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Biochimica et Biophysica Acta 1271 (1995) 141–151

BBA Biochimica et Biophysica Acta

Mitochondrial DNA mutations in human degenerative diseases and aging

Douglas C. Wallace *, John M. Shoffner, Ian Trounce, Michael D. Brown, Scott W. Ballinger, Marisol Corral-Debrinski, Terzah Horton, Albert S. Jun, Marie T. Lott

Department of Genetics and Molecular Medicine, Emory University School of medicine, 1462 Clifton Road, room 446, Atlanta, GA 30322, USA

Abstract

A wide variety of mitochondrial DNA (mtDNA) mutations have recently been identified in degenerative diseases of the brain, heart, skeletal muscle, kidney and endocrine system. Generally, individuals inheriting these mitochondrial diseases are relatively normal in early life, develop symptoms during childhood, mid-life, or old age depending on the severity of the maternally-inherited mtDNA mutation; and then undergo a progressive decline. These novel features of mtDNA disease are proposed to be the product of the high dependence of the target organs on mitochondrial bioenergetics, and the cumulative oxidative phosphorylation (OXPHOS) defect caused by the inherited mtDNA mutation together with the age-related accumulation mtDNA mutations in post-mitotic tissues.

Keywords: mtDNA mutation; Oxidative phosphorylation; Mitochondrion

1. Mitochondrial bioenergetics and biogenesis

Mitochondrial OXPHOS plays a major role in human cellular bioenergetics through generation of energy in the form of ATP and by regulation of the cellular redox state via the reoxidation of NADH. Most human cells contain hundreds of mitochondria, and each mitochondrion contains multiple mtDNAs. The mtDNAs code for essential polypeptides of the OXPHOS pathway plus the structural RNAs for mitochondrial protein synthesis. Hence, deleterious mutations of the mtDNA adversely affect cellular bioenergetics through the reduction in ATP availability and/or the increase in the cytosolic NADH/NAD^+ ratio.

Mitochondrial OXPHOS is assembled within the mitochondrial inner membrane from gene products encoded by both the nuclear DNA (nDNA) and mtDNA. The mtDNA encodes 13 polypeptides which include seven subunits (ND1, 2, 3, 4L, 4, 5, 6) of respiratory complex I (NADH:ubiquinone oxidoreductase), one subunit (cytochrome *b*, cyt*b*) of respiratory complex III (ubiquinol:ferricytochrome *c* oxidoreductase), three subunits (CO1, CO2, and CO3) of respiratory complex IV (ferrocytochrome *c*:oxygen oxidoreductase), and two subunits (ATP6 and 8) of respiratory complex V (H^+-translocating ATP synthase) (Fig. 1). In addition, complex I encompasses about 34 nDNA subunits, complex II (succinate:ubiquinone oxidoreductase) four nDNA subunits, complex II and IV include about 10 nDNA subunits, and complex V about 14 nDNA subunits.

Complexes I to IV compose the electron transport chain in which electrons are collected from NADH by complex I and from succinate by complex II and transmitted to coenzyme Q (CoQ or ubiquinone). From $CoQH_2$ (reduced CoQ or ubquinol) the electrons are transmitted successively to complex III, cytochrome *c*, complex IV, and finally oxygen to give water. The energy that is generated is used to pump protons across the mitochondrial inner membrane at complexes I, III, and IV. This electron transport chain-generated proton gradient can subsequently be dissipated by transport back through the ATP synthase proton channel with the energy that is released used to condense ADP and Pi to make ATP. The mitochondrial ATP is then exchanged for cytosolic ADP by the adenine nucleotide translocator (ANT) [1,2].

While most of the oxygen that is consumed by the mitochondrion is converted to water at complex IV, about 4 to 5% of the oxygen picks up electrons directly from the flavin dehydrogenases and $CoQH_2$ to generate superoxide anion. Superoxide anion is converted to H_2O_2 by mitochondrial manganese superoxide dismutase, and on to the highly reactive hydroxyl anion in the presence of transition metals. Inhibition of mitochondrial electron transport increases the electronegativity of the electron transport chain and accentuates the production of reactive oxygen species

* Corresponding author. Fax: + 1 (404) 727 3949.

SSDI 0925-4439(95)00021-6

[1–3]. These mitochondrially generated reactive oxygen species can then damage the mitochondrial membranes, proteins and DNA, further inhibiting OXPHOS.

The average human cell harbors thousands of mtDNA molecules. The mtDNA is a closed circular molecule of 16 568 nucleotide pairs (nps) which is replicated from two origins, O_H of the G-rich heavy (H)-strand and O_L of the C-rich light (L)-strand, separated by 2/3 of the mtDNA in the direction of H-strand replication. The mtDNA codes for 37 genes: the 13 OXPHOS polypeptides, 12*S* and 16*S* rRNAs, and 22 tRNAs that punctuate the polypeptide and rRNA genes (Fig. 1) [4].

The high copy number and cytoplasmic location of the mtDNA result in a unique genetics. The mtDNA is maternally inherited, and when a mutation arises in a mtDNA, it creates an intracellular mixture of mutant and normal molecules, known as heteroplasmy. As the cell undergoes mitotic or meiotic division, the mutant mtDNAs are ran-

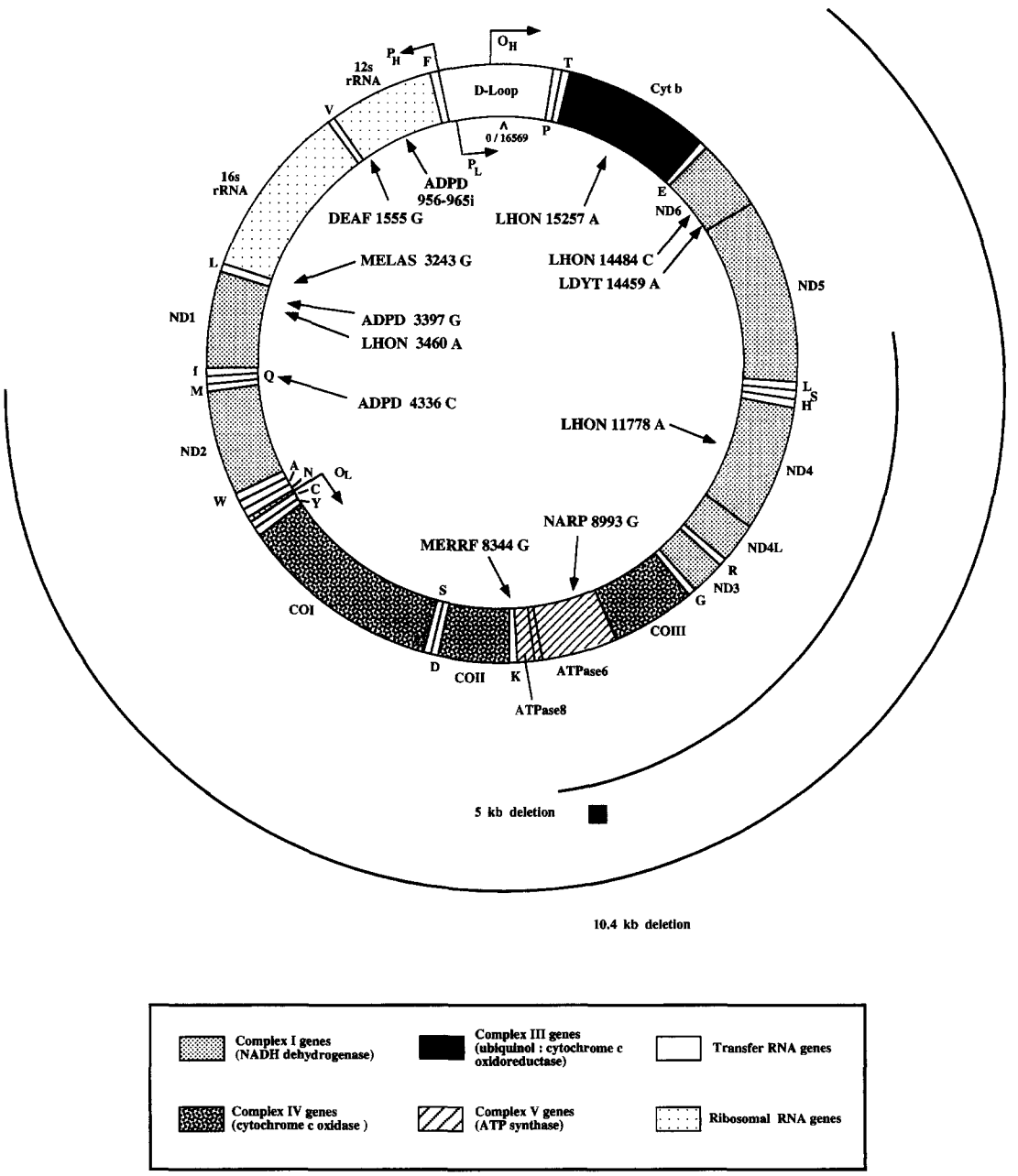

Fig. 1. The human mtDNA map showing the location of the genes and common disease mutations. The polypeptide and rRNA gene identifiers are defined in the text. The punctuating tRNA genes are identified by their cognate amino acid, given in single letter code. Genes whose designators are outside the circle are encoded by the H-strand. Those whose designators are inside are encoded by the L-strand. The positions of the base substitution mutations which cause diseases are identified using a four or five letter clinical designator, the nucleotide position of the mutation, and the mutant base. The positions of two insertion-deletion mutations are shown by the outer arcs. For the diabetes mellitus and deafness insertion-deletion mutation, the 10.4 kb deletion is shown. It has the same breakpoint junction as the 6.1 kb insertion.

domly distributed into the daughter cells such that the mtDNA genotype drifts toward pure mutant or normal molecules, homoplasmy. The brain, heart, muscle, kidney and endocrine systems are highly reliant on mtDNA bioenergetics. Hence, as the mitochondrial bioenergetic capacity declines, it ultimately drops below the minimum threshold necessary for tissues to function and clinical manifestations ensue [1,2,4].

The mtDNA has a much higher mutation rate than the nuclear genome. Both rearrangement and base substitution mutations occur in both germline and somatic tissues. Deleterious mutations in germline cells result in maternally transmitted diseases, while the accumulation of somatic mutations in post-mitotic tissues results in the progressive decline of mitochondrial bioenergetics during inherited disease progression and aging [1,2,4].

2. Diseases resulting from mtDNA rearrangements

mtDNA rearrangements have been associated with the ocular myopathies including chronic progressive external ophthalmoplegia (CPEO) and the Kearns-Sayre Syndrome (KSS), the Pearson Marrow/Pancreas Syndrome, and adult-onset diabetes mellitus and deafness [1,2]. All of these conditions result from a single mutational event which occurred either in the female germline or early in development, and all progress with age. In the ocular myopathies disease progression has been associated with the age-related accumulation of deleted molecules in the post-mitotic muscle [5] due to the clonal expansion of the deleted molecules in segmental zones of the muscle fibres. These segmental zones experience a proliferation of abnormal mitochondria which stain red by Gomori trichrome, giving the characteristic ragged red muscle fibres (RRFs) [6,7].

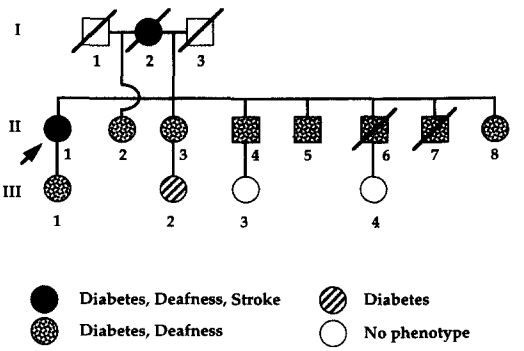

Fig. 2. Diabetes, deafness and stroke pedigree.

A similar pathophysiological mechanism may underlie the late-onset and progression of maternally-inherited diabetes mellitus and deafness resulting from a complex mtDNA rearrangement [8,9]. We first demonstrated that mtDNA mutations can cause adult-onset (Type II) diabetes in a large three generation pedigree (Fig. 2). The mother (I-2) transmitted her diabetes and deafness to all of her offspring in two separate; marriages and her daughters, but not her sons, transmitted the disease to the next generation. The proband (II-1) and her mother (I-1) also experienced strokes involving cortical and subcortical structures.

Four maternal relatives were muscle biopsied. All had significant defects in OXPHOS, but none exhibited RRFs. Endocrine analysis of two of the proband's brothers who are prone to diabetic ketoacidosis (II-4 and II-5) revealed that one of the brothers produced normal levels of insulin during euglycemia, while the other had very low levels of euglycemic insulin production. Following sustacal-induced

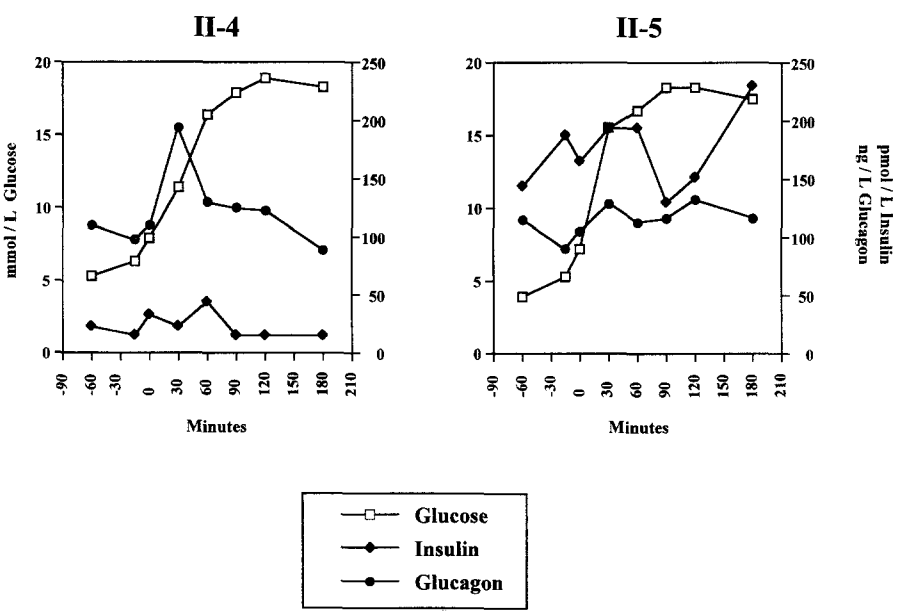

Fig. 3. Endocrine function of mitochondrial diabetes and deafness patients. The time is given in minutes relative to sustacal challenge.

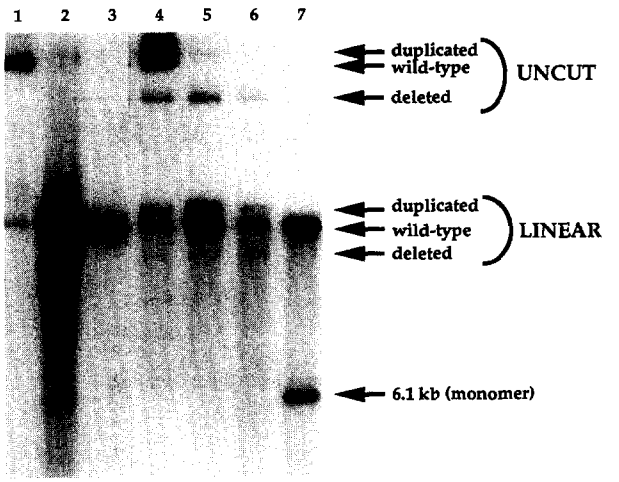

Fig. 4. Duplicated, deleted and normal mtDNA is maternally-transmitted diabetes mellitus and deafness. Skeletal muscle DNA from the proband's daughter (individual III-1 of Fig. 2) (Lanes 4–7) and leukocyte DNA from a control (Lanes 1–3) were digested with various restriction endonucleases, the fragments separated on an agarose gel, and the Southern blot hybridized with total mtDNA probe [9].

hyperglycemia, both brothers failed to respond by increasing insulin release or decreasing glucagon release. Thus, both brother had a defect in their pancreatic α and β cell glucose sensors (Fig. 3).

Analysis of the mtDNAs of multiple maternal relatives revealed that all harbored a complex mtDNA rearrangement involving related deleted and inserted mtDNA molecules co-inherited with normal moleculars in a unique trimolecular heteroplasmy. This was demonstrated by the Southern analysis of the mtDNA from the proband's daughter (III-1), as compared to a normal control (Fig. 4). Undigested control mtDNAs gave two bands, a slower-migrating (upper) supercoiled form and a faster-migrating (lower) nicked and linear 16.5 kb form (Lane 1). Undigested patient mtDNA, by contrast, gave six bands, three upper and three lower, with the middle band of each set of three bands corresponding to the control band (Lane 4). Hence, this patient harbors, in addition to the normal mtDNA, both a larger (duplicated) and a smaller (deleted) mtDNA molecules. Digestion of the control's mtDNA with either *Bam*HI (single site at np 14258) (Lane 2) or *Pvu*II (single site at np 2650) (Lane 3) linearized all of the mtDNA to give a 16.5 kb fragment. By contrast, digestion of the patient's mtDNA with *Bam*HI (Lane 5) or *Sna*BI (single site at np 10734) (Lane 6) cleaved the top two bands of the upper triplet and enhanced the upper two linear bands of the lower triplet. However, the lowest of the upper three bands remained intact. This demonstrated that the top two bands of the patient's upper triplet each contained one *Bam*HI and one *Sna*BI site, but that this region was deleted in the lower band of the upper triplet. Digestion of the patient's mtDNAs with *Pvu*II reduced all of the bands to two fragments, a 16.5 kb full length linear fragment and a smaller 6.1 partial fragment (Lane 7). This demonstrates that the patient's duplicated mtDNA must harbor two *Pvu*II sites separated by 6.1 kb and that both the duplicated and deleted molecules must share the same 6.1 kb region of the mtDNA. Further, the patient's undigested deleted mtDNA (Lanes 4–6) migrates at about 12 kb, suggesting that it is a dimer of 6.1 kb fragments. Sequence analysis of the duplicated and deleted molecules revealed that the molecules shared the same breakpoint junction, which was flanked by a 10 np direct repeat. Hence, these molecules must have generated a common original event [8,9].

Analysis of the proportions of normal, duplicated and deleted molecules in the muscle and blood cells of multiple maternal relatives revealed that these tissues had all three molecular forms, but the relative proportion varied between individuals and tissues. Moreover, the deletion was favored in the post-mitotic muscle tissue, while the duplicated molecule was favored in the mitotically active blood cells. Propagation of lymphoblastoid cell lines from three patients resulted in the loss of the deleted molecules and enrichment of the duplicated molecules [9]. These observations suggest that the duplicated molecule may be the maternally transmitted form and that the duplicated molecule is converted to the deleted molecule over time in post-mitotic tissues. If this model is correct, then it is the progressive accumulation of the deleted molecules with age that erodes tissue energy metabolism, ultimately crossing expression thresholds and causing tissue and organ failure.

Complex mtDNA rearrangements have also been observed in the ocular myopathies [10], and a similar mtDNA duplication has been reported in a family with ocular myopathy and diabetes [11]. Moreover, diabetes and deafness has also been associated with the $tRNA^{Leu(UUR)}$ mutation at np 3243 [the MELAS (mitochondrial encephalomyopathy, lactic acidosis and stroke-like episodes) mutation] in a large maternal pedigree [12] as well as in a portion of randomly selected Type II diabetes patients [13]. Hence, OXPOS defects caused by the inheritance of complex mtDNA rearrangements and mtDNA point mutations are an important mechanism for producing diabetes mellitus.

Maturity-Onset Diabetes of the Young (MODY) has also been linked to mutations in the pancreatic β cell glucokinase gene. Glucokinase has a higher K_m than other cellular hexokinases, and is only active when serum glucose levels are high. Hence, glucokinase provides the glucose sensor for the pancreas endocrine system. MODY mutations increase the glucokinase K_m, inactivating the glucose sensor and rendering individuals unable to respond to hyperglycemia with increased insulin production [14–16]. Recently, pancreatic glucokinase has been shown to be predominantly bound to the mitochondrial outer membrane through the outer membrane pore protein porin. Porin is thought to interact with the inner membrane ANT, permitting rapid exchange of ATP and ADP between the matrix and the cytosol. The resulting macromolecular com-

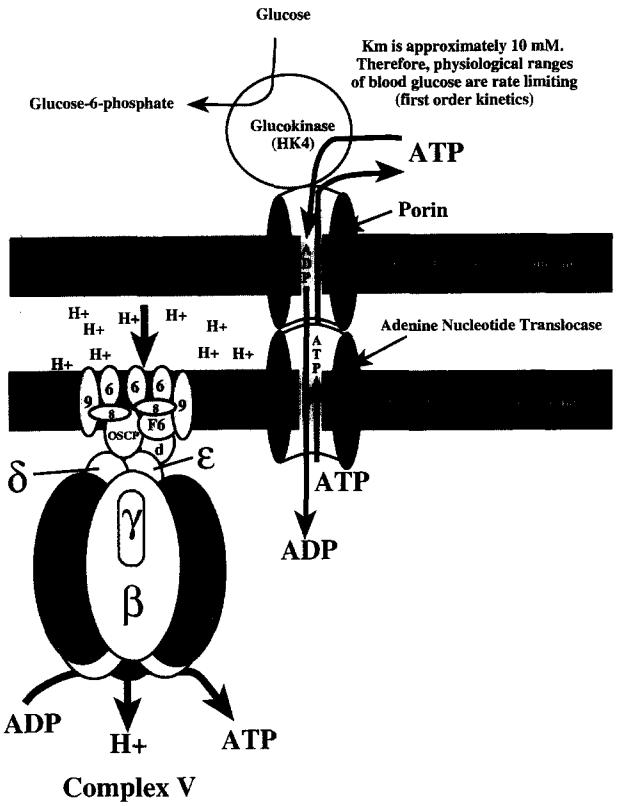

Fig. 5. Cellular energetics and glucose sensing.

plex within the pancreatic β cell of glucokinase, porin, ANT and H^+-translocating ATP synthase (Fig. 5) would link the cytosolic glucose concentration with mitochondrial ATP production, through the two substrates of glucokinase, glucose and ATP. In this manner, the glucose sensor would be sensitive to mutations in either glucokinase or the mtDNA OXPHOS genes [9,17,18].

The insertion-deletion, diabetes and deafness pedigree demonstrates that adult-onset diabetes and deafness can result from mtDNA mutations, and suggests that the delayed onset of the insulin defect could be the result of the age-related accumulation of deleted mtDNAs in the post-mitotic target tissue. The predilection to diabetes and deafness caused by the np 3243 $tRNA^{Leu(UUR)}$ mutation must also be the result of the inheritance of a partial OXPHOS defect. However, the delayed-onset of the disease must be the product of some secondary factor, possibly the age-related accumulation of somatic mtDNA mutations in the beta cells. The linkage of diabetes mellitus to two very different mtDNAs mutations suggests that defects in OXPHOS may be a common cause of diabetes.

3. Diseases resulting from mtDNA base substitutions

MtDNA nucleotide substitution mutations can adversely affect either polypeptide genes or protein synthesis (tRNA and rRNA) genes. Deleterious polypeptide missense mutations which occur in the H^+-translocating ATP synthase (complex V) have been associated with neurogenic muscle weakness, ataxia, and retinitus pigmentosa (NARP) as well as the Leigh's syndrome; while missense mutations that alter the subunits of the electron transport chain (complexes I, III, and IV) frequently cause a form of mid-life, acute-onset, central vision loss called Leber's hereditary optic neuropathy (LHON) and/or dystonia [2,19,20]. MtDNA tRNA mutations are frequently associated with RRFs and include the np 3242 mutation of the MELAS syndrome and the np 8344 mutation in $tRNA^{Lys}$ of the myoclonic epilepsy and ragged red fiber (MERRF) disease. A mtDNA 12*S* rRNA mutation at np 1555 has been associated with deafness, both maternally-inherited and aminoglycoside-induced [19,20].

3.1. Neurodegenerative disease caused by the ATP6 np 8993 mutation

A T to G transversion at np 8993 converts the highly conserved leucine 156 in the ATP6 gene to an arginine. This mutation is invariably heteroplasmic and has been shown to cause a spectrum of clinical manifestations [21]. This is clinical variability is exemplified by one three generation pedigree (Fig. 6) in which the proband (IV-3) presented at 18 months with subacute necrotizing encephalopathy (Leigh's syndrome) and associated basal gangliar degeneration. His brother (IV-2) exhibited cerebellar dysfunction, and his half brother (IV-1) through his mother died of Leigh's syndrome at age 5 1/2 years. Their mother (III-4), grandmother (II-4) and two maternal aunts (III-1 and III-2) all had salt-and-pepper retinopathy and migraine, while the proband's great uncle (II-1) presented

Table 1
OXPHOS capacity of isolated mitochondria from lymphoblasts and rho^0 transformants carrying the mtDNA $8993^{T \rightarrow G}$ mutation

	State III	State IV	ADP/O	+DNP	III/DNP
Lymphoblasts					
Controls ($n = 9$)	164 ± 27	57 ± 19	2.55 ± 0.14	174 ± 28	0.88 ± 0.02
8993 T → G ($n = 2$)	101 ± 28	71 ± 42	1.78 ± 0.40	193 ± 35	0.52 ± 0.06
Cybrids					
Controls ($n = 3$)	75 ± 5.6	20 ± 2.9	2.35 ± 0.09	86 ± 7.8	0.92 ± 0.07
8993 WT ($n = 3$)	70 ± 21.7	17 ± 2.5	2.32 ± 0.12	81 ± 30	0.88 ± 0.07
8993 T → G ($n = 5$)	44 ± 8.7	22 ± 3.9	1.70 ± 0.30	83 ± 22	0.54 ± 0.05

Substrate is pyruvate + malate.

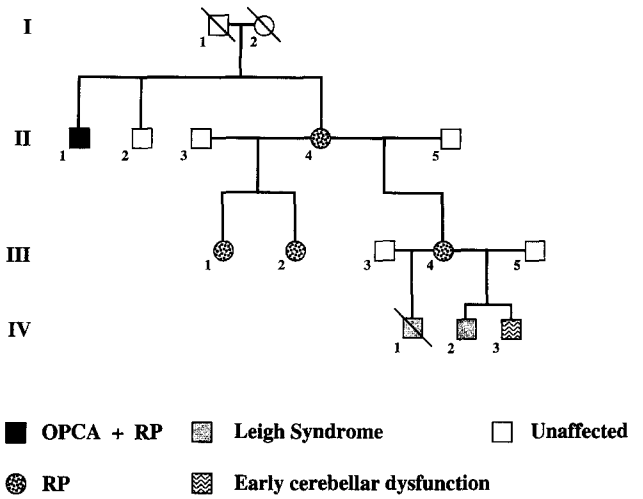

Fig. 6. OPCA + RP and Leigh syndrome associated with mtATP68993 mutation.

with mental retardation, hearing loss, macular degeneration, and olivopontocerellar atrophy on MRI [22].

The biochemical defect associated with the np 8993 T to G mutation was indentified by quantitating mitochondria respiration from two patient lymphoblastoid cell lines (Table 1). When oxidizing NADH-linked substrates, the state IV and uncoupler dinitophenol (DNP)-stimulated respiration rates of the patient and control lymphoblastoid cell lines were essentially the same. However, the ADP-stimulated state III respiration rate of the patient cell lines was 38% less than the controls, reducing the state III/DNP-stimulated rate ratio by 40% (Table 1, upper two lines). This implies that these patients harbor a defect in the proton channel of their H^+-translocating ATP synthase, a conclusion supported by a 30% reduction in the ADP/O ratio of the patient mitochondria [23].

To confirm that the observed defect was the product of the np 8993 T to G mutation, the mitochondria and mtDNAs of the patient's lymphoblasts were transferred to a different nuclear background by the cytoplasmic hybrid or cybrid method [24]. The patient lymphoblastoid cells were enucleated by incubation in cytochalasin B and centrifugation in a percoll gradient. The resulting cytoplasts containing the patient's mitochondria and mtDNAs were then fused by electric shock to human osteosarcoma cells which had previously been depleted of their mtDNAs by extended cultivation in eithidium bromide [25]. Cybrids were isolated by selecting for the osteosarcoma cell's thymidine kinase (TK)-deficient nuclei by growth in bromodeoxyuridine (BrdU) and for acquisition of the patient's mitochondria by growth in the absence of uridine. Transmitochondrial cybrids were clonally isolated and tested for their mtDNA genotype. Since the patient's donor cell lines were heteroplasmic at np 8993, some of the cybrid clones were found to be homoplasmic for the mutant base (G), while others were homoplasmic for the normal base (T). The complex V activities of these cybrids were then compared to each other and to comparable cybrids prepared using normal lymphoblastoid cell lines as cytoplasmic donors (Table 1, lines 3–5). As with the lymphoblastoid cell lines, the state IV and DNP-uncoupled rates of the patient and control transmitochondrial cybrid cell lines were all comparable. Similarly, the ADP-stimulated state III rates, state III/DNP ratios and ADP/O ratios were comparable for the control cybrids and the patient cybrids that were homoplasmic for normal base (T) at np 8993. By contrast, the state III rates of the patient cybrids which inherited the mutant G at np 8993 were reduced 37% and the state III/DNP ratios were reduced 41% relative to those of control cybrids and the patient cybrids which had acquired mtDNAs with the normal base. The ADP/O ratios of the cybrids with the mutant base were similarly reduced by 27% relative to control cybrids and patient mitochondria with the normal base. Thus the patient defects in the state III (ADP-stimulated) respiration and ADP/O ratio are linked to and solely the consequence of the np 8993 T to G mutation [23].

These studies prove that a single nucleotide change can specifically reduce the efficiency of mitochondrial energy production, and have profound effects on the physiology and integrity of the brain. Consequently, these studies prove that systemic mitochondrial bioenergetic defects can lead to tissue-specific degenerative diseases.

3.2. LHON and dystonia caused by electron transport chain mutations

LHON is most commonly associated with four primary mtDNA mutations. In order of severity and frequency, these are np 11778, np 3460, np 14484, and np 15257. The most common cause of LHON is a mutation in the ND4 gene at np 11778. This G to A transition converts the highly conserved arginine 340 to a histidine and accounts for about 50% of European LHON cases [26]. In rare instances, the np 11778 mutation has been associated with adult-onset rigidity and bilateral basal ganglia degeneration [27], suggesting that mtDNA missense mutation can cause movement disorders and that the np 11778 mutation is the most severe of the common LHON mutations. The next most common cause of LHON is a mutation in the ND1 gene at np 3460. This G to A transition converts the moderately conserved alanine 52 to a threonine [28,29]. A comparably frequent, but still milder mutation is the ND6 mutation at np 14484. This T to C transition converts the mildly conserved methionine 63 to a valine [30]. The least severe of the primary LHON mutations is the cytb mutation at np 15257. This G to A transition converts the highly conserved asparate 171 to an asparagine [31]. Together, these mutations account for over 80% of LHON cases.

mtDNA haplotype analysis of LHON patients harboring the four common primary mutations has demonstrated that the np 11778 and 3460 mutations have occurred multiple

times giving rise to LHON, and that these mutations show little if any association with particular mtDNA haplotypes [32,33]. By contrast, the np 14484 and 15257 mutations are most frequently associated with a particular mtDNA lineage defined by nucleotide substitutions at np 4216 and 13708. The np 4216 mutation converts a unconserved tyrosine to a histidine, while the np 13708 mutation converts a moderately conserved alanine to a threonine [19]. The np 14484 mutation appears to have arisen in this mtDNA lineage several independent times and consistently resulted in LHON. The np 15257 mutation appears to have arisen once in this mtDNA lineage [33] and once in an unrelated mtDNA lineage [34]. In the np 4216 and np 13708 lineage, the np 15257 is frequently associated with the np 14484 mutation. It has also been associated with a heteroplasmic G to A transition at np 5244 which converts the totally conserved glycine 259 in the ND2 gene to a serine [31], but has been found in other patients without other identifiable pathogenic mutations [33]. Hence, the np 14484 and np 15257 mutations appear to be enhanced in their pathogenicity by a synergistic interaction with other mild LHON mutations and by certain background mtDNA haplotypes [19,20,33].

In addition to these LHON mutations, we have recently identified a more severe electron transport chain mutation which can cause either LHON or severe generalized dystonia. This G to A transition at np 14459 in the ND6 gene converts the highly conserved alanine 72 to a valine [35]. The mutation was first indentified in a large Hispanic family (Fig. 7) in which both adolescent-onset LHON and childhood-onset dystonia were found among maternal relatives. For the dystonic children, the mean age of onset was 4 years, and asymmetric dystonic rigidity and pseudobulbar syndrome were associated with symmetrical basal gangliar lesions on MRI [36]. Overall, 19% of the 42 maternal relatives manifested LHON, 31% presented with dystonia, and 2% exhibited both LHON and dystonia. Moreover, LHON predominated in the early generations of this pedigree, while dystonia came to predominate in later generations [36]. The proband with dystonia (IV-36), whose mtDNA was sequenced, was homoplasmic for this mutation, as was her dystonic half brother (IV-35). However,

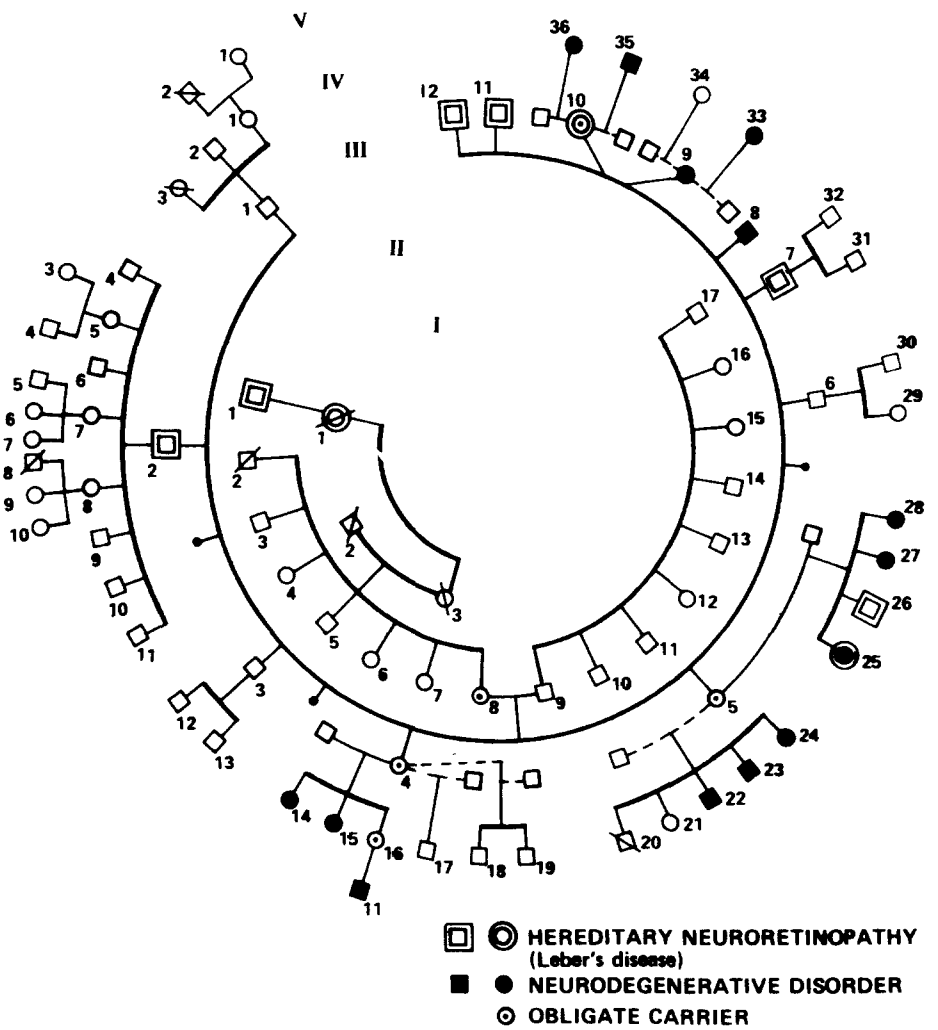

Fig. 7. LHON and dystonia pedigree from Ref. [36].

their mother with LHON was heteroplasmic, suggesting that the severity of the phenotype might be a function of the proportion of mutant mtDNAs in the target organs. Comparison of the mtDNA haplotype of this family with the mtDNA haplotypes commonly found in the various continents revealed that the np 14484 mutation in the family had arisen on a Native American haplogroup B mtDNA, consistent with the Hispanic origin of the family [35]. Screening additional LHON and dystonia patients for this mutation revealed two additional cases, an independent LHON pedigree and in an isolated dystonia case. The LHON pedigree involved an affected mother and daughter, with an MRI of the daughter revealing basal ganglia lesions. Skeletal muscle mitochondria OXPHOS enzyme analysis revealed a complex I defect consistent with the ND6 mutation. The dystonic child exhibited symmetrical bilateral basal ganglia degeneration, but had no other family history. The mtDNAs from these families were not of Native American origin, confirming that the np 14459 mutation has arisen multiple times and that the different clinical presentations are the product of the mutation and not the background mtDNA haplotype [37].

The np 14484 and np 14459 LHON mutations both occur in the ND6 gene and are only nine codons apart, yet the np 14484 mutation is associated only with LHON and the np 14459 mutation with LHON and dystonia. These mutations occur in the most highly conserved region of ND6. Comparison of the sequence conservation of these two codons (Fig. 8) revealed that the np 14484 mutation substitutes a valine for a methionine which is conserved only in human and bovine, but the np 14459 mutation substitutes a valine for an alanine which is conserved from human to sea urchin and for which either an alanine or a serine is present in all species that have been examined. This suggests that the severity of a mtDNA mutation can markedly effect the clinical manifestations of the disease, and that the more sever electron transport chain missense mutations can preferentially affect the basal ganglia and impair motor control.

Since the relatively sever np 14459 mutation causes pediatric onset movement disorders, it follows that milder mtDNA mutations might cause later-onset disease. Support for this hypothesis has been supported by screening Alzheimer and Parkinson disease patients for potentially deleterious mtDNA mutations.

3.3. Alzheimer and Parkinson disease associated mtDNA mutations

A collection of 74 neuropathologically confirmed Alzheimer disease or Alzheimer plus Parkinson disease brains were screened for associated mtDNA sequence variants by regional polymerase chain reaction (PCR) amplification and high density restriction endonuclease analysis. Three mutations were identified which may contribute to the etiology of these diseases. The first was a base substitution in the $tRNA^{Gln}$ at np 4336 which converted a moderately conserved G to a A at the intersection between the amino acid acceptor and TψC stems. This mutation was found in 5% of patients, as compared to 0.4% of controls, and defined a Caucasian mtDNA lineage at increased risk for Alzheimer and Parkinson disease. The second mutation was an ND1 missense mutation at np 3397. This mutation converted a highly conserved methionine to a valine, and was found in two independent mtDNA haplotypes, one within the $tRNA^{Gln}$ np 4336 mtDNA lineage and the other outside. In both instances, this mutation was associated with more than one affected maternal relative. The third mutation was an approximately five nucleotide insertion in the 12S rRNA gene between nps 956 and 965. This novel mutation was also found in a the np 4336 mtDNA lineage. The accumulation of additional deleterious mtDNA mutations in the Alzheimer and Parkinson disease-prone $tRNA^{Gln}$ mtDNA lineage, is remi-

	Amino Acid Position																
	60	61	62	63	**64**	65	66	67	68	69	70	71	**72**	73	74	75	76
LHON + Dystonia (USA)	L	G	G	M	**M**	V	V	F	G	Y	T	T	**V**	M	A	I	E
LHON	L	G	G	M	**V**	V	V	F	G	Y	T	T	**A**	M	A	I	E
Human	L	G	G	M	**M**	V	V	F	G	Y	T	T	**A**	M	A	I	E
Cow	L	G	G	M	**M**	V	V	F	G	Y	T	T	**A**	M	A	T	E
Fin whale	L	G	G	M	**L**	V	V	F	G	Y	T	T	**A**	M	A	T	E
Mouse	L	G	G	M	**L**	V	V	F	G	Y	T	T	**A**	M	A	T	E
Rat	L	G	G	M	**L**	V	V	F	G	Y	T	T	**A**	M	A	T	E
Xenopus	L	G	G	M	**L**	V	V	F	A	Y	S	A	**A**	R	A	-	K
Sea Urchin	I	G	G	M	**L**	V	V	F	P	Y	S	S	**A**	I	S	P	E
Chicken	L	G	G	M	**L**	V	V	F	V	Y	S	V	**S**	L	A	A	D
Quail	L	G	G	M	**L**	V	V	F	V	Y	S	V	**S**	L	A	A	D
Fruit fly	L	G	G	M	**L**	V	L	F	I	Y	V	T	**S**	L	A	S	N
C. elegans	L	S	G	I	**F**	V	I	L	V	Y	F	S	**S**	L	S	K	I
A. suum	L	S	G	I	**F**	V	I	L	V	Y	F	S	**S**	L	S	K	I

Fig. 8. Evolutionary conservation of ND6 codon 64 altered by the np 14484 LHON mutation of codon 72 altered by the np 14459 LHON and dystonia mutations.

Table 2
Age-related mtDNA damage in Alzheimer's disease and control brains

	Frontal cortex			All cortex		
	< 75 year	> 75 year	> 75/< 75	< 75 year	> 75 year	> 75/< 75
Controls	0.00096 ± 0.0008	0.011 ± 0.010	11	0.00072 ± 0.00071	0.012 ± 0.01	17
	$n = 12$	$n = 7$		$n = 19$	$n = 17$	
AD	0.012 ± 0.015	0.0025 ± 0.002	0.21	0.011 ± 0.013	0.0026 ± 0.0022	0.24
	$n = 10$	$n = 10$		$n = 27$	$n = 27$	
AD/Control	13	0.23	-	15	0.22	-

niscent of the clustering of potentially pathologic mutations in LHON lineages, and suggests that these mutations may synergistically interact to increase to probability of clinical manifestation [38].

4. Somatic mtDNA mutations in aging and disease progression

These studies demonstrate that maternally-inherited mtDNA mutations can predispose individuals to a spectrum of neurodegenerative diseases, with the severity of the mutation correlating with the age of onset of the disease. However, all maternally transmitted mtDNA mutations are present at birth, so some additional factor must account for the delayed onset of the symptoms. That factor is likely to be the age-related accumulation of somatic mtDNA mutations in post-mitotic tissues. These would progressively erode the patient's mitochondrial OXPHOS capacity untill it drops below tissue-specific bioenergetic thresholds leading to disease.

Analysis of mitochondrial OXPHOS enzymes in post-mitotic tissues has revealed that the specific activities of the respiratory complexes decline with age [39,40]. This decline has been correlated with the progressive accumulation of somatic mtDNA mutations in post-mitotic tissues. For example, quantitation of the common 5 kb mtDNA deletion in normal brain has revealed that the cerebellum accumulates relatively little mtDNA damage with age. By contrast, the various cortical regions of the brain accumulate appreciable mtDNA damage after age 75 (Table 2), and the basal ganglia accumulates the highest levels of somatic mtDNA mutations, with over 10% of the basal ganglia mtDNAs containing the 5 kb deletion by age 80 [41,42].

If the somatic accumulation of mtDNA mutations is the precipitating factor in mtDNA diseases, then the severity of the inherited mtDNA mutation would set the initial bioenergetic capacity at birth and thus the relative age of onset of the disease. Individuals with an initially high mitochondrial bioenergetic capacities would require extensive somatic mtDNA damage to cross expression thresholds and thus remain functional until late in life, while individuals which inherit significantly deleterious mtDNA mutations would start at a lower initial capacity and require fewer somatic mtDNA mutations to have the same effect and would develop symptoms earlier (Fig. 9).

This differential effect of the somatic mutation-induced bioenergetic decline in mitochondrial disease patients could be further accentuated by an elevation of the mtDNA somatic mutation rates in individuals with partial OXPHOS defects. One cause of somatic mtDNA mutations is

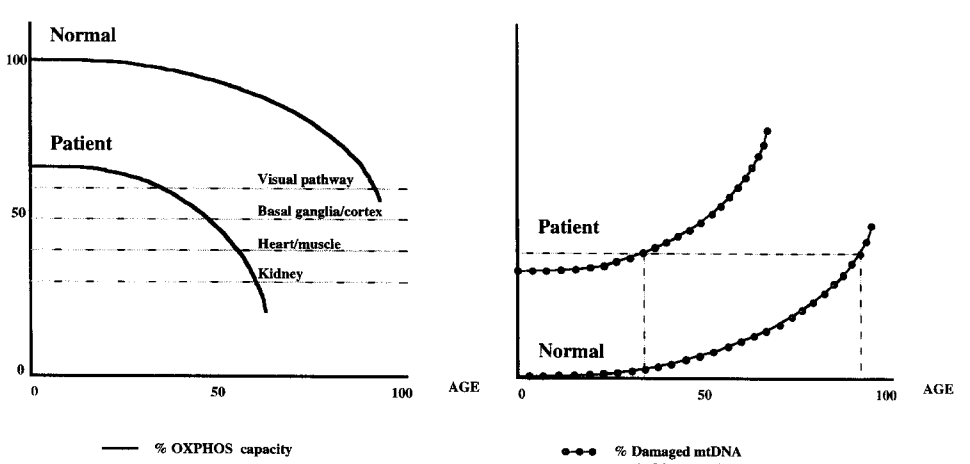

Fig. 9. Hypothesis proposing the relationship between OXPHOS diseases and aging. The cumulative genetic load resulting from inherited (germline) and acquired (somatic) mutations is shown in the right panel. The associated age-related decline in OXPHOS relative to clinical expression thresholds is shown in the right panel.

mitochondrially-generated reactive oxygen damage, which is increased when OXPHOS is inhibited. The importance of reactive oxygen species for mtDNA damage has been dramatically demonstrated in patients with chronic cardiac ischemia and reperfusion due to atherosclerotic plaques in their coronary arteries. In these patients, as the partially occluded coronary artery constricts, the heart mitochondria are deprived of oxygen, while retaining an excess of the reducing substrates, fatty acids and carbohydrates. Consequently, the electron transport chain becomes fully reduced. On subsequent dilation of the coronary artery, the heart tissue is rapidly reperfused with oxygen, thus pulling the excess electrons directly from the reduced flavoproteins and ubiquinol generating reactive oxygen species. Within one minute of repurfusion, 95% of the highly-reactive hydroxyl radical is present in the mitochondria in model systems [43].

Quantitation of the common 5 kb mtDNA deletion in hearts from normal subjects and patients with chronic ischemia and reperfusion demonstrated that the ischemic hearts had a markedly increased level of mtDNA deletions relative to age-matched controls. The hearts of normal subjects accumulated little mtDNA damage up to age 30, and subsequently experienced a slow but steady increase in mtDNA deletions. By contrast, the chronically ischemic hearts accumulated between 8- and 2000-fold higher deletion levels than the age-matched controls. Individuals who died of acute cardiac disease have less deletions than those with chronic illness suggesting that the effect is cumulative [44,45]. These results support the conclusion that reactive oxygen damage is an important cause of mtDNA damage and mutations.

While ischemia inhibits electron transport by removing the terminal electron acceptor, mtDNA mutations block electron transport by inactivating one or more of the respiratory complexes. Thus, deleterious mtDNA mutations would also increase the electronegativity of the of the initial steps of the electron transport chain, increasing the potential for reactive oxygen species generation and somatic mtDNA damage.

To determine if increased somatic mtDNA mutations are associated with progressive neurodegenerative diseases, we have quantitated the levels on the common 5 kb deletion in the brains of Alzheimer disease patients (Table 2). We found that the cortical regions of the brains of patients who died prior to age 75 had over 15-fold more mtDNA deletions than comparably aged controls. This demonstrates that Alzheimer disease brains do have increased levels of somatic mtDNA mutations. By contrast, the cortical regions of Alzheimer's disease patients who died after age 75 had only one quarter of the deletion levels of age-matched controls [46]. While we still do not know why the mtDNA deletion levels decline in older Alzheimer disease brains, one possibility is that the mutant mtDNAs are clonally expanded in the neurons in which they occur, in a manner analogous to the regional expansion of the deleted mtDNA is muscle segments of KSS patients. If this is the case, then the level of deletion would accumulate within the neuron until there was insufficient mitochondrial bioenergetic capacity to sustain viability, thus causing the neuron to die. When the neuron dies the resident deleted mtDNAs would be lost resulting in a decline in the average deletion levels. Thus, the decline in deleted mtDNA may be an indicator of the neuronal dysfunction and death responsible the age-related dementia Alzheimer's disease patients.

Acknowledgements

This work was supported by National Institutes of Health grants NS21328, NS30164, HL45572, GM46915, AG10139, and DK45215: and a Muscular Dystrophy Foundation Clinical grant awarded to D.C.W. and the Emory Clinical Research Center Grant BR00039.

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