Progressive myoclonic epilepsy as an adult-onset manifestation of Leigh syndrome due to m.14487T>C

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ABSTRACT

Background: m.14487T>C, a missense mutation (p.M63V) affecting the ND6 subunit of complex I of the mitochondrial respiratory chain, has been reported in isolated childhood cases with Leigh syndrome (LS) and progressive dystonia. Adult-onset phenotypes have not been reported.

Objectives: To determine the clinical–neurological spectrum and associated mutation loads in an extended m.14487T>C family.

Methods: A genotype–phenotype correlation study of a Belgian five-generation family with 12 affected family members segregating m.14487T>C was carried out. Clinical and mutation load data were available for nine family members. Biochemical analysis of the respiratory chain was performed in three muscle biopsies.

Results: Heteroplasmic m.14487T>C levels (36–52% in leucocytes, 97–99% in muscle) were found in patients with progressive myoclonic epilepsy (PME) and dystonia or progressive hypokinetic-rigid syndrome. Patients with infantile LS were homoplasmic (99–100% in leucocytes, 100% in muscle). We found lower mutation loads (between 8 and 35% in blood) in adult patients with clinical features including migraine with aura, Leber hereditary optic neuropathy, sensorineural hearing loss and diabetes mellitus type 2. Despite homoplasmic mutation loads, complex I catalytic activity was only moderately decreased in muscle tissue.

Interpretation: m.14487T>C resulted in a broad spectrum of phenotypes in our family. Depending on the mutation load, it caused severe encephalopathies ranging from infantile LS to adult-onset PME with dystonia. This is the first report of PME as an important neurological manifestation of an isolated mitochondrial complex I defect.

Mitochondrial disorders of oxidative phosphorylation (OXPHOS disorders) affect ~1/5000 individuals in the general population[1 2] and can present with a surprisingly wide range of multisystemic and neuromuscular phenotypes.[3]

m.14487T>C is a known pathogenic mtDNA mutation[4] resulting in an amino acid substitution (p.M63V) in NADH dehydrogenase 6 (ND6), a complex I subunit of the mitochondrial respiratory chain. Thus far, the m.14487T>C mutation has been found in isolated cases with infantile Leigh syndrome (LS) (onset ages between 5 months and 4 years) and progressive dystonia (onset ages between 4 and 6 years).[5–10] A milder phenotype was reported in a 16-year-old patient with cerebellar ataxia and optic atrophy.[7] Adult and late-onset phenotypes have not been reported, and it is not known to what extent m.14487T>C mutation loads correlate with phenotypic severity and complex I activity.

PATIENTS AND METHODS

Family description

The Belgian family comprises a total of 12 family members with clinical features suggestive of a mitochondrial disease (fig 1A). For patients II-3, III-2, III-3, III-5, III-6 and IV-1, limited clinical information could be obtained from hetero-anamnesis. More detailed clinical information was available for five probands (II-1, IV-3, IV-4, IV-5 and V-1). Blood samples were available for nine individuals (II-3, III-4, III-6, IV-2, IV-3, IV-4, IV-5, V-1 and V-2).

Biochemical and histopathological analysis

Muscle biopsies of patients IV-3, IV-5 and V-1 were performed for routine histochemical, spectrophotometrical and blue native–polyacrylamide gel electrophoresis (BN-PAGE) with in-gel activity staining as described previously.[11]

DNA analysis

Total DNA was prepared from leucocytes (individuals II-3, III-4, III-6, IV-2, IV-3, IV-4, IV-5, V-1), muscle tissue (individuals IV-3 and IV-5) and cultured skin fibroblasts (IV-4) following standard procedures. Southern blotting was used to investigate the presence of deletions or duplications in the mtDNA. All mtDNA-encoded complex I genes were investigated for nucleotide alterations using polymerase chain reaction (PCR) denaturing high-performance liquid chromatography (dHPLC) analysis (WAVE, Transgenomic) and subsequent direct sequencing (Big-Dye Terminator version1.1 Cycle Sequencing kit on the ABI3130xl genetic analyser; Applied Biosystems, Lennik, Belgium). To determine heteroplasmy levels last, hot cycle PCR–restriction fragment length polymorphism was performed as previously described.[12]

RESULTS

Clinical–genetic features of the m.14487T>C family

The inheritance pattern was consistent with maternal transmission (fig 1). m.14487T>C (p.M63V in the ND6 subunit of complex I) was found in seven affected and two currently unaffected family

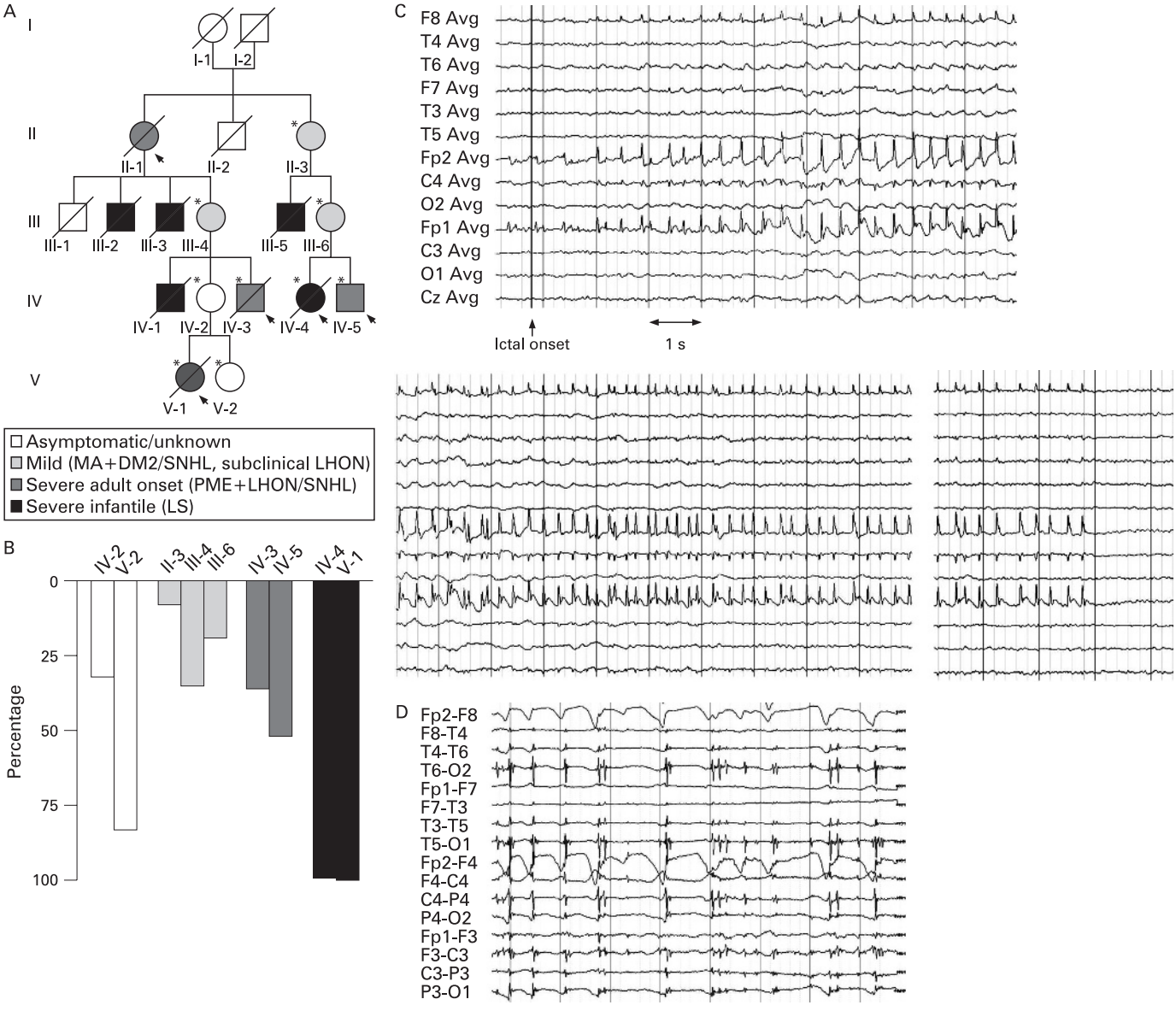

Figure 1 (A) Abbreviated pedigree structure of the Belgian family showing maternal transmission of the m.14487T>C complex I mutation. Squares: male subjects; circles: female subjects; filled symbols: subjects with clinical features suggestive of mitochondrial disease; slashed symbols: deceased subjects; arrows: probands. The asterisks indicate the presence of m.14487T>C. Grey-scale coding corresponds to four categories with increasing phenotypic severity. Question marks indicate lack of clinical information but that the presence of severe neurological disease is likely. (B) m.14487T>C heteroplasmy levels (%) in leucocytes of nine mutation carriers grouped and ordered in four categories with increasing phenotypic severity from left to right. Grey-scale coding and patient numbering are identical to those in fig 1A. (C) Ictal EEG of individual IV-3. High-voltage spikes were recruited mainly at the frontopolar electrodes lasting 22 s. Facial twitches coincided with the spikes. Although the wave configuration looked epileptical, we cannot fully exclude movement artefacts. Time base: 30 mm/s; sensitivity (of original recording): 150 μV/cm; high cut: 15.0 Hz; low cut: 0.53 Hz. Referential montage. (D) EEG of individual IV-5 during myoclonic status epilepticus. There were multifocal spikes and polyspikes on a slow background, which were often, but not always, associated with myoclonic jerks. Time base: 30 mm/s; sensitivity (of original recording): 100 μV/cm; high cut: 30 Hz; low cut: 0.5 Hz. Midtemporal referential montage. DM2, diabetes mellitus type 2; LHON, leber hereditary optic neuropathy; LS, Leigh Syndrome; MA, migraine with aura; PME, progressive myoclonic epilepsy; SNHL, sensorineural hearing loss.

members (fig 1A). The core clinical features and tissue-specific mutation loads are summarised in table 1. Five patients (probands in fig 1A) had a severe encephalopathy ranging from severe infantile LS (individuals IV-4 and V-1) to adult-onset progressive myoclonic epilepsy (PME) (individuals II-1, IV-3 and IV-5). Available hetero-anamnestic information suggests that LS was also the diagnosis in individuals III-5 and IV-1. Two patients with LS presented with a cardiopathy. While patient V-1 had a mitochondrial hypertrophic cadiomyopathy, patient IV-4 presented with a congenital structural heart defect probably unrelated to the mitochondrial defect. Additional phenotypes included migraine with aura (MA), Leber hereditary optic neuropathy (LHON), sensorineural hearing loss (SNHL) and diabetes mellitus type 2 (DM2) (table 1).

PME in patients II-1, IV-3 and IV-5

Individual II-1 suffered from a progressive neurological disorder which started around the age of 40 years with sudden bilateral vision loss and ataxic gait problems. At 68 years, she had severe dementia, prominent myoclonus, cerebellar ataxia, epilepsy, generalised rigidity, brisk deep tendon and brainstem reflexes, consistent with PME. Brain computed tomography (CT) scans

Table 1 Clinical features and m.14487T>C heteroplasmy levels in eight family members

Patient	Neurological diagnosis	Onset	Other diagnoses	Onset	Current age/ death	MRI fluid-attenuated inversion recovery/ T2 hyperintensities	Muscle biopsy	Heteroplasmy (%)
II-1	PME	<60 years	Visual loss	40 years	74 years†	ND	ND	ND
			Dementia	<60 years				
II-3	MA	~22 years	Diabetes mellitus type 2	57 years	72 years	ND	ND	8% (L)
III-4	–	–	Subclinical LHON	–	56 years	ND	ND	35% (L)
III-6	MA	14 years	SNHL	35 years	48 years	ND	ND	19% (L)
IV-2	–	–	–	–	34 years	–	ND	32% (L)
IV-3	PME	27 years	LHON	19 years	32 years	+	LM: normal, no RRFs	36% (L)
								97% (M)
IV-4	LS	1 year	Structural cardiopathy	11 days	2 years†	+	ND	99% (L)
								100% (F)
IV-5	PME	24 years	Dysarthria	10 years	28 years	+	LM: normal, no RRFs	52% (L)
			SNHL	17 years			EM: rare enlarged mitochondria, rare mitochondrial clusters	99% (M)
V-1	LS	1.5 month	Cardiomyopathy	3.5 m	3.5 months†	+	LM: normal, no RRFs	100% (L)
							EM: multiple clusters of enlarged mitochondria	100% (M)
V-2	–	–	–	–	19 months	ND	ND	83% (L)

†Age at death. EM, electron microscopy; LHON, Leber hereditary optic neuropathy; LM, light microscopy; LS, Leigh syndrome; MA, migraine with aura; PME, progressive myoclonic epilepsy; RRF, ragged red fibres; SNHL, sensorineural hearing loss.

showed cortical and subcortical atrophy and a hypodense lesion in the basal ganglia on the left side. EEG recordings showed slowing of background activity (7–8 Hz) with increased theta-wave activity bilaterally. Cardiac ultrasonography demonstrated concentric hypertrophy of the left ventricle. The patient died at age 74 years.

Individual IV-3 is a 31-year-old man who experienced transient bilateral visual loss due to bilateral optic neuropathy at the age of 19 years. LHON was suspected. Aged 27 years, he developed myoclonus and dystonia in the arms and lower limbs, and a few months later he had two generalised epileptic seizures. Brain MRI showed symmetrical fluid-attenuated inversion recovery (FLAIR) and T2-weighted hyperintensities in the substantia nigra. Around the age of 30 years, abnormal movements in the left arm reappeared regularly and gradually increased in frequency and intensity. At age 31, he developed episodes of rithmic clonic movements of head and twitching of facial muscles lasting around 30 s and recurring every 5 min, which we clinically interpreted as epileptic seizures. The ictal EEG is shown in fig 1C. The interictal EEG showed generalised slow but no epileptic activity. There was no photosensitivity. After several weeks, these seizures were controlled on levetiracetam 3000 mg, phenytoin 250 mg and phenobarbital 300 mg. He has remained seizure free for 1 year now.

Besides the lesions in the substantia nigra, brain MRI showed multiple lesions in the basal ganglia and brain stem, which were progressive over time. Most recently the patient has developed a progressive hypokinetic-rigid syndrome and is cognitively slowed.

Individual IV-5 is a 27-year-old man, who developed a progressive dysarthria since the age of 10 years. At the age of 17 years, he was diagnosed as having bilateral SNHL. Aged 22 years, he developed myoclonus in the right arm and a progressive right hemiparesis. At the age of 24 years, he started to have epileptic seizures characterised by tonic posturing of the four limbs and loss of consciousness. In later years, the neurodegenerative process has further progressed to a right-sided spastic hemiparesis, stimulus-sensitive medically refractory myoclonus in the face and left arm and leg, left-sided dystonic posturing of the neck, severe dysarthria, dysphagia leading to aspiration pneumonias, fluctuating aphasia and cognitive deterioration. Repeated brain MRI scans documented multiple FLAIR and T2-weighted hyperintens lesions in both hemispheres and the brainstem, with a progressive increase in size and number over several years. Video-EEG monitoring showed continuous multifocal epileptic spikes and polyspikes (fig 1D) with concomitant multifocal myoclonic jerks. There was no photosensitivity. The epilepsy was not controlled with carbamazepine, levetiracetam, pregabalin, clonazepam and topiramate.

m.14487T>C mutation loads

Mutation loads correlated with phenotypic severities (fig 1B). LS (individuals IV-4 and V-1) was associated with m.14487T>C homoplasmy in leucocytes (≥99%), while heteroplasmy levels between 8 and 35% were associated with mild symptoms as MA+DM2 (individual II-3), MA+SNHL (individual III-6), subclinical LHON (individual III-4) or no symptoms (individual IV-2). The severe adult-onset PME phenotype was associated with leucocytic heteroplasmy levels of 36% in individual IV-3 and 52% in individual IV-5. Mutation load data for PME patient II-1 were not available. A high leucocytic mutation load (83%) was found in patient V-2, who is presently 19 months old but clinically unaffected. Both patient IV-2 and V-2 are at increased risk of developing a progressive encephalopathy.

Muscle biopsy findings

In skeletal muscle, mutation loads were almost homoplasmic in PME patients IV-3 and IV-5 and homoplasmic in LS patient V-1. Spectrophotometrical analysis showed modestly decreased complex I activity in the three patients. However, in-gel activity staining for complex I following BN-PAGE showed no significant decrease in intensity of the bands in the patients versus controls.

DISCUSSION

We present a family segregating m.14487T>C, a mitochondrial complex I mutation previously only reported in isolated cases

with severe infantile phenotypes such as LS and progressive dystonia.[5–10] Neurological manifestations ranged from severe infantile LS to adult-onset PME and dystonia or progressive hypokinetic-rigid syndrome. Other mitochondrial features included LHON (as expected for ND6), SNHL, MA, DM2 and hypertrophic cardiomyopathy. In addition, the severity of the phenotypes correlated with leucocytic heteroplasmy levels. Although currently unaffected, our results suggest that individual V-2, who is presently 19 months old and has a leucocytic mutation load of 83%, is at increased risk of developing a severe encephalopathy.

A striking feature was the occurrence of PME as an adult manifestation of m.14487T>C in three patients. PME, which is characterised by myoclonic seizures, tonic–clonic seizures, progressive neurological and cognitive deterioration and ataxia, is a classical neurological feature in myoclonic epilepsy with ragged red fibres (MERRF). In addition, PME has also been reported in mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke (MELAS) or MERRF/MELAS overlap syndromes[13] and, most recently, in muscle weakness, ataxia and retinitis pigmentosa (NARP).[14] While MERRF and MELAS mutations typically affect general mitochondrial protein synthesis, and NARP is caused by an isolated complex V defect, isolated complex I defects have, to our knowledge, not been associated with PME.

Interestingly, the occurrence of MA in two patients with very low mutation loads (at least in leucocytes) suggests that MA might be the mildest manifestation of m.14487T>C-induced cortical hyperexcitability.[15] This finding agrees with a report demonstrating migraine as an important manifestation of the nearby stereotypical m.14484T>C LHON mutation[16] and supports the concept that migraine and epilepsy share a common pathological mechanism.[15]

In accordance with its severe homoplasmic phenotypes, m.14487T>C affects a residue (p.M63V) in the most conserved transmembrane region of ND6.[10] In contrast, the associated catalytic complex I activity measured in the available (nearly) homoplasmic muscle homogenates was only moderately decreased. Interestingly, this observation agrees with recent findings in a bacterial model suggesting that LHON mutations in ND6 affect ubiquinone reduction kinetics rather than the catalytic properties of the enzyme.[17]

In conclusion, our results show that homoplasmic m.14487T>C mutation loads, while resulting in moderately lowered complex I activity, do lead to severe infantile-onset LS phenotypes. In contrast, lower heteroplasmy levels resulted in adult-onset PME. This is the first report of PME as an important neurological manifestation of an isolated mitochondrial complex I defect.

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