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Inherited Mitochondrial Diseases of DNA Replication

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Abstract

Mitochondrial genetic diseases can result from defects in mitochondrial DNA (mtDNA) in the form of deletions, point mutations, or depletion, which ultimately cause loss of oxidative phosphorylation. These mutations may be spontaneous, maternally inherited, or a result of inherited nuclear defects in genes that maintain mtDNA. This review focuses on our current understanding of nuclear gene mutations that produce mtDNA alterations and cause mitochondrial depletion syndrome (MDS), progressive external ophthalmoplegia (PEO), ataxia-neuropathy, or mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). To date, all of these etiologic nuclear genes fall into one of two categories: genes whose products function directly at the mtDNA replication fork, such as *POLG*, *POLG2*, and *TWINKLE*, or genes whose products supply the mitochondria with deoxynucleotide triphosphate pools needed for DNA replication, such as *TK2*, *DGUOK*, *TP*, *SUCLA2*, *ANT1*, and possibly the newly identified *MPV17*.

Keywords

DNA polymerase γ ; nucleotide pools; mitochondrial DNA depletion syndrome; progressive external ophthalmoplegia; ataxia-neuropathy

INTRODUCTION

Mitochondrial diseases can be caused by genetic defects in mitochondrial DNA (mtDNA) or in nuclear genes that encode proteins that function in the mitochondria (1). The mitochondrial genome contains 37 genes, all of which are directly or indirectly involved in the production of ATP. Thirteen of these genes encode protein subunits involved in electron transport to carry out oxidative phosphorylation. The remaining 24 genes encode the transfer RNAs (22 genes) and ribosomal RNAs (2 genes) required for mitochondrial protein synthesis. The mtDNA copy number is high; a cell contains between 1000 and 10,000 copies. MtDNA is replicated by the concerted action of DNA polymerase γ (pol γ), its accessory subunit p55 (encoded by *POLG2*), and replication factors, such as the mitochondrial single-stranded DNA binding protein and the Twinkle helicase. Pol γ is the only known DNA polymerase found in mammalian mitochondria and thus bears the burden of DNA replication and DNA repair functions (2). Since 1999, there has been a flurry of discoveries involving nearly a dozen genes linked to mitochondrial depletion syndrome (MDS) and related disorders (Table 1). MDS includes not only commonly known disorders such as progressive external ophthalmoplegia (PEO) and ataxia but also some very rare tricarboxylic acid (TCA) cycle abnormalities. Mutations in *POLG*, *POLG2*, *TWINKLE*, and *ANT1* are associated with PEO, and mutations

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RELATED RESOURCES Human DNA polymerase mutation database: <http://dir-apps.niehs.nih.gov/polg/>

United Mitochondrial Disease Foundation: <http://www.umdf.org>

Mitomap, a human mitochondrial DNA mutation database: <http://www.mitomap.org>

in several nuclear genes encoding enzymes involved in mitochondrial nucleotide metabolism can cause point mutations, deletions, or depletion in mtDNA, resulting in mitochondrial syndromes. Mitochondria depend heavily on either mitochondrial transport proteins or salvage pathway enzymes to supply deoxynucleotide triphosphates (dNTPs) required for mtDNA replication.

DNA POLYMERASE γ , THE *POLG* GENE

Diseases resulting from mutations in *POLG*, the gene encoding the catalytic subunit of pol γ , are highly heterogeneous (2–5). Mutations in *POLG* are associated with such diverse disorders as PEO, parkinsonism, premature menopause, Alpers syndrome, mitochondrial neurogastrointestinal encephalomyopathy (MNGIE), and sensory ataxic neuropathy, dysarthria, and ophthalmoparesis (SANDO) (2,4–6). Nearly 90 pathogenic mutations have been found in *POLG* (Figure 1) (6) (see also the Human Polymerase Gamma Mutation Database, <http://dir-apps.niehs.nih.gov/polg>).

POLG and Progressive External Ophthalmoplegia

Mutations in *POLG* were first identified as a locus for PEO in 2001 (7). PEO is a mitochondrial disorder associated with mtDNA depletion and/or accumulation of mtDNA mutations and deletions (7–9). PEO is characterized by late onset (between 18 and 40 years of age), bilateral ptosis, and progressive weakening of the external eye muscles, resulting in blepharoptosis and ophthalmoparesis. Proximal muscle weakness and wasting, as well as exercise intolerance, are also associated with PEO. Skeletal muscles of PEO patients have decreased respiratory chain enzyme activity and show ragged red fibers pathologically. Multiple large-scale deletions of mtDNA isolated from muscle biopsies were first demonstrated in Italian families with heritable autosomal dominant PEO (adPEO) (8).

To date, with one exception, all autosomal dominant *POLG* mutations responsible for PEO are in the polymerase domain of pol γ . Two of the substitutions, R943H and Y955C, change side chains that interact directly with the incoming dNTP (10). These enzymes retain <1% of the wild-type polymerase activity and display a severe decrease in processivity. The extremely low catalytic activities and resultant stalling of DNA synthesis are the two most likely causes of the severe clinical presentation in R943H and Y955C heterozygotes (10). The Y955C substitution also increases nucleotide misinsertion errors 10–100-fold in the absence of exonucleolytic proofreading (11). In a yeast model developed to evaluate the homologous mutation in the yeast *MIPI* gene, we found that a Y757C mutant (Y955C in humans) demonstrated mtDNA abnormality indicative of oxidative damage and very high petite frequency (12). In a related study, Baruffini et al. showed that this high petite frequency could be rescued by treatment with antioxidants or upregulation of ribonucleotide reductase (13). In a mouse transgenic model in which the Y955C *POLG* was targeted to the heart, the mice developed cardiomyopathy, loss of mtDNA, and increased levels of 8-oxo-dG in their mtDNA (14). Collectively, these phenotypes suggest that patients harboring the Y955C mutation may have elevated oxidative damage and may benefit from antioxidant therapy.

Co-occurrence of parkinsonism and adPEO was described in two individuals with mutations in *POLG* (15). Parkinsonism manifested several years after initial PEO symptoms. Women with PEO from *POLG* mutations may experience premature menopause and suffer from high gonadotropin and low estrogen concentrations indicative of premature ovarian failure (15, 16).

Most *POLG* mutations are associated with autosomal recessive PEO (arPEO), and patients with PEO are often compound heterozygotes with two different mutant PEO alleles. For example, the A467T mutation has been found in *trans* with other *POLG* missense mutations

in PEO, ataxia-neuropathy, and Alpers syndrome (17,18). The A467T mutation was found in two pedigrees as a homozygous mutation and associated with severe ataxia in mid-life (19). Biochemical analysis indicates that the A467T mutant pol γ possesses only ~4% of the wild-type polymerase activity with only a modest effect on the exonuclease (20). Additionally, the A467T pol γ protein fails to interact with its accessory subunit, p55, which is normally required for highly processive DNA synthesis (20). Nevertheless, A467T is a common mutation, present in 0.6% of the Belgian population (17).

***POLG* and Alpers Syndrome**

Alpers syndrome is a rare but severe autosomal recessive MDS disease that afflicts young children. Within the first few years of life, patients exhibit progressive spastic quadri-paresis, progressive cerebral degeneration leading to mental deterioration and seizures, cortical blindness, deafness, liver failure, and eventual death. Naviaux et al. reported an Alpers patient with reduced electron transport chain function, dicarboxylic aciduria, fulminant hepatic failure, refractory epilepsy, and lactic acidosis that resulted in death at 42 months (21). Skeletal muscle biopsy indicated a reduction of mtDNA content to 30% of wild-type levels with no detectable pol γ activity (21). Sequencing of the *POLG* gene in these pedigrees revealed a heterozygous G-to-T nonsense mutation in *POLG* that converts Glu873 (GAG) to a stop codon and the heterozygous A467T substitution just after the exonuclease domain in the linker region (18). Pol γ mRNAs with the E873 stop mutation are removed from the pool of mRNAs by nonsense-mediated decay, resulting in mono-allelic expression of *POLG* containing only the A467T mutation (22). To date, the number of reported Alpers-associated *POLG* mutations has risen to >35, from 46 different probands (23–25). In all cases, the *POLG* mutations in Alpers patients are recessive, and many of these same mutations may also cause arPEO. The A467T mutation commonly found as a compound mutation in arPEO is the most frequent Alpers mutation and is found as either a homozygous or a heterozygous mutation combined with other mutations.

***POLG* and Ataxia-Neuropathy**

Mutations in *POLG* can also cause ataxia-neuropathy syndrome with onset in the early teens to late thirties. This ataxia, also termed mitochondrial-associated ataxia syndrome (MIRAS) (26), spino-cerebellar ataxia-epilepsy syndrome (SCAE), or SANDO (19), is caused by autosomal recessive mutations in *POLG* producing multiple mtDNA deletions in the affected individuals. Symptoms include peripheral neuropathy, dysarthria, mild cognitive impairment, involuntary movements, psychiatric symptoms, myoclonus, and epileptic seizures. Ataxic patients who are homozygous for the A467T mutation present with symptoms in their early to late teens (19,27). SANDO patients have also been found to have compound heterozygous mutations with the A467T mutation in one *POLG* allele and R3P, L304R, or R627W in the other (17). One patient with ataxia-myopathy syndrome was shown to have the A467T mutation in one *POLG* allele and R627Q and Q1236H mutations in the other *POLG* allele (28). Other patients with ataxia were found to be heterozygous, with the A467T mutation in one allele and W748S in *cis* with the E1143G mutation in the other allele. The E1143G mutation was originally identified as a single-nucleotide polymorphism (SNP) in 4% of the general population (6). However, the accumulated reports of this mutation with other *POLG* mutations in mitochondrial disease suggest that E1143G may augment the disease process (5,29). The W748S mutation in combination with E1143G has been found to be a frequent cause of ataxia (26,27). Haplotype analysis of the Finnish population demonstrates a carrier frequency of 1:125 for the W748S mutation, with a common-ancestor origin of this allele in the ancient European population (26). We have found that the W748S mutation alone causes the polymerase to have a low catalytic activity and a severe DNA-binding defect (30). The E1143G substitution partially rescues the deleterious effects of the W748S mutation and appears to modulate the effects of disease mutations in *POLG*.

***POLG* and Male Infertility**

The human *POLG* gene contains a 10-unit CAG trinucleotide tract encoding a polyglutamine stretch near the N terminus of the mature protein (31). Although deletion of the CAG repeat has no detectable effect on mitochondrial function in tissue culture cells (32), some studies suggest that alteration of the CAG repeat is associated with loss of sperm quality and contributes to 5%–10% of the male infertility cases in the European population (33,34). In contrast to these studies, two independent studies failed to confirm a relationship between the polymorphic CAG repeat in *POLG* and male infertility (35,36).

***POLG2*, THE ACCESSORY SUBUNIT OF POL γ**

Recently, a single mutation in the gene encoding the accessory subunit, *POLG2*, was reported in a patient with adPEO (37). This mutation results in G451E substitution in a loop region not involved in p55 dimerization. Characterization of the recombinant G451E mutant of p55 demonstrates that the mutant accessory subunit is defective in binding with the pol γ catalytic subunit and fails to stimulate processive DNA synthesis. The failure to enhance processivity in the catalytic subunit would cause the complex to stall during DNA replication and is consistent with the accumulation of mtDNA deletions detected in PEO.

THE MITOCHONDRIAL DNA HELICASE, THE *TWINKLE* OR *PEO1* GENE

The mtDNA helicase encoded by the *TWINKLE* gene, also known as *PEO1* is related to the phage T7 gp4 helicase-primase. This gene was first isolated as a locus for PEO on chromosome 10, C10orf2 (38). The derived amino acid sequence has significant sequence homology to the C-terminal end of T7 gp4 helicase but lacks critical primase-associated sequences found in T7 gp4 (38,39). Screening of the *TWINKLE* gene in individuals with adPEO, associated with multiple mtDNA deletions, identified 11 different coding-region mutations that cosegregated with the disorder in 12 affected families (38). The majority of these mutations reside in a linker region between the helicase domain in the C terminus and the N-terminal region. This region is thought to be involved in subunit interaction between the monomers to form a functional hexamer.

Mutations in *TWINKLE* are mainly associated with adPEO, but one report has described a recessive *TWINKLE* mutation as a cause of SANDO (40). Mouse transgenic models that overexpressed several of the PEO mutations in *TWINKLE* recapitulated many of the characteristics of human PEO, including multiple mtDNA deletions, progressive respiratory dysfunction, and cytochrome c oxidase deficiency (41).

ADENINE NUCLEOTIDE TRANSLOCATOR, *ANT1*

ANT1 is one of three adenine nucleotide translocator proteins found as inner-transmembrane mitochondrial proteins and is the most abundant protein in the mitochondria. ANT1 functions as a homodimer composed of 30-kDa monomers and is highly expressed in heart, kidney, liver, and skeletal muscle. Its principal function is to transport ATP out of the mitochondrial matrix in exchange for ADP. Kaukonen et al. (42) used positional cloning in one adPEO family to narrow the locus for adPEO to 4q, which includes *ANT1* and 64 other genes. Sequence analysis of *ANT1* in five families and one patient with sporadic adPEO identified two heterozygous missense mutations in *ANT1* (43). The autosomal mutation A114P and the sporadic mutation V289M are both found within transmembrane domains in the structure of the protein. The analogous A114P mutation in the yeast ortholog, *AAC2*, caused respiratory deficiency (43). A heterozygous T293C *ANT1* mutation was found in a Greek family with adPEO (44), a heterozygous V289M mutation in an Italian patient with sporadic PEO (45), and a heterozygous A90D mutation in a German family with adPEO. In the German family, although microsatellite

markers showed that the allele was dominant and inherited from the mother, the patient did not carry the mutation in blood, indicating germ-line mosaicism (46).

Expression of several other equivalent mutations in the yeast gene *AAC2* in an *aac2*-defective haploid strain of yeast resulted in a marked growth defect on nonfermentable carbon sources, as well as a reduction in cellular respiration (47). The *AAC2* pathogenic mutants showed a significant defect in ADP versus ATP transport compared to wild type.

THYMIDINE PHOSPHORYLASE, *ECGF1*

Thymidine phosphorylase (TP) is part of the pyrimidine salvage pathway required for the conversion of thymidine and phosphate to thymine and deoxyribose-1-phosphate (Figure 2). A defect in *ECGF1*, the gene encoding TP, causes the accumulation of thymidine and uracil in the blood. Because mitochondria rely heavily on salvage pathways for generating intramitochondrial dNTP pools, the mitochondria take up the excess thymidine, which in turn stimulates the synthesis of excess deoxythymidine triphosphate (dTTP) by thymidine kinase 2 in the mitochondria. The resulting unbalanced mitochondrial deoxynucleotide pools cause mtDNA depletion, multiple deletions, and point mutations.

MNGIE is an autosomal recessive disorder caused by mutations in *ECGF1* (48,49). To date, more than 30 mutations in *ECGF1* are known to be associated with MNGIE (49). Like PEO, the disease is associated with multiple deletions and depletion of mtDNA (9). Onset is usually between the second and fifth decades of life, and typical clinical features include ptosis, PEO, gastrointestinal dysmotility, cachexia, peripheral neuropathy, myopathy, and leukoencephalopathy (50,51). TP deficiency leads to increased concentrations of circulating deoxythymidine (52) and deoxyuridine (53). These increases result in imbalanced mitochondrial deoxyribonucleoside triphosphate pools, the effect of which can increase mtDNA mutagenesis (54).

More than 80% of mtDNA mutations found in tissues from MNGIE patients are T-to-C transitions preceded by a short run of As (55). This signature mutation suggests a “next-nucleotide effect” caused by the more common misinsertion T:dGMP (deoxyguanosine monophosphate) (56), which is quickly extended by the elevated dTTP concentration resulting from TP deficiency in the mitochondria of MNGIE cells (55). Additionally, elevated concentrations of dTMP (deoxythymidine monophosphate) derived from the increased thymidine can inhibit the exonuclease activity of pol γ (57). HeLa cells grown in media supplemented with 50 μ M thymidine demonstrated mtDNA deletions and elevated mitochondrial pools of dTTP and dGTP, a result that recapitulated many of the genetic effects seen in MNGIE (54). These results support a mutagenic mechanism involving competition between dGTP and dATP for incorporation opposite to template T (55).

Several exciting avenues of research are showing potential for the treatment of MNGIE. Hemodialysis has been shown to transiently reduce thymidine levels in blood (58). Allogeneic stem cell transplantation has had some success in restoring TP activity and lowering plasma thymidine levels (59). In addition, repeated platelet infusions can reduce thymidine levels in blood in MNGIE patients (60).

MITOCHONDRIAL THYMIDINE KINASE, *TK2*

In a study of four unrelated patients with fatal myopathy during infancy and mitochondrial depletion syndrome, Saada et al. (61) found reduced mitochondrial respiratory chain function and reduced amounts of mtDNA in muscle. Sequence analysis of the thymidine kinase 2 (*TK2*) gene identified either of two homozygous mutations, H90R or I181D. Activity analysis in mitochondrial lysates from these patients revealed reduced TK2 activity (61). DNA

sequences by other groups have identified a total of 14 missense mutations found either as recessive homozygous or as compound heterozygous mutations and one stop codon from a collection of 12 probands with muscle weakness and hypotonia (62–67). Mitochondrial dNTP pools arise either through active transport of cytosolic dNTPs or through salvage pathways by the action of two mitochondrial deoxyribonucleoside kinases, TK2 and deoxyguanosine kinase (Figure 2). In nondividing cells, cytosolic TK1 and dNTP synthesis is downregulated, forcing the burden of mitochondrial dNTP pool synthesis on the two mitochondrial deoxyribonucleoside kinases. *TK2* mutations primarily affect muscle tissue with no effect on liver, brain, heart, or skin. Quantitation of TK2 activity in various tissues relative to mtDNA or cytochrome c oxidase activity helps to explain the tissue specificity of TK2 deficiency (68).

DEOXYGUANOSINE KINASE, *DGUOK*

Deoxyguanosine kinase is the other mitochondrial deoxyribonucleoside kinase that phosphorylates the purine nucleosides into nucleotide monophosphates (Figure 2). Homozygosity mapping in three consanguineous kindreds with hepatocerebral MDS identified a region on chromosome 2p13 that included the deoxyguanosine kinase gene, *DGUOK* (69). Sequence analysis of this gene identified a nucleotide deletion (204delA) that segregated with the disease (69). In a screen of 21 patients with MDS, Salviati et al. (70) identified three patients (14%) with *DGUOK* mutations. Phenotypes of these three patients were highly variable, including one patient who developed liver failure but responded well to liver transplant (70). To confirm that the *DGUOK* mutations did indeed disrupt enzymatic function, Wang et al. (71) characterized recombinant deoxyguanosine kinase with these substitutions. They found that the R142K variant had very low activity with deoxyguanosine (dG) and no activity with deoxyadenosine (dA). The E227K protein had normal affinity for dG and dA but low catalytic efficiency, indicating that this mutation disrupts catalysis without affecting substrate binding. The C-terminal truncated variants were inactive. Further analysis found that one patient with MDS, muscular weakness, and exercise intolerance due to a severe mitochondrial myopathy harbored the L250S mutation in *DGUOK* (65). Examination of the recombinant L250S-dGuoK protein revealed <1% activity compared to the wild-type enzyme with differential competition between deoxycytidine (dC) and deoxythymidine (dT) as substrates (65). Freisinger et al. (72) also identified five new mutations and two previously described mutations in six children with infantile hepatoencephalopathies and MDS. To date, 13 mutations have been described in the *DGUOK* gene, most presented as homozygous mutations from 14 probands (67,72).

DEOXYNUCLEOTIDE CARRIER, *SLC25A19*

In 2002, Kelley et al. (73) described a metabolic disorder among the Old Order Amish of Lancaster County, Pennsylvania, characterized by severe congenital microcephaly, severe 2-ketoglutaric aciduria, and death usually within the first year. Amish lethal microcephaly (MCPHA), an autosomal recessive disorder, has an unusually high incidence of at least 1 in 500 births in this population (73). By using a pedigree analysis in 23 ancestrally related families combined with whole-genome scanning, Rosenberg et al. (74) localized the gene for MCPHA to chromosome 17q25. Contained within this region is the mitochondrial deoxynucleotide carrier gene (*DNC* or *SLC25A19*), which was found to harbor a missense mutation that alters Gly177 to Ala and was not found in 252 control chromosomes. Functional analysis of the G177A mutant *DNC* confirmed that the mutant protein was defective in transport activity (74).

A *DNC* knockout in mouse causes 100% prenatal lethality by day 12 with neural-tube closure defects and elevated α -ketoglutarate (75). However, mouse *DNC*^{−/−} cells did not show any reduction in mtDNA levels. Furthermore, mitochondrial ribo- and deoxyribonucleoside triphosphate levels were normal, suggesting that nucleotide transport may not be the primary

role of DNC. A protein with similar amino acid homology in yeast, Tpc1p, was found to transport thiamine pyrophosphate (ThPP) into mitochondria in exchange for thiamine monophosphate (76). Indeed, in vitro transport assays confirmed that the G177A *DNC* did not cause a defect in ribonucleotide or deoxynucleotide transport into the mitochondria. Furthermore, the mitochondria from the mouse knockout had no detectable ThPP and mitochondria from MCPHA cells had reduced levels of ThPP. Thus, reduction of ThPP levels causes the inability of the α -ketoglutarate dehydrogenase complex to function properly, which leads to the high levels of α -ketoglutaric acid in these patients.

SUCCINYL-COA SYNTHETASE, *SUCLA2*

A small Muslim pedigree with autosomal recessive encephalomyopathy associated with mtDNA depletion was reported in 2005 (77). Genome-wide linkage mapping identified a 20-Mb region on chromosome 13, which was narrowed down to three genes by mitochondrial import prediction programs. DNA sequencing of one of these, the *SUCLA2* gene, revealed a homozygous deletion of 43 nt at the 3' end of exon 6. This mutation results in deletion of exon 6 and part of exon 7 in the mRNA of the affected patients (77). Succinyl-CoA synthetase is a mitochondrial matrix enzyme that catalyzes the reversible synthesis of succinate and ATP (or GTP) from succinyl-CoA and ADP in the tricarboxylic acid (TCA) cycle. *SUCLA2* encodes the β -subunit of succinyl-CoA synthetase. The reverse reaction occurs in the Krebs cycle, whereas the forward reaction may produce succinyl-CoA for activation of ketone bodies and heme synthesis.

Following this initial finding, 12 patients with autosomal recessive mitochondrial encephalomyopathy and elevated methylmalonic acid were identified in the Faroe Islands population (78). Elevated methylmalonic acid is characteristic of methylmalonic acidemia (MMA), a heterogeneous group of disorders with symptoms that include vomiting, dehydration, lethargy, seizures, failure to thrive, progressive encephalopathy, and developmental delays. The increased level of methylmalonic acid impairs the conversion of vitamin B12 into its two metabolically active forms. The accumulated succinyl-CoA inhibits the metabolism of methylmalonyl-CoA to succinyl-CoA, causing the accumulation of methylmalonyl-CoA and MMA. Mutation analysis identified a novel splice site mutation in *SUCLA2* leading to skipping of exon 4. A founder effect caused the high incidence (1 in 1700) in the Faroe Islands population, with a carrier frequency of 1 in 33 (78). In a related study, DNA sequencing of 14 patients from southern Italy and the Faroe Islands confirmed *SUCLA2* mutations in all patients and led to the identification of three novel mutations (79). The frequency of these mutated alleles in the Faroe Islands population was 2% corresponding to an estimated homozygote frequency of 1:2500 (79).

How a defect in the citric acid cycle and accumulation of methylmalonic acid cause depletion of mtDNA is not clear. However, immunoprecipitation experiments have found succinyl-CoA synthetase in complex with mitochondrial nucleotide diphosphate kinase (80). Elpeleg et al. (77) propose that the defect in *SUCLA2* disrupts association with the nucleotide diphosphate kinase, causing a defect in the last step of mitochondrial dNTP salvage by nucleotide diphosphate kinase, which leads to decreased dNTPs and subsequent mtDNA depletion. Mutations in the succinyl-CoA gene should be considered in patients with early/neonatal-onset encephalomyopathy, dystonia, deafness, and Leigh-like MRI abnormalities mainly affecting the putamen and the caudate nuclei (79).

MPV17

One of the latest genetic loci to be added to the list of mtDNA instability disorders is the *MPV17* gene (81). Using enhanced computational algorithms designed to identify mitochondrial targeted gene products, Calvo et al. (82) identified 1080 gene products, including

368 that were not previously predicted to localize to mitochondria. Eight of these new genes, including *MPV17*, were identified as candidate loci for mitochondrial disorders (82). In an accompanying article, Spinazzolla et al. (81) reported several families with MDS in which patients suffered hepatic failure early in life. Genome-wide linkage analysis mapped this gene to the same region as *MPV17*, and DNA sequence analysis identified a common mutation at Arg50, which was mutated to either Gln or Trp in these patients. The protein MPV17 was originally presumed to be localized to peroxisomes, but confocal immunofluorescence in human and monkey cells demonstrates colocalization of MPV17 with mitochondria (81). A parallel study identified the same R50Q mutation in Navajo neurohepatopathy in six patients (83). Navajo neurohepatopathy is an autosomal recessive multisystem disorder found in the Navajo of the southwestern United States. It is characterized by liver failure, severe sensory neuropathy, corneal anesthesia and scarring, cerebral leukoencephalopathy, failure to thrive, and acidosis (83 and references therein).

The *MPV17* homolog in yeast, *SYM*, is localized to the inner mitochondrial membrane. *SYM1* deletion or mutations in *SYM1* that produce the R51Q or R51W substitution (equivalent to R50Q or R50W in human) cause respiration deficiency and mtDNA rearrangement (81). *MPV17*^{-/-} mice develop age-dependent hearing loss and glomerular sclerosis (84), along with a decrease of mtDNA in liver, muscle, brain, and kidney, and decreased respiratory function (81). Although the function of MPV17 remains to be elucidated, the finding of this protein through integrative genomic analysis paves the way for the identification of other genes involved in MDS. Like the defect in *SUCLA2*, the defect in *MPV17* may result in a destabilized protein complex with a nucleotide metabolism protein, or destabilization of the membrane-associated protein-DNA nucleoid complex where mtDNA replication is expected to take place.

P53-INDUCIBLE RIBONUCLEOTIDE REDUCTASE, *RRM2B*

The nucleotide precursors required for DNA replication can be directly obtained by reduction of ribonucleoside diphosphates to deoxyribonucleoside diphosphates by ribonucleotide reductase. Ribonucleotide reductase is made up of two subunits: a large catalytic subunit, R1, and the smaller R2 subunit. Cells have two forms of the R2 subunit: a cell cycle regulated form that is maximally expressed in S-phase, and a p53-inducible form known as p53R2. The p53R2 form is required for a basal level of DNA repair and mtDNA synthesis in nonproliferating cells. Recently, Bourdon et al. (85) identified the *RRM2B* gene encoding p53R2 as a candidate disease gene from a genome-wide linkage analysis in a family with severe muscle mtDNA depletion. Sequence analysis of *RRM2B* in this family and three other affected families identified nonsense, missense, and splice-site mutations and inframe deletions within the *RRM2B* gene that were not found in control chromosomes. Severe mtDNA depletion from *RRM2B* disruption was also confirmed in *Rrm2b*^{-/-} mice, demonstrating the essential role of this gene in mtDNA nucleotide metabolism and mitochondrial disease (85).

CONCLUSION

Diseases of mtDNA stability have been traced to core proteins of mtDNA replication or genes involved in supplying the mitochondrial nucleotide precursors needed for mtDNA replication (Figure 2). Mutations in the *POLG* gene are responsible for several mitochondrial disorders, including fatal childhood diseases such as Alpers syndrome, PEO, ataxia-neuropathy, and possibly male result in inactive and/or truncated proteins, the dominant mutations in *POLG* and *TWINKLE* commonly found in PEO produce “dominant-negative” proteins that interfere with their wild-type counterparts. The fact that many genes involved in nucleotide salvage pathways and nucleotide transport are responsible for mitochondrial diseases suggests that imbalanced nucleotide pools are detrimental to mtDNA replication. Mouse models, yeast

genetics, and in vitro biochemical analyses of mutant proteins have become invaluable for understanding the in vivo consequences of heritable mutations in these genes.

SUMMARY POINTS

1. Disease of mtDNA replication falls primarily into two genetic categories, those that directly affect DNA replication proteins, and those that affect the mitochondrial nucleotide pools.
2. These diseases can affect a variety of organs, with variable ages of onset. The specific manifestations of mtDNA replication diseases are usually determined by the types of mutations within these sets of genes.
3. There is generally a correlation with age of onset and tissue specificity for many symptoms. For example, the hepatopathy usually occurs early in life whereas skeletal muscle myopathy presents later in life. However, neuropathy appears to occur any time in these patients.
4. *POLG* is frequently mutated in this collection of diseases.

FUTURE ISSUES

1. A better understanding of the pathogenesis of these diseases and their tissue specificity.
2. The need to develop suitable animal models that emulate diseases of mtDNA replication to elucidate tissue presentation and pathology.
3. Development of therapies that may slow the progression of these diseases.

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Glossary

mtDNA

mitochondrial DNA

pol γ	DNA polymerase γ , encoded by <i>POLG</i> gene
MDS	mitochondrial DNA depletion syndrome
PEO	progressive external ophthalmoplegia
<i>TWINKLE</i>	gene encoding mitochondrial helicase
dNTP	deoxynucleotide triphosphate
MNGIE	mitochondrial neurogastrointestinal encephalomyopathy
SANDO	sensory ataxic neuropathy, dysarthria, and ophthalmoparesis
ad	autosomal dominant
ar	autosomal recessive
TP	thymidine phosphorylase

Figure 1.

Schematic diagram of human pol γ protein showing locations of disease-causing amino acid substitutions and polymorphisms. The protein is arranged with the exonuclease domain in the first third, followed by a long linker region, with the polymerase domain in the C-terminal third. The disease substitutions are represented by boxes: Light blue boxes, autosomal dominant PEO; light green boxes, autosomal recessive mutations; gray boxes, sporadic PEO; pink boxes, Alpers syndrome; yellow boxes, ataxia-neuropathy-like syndromes; and orange boxes, male infertility. Striped boxes represent disease substitutions found in more than one disease. Red arrows depict the nonsynonymous polymorphic amino acid changes.

Figure 2.
Schematic diagram of the mitochondrion, showing enzyme pathways that cause mtDNA mutations or depletion when disrupted. Gene products associated with MDS or mtDNA mutations are in green boxes.

Table 1
Nuclear loci that affect the stability of mitochondrial DNA

Gene	Disorder ^a	Chromosomelocus	Function
<i>POLG</i>	PEO/Alpers/ataxia	15q25	mitochondrial DNA polymerase
<i>POLG2</i>	PEO	17q23–24	pol γ accessory subunit
<i>TWINKLE (PEO1)</i>	PEO/ataxia	10q24	mitochondrial DNA helicase
<i>ANT1</i>	PEO	4q34–35	adenine nucleotide translocator
<i>TP (ECGF1)</i>	MNGIE	22q13.32	thymidine salvage
<i>DGUOK</i>	MtDNA depletion	2p13	deoxyguanosine kinase
<i>TK2</i>	MtDNA depletion	16q22	mitochondrial thymidine kinase
<i>DNC^b</i>	MCPHA	17q25.3	deoxynucleotide carrier (thiamine pyrophosphate transport)
<i>MPV17</i>	mtDNA depletion	2p21–23	mitochondrial inner membrane protein
<i>SUCLA2</i>	mtDNA depletion	13q12.2–q13.3	succinate-CoA ligase
<i>RRM2B</i>	mtDNA depletion	8q23.1	ribonucleotide reductase

^a Abbreviations: PEO, progressive external ophthalmoplegia; MNGIE, mitochondrial neurogastrointestinal encephalomyopathy; MCPHA, Amish lethal microcephaly.

^b *DNC* mutations have not been associated with mitochondrial DNA depletion syndrome, and recent evidence suggests the gene product functions to transport thiamine pyrophosphate.