Heterogeneous presentation in A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene

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Abstract

Aims—To clarify the phenotype–genotype relation associated with the A3243G mitochondrial DNA mutation.

Methods—Five unrelated probands harbouring the A3243G mutation but presenting different clinical phenotype were analysed. Probands include Leigh syndrome (LS³²⁴³), mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS³²⁴³), progressive external ophthalmoplegia (PEO³²⁴³), and mitochondrial diabetes mellitus (MDM³²⁴³). Extensive clinical, histological, biochemical, and molecular genetic studies were performed on five families.

Results—All patients showed ragged red fibres (RRF), and focal cytochrome c oxidase (COX) deficiency except for the patient with MDM³²⁴³. The mutation load was highest in the proband with LS³²⁴³ (>90%), who also presented the highest proportion of RRF (68%) and COX negative fibres (10%), and severe complex I plus IV deficiency. These proportions were lower in the probands with PEO³²⁴³ and with MDM³²⁴³.

Conclusion—The most severe clinical phenotype, LS³²⁴³, was associated with the highest proportion of the A3243G mutation as well as the most prominent histological and biochemical abnormalities.

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Keywords: MELAS; Leigh syndrome; progressive external ophthalmoplegia; mitochondrial DM

An A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene has been associated with various types of mitochondrial multisystem disorder, such as mitochondrial myopathy, encephalopathy, lactic acidosis and stroke like episodes (MELAS),^{1,2} myoclonus epilepsy with ragged red fibres (MERRF),³

MELAS+MERRF,⁴ maternally inherited progressive external ophthalmoplegia (PEO),⁵ MELAS+PEO,⁶ mitochondrial diabetes mellitus (MDM),⁷ hypertrophic cardiomyopathy,⁸ cluster headache,⁹ pancreatitis,¹⁰ and a subtype of Leigh syndrome (LS).¹¹

Here we report the five unrelated probands having the A3243G mutation in the mitochondrial tRNA^{Leu(UUR)} gene, including clinically and pathologically defined LS³²⁴³, MELAS³²⁴³, PEO³²⁴³, and MDM³²⁴³. Our LS proband is the second case found to be associated with A3243G mutation. Though the muscle is not the main target of any of the four clinical phenotypes studied here (except, perhaps, PEO), we analysed the variations in the distribution pattern of the A3243G mutation in muscle in order to clarify the phenotype–genotype relation in A3243G disorders.

Patients and methods

PATIENTS

Figure 1 shows the pedigrees of LS³²⁴³ (A), MELAS³²⁴³ (B and C), PEO³²⁴³ (D), and MDM³²⁴³ (E). Table 1 summarises the clinical findings of all these probands and their family. All probands and their family members gave their informed consent and the study was approved by the local ethics committee.

LS³²⁴³ FAMILY (A)

The proband, subject AIII3 died at 10 years of age. He had been clinically and pathologically described previously as having Leigh disease.¹² He was born from non-consanguineous parents, at 39 weeks gestation, with an uneventful delivery. The early developmental milestones were normal. However, no further developmental progress occurred until after 20 months of age. At 38 months old, he was admitted for generalised seizure, generalised ataxia, and diffuse muscle weakness. His height was 89 cm (–2.7 SD below normal), body weight 10.4 kg (–3SD below normal), and his developmental

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Table 1 Patients with A3243G mutation

Pedigree (fig 1)	LS ³²⁴³						MELAS ³²⁴³			MELAS ³²⁴³			PEO ³²⁴³					MDM ³²⁴³	
	AII4	AII2	AIII1	AIII2	AIII3	AIII4	BI2	BII1	BII2	CI2	CII1	CII2	DII4	DII4	DII5	DIII2	DIII3	EIII3	
Age at biopsy (y)	ND	ND	ND	ND	4	ND	ND	ND	13	ND	ND	26	ND	ND	ND	ND	42	27	
Age of onset (y)	42	46	16	N	1	N	24	15	3	24	16	12	24	26	23	32	18	15	
Muscle weakness	+	+	–	–	+	–	–	–	+	–	–	+	–	–	–	–	+	+	
Hearing loss	–	–	+	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	
Stroke	–	–	–	–	+	–	–	–	+	–	–	+	–	–	–	–	–	–	
Ophthalmoplegia	–	–	–	–	+	–	–	–	–	–	–	–	+	+	+	+	+	–	
Seizure	–	–	+	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	
Migraine headache	+	+	+	–	+	–	+	+	+	+	+	+	–	+	–	–	+	+	
Mental retardation	–	–	+	–	–	–	–	–	+	–	–	+	–	–	–	–	–	–	
Short stature	+	+	+	+	+	+	+	–	+	+	+	+	–	–	–	+	+	+	
Lactic acidosis	–	–	–	–	+	–	–	–	+	–	–	+	–	–	–	–	–	–	

ND, not done; N, normal or asymptomatic.

Figure 1 Pedigrees of five families with A3243G mutation: family A (LS^{2433}); families B and C ($MELAS^{3243}$); family D (PEO^{3243}); family E (MDM^{243}). The proportions of mutant mtDNA in peripheral blood lymphocytes (B), hair follicles (H), and muscle (M) are shown. Arrows denote the probands. Solid symbols denote patients having each symptom; shaded symbols denote oligosymptomatic relatives.

quotient (DQ) was 68. A Levine 2/6 ejection systolic murmur was audible on the fourth left intercostal line. Deep tendon reflexes were diminished. A chest x ray showed an enlargement in the cardiothoracic ratio (68%). An echocardiogram showed hypertrophic cardiomyopathy. On laboratory examination, hepatic transaminase and blood urea nitrogen were within the normal range. The concentrations of lactate and pyruvate were increased to 6.0 mmol/l (normal 0.3–1.3 mmol/l) and 0.17 mmol/l (0.03–0.08 mmol/l) in blood, and 8.45 and 0.26 in CSF, respectively. A muscle biopsy was taken at the age of 3 years 9 months.

His condition remained stable until 9 years of age when he developed slight but increasing impairment in visual acuity. Progressive muscle weakness occurred. At the age of 10 years 3 months, he developed intractable epilepsy characteristic of Lennox–Gastaut syndrome. A computed tomography (CT) scan of the brain revealed notable cortical atrophy, ventricular dilatation, and enlargement of the fourth ventricle. At the age of 10 years 10 months, he underwent an operation for paralytic ileus; 10 days later he developed respiratory insufficiency. He died with complications of respiratory distress and severe infection. An autopsy was performed after receiving informed consent.

The mother (AII2), a 42 year old woman, had been healthy until about 10 years previously, when she first noticed progressive

muscle weakening; since then she has also suffered migraine headaches. However, she has never been admitted for any detailed examination.

The grandmother (AI4), a 78 year old woman, had developed muscle weakness and easy fatigability for the past 25 years. However, she declined to undergo any clinical or other further examination.

The proband's elder brother (AIII1) has noticed migraine headache. However, all other relatives presented no symptoms suggesting any mitochondrial disorders.

MELAS³²⁴³ FAMILY (B)

The proband, MELAS³²⁴³ (BII2) was admitted to our hospital at the age of 10 years for generalised muscle weakness, migraine headache, periodic vomiting, and right side hemiparesis. On admission, her height was 121 cm (−2.7 SD below normal), body weight 17 kg (−3SD below normal), and her IQ was 102. She had bilateral sensorineural hearing loss (below 40 db), severe muscle weakness, and muscle atrophy. CT and magnetic resonance imaging (MRI) scans showed cortical atrophy and calcification of the brain stem. She fulfilled the clinical criteria of MELAS. She underwent a muscle biopsy at the age of 13 years.

The elder sister (BII1) and the mother (BI2) have been suffering migraine headaches for 10 years.

MELAS³²⁴³ FAMILY (C)

The proband, MELAS³²⁴³ (CII2) was admitted to our hospital at the age of 23 years for generalised muscle weakness, periodic vomiting, and hemiparesis. On admission, her height was 146 cm (−1.5 SD below normal), body weight 45 kg (−1.3 SD below normal), and her IQ was 106. She had sensorineural hearing loss (below 30 db), severe muscle weakness, and muscle atrophy. CT and MRI scans showed cortical atrophy and calcification in the brain stem. She fulfilled the clinical criteria of MELAS. She underwent a muscle biopsy at the age of 26 years.

The elder brother (CII1) and the mother (CI2) suffered migraine headaches.

PEO³²⁴³ FAMILY (D)

The proband, PEO³²⁴³ (DIII3) was admitted to our hospital at the age of 42 years for progressive external ophthalmoplegia (PEO) and generalised muscle weakness. His height was 168 cm (mean), body weight 52 kg (−1.3 SD below normal), and his IQ was 98. CT and MRI scans revealed no abnormality. He fulfilled the clinical criteria of maternally transmitted PEO. He underwent a muscle biopsy at the age of 42 years.

Though his maternal relatives (DI4, DII4, DII5, and DIII2) showed PEO, no family member had diabetes, muscle weakness, short stature, or any hearing loss suggesting mitochondrial disorder.

MDM³²⁴³ FAMILY (E)

The proband, MDM³²⁴³ (EIII3) was admitted to our hospital at the age of 26 years for the control of insulin dependent diabetes mellitus. On admission, he had short stature (−2.0 SD below normal), mild sensorineural hearing loss, and mild muscle weakness. He showed no stroke like episodes and no other symptoms.

Table 2 Pathological and biochemical analysis of muscle biopsy specimens from LS³²⁴³, MELAS³²⁴³, and PEO³²⁴³

	LS ³²⁴³ (AIII4)	MELAS ³²⁴³ (BII2)	MELAS ³²⁴³ (CII2)	PEO ³²⁴³ (DIII3)
<i>Muscle pathology</i>				
RRF (%)	68	5	6	0.5
COX (−) fibres (%)	10	0.2	0.1	0.2
SSVs	+	+	+	−
<i>Biochemistry</i>				
Complex I–III	28.4	63.8	70.8	202.6
226.9 (119.6) (n = 26)	12.5%	28.1%	31.2%	89.3%
Complex II–III	438.1	386.6	471.4	455.4
432.9 (134.7) (n = 32)	101.2%	89.3%	108.9%	105.2%
Complex IV	39.1	162.6	157.4	454.4
471.4 (112.5) (n = 28)	8.3%	34.5%	33.4%	96.4%
CS	1222.1	908.8	762.1	863.6
853.4 (132.6) (n = 32)	143.2%	106.5%	89.3%	101.2%
Complex I–III/CS	0.02	0.07	0.09	0.23
Complex II–III/CS	0.36	0.43	0.62	0.53
Complex IV/CS	0.03	0.18	0.21	0.53
Cytochrome b	858.9	851.0	976.3	1044.9
1128.7 (506.6) (n = 28)	76.1%	75.4%	86.5%	92.6%
Cytochrome c+c ₁	832.9	789.3	949.0	1290.2
1148.9 (174.4) (n = 32)	72.5%	68.7%	82.6%	112.3%
Cytochrome a+a ₃	90.8	451.9	534.2	1054.2
1068.4 (366.8) (n = 28)	8.5%	42.2%	50.0%	98.7%

The control values of the respiratory chain enzyme activities, expressed as nmol/min/mg of mitochondrial protein, were expressed as mean (SD).

Activities of complex I–III (rotenone sensitive NADH–cytochrome c oxidoreductase), complex II–III (succinate–cytochrome c oxidoreductase), complex IV (cytochrome c oxidase), and CS (citrate synthetase) were measured. Control values of cytochrome contents were calculated using the extinction coefficients after reduction by the addition of 10 mM succinate in the presence of 1.0 mM KCN. Control values, expressed as pmol/mg of mitochondrial protein, are expressed as mean (SD).

His mother had been diagnosed as having diabetes mellitus and had earlier died at the age of 49 years, before the proband was diagnosed. We did not have any opportunity to examine the mother.

BIOCHEMICAL ANALYSIS

Respiratory chain enzyme activities were measured in muscle biopsy specimens from patients with LS³²⁴³ (AIII3), MELAS³²⁴³ (BII2, CII2), PEO³²⁴³ (DIII3), and MDM³²⁴³ (EIII3), and in tissues taken at postmortem examination from patient LS³²⁴³ (AIII3), according to established procedures.¹³

DNA ANALYSIS

Total DNA was extracted from leucocytes, hair follicles, and muscle biopsy specimens. The A3243G mutation was analysed by polymerase chain reaction (PCR)–restriction fragment length polymorphism according to methods described previously.¹⁴ Tissues from postmortem examination of LS³²⁴³ were also subjected to DNA analysis. The DNA signals were quantified using a BAS 2000II Bioimage Analyser (Fujix Inc., Tokyo, Japan).

Results

MUSCLE PATHOLOGY IN LS³²⁴³ (AIII3), MELAS³²⁴³ (BII2), MELAS³²⁴³ (CII2), PEO³²⁴³ (DIII3), AND MDM³²⁴³ (EIII3)

There was mild to moderate variation in fibre size in both type 1 and type 2 fibres in all patients examined. Scattered ragged red fibres (RRF) were seen in all probands, except the probands with MDM³²⁴³. RRF showed speckled, patchy, or heterogeneous pattern, stronger at the subsarcolemmal regions, while non-RRF showed homogeneous on cytochrome c oxidase (COX) staining. In LS³²⁴³, 10% of RRF showed a homogeneously negative pattern on COX. The intramuscular small arteries showed hyperactivity on SDH staining; strongly succinate dehydrogenase reactive blood vessels (SSVs) were seen in all patients, except PEO³²⁴³. SSVs were always COX positive. Table 2 summarises the percentages of RRF and of COX negative fibres.

HISTOPATHOLOGICAL FINDINGS IN BRAIN AND HEART OF LS³²⁴³ (AIII3)

An autopsy was performed four hours after death at the age of 10 years 10 months. The brain was oedematous and increased in softness with leptomeningeal turbidity. Microscopically, there were extensive symmetric necrotic lesions in the brain stem in which the bilateral putamen and thalamus showed necrotic softening. Some symmetrical necrotic lesions were noted around the midbrain extending down to the pons, where demyelination, proliferation of capillaries, and gliosis were noted. These findings were consistent with those reported as characteristic lesions of LS. The heart muscle showed increased vacuolation in the myocardium, and also increased fibrosis in the perimysium and endomysium area, showing characteristic features of cardiomyopathy.

A3243G MUTATION IN FAMILY MEMBERS

Figure 1 shows the percentage of the A3243G mutation in the muscle of the proband, and also percentages in the leucocytes and/or hair follicles of the family members. Maternal transmission of A3243G mutation was shown in all five families studied, except for MDM³²⁴³. In the MDM³²⁴³ family, we could not detect the mutation in the relatives.

BIOCHEMICAL ANALYSIS

Table 2 summarises results from the biochemical analysis of the muscle biopsy specimens from each of the probands. Enzyme activities of the respiratory chain complexes IV and I were greatly decreased in all probands, except in PEO³²⁴³ and in MDM³²⁴³ whose activities remained normal. The concentrations of cytochrome a+a₃ were decreased in LS³²⁴³ and MELAS³²⁴³, consistent with the decreased activities of COX. In the tissues from postmortem examination of LS³²⁴³ (AIII3), all respiratory chain enzyme activities were considerably decreased, except for the activity of complex II which remained normal (data not shown).

A3243G MUTATION IN TISSUES FROM POSTMORTEM EXAMINATION OF LS³²⁴³ (AIII3)

The percentage of the A3243G mutation determined in the tissues from postmortem examination, including muscle, heart, liver, kidney, and the cerebrum was 92, 91, 90, 97, and 91%, respectively.

Discussion

We have performed a comparative study of the clinical symptoms, morphology, and mitochondrial genetics of LS³²⁴³, MELAS³²⁴³, PEO³²⁴³, and MDM³²⁴³ in order to understand the phenotype-genotype relation in these four disorders associated with the A3243G mutation. We have found a direct correlation between the percentage mutation and an impairment in the respiratory chain function of the muscle tissues. LS³²⁴³ harboured the highest percentage of A3243G mutation among these four phenotypes and showed the most severe impairment of respiratory dysfunction. The most striking morphological difference among the muscle specimens from these four phenotypes was the higher percentage of RRF (68% of total muscle fibres), and COX negative RRF in LS³²⁴³ (10% of total muscle fibres) than that in MELAS³²⁴³ (average 0.15%) and PEO³²⁴³ (0.2%). Unlike in LS³²⁴³, LS caused by COX deficiency or pyruvate dehydrogenase complex deficiency has usually not been associated with RRF. The reduction in COX activity in muscle fibre of the MELAS patient¹⁵ appeared to be less severe than that described in MERRF¹⁶ or Kearns-Sayre syndrome (KSS)/PEO¹⁷ caused by different mtDNA mutations. In MERRF and KSS/PEO, RRF were consistently COX negative, while in MELAS patients, RRF often showed positive COX staining. Our findings were consistent with those of Petruzzella *et al.*¹⁸ who found that even though PEO³²⁴³ muscle contained less mutant mtDNA overall, these were distributed more heterogeneously in such a way that many fibres had amounts of mutant

genomes sufficiently high to cause COX deficiency. In our LS³²⁴³, the percentage of mutant in crude homogenate was so high that the percentage mutation in RRF may be much higher than the threshold effect of COX negative change. This may be clarified by single muscle fibre PCR analysis. As SSVs showed positive COX activities, the COX may be not the primary enzyme defect in A3243G mutation. When the percentage mutation is lower than threshold (<95%), the extensive mitochondrial proliferation is able to compensate for the local needs of oxidative phosphorylation in an affected muscle domain. According to the pedigree of these four clinical phenotypes, a striking inherited difference in the heteroplasmic condition in the family members between LS³²⁴³ and the others was the higher percentage of A3243G mutation in LS³²⁴³. In LS³²⁴³, though the percentages of mutant mtDNA from the mother (AII2) and from the grandmother (AI4) were each less than 15% in blood and hair follicle, all the sisters including the proband had a rather higher percentage of mutant mtDNA in muscle, blood, and hair follicle. As the percentages of mutant mtDNA in the autopsy tissues of the proband (AIII3) were all high (>92%) and homogeneously distributed, we have hypothesised that the proportion of mutant genome in each of the fertilised eggs from the mother of LS³²⁴³ was much more significant than the other clinical features.

The pathogenic mechanism by which an individual having the A3243G mutation in the tRNA^{Leu(UUR)} gene leads to LS and cardiomyopathy, while in others leads to MELAS³²⁴³, PEO³²⁴³, or the MDM³²⁴³ phenotype, remains to be elucidated. Heterogeneous clinical expression of the individual pathogenic mtDNA mutation has been reported; the T8993G mutation in ATPase 6 was associated with neuropathy, ataxia, and retinitis pigmentosa (NARP)¹⁹ at moderately high heteroplasmy (about 70%), and extremely high maternally inherited LS (>95%),²⁰ while the A8344G mutation in the mitochondrial tRNA^{Lys} gene was associated with MERRF,²¹ and with LS²² in extremely high amounts.

The findings reported here of the different concentrations and different spatial distributions of a single mtDNA point mutation extend this concept to the cellular level, and probably have relevance to other mitochondrial diseases. Moreover, our analyses have suggested that the LS³²⁴³ with cardiomyopathy phenotype was the most severe outcome in the clinical spectrum associated with this point mutation of A3243G in the mitochondrial tRNA^{Leu(UUR)} gene.

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