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Delineating *MT-ATP6*-associated disease

From isolated neuropathy to early onset neurodegeneration

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Abstract

Objective

To delineate the phenotypic and genotypic spectrum in carriers of mitochondrial *MT-ATP6* mutations in a large international cohort.

Methods

We analyzed in detail the clinical, genetical, and neuroimaging data from 132 mutation carriers from national registries and local databases from Europe, USA, Japan, and China.

Results

We identified 113 clinically affected and 19 asymptomatic individuals with a known pathogenic *MT-ATP6* mutation. The most frequent mutations were m.8993 T > G (53/132, 40%), m.8993 T > C (30/132, 23%), m.9176 T > C (30/132, 23%), and m.9185 T > C (12/132, 9%). The degree of heteroplasmy was high both in affected (mean 95%, range 20%–100%) and unaffected individuals (mean 73%, range 20%–100%). Age at onset ranged from prenatal to the age of 75 years, but almost half of the patients (49/103, 48%) became symptomatic before their first birthday. In 28 deceased patients, the median age of death was 14 months. The most frequent symptoms were ataxia (81%), cognitive dysfunction (49%), neuropathy (48%), seizures (37%), and retinopathy (14%). A diagnosis of Leigh syndrome was made in 55% of patients, whereas the classic syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP) was rare (8%).

Conclusions

In this currently largest series of patients with mitochondrial *MT-ATP6* mutations, the phenotypic spectrum ranged from asymptomatic to early onset multisystemic neurodegeneration. The degree of mutation heteroplasmy did not reliably predict disease severity. Leigh syndrome was found in more than half of the patients, whereas classic NARP syndrome was rare. Oligosymptomatic presentations were rather frequent in adult-onset patients, indicating the need to include *MT-ATP6* mutations in the differential diagnosis of both ataxias and neuropathies.

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ATP6 Study Group coinvestigators are listed in the appendix 2 at the end of the article.

Glossary

LS = Leigh syndrome; **mtDNA** = mitochondrial DNA; **NARP** = neuropathy, ataxia, and retinitis pigmentosa; **NGS** = next-generation sequencing; **SARA** = scale for assessment and rating of ataxia.

The *MT-ATP6* gene of the mitochondrial DNA (mtDNA) (further named *ATP6*) encodes a subunit of the F_1F_0ATP-synthase complex, a key enzyme of mitochondrial energy metabolism.

Variants in *ATP6* have long been recognized as a cause of mitochondrial disease.[1] Since the first description of a pathogenic m.8993 T > G variant in 1992,[1] more than 500 cases of *ATP6*-associated disease have been reported.

The initially described phenotype is today referred to as the syndrome of neuropathy, ataxia, and retinitis pigmentosa (NARP). A more severe form of ATP6-associated disorders, traditionally associated with high degrees of mutation heteroplasmy,[2,3] is maternally inherited Leigh syndrome (LS), with early onset psychomotor developmental delay and symmetrical lesions in basal ganglia and brainstem, often leading to premature death.[4] With the advent of next-generation sequencing (NGS), less typical manifestations have been observed, e.g., nonsyndromic sensorimotor neuropathy;[5] any combinations of ataxia, neuropathy, diabetes mellitus, and hypergonadotropic hypogonadism;[6] and mitochondrial myopathy, lactic acidosis, and sideroblastic anemia.[7] Hence, the phenotypic spectrum of *ATP6*-associated mitochondrial disorders seems much broader than previously assumed. So far, no systematic study on the clinicogenetic spectrum of an extended cohort has been performed.

We report a cohort of 132 *ATP6* mutation carriers from 11 countries, delineate phenotype and genotype, and evaluate the role of heteroplasmy as a disease modifier.

Methods

Probands and design

This is a multicenter, retrospective, cross-sectional cohort study. Probands with known pathogenic *ATP6* variants were identified from the registry of the German network for mitochondrial disorders (mitoNET, n = 22) and 33 further diagnostic and research centers from Europe (n = 55), the USA (n = 6), China (n = 37) and Japan (n = 18).

For data collection, patients' physicians were asked to complete a questionnaire which includes signs, symptoms, and laboratory parameters. Detailed clinical histories of individual patients are provided in table e-1, links.lww.com/NXG/A213. Examinations and diagnostic procedures were performed and evaluated by experienced medical professionals (neurologists, pediatricians, child neurologists, pathologists, radiologists, and clinical geneticists).

Review of the literature

A systematic review of the literature was conducted by searching PubMed for all articles published from 1990 with the terms "ATP6" and "ATPase6."

Variants classified as confirmed disease-associated and reported mutations in MITOMAP (mitomap.org) were included in the analysis, for a total of 502. We reviewed the reported variants for the range of heteroplasmy in affected individuals and unaffected carriers, age at onset and phenotypic association (table e-2, links.lww.com/NXG/A214).

Molecular genetics

Individuals were screened for *ATP6* mutations by using different approaches depending on the local facilities and year of analysis, including targeted single-gene sequencing, high-throughput panel sequencing, or whole-exome sequencing.

Levels of heteroplasmy in patients were assessed in leukocytes (n = 114), skeletal muscle (n = 20), urine (n = 23), and fibroblasts (n = 5).

Statistical analysis

Datasets were collected and organized in Microsoft Excel and LibreOffice Calc and subjected to a basic descriptive analysis. Deeper descriptive and explorative data analysis as well as statistical testing was then performed in R (version 3.5.1) and SPSS (version number 25). A group analysis of parametric data was performed by one-way analysis of variance (ANOVA) for comparison of means or by Welch test when Levene test for homogeneity of variances failed. A post hoc group-wise analysis was performed with Tukey Honestly Significant Difference test. Although ANOVA is considered comparably robust to the violation of the assumption of normality, we additionally performed Kruskal-Wallis test with a post hoc group-wise analysis with Wilcoxon rank sum test as a nonparametric alternative when Shapiro-Wilk test for normal distribution of residues failed. Analysis of nominal and ordinal data was performed with χ^2 test where applicable, and Fishers exact test was used where the sample size did not permit for evaluation with χ^2 test. The significance level was corrected with the Bonferroni method for multiple comparisons with $p = 0.00094$.

Standard protocol approvals, registrations, and patients consents

All biological materials (tissue samples including blood, urine, skin, muscle, and nerve and derivatives including DNA extracted from tissues and cell lines), brain MRI scans, and medical and neurophysiologic reports were obtained with written informed consent of the probands, their parents, or legal guardians. The local institutional review boards approved the study.

Data availability

Anonymized data presented in this study will be made available on request by a qualified investigator. Requests should be made to Thomas Klopstock.

Results

Our cohort of 132 mutation carriers consisted of 113 patients and 19 asymptomatic relatives. Twenty-three cases have been previously published.[5,8–13] Because not every clinical feature was assessed in every patient, we provide the number of patients checked for a specific trait in the denominator and the number of patients positive for this feature in the numerator. Of all patients, 55% (62/112) had classic LS, 18% (20/112) a Leigh-like phenotype, 8% (9/112) NARP syndrome, and 19% (21/112) other phenotypes, e.g., multisystemic ataxia, polyneuropathy, or cardiomyopathy (table e-1, links.lww.com/NXG/A213).

Mutational spectrum

We identified 8 previously reported pathogenic *ATP6* variants and 1 variant in the overlapping region of the *ATP6* and *ATP8* gene. Recurrent known pathogenic mutations found in our cohort were m.8993 T > G (53/132 40%), m.8993 T > C (30/132, 23%), m.9176 T > C (30/132, 23%), m.9185 T > C (12/132, 9%), and m.9035 T > C (3/132, 2%). Single patients (0.7%) carried the mutations m.8528 T > C, 8611_8612insC, 8969 G > A, and m.9134 A > G.

Degrees of heteroplasmy

In 105 patients and 19 asymptomatic mutation carriers, degrees of heteroplasmy were determined in at least one of 5 tissues (leukocytes, skeletal muscle, fibroblasts, urothelium, or buccal swabs) (table e-1, links.lww.com/NXG/A213). In 21 probands, more than 1 tissue was tested and there was a high correlation of heteroplasmy degrees between tissues (table e-1). Degrees of heteroplasmy ranged from 20% to 100% in patients with a median of 100% and average of 95% (figure 1A). In 88 of 105 affected individuals (84%), the mutation was detected with a high degree of heteroplasmy (≥ 90% in at least one tissue), in 73 patients (70%) even homoplasmic. In the group of asymptomatic carriers, heteroplasmy ranged from 20% to 100% with a median of 77% and average of 73%. High degrees of heteroplasmy (≥90% in at least one tissue) were found in 37% (7/19) of unaffected carriers, 4 being homoplasmic for a known pathogenic mutation (m.8993 T > C, n = 1; and m.9176 T > C, n = 3). Of note, 5 of 70 patients with LS (7%) showed a degree of heteroplasmy below 90%. Patient 54-1 with a Leigh phenotype harbored the variant m.8993 T > C even only at 67.8% heteroplasmy in leukocytes and 64.7% in urine cells (figure 1B).

Age at onset and time to diagnosis

Age at onset ranged from the prenatal period (e.g., microcephaly or cardiomyopathy in fetal ultrasound imaging) to the age of 75 years. Distribution by age categories is shown in figure 2A. Among the 4 most frequent genotypes in our cohort (m.8993 T > G, m.8993 T > C, m.9176 T > C, and 9185 T > C), the m.8993 T > G mutation had a significantly earlier onset than the other genotypes ($p = 0.0003$) (figure 2B).

At the time of writing, 28% (28/99) of the patients were deceased with a median age of death of 12 months (range: 5 months to 68 years, mean 4.7 years). All deceased patients had become symptomatic during infancy or childhood, and more than half of them died within the first 2 years of life (57%, 16/28). All except one suffered from Leigh or Leigh-like syndrome (96%, 27/28), and half of them harbored the m.8993 T > G mutation (14/28).

We investigated the lag time between onset of symptoms and molecular diagnosis in 3 subgroups: (1) age at onset in infancy (47/ 101), (2) in early childhood (age 1–6; 28/ 101), and (3) above the age of 6 years (26/ 101). The mean delay in diagnosis was 1.79 years (range 0–19; SD 3.31; median: 0.58) in group 1, 5.39 years (range: 0–30; SD 8.33; median: 1) in group 2, and 12.2 years (range: 0–60; SD 14.6; median: 7) in group 3, implying a correlation of time to diagnosis with the age at onset ($p = 0.00085$). This was further specified when comparing subgroups individually, with statistical significance reached for comparison of groups 1 and 3 ($p = 0.00019$), and a tendency showing when comparing groups 2 and 3 ($p = 0.038$), indicating that later onset of symptoms may lead to a longer delay until molecular diagnosis (figure 2C).

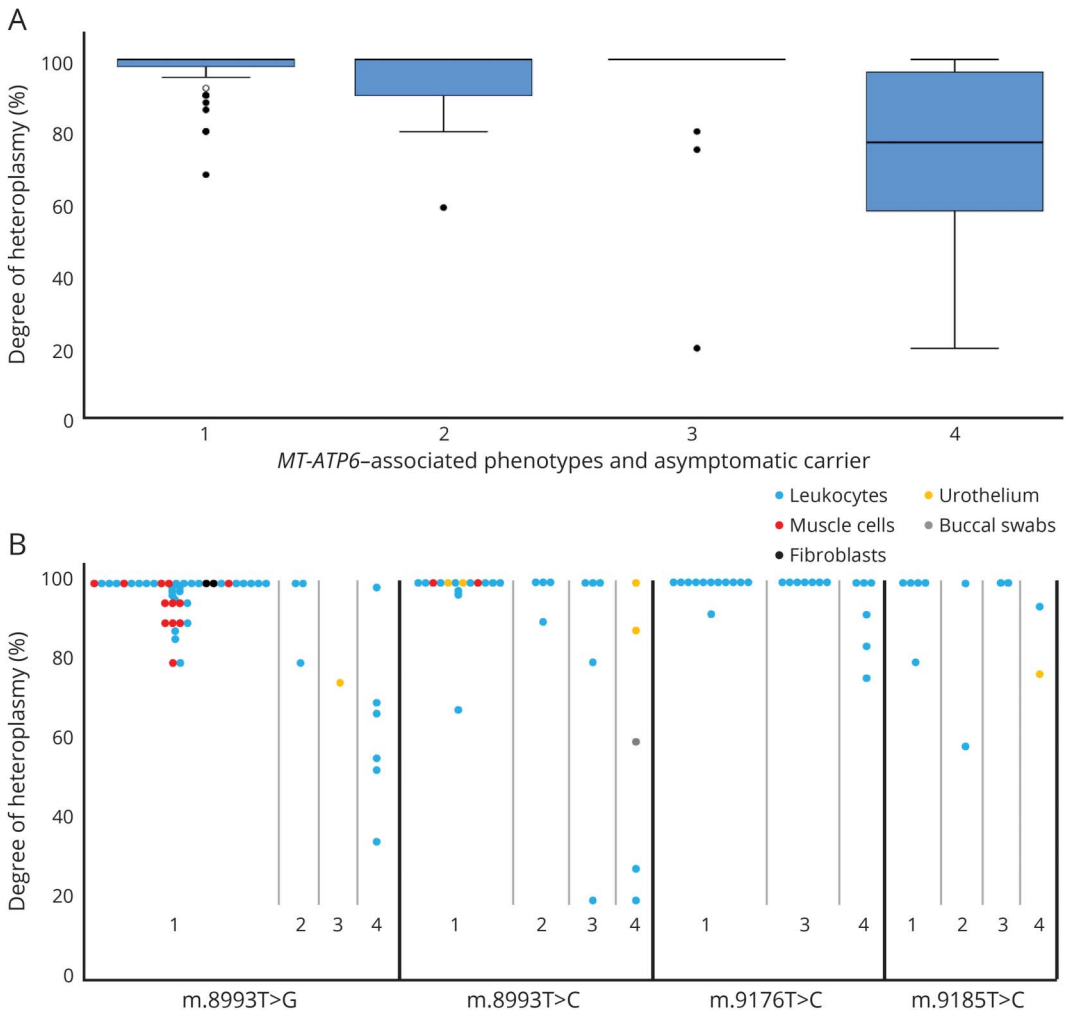
Phenotypic spectrum

Clinical findings in our cohort are summarized in figure 3 and table e-1, links.lww.com/NXG/A213.

LS was the most frequent phenotype (62/112; 55%). In this subgroup, nearly half of the patients (30/62; 48%) harbored the m.8993 T > G variant, followed by m.9176 T > C (14/62; 23%), m.8993 T > C (12/62; 19%), and m.9185 T > C (5/62; 8%) and 1 patient with the rare variant m.8969 G > A. Most of the patients with LS became symptomatic in infancy and early childhood (55/ 60; 92%); oldest age at onset was 16 years (patient 29-1). In addition, 20 children showed clinical symptoms compatible with the diagnosis of LS, but brain MRI was either not available (11/ 20; 55%) or MRI findings were distinct from classic LS, showing no basal ganglia or brainstem lesions but delayed myelination (3/20; 15%), cerebral atrophy/microcephaly (3/20; 15%), or no pathology at all (3/20; 15%). Only 9 patients in our cohort exhibited a NARP phenotype (8%), 6 of those with additional signs (e.g., cognitive dysfunction, global developmental delay, and seizures). In 6/9 patients (67%), the degree of heteroplasmy was 100%, whereas it was lower in the other 3 (90%, 80%, and 59%, respectively).

Most patients evaluated for cerebellar symptoms showed cerebellar ataxia (68/84; 81%), 26% of those in combination with a sensory ataxia (18/68; 26%). One patient showed signs of sensory ataxia without having additional cerebellar signs. Symptoms in 16% of adult patients (18/112) were rated using

Figure 1 Distribution of the degrees of heteroplasmy



(A) Distribution of the degrees of heteroplasmy in different *MT-ATP6*-associated phenotypes and asymptomatic carriers. 1 = Leigh/Leigh-like syndrome; 2 = NARP syndrome; 3 = other phenotypes; 4 = asymptomatic carrier. (B) Heteroplasmy grades in every single patients. Each circle represents an individual with a *MT-ATP6* mutation. The different tissues used for heteroplasmy assessment are represented by different colors. In probands, where more than one tissue was evaluated for heteroplasmy (n = 21), the highest value was selected. All values in all available tissues are shown in table e-1, links.lww.com/NXG/A213. 1 = Leigh/Leigh-like syndrome; 2 = NARP syndrome; 3 = other phenotypes; 4 = asymptomatic carrier. NARP = neuropathy, ataxia and retinitis pigmentosa.

a scale for assessment and rating of ataxia (SARA). The median score was 8.5/40 (mean 10/40, range 1–40/40).

Peripheral neuropathy was suspected in 36 of 40 (90%) patients assessed for clinical symptoms and signs of peripheral nerve dysfunction; in 19 patients (19/40, 48%), nerve conduction studies and EMG confirmed the diagnosis. One patient without clinically apparent neuropathy showed neurophysiologic findings of a peripheral neuropathy. Neuropathy was predominantly axonal and sensory in 10 patients, whereas in the other 9 patients, detailed electrophysiologic data were not available.

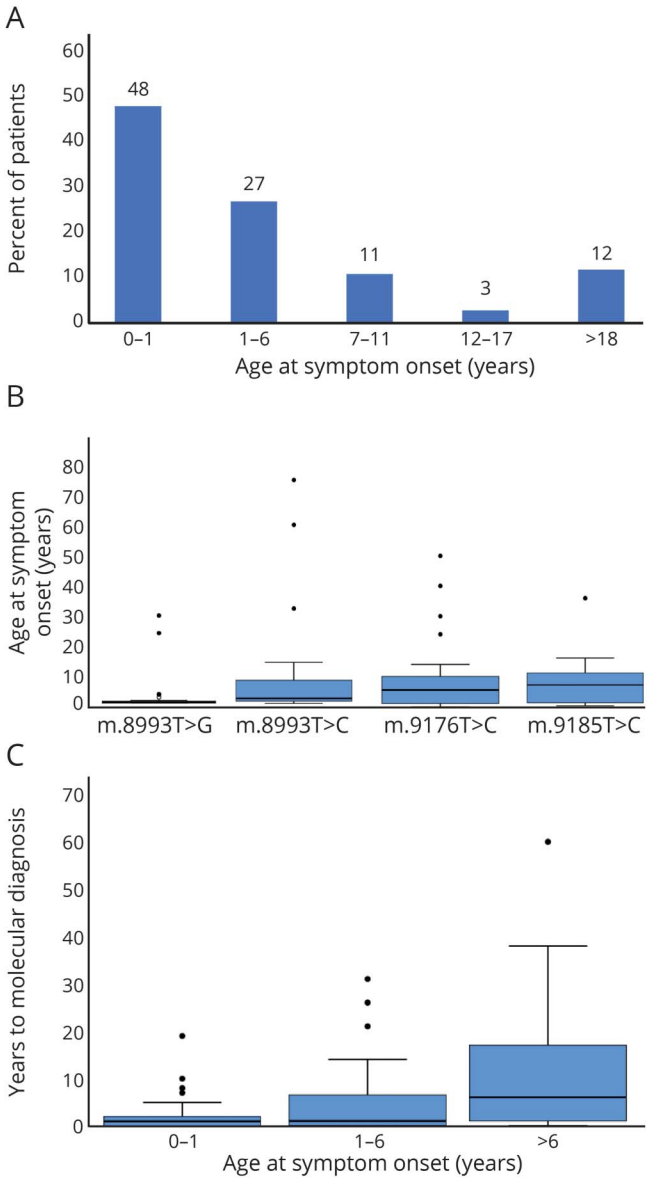
Any form of cognitive dysfunction was reported in 49% (43/87) of the patients, ranging from mild learning disabilities to severe mental retardation or secondary cognitive decline.

Epileptic seizures were reported in 37% (35/95) of patients, mostly in Leigh or Leigh-like syndrome (30/35, 86%) but also in NARP syndrome (4/9, 44%).

Overall, 14 of 70 patients (20%) with an ophthalmologic assessment showed (nonmuscular and noncerebellar) ocular symptoms. Ten patients (10/70, 14%) had a retinopathy, mostly (9/10, 90%) in the context of NARP syndrome. Other ocular symptoms included optic atrophy in 4/70 (6%) and cataract in 3/70 (4%) patients.

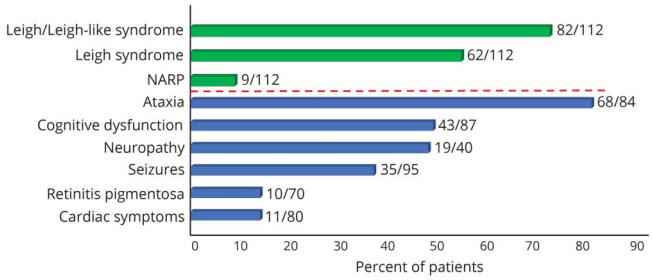
Cardiac symptoms were reported in 11/80 patients (14%). Three had cardiac arrhythmia, which manifested as Wolff-Parkinson-White syndrome in 2 (carrying the mutations m.9185 T > C and m.8993 T > C). Six patients had cardiomyopathy.

Figure 2 Distribution of age at onset and time diagnosis



(A) Age at symptom onset (years). (B) Distribution of age at symptom onset grouped by mutation. (C) Lag time between age at symptom onset and age at molecular diagnosis in 3 different age groups in years (0–1 1–6, >6).

Figure 3 Distribution and frequency of phenotypes and symptoms in our cohort



Bars indicate the percentage of patients with a given phenotype (green bars) or symptom (blue bars) of the total assessed for this phenotype or symptom.

One patient (85-1) carrying the m.9176 T > C variant had a chronic progressive external ophthalmoplegia as a first sign, which is unusual in reported *ATP6* patients.

We examined correlation of distinct genotypes with the occurrence of specific phenotypes or symptoms. A Leigh/Leigh-like syndrome (43/47; 91%) was more common in patients harboring the m.8993 T > G variant (22/39; 56%) than in patients carrying other variants. No other pathogenic variant appeared to be predictive of distinct symptoms or signs (table 1). There was, however, a tendency toward a higher proportion of seizures in patients with the m.8993 T > G variant (22/39 patients, 56%) as compared with patients with other variants (12/51 patients, 24%) (p = 0.0021) and a trend toward lower proportion of cognitive dysfunction in patients with the m.9176 T > C variant (4/21 patients, 19%) as compared with patients with other variants (34/59 patients, 58%) (p = 0.0044).

Twelve of our patients manifested in adulthood were monosymptomatic or oligosymptomatic and had a relatively mild disease course. One patient showed isolated sensorimotor neuropathy (patient 2-3) and another had pure cerebellar ataxia (patient 96-1). Six patients exhibited signs of neuropathy plus cerebellar ataxia, spasticity, or cognitive dysfunction. Two adult patients presented with a NARP phenotype (patients 25-1 and 28-1).

Neuroimaging data

Brain MRI findings in *ATP6* patients are presented in figure 4, A–F. Brain MRI data were available for 85 patients, which showed abnormalities in 81 (95%) patients. Basal ganglia and brain stem lesions, usually found in classic LS, were reported in 65% (55/85) and 32% (27/85) of the patients, respectively, with 22% (19/85) patients showing both alterations. Cerebellar atrophy was present in 16% (14/85), and cortical/subcortical atrophy was identified in 13% (11/85). Other less frequent signs were white matter lesions in 5 patients, delayed myelination in 4 patients, and microcephaly in 2 patients. Brain MRI was reported normal in 4 patients.

In 20 cases, muscle biopsy specimens were studied histologically, and evaluation was either normal (9/20 cases) or disclosed unspecific changes (11/20 cases). Ragged red fibers were found only in 1 adult patient carrying a m.9035 T > C mutation who presented with ataxia in his late 50s and displayed no clinical signs of myopathy.

Discussion

Our study presenting the currently largest series of patients with mitochondrial disease has 2 major results: First, it broadens the clinical spectrum of *ATP6*-associated mitochondrial disorders

Table 1 Comparison of the frequency of phenotypes and symptoms among carriers of different *MT-ATP6* mutations

Variant	Leigh/Leigh-like syndrome	LS	Ataxia	Cognitive dysfunction	Neuropathy	Seizures	Retinitis pigmentosa	Cardiac symptoms
m.8993T > G vs m.8993T > C, m.9176T > C, m.9185T > C	0.0005[a]	0.4274[a]	0.7711[a]	0.022[a]	0.0019[a]	0.0021[a]	1.0[b]	0.4732[b]
m.8993T > C vs m.8993T > G, m.9176T > C, m.9185T > C	0.1126[a]	0.3492[a]	0.3329[b]	0.7931[a]	0.2742[b]	0.8011[a]	0.4375[b]	0.6771[b]
m.9176T > C vs m.8993T > G, m.8993T > C, m.9185T > C	0.1126[a]	0.8146[a]	0.1025[a]	0.0044[a]	0.6618[b]	0.0191[a]	0.1868[a]	0.6771[b]
m.9185T > C vs m.8993T > G, m.8993T > C, m.9176T > C	0.2593[b]	0.7370[b]	0.1921[2]	1.0[b]	0.0473[b]	0.3084[b]	0.6255[b]	0.2878[b]

Abbreviation: LS = Leigh syndrome.
p values of χ^2 Pearson or Fishers exact are shown. Significance level was defined as $p < 0.0009$ after Bonferroni correction for multiple testing.
[a] Chi square Pearson (2-sided *p* values).
[b] Fishers Exact (2-sided *p* values).

and shows that the clinical spectrum is more heterogeneous than previously reported. Second, it shows that the degree of mutation heteroplasmy does not strictly correlate with disease severity, with homoplasmic mutations found not only in LS but also in milder cases and even asymptomatic probands.

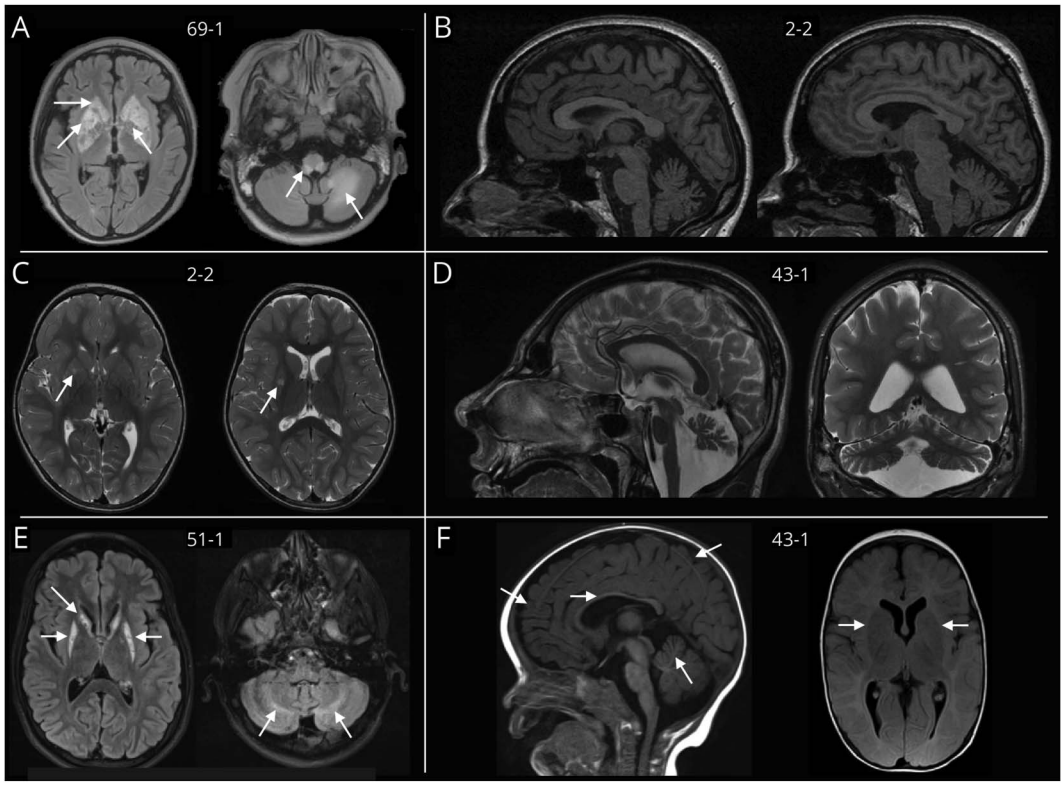
The phenotypic spectrum in our cohort ranged from asymptomatic mutation carriers to fatal, early onset, and multisystemic neurodegenerative disease with severe disability. Most affected children presented with an overall homogeneous Leigh or Leigh-like syndrome phenotype (73%; 82/112) with developmental delay and hypotonia as first signs. One exception is patient 29-1 who showed first symptoms at the age of 16 years with acute brainstem dysfunction with dysarthria, dysphagia, tetraparesis, and ophthalmoplegia, followed by respiratory insufficiency and coma. Brain MRI revealed extensive lesions in the basal ganglia, brainstem, and cerebellum. Patient 85-1 showed isolated chronic progressive external ophthalmoplegia at the age of 10 years, followed by respiratory and cardiac failure. Brain MRI revealed Leigh-like abnormalities in the dorsal midbrain and hypothalamus. About half of the deceased patients in our cohort, mostly severely affected children, carried the mutation m.8993 T > G. Together with the higher proportion of seizures in the m.8993 T > G group, this implies that the m.8993 T > G variant might be associated with a more severe course of disease.[14]

Only 9 patients in our cohort (8%) presented with a NARP phenotype, and 6 of those showed severe additional signs and symptoms such as epilepsy, cognitive dysfunction, delayed myelination, and Leigh-like changes. This low frequency of the NARP phenotype was unexpected because NARP has been regarded as a frequent *ATP6*-associated disorder.[15] All 6 of our patients with NARP with additional features (patient 4-1, 5-1, 5-2, 8-1, 28-1, and 43-1) showed some overlap to a Leigh-syndrome phenotype as they exhibited a global developmental delay, mental retardation, and/or seizures. Patients 8-1 and 28-1 displayed, besides a severe cognitive impairment (mental retardation), Leigh-like alterations in brain MRI scans.

On the mild end of the spectrum, 13% (15/112) of our patients exhibited a monosymptomatic or oligosymptomatic disease (mainly cerebellar ataxia and neuropathy), including all patients with adult onset (12/103). Early manifestation (<1 year) was observed in almost half of the patients (48%, 49/103). However, adult onset was observed in 12% (12/103), stressing that *ATP6*-associated mitochondrial disorder is not restricted to infancy or childhood-onset. Early onset was observed mostly in patients with a severe course of disease (Leigh or Leigh-like syndrome). Later onset showed a significant correlation with a larger delay until the molecular diagnosis was established. One obvious explanation for this finding is that there is a clear need for a fast and broad diagnostic workup if the disease starts early in life and takes a life-threatening course such as in children with LS, whereas staged diagnostic approaches for milder presentations such as neuropathy and ataxia rarely include *ATP6* diagnostics. To avoid this unnecessary delay in diagnosis, *ATP6*-associated mitochondrial disorders should be considered in monosymptomatic or oligosymptomatic presentations that have not previously been considered typical for *ATP6*-associated disease, such as isolated neuropathy or ataxia. The *ATP6* gene should be included in routine NGS panel diagnostics for the differential diagnosis of both ataxias and neuropathies and comprehensive genetic testing via whole-exome sequencing should cover mtDNA in addition to nuclear DNA in a clinical diagnostic setting.[16]

Clinical variability between patients with the same genotype was high in our cohort, not only interfamilial but also intrafamilial. Of note, the monozygotic twin sisters 3-1 and 3-2, both harboring the homoplasmic m.8993 T > C mutation show very different disease courses. Patient 3-1 showed a severe gait ataxia because of a combination of sensorimotor polyneuropathy and cerebellar ataxia starting at age 2 years and has been has been using a wheelchair from the age of 16 years (SARA score 18.5/40), whereas her sister was only slightly affected with mild gait ataxia from the age 7 of years. She was able to walk unaided at age 16 (SARA score 3/40).

Figure 4 Examples of brain MRI changes detected in patients with *MT-ATP6*-associated disease



Number of the patient is given in the middle of the images. (A) FLAIR sequence of axial view showing symmetrical bilateral hyperintensities and partly cystic degeneration in the caudate nuclei and putamen (arrows on the left image), as well as bilateral hyperintensities in the medulla oblongata and hyperintensities on the left cerebellar hemisphere (arrows on the right image). (B) Sagittal view of T1 sequence shows a pronounced fronto-parietal and cerebellar atrophy. (C) Axial view of T2 sequence shows subtle unilateral hyperintensities in the left putamen (arrow on left and right image). (D) T2 sequence of sagittal view reveals a microcephaly and hypoplasia of the brainstem and cerebellum. (E) axial view of FLAIR sequence shows bilateral putaminal and caudate head signal alterations, atrophy and cystic changes (arrows on left image). Axial view of T2 sequence reveals a slight cerebellar atrophy and bilateral T2 hyperintensities within the cerebellar peduncles (right image). (F) T1 sequence of sagittal view shows cerebellar and cerebral atrophy and thinning of the corpus callosum (arrows on the left image), whereas basal ganglia structure appeared normal (arrows on right side). FLAIR = fluid-attenuated inversion recovery.

A generally accepted preconception about mitochondrial disease because of mtDNA variants is a direct correlation of symptom severity to degree of heteroplasmy. It is widely believed that a percentage of heteroplasmy >90% for the m.8993 T > C/G variant leads to LS, whereas a heteroplasmy load between 70% and 90% results in a NARP phenotype.[17,18] This idea has recently been challenged.[19] Our findings of a high grade of heteroplasmy in the asymptomatic carrier group (7/17 ≥ 90%, 4/17 homoplasmic for known pathogenic mutations) and the observation of moderate degrees of heteroplasmy in several early onset symptomatic patients support the view that heteroplasmy load alone is insufficient to explain disease severity. For instance, patient 54-1 presenting with a LS showed onset in infancy and harbored the m.8993 T > C variant at moderate degrees of heteroplasmy (68% in leukocytes and 65% in urine cells). By contrast, the asymptomatic mother (proband 59-2) of a patient with LS harbored the same variant even at higher degrees of heteroplasmy (78% leukocytes and 88% urine cells). In 3 asymptomatic carriers harboring the m.8993 T > G (proband 49-1) and the m.9176 T > C (proband 69-3, 69-4) variants, the high degrees of heteroplasmy have to be interpreted with caution because they are children and might still develop symptoms later in life.

Degrees of heteroplasmy in our multicentric study were determined in different tissues, at different ages, and with different methods, warranting careful interpretation. However, age differences in measurements of heteroplasmy may have a negligible impact at least for the m.8993 variant because it has been shown that heteroplasmy of m.8993 T > G/C does not change significantly over time.[20] Moreover, we found a very high correlation of degrees of heteroplasmy between different tissues in 21 probands (table e-1, links.lww.com/NXG/A213).

Further modifying factors determining disease manifestation may include the mtDNA background, which has been shown to play an important role in modulating the biochemical defects and clinical outcome.[21] For example, the variants m.8741 T > G and m.8795 A > G have been reported to be

protective factors for the m.8993 T > G mutation causing a LS.[22] mtDNA copy number may be another modifying factor of disease severity because it has recently been shown for the m.3243 A > G mutation,[23] but so far, there are no data on this in *ATP6*-associated disorders. Furthermore, *ATP6* variants may not be fully penetrant, even when homoplasmic.[8]

In essence, our study demonstrates that *ATP6* variants cause a continuous disease spectrum rather than a group of distinct clinical syndromes. Early onset was associated with a more severe course of disease, especially in patients carrying the m.8993 T > G variant. Onset after the age of 6 years leads to a considerable delay in diagnosis, most likely because of monosymptomatic and oligosymptomatic presentations, especially in the adult group. Including *ATP6* mutation screening in routine diagnostics for neuropathy and ataxia will benefit patients regarding a prompter and correct diagnosis and genetic counseling.

Our observations strongly support the notion that the degree of heteroplasmy alone cannot reliably predict disease severity. Further studies are needed to identify factors that modulate prognosis.

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Disclosure

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Appendix 1 *(continued)*

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Continued

Appendix 2 *(continued)*

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Appendix 2 *(continued)*

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