Two novel POLG1 mutations in a patient with progressive external ophthalmoplegia, levodopa-responsive pseudo-orthostatic tremor and parkinsonism

Federica Invernizzi a,1, Sara Varanese b,c,1 , Astrid Thomas b,c , Franco Carrara a ,

Marco Onofrj b,c, Massimo Zeviani a,\*.a Unit of Molecular Neurogenetics, Pierfranco e Luisa Mariani Center for the Study of Children’s Mitochondrial Disorders, ‘‘C. Besta” Neurological Institute Foundation-IRCCS, Milan, Italy b Department of Oncology, Neuroscience and Neurophysiopathology, University ‘‘G. D’Annunzio,” Chieti-Pescara, Italy

c Aging Research Center, Ce.S.I. ‘‘G. D’Annunzio” University Foundation, Chieti-Pescara, Italy

Received 31 January 2008; received in revised form 31 January 2008; accepted 2 April 2008

Abstract

Different mutations, or combinations of mutations, in POLG1, the gene encoding pol cA, the catalytic subunit of mitochondrial DNA polymerase, are associated with a spectrum of clinical presentations including autosomal dominant or recessive progressive external ophthalmoplegia (PEO), juvenile-onset ataxia and epilepsy, and Alpers–Huttenlocher syndrome. Parkinsonian features have been reported as a late complication of POLG1-associated dominant PEO. Good response to levodopa or dopamine agonists, reduced dopamine uptake in the corpus striatum and neuronal loss of the Substantia Nigra pars compacta have been documented in a few cases. Here we report two novel mutations in POLG1 in a compound heterozygous patient with autosomal recessive PEO, followed by pseudo-orthostatic tremor evolving into levodopa-responsive parkinsonism. These observations support the hypothesis that mtDNA dysfunction is engaged in the pathogenesis of idiopathic Parkinson’s disease. Ó 2008 Elsevier B.V. All rights reserved.

Keywords: Progressive external ophthalmoplegia; Parkinsonism; Polymerase gamma; Mitochondrial DNA; Multiple mtDNA deletions

1. Introduction

Mitochondrial DNA (mtDNA) is faithfully replicated by DNA polymerase V (pol V), an enzyme composed of a single, 145 kDa catalytic subunit (pol cA) bound to two, 55 kDa accessory subunits (pol cB). Pol cA is encoded by POLG1, a 23-exon gene located on human chromosome 15. Functional genetic variants of POLG1 are present inapproximately 0.5% of the general population. Numerous POLG1 mutations have been associated with a spectrum

of clinical presentations including autosomal dominant or recessive progressive external ophthalmoplegia (PEO), juvenile-onset ataxia and epilepsy, and Alpers–Huttenl-

ocher syndrome. At the molecular level, PEO patients are characterized by the accumulation of a collection of deleted mtDNA species in affected tissues, notably skeletal muscle

and brain. Additional symptoms, including migraine, epi-lepsy, peripheral neuropathy, ataxia, premature ovarian failure, hypogonadism and male hypofertility, may occur

in different PEO families and with different POLG1 mutations [1–4]. Parkinsonism is a rare and late-onset complication of POLG1-associated PEO [3–5]. We report here on a

patient presenting progressive external ophthalmoplegia followed by pseudo-orthostatic tremor and parkinsonism. Molecular analysis showed that this patient is a compound

heterozygous for two novel mutations of the POLG1 gene.

0960-8966/$ - see front matter Ó 2008 Elsevier B.V. All rights reserved.

doi:10.1016/j.nmd.2008.04.005\* Corresponding author. Fax: +39 02 2394 2619.

E-mail address: zeviani@istituto-besta.it (M. Zeviani).

1 These authors contributed equally to this work.

www.elsevier.com/locate/nmd

Neuromuscular Disorders 18 (2008) 460–464

2. Case description

The patient is a 54 year-old (yo) man from Central Italy. His father died at 71 years of lung cancer, had non-insulin dependent diabetes mellitus but was free from any neuro-

logical symptom. The 82 yo mother, a 47 yo brother and two 29 and 24 yo daughters are all alive and well. The pro-positus was born at term after an uneventful pregnancy. In contrast to what was reported previously [6], this patient developed PEO with bilateral ptosis at 30 years of age, followed by progressive dysphonia with rhinolalia, dysphagia, proximal muscle weakness and wasting, and tendon are-flexia. Reduction of caloric intake, caused by severe dysphagia and the onset of chronic diarrhoea, resulted insevere body weight loss. At 50 years of age the patient developed tremor in the standing position (dominant frequency at 6.2 Hz) initially affecting the left lower limb, then both lower limbs, with occasional mild resting tremor of the hands. This patient was included in a recent report on 4 cases affected by levodopa-responsive pseudo-orthostatic tremor in parkinsonism (case 3 in [6]). In the following 2 years he developed extra-pyramidal rigidity with a cogwheel sign, reduced tapping (left > right) and postural instability (Unified Parkinson’s Disease Rating Scale-

UPDRS motor score 21 points). Physical examination showed a hypomimic face, hypophonia, bilateral ptosis, ophthalmoplegia, mild symmetrical proximal muscle weakness (Medical Research Council grade 4+) and absence of lower limb tendon reflexes. An ECG and structural brain MRI were both normal. A Single-Photon Emission Computed Tomography (SPECT) using ([(123)I]-2b-carbome-thoxy-3b-(-4-iodophenyl)-N-(3-fluoropropyl)-nortropane (I-FP-CIT SPECT) revealed a bilaterally reduced dopamine uptake in the corpus striatum (right > left). Serum creatine kinase (CK) level was 2156 U/L (normal < 140U/L). An EMG was compatible with axonal sensory neuropathy associated with myopathic features. The patient was initially treated with low doses of dopamine agonists (pramipexole 2.1 mg/day) associated with levodopa up to 400 mg/day with disappearance of standing legs tremor

and reduction in UPDRS motor score. Two years later he complained of end-of-dose deterioration, with re-occurrence of the disabling standing tremor, which appeared 1.5–2.5 h after each single levodopa (UPDRS motor score was 27 in ‘‘on” state and 42 in ‘‘off”

state) and was associated with severe anxiety and panic attacks during the ‘‘off” states. Levodopa was increased to 600 mg/day and entacapone was added (1200 mg/day).

The patient did not report further fluctuations for the next 6 months, when he developed peak-dose dyskinesias, reoccurrence of end-of-dose deterioration and severe stand-

ing tremor. Entacapone was substituted with tolcapone 100 mg t.i.d. with reduction of dyskinesias and ‘‘off” state. Since then, dysphagia has progressively worsened, with fur-

ther loss of weight because of malnutrition, requiring the positioning of a percutaneous endoscopic gastrostomy for enteral nutrition. Onset of ventilatory failure has required

mechanical ventilation. The serum CK level remains high (6143 U/L), with worsening and spreading of the muscle weakness. High serum CK is an unusual, but occasionally

reported, finding in POLG1-associated PEO with multiple mtDNA deletions: for instance, Hudson et al. [5] report a patient (individual II:8) with serum CK levels > 2500 U/l.

The tremor and the ‘‘off” state now occur 1.5–2 h after levodopa administration, which must be repeated 7–8 times per day. In the last months the patient has also developed

progressive cognitive dysfunction associated with psychotic features.

3. Methods

Southern blot analysis of mtDNA was carried out on DNA extracted from skeletal muscle following standard procedures [7] and using the ECL chemiluminescence as the detection method. DNA from peripheral blood lymphocytes was used as a template to amplify the 22 coding exons of the POLG1 gene (exons 223). Polymerase chain reaction (PCR) conditions were 96 °C for 30 s, 56 °C for 20 s and 72 °C for 60 s, for 30 cycles plus as initial denaturation step at 96 °C for 2 min. PCR fragments were analyzed by automated nucleotide sequencing using the Big Dye terminator Ready Reaction Kit version 2 on a 3100 Genetic Analyzer Automated Sequencer (Applied Biosystems). The sequence variants found in the patient were searched for in available relatives as well as in 100 consecutive control subjects (200 alleles).

4. Results

A skeletal muscle biopsy taken when the patient was 32 years old showed the presence of several ragged-red fibers with variation of fiber caliber and nuclear centralization, and reduced or absent reactivity to cytochrome c oxidase (Fig. 1A). Sequence analysis of mtDNA excluded the presence of point mutations commonly associated with MERRF and MELAS. However, Southern blot analysis revealed the presence of a collection of deleted mtDNA

species in addition to wild-type mtDNA (Fig. 1B) [6]. These results prompted us to consider the possibility of mutation(s) in one of the nucleus-encoded genes commonly associated with mtDNA instability and PEO, namely Twinkle, ANT1 and POLG1. Sequence analysis of the first two genes ruled out the presence of pathogenic mutations. However, sequence analysis of the entire coding region of the POLG1 gene led us to the identification of two novel heterozygous mutations. One is a c.1288A > T transversion, predicting a p.M430L amino-acid substitution. The second is a c.2752T > C transition, determining the aminoacid substitution p.W918R (Fig. 2A). Segregation analysis, carried out in available members of the family (Fig. 2B), revealed the presence of the 2752T > C heterozygous mutation in the mother of the patient and in the two healthy daughters, while no mutation was detected in his brother.

F. Invernizzi et al. / Neuromuscular Disorders 18 (2008) 460–464 461

5. Discussion

We found two novel heterozygous POLG1 mutations in a compound heterozygous patient characterized by PEO, muscle weakness and later onset levodopa-responsive

pseudo-orthostatic tremor and parkinsonism [6]. The maternal allele predicts a p.W918R aminoacid sub-stitution in a highly conserved position in the pol cA polymerase domain (http://dir-apps.niehs.nih.gov/polg/) [8]. The paternal allele harbors a c.1288A > T transversion predicting the replacement of the p.M430 residue into an L residue. M430 is contained within the so-called spacer Fig. 1. Morphological and molecular findings in skeletal muscle. (A) Top panel, NADH dehydrogenase staining shows RRF (asterisks); bottom panel, cytochrome c oxidase staining of a serial muscle section shows several fibers with reduced or absent reactivity, including the same two RRF shown in the top panel (asterisks). (B) Southern blot analysis on PvuII-linearized mtDNA performed on the muscle biopsy of the patient (P) and a control (C). Both P and C samples show a 16.6 kb band corresponding to wild-type mtDNA (arrow). In addition, the P sample shows a collection of smaller bands

corresponding to deletion-containing mtDNA species. Fig. 2. (A) Identification of POLG1 mutations by sequence analysis. The electropherograms show the c.1288A > T transversion, predicting a p.M430L aminoacid substitution and the c.2752T > C transition, predicting a p.W918R aminoacid substitution. (B) Family pedigree. The solid black square symbol

indicates the proband; open symbols indicate unaffected individuals. The ‘‘+” and ‘‘” symbols indicate mutant vs. wild-type alleles. (C) Multiple alignment of mutation-containing pol cA regions in different species. The red boxes indicate the aminoacid substitutions found in the patient. 462 F. Invernizzi et al. / Neuromuscular Disorders 18 (2008) 460–464

domain, a region encompassing the midportion of pol cA, which in multicellular eukaryotes interacts with two pol cB accessory subunits to form a functionally active trimeric holoenzyme. Pol cB orthologs are absent in fungi, which can explain why M430 is conserved in metazoan pol cA sequences but not in fungal orthologs (for instance, MIP1 in Saccharomyces cerevisiae) (Fig. 2C). Segregation analysis in the family and absence of either mutation in 200 control alleles further support the pathogenic role of

the mutations. The role of POLG1 genetic variants in the pathogenesis of parkinsonism is suggested by several reports. Parkinsonism was documented in 13 affected members of five out of seven POLG1 mutant PEO families reported by Luoma et al. [3]. Three of these families displayed dominant transmission of the trait and were found to carry a heterozygous c.2864A > G (p.Y955C) mutation, one of the most common pathogenic variants of POLG1 [9]. In two additional families both parkinsonism and PEO were transmitted as an allegedly recessive trait. However, while the three affected members of the first of these families carried two allelic POLG1 mutations, namely c.1402A > G (p.N468D) and c.3313G > A (p.A1105T), only the latter was found in heterozygosity in the single patient of the second family. Good response to levodopa was documented in all patients to whom this treatment had been given. Five additional POLG1 mutant patients belonging to three families were described with a combination of PEO and parkinsonism, but in some of these cases the pathogenic role of the mutations remains controversial. This is the case for a family including a 49 yo woman with autosomal dominant PEO, neuropathy, hypogonadism and par-

kinsonism and her 59 yo brother with overt parkinsonian features [4]. Multiple mitochondrial DNA deletions in muscle and a heterozygous POLG1 missense mutation c.2492A > G, leading to p.Y831C aminoacid replacement, were found in the proband and her affected brother. The patient was successfully treated with levodopa. However,

the c.2492A > G transition has been reported in about 2% of control subjects in Poland and in normal controls in Finns [10,11] and Italians (MZ and FI, personal observation). These observations suggest that the p.Y831C aminoacid substitution is a non-pathogenic variant in different European populations. A second family included several PEO patients, one also being affected by parkinsonism. In this family autosomal dominant PEO segregated with a c.1532G > A heterozygous missense mutation (p.S511N) in exon 8, in combination with an intronic variant c.2070 + c.158G > A in cis [5]. The subject also affected by parkinsonism was found to carry an allelic in-trans change c.1389G > T (p.L463F) the pathogenic role of which is as yet not determined, although the latter was the only change detected in an

unaffected sibling. A third family was composed of two sisters with early- onset parkinsonism and signs of sensorimotor axonal peripheral neuropathy, without PEO; both were compound heterozygous, carrying the already reported c.2557C > T

(p.R853W) in exon 16 [12] of one allele, while the other allele carried a second, novel mutation c.2209C > A (p.G737R) in exon 13. One sister was treated with levodopa

at low dosage but she developed early oromandibular dyskinesias, the other was treated with dopamine agonists. Besides the POLG1 mutant patients mentioned above, a

heterozygous mutation c.1121G > A (p.R374Q) was reported in exon 1 of the Twinkle in a single family composed of 10 PEO patients, three of whom had also parkinsonism [13]. Finally, a PEO patient with a levodoparesponsive parkinsonism, dyskinesias and motor fluctuations [14] was shown to harbor mtDNA deletions in muscle but no mutation was found in either of the known PEO-associated genes. A comparative study of 420 Parkinson’s disease patients and controls did not support the role for common POLG1 genetic variants in parkinsonism, concluding that dominant POLG1 mutations are a rare cause of parkinsonism [15]. The hypothesis that length variants of the pol cA polyglutamine tract may predispose to idiopathic sporadic Parkinson’s disease was suggested by studies on Finns [3,11], but has not as yet been confirmed in other populations. On the other hand, POLG1 mutations are not the only cause of mtDNA defects associated with parkinsonism [16]. Interestingly, high levels of deleted mtDNAs were recently reported in neurons of the Substantia Nigra parscompacta in aged individuals. Patients with Parkinson’s disease showed levels of deleted mtDNA that were significantly higher than in age-matched control brains [17]. These recent results support the long-lasting hypothesis that mitochondrial dysfunctions play a role in the patho-

genesis of Parkinson’s disease, as well as other adult-onset neurodegenerative diseases including Alzheimer’s disease, Huntington’s disease and amyotrophic lateral sclerosis[18]. Interestingly, in two patients [12], including ours, dyskinesias heralded the parkinsonism associated with POLG1 mutations. The latter can be concealed by the presence of peripheral neuropathy and muscle weakness. The identification of two novel POLG1 mutations expressing a phenotype characterized by PEO and parkinsonism supports the hypothesis that loss of mtDNA integrity is implicated in the pathogenesis of Parkinson’s disease. Combination of myopathic and neuromuscular symptoms with parkinsonism should prompt to include the screening of the POLG1 gene in the diagnostic work-out, especially, but not exclusively, when the syndrome is inherited.

Acknowledgments

This work was supported by the Pierfranco and Luisa

Mariani Foundation, the Telethon-Italy Foundation

(Grant No. GGP07019) the Italian Ministry of University

and Research (FIRB 2003 – project RBLA038RMA), the

Italian Ministry of Health (RF2006 ex 56/05/21), MITO-

F. Invernizzi et al. / Neuromuscular Disorders 18 (2008) 460–464 463

CIRCLE and EUMITOCOMBAT (LSHM-CT-2004-

503116) network Grants from the European Union frame-

work program 6. The authors are grateful to the patient

and his family for their collaboration in the study.

References

[1] Graziewicz MA, Bienstock RJ, Copeland WC. The DNA polymerase

c Y955C disease variant associated with PEO and parkinsonism

mediates the incorporation and translesion synthesis opposite 7,8-

dihydro-8-oxo-20-deoxyguanosine. Hum Mol Genet 2007;16:2729–39.

[2] Hudson G, Chinnery PF. Mitochondrial polymerase-c and human

disease. Hum Mol Genet 2006;15:R244–52.

[3] Luoma P, Melberg A, Rinne JO, et al. Parkinsonism, premature

menopause, and mitochondrial DNA polymerase c mutations:

clinical and molecular genetic study. Lancet 2004;364:875–82.

[4] Mancuso M, Filosto M, Oh SJ, DiMauro S. A novel polymerase c

mutation in a family with ophthalmoplegia, neuropathy, and

parkinsonism. Arch Neurol 2004;61:1777–9.

[5] Hudson G, Schaefer AM, Taylor RW, et al. Mutation of the linker

region of the polymerase c-1 (POLG1) gene associated with progres-

sive external ophthalmoplegia and parkinsonism. Arch Neurol

2007;64:553–7.

[6] Thomas A, Bonanni L, Antonini A, Barone P, Onofrj M. Dopa-

responsive pseudo-orthostatic tremor in parkinsonism. Mov Disord

2007;22:1652–6.

[7] Zeviani M, Servidei S, Gellera C, Bertini E, Di Mauro S, Di Donato

S. An autosomal dominant disorder with multiple deletions of

mitochondrial DNA starting at the D-loop region. Nature

1989;339:309–11.

[8] Longley MJ, Graziewicz MA, Bienstock RJ, Copeland WC. Conse-

quences of mutations in human DNA polymerase c. Gene

2005;354:125–31.

[9] Lamantea E, Tiranti V, Bordoni A, et al. Mutations of mitochondrial

DNA polymerase cA are a frequent cause of autosomal dominant or

recessive progressive external ophthalmoplegia. Ann Neurol

2002;52:211–9.

[10] Stopin´ska K, Grzybowski T, Malyarchuk BA, Derenko MV,

Mis´cicka-Sliwka D. Optimization of the Y831C mutation in human

DNA polymerase c by allelic discrimination assay. Acta Biochim Pol

2006;53:591–5.

[11] Luoma PT, Eerola J, Ahola S, et al. Mitochondrial DNA polymerase

gamma variants in idiopathic sporadic Parkinson disease. Neurology

2007;69:1152–9.

[12] Davidzon G, Greene P, Mancuso M, et al. Early-onset familial

parkinsonism due to POLG mutations. Ann Neurol 2006;59:859–62.

[13] Baloh RH, Salavaggione E, Milbrandt J, Pestronk A. Familial

parkinsonism and ophthalmoplegia from a mutation in the mito-

chondrial DNA helicase twinkle. Arch Neurol 2007;64:998–1000.

[14] Wilcox RA, Churchyard A, Dahl HH, Hutchison WM, Kirby DM,

Thyagarajan D. Levodopa response in parkinsonism with multiple

mitochondrial DNA deletions. Mov Disord 2007;22:1020–3.

[15] Tiangyou W, Hudson G, Ghezzi D, et al. POLG1 in idiopathic

Parkinson disease. Neurology 2006;67:1698–700.

[16] Horvath R, Kley RA, Lochmuller H, Vorgerd M. Parkinson

syndrome, neuropathy, and myopathy caused by the mutation

A8344G (MERRF) in tRNALys

. Neurology 2007;68:56–8.

[17] Bender A, Krishnan KJ, Morris CM, et al. High levels of mitochon-

drial DNA deletions in substantia nigra neurons in aging and

Parkinson disease. Nat Genet 2006;38:515–7.

[18] Schapira AH. Mitochondrial disease. Lancet 2006;368:70–82.

464 F. Invernizzi et al. / Neuromuscular Disorders 18 (2008) 460–464