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Mitochondrial DNA polymerase-γ and human disease

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The maintenance of mitochondrial DNA (mtDNA) is critically dependent upon polymerase-γ (pol-γ), encoded by the nuclear gene *POLG*. Over the last 5 years, it has become clear that mutations of *POLG* are a major cause of human disease. Secondary mtDNA defects characterize these disorders, with mtDNA depletion, multiple mtDNA deletions or multiple point mutations of mtDNA in clinically affected tissues. The secondary mtDNA defects cause cell and tissue-specific deficiencies of mitochondrial oxidative phosphorylation, leading to organ dysfunction and human disease. Functional genetic variants of *POLG* are present in up to ~0.5% of the general population, and pathogenic mutations have been described in most exons of the gene. Clinically, *POLG* mutations can present from early neonatal life to late middle age, with a spectrum of phenotypes that includes common neurological disorders such as migraine, epilepsy and Parkinsonism. Transgenic mice and biochemical studies of recombinant mutated proteins are helping to unravel mechanisms of pathogenesis, and patterns are beginning to emerge relating genotype to phenotype.

INTRODUCTION

The production of adenosine triphosphate (ATP) by mitochondrial oxidative phosphorylation is critically dependent upon the structural integrity of the mitochondrial genome (mtDNA). MtDNA codes for 13 essential polypeptide subunits of the respiratory chain, which combine with over 70 nuclear subunits to form the final common pathway for energy metabolism (1–3). Pathogenic mutations of mtDNA lead to relative ATP depletion, cellular dysfunction and ultimately cell death. Until recently, it was thought that primary mtDNA mutations were the major cause of mitochondrial disease in humans (4,5), particularly those presenting in adult life (6). However, an emerging class of autosomal mitochondrial diseases account for a rapidly growing clinical group: disease due to mutations in the gene coding for the mtDNA polymerase-γ (*POLG*), which cause disease through a secondary effect on mtDNA.

This review focuses on recent advances in our understanding of inherited *POLG* mutations and human disease. An up-to-date mutation database can be found at http://dir-apps.niehs.nih.gov/polg/. In this article, we will initially refer to substitutions using conventional numbering from the codon initiating translation on the cDNA (NM 002693) and the predicted amino acid substitution. Subsequent reference to the same substitution will only refer to the amino acid change. Polymerase-γ (pol-γ) has been implicated in a number of non-inherited disorders, including nucleoside analogue toxicity (7), which are not the topic of this review.

POLYMERASE-γ AND THE MTDNA REPLISOME

Unlike nuclear DNA, which only replicates once during cell division, mtDNA is continuously recycled, independent of the cell cycle (see Fig. 1 for details) (8,9). MtDNA replication is achieved by a number of nuclear-encoded proteins (Fig. 1; Table 1), including the only DNA polymerase present in the mammalian mitochondria: pol-γ (10). In humans, mtDNA is copied by a 195 kDa heterotrimer consisting of a catalytic subunit (p140, coded by *POLG* on chromosome 15q25) and two identical accessory subunits (p55, coded by *POLG2* on chromosome 17q) (11). The catalytic subunit (p140) possesses DNA polymerase (11), 3′→5′ exonuclease (12) and 5′dRP lyase activities (13), whereas the accessory subunit (p55) is a DNA binding factor that confers high processivity by increasing the affinity of the heterotrimer for template DNA (12). Pol-γ has a high base-substitution fidelity ($<2 \times 10^{-6}$ errors per nucleotide) and is relatively accurate over short

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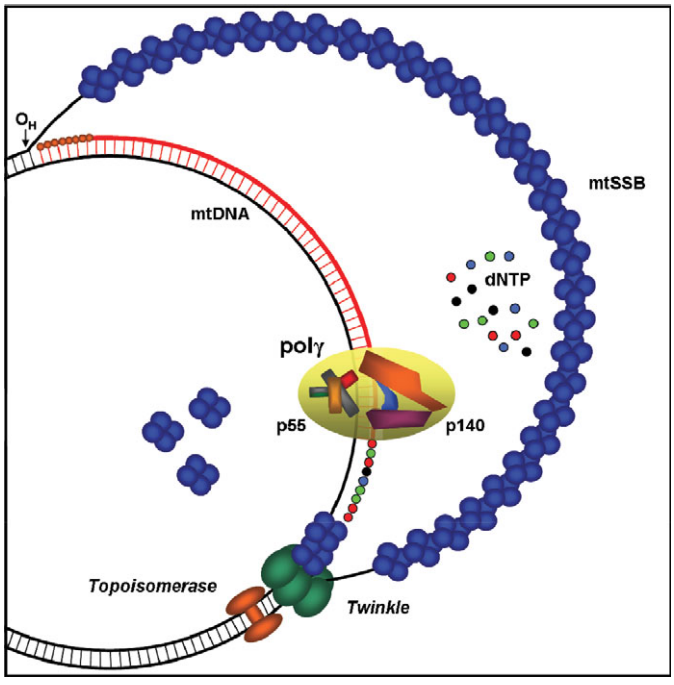


Figure 1. The human mtDNA replisome. Schematic diagram showing the key components of mtDNA replication. The overall amount of mtDNA varies from cell type (79,80) and also depends on local energy requirements (81). Two models of mtDNA replication have been proposed. In the 'strand displacement' or 'asynchronous' model (82), replication is initiated by transcription with the non-coding mtDNA displacement loop and proceeds in an anticlockwise direction from the origin of heavy-strand replication (O_H) until the origin of light-strand replication (O_L) is exposed. This allows light-strand synthesis to proceed in the clockwise direction until the entire molecule is copied. Recent work has shown that symmetric strand-coupled replication may be the dominant mechanism (83,84). According to this model, replication is initiated from multiple origins distributed across a 4 kB fragment 3′ from the non-coding displacement loop and proceeds in both directions in replication 'bubbles' (85). Replication is arrested at O_H (also referred to as OriH), allowing the rest of the molecule to be copied in one direction. The initiation of replication may be different under different circumstances, with intiation at position 57 under quiescent conditions, allowing the steady-state maintenance of mtDNA copy number, but with multiple origins of replication after recovery following depletion (86). Although the figure is based upon the strand displacement model for simplicity, the actual proteins involved in the strand-symmetric model would be the same. dNTP, nucleotides; O_H, origin of heavy strand replication, also referred to as OriH by some authors; pol-γ, polymerase-gamma; p55, accessory subunit; p140, catalytic subunit; mtSSB, mtDNA single-stranded binding protein and Twinkle, mtDNA helicase.

repeat sequences, but longer homopolymeric tracts (>4 bp) lead to slippage during replication (14).

Other components of the mtDNA replisome include the mitochondrial single-stranded binding protein (mtSSB), which probably stabilizes single-stranded regions of DNA at replication forks, enhancing pol-γ activity (15). Twinkle is a 5′→3′ helicase which unwinds double-stranded mtDNA at the replication fork, facilitating mtDNA synthesis (16). Twinkle may also regulate mtDNA copy number within cells (17) and act as an mtDNA primase (18). Both RNase MRP and endonuclease G may modify the D-loop RNA primer that precedes heavy strand replication (19). A small proportion of cellular RNase H1 localizes to mitochondria and might be involved in the processing of Okazaki fragments in strand symmetric replication (Fig. 1). Several topoisomerases have been identified in humans, including mitochondrial topoisomerase 1 (TOPOImt) (20) and IIIα (hTOP3α) (21) regulating supercoiling of mtDNA, and DNA ligase III is also present in the mitochondria (22). Finally, the close relationship between the mitochondrial transcription factor A (mtTFA) and mtDNA levels (23) suggests that mtTFA binds to mtDNA as a chaperone, protecting against oxidant damage (24).

Factors regulating the expression of the replisome are poorly understood. Nuclear respiratory factor-1 (NRF-1) is a transcription factor that regulates the expression of many mitochondrial proteins (25). NRF-1 expression is related to cellular ATP levels and binds to promoter regions of *POLG, POLG2* and mtTFA (25).

PATHOGENIC MUTATIONS OF *POLG*

Autosomal dominant syndromes

The first pathogenic mutations in *POLG* were identified in families with autosomal dominant chronic progressive external ophthalmoplegia (adPEO, MIM 157640) (26). Affected individuals had multiple different secondary deletions of mtDNA in skeletal muscle causing a mosaic cytochrome *c* oxidase (COX) defect (complex IV of the mitochondrial respiratory chain) (27). A high incidence of psychiatric disease, a Parkinsonian syndrome, and primary gonadal failure have also been documented in some families transmitting dominant *POLG* mutations (28,29).

Autosomal recessive syndromes

Compound heterozygous *POLG* mutations were also identified in patients with sporadic and recessive PEO (26). Some cases have a profound peripheral neuropathy and ataxia, similar to the previously described SANDO syndrome (sensory ataxic neuropathy with dysarthria and ophthalmoparesis) (30). Recessive *POLG* mutations also present with adult-onset ataxia without ophthalmoplegia [also called mitochondrial recessive ataxia syndrome (MIRAS)] (31,32), which appears to be common in Scandinavia because of a founder effect (32). Parkinsonism and peripheral neuropathy have recently been described as the presenting feature of recessive *POLG* mutations (33).

Recessive *POLG* mutations are also a major cause of the Alpers–Huttenlocher syndrome (MIM 203700), which is a severe hepatoencephalopathy with intractable seizures and visual failure which presents in early childhood and is associated with depletion (loss) of mtDNA in affected tissues (33–38).

MOLECULAR PATHOPHYSIOLOGY

Biochemical consequences of *POLG* substitutions

Recombinant proteins have been generated for a limited number of *POLG* mutations and studied biochemically. The dominant mutations c.2828G→A (R943H) and c.2864A→G (Y955C) alter amino acid side chains that

Table 1. Proteins known to be involved in mtDNA replication and repair

	Gene	Chromosome	Product	Size (kDa)
mtDNA replication	*POLG* 1	15q25	Catalytic unit	140
	POLG 2	17q23–24	Accessory subunit	55
	SSBP 1	7q34	Single-stranded binding protein	15
	PEO 1	10q23.3	T7-primase/helicase (*twinkle*)	77
	TOP1MT	8q24.3	Topoisomerase (DNA) I, mitochondrial	67
	*TOP*3a	17p12–11.2	Topoisomerase (DNA) III-alpha	112
	*LIG*3	17q11.2–12	Ligase III, DNA, ATP-dependent	96
RNA transcription	*POLR2E*	19q13.3	Core RNA polymerase	150
	TFAM	10.21	Transcription factor A	24
	TFB1M	6q25.1	Transcription factor B1	40
	RMRP	9p21–p12	Component of RNase MRP RNA	275–285 nt
	RNASEH1	2p25	Ribonuclease H1	32
mtDNA repair	*UDG*	12q23–q24.1	Uracil-DNA-glycosylase	27.5
	*OGG*1	3p26.2	8-Oxygaunine-DNA-glycosylase	38
	*NTHL*1	16p13.3	Nth Endonuclease III-like (*Escherichia coli*)	34
	MUTYH	1p34.3–p32.1	mutY homologue (*E. coli*)	60
	*APEX*2	xp11.21	APEX nuclease (apurinic/apyrimidinic endonuclease) 2	57
	ENDOG	9q34.1	Endonuclease G	30

Adapted from Graziewicz *et al.* (87).

interact with dinucleotide triphosphates and have <1% of wild-type polymerase activity (39), whereas the c.2768G→A (G923D) and c.2869G→C (A957S) have <50% of wild-type polymerase activity (39). G923D, R943H and A957S show a slightly increased DNA binding capacity, in keeping with the proposed dominant negative mechanism, where the mutated polymerase competes with the wild-type polymerase for the mtDNA template (39).

c.1399G→A (A467T) has been described in homozygous or heterozygous individuals with autosomal recessive Alpers–Huttenlocher syndrome or late-onset ataxia (32,35,40,41). Recombinant A467T protein has <5% of the wild-type polymerase activity (42) and impairs interaction between the catalytic (p140) and accessory (p55) subunits (43).

Some recessive *POLG* mutations are predicted to truncate the pol-γ protein. Two children homozygous for a predicted stop codon mutation c.2617G→T (E873X) had a normal birth and early infancy (34). Some full-length transcript must, therefore, have been expressed in these individuals, possibly through mRNA editing or because some ribosomes read through the stop codon (34). Shorter mRNA species generated by stop codon mutations are removed by nonsense mediated decay (44).

Secondary mtDNA defects due to *POLG* mutations

A high frequency of mtDNA point mutations in the non-coding mtDNA control region in muscle from *POLG* patients (45) raised the possibility that the point mutations are instrumental in the formation of mtDNA deletions. However, point mutations are equally present in both deleted and non-deleted molecules (46), do not cluster around the deletion breakpoints (47) and are generally not found in COX negative muscle fibres (47), questioning their role in pathogenesis. The preferential localization of deletion breakpoints around homopolymeric tracts points towards replication stalling as a mechanism (46), but homopolymeric tracts are common in mtDNA (48), and a much larger study is required before firm conclusions can be drawn.

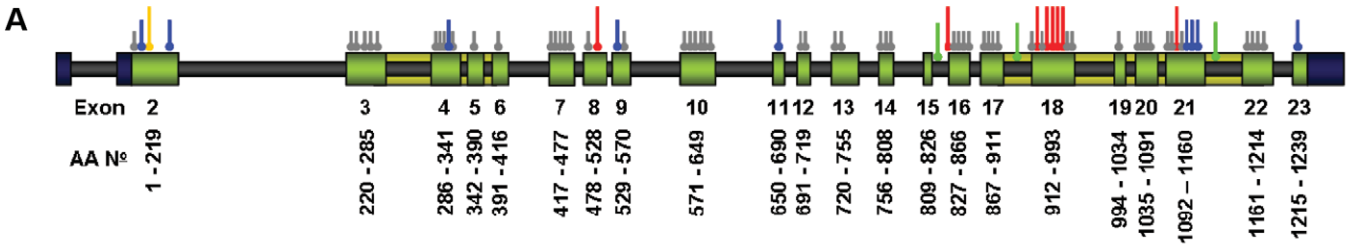
It is intriguing that the heterozygous *POLG* point mutations that cause point mutations and deletions of mtDNA can also cause mtDNA depletion when there are two mutated alleles. Moreover, the same *POLG* mutations can cause an early onset encephalomyopathy with severe mtDNA depletion or late-onset PEO with ataxia [for example, A467T homozygotes (35,40,41)]. The reasons for this are not fully understood.

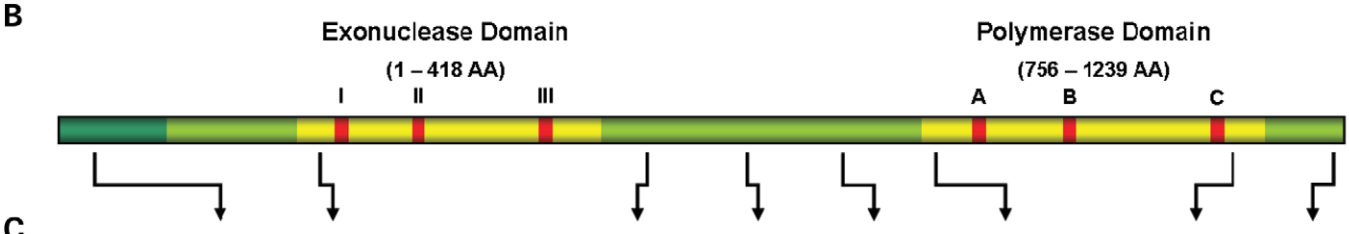
Transgenic mice

Mice with a homozygous mutation in the second exonuclease domain of pol-γ (D257A) developed increased levels of both mtDNA point mutations and mtDNA deletions when compared with controls, associated with a reduced life span and classical ageing phenotypes including hair loss, kyphosis, osteoporosis and cardiac enlargement (49). The accumulation of secondary mtDNA point mutations was linear during life and not associated with the increased production of oxygen-free radical species (50). These observations confirm the potential importance of mitochondrial mechanisms in ageing, but cast doubt on the 'vicious circle' hypothesis, whereby the mutations lead to respiratory chain dysfunction, which further compromises the mitochondrial genome. In the second model, a 2 bp substitution corresponding to positions 1054 and 1055 of mouse *POLG* cDNA was introduced, resulting in a conserved amino acid substitution in the exonuclease domain (D257A) (51). This also led to the accumulation of somatic mtDNA mutations during life, associated with the induction of apoptotic markers, particularly in rapidly dividing tissues (51).

PHENOTYPIC CONSEQUENCES OF *POLG* SUBSTITUTIONS

Over 70 different substitutions have been described in *POLG* in over 200 published cases (June 2006), including missense





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	CAG(n)*	T251I	A467T	K662E	G737R	T885S	E1143G	H1236Q
Exon	2	3	7	11	13	17	21	23
SNP ID	-	-	-	rs2307480	-	-	rs2307441	rs3087374
UK	70 : 23 : 7	-	0.69 (0.14-2.02)	-	-	2.86 (0.06-5.28)	2.08 (0.57-5.25)	4.30 (1.3-11.0)
Finland	74 : 24 : 2	-	0.17 (0.0-0.92)	-	-	-	2.67 (1.16-5.19)	-
Italy	66 : 31 : 3	1.11 (0.36-2.57)	0 (0.0-0.44)	-	-	-	-	-
Germany	-	-	0 (0.0-1.86)	-	0.3 (0.04-1.08)	-	-	-
Asian	94 : 5 : 1	-	-	0 (0.0-0.09)	-	-	0 (0.0-0.06)	0 (0.0-0.05)
E. Eura	78 : 17 : 5	-	-	-	-	-	-	-
Global	78 : 17 : 5	-	-	0.07	-	-	0.07	0.08

Figure 2. Nucleotide substitutions in *POLG*. (**A**) Human *POLG* gene on chromosome 15q25 showing the 22 coding exons (exons 2–23) with corresponding exon–intron boundaries shown below. Documented base substitutions are shown with coloured vertical lines above the corresponding exon. Long red markers, dominant mutations; short grey markers, recessive mutations (includes Alpers–Huttenlocher syndrome and other phenotypes); blue markers, single nucleotide polymorphisms; yellow, exon 2 CAG repeat and green, intronic variants. The details of each mutation can be found on http://dir-apps.niehs.nih.gov/polg/ (**B**) Pol-γ protein showing the different domains of the protein and their amino acid (AA) boundaries. The linker region resides between the exonuclease and the polymerase domains. In yeast, 3′→5′ exonuclease activity is dependent upon three N-terminal motifs, which are highly conserved across a range of species [see Table 3 in (87)], and mutation of these motifs leads to increased rates of base substitution in mice and humans (45,49,51). Both the polymerase activity and the 3′→5′ exonuclease activity of pol-γ require a divalent metal cation (Mg^{2+} for DNA templates and Mn^{2+} for RNA templates), are stimulated by salt (with optimal activity from 75 to 175 mM NaCl for the p140·p55 complex) and are stable across a broad pH range (11). (**C**) Frequency of alleles in control subjects. Table showing percentage allele frequencies of control subjects. For the exon 2 CAG repeat, values are expressed in percentage of the control population in the following way: 10/10: POLG10/ ≠ 10: POLG ≠ 10/ ≠ 10. For the other alleles, values are shown for the percentage frequency of the substitution in control subjects, with the exact 95% confidence interval below. It was not possible to calculate the confidence intervals for the NIH data set. Asian = Korean, Mongolian; E. Eura is East Eurasian: Polish, Russian, does not contain previously mentioned, e.g. Germany (59). Global = www.hapmap.org; and NIH Polymorphism Discovery Resource (40,59–62,88).

mutations, nonsense mutations and splice-site variants (Fig. 2). Although there is no direct relationship between genotypes, some patterns are beginning to emerge.

To date, the vast majority of dominant mutations directly affect the polymerase domain of the protein, although there are exceptions. A467T alters the linker region of the protein and segregated with autosomal dominant ptosis in an Austrian family (42). However, because mutation also affects processivity of the enzyme, a categorical subdivision of the gene is probably an over simplification (43). This is supported by our recent observation of another heterozygous linker-region mutation segregating with adPEO (52).

In contrast, the vast majority of patients with recessive disease have one heterozygous mutation in the linker region and one in the polymerase region (Fig. 2A). A467T is present at high frequencies in some European populations (Fig. 2C), acting as a reservoir for recessive disease. In two series, ∼60% of childhood onset cases harboured A467T (35,40), but only ∼20% of the adult-onset recessive cases (40). Intriguingly, compound heterozygotes with A467T have a significantly shorter survival than homozygotes for either A467T or c.2243G→C (W748S) (41). This supports a relative dominant negative effect for these alleles, possibly through a quaternary interaction between catalytic subunits in different heterotrimers (41).

Table 2. Clinical features in patients with *POLG* mutations

System	Feature	Notes/references
Psychiatric	Depression	(28)
	Psychosis	(32,40)
	Dementia	(40,53)
Seizures	Myoclonus	Common in children (40) and adults with ataxia (32,41,53)
	Focal motor seizures	(41)
	Generalized seizures	(31,32,40)
	Status epilepticus	(41)
Extrapyramidal	Parkinsonism	Responds to levodopa (28,58)
	Chorea	(32)
Cerebellum	Ataxia	(31,32,40,53)
'Cerebrovascular'	Migraine	May precede other features by many years (32,41)
	Stroke-like episodes	Usually asymptomatic in children, diagnosed on imaging (40)
Special sensory	Sensorineural deafness	(32,40,89,90)
	Retinopathy	(28,32,90)
	Cataract	(28)
Muscle	Ptosis and external ophthalmoplegia	May be isolated ptosis (42)
	Proximal myopathy	Distal myopathy reported (40)
	Exercise intolerance	(28,40)
Peripheral nerve	Sensory neuronopathy/ganglionopathy	Corresponds to SANDO (30). Profound sensory ataxia
	Axonal sensorimotor neuropathy	(33,40)
Endocrine	Diabetes	(40)
	Primary ovarian failure	(28,32)
	Primary testicular failure	(89)
Gastrointestinal	Liver failure	Spontaneous or precipitated by sodium valproate in children (34,36,40), also in adults with ataxia (26,41)
	Gastrointestinal dysmotility	(53,89)
Cardiac	Cardiomyopathy	(40,53)

There are now numerous case reports describing acute deterioration in both cerebral and hepatic functions shortly after exposure to valproate, both in children and in adults (36,40,41,53). The underlying mechanisms are not clear at present. Sodium valproate is known to inhibit mitochondrial fatty acid oxidation (54), but its effect on mtDNA replication has not been investigated.

Non-specific headache or migraine may precede ataxia or PEO by many years, and PEO may be a late feature if at all (32,40,41). Myoclonus and epilepsy may be the presenting features in both children and adults, including focal motor seizures and status epilepticus (41). In addition to the fluctuating encephalopathy in adults (41), stroke-like episodes have been described in children (40). Hyperkinetic movement disorders (32) have been documented in addition to Parkinsonism (28), and early onset dementia is well recognized (40,41). The psychiatric features also include severe depression and psychosis (32). Dysphagia, diabetes and deafness are well recognized, and muscle pain or isolated myopathy may be the presenting feature (40) (Table 2). A key clinical point is that at least some patients do not have evidence of mitochondrial dysfunction in muscle and may not have multiple deletions of mtDNA in muscle (31,53). This places great emphasis on clinical intuition in deciding which patients to screen for *POLG* mutations.

MULTIPLE SUBSTITUTIONS: PHENOTYPIC MODIFIERS?

In one series, ~30% of the patients had more than one substitution in *POLG* (40). Although some of these variants are well established single nucleotide polymorphisms (Fig. 2C, http://www.genome.utah.edu/genesnps/), for others it can be less clear [for example, c.752C→T (T251I) and c.1760C→T (P587L) discussed in (40)]. c.3428A→G (E1143G) is present in ~4% of controls (Fig. 2C) and alters a highly conserved residue (55). The frequency of E1143G in patients with sporadic PEO is greater than would be expected by chance (55), and segregation analysis in families with other primary pathogenic *POLG* mutations suggests that E1143G interacts with other pathogenic alleles to modify the disease phenotype (40,56). On the contrary, E1143G homozygotes have been detected in healthy control subjects (40). Further clinical and functional studies are needed to resolve this important issue.

Given the intimate relationship between the various proteins involved in mtDNA replication (Fig. 1), the possibility of mutations in two genes with interacting gene products is an attractive hypothesis. In keeping with this, a novel mutation in the *PEO1* gene (c.1031G→A, R334Q, formerly C10Orf2) and a novel mutation in *POLG* were identified in a man with PEO (57). We also detected a novel heterozygous substitution in *POLG* c.1282G→C (L428V) and a novel *PEO1* mutation c.2038T→C (S680T) (Hudson and Chinnery, unpublished data). However, the same *PEO1* mutation that was identified in the published family has also been identified in a family with adPEO in the absence of a *POLG* mutation, demonstrating that R334Q is capable of causing adPEO on its own (40). The majority of patients with confirmed pathogenic mutations in *POLG* do not have mutations in *PEO1* (40), questioning the role of digenic inheritance in PEO.

The identification of multiple mutations in the same individual reaffirms the polymorphic nature of *POLG* and stresses the

importance of screening large ethnically matched control groups before reporting an apparent disease association. This is particularly important for large-scale screening studies searching for *POLG* mutations in common conditions such as idiopathic Parkinson's disease. A novel heterozygous substitution (c.2492A→G, Y831C) was detected in two siblings with Parkinsonism in a family with PEO (58) and not detected in 130 control subjects, but we recently detected the same substitution in control subjects in the UK, questioning its role as a dominant mutation with reduced penetrance (Tiangyou *et al*. submitted for publication).

Given emerging evidence that the carrier frequency of established recessive mutations varies throughout Europe (Fig. 2C), the choice of control panels must be done with great care. Recurrence of the same mutation in different families, the absence of heterozygous asymptomatic carriers of putative dominant mutations and the absence of homozygous healthy controls would clearly support a pathogenic role. Ideally, functional studies should be carried out, either *in vitro* or in animal models, before reaching a firm conclusion—but this is rarely possible in a diagnostic setting. This makes the screening of appropriate control subjects and a single mutation database all the more important.

POL-γ CAG REPEAT AND MALE INFERTILITY

In humans, there is a $CAG_{(n)}$ repeat sequence in exon 2 of *POLG* coding for a polyglutamine tract (Fig. 2). About 88–96% of Eurasian alleles have 10 CAG repeats (59). Two studies reported a difference in allele distributions between normal controls and males with male infertility (60,61), but this has not been replicated by others (62–64). Deleting the CAG repeat has no effect on respiratory chain activity *in vitro* (65), and it remains to be seen whether genetic variation in the repeat region has any functional consequences *in vivo*.

RELATED DISEASE GENES AND NEW CANDIDATES

Dominant PEO

Dominant PEO with multiple deletions can also be due to mutations in the gene coding for the mtDNA helicase Twinkle (*PEO1*) (66) and the adenine nucleotide translocase (*ANT1*) (67). Convincing recessive mutations in these genes have not been described, although sporadic cases have been identified (55).

A single heterozygous substitution in *POLG2* impaired interaction between the accessory subunit and the catalytic subunit and compromised interaction between the heterotrimer and a DNA template *in vitro*, leading to incomplete stimulation of DNA synthesis and replication stalling, potentially generating mtDNA deletions *in vivo* (68). It was not possible to demonstrate a dominant negative effect of the mutant protein *in vitro*, pointing towards haploinsufficiency as a mechanism, possibly through heterodimerization (68). *POLG2* mutations appear to be rare, accounting for ~1% of adult onset cases of PEO with multiple deletions (68).

Recessive disorders with multiple mtDNA deletions

Recessive mutations in the gene coding for thymidine phosphorylase (TP) cause mitochondrial neurogastrointestinal encephalomyopathy (69,70) and secondary mtDNA defects including depletion, multiple deletions and point mutations (71,72).

MtDNA depletion

MtDNA depletion has also been observed in a number of recessive disorders of nucleoside metabolism, including thymidine kinase deficiency (*TK2*) (73) and deoxyguanosine kinase deficiency (*DGUOK*) (74). These two disorders present in childhood with hypotonia because of myopathy, with hepatic involvement in patients with *DGUOK* mutations (75). Mutations in the ADP-forming succinyl-CoA sythase (*SUCLA2*) were also described in a family with autosomal recessive encephalopathy with mtDNA depletion (76). In addition, mutations in *MPV17*, which codes for a protein which localizes to the inner mitochondrial membrane, also cause mtDNA depletion and infantile hepatic encephalomyopathy (77).

Despite the massive international effort to identify disease genes involved in mtDNA maintenance, a large proportion of patients with secondary mtDNA defects still elude diagnosis. There is a growing list of proteins known to be involved in mtDNA replication and repair in humans and other organisms (Table 1), forming an attractive list of candidate genes for these disorders.

CONCLUSION

The first pathogenic mutations in *POLG* were reported only 5 years ago in patients with a 'classical' mitochondrial phenotype: autosomal dominant PEO. The breadth of clinical phenotypes that have subsequently been linked to this gene is remarkable, from severe childhood encephalopathy to late-onset mild ptosis. Crude estimates indicate that *POLG* mutations will be a major cause of human mitochondrial disease, possibly more prevalent than primary mtDNA mutations in adults (A.M. Schaefer, unpublished data).

From the clinical perspective, mitochondrial disorders are generally considered to be difficult to recognize and diagnose. Paradoxically, the work on *POLG* in the last 5 years has made diagnosis even more difficult, overlapping with common neurological traits such as Parkinsonism and epilepsy. Although patients with *POLG* mutations currently seem to fall into discrete categories, the boundaries are starting to merge, pointing towards an overlapping spectrum of disease with multiple substitutions with functional consequences *in vitro* that may interact and modify the clinical phenotype. The public health implications of *POLG* mutation are potentially massive. Fortunately, a number of groups have developed a means of assessing the function of novel genetic variants [for example (39,42,78)], and two mouse models have been generated (50,51). Hopefully, these resources will provide the necessary tools to develop treatments in the near future.

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