Epilepsy in MT-ATP6 - related mils/NARP: correlation of elettroclinical features with heteroplasmy

Laura Licchetta 1,2, Lorenzo Ferri 2, Chiara La Morgia 1,2, Corrado Zenesini 1, Leonardo Caporali 1,2, Maria Lucia Valentino 1,2, Raffaella Minardi 1, Daniela Fulitano 3, Lidia Di Vito 1, Barbara Mostacci 1, Lara Alvisi 1,2, Patrizia Avoni 1,2, Rocco Liguori 1,2, Paolo Tinuper 1,2, Francesca Bisulli 1,2,a & Valerio Carelli 1,2,a

1IRCCS Istituto delle Scienze Neurologiche di Bologna, Full Member of the ERN EpiCARE, Bologna, Italia
2Department of Biomedical and Neuromotor Sciences, University of Bologna, Bologna, Italia
3Neurology Unit, Rovigo, Italy

Correspondence
Francesca Bisulli, IRCCS Istituto delle Scienze Neurologiche di Bologna, Bologna, Italy, Department of Biomedical and Neuromotor Sciences, University of Bologna, Epilepsy Center - Reference Centre for Rare and Complex Epilepsies (EpiCARE), Bellaria Hospital, Via altura 3 Bologna, Italy 40139. Tel: +39 0514966937; Fax: +39 0514966080; E-mail: francesca.bisulli@unibo.it

Funding Information
The authors thank the “Ricerca Corrente” funding (to L Caporali and V Carelli) from the Italian Ministry of Health for supporting the study.

Introduction
Mutations in mitochondrial ATP6 gene (MT-ATP6) cause a spectrum of progressive neurodegenerative disorders ranging from MILS (maternally inherited Leigh’s syndrome)1 to NARP (neuropathy, ataxia, retinitis pigmentosa).2 Milder manifestations further broaden the clinical spectrum to include oligosymptomatic cases (incomplete NARP). Different phenotypes can occur in the same family.3-4

MT-ATP6 encodes the ATP6 subunit of the F1F0-ATPase (ATP synthase/complex V). The most common mutation is the heteroplasmic m.8993T>G.5,6 The wide variability of disease severity and age at onset has been mainly attributed to the degree of heteroplasmy, the higher the percentage of mutant mtDNA, the more severe the phenotype.1-4,7 Epileptic seizures are reported in up to 86% and 44% of patients with MILS and NARP, respectively.6 Ictal

1. BRIEF COMMUNICATION

Abstract
The study aims to characterize the epilepsy phenotype of maternally inherited Leigh’s syndrome (MILS) and neuropathy, ataxia, retinitis pigmentosa (NARP) due to mutations in the mitochondrial ATP6 gene and to correlate electroclinical features with mutant heteroplasmy load (HL). We investigated 17 individuals with different phenotype, from asymptomatic carriers to MILS: 11 carried the m.8993T>G mutation, 5 the m.8993T>C and one the novel, de novo m.8858G>A mutation. Seizures occurred in 37.5% of patients, EEG abnormalities in 73%. We ranked clinical and EEG abnormalities severity and performed quantitative EEG to estimate Abnormality Ratio (AR) and Spectral Relative Power (SRP). Spearman’s rho and Kruskal–Wallis test were used for correlation with heteroplasmy load (HL). HL correlated with disease severity (Rho = 0.63, P = 0.012) and was significantly higher in patients with seizures or EEG abnormalities (P = 0.014). HL correlated with EEG severity score only for the m.8993T>G (Rho = 0.73, P = 0.040), showing a trend toward a positive correlation with AR and delta SPR, irrespective of the mutation.
manifestations range from intractable generalized and focal seizures in MILS to occasional generalized tonic-clonic seizures (GTCS) in adult onset-NARP. In the latter, epileptiform EEG abnormalities preceding the onset of epilepsy by several years have also been reported, with potential implications in terms of seizure surveillance.

The disease severity of NARP and MILS appears to be related to the occurrence of seizures, the age at seizures onset and the epilepsy phenotype. However, no studies have explored the association between epilepsy and the heteroplasmy load (HL) of mtDNA mutations so far.

We here characterized the epilepsy phenotype of patients with mitochondrial diseases due to MT-ATP6 mutations. The electro-clinical study, extended to asymptomatic carriers, included quantitative EEG (qEEG) spectral analysis in a subgroup. We aimed to provide prevalence esteem of seizures and EEG abnormalities in our case-series and investigate the correlation of HL with electroclinical and qEEG findings.

**Methods**

The study was approved by the Local Ethics Committee (13036).

We investigated patients with MILS or NARP referred to our Institute between 1971 and 2019. All index-cases and available affected/unaffected relatives underwent clinical and neurophysiological assessment including EEG and/or polygraphic recording. Clinical information on unreachable or deceased family members was collected for pedigree reconstruction. We focused on epileptic phenotype, referring to the ILAE guidelines (www.epilepsydiagnosis.org).

Genetic analysis was performed by direct sequencing of MT-ATP6. Since mutant mtDNA shows fairly uniform tissue distribution, HL was quantified in DNA extracted from blood in all index-cases and maternal relatives by SNaPshot assay (detailed in Appendix S1).

All individuals carrying MT-ATP6 mutations were included. Based on clinical and molecular findings we distinguished different affection status, scoring disease severity as follows: 0 = asymptomatic carrier; 1 = incomplete NARP with one symptom of the triad; 2 = incomplete NARP with two symptoms; 3 = NARP; 4 = NARP with encephalopathy and/or transient basal ganglia lesions (labeled "NARP-MILS"); 5 = MILS as defined by bilateral striatal lesions. EEG patterns were categorized according to a 0-5 ranking scale, with 0 = normal trace/non-specific changes and 5 = combination of slow background activity (BA), burst of slow-waves and spike-wave discharges (Appendix S1-S2). Quantitative EEG (qEEG) spectral analysis was performed in all patients who underwent a 19-electrodes-digital registration according to a standardized protocol. Abnormality Ratio (AR) and Spectral Relative Power (SRP) of Delta, Theta, Alpha, and Beta band were obtained for each patient (Appendix S1).

Statistical analysis was performed using statistical package Stata SE 14.2. Spearman’s rank correlation (Rho) was used to evaluate the strength and direction of the relationship between HL and continuous variables, whereas Kruskal–Wallis test to compare the median level (and Interquartile Range, IQR) of HL for dichotomic categories. A sub-group analysis was performed for the m.8993T>G mutation.

**Results**

We studied seven unrelated index-cases with MILS or NARP syndrome (five familial and two isolated) and 10 family members carrying various HL of MT-ATP6 mutations. Of the 17 individuals (M/F:5/12), 15 were affected (four with MILS, six with NARP-MILS, one with NARP, four with incomplete NARP) and two were asymptomatic carriers. Figure 1A shows the pedigrees, three of which previously reported (pedigree 2, 3, 6).

Twelve individuals were directly assessed (mean age at last assessment: 41.3 ± 13.6 years; mean follow-up: 12.2 ± 13.2 years). The remaining five include two children with MILS (pedigree 1/III.1; 6/II.1) deceased at age 3 years following an acute metabolic crisis. All electroclinical and genetic findings are detailed in Table 1.

Genetic analysis highlighted the m.8993T>G mutation in 11 individuals (64.7%), 10 belonging to three families and one sporadic case (pedigrees 1-4). Among them, nine were affected (two had MILS, four NARP-MILS, one NARP and two incomplete NARP; HL between 48%-98%) and two were asymptomatic carriers (HL 44%-74%). Epileptic seizures occurred in all the four patients with NARP-MILS, while EEG abnormalities were detected in six out of the eight individuals with available EEG, including two without seizures (Table 1).

Five affected patients from two families (pedigree 5-6) disclosed the MT-ATP6 m.8993T>C mutation (HL 29%-99%). Of them, two patients with MILS and NARP-MILS had GTCS and brief seizures with impaired awareness, respectively. BA slowing was recorded in two patients (EEG not available in three).

Last, a novel m.8858G>A variant was found de novo in a sporadic case with NARP-MILS (HL 34%, pedigree 7) without seizures neither EEG changes (Table 1).

The HL could not be assessed in the two individuals deceased (1/III.1; 6/II.1). Overall, epileptic seizures occurred in 37.5% of patients with severe phenotype (6/16, information not available in one patient deceased) carrying the m.8993T>G or the m.8993T>C mutation. The mean age at seizure onset was 8.9 ± 9.4 years.
Seizures, associated with psychomotor deterioration and/or alteration of consciousness during hyperthermia, were the presenting manifestation in two individuals. Four patients had rare GTCS alone (2) or associated with myoclonic (1) or atonic seizures (1). One patient had progressive myoclonus epilepsy (PME), one experienced brief seizures with impaired awareness.

EEG and/or polygraphic records were available in 11 patients (four with seizures, seven without). EEG abnormalities were detected in eight individuals (72.7%) with MILS (2), NARP-MILS (4), NARP (1) or incomplete NARP (1). Half of the patients with abnormal EEG had never had seizures after a mean follow-up of 14.5 ± 22.5 years. Two of them showed epileptic discharges, possibly associated with bursts of monomorphic slow waves (Fig 1B-a, b), one had a burst of high-amplitude, delta waves and one slow BA.

Surface EMG documented myoclonic bursts associated with epileptiform discharges in individual 2/II.1 (Fig 1B-c). QEEG analysis was performed in six affected individuals, five carrying the m.8993T>G variant and one the m.8858G>A. Their features are detailed in Appendix S2.
Epilepsy in MILS/NARP due to MT-ATP6 mutations

Table 1. Electroclinical features of the 17 individuals with MT-ATP6 pathogenic variants included in the study

| MTATP6 variant | Phenoype (#) | HL (%) range | Age at onset | Symptom at onset | Other signs/symptoms | Seizure type | EEG/polygraphy | Neuroimaging | ASM |
|----------------|--------------|--------------|--------------|------------------|----------------------|--------------|----------------|--------------|-----|
| 8993T>G | MILS (2) | 90-na | 2 m-na | 1 acute E | 1 cer/pyr, M, ID | 1 none | 1 sW> post, S-W | 1 BG, CA | 1 None |
| 8993T>G | NARP-MILS (4) | 96-98 | birth-1 y | 2 psychomotor delay | 1 na (deceased at 3 y) | 1 na | 1 na | 1 na (deceased) |
| 8993T>G | NARP (1) | 88 | 29 y | 1 RP, A | None | slow BA, sW | 1 WM, CA | None |
| 8993T>G | Incomplete NARP (2) | 48-90 | 17-32 y | 2 RP | 1 N, mig, M, fat; 1 A, mig, M | None | 1 S-W; 1 n/s | 1 normal; 1 WM | 2 None |
| 8993T>G | Carrier (2) | 44-74 | n appl | n appl | 1 n/s | None | 1 normal; 1 na | 1 na | 2 None |
| 8993T>C | MILS (2) | 99-na | 2 m-3 y | 1 psychomotor delay | 1 ID, cer/pyr | 1 none | 1 slow BA | 1 BG | 1 None |
| 8993T>C | NARP-MILS (1) | 97 | 7 y | 1 A | regression, hyp, fat, mig, pyr | UOs | slow BA | BG (t), CA, WM | 1 VPA |
| 8993T>C | Incomplete NARP (2) | 29-95 | na | 2 N | 1 pyr; 1 none | 2 none | 2 na | 2 na | 2 None |
| 8858G>A | NARP-MILS (1) | 34 | 6 y | RP | hyp, fat, M, pyr | none | n/s | WM, CA, BG (t) | None |

A, ataxia; AF, Atrial Fibrillation; As, Atonic seizures; ASM, Anti Seizure Medication; BA, background activity; BDZ, Benzodiazepines; BG, basal ganglia lesions (hypertensive signal on T2-weighted, hypointense on T1-weighted MRI); CA, cerebellar; cerebral atrophy; CBD, cannabidiol; cer: cerebellar signs; E, encephalopathy; ext, extrapyramidal signs; fat, fatigue; GTCS, Generalized Tonic-Clonic Seizures; HL, heteroplasmy load %; hyp, hypoacusia; ID, intellectual disability; LEV, levetiracetam; m, months; M, Myoclonias; mig: migraine; MILS, Maternally inherited Leigh’s syndrome; Ms, Myoclonic seizures; n appl, not applicable; n/s: not specific EEG abnormalities; N, neuropathy; na, not available; NARP, neuropathy, ataxia, retinitis pigmentosa; PB, phenobarbital; PME, Progressive Myoclonic Epilepsy; pyr, pyramidal signs; RP, retinitis pigmentosa; sW, runs of sharply-contoured slow waves; S-W, spike-wave discharges; (t), transient; THC, tetrahydrocannabinol; UOs, unknown onset seizures; VPA, valproate; WM, white matter hyperintensities; y, years.

1de novo, never reported.

Statistical analysis showed a correlation between HL and disease severity (Rho = 0.63, P = 0.012), with a stronger correlation only considering the most frequent mutation, the m.8993T>G (Rho = 0.76, P = 0.011; Table 2). Seizure occurrence and the presence of EEG abnormalities were associated with a significantly higher HL (median 97% versus 81%, P = 0.014; median 95.5% versus 44%, P = 0.014). These findings are confirmed in the m.8993T>G subgroup analysis (Table 3). EEG abnormalities severity score showed a significant correlation with HL for patients carrying the m.8993T>G (Rho = 0.73, P = 0.040), with only a trend considering all the mutations (Rho = 0.52, P = 0.099). Similarly, on qEEG we found a trend towards positive correlations between HL and the AR (Rho = 0.75, P = 0.084). On qEEG spectral analysis, no noteworthy findings emerged, except for a weak, positive correlation between HL with SRP of delta band (Table 4; Appendix S3).

Discussion

This study focused on the epilepsy phenotype of patients with MT-ATP6-related MILS and NARP syndrome and investigated the correlation of mutant HL with seizures, EEG, and qEEG findings.

The prevalence of epileptic seizures in our case series was 37.5%, in line with literature data. Epilepsy occurs in patients with a severe phenotype (MILS or NARP-MILS), most carrying the m.8993T>G change. Similarly, in a large published cohort of patients with different MT-ATP6 mutations, this mutation was associated with a significant higher proportion of seizures and a more severe course.6

Generalized seizures represent the most common seizure type; notably, one patient showed PME. Among mitochondrial disorders, PME is a recognized manifestation of MERRF (myoclonic epilepsy with ragged red
fibres\textsuperscript{13} whereas it is extremely rare in NARP syndrome. In fact, to date only another individual with NARP-MILS carrying the 8993T\textgreater{}G change\textsuperscript{14} has been reported, besides our patient.

Statistical analysis confirmed a correlation between disease severity and HL, especially for the m.8993T\textgreater{}G mutation (\(\rho = 0.76, P = 0.011\)). HL correlated also with seizure occurrence for all mutations (\(P = 0.014\)), thus confirming epilepsy phenotype as an index of disease severity and further highlighting that the proportion of mtDNA mutation in peripheral tissues faithfully reflects the HL distribution in the brain. The best HL threshold for seizure occurrence was set at \(> 95\%\) (sensitivity 100%, specificity 90%; Fig. 2).

In our series, up to 50\% of affected individuals without seizures showed EEG abnormalities (from slow BA to variable combinations of paroxysmal/epileptiform activities). Remarkably, among them even those showing epileptiform discharges have never experienced seizures after a long-term follow-up. This observation does not support the role of EEG abnormalities in predicting seizures, as reported.\textsuperscript{10} HL correlated with the presence of any EEG abnormalities (\(P = 0.014\)) with only a trend towards a correlation with the EEG qualitative severity score and the AR (i.e. the amount of paroxysmal activity including both, delta bursts and epileptiform discharges). Generalized background slowing and bursts of slow paroxysmal activities are common in mitochondrial diseases\textsuperscript{15} but no qEEG studies are available. QEEG studies on neurodegenerative diseases demonstrated a correlation of theta/delta band powers with cognitive deterioration.\textsuperscript{16} Thus, we interpreted the correlation of HL with EEG abnormalities (not necessarily epileptiform) and the weak correlation of HL with the relative delta power at SPR analysis, as expression of the severity of the underlying encephalopathy in patients with higher HL. Thus, as no reliable biomarkers of mitochondrial disease severity are currently available, EEG could represent a useful tool to assess disease severity, especially in patients with the m.T8993G mutation. QEEG studies on larger samples with long follow-up, possibly including other mutational categories and clinical phenotypes, could confirm a role of relative delta power as a biomarker in mitochondrial diseases, useful to evaluate natural history and assess therapeutic efficacy in multicenter clinical trials.

### Acknowledgments

The authors thank the "Ricerca Corrente" funding (to L Caporali and V Carelli) from the Italian Ministry of Health for supporting the study. The authors thank all the patients participating in this study. The authors thank

### Table 2. Correlation between heteroplasmy load (HL, \%), disease severity and EEG abnormalities score in the all variants and in the 8993T\textgreater{}G only

| Heteroplasmy load | Spearman’s Rho | Number | \(P\)-value |
|-------------------|---------------|--------|------------|
| disease severity  | 0.63          | 15     | 0.012      |
| score – all variants | 0.76        | 10     | 0.011      |
| disease severity  | 0.52          | 11     | 0.099      |
| score – only 8993T\textgreater{}G | 0.73      | 8      | 0.040      |
| EEG abnormalities | 0.73          | 8      | 0.040      |
| score – all variants | 0.73      | 8      | 0.040      |
| EEG abnormalities | 0.73          | 8      | 0.040      |
| score – only 8993T\textgreater{}G | 0.73      | 8      | 0.040      |

Statistically significant values (\(P\) values <0.05) in bold.

### Table 3. Comparison of HL median level and Interquartile Range (IQR) between presence (or not) of EEG abnormalities and seizures in the all variants and in the 8993T\textgreater{}G only

| Heteroplasmy load | Median (IQR) | Number | \(P\)-value |
|-------------------|--------------|--------|-------------|
| EEG abnormalities – all variants: | 95.5 (90–97.5) | 8 | 0.014 |
| Yes | 44 (34–48) | 3 | |
| No | 46 (44–48) | 2 | |
| EEG abnormalities – only 8993T\textgreater{}G: | 93 (90–97) | 6 | 0.046 |
| Yes | 46 (44–48) | 2 | |
| No | 46 (44–48) | 2 | |
| Seizures – all variants: | 97 (97–97) | 5 | 0.014 |
| Yes | 81 (44–90) | 10 | |
| No | 81 (48–90) | 6 | |
| Seizures – only 8993T\textgreater{}G: | 97 (96.5–97.5) | 4 | 0.010 |
| Yes | 81 (48–90) | 6 | |
| No | 81 (48–90) | 6 | |

Statistically significant values (\(P\) values <0.05) in bold.

### Table 4. Correlation between heteroplasmy load (%) and abnormalities ratio and sub-band spectral relative power in six patients undergone standardized EEG protocol.

| Heteroplasmy load | Spearman’s Rho | \(P\)-value |
|-------------------|---------------|-------------|
| Abnormalities/background ratio | 0.75 | 0.084 |
| Delta relative | 0.49 | 0.32 |
| Theta relative | 0.46 | 0.35 |
| Alfa relative | 0.41 | 0.42 |
| Beta relative | -0.41 | 0.43 |
| Gamma relative | -0.46 | 0.35 |

\(\text{L. Licchetta et al.}\)
the Neurogenetics Laboratory staff of our Institute, led by Prof Valerio Carelli. The authors thank Dr. Martina Romagnoli and Davide Morosinotto for their help, to the technicians of the Epilepsy Centre of our Institute, and to Stefania Gamberini for her secretarial help.

Conflicts Of Interest

None of the authors has any conflict of interest to disclose.

REFERENCES

1. Tatuch Y, Christodoulou J, Feigenbaum A, et al. Heteroplasmic mtDNA mutation (T-G) at 8993 can cause Leigh disease when the percentage of abnormal mtDNA is high. Am J Hum Genet 1992;50:852–858.
2. Holt IJ, Harding AE, Petty RK, Morgan-Hughes JA. A new mitochondrial disease associated with mitochondrial DNA heteroplasmy. Am J Hum Genet 1990;46:428–433.
3. Mäkelä-Bengs P, Suomalainen A, Majander A, et al. Correlation between the clinical symptoms and the proportion of mitochondrial DNA carrying the 8993 point mutation in the NARP syndrome. Pediatr Res 1995;37:634–639. https://doi.org/10.1203/00006450-19950500-00014.
4. Uziel G, Moroni I, Lamantia E, et al. Mitochondrial disease associated with the T8993G mutation of the mitochondrial ATPase 6 gene: a clinical, biochemical, and molecular study in six families. J Neurol Neurosurg Psychiatry 1997;63:16–22. https://doi.org/10.1136/jnnp.63.1.16.
5. Thorburn DR, Rahman J, Rahman S. Mitochondrial DNA-Associated Leigh Syndrome and NARP. 2003 Oct 30
6. Stendel C, Neuhofer C, Floride E, et al. Delineating MT-ATP6-associated disease: From isolated neuropathy to early onset neurodegeneration. Neurol Genet 2020;6(1):e393. https://doi.org/10.1212/NXG.000000000000393.
7. Carelli V, Baracca A, Barogi S, et al. Biochemical-clinical correlation in patients with different loads of the mitochondrial DNA T8993G mutation. Arch Neurol 2002;59:264–270. https://doi.org/10.1001/archneur.59.2.264.
8. Sofou K, De Coo IF, Isohanni P, et al. A multicenter study on Leigh syndrome: disease course and predictors of survival. Orphanet J Rare Dis 2014;9:52. https://doi.org/10.1186/1750-1172-9-52.
9. Santorelli FM, Shanske S, Macaya A, et al. The mutation at nt 8993 of mitochondrial DNA is a common cause of Leigh’s syndrome. Ann Neurol 1993;34:827–834. https://doi.org/10.1002/ana.410340612.
10. Keränen T, Kuusisto H. NARP syndrome and adult-onset generalised seizures. Epileptic Disord 2006;8:200–203.
11. White SL, Shanske S, McGill JJ, et al. Mitochondrial DNA mutations at nucleotide 8993 show a lack of tissue or age-related variation. J Inherit Metab Dis 1999;22:899–914. https://doi.org/10.1023/a:1005639407166.
12. Sciacco M, Prelle A, D’Adda E, et al. Familial mtDNA T8993C transition causing both the NARP and the MILS phenotype in the same generation. A morphological, genetic and spectroscopic study. J Inherit Metab Dis 1999;22:899–914. https://doi.org/10.1023/a:1005639407166.
13. Shahwan A, Farrell M, Delanty N. Progressive myoclonic epilepsies: a review of genetic and therapeutic aspects. Lancet Neurol 2005;4:239–248. https://doi.org/10.1016/S1474-4422(05)70043-0.
14. Jung J, Mauguière F, Clerc-Renaud P, et al. NARP mitochondrialopathy: an unusual cause of progressive myoclonic epilepsy. Neurology 2007;68:1429–1430. https://doi.org/10.1212/01.wnl.000024019.53959.10.
15. Chevallier JA, Von Allmen GK, Koenig MK. Seizure semiology and EEG findings in mitochondrial diseases. Epilepsia 2014;55:707–712. https://doi.org/10.1111/epi.12570.
16. Caviness JN, Hentz JG, Belden CM, et al. Longitudinal EEG changes correlate with cognitive measure deterioration in Parkinson’s disease. J Parkinsons Dis 2015;5:117–124. https://doi.org/10.3233/JPD-140480.

Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Appendix S1. Supplemental Methods for SNaPshot assay, EEG standardized protocol, EEG qualitative severity score and EEG quantitative Analysis

Appendix S2. Supplemental Table: clinical features of the six patients with EEG quantitative study

Appendix S3. Supplemental Figure: Scalp EEG topographic band maps in three individuals with different heteroplasmy load (%).