdeletion in *ND2* had inherited his muscle mtDNA (but not the pathogenic mutation) mostly from his father [61]. This prompted several groups, including the authors of the "heretic" paper, to study other myopathic patients with muscle-restricted mtDNA mutations, but none of them showed anything but maternally inherited mtDNA [62–64], thus preserving this principle as a rule—if not as the dogma. However, the freak coexistance of paternal and maternal DNA in the muscle of one individual allowed to document unequivocally that mtDNA molecules can recombine [65].

2.1.1.4. The significance of mtDNA haplotypes. There is evidence (discussed above) that the expression of pathogenic mtDNA mutations can be modulated by the rest of the mtDNA sequence, i.e., by the mtDNA haplotype. More generally, the conventional wisdom is that certain mtDNA haplotypes may affect oxidative phosphorylation, thus predisposing to—or protecting from—disease [66]. Thus, mtDNA haplotypes have been associated with cardiomyopathy [67], Alzheimer disease and dementia with Lewy bodies [68], and multiple sclerosis [69]. Interestingly, they have also been associated with IQ [70], adaptation to cold climates [71], and spermatozoa motility [72]. While these associations are certainly interesting and potentially important, they need to be bolstered by better evidence that different haplotypes cause differences in mitochondrial function.

2.1.1.5. Pathogenic mechanisms. Sixteen years after the discovery of mtDNA mutations, the detailed pathogenic mechanisms of the various diseases, and especially of those due to mutations in tRNA genes, remain elusive. Consider the three major syndromes attributed to impaired mitochondrial protein synthesis, KSS (single large-scale mtDNA deletion), MELAS (typically, A3243G mutation in $tRNA^{Leu(UUR)}$), and MERRF (typically, A8344G in $tRNA^{Lys}$). The conventional pathogenic interpretation, supported by numerous studies in cybrids, is that oxidative phosphorylation and mitochondrial protein synthesis are impaired in all three conditions to similar extents, and all three syndromes are due to impaired energy supply. If this were so, however, one would expect similar consequences, i.e., similar phenotypes. While there is some overlap of symptoms and signs, the syndromes are distinctive enough to make the differential diagnosis relatively easy. Thus, epilepsy is typical in MELAS and MERRF, but rare in KSS; strokes are typical of MELAS but only rarely seen in KSS; myoclonus is typical of MERRF and unusual in the other two conditions; heart block is a characteristic feature of KSS; and multiple lipomas have been described only in MERRF. The easiest explanation is a spatial one: symptoms reflect the tissues in which a specific mutation surpasses the pathogenic threshold. There is some support for this hypothesis: MELAS mutations are abundant in the walls of cerebral arterioles and MERRF mutations are abundant in the dentate nucleus of the cerebellum, which, however, begs

the question of what "directs" them to those particular tissues. Mutations in some tRNAs seem to associate with certain tissue vulnerability: MERRF is almost invariably due to mutations in $tRNA^{Lys}$; cardiopathy is often associated with mutations in $tRNA^{Ile}$; diabetes is almost always caused by the T14709C mutation in $tRNA^{Glu}$. But, again, the bases for this tRNA "tissue-specificity" escape us. Detailed biochemical studies are beginning to reveal subtle differences among the mitochondrial tRNA mutations. For example, modifications in taurine-containing uridines (which are normal components of mtDNA tRNAs) are lacking in the common MELAS and MERRF mutations [73]. Nevertheless, pathogenesis is still largely terra incognita, which explains why we are so clumsy in our therapeutic attempts.

2.2. Mutations in nDNA

Recent advances regarding the first two groups of diseases in Table 1 have been mentioned in Historical Considerations.

2.2.1. Defects of intergenomic signaling

Our understanding of the disorders due to defects of intergenomic signaling has progressed significantly after the discovery of the gene responsible for MNGIE (*m*itochondrial *n*eurogastro*i*ntestinal *e*ncephalomyopathy), thymidine phosphorylase (TP) [74]. Although *TP* is a cytoplasmic enzyme, mutations in *TP* called attention to the nucleotide pool in the pathogenesis of multiple deletions and depletion of mtDNA, as both alterations coexist in muscle from patients with MNGIE. Thus, in short order, mutations in *ANT1*, encoding one isoform of the adenine nucleotide transporter, and in *Twinkle*, encoding a helicase, were identified in patients with autosomal dominant PEO (adPEO) and multiple mtDNA deletions [75,76], whereas mutations in *POLG*, the gene encoding the mitochondrial polymerase γ, were found in families with either adPEO or autosomal recessive PEO (arPEO) [77,78]. Mutations in two other genes controlling the nucleotide pool, *dGK* (encoding deoxyguanosine kinase) and *TK2* (encoding thymidine kinase), were associated with the hepatocerebral and myopathic forms of mtDNA depletion [79,80]. It is important to note that—despite these advances—there are still patients with PEO and multiple mtDNA deletions as well as patients with mtDNA depletion in whom the molecular defects remain unknown. It would not be surprising if new information in this regard will become available at this meeting.

An interesting recent paper from the San Diego group reported *POLG* mutations in a child with Alpers syndrome, a multisystem disorder of infancy or childhood described by Bernard Alpers over 70 years ago and characterized by the association of liver disease and symmetrical lesions of the brain grey matter, a poliodystrophy [81]. Clinically, Alpers syndrome and the hepatocerebral presentation of the mtDNA depletion syndrome are very similar, and it stands to reason, therefore, that mutations in *POLG* might be one other important cause of Alpers syndrome besides mutations in *dGK*. This concept is bolstered by recent findings of our group in collaboration with that of David Thorburn in Melbourne.

POLG is in the limelight not only because of the variety of human diseases—some transmitted as autosomal recessive, others as autosomal dominant traits—associated with it, but also because homozygous knock-in mice expressing a proof-reading-deficient version of POLG show dramatic evidence of premature *aging* [82]. As these animals accumulate excessive numbers of mtDNA deletions and point mutations in all tissues, they are living (albeit short-living) proof that the mitochondrial theory of aging is alive [37]. Further studies of these "progeric" mice will help in: (i) dissecting the contribution of nuclear and mitochondrial factors to the process of aging; (ii) better understanding the pathogenesis of age-related degenerative phenomena; and (iii) perhaps finding ways to slow down the aging process (an unrequited human dream).

2.2.2. New pathogenic mechanisms

New and exciting pathogenic mechanisms are emerging. For example, alterations of the respiratory chain may be due to abnormal phospholipid composition of the inner mitochondrial membrane. This has been proposed in Barth syndrome, an X-linked recessive disorder characterized by mitochondrial myopathy, cardiomyopathy, and cyclic neutropenia [83]. The gene for Barth syndrome, *G4.5*, encodes a family of proteins named tafazzins, which share conserved regions with acyltransferases of diverse organisms, suggesting the possibility that Barth syndrome may be due to the defect of a specific mitochondrial acyltransferase [84]. Indirect support for this concept comes from the demonstration that cardiolipin, the most abundant phospholipid component of the inner mitochondrial membrane, is markedly decreased in tissues from patients with Barth syndrome [85–87]. Because cardiolipin is essential for the correct assembly and function of the respiratory chain [88], abnormalities in cardiolipin concentration or composition could lead to respiratory chain dysfunction in Barth syndrome, though this remains to be conclusively documented.

One mitochondrial property that has been neglected until recently is the dynamic nature of these organelles, which we—at least the myologists among us—tend to view as tiny beans more or less trapped within the contractile mesh of the muscle fiber. In reality, mitochondria fuse, split, and move within the cell. In non-muscle cells, such as neurons, mitochondria form tubular networks, which favor a uniform distribution of energy within the cell [89]. Mitochondria move on microtubular rails propelled by motor proteins called kinesins (KIFs), one of which, KIFBα, is specific for mitochondria [90].

The machinery for mitochondrial fission in mammals requires a number of proteins acting in concert, mainly

dynamin-related protein 1 (DRP-1), a GTPase. One proposed mechanism of action of DRP-1 is that it oligomerizes into rings, which, in the presence of GTP, constrict and split mitochondrial tubules [89]. As DRP-1 is a cytoplasmic protein, it is attracted to mitochondria by an outer mitochondrial membrane protein called Fis1. The process of fission is probably triggered by a transient increase of cytoplasmic calcium. At present, no human diseases have been attributed to defects of mitochondrial fission, a situation that probably will not last long.

The machinery of mitochondrial fusion also requires several proteins, including two outer membrane GTPases, mitofusin 1 (MFN 1) and mitofusin 2 (MFN 2), and a third dynamin-related GTPase, OPA-1, which is embedded in the inner mitochondrial membrane [89]. OPA-1 is probably activated through partial proteolysis by a presenilin-associated rhomboid-like (PARL) protease. There are already two human diseases due to impaired mitochondrial fusion. The first to be described is autosomal dominant optic atrophy, an important cause of blindness in young adults (as it were, the mendelian counterpart of LHON), in which mutations in *OPA-1* gene have been documented [91,92]. The second disorder is CMT type 2A, where mutations in the gene for mitofusin 2 (*MFN2*) have been documented in seven families of diverse ethnic origins [93].

This brings me to the conclusion of this "annotated review" of the genetic classification of the primary mitochondrial diseases (or—as Canio sings in the last scene of *I Pagliacci*—"La commedia è finita"). Many topics are left out, such as mitochondrial involvement in neurodegenerative diseases, animal models of mitochondrial disorders, secondary mitochondrial diseases (iatrogenic or environmental), the role of mitochondria in apoptosis and—conversely—the occurrence of apoptosis in mitochondrial diseases. Most importantly, I did not discuss therapy, which is still dismally limited. However, we will hear about ingenious therapeutic strategies—both pharmacologic and genetic—which bode well for the future.

Acknowledgement

Part of the work described here was supported by NIH grants NS11766 and HD32062, by a grant from the Muscular Dystrophy Association, and by the Marriott Mitochondrial Disorders Clinical Research Fund (MMDCRF).

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