

Mitochondrial encephalomyopathy with elevated methylmalonic acid is caused by *SUCLA2* mutations

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We have identified 12 patients with autosomal recessive mitochondrial encephalomyopathy with elevated methylmalonic acid. The disorder has a high incidence of 1 in 1700 in the Faroe Islands due to a founder effect, and a carrier frequency of 1 in 33. The symptoms comprise hypotonia, muscle atrophy, hyperkinesia, severe hearing impairment and postnatal growth retardation. Neuroimaging showed demyelination and central and cortical atrophy, including atrophy of the basal ganglia, and some of the patients fulfilled the criteria for Leigh syndrome. Urine and plasma methylmalonic acid were elevated. Homozygosity mapping with the Affymetrix 10 K array revealed a homozygous region on chromosome 13q14 harbouring the *SUCLA2* gene. Mutations in *SUCLA2* were recently shown to cause a similar disorder in a small Israeli family. Mutation analysis identified a novel splice site mutation in *SUCLA2*, IVS4 + IG → A, leading to skipping of exon 4. The *SUCLA2* gene encodes the ATP-forming β subunit of the Krebs cycle enzyme succinyl-CoA ligase. The hallmark of the condition, elevated methylmalonic acid, can be explained by an accumulation of the substrate of the enzyme, succinyl-CoA, which in turn leads to elevated methylmalonic acid, because the conversion of methylmalonyl-CoA to succinyl-CoA is inhibited.

Keywords: Methylmalonic acid; Leigh syndrome; mitochondrial encephalomyopathies; mitochondrial diseases

Abbreviations: MMA = methylmalonic acid; PDH = pyruvate dehydrogenase

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Introduction

The incidence of mitochondrial encephalomyopathies in childhood is around 1 in 10 000 (Darin *et al.*, 2001; Skladal *et al.*, 2003). The disorders can present with a number of different clinical phenotypes, often with neurological symptoms, but other organs may also be affected, most frequently heart, liver and pancreas. A decreased enzyme activity of one or more of the respiratory chain complexes is usually found. Combined respiratory chain deficiencies constitute around 30% of cases with decreased respiratory chain enzyme activity (Kleist-Retzow *et al.*, 1998), and are caused by mutations in mitochondrial DNA (mtDNA) or nuclear DNA (nDNA) genes. Some nDNA mutations affect the amount or stability of mtDNA, resulting in multiple mtDNA deletions or mtDNA depletion. Multiple

mtDNA deletions have been reported in mitochondrial neuro-gastro-intestinal encephalopathy (MNGIE), caused by mutations in thymidine phosphorylase (Nishino *et al.*, 1999), and in autosomal dominant progressive external ophthalmoplegia (PEO), due to mutations in *c10orf2* (Spelbrink *et al.*, 2001) or *ANT* (Kaukonen *et al.*, 2000). Mutations in *POLG* may give rise to various phenotypes associated with mtDNA deletions, including autosomal dominant or recessive PEO (Van Goethem *et al.*, 2001) and mitochondrial recessive ataxia syndrome (MIRAS) (Hakonen *et al.*, 2005), as well as Alpers syndrome (Naviaux *et al.*, 2004), where mtDNA depletion may be found. MtDNA depletion is also found in hepatoencephalopathy caused by *DGUOK* mutations

(Mandel *et al.*, 2001) and in encephalomyopathy due to thymidine kinase 2 mutations (Saada *et al.*, 2001).

Recently, a mutation in the *SUCLA2* gene (MIM 603921) was reported in a small consanguineous family with combined complex I, III and IV deficiency and mtDNA depletion in muscle (Elpeleg *et al.*, 2005). The clinical features included psychomotor retardation, muscle hypotonia, hearing impairment, seizures and neuroimaging suggestive of Leigh syndrome. No organic acid results were reported.

The *SUCLA2* gene encodes the β subunit of the Krebs cycle enzyme succinyl-CoA ligase that catalyses the formation of succinate and ATP from succinyl-CoA and ADP in a reversible manner. It is a mitochondrial matrix enzyme that shares its α subunit, *SUCLG1*, with another β subunit, the GTP-forming succinyl-CoA ligase *SUCLG2*; hence the β subunit determines substrate specificity (Johnson *et al.*, 1998).

Here we report an autosomal recessive encephalomyopathy with elevated methylmalonic acid (MMA) levels. The disorder has a high incidence in the Faroe Islands. By SNP homozygosity mapping, we identified a homozygous locus on chromosome 13q14, among others harbouring the *SUCLA2* gene. Sequencing revealed a splice site mutation that leads to skipping of exon 4 of the *SUCLA2* gene in all 12 affected patients.

Material and methods

Subjects

We identified 10 patients, including two sib pairs, from the Faroe Islands, born in the period from 1980 to 2003. DNA was available from all 10 patients, 10 parents and three healthy siblings. Genealogical studies revealed a familial relationship between five of the patients (Fig. 1). In addition, two Icelandic siblings with a

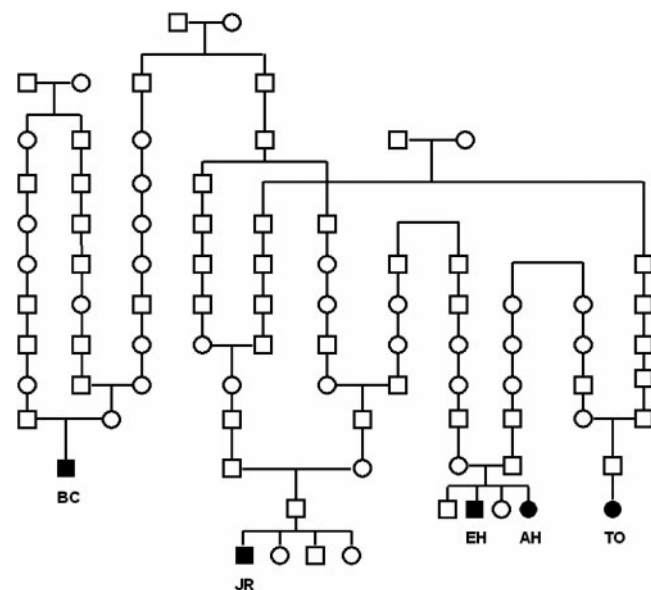


Fig. 1 Pedigree showing the relationship between five Faroese patients with methylmalonic encephalomyopathy.

similar phenotype and parents of Faroese origin were included. Informed consent was obtained from the patients or their parents.

DNA from 200 anonymous Guthrie cards from the Faroe Islands was analysed to assess the carrier frequency of the mutation found in this study.

Genotyping

DNA was extracted from blood, muscle, fibroblasts or Guthrie cards using standard methods. DNA from 9 of the 12 patients was used for a genomewide search for homozygosity with the Affymetrix GeneChip 10 K array, version 2.0 (Affymetrix Inc., Santa Clara, CA). In brief, 250 ng of DNA was digested with the restriction enzyme *Xba*I, mixed with *Xba* adapters and ligated with the T4 DNA ligase. The ligated DNA was PCR-amplified in four PCR reactions, pooled and purified. The purified PCR product was fragmented with DNase I and end-labelled with biotin. The samples were hybridized to an array for 18 h in a hybridization oven. The array was washed, stained and scanned with an Affymetrix GeneChip scanner 3000. Affymetrix software was used to analyse the data, which were exported to an Excel file.

For the haplotype analyses, we used six microsatellite markers flanking the *SUCLA2* locus (primer sequences are listed in supplementary Table 1 online). The forward primer was labelled with FAM or HEX. PCR was performed with the Promega kit and the following conditions: 0.2 mM dNTPs, 1 \times buffer, 1.5 mM MgCl₂, 0.5 mM of each primer, 10 ng template and 1.5 U polymerase in a total volume of 50 μ l. The PCR program was: 94°C for 2 min, 35 cycles of denaturing at 94°C for 30 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, and a final extension step of 72°C for 7 min. The PCR products were analysed on an ABI3100.

Mutation analysis

The 11 exons of the *SUCLA2* gene were amplified in eight fragments. To assess the functional effect of the mutation on splicing, RNA was extracted from fibroblasts and reverse transcribed to cDNA with the SuperScript II Reverse Transcriptase kit (Invitrogen), and PCR of a 876 bp cDNA fragment encompassing exons 4–8 was performed with the previously reported primers (Elpeleg *et al.*, 2005). The same PCR conditions as for microsatellite analysis were used for amplification of genomic DNA and cDNA. The products were sequenced with the Big Dye Terminator Ready Reaction mix version 1.0.

To screen for the IVS4 + 1G \rightarrow A mutation, a TaqMan assay was developed (Applied Biosystems, Foster City, CA). The PCR conditions were: 10 μ l Universal PCR Master Mix, 0.5 μ l 40 \times assay mix and 20–100 ng DNA in a total volume of 20 μ l. The PCR programme was 95°C for 10 min, and 50 cycles at 92°C for 15 s and 60°C for 1 min. The samples were run on an ABI Prism 7000 and analysed with ABI SDS software.

Quantification of mtDNA

The mtDNA copy number was determined by Southern blot analysis. Total DNA was digested with *Pvu*II. Nuclear-encoded 28S rDNA was detected with a 345 bp ³²P-labelled probe, and mtDNA was detected with a mixture of two ³²P-labelled probes, tRNA-Pro and ND2 (1139 bp; nt 15978–547 and 615 bp; nt 5289–5903). The radioactive signals were quantified with Imagequant software (GE Healthcare).

Enzyme activity

Analysis of respiratory chain enzyme activity in muscle and fibroblasts was carried out as described (Birch-Machin *et al.*, 1994; Krahenbuhl *et al.*, 1994) with a few modifications.

Western blotting

Mitochondrial protein was isolated from fibroblasts as previously reported (Birch-Machin *et al.*, 2001). The samples (10 µg protein/lane) were run on a 12% SDS polyacrylamide gel and transferred to nitrocellulose membranes. The membranes were probed with polyclonal antibody against *SUCLA2* at a 1:1000 dilution and developed with a 1:5000 dilution of goat anti-rabbit antibody (Dako). An antibody against the pyruvate dehydrogenase (PDH) E2 subunit (Molecular Probes) was used as a loading control at a 1:5000 dilution. The secondary antibody was goat anti-mouse at a 1:10 000 dilution (Dako). The bands were visualized with the Supersignal West Pico substrate (Pierce) and the blots were exposed to film.

Determination of MMA

MMA was extracted from plasma with solid phase extraction columns and determined by an isotope dilution assay (Rasmussen, 1989). After derivatization, the dicyclohexyl derivative of MMA was quantified by selected ion monitoring using deuterated MMA as internal standard. MMA in urine was quantified by an assay modified from Christensen *et al.* (1981) by ion extraction of the total ion chromatogram using 3-phenylbutyric acid as internal standard.

Results

Clinical presentation

The clinical data of 12 patients from nine families are summarized in Table 1.

Pregnancy and birth were mostly unremarkable, except for patient FT, where delivery was induced because of dysmaturity and oligohydramnios. Patients BC and DJ were dysmature. Birth weights and birth lengths were within normal values.

The presenting symptom in most patients was muscle hypotonia, with onset from birth to 5 months of age. Some patients had onset with fatigue, reduced muscle mass or motor retardation. All patients developed severe hypotonia with lack of head and trunk control and none of them achieved ambulation. The muscles were severely atrophic (Fig. 2). Scoliosis or kyphosis was found in most patients and all patients wore a corset. Severe postnatal growth retardation with postnatal weight at or below the 3rd centile was found in nearly all patients, and most patients were tube-fed through a percutaneous endoscopic gastrostomy. Recurrent airway infections were often seen. In four patients hyperhidrosis was reported. Five patients are alive, while the remaining seven patients died between the ages 8 months and 21 years, most often during an acute infection.

All patients had dystonia and most of them hyperkinesias as athetoid or choreiform movements. The dyskinesias were

treated with various drugs; baclofen alone or in combination had the best and longest lasting effect. One patient (TD) had epilepsy from birth, spasm like, with an EEG that was severely abnormal, but not hypsarrhythmic. At 18 months the EEG was normal and the antiepileptics were discontinued. None of the other patients had epilepsy, and a normal EEG was found in three patients.

Nearly all patients had severe hearing impairment, most often diagnosed by brainstem audiometry. The age at diagnosis of hearing impairment was from 8 months to 4 years. Patients DJ, FT and BN had a cochlear implant operation and they all had considerable improvement in their communication. Patient DJ died a few years after, but his younger brother FT had a remarkable improvement, and patient BN, who had intensive communicative training, is now considered to be of normal intellectual capacity. The non-implanted patients make use of all senses except hearing, in a way that leaves the impression of considerable, or even normal, intellectual capacities.

Ophthalmological investigations were normal in most patients, except for one patient with strabismus and two patients with ptosis.

Autopsy of patient JR showed an atrophic brain with severely dilated ventricles and decreased white matter volume. There was severe basal ganglia atrophy, especially of the caudate nuclei, and loss of medullar anterior horn motor neurons.

Brain MRI was done in five patients; four patients had central and cortical atrophy, and in two patients delayed myelination was found. No basal ganglia abnormalities were reported. Brain CT in five patients showed central and cortical atrophy including caudate nuclei atrophy. A diagnosis of Leigh syndrome was made only for patient BD based on neuroimaging.

Sensory and motor nerve conduction velocities were normal. Electromyography in three patients showed prolonged mean potential durations (21–46%) and increased mean amplitudes of motor unit potentials (46–205%), suggesting affection of medullar motor neurons.

Histology of muscle tissue showed a very consistent and characteristic pattern in all seven patients from whom a muscle biopsy was available. The findings included (i) increased variability of fibre diameter with scattered hypertrophic, spherical fibres with an increased mitochondrial content, (ii) a marked type I fibre predominance (>95%) and (iii) extensive intracellular fat accumulation in type I fibres (Fig. 3).

In three patients the level of mtDNA was investigated; all three had muscle mtDNA depletion (Fig. 4E), with mtDNA:nDNA ratios between 15–41% of mean control.

Most patients (5/8) had clear lactic acidosis. There was a consistently elevated urinary excretion of MMA, and as in classical methylmalonic aciduria, the MMA excretion was accompanied by an increased methylcitrate excretion in periods of exacerbation as well as by an inconsistent excretion of beta-hydroxypropionic acid, lactate, and Krebs

Table 1 Clinical and laboratory data of 12 patients with methylmalonic encephalomyopathy

Patient initials	BN	TO	BC	DJ	FT ^a	JR	RJ	AH	EH ^b	TD	LD	BD ^c
Gender	M	F	M	M	M	M	F	F	M	M	M	F
Pregnancy and birth	CS	CS 2–3 wks post-term	Dysm	Dysm	Dysm, oligo	N	N	N	N	N	N	Twin birth
Birth weight	3055 g	3500 g	2800 g	2900 g	N.a.	N.a.	3750 g	3650 g	3400 g	N.a.	3905 g	2410 g
Birth length	50 cm	54 cm	49 cm	53 cm	N.a.	N.a.	N.a.	53 cm	53 cm	N.a.	55 cm	48 cm
Age at onset	1 month	2–3 months	3½ months	5 months	2 months	4 months	5 months	3 months	Birth	Birth	Birth	Birth
Outcome	Alive at 10 years	Alive at 16 years	Died 21 years old	Died 10 years old	Alive at 6 years	Died 15 years old	Alive at 2 years	Died 10 years old	Died 18 years old	Died 4 years old	Died 8 months old	Alive at 5 years
Presenting symptom	Hyp, Musc Atr, FTT	Hyp, Fat	Mot, Hyp	Mot	Hyp, HI, Hid	Hyp	Hyp	Hyp	Hyp	IS	Hyp	Hyp
Clinical												
Hypotonia	+	+	+	+	+	+	+	+	+	+	+	+
Motor retardation	+	+	+	+	+	+	+	+	+	+	+	+
Muscle atrophy	+	+	+	+	+	+	+	+	+	+	+	+
Hyperkinesia/dystonia	+	+	+	+	+	+	+	+	+	+	+	+
Hyperhidrosis	–	+	+	+	–	–	–	–	–	+	–	–
Hearing impairment	+, CI	+	+	+, CI	+, CI	+	+	+	+	+	?	–
Neuroimaging	MRI: Gen Atr, Abn Bas Gang	N.a.	CT: severe Cent + Cort Atr	N.a.	MRI: slight Cent + Cort Atr	CT: Dil Ant horn, Bas Gang Atr	MRI: slight Cent Atr	N.a.	CT: slight Ant horn Atr, Bas Gang Atr	MRI: Enl Subarach space, severely Delay Myelin	N.a.	MRI: severe Atr, Abn Bas Gang
Postnatal growth retardation												
Height	+	+	+	+	+	+	–	+	N.a.	–	–	+
Weight	+	+	+	+	+	+	+	+	N.a.	+	–	+
Laboratory investigations												
Plasma lactate (in mmol/l). Reference <2.0 mmol/l.	1.9–3.0	8.6	5.4–5.9	0.9–1.4	N.a.	N.a.	4.9	N.a.	N.a.	3.7–11.4	2.1	5.4–5.6
CSF lactate (in mmol/l). Ref. 0.8–1.2 mmol/l.	2.2–3.3	N.d.	2.9	N.d.	6.3	N.d.	N.d.	N.d.	N.d.	N.d.	N.d.	3.1
Urine MMA (in µmol/mmol creatinine). Ref. <3.6 µmol/mmol creatinine	183	↑	153–212	N.d.	↑	↑	62–127	N.d.	↑	51	↑	160
Plasma MMA (in µmol/l). Ref. <0.28 µmol/l	2.4	2.5	2.7	N.d.	N.d.	N.d.	0.8	N.d.	N.d.	N.d.	N.d.	33.0
Urine methylcitrate	↑	N	↑	N.d.	N	N	↑	N.d.	↑	↑	N	↑

^aSibling of DJ, ^bsibling of AH, ^csibling of LD. CS = Caesarean section; Dysm = dysmaturity; Oligo = oligohydramnion; Hyp = hypotonia; Musc = muscular; Atr = atrophy; FTT = failure to thrive; Fat = fatigue; Mot = motor retardation; HI = hearing impairment; Hid = hyperhidrosis; IS = infantile spasms; Pto = ptosis; Strab = strabismus; CI = cochlear implant, Gen = generalized; Cent = central, Cort = cortical; Dil = dilated; Ant = Anterior; Abn = abnormal; Bas Gang = basal ganglia; Enl Subarach = enlarged subarachnoid; Delay Myelin = delayed myelination. N = normal; N.d. = not determined; N.a. = not available. ↑: slightly elevated (not quantified).



Fig. 2 Patient TO at age 15 years showing dystonic posture of the extremities (**A**) and severe muscular atrophy (**B**).

cycle intermediates such as succinic acid, citric acid and alpha-ketoglutaric acid. Plasma MMA was quantified in five patients and found to be elevated in all. The levels were not as high as in methylmalonic aciduria caused by defects in the methylmalonyl-CoA mutase gene. In contrast, the MMA concentrations were comparable with those seen in transcobalamin 2 or cobalamin C deficiency (unpublished data, E. Christensen). The propionate pathway was studied in fibroblasts from two patients and showed normal methylmalonyl-CoA mutase activity and normal ^{14}C -propionate fixation (Dr Brian Fowler, Basel). B_{12} therapy had no effect on the course of the disease and no effect on the excretion of methylmalonate.

SNP genotyping and microsatellite marker results

SNP genotyping in nine patients showed several small homozygous areas. Additional genotyping with microsatellite markers allowed us to exclude all areas except one on chromosome 13q14, where all patients shared a short, common core haplotype of 1.4 Mb between markers D13S287 and D13P4819 (Table 2). The shared homozygous haplotype extended to around 8.2 Mb in some patients (data not shown). The common core haplotype was also found in the three patients where SNP mapping had not been performed, including the two Icelandic patients.

The 1.4 Mb locus harboured ten genes, among these the *SUCLA2* gene, which encodes a Krebs cycle enzyme, and *SUCLA2* was therefore considered a candidate gene.

Mutation analysis

Mutation analysis of the *SUCLA2* gene showed that all patients were homozygous for a novel splice site mutation in intron 4, IVS4+1G \rightarrow A (Fig. 4A). DNA was available from the parents of patients JR, BC, TO, BN, EH and AH, and they were all heterozygous for the mutation. Three siblings (of EH and AH, and JR), were tested for the mutation, and they were found to be either heterozygous carriers or homozygous for the wild-type.

cDNA analysis identified a transcript 163 bp shorter than the control (Fig. 4B). Sequencing of the cDNA showed that the mutation caused skipping of exon 4 and a change of the reading frame, creating a premature stop codon (Fig. 4D).

Founder mutation

The incidence of the disorder in the Faroe Islands is estimated to be 1 in 1700 (95% confidence interval 1:1290–1:2480), based on 10 patients born from 1980 to 2003, divided by the total number of Faroese born in this period ($\sim 708/\text{year}$: 16 992 persons). Using the

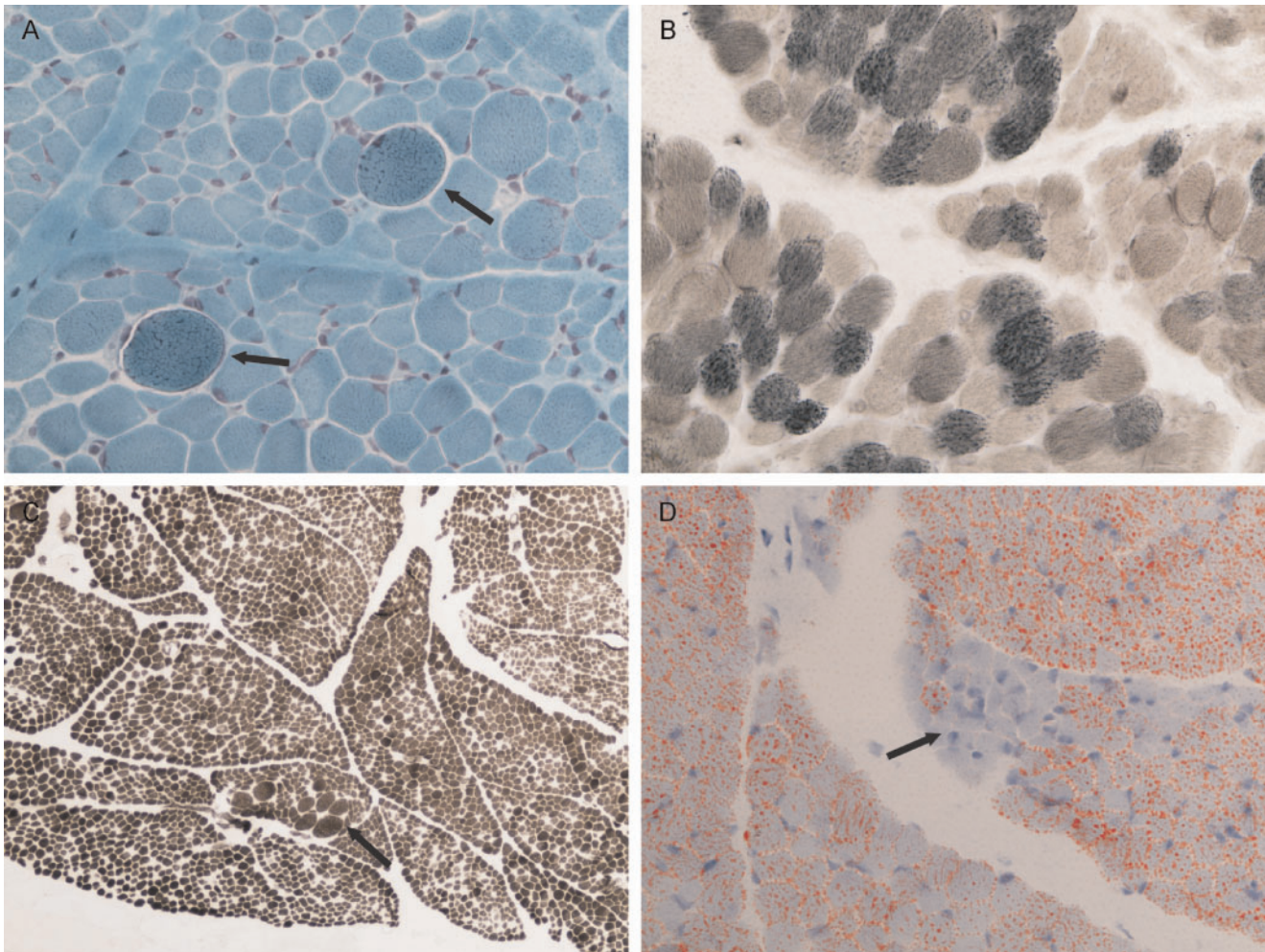


Fig. 3 Histology of muscle tissue from patients with methylmalonic encephalomyopathy. **(A)** Gomori trichrome staining showing two large spheric muscle cells (arrows) with increased mitochondrial content. **(B)** Sudan black staining showing abnormal lipid accumulation as black granules. **(C)** ATPase staining at pH 4.3 showing a pronounced type I fibre preponderance (black fibres). A small group of hypertrophic, spherical cells are seen (arrow). **(D)** Oil red staining showing a predominance of type I fibres with severely increased lipid content as red granules, and a few type II fibres (arrow) with no lipid accumulation.

Hardy–Weinberg equation, the carrier frequency is estimated to be 1 in 21.

This frequency was verified by testing 200 Faroese controls for the IVS4+1G → A mutation. Of these, six were heterozygous for the mutation, corresponding to a carrier frequency of 1 in 33 (95% confidence interval 1 : 23–1 : 55).

Enzyme activity and western blotting

Respiratory chain enzyme analysis of muscle tissue from patient RJ showed a moderately decreased complex I, III and IV activity, whereas complex II, which is encoded exclusively by nDNA genes showed normal activity (Supplementary Table 2 online). Fibroblasts were analysed from three patients and showed normal activity or a slightly decreased complex IV activity.

Western blot analysis of mitochondrial proteins isolated from fibroblasts showed absence of *SUCLA2* protein in the patients (Fig. 4B).

Discussion

Mutations in the *SUCLA2* gene are responsible for a specific mitochondrial encephalomyopathy with a high incidence in the Faroe Islands. The patients had a homogeneous clinical phenotype, encompassing severe muscle hypotonia, muscle atrophy, dyskinesia, postnatal growth retardation, severe hearing impairment and elevated MMA. Some patients fulfilled the criteria for Leigh syndrome, since they had a progressive disorder with basal ganglia affection and elevated lactate. Neuroimaging performed early in life may be the cause of not detecting basal ganglia lesions in some patients; all patients developed signs of basal ganglia

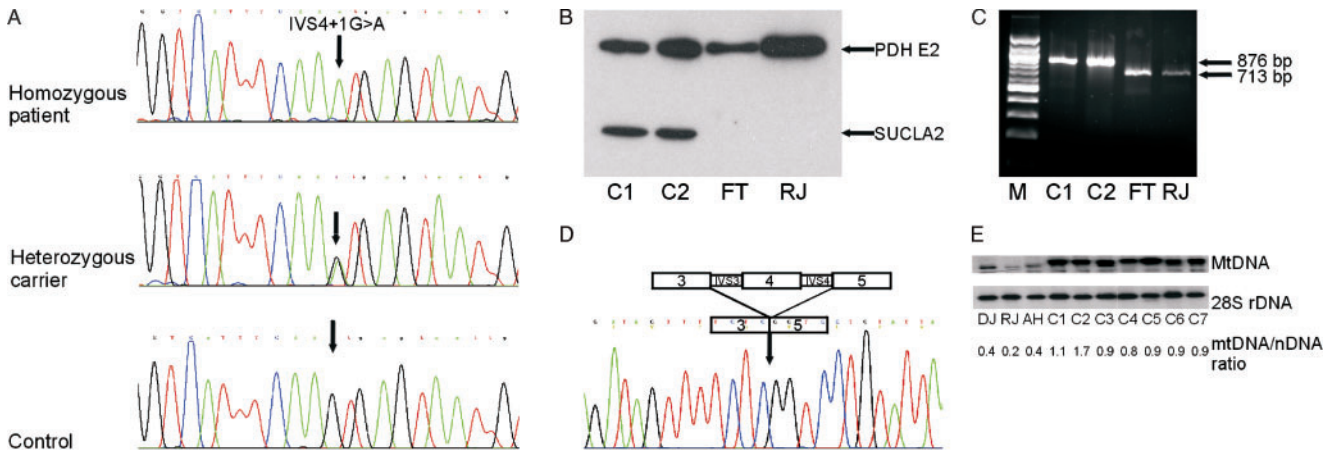


Fig. 4 Characterization of the *SUCLA2* gene and protein, and mtDNA levels in patients with methylmalonic encephalomyopathy. **(A)** Sequence analysis of *SUCLA2* in a control, a heterozygous carrier (mother of patient TO) and a homozygous patient (EH) shows the IVS4+1G → A mutation. **(B)** Western blot analysis of protein from fibroblast mitochondria shows absence of *SUCLA2* protein in patients RJ and FT compared with controls (C1 and C2). PDH E2 was used as a loading control. **(C)** Agarose gel of a PCR-amplified cDNA fragment of *SUCLA2* shows a fragment of 876 bp in the controls (C1 and C2) and a 163 bp shorter fragment in patients RJ and FT who are homozygous for the IVS4+1G → A mutation. M = molecular weight marker. **(D)** Sequencing of the cDNA fragment in **A** from patient RJ shows skipping of exon 4, resulting in a change of the reading frame and a premature stop codon. **(E)** Southern blot analysis showing decreased muscle mtDNA levels in three *SUCLA2* patients (DJ, RJ and AH) compared with 7 age-matched controls (C1–C7).

Table 2 Haplotypes of 12 patients with methylmalonic encephalomyopathy

Microsatellite marker	Position	Distance from <i>SUCLA2</i>	TO	AH	EH	BC	JR	DJ	FT	TD	RJ	BN	LD	BD
DI3P4586	45.8 Mb	1.6 Mb		2	2	2		3	3		4	3		
DI3SI6I	46.7 Mb	759 kb												
DI3S287	46.8 Mb	655 kb				2								
DI3P47I3	47.1 Mb	311 kb												
	47.4 Mb	<i>SUCLA2</i>												
DI3SI53	47.8 Mb	348 kb												
DI3P48I9	48.2 Mb	755 kb	2			2	2			2	2	2	2	2

The core haplotype is shaded.

involvement with dystonia and hyperkinesias. The two patients described in the first article reporting *SUCLA2* mutations had a phenotype that was similar to the Faroese patients, i.e. onset of disease within the first 2 months of life with muscle hypotonia, severe psychomotor retardation and hearing impairment. They did, however, have a low birth weight, below the 3rd centile, whereas the Faroese patients had normal birth weight. Both patients had generalized seizures with onset at age 1 and 3 years, respectively. Seizures were reported in only one of the Faroese patients, and this patient had infantile spasms that disappeared around age 1½ years. As in some of the Faroese patients, the brain MRI/CT of the first two patients was suggestive of Leigh syndrome.

Muscle histology was reported to be normal in the first two patients reported. We found a characteristic muscle histology pattern in all seven Faroese patients studied with a predominance of type I fibres, some very large fibres with mitochondrial accumulation and a pronounced accumulation of intracellular lipid. All patients in both studies had

mtDNA depletion in muscle. As suggested (Elpeleg *et al.*, 2005), a likely explanation of muscle mtDNA depletion in patients with *SUCLA2* mutations is the interaction of succinyl-CoA ligase with the mitochondrial nucleoside diphosphate kinase (NDPK), which is involved in the maintenance of the nucleotide pool in the mitochondria (Kowluru *et al.*, 2002).

The Faroese patients had elevated MMA in urine and plasma and some patients also excreted methylcitrate. The elevated MMA may be explained by an accumulation of succinyl-CoA due to the deficiency of the enzyme succinyl-CoA ligase (Fig. 5). The accumulated succinyl-CoA inhibits the metabolism of methylmalonyl-CoA to succinyl-CoA, and thereby leads to accumulation of methylmalonyl-CoA, which is converted to MMA. The methylcitrate elevation is caused by accumulation of propionyl-CoA due to product inhibition of propionyl-CoA carboxylase. When propionyl-CoA is in excess amounts it is metabolized to methylcitrate by citrate synthase, which normally converts acetyl-CoA to citrate (Fig. 5).

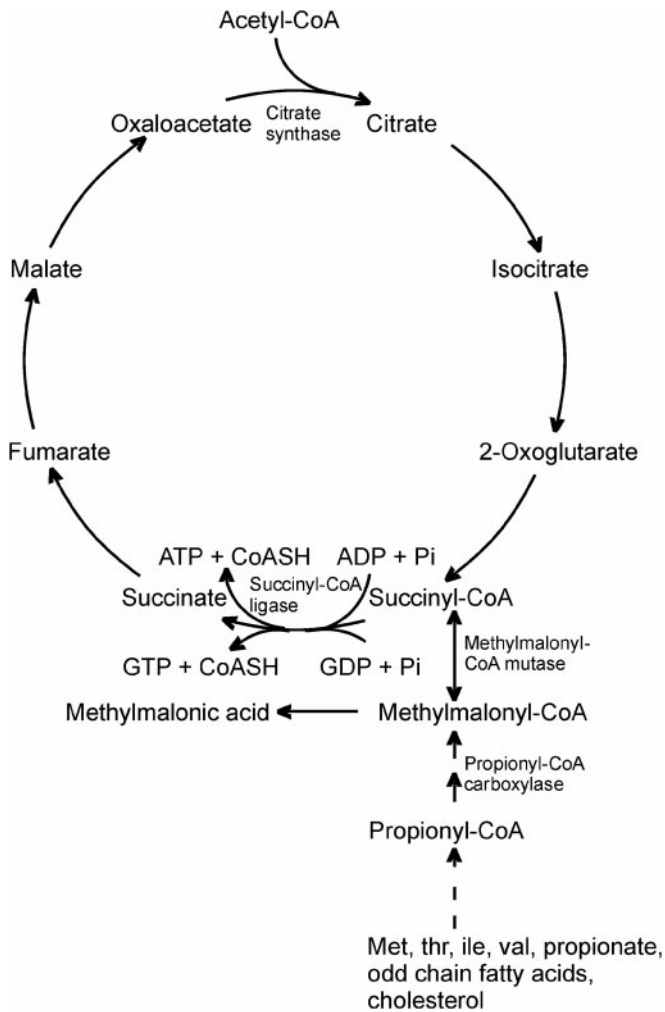


Fig. 5 The Krebs cycle and methylmalonic acid metabolism.

This study demonstrates another cause of elevated MMA in addition to the disorders caused by deficiency of methylmalonyl-CoA mutase or defects in B₁₂ metabolism. In the literature, ‘atypical’ methylmalonic aciduria has been reported in three papers (Stromme *et al.*, 1995; Mayatepek *et al.*, 1996; Yano *et al.*, 2003); These patients typically had hypotonia, muscle weakness and developmental delay, which are symptoms similar to those found here, and an increased urinary excretion of MMA in the range 200–400 $\mu\text{mol}/\text{mmol}$ creatinine. In one article, a partial complex IV deficiency and mtDNA depletion was found; no enzyme or depletion studies were performed in the other reports. Based on the clinical and laboratory results, these cases may well be caused by *SUCLA2* mutations.

In some patients with respiratory chain disorders, urinary excretion of tricarboxylic acid cycle metabolites or other organic acids is found (Barshop, 2004), but these findings are usually unspecific and do not point towards a specific genetic defect. There is, however, one exception; the elevated urine excretion of ethylmalonic acid found in ethylmalonic encephalopathy, which is caused by mutations

in the *ETHE1* gene (Tiranti *et al.*, 2004). *ETHE1* encodes a mitochondrial matrix enzyme of yet unknown function, and, for unknown reasons, the patients have a complex IV deficiency in muscle. This study suggests that in parallel with ethylmalonic acid in ethylmalonic encephalopathy, MMA excretion is a useful marker of decreased succinyl-CoA ligase activity; this is supported by the fact that we have subsequently ascertained a patient of Pakistani descent with a different *SUCLA2* mutation through elevated MMA and methylcitrate (unpublished data, E. Ostergaard). Initially, some of the Faroese patients were reported to have normal urine screening results, but re-examination of the chromatograms showed elevated MMA excretion. Hence, when using qualitative instead of quantitative methods, a slightly elevated MMA excretion may not be identified; this has important implications for those involved in the interpretation and reporting of metabolic data.

In the Faroe Islands, mitochondrial encephalomyopathy with elevated MMA has a high incidence, 1 in 1700, and a carrier frequency of 1 in 33. We found a clear founder effect with all patients sharing a common core haplotype around the *SUCLA2* locus, including two Icelandic patients with Faroese parents. The Faroe Islands are situated in the North Atlantic Ocean between Iceland and Norway. The ancestors of the present population settled around 825 AD by migration from the Western part of Norway (Young, 1979). There has been little migration to the Faroe Islands since then. The population remained small for a long time with an estimated size of 4000 in the 14th century, 9000 in the 19th century, but it has expanded during the last 150 years to the present population size of around 50 000 (West, 1972). No major bottlenecks are thought to have occurred (Jorgensen *et al.*, 2002). A founder effect is responsible for a high incidence of some disorders in the Faroe Islands, e.g. cystic fibrosis (incidence 1 in 1775) (Schwartz *et al.*, 1995) and glycogen storage disease type IIIA (incidence 1 in 3600) (Santer *et al.*, 2001). This study adds mitochondrial encephalomyopathy with elevated MMA to the presently known disorders caused by founder effects in the Faroe Islands. The small size of the common core haplotype indicates that the mutation is old, and it may date back to the first immigrants in the 9th century.

One of the obstacles for the investigation of respiratory chain disorders is the large number of genes involved. Combined respiratory chain deficiencies may be caused by mutations in the mtDNA tRNA genes or in a number of nuclear genes, and several are probably still to be identified. The clinical presentation may not always be a good clue to which genes to investigate, due to the considerable genetic and phenotypic heterogeneity in mitochondrial disorders. In some cases though, e.g. Leigh syndrome with complex IV deficiency where *SURF1* mutations are frequently found, the clinical and laboratory findings are very helpful in this decision (Bohm *et al.*, 2006). In parallel with this, the phenotype of mitochondrial encephalomyopathy with

elevated MMA appears to be quite homogeneous, and the identification of this phenotype and/or a slightly elevated MMA should prompt a *SUCLA2* mutation analysis.

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