“Mitochondrial neuropathies”: A survey from the large cohort of the Italian Network

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Abstract

Involvement of the peripheral nervous system in mitochondrial disorders has been previously reported. However, the prevalence of peripheral neuropathy in mitochondrial disorders is still unclear. Based on the large database of the “Nation-wide Italian Collaborative Network of Mitochondrial Diseases”, we reviewed the clinical data of 1200 patients, with special regard to peripheral neuropathy (mean age at onset 24.3 ± 20.1 years; age at last evaluation 39.8 ± 22.3 years; females 52.7%; childhood onset [before age 16 years] 43.1%). Peripheral neuropathy was present in 143/1156 patients (12.4%), being one of the ten most common signs and symptoms. POLG mutations cause a potentially painful, axonal/mixed, mainly sensory polyneuropathy; TYMP mutations lead to a demyelinating sensory-motor polyneuropathy; SURF1 mutations are associated with a demyelinating/mixed sensory-motor polyneuropathy. The only mtDNA mutation consistently associated with peripheral neuropathy (although less severely than in the above-considered nuclear genes) was the m.8993T > G (or the rarer T > C) changes, which lead to an axonal, mainly sensory polyneuropathy. In conclusion, peripheral neuropathy is one of the most common features of a mitochondrial disorder, and may negatively impact on the quality of life of these patients. Furthermore, the presence or absence of peripheral neuropathy, as well as its specific forms and the association with neuropathic pain (indicative of a POLG-associated disease) can guide the molecular analysis.

Keywords: Disease registry; Mitochondrial myopathies; mtDNA; Neuropathy; Peripheral nerve

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1. Introduction

The central task of mitochondria is to generate energy in the form of adenosine triphosphate, through the electron transport chain and oxidative phosphorylation (OXPHOS) [1]. This pathway is under control of both nuclear and mitochondrial (mtDNA) genomes. Mitochondrial diseases are a group of disorders caused by OXPHOS impairment. They are one of the commonest inherited neuromuscular diseases, with an estimated prevalence of 1–2 in 10,000. The genetic classification distinguishes the disorders due to defects in mtDNA from those due to defects in nuclear DNA.

Both mtDNA and nuclear mutations affecting OXPHOS usually result in multisystem clinical phenotypes, including peripheral neuropathy [2]. Peripheral neuropathy is a defining feature in some mitochondrial disorders such as SANDO (sensory ataxic neuropathy, dysarthria and ophthalmoplegia), NARP (neuropathy, ataxia and retinitis pigmentosa), or MNGIE (mitochondrial neuro-gastro-intestinal encephalomyopathy) [3], and occasionally is the prevalent disease manifestation [4,5]. However, the exact prevalence of peripheral neuropathy in mitochondrial disorders is still unknown [6].

The aim of this retrospective study is to evaluate the minimum prevalence of peripheral neuropathy in patients with mitochondrial disorders, taking advantage of the large database built by the "Nation-wide Italian Collaborative Network of Mitochondrial Diseases". Specific genotype–phenotype correlations are highlighted.

2. Methods

We reviewed the clinical data of all of the 1200 histologically, biochemically and/or molecularly defined patients present in our "Nation-wide Italian Collaborative Network of Mitochondrial Diseases" database (updated on December 31, 2014) and followed up by the involved centers, focusing on peripheral neuropathy as a clinical feature of a mitochondrial disorder.

The database establishment (and its use for scientific purposes) was permitted by the local Ethical Committees of the single centers, which obtained written informed consent from all patients or their tutors, and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. This project has been supported by a Telethon grant (GUP09004).

This is a retrospective study; all the involved centers have specific expertise in mitochondrial disorders. The clinical section of our web-based database includes "yes or no" dichotomic items agreed by all centers in a preliminary consensus phase. The consensus phase was specifically designed to include the clinical features known to be relevant in mitochondrial medicine [1]. The presence or absence of neuropathy, and its specific type (i.e., axonal versus demyelinating, motor versus sensory, polyneuropathy versus multiplex neuropathy – including mixed forms [2]) was requested to all centers for data elaboration. Clinical symptoms/signs potentially suggestive of peripheral nerve dysfunction (i.e. numbness, paresthesia, hypotonia, weakness, muscle atrophy) have also been collected. Pure hereditary neuropathies (Charcot–Marie–Tooth disease – CMT) due to mutations in mitochondrial proteins, such as *MFN2* and *GDAP1* [3], have not been considered in this study, which addresses the classic OXPHOS disorders [4].

Results were expressed as means ± standard deviation. Comparisons of proportions have been performer by two-tailed Fischer's exact test. A P value <0.05 was considered as significant. Data analysis was carried out using MedCalc® Version 7.3.0.1.

3. Results

The clinical picture was fully available for 1156 patients (mean age at onset 24.3 ± 20.1 years; age at last evaluation 39.8 ± 22.3 years; females 52.7%; childhood onset [before age 16 years] 43.1%). Peripheral neuropathy was part of the clinical picture in 143/1156 patients (12.4%), being the tenth most frequent clinical manifestation (Table 1). In 33 patients, neuropathy was present at the onset of the disease.

In order to understand if the presence (or absence) of peripheral neuropathy was linked to specific molecular or clinical features, the patients of the Registry were divided in two groups, with and without neuropathy. Age at onset, age at last evaluation and gender ratio did not significantly differ between the two groups.

From a genotype-based point of view (Table 2), the mtDNA mutations associated with Leber hereditary optic neuropathy (LHON) had the lowest frequency in the group with neuropathy. The same was also observed in those patients harboring single mtDNA deletions ($p < 0.000003$). In contrast, mutations in the nuclear genes *POLG*, *SURF1* and *TYMP* were those most consistently associated with peripheral neuropathy (Table 2).

Table 1
Clinical features at the last evaluation in mitochondrial patients. The table shows the 15 most common features, present in at least 100 cases. Migraine, cardiomyopathy, retinopathy, respiratory impairment, gastrointestinal dysmotility, cataract, stroke-like episodes, myoclonus, tremor, parkinsonism, dystonia, dyskinesias, psychiatric involvement, vomiting, hypothyroidism, anemia were rarer (2–8%).

	Patients (n = 1156)	%
Ptosis/ophthalmoparesis	617	53.4
Muscle weakness	446	38.6
Hearing loss	279	24.1
Exercise intolerance	239	20.7
Optic neuropathy	214	18.5
Muscle wasting	212	18.3
Cerebellar ataxia	186	16.1
Cognitive involvement	180	15.6
Hypotonia	179	15.5
Neuropathy	143	12.4
Swallowing impairment	137	11.9
Epileptic seizures	131	11.3
Muscle pain	124	10.7
Pyramidal involvement	112	9.7
Diabetes	102	8.8

Table 2
Genotype-based approach. The patients have been divided in two groups, with and without neuropathy. Genotypes with less than ten patients have not been considered and are not shown. Significance levels after Bonferroni's correction 0.0042. Significant differences are represented in bold. LHON, Leber hereditary optic neuropathy. n.s., not significant difference.

	Neuropathy: No (n = 1013)	Neuropathy: Yes (n = 143)	*P*
mtDNA single deletion	218 (21.5%)	9 (6.3%)	**0.000003**
mtDNA A3243G mutation	90 (8.9%)	12 (8.4%)	n.s.
mtDNA A8344G mutation	30 (3.0%)	5 (3.5%)	n.s.
mtDNA T8993G (or C) mutation	12 (1.2%)	6 (4.2%)	0.017
mtDNA LHON mutations	103 (10.2%)	1 (0.7%)	**0.00002**
Other mtDNA mutations	53 (5.2%)	7 (4.8%)	n.s.
OPA1 mutations	72 (7.1%)	5 (3.5%)	n.s.
POLG mutations	26 (2.6%)	19 (13.3%)	**<0.000001**
Twinkle mutations	22 (2.2%)	6 (4.2%)	n.s.
SURF1 mutations	10 (1.0%)	10 (7.0%)	**0.00004**
PDHA1 mutations	11 (1.1%)	1 (0.7%)	n.s.
TP mutations	2 (0.2%)	9 (6.3%)	**<0.000001**

From a phenotype-based point of view (Table 3), patients with neuropathy had an increased prevalence of ataxia, hearing loss, muscle weakness and muscle wasting. There was neither association with other neuromuscular symptoms, such as exercise intolerance and muscle pain, nor with other multisystem features, in particular diabetes.

In those patients for whom muscle biopsy was available, ragged red fibers were equally frequent in the group with (67/96, 69.8%) or without (423/618, 68.4%) peripheral neuropathy. The same was observed for cytochrome c oxidase (COX)-negative fibers (81/96 [84.4%] and 470/618 [76.1%] respectively). Finally, considering the patients for whom plasma lactate levels were available, this biomarker was increased in 58/98 (59.2%) patients with and in 322/632 (50.9%) patients without neuropathy, again a non-significant difference.

Table 3
Phenotype-based approach. The patients have been divided in two groups, with and without neuropathy. Genotypes with less than ten patients have not been considered and are not shown. Significance levels after Bonferroni's correction 0.0036. Significant differences are represented in bold. LHON, Leber hereditary optic neuropathy. n.s., not significant difference.

	Neuropathy: No (n = 1013)	Neuropathy: Yes (n = 143)	*P*
Ptosis/ophthalmoparesis	536 (52.9%)	81 (56.6%)	n.s.
Muscle weakness	362 (35.7%)	84 (58.7%)	**<0.0001**
Hearing loss	220 (21.7%)	59 (41.3%)	**<0.0001**
Exercise intolerance	203 (20.0%)	36 (25.2%)	n.s
Optic neuropathy	195 (19.2%)	19 (13.3%)	n.s
Muscle wasting	169 (16.7%)	43 (30.1%)	**0.0003**
Ataxia	131 (12.9%)	55 (38.5%)	**<0.000001**
Cognitive involvement	148 (14.6%)	32 (22.4%)	0.0045
Hypotonia	152 (15.0%)	27 (18.8%)	n.s.
Swallowing impairment	119 (11.7%)	18 (12.6%)	n.s.
Epileptic seizures	110 (10.9%)	21 (14.7%)	n.s.
Muscle pain	104 (10.3%)	20 (14.0%)	n.s.
Pyramidal involvement	100 (9.9%)	12 (8.4%)	n.s.
Diabetes	85 (8.3%)	17 (11.9%)	n.s.

Table 4
Tentative phenotype-genotype correlations in mitochondrial neuropathy.

***POLG*-associated neuropathy**
Prevalence 19/45 (42.2%), in five cases from the onset
High prevalence of neuropathic pain (>1/3 – see Table 5)
9 pure axonal, 1 pure demyelinating, 9 had mixed/undefined pattern
1 pure motor, 6 pure sensitive, 12 mixed/undefined pattern
11 length-dependent polyneuropathy, 1 multiplex neuropathy, 7 different/undefined pattern
(axonal/mixed mainly sensory polyneuropathy)
***TP* (*TYMP*)-associated neuropathy**
Prevalence 9/11 (81.8%), in no case from the onset
Neuropathic pain is absent
2 pure axonal, 5 pure demyelinating, 2 had a mixed pattern
1 pure motor, 8 mixed sensori-motor pattern
8 length-dependent polyneuropathy, 1 different/undefined pattern
(demyelinating sensory-motor polyneuropathy)
***SURF1*-associated neuropathy**
Prevalence 10/20 (50.0%), in no case from the onset
3 pure demyelinating, 7 had a mixed/undefined pattern
1 pure motor, 9 mixed sensori-motor/undefined pattern
6 length-dependent polyneuropathy, 4 different/undefined pattern
(demyelinating/mixed sensory-motor polyneuropathy)
m.8993T > G or T > C mutation-associated neuropathy
Prevalence 6/18 (33.3%), in one case from the onset
5 pure axonal, 1 mixed/undefined
3 pure sensory, 3 mixed/undefined
5 length-dependent polyneuropathy, 1 different/undefined pattern
(axonal, mainly sensory polyneuropathy)

Among our patients with neuropathy, the "pure" forms were rare. Specifically, 55 patients had a pure axonal neuropathy, 18 pure demyelinating, whereas 70 had a mixed/undefined pattern. Furthermore, 14 patients had a motor neuropathy, 26 a sensitive form, whereas the remaining patients had a mixed/undefined pattern. Finally, 94 patients have been defined as having a length-dependent polyneuropathy, three as a multiplex neuropathy, and the remaining 46 as a different/undefined pattern. Only 15 patients suffered of neuropathic pain.

The relative rarity of the pure forms precluded a strict genotype–phenotype analysis, but some indications (shown in Table 4) were noted; among the POLG-mutated patients, 9 had a pure axonal – sensory or sensory-motor – neuropathy and only one a pure demyelinating sensory neuropathy; the prevalence of neuropathic pain seemed more frequent in POLG patients than in mitochondrial patients with neuropathy due to different genetic causes (see Table 5).

Table 5
Neuropathic pain and *POLG*-associated neuropathy. $P = 0.000806$ (Fisher's exact test).

	POLG mutations	Other etiologies	
Neuropathic pain: yes	7	8	15
Neuropathic pain: no	12	116	128
	19	124	

4. Discussion

Despite the great progress in our genetic and clinical understanding of mitochondrial disorders, the prevalence of peripheral neuropathy is still unknown. Most of the published studies have examined single cases or families, or have been conducted by recruiting a limited number of patients [7]. Girlanda et al. analyzed 27 patients with unselected mitochondrial disease, and observed axonal neuropathy in six cases (18%) [8]. Mancuso et al. [9] showed electrophysiologically that peripheral neuropathy was present in about 35% of unselected mitochondrial patients, even if the peripheral nerve involvement was frequently subclinical. In the present study, taking advantage of a very large unselected cohort of mitochondrial disorders patients, including LHON and oligosymptomatic forms, we found a lower prevalence of peripheral neuropathy (12,4%), which could represent the minimum prevalence of peripheral neuropathy in mitochondrial disorders (13.5% [142/1052] if we exclude the patients with LHON).

This preliminary survey presents the typical limitations of all retrospective studies, and may underestimate the real prevalence of neuropathy in patients with mitochondrial disorders. However, all the involved centers have strong expertise in clinical and electrophysiological assessment of neuromuscular disorders, which should minimize false negative or positive cases. Previous surveys [7–9], specifically designed for assessing the involvement of peripheral nervous system, also included patients with subclinical neuropathy, a criterion which can partially account for the discrepancy with the data presented here.

Recently, Horga et al. [10] reported that peripheral neuropathy might suggest a nuclear gene defect in patients with mitochondrial progressive external ophthalmoplegia (PEO). In this study, 67% of PEO patients had a single mitochondrial DNA deletion, whereas 10% had a point mutation of mtDNA and 22% had mutations in either POLG, C10orf2, RRM2B, or had multiple mtDNA deletions in muscle without an identified nuclear gene defect. The prevalence of peripheral neuropathy was significantly lower in patients with single, sporadic mitochondrial DNA deletion (2%) as compared to those with a mtDNA point mutation or with a nuclear DNA defect (44% and 52%, respectively; $P < 0.001$) [10]. Therefore, our results confirm the suggestion by Horga et al., that in PEO patients peripheral neuropathy is rarely found in association with single mtDNA deletions, whereas it is highly predictive of an underlying nuclear DNA defect [10,11]. Indeed, our data extend this concept to the entire population of mitochondrial patients, in addition to those with PEO; furthermore, we show that a "mitochondrial neuropathies" are predominantly associated with a few specific nuclear genes associated: POLG, TYMP and SURF1. This observation may facilitate the development of diagnostic algorithms suggesting nuclear gene testing in patients with mitochondrial disease and peripheral neuropathy, for a more rapid diagnosis, which can be achieved without performing a muscle biopsy.

Furthermore, peripheral neuropathy with anesthesia leading to corneal scarring and acral mutilations is one of the major clinical features of Navajo neurohepatopathy, an autosomal recessive hepato-cerebral mtDNA depletion syndrome caused by a mutation in the *MPV17* gene [12].

In our cohort, the subjects with neuropathy had an increased prevalence of ataxia, hearing loss, muscle weakness and muscle wasting. Therefore, neuropathy can cause and/or exacerbate neuromuscular symptoms that have a strong impact on the quality of life of these patients.

Some phenotype–genotype correlations were highlighted in our study. In particular, POLG mutations can cause a potentially painful, axonal/mixed, mainly sensory polyneuropathy; TYMP mutations can lead to a demyelinating sensory-motor polyneuropathy (usually in the context of the MNGIE syndrome); SURF1 mutations lead to a Leigh (or Leigh-like) phenotype frequently associated with a demyelinating/mixed sensory-motor polyneuropathy.

In our cohort, the only mtDNA mutation potentially associated with neuropathy is the m.8993T > G (or T > C) change, which can lead to an axonal, mainly sensory polyneuropathy.

In conclusion, peripheral neuropathy is one of ten most common features of mitochondrial disease that potentially have a strong impact on the quality of life of these patients. Some genotype–phenotype correlations were highlighted, and the presence or absence of peripheral neuropathy, as well as its specific form and the association with neuropathic pain (indicative of a POLG-associated disease) can guide the molecular analysis.

Prospective studies on clinical features in mitochondrial disorders are strongly needed; however, large multicenter retrospective studies help to characterize the clinical picture and natural history of rare diseases such as mitochondrial disease, and represent the basis for planning more rigorous longitudinal studies.

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References

[1] DiMauro S, Schon EA. Mitochondrial respiratory-chain diseases. N Engl J Med 2003;348:2656–68.
[2] Mancuso M, Orsucci D, Coppede F, Nesti C, Choub A, Siciliano G. Diagnostic approach to mitochondrial disorders: the need for a reliable biomarker. Curr Mol Med 2009;9:1095–107.
[3] Pareyson D, Piscosquito G, Moroni I, Salsano E, Zeviani M. Peripheral neuropathy in mitochondrial disorders. Lancet Neurol 2013;12:1011–24.
[4] Pineda M, Solano A, Artuch R, et al. Peripheral neuropathy with ataxia in childhood as a result of the G8363A mutation in mitochondrial DNA. Pediatr Res 2004;56:55–9.
[5] Hisama FM, Mancuso M, Filosto M, DiMauro S. Progressive external ophthalmoplegia: a new family with tremor and peripheral neuropathy. Am J Med Genet A 2005;135:217–19.
[6] Finsterer J. Mitochondrial neuropathy. Clin Neurol Neurosurg 2005;107:181–6.

[7] Chu CC, Huang CC, Fang W, Chu NS, Pang CY, Wei YH. Peripheral neuropathy in mitochondrial encephalomyopathies. Eur Neurol 1997; 37:110–15.
[8] Girlanda P, Toscano A, Nicolosi C, et al. Electrophysiological study of neuromuscular system involvement in mitochondrial cytopathy. Clin Neurophysiol 1999;110:1284–9.
[9] Mancuso M, Piazza S, Volpi L, et al. Nerve and muscle involvement in mitochondrial disorders: an electrophysiological study. Neurol Sci 2012;33:449–52.
[10] Horga A, Pitceathly RD, Blake JC, et al. Peripheral neuropathy predicts nuclear gene defect in patients with mitochondrial ophthalmoplegia. Brain 2014;137:3200–12.
[11] Mancuso M, Orsucci D, Angelini C, et al. Redefining phenotypes associated with mitochondrial DNA single deletion. J Neurol 2015; 262:1301–9.
[12] Karadimas CL, Vu TH, Holve SA, et al. Navajo neurohepatopathy is caused by a mutation in the MPV17 gene. Am J Hum Genet 2006;79:544–8.