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The wide *POLG*-related spectrum: An integrated view

M. Béreau [a,1], M. Anheim [b,c,*,1], A. Echaniz-Laguna [b,c,d], A. Magot [e], C. Verny [f], M. Goideau-Sevrain [f], M. Barth [g], P. Amati-Bonneau [g], S. Allouche [h], X. Ayrignac [i], A.-L. Bédat-Millet [j], L. Guyant-Maréchal [k], T. Kuntzer [l], F. Ochsner [l], P. Petiot [m], C. Vial [n], S. Omer [o], G. Sole [p], G. Taieb [q], N. Carvalho [r], G. Tio [s], S. Kremer [t], C. Acquaviva-Bourdain [u], B. Mousson de Camaret [u,2], C. Tranchant [b,c,2]

[a] *Département de Neurologie, Hôpital Jean Minjoz, Besançon, France*
[b] *Unité des Pathologies du Mouvement, Service de Neurologie, Hôpital de Hautepierre, Strasbourg, France*
[c] *Fédération de Médecine Translationnelle (FMTS), Faculté de Médecine, Strasbourg, France*
[d] *Centre de Référence Neuromusculaire Grand Est (CERNEST), France*
[e] *Centre de Référence des Maladies Neuromusculaires, CHU de Nantes – Hôtel Dieu, Nantes, France*
[f] *Centre de Référence des Maladies Neurogénétiques, CHU d'Angers, Angers, France*
[g] *Département de Biochimie et Génétique, CHU d'Angers, Angers, France*
[h] *Service de Biochimie, Hôpital de la Côte de Nacre, CHU de Caen, France*
[i] *Département de Neurologie, CHRU de Montpellier, Montpellier, France*
[j] *Département de Neurologie, Hôpital Charles Nicolle, Rouen, France*
[k] *Unité de Génétique Clinique, Hôpital Charles Nicolle, Rouen, France*
[l] *Department of Clinical Neuroscience, Lausanne University Hospital (CHUV), Lausanne, Switzerland*
[m] *Neurologie et Explorations Fonctionnelles Neurologiques, Centre de référence maladies neuromusculaires région Rône-Alpes, Hôpital de la Croix-Rousse, Lyon, France*
[n] *Service ENMG et Pathologies NeuroMusculaires, Hôpital Neurologique, Lyon-Bron, France*
[o] *Department of Neurology, St. George's Hospital, London, UK*
[p] *Département de Neurologie, CHU de Bordeaux, Bordeaux, France*
[q] *Département de Neurologie, Hôpital Universitaire Carémeaux, Nîmes, France*
[r] *E.A. 481, Laboratoire de Neurosciences, Université de Franche-Comté, Besançon, France*
[s] *Département de Statistiques, Centre d'Investigation Clinique et d'Innovations Technologiques, INSERM CIT-808, CHRU de Besançon, Besançon, France*
[t] *Imagerie 2-Hôpital de Hautepierre-CHU de Strasbourg-Université de Strasbourg-Fédération de Médecine Translationnelle-I Cube, France*
[u] *Service des Maladies Héréditaires du Métabolisme, Centre de Biologie et de Pathologie Est, CHU Lyon, 69677 Bron, France*

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ABSTRACT

The aims of this study were to describe the spectrum of recessively inherited *POLG*-related disorders, to report new *POLG* mutations and to discuss genotype-phenotype correlations in order to propose a strategy for diagnosis. Twenty eight patients diagnosed with two *POLG* mutations at 12 tertiary European centers of adult neurology were studied. Exhaustive phenotypic data, brain MRI, muscle analysis, mitochondrial DNA and *POLG* analysis findings were collected. Five distinct phenotypes were observed: Sensory Ataxic Neuropathy, Dysarthria and Ophthalmoparesis (SANDO), autosomal recessive Progressive External Ophthalmoplegia (arPEO), Spino Cerebellar Ataxia with Epilepsy (SCAE), Mitochondrial Neuro Gastro Intestinal Encephalopathy (MNGIE)-like phenotype and Sensory Ataxic Neuropathy with Ophthalmoparesis but without dysarthria which we propose to name SANO. An increasing gradient of functional severity was appreciated from PEO with the best prognosis, to SANO, SANDO and finally SCAE respectively. Four new missense mutations were found. Regarding genotype/phenotype correlations, P587L mutation was associated with SANO rather than with SANDO ($p < 0.005$) and W748S mutation was associated with SANDO or SCAE (with more severe disease progression), rather than with SANO or PEO ($p < 0.004$). Distinguishing between various phenotypes can have important diagnosis and prognosis implications. *POLG* mutations should be priority searched for in cases of SANDO or SANO. Mitochondrial respiratory chain and mitochondrial DNA studies should be considered in the case of negative *POLG* analysis or other phenotypes.

* Corresponding author at: Département de Neurologie, Hôpital Civil de Strasbourg, 1, place de L'hôpital BP 426, 67091 Strasbeourg Cedex, France.
E-mail address: mathieu.anheim@chru-strasbourg.fr (M. Anheim).
[1] Both authors contributed equally to this work and should be considered as first equal.
[2] Both authors contributed equally to this work and should be considered as last equal.

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1. Introduction

Nuclear *POLG* gene mutations are responsible for multiple mtDNA deletions or mtDNA depletion and constitute a major cause of inherited mitochondrial disorders. More than 100 pathogenic *POLG* variations associated with numerous clinical phenotypes and two modes of inheritance have been reported (Human DNA Polymerase Gamma Mutation Database http://tools.niehs.nih.gov/polg/). With the exception of Alper's syndrome and other rare multi-systemic syndromes, usually occurring in children, many other syndromes have been reported for adults carrying mutations in *POLG*: autosomal dominant (ad) or autosomal recessive (ar) progressive external ophthalmoplegia (PEO) [1,2], Sensory Ataxic Neuropathy, Dysarthria and Ophthalmoparesis (SANDO) [3], Mitochondrial Recessive Ataxia Syndrome (MIRAS) [4], or Spino Cerebellar Ataxia with Epilepsy (SCAE) [5]. Multiple different clinical phenotypes are associated with POLG mutations, including Parkinsonism [6], optic neuropathy [7], psychiatric disorders and dementia. A few series of *POLG* mutations have been reported [3,5,8–11], though no robust phenotype-genotype correlations have been found. However one study, considered controversial, did find a genotype-phenotype correlation by defining five functional clusters in the catalytic core of the POLG protein [12]. Our aim was to delineate the spectrum of *POLG*-related phenotypes and genotypes from 28 patients carrying bi-allelic *POLG* mutations. On the basis of these results, we provide genotype-phenotype correlations in order to propose an original integrated view of *POLG*-related spectrum and to recommend a strategy for diagnosis.

2. Methods

Retrospectively clinical and paraclinical data were collected of 28 adult patients referred to 12 adult tertiary centers of neurology between January 2003–June 2013 and diagnosed as carrying bi-allelic *POLG* mutations. The patients had undergone *POLG* sequencing during the diagnosis work as *POLG*-related spectrum had been suspected given the combination of a variety of neurological signs, in particular movement and/or neuromuscular disorders.

2.1. Clinical and paraclinical tests

Family history, age of onset, first signs of the disease, current clinical signs, results of electromyography and nerve conduction studies, as well as brain MRI were collected.

2.2. Morphological and biochemical analysis

Muscle biopsies were processed using standard histological and histochemical methodologies. Enzymatic activities of the mitochondrial respiratory chain (MRC) complexes were measured in muscle [13].

2.3. Molecular investigations

POLG (gene ID 6834, MIM#17463) exons 2–23 as well as intron/exon boundaries were amplified and sequenced from genomic DNA of patients. Mitochondrial DNA deletions were detected by a screening procedure using the polymerase chain reaction [14]. Quantification of mitochondrial DNA deletion, depletion and over-replication was assessed [15]. The pathogenicity of mutations was reviewed by different bioinformatics tools (Alamut Visual: http://www.interactive-biosoftware.com/alamut-visual/; SIFT: http://sift.jcvi.org/, Mutation Taster: http://www.mutationtaster.org/). Known mutations in the coding region of the *POLG* gene and associated diseases were listed in the Human DNA Polymerase Gamma Mutation Database (http://tools.niehs.nih.gov/polg/). Moreover, for the general population, the carrier frequency of the recently identified mutations was analyzed using Exome Aggregation Consortium (ExAC) browser (http://exac.broadinstittute.org/). Segregation studies were performed in 15 patients to assess the pathogenicity of the identified mutations.

2.4. Statistical analysis

All computations were performed using SAS software 9.3 TS1M2. All statistical tests' significance were set at the 0.05 level. Logistic regression for repeated measures data was used to analyze clinical signs for phenotype parts. Association between the clinical features and the three most frequent mutations P587L, A467T, W748S was compared using the Student *t*-test or Wilcoxon-test, depending upon the normality of distribution.

2.5. Ethical considerations

All patients gave written informed consent and the local ethics committee approved the study.

3. Results

3.1. Clinical manifestations

The 28 patients comprised 11 male and 17 female. Mean age of onset was 35.7 years (SD = 18.3). At time of diagnosis, mean disease duration was 18.5 years (ranging from 9 to 71; SD = 13.4). Initial clinical features were ptosis (50% of cases), gait imbalance (22.7%), sensory disorders with dysesthesia or hypoesthesia (13.6%), seizure (9.1%) and exercise intolerance (4.5%). At the time of diagnosis, ophthalmoparesis (89.3%), ptosis (82.1%), sensory loss (82.1%) and ataxia (82.1%), either proprioceptive (28.6%), cerebellar (3.6%), or mixed (i.e. both proprioceptive and cerebellar) ataxia (50%), were significantly more frequent than the other clinical features ($p < 0.02$). For 19 patients (67.9%), ophthalmoparesis, ataxia, ptosis and sensory loss were combined. Other clinical features included: dysarthria (50%), dysphagia (39.3%), proximal muscle weakness (32.1%), migraine (25%), exercise intolerance (21.4%), depression (21.4%), epilepsy (14.3%), cognitive impairment (14.3%), movement disorders (14.3%), deafness (10.7%), and gastrointestinal dysmotility (3.6%).

3.2. POLG phenotypes (Table 1)

Five distinct phenotypes were identified. 4 patients (10, 21, 25, 26), with PEO (14.3%), 12 patients (2, 6, 9, 11, 12, 14, 15, 16, 17, 19, 20, 27), with SANDO (42.9%), 7 patients (1, 7, 18, 22, 23, 24, 28), with Sensory Ataxic Neuropathy with Ophthalmoparesis (but without dysarthria, due to the lack of cerebellar involvement), that we named SANO (25%), 4 patients (3, 4, 8, 13), with SCAE (14.3%) and one (patient 5) with an MNGIE-like syndrome (3.6%). Of the 4 PEO patients, one (patient 10), displayed a classical autosomal recessive PEO phenotype including ptosis, ophthalmoplegia and mild proximal weakness while the other three (21, 25, 26), expressed an initial oculopharyngeal muscular dystrophy-like phenotype with dysphagia and ptosis but without ophthalmoplegia, proximal muscle weakness, or *PABPN1* mutation. Patients 21 and 25 developed ophthalmoplegia and proximal muscle weakness subsequently. At the last examination, patient 26 displayed neither strabismus nor proximal muscle weakness. All SANO patients presented a proprioceptive ataxia due to an axonal sensorimotor neuropathy, while SANDO patients displayed a mixed cerebellar and proprioceptive ataxia. 7/12 SANDO patients (6, 9, 11, 15, 16, 17, 19), had an axonal sensorimotor neuropathy and 5/12 patients (2, 12, 14, 20, 27), had a dorsal root ganglionopathy. The MNGIE-like phenotype was characterized by progressive external ophthalmoplegia, sensory neuropathy and gastrointestinal dysmotility without either leukoencephalopathy or *TYMP* gene mutation. SCAE patients displayed a more severe functional impairment: patient 13 having severe ataxia became wheelchair-bound; patient 8 had diffuse cognitive deficits due to status epilepticus