diagnosis of a complex II abnormality. Similarly, according to the Australian data, the most common abnormality was in complex I (45%), followed by abnormalities in multiple complexes (28%), complex IV (21%), and complex III (5%); only one patient had a complex II abnormality.

Manifestations, genetic diagnoses, and prognoses of MTDPS

A qPCR-based diagnosis of MTDPS was made for 16 of the 80 patients with an enzymatic diagnosis of a multiple complex abnormality, and for seven of the 105 patients with an enzymatic diagnosis of a respiratory chain complex I abnormality. Three of these 23 patients died due to sudden infant death syndrome and thus had no available records of clinical findings; the clinical findings from the remaining 20 patients were further analyzed.

The disease types among these 20 patients were compared with those reported by Sarzi *et al.*[4] (Fig. 4). Among the Japanese patients, 13 (65%) had acute hepatocerebral MTDPS, two (10%) had Alpers-like syndrome (delayed-onset hepatocerebral MTDPS), and five (25%) had encephalomyopathic MTDPS. This distribution is similar to that reported by Sarzi *et al.* We must note here that "Alpers-like" refers simply to delayed-onset hepatocerebral MTDPS. This is because no true case of Alpers syndrome has yet been identified in Japan. The results of analyses of the three main genes responsible for MTDPS are shown in Figure 5. Causative genetic anomalies were identified in six of the 20 Japanese patients (30%). No abnormality was identified in the three genes of the remaining 14 patients (70%). The responsible genes were *DGUOK* in three patients whose clinical reports are described in the previous section, *MPV17* in two patients,[7] and *POLG* in one patient whose clinical report will be published elsewhere. The individual genetic abnormalities are listed with the clinical findings in Table 1. Although three of the patients

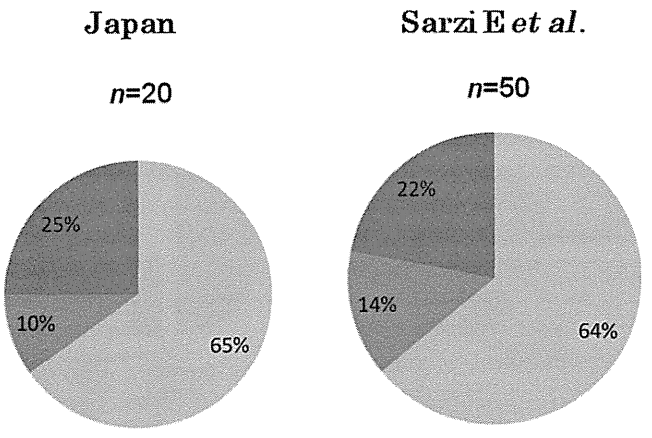

Fig. 4 Percentage distribution of disease types of mitochondrial DNA depletion syndrome (MTDPS) in Japan and those reported by Sarzi *et al.* "Alpers-like" refers simply to delayed-onset hepatocerebral MTDPS, because no true case of Alpers syndrome has yet been identified in Japan. The distribution of disease types in the present study did not differ from that reported by Sarzi *et al.*[4]. (▒) Hepatocerebral, (▒) Alpers–like syndrome, (▒) Encephalomyopathic.

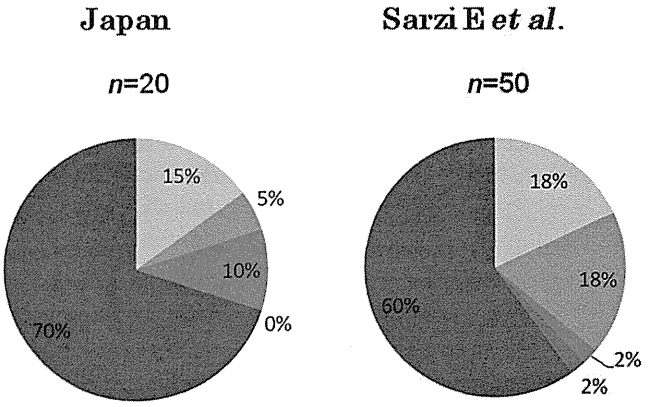

Fig. 5 Percentage distribution of responsible genes for mitochondrial DNA depletion syndrome (MTDPS) in Japan and those reported by Sarzi *et al.* The causative gene was not identified in the majority of patients in each population. Four genes, *DGUOK*, *POLG*, *MPV17*, and *TK2*, contained 40% of the causative genetic abnormalities identified by Sarzi *et al.*,[4] while three genes, *DGUOK*, *POLG*, and *MPV17*, contained 30% of the abnormalities found in the Japanese patients. (▒) *DGUOK*, (▒) *POLG*, (▒) *MPV17*, (▒) *TK2*, (▒) *unknown*. DGUOK, deoxyguanosine kinase; POLG, DNA polymerase γ.

underwent liver transplantation during infancy, five of them died before 2 years of age. Patient 5 lived longer than the others because of dietary and pharmaceutical treatment targeting the mitochondrial respiratory chain complex II.[7]

The *DGUOK*-related patients were two sisters, with a homozygous 335 bp deletion (Fig. 1a; g.11692_12026del335; encompassing 308 bp of intron 1 and 27 bp at the start of exon 2), and a compound heterozygote patient, genetically unrelated to these sisters, with the same deletion and a c.743T>C (p.L248P) missense mutation. The large 335 bp deletion encompassing from intron 1 to exon 2 causes the complete skipping of exon 2, and the resultant mRNA has a premature termination codon (p.A48fsX90). Each parent and healthy sister is heterozygous for this mutation (Fig. 1b). The p.L248P variation is not listed as a polymorphism in the ensembl_mart_47 database (martdb.ensembl.org) and has not been reported as a disease-causing mutation. Moreover, the alignment shows that Leu248 is absolutely conserved in all species (Fig. 6).[18]

The *MPV17* patients were previously reported compound heterozygote siblings.[7] The *POLG* patient was a compound heterozygote. The genetic mutations noted in these six patients were confirmed to be absent in DNA of 100 healthy Japanese controls (data not shown).

Like Sarzi *et al.*, who did not find the responsible gene or genes in 60% of the patients, we were unable to identify the responsible gene or genes in a majority of the cases. We sequenced the whole exome of all the MTDPS patients to identify the underlying nuclear disease genes using next-generation sequencing system (data not shown). This did not identify pathogenic mutations in any of the known genes associated with MTDPS (*TK2, SUCLA2, RRM2B, SUCLG1, MGME1, C10orf2, TYMP, and AGK*) in the present MTDPS patients.

Table 1 Clinical and molecular characteristics for Japanese hepatocerebral MTDPS patients

Patient	Sex	Initial symptoms (age)	Outcome (age)	Clinical diagnosis	Complications	Liver transplantation	Blood lactate/ pyruvate (mmol/L)	%mtDNA in liver	Identified mutations	Ref
1	F	Failure to thrive (3 months)	Dead (1 year)	Hereditary tyrosinemia	Developmental delay	Done	Not available	3	*DGUOK* (g.11692_12026del335 (p.A48fsX90) homozygote)	
2	F	Tachypnea (2 days)	Dead (9 months)	Mitochondrial hepatopathy	Hypoglycemia	Not done	20.9/0.27	6	*DGUOK* (g.11692_12026del335 (p.A48fsX90) homozygote)	
3	F	Feeding difficulty (8 days)	Dead (1 year)	Mitochondrial hepatopathy	Developmental delay, failure to thrive	Done	2.9/0.14	2	*DGUOK* (g.11692_12026del335 (p.A48fsX90) / c.743T>C (p.L248P))	
4	M	Failure to thrive, acholic stool (3 months)	Dead (1 year)	Hepatic failure	Developmental delay	Done	Not available	8	*MPV17* (c.451insC (p.L151fsX189)/ c.509C>T (p.S170F))	7
5	M	Failure to thrive, vomiting (8 months)	Dead (6 years)	Hepatic failure	Developmental delay, gastroesophageal reflux, respiratory failure	Done (at 6 years)	Normal	7	*MPV17* (c.451insC (p.L151fsX189)/ c.509C>T (p.S170F))	7
6	F	Failure to thrive (4 months)	Dead (7 months)	Hepatic failure	Hypotonia	Not done	1.76/0.1	3	*POLG* (c.2869G>C (p.A957V) / c.3354T>C (p.I1185T))	

Shaded columns, two pairs of siblings. MTDPS, mitochondrial DNA depletion syndrome.

```
Human          241   -----ALMNIPVLVLDV--NDDFSEEVTKQEDLMREVNTFVKNL------   277
Pan Trog       241   -----ALMNIPVLVLDV--NDDFSEEVTKQEDLMREVNTFVKNL------   277
Canis          241   -----ALLNIPVLVLDV--NDDFSEEVTKQEELMKKVNIFVKNL------   277
Bos            241   -----ALLNIPVLVLDV--NDDFSEEVTIQEELMRRVNTFVKNL------   277
Mus            241   -----ALQHVPVLVLDV--TEDFSENAARQEELMGQVNTFMRNL------   277
Rat            241   -----ALRHVPVLVLNI--SEDFSENAAKQEELMGQVNTFMRNL------   277
Danio          233   -----QLMKVPVLVLDA--EVAFEQNPEVQDCLLSKVRDFLSQL------   269
Arabidopsis    483   NHMHSSIQKVPALVLDCEPNIDFSRDIEAKTQYARQVAEFFEFVKKKQET   532
Oryza          408   DHMHSSIQKVPALVLDCEHDIDFNKDIEAKRQ-----------------   439
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Fig. 6 ClustalW multiple sequence alignment of deoxyguanosine kinase (*DGUOK*) orthologs. The alignment shows that amino acid 248Leu mutated in the patient is absolutely conserved in all species. URLs: HomoloGene, http://www.ncbi.nlm.nih.gov/homologene (for the DGUOK ortholog amino acid sequences of human [accession no. NP_550438.1], Pan [accession no. XP_001153473.1], Canis [accession no. XP_533001.2], Bos [accession no. NP_001014888.2], Mus [accession no. XP_001107072.1], Rat [accession no. NP_001100072.1], Danio [accession no. XP_001093561.1], Arabidopsis [accession no. NP_565032.2], Oryza [accession no. NP_001044956.1]). ClustalW, http://www.ebi.ac.uk/Tools/clustalw/.[18]

Of the genetic mutations identified, *POLG* mutations were less prevalent than in Caucasian subjects. Only one of the present 15 cases of Alpers syndrome or hepatocerebral MTDPS were caused by recessive *POLG* mutations, compared with eight of 39 such cases diagnosed in France.

Sixteen of the 20 Japanese MTDPS patients were deceased. Sarzi *et al.* reported that 29 of the 50 MTDPS patients they analyzed were deceased. The data of the deceased patients were plotted to obtain curves of the ages of death (in months) in the two groups for comparison (Fig. 7). MTDPS patients had a short life in both study groups; many died during or before reaching early childhood. On log-rank test and Gehan-Breslow-Wilcoxon test no significant differences were seen between the survival data.

Discussion

We started an enzyme diagnosis referral service for children suspected of MRCD in 2007 and have diagnosed MRCD in 30–40 patients from around Japan annually since then. In the last year we have made >100 new MRCD diagnoses. Approximately half of the diagnoses are for neonates. There are approximately one million births in Japan annually. Under the assumption that the patients referred for enzyme diagnosis represent approximately half of all Japanese MRCD patients, the prevalence of neonatal-onset MRCD becomes $50 \times 2/1\,000\,000 = 1/10\,000$. When patients with juvenile-onset and adult-onset mitochondrial disease are factored in, the prevalence of these diseases in Japan becomes one in several thousand, which is comparable to the prevalence in Western countries.[1]

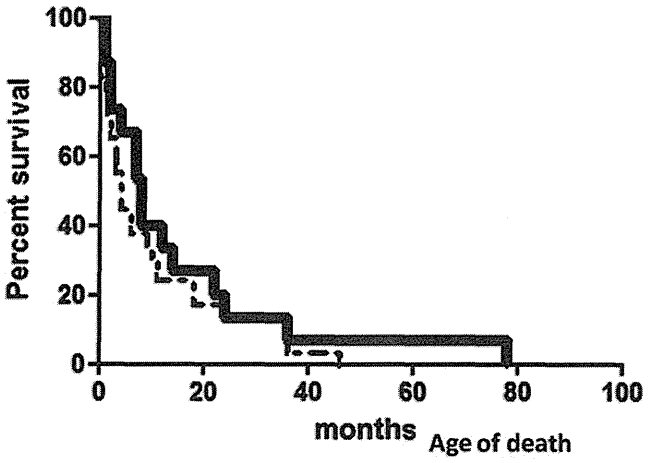

Fig. 7 Comparison of the ages of death (in months) in the two studies. A commonality between the Japanese patients and the Sarzi *et al.* patients[4] was observed. No significant difference in disease severity was identified (log-rank test, $P = 0.3637$; Gehan-Breslow-Wilcoxon test, $P = 0.2667$). (▬) Japanese, $n = 16/20$; (-•-) Sarzi *et al.*, $n = 29/50$.

It is noteworthy that >10% of the patients lacked lactic acidemia, which many physicians still regard as synonymous with mitochondrial disease. Hence, mitochondrial disease must also be considered in lactic acidemia-free patients with unexplained signs and symptoms in multiple organs.

The enzymatic diagnosis of MRCD showed similar trends in prevalence between Japanese and Australian patients, with respiratory chain complex I being the most common type of MRCD, followed by abnormalities in multiple complexes, complex IV abnormalities, and complex III abnormalities. Complex II abnormalities were very rare in both populations.

Twenty percent of the patients with multiple respiratory chain disorders in the present study and 50% of the patients in the Sarzi *et al.* study[4] had MTDPS. Although MTDPS was the leading cause of MRCD in both groups, MTDPS represented a smaller proportion of the MRCD in Japan. According to the Online Mendelian Inheritance in Man database, MTDPS can be classified as encephalomyopathic, hepatocerebral, or specific (a classification that includes mitochondrial neurogastrointestinal encephalopathy [MNGIE] and Sengers syndrome). Encephalomyopathic MTDPS features respiratory failure and myopathy. Hepatocerebral MTDPS is characterized by liver disorders, growth disorders, and hypoglycemia. The distribution of the disease type classifications of the Japanese patients did not differ from the distribution reported by Sarzi *et al.*

Four genes, *DGUOK*, *POLG*, *MPV17*, and *TK2*, contained 40% of the causative genetic abnormalities in the Sarzi *et al.* study, while three genes, *DGUOK*, *POLG*, and *MPV17*, contained 30% of the abnormalities found in the Japanese patients. The causative gene, however, was not identified in the majority of patients in each study. The six Japanese hepatocerebral MTDPS patients in whom the responsible gene was identified are listed in Table 1. The serious nature of this disease is evident, given that all six experienced onset as neonates or infants and died during or before reaching early childhood.

Deoxyguanosine kinase deficiency was originally described as the cause of infantile-onset hepatocerebral mitochondrial disease, typically featuring hepatic failure, nystagmus and hypotonia.[19] Recently it has been shown that patients with *DGUOK* mutation may present with neonatal hemochromatosis[20] or adult-onset myopathy and mitochondrial DNA multiple deletions, with or without liver involvement.[21,22] We found two novel *DGUOK* mutations in two apparently unrelated Japanese families. Three patients in two families had typical signs and symptoms of hepatocerebral MTDPS, and both parents in each family were

heterozygous for these mutations. A 335 bp deletion in *DGUOK* was found in both families, and may therefore be a common mutation in the Japanese population.

The present analysis of MTDPS patients concludes with a comparison of the ages of death (in months) in the two groups (Fig. 7). A commonality between the Japanese patients and the Sarzi *et al.* patients was the early age of death: most patients died during or before reaching early childhood. *DGUOK* deficiency was most serious in both studies. Likewise, many patients in each study experienced onset as neonates or infants. No significant difference in disease severity was identified between the two studies.

The present results indicate a lower prevalence of *POLG* mutations in the Japanese population, which is likely attributable to several common mutations found in Caucasian people that appear to be ancient European founder mutations (p.A467T, p.G848S, and p.W748S).[23] In children with recessive *POLG* mutations, these three mutations represented seven of 16 mutant alleles reported by Sarzi *et al.*[4] A recent study collated the prevalence of these three mutations in 10 studies reporting a total of 249 POLG patients and found that they represented 49% of mutant alleles in predominantly Caucasian patients.[24] Most Caucasian POLG patients will thus have at least one allele carrying one of these three founder mutations, and Hakonen *et al.* suggested that they may have been spread during Viking times.[23] The carrier frequency of these mutations is as high as 2% in some European countries. Their expected absence in Asian patients likely explains a lower prevalence of recessive POLG disease in Asian populations.

Conclusion

Mitochondrial DNA depletion syndrome and other mitochondrial respiratory chain disorders are common, but serious, diseases that occur across all races.

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A rapid screening with direct sequencing from blood samples for the diagnosis of Leigh syndrome

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ABSTRACT

Large numbers of genes are responsible for Leigh syndrome (LS), making genetic confirmation of LS difficult. We screened our patients with LS using a limited set of 21 primers encompassing the frequently reported gene for the respiratory chain complexes I (ND1–ND6, and ND4L), IV(SURF1), and V(ATP6) and the pyruvate dehydrogenase E1α-subunit. Of 18 LS patients, we identified mutations in 11 patients, including 7 in mDNA (two with ATP6), 4 in nuclear (three with SURF1). Overall, we identified mutations in 61% of LS patients (11/18 individuals) in this cohort. Sanger sequencing with our limited set of primers allowed us a rapid genetic confirmation of more than half of the LS patients and it appears to be efficient as a primary genetic screening in this cohort.

1. Introduction

Leigh syndrome (LS) (OMIM 256000) is an early onset, devastating neurodegenerative disease of the central nervous system (CNS) characterized by symmetrical necrotic lesions in the brainstem, basal

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ganglia and thalamus [1,2]. The symptoms of LS include psychomotor retardation, respiratory difficulties, nystagmus, hypotonia, seizures, myoclonus, ataxia, dystonia, ptosis, ophthalmoplegia and high lactate levels in the blood and cerebrospinal fluid. Mutations in both mitochondrial DNA (mDNA) and nuclear DNA cause LS [3].

LS arises from a deficiency in the enzymes relating to energy production in the mitochondria, such as the respiratory chain complexes I–V, and the pyruvate dehydrogenase complex. Among the enzymes, isolated complex I deficiency is the most frequent oxidative phosphorylation (OXPHOS) defect in children with LS [4,5], followed by a deficiency of complex IV (cytochrome C oxidase) and complex V (ATP synthase). Complex I is composed of seven mDNA encoded NADH dehydrogenase (ND) subunits (ND1–6, ND4L) and at least 38 nuclear DNA subunits [4]. An isolated generalized defect of complex IV is the second most common biochemical abnormalities found in patients with Leigh syndrome [6,7]. *SURF1* mutations, which encode the putative assembly protein of complex IV, have been repeatedly reported [6].

Since a large number of genes are reportedly related to LS, molecular diagnosis appears challenging. However, emerging drugs for LS demand prompt diagnostic confirmation of LS. Although exome sequencing is a powerful method of suspected mitochondrial disorders, it is time and cost consuming, and impractical to be applied to all patients with LS. Based on the reported mutation information, we designed a small set of 21 primers that cover the gene in which LS mutations have been frequently reported [3]. In this study, we have examined the efficacy of our Sanger sequencing method as a genetic screening for LS in 18 unrelated LS cases from one children's hospital. We identified 7 patients with point mutations in mDNA including 2 cases in the *ATP6* gene and five in the *ND* genes. We also elucidated 4 mutations in the nuclear encoded gene, including 3 patients with a mutation in *SURF1* and 1 patient with a mutation in *PDHA1* (pyruvate dehydrogenase E1α-subunit). Our data suggest that Sanger screening using limited sets of primers is useful as first line screening for LS.

2. Methods

We identified 18 patients from 16 families that met the criteria of LS at our institution (2005–2012). Diagnoses of LS were defined as presenting progressive neurologic disease with signs and symptoms of brain stem and/or basal ganglia abnormalities revealed on MR images. The clinical courses are summarized in Table 1 and Supplementary text. We have designed 7 sets of primers encoding mitochondrial derived subunits for complex I (*ND1-6, ND4L*) [3]. Primers were also designed on frequently reported gene *SURF 1* from complex IV [7] and *ATP synthase* from complex V [8]. If the blood lactate/pyruvate ratio is less than 10, we first sequenced the *PDHA1* gene (Suppl. Fig. 1) [8]. Methods of genetic analysis, enzyme assays and determination of heteroplasmic rate and associated references are available in the online version of the paper (Suppl. text).

3. Results (Table 1, Suppl. Fig. 2)

Of 18 LS patients, we identified gene mutations in 11 patients from 11 families. mDNA mutations were identified in 7 patients. An *ND1* mutation of complex I (m3697G>A, p.Gly131Ser) was identified in 2 individuals with homoplasmy. Mutations in *ND3* (m10158T>C, p.Ser34Pro; mutant rate 90% in white blood cell), *ND5* (m13513G>A, p.Asp393Asn; mutant rate 50% in white blood cell) and *ND6* (m14459G>A, p.Ala71Val, homoplasmic state) were identified in a single patient, respectively. One severe patient died at 1 year, and carried a mutation in *ATP6* (m8993T>G, p.Leu156Arg) of complex V of OXPHOS as a homoplasmic state. Instead of T>G, T>C mutation of the same nucleotide, m8993T>C p.Leu156Pro, was observed with homoplasmy in a milder case.

Four patients were identified with mutations in nuclear DNA. *SURF1* mutations were identified in 3 cases, including 2 cases that were compound heterozygous (c.49+1G>T/c.752_753delAG) and (c.574C>T, p.Arg192Trp and c.743C>A, p.Ala248Asp) and 1 case that was homozygous (c.743C>A, p.Ala248Asp). One male patient was identified with a hemizygous mutation (c.121T>C, p.Cys41 Arg) in *PDHA1*. Overall, we identified mutations in 61% of LS patients (11/18 individuals) in this cohort.

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4. Discussion

Molecular elucidation of LS at the DNA level is challenging. LS has been associated with a variety of genes in either mitochondrial or nuclear encoded DNA [3]. Surprisingly, we could reveal mutations in 61% of LS patients (11/18 individuals).

We disclosed 7 patients with mDNA mutations. From mitochondrial *ND1*, we identified an m3697G>A mutation in 2 unrelated patients, which has been reported previously in association with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) [9] and Leber's hereditary optic neuropathy (LHON) [10]. To our knowledge, this is the first report of the m3697G>A/*ND1* gene mutation causing Leigh syndrome. The heteroplasmy rate is reportedly 80% in patients with MELAS (skeletal muscle) and was 56% with LOHN [9,10]. A high mutation load (100%), found in the blood of Patients 1 and 2 may be associated to severe phenotype in our patients [11]. Low level of m3697G>A mutation (~40%) was found in the blood from an asymptomatic mother of Patient 1 (Suppl. Figs. 3 and 4).

For ND3, we found a mutation of m10158T>C with 90% of heteroplasmic rate in one patient showing an early onset and very rapid progress. Severe clinical course and high mutant loads are consistent with reported cases with rapid progression and lethal consequences at early childhood [12]. A mutation of m10158T>C was not detected in the mother of Patient 3 in several tissues examined. Q5

We found one patient with ND5 mutation, m13513G>A which has been described as causing MELAS, LS or overlapping features of the two syndromes [13–15]. We also found one LS patient with m14459G>A/*ND6* mutation that was reported in patients with LHON, dystonia [16] and LS [17]. So far, the phenotype of these two patients is LS without MELAS, LHON.

We found two patients with *ATPase6* mDNA mutations, m8993T>G and T>C, that are frequently reported in the literature [8]. A patient with a T>G mutation usually exhibits earlier onset and more rapid progression compared to T>C mutation at m8993 that was compatible with our patients (Table 1). Q6

We found 4 patients carrying nuclear encoded gene mutations. SURF1 deficiency is the most frequent cause of LS with complex IV (cytochrome C oxidase) deficiency [7]. We identified 3 patients with the *SURF1* mutations [18]. Pyruvate dehydrogenase deficiency (PDH) is a common cause of primary congenital lactic acidosis. The biochemical features of PDH deficiency is elevated blood lactate and pyruvate levels with a normal lactate/pyruvate ratio [19]. According to the genetic screening flowchart for Leigh syndrome (Suppl. Fig. 1), we confirmed 1 patient with a hemizygous mutation in the *PDHA1* gene with 7 sets of primers.

Recently, new drugs such as EPI-743 have been shown to improve neurological and neuromuscular symptoms in LS [20,21]. Rapid genetic confirmation of mitochondrial disease may help initiate such treatment early. Next gene sequencing is revealing a wide range of dual mutations both mitochondrial and nuclear gene from patients with mitochondrial disorders [22–24]. However, it is costly and time consuming. Aiming to elucidate genetic basis of LS patients, we screened with our limited set of primers. Surprisingly, it allowed us confirmation for more than half of the patients. Therefore, this method appears to be efficient as a primary genetic screening. Our data also implicates that LS consisted of few "common" causative genes and a large number of "rare" genes. We are now undertaking whole mDNA and exome sequencing for negative cases of this method [22–24]. These data, together with increasing data of mutations, would help us improve our screening method. Q7 Q8

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2014.02.006.

Conflict of interest statement

We have no conflict of interest to disclose.

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Table 1
Genetically determined Leigh syndrome in our institution (2005–2012).

Patient	1	2	3	4	5	6	7	8	9	10	11
Age, gender	6 y, M	0 y, F	9 m, F	7 y, M	11 y, M	1 y†, M	2 y, M	4 y, F	9 y, M	25 y†, M	17 y, M
Type of gene	Mito	Mito	Mito	Mito	Mito	Mito	Mito	Nuclear	Nuclear	Nuclear	Nuclear
Gene	*ND1*	*ND1*	*ND3*	*ND5*	*ND6*	*ATPase6*	*ATPase6*	*SURF1*	*SURF1*	*SURF1*	*PDHA1*
Complex	I	I	I	I	I	V	V	IV	IV	IV	
Mutations	m3697G>A (p.G131S) Homo (b)	m3697G>A (p.G131S) Homo (b,s,h,n)	m10158T>C (p.S34P) Hetero (90%) (b)	m13513G>A (p.D393N) Hetero (50%) (b)	m14459G>A (p.A71V) Homo (b)	m8993T>G (p.L156R) Homo (b)	m8993T>C (p.L156P) Homo (b)	c.49+1G>T c.752–753delAG	c.743 C> A p.A248D c.743C> A p.A248D	c.574C>T p.R192 W c.743C>A p.A248D	c.121T>C p.C41R
Consanguinity	N	N	N	N	N	N	N	N	Y	N	N
Inheritance	Maternal* hetero:40%	N.A.	De novo	N.A.	N.A.	N.A.	N.A.	Maternal/ paternal	Maternal/ paternal	N.A.	N.A.
Age at onset	3 y 9 m	3 y 0 m	0 y 5 m	1 y 6 m	2 y 0 m	6 m	1 y 0 m	1 y 7 m	1 y 9 m	2 y	1 y 0 m
Initial Symptoms	Hypertonia Walk regre	Ataxic gait Walk regre Tremor	Hypotonia Strabismus	Dev. delay	Fever → lethargy	Dev. delay/ seizure Hypotonia/ nystagmus	Fever → lethargy	Ataxic gait	Ataxic gait	Dev. delay Ataxia	Dev. delay
Status	Walk Normal class	Wheelchair Special class	Tracheo Mech. venti	Walk	Wheelchair Normal class	(Respiratory failure)	No sitting	Tracheo Mech. venti	Tracheo Mech. venti	(Respiratory failure)	Walk Special school
RC enzymes ↓	I, IV (m)	I, III, IV (m)	I (f)	Normal (m/f)	I, III (m)	I, IV (m)	N.A.	N.A.	IV (f)	IV (m)	N.A.
Morphological findings in muscle	No RRF	No RRF	N.A.	No RRF	RRF	N.A.	N.A.	N.A.	N.A.	RRF	N.A.

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MRI											
Basal ganglia hyperintensities	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Brainstem hyperintensities	N	Y	Y	N	N	N	Y	Y	Y	Y	N
Cerebellar atrophy	N	N	N	Y	N	N	N	N	N	Y	Y
Symptoms											
Dysmorphisms	N	N	N	N	N	N	N	Y	Y	N	N
Developmental delay	N	N	Y	Y	N	N	Y	Y	Y	Y	N
Regression	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N
Feeding problems	N	N	Y	N	N	Y	N	N	N	N	N
Ptosis	N	N	N	N	N	N	N	Y	N	N	N
Ophthalmople	N	N	Y	N	N	N	N	Y	N	Y	N
Pyramidal symptoms	Y	Y	Y	Y	Y	N	N	Y	Y	N	Y
Extrapyramidal symptoms	Y	Y	Y	Y	N	Y	N	Y	Y	N	Y
Dystonia	Y	Y	Y	N	N	N	N	Y	Y	N	Y
Hypotonia	N	N	Y	Y	N	Y	Y	Y	Y	N	Y
Ataxia	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y
Neuropathy	N	N	N	N	N	N	N	Y	Y	Y	Y
Others				WPW syndrome		West syndrome					Nystagmus

y: year, m: month, M: male, F: female, mito: mitochondria, Complex: complex in oxidative phosphorylation, b: blood, s: saliva, h: hair, n: nail, RC: respiratory chain, m: muscle, f: fibroblast, RRF: ragged red fibers, N.A.: not analyzed/not determined, N: no, negative, Y: yes, positive, regre: regression, Dev. delay: Developmental delay, Mech.venti: Mechanically ventilated, Ophthalmople: Ophthalmoplegia, *: asymptomatic.

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ANNALS of Clinical and Translational Neurology
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RESEARCH ARTICLE

Deficiency of ECHS1 causes mitochondrial encephalopathy with cardiac involvement

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Abstract

Objective: Short-chain enoyl-CoA hydratase (ECHS1) is a multifunctional mitochondrial matrix enzyme that is involved in the oxidation of fatty acids and essential amino acids such as valine. Here, we describe the broad phenotypic spectrum and pathobiochemistry of individuals with autosomal-recessive ECHS1 deficiency. **Methods:** Using exome sequencing, we identified ten unrelated individuals carrying compound heterozygous or homozygous mutations in *ECHS1*. Functional investigations in patient-derived fibroblast cell lines included immunoblotting, enzyme activity measurement, and a palmitate loading assay. **Results:** Patients showed a heterogeneous phenotype with disease

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onset in the first year of life and course ranging from neonatal death to survival into adulthood. The most prominent clinical features were encephalopathy (10/10), deafness (9/9), epilepsy (6/9), optic atrophy (6/10), and cardiomyopathy (4/10). Serum lactate was elevated and brain magnetic resonance imaging showed white matter changes or a Leigh-like pattern resembling disorders of mitochondrial energy metabolism. Analysis of patients' fibroblast cell lines (6/10) provided further evidence for the pathogenicity of the respective mutations by showing reduced ECHS1 protein levels and reduced 2-enoyl-CoA hydratase activity. While serum acylcarnitine profiles were largely normal, in vitro palmitate loading of patient fibroblasts revealed increased butyrylcarnitine, unmasking the functional defect in mitochondrial β-oxidation of short-chain fatty acids. Urinary excretion of 2-methyl-2,3-dihydroxybutyrate – a potential derivative of acryloyl-CoA in the valine catabolic pathway – was significantly increased, indicating impaired valine oxidation. **Interpretation:** In conclusion, we define the phenotypic spectrum of a new syndrome caused by ECHS1 deficiency. We speculate that both the β-oxidation defect and the block in L-valine metabolism, with accumulation of toxic methacrylyl-CoA and acryloyl-CoA, contribute to the disorder that may be amenable to metabolic treatment approaches.

Introduction

Short-chain enoyl-CoA hydratase (ECHS1, synonym: crotonase, EC 4.2.1.17), encoded by *ECHS1* (cytogenetic location: 10q26.3; GenBank accession number: NM_004092.3; OMIM*602292), is a mitochondrial matrix enzyme that catalyzes the second step of the β-oxidation spiral of fatty acids, that is, the hydration of chain-shortened α,β-unsaturated enoyl-CoA thioesters to produce β-hydroxyacyl-CoA.[1] For each turn of this spiral pathway, one acetyl-CoA molecule is released and utilized for either the formation of citrate (tricarboxylic acid [TCA] cycle) or ketone bodies (ketogenesis). Decreased activity of mitochondrial β-oxidation of fatty acids thus decreases the formation of important energy substrates. Decreased formation of acetyl-CoA results in increased susceptibility to energy deficiency during catabolic states and to the dysfunction of organs that particularly rely on fatty acids and ketone bodies as their energy source (e.g., cardiac tissue). In addition, decreased formation of acetyl-CoA, and hence limited availability of acetate, hampers myelination because acetate is required for cholesterol biosynthesis. Moreover, decreased formation of acetyl-CoA may hamper posttranslational acetylation of mitochondrial proteins, a mechanism that is emerging as a critical regulator of mitochondrial function.[2] Evidence is increasing that ECHS1 has a wide substrate specificity and thus also plays an important role in amino acid catabolism, in particular of valine, where it converts methacrylyl-CoA to (S)-3-hydroxyisobutyryl-CoA and acryloyl-CoA to 3-hydroxypropionyl-CoA (Fig. 1), the fourth step of valine oxidation.[3] Accumulation of toxic methacrylyl-CoA and acryloyl-CoA, two highly reactive intermediates that spontaneously react with sulfhydryl groups of, for example, cysteine and cysteamine, is suspected to cause brain pathology and biochemical phenotype in β-hydroxyisobutyryl-CoA hydrolase (HIBCH) deficiency, a disorder of the fifth step of valine oxidation with a Leigh-like phenotype and deficiency of multiple mitochondrial enzymes.[4,5] Very recently, *ECHS1* mutations were reported in two siblings with Leigh disease and remarkable clinical and biochemical similarities to HIBCH deficiency.[6] Both presented soon after birth with generalized hypotonia, poor feeding, respiratory insufficiency, and developmental delay. They suffered a severe clinical course and died at the age of 4 and 8 months.

Here, we report 10 unrelated individuals, identified by exome sequencing, who carry compound heterozygous or homozygous *ECHS1* mutations and present with a combination of (Leigh-like) mitochondrial encephalopathy, deafness, epilepsy, optic nerve atrophy, and cardiomyopathy. This work confirms *ECHS1* mutations as a cause of mitochondrial disease, and defines the broad phenotypic spectrum of this new disorder which ranges from fatal neonatal courses to survival into adulthood.

Patients and Methods

Written informed consent was obtained from all patients investigated or their guardians and the ethics committee

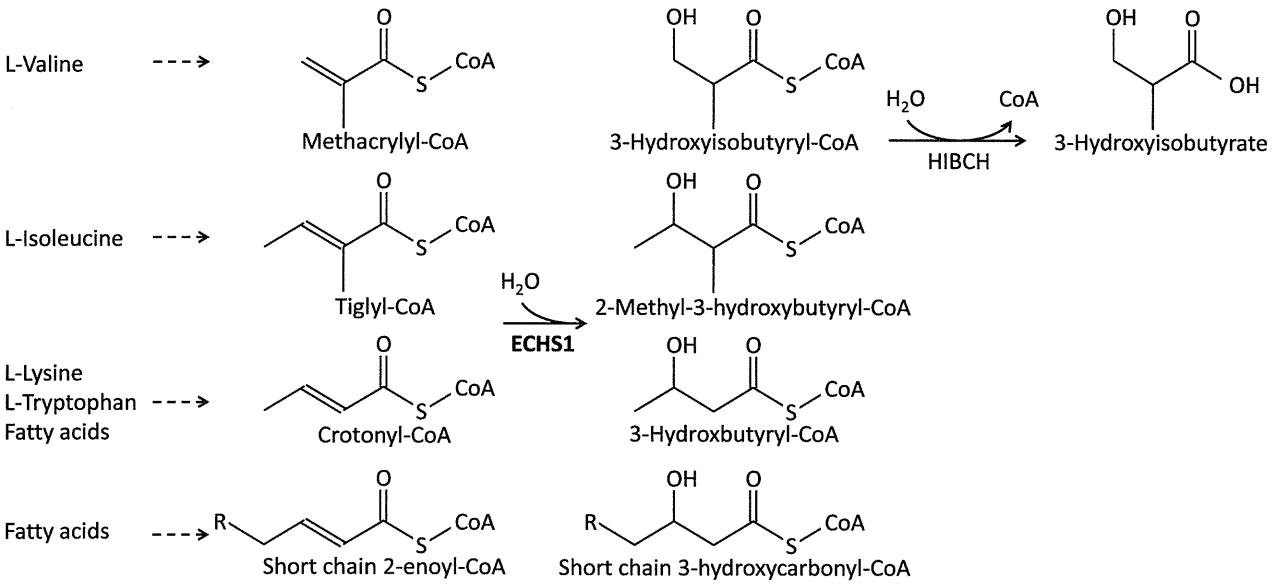

Figure 1. Short-chain enoyl-CoA hydratase (ECHS1) functions. Proposed functions of ECHS1 in the mitochondrial amino acid and fatty acid metabolism with illustration of the level of HIBCH (3-hydroxyisobutyryl-CoA hydrolase) deficiency.

of the Technische Universität München approved the study. The patients tested positive for *ECHS1* mutations are part of a large cohort of cases with suspected mitochondrial disorders. DNA samples have been collected for genetic analyses in three different centers including Bern (Switzerland, 47 cases including family F2), Saitama (Japan, 180 cases including families F1 and F6), and Munich (Germany, 435 cases including families F3-5 and 7-10; Fig. 2). Clinical and biochemical findings of *ECHS1* mutation-positive patients are summarized in Table 1 and representative abnormal magnetic resonance imaging (MRI) findings are shown in Figure 3. In addition, we report on four older siblings who have died undiagnosed with a clinical picture similar to the mitochondrial encephalocardiomyopathy described in their younger siblings with a confirmed diagnosis of ECHS1 deficiency.

Regarding terminology, we avoided the term "Leigh syndrome" or "Leigh-like syndrome" for the whole group of our patients with ECHS1 deficiency, because these are ill-defined entities and many of our patients did not fulfill the criteria suggested by Rahman et al.[7] In those individual cases that fulfilled the definition by Rahman, we preferred to use the more neutral term "Leigh-like syndrome" or "Leigh-like pattern in MRI".

Case reports

Patient #346 (F1, II:2, c.[176A>G];[476A>G], p.[Asn59Ser];[Gln159Arg]), a girl, was born after a normal pregnancy at 39 weeks of gestation with normal birth measurements (weight 2935 g, length 50.5 cm) as the second child of unrelated Japanese and American parents. Soon after birth, she was admitted to a neonatal medical center for severe respiratory and cardiac failure with hypertrophic cardiomyopathy (HCM) and suspected deafness. There was profound lactic acidosis in blood (21–43 mmol/L, lower limit of normal 1.8 mmol/L), but metabolic profiling (amino acid analysis, urine organic acid analysis, acylcarnitine analysis) was unremarkable. Brain MRI at day 8 showed low intensity in cerebral white matter, and moderate brain atrophy at day 58. She died at the age of 4 months and autopsy was performed. Respiratory chain analysis showed mild deficiency of complex I in liver, but normal activities in muscle and heart. Her older sister died due to respiratory failure and severe lactic acidosis on her first day of life.

Patient #42031 (F2, II:1, c.[197T>C];[449A>G], p.[Ile66Thr];[Asp150Gly]), a boy, is the first child of healthy nonconsanguineous Swiss parents. After a normal pregnancy, he was born at 42 weeks of gestation with normal birth measurements. Postnatally, he developed lactic acidosis and neonatal seizures. Analysis of fibroblasts showed reduced pyruvate oxidation compatible with a complex I or pyruvate dehydrogenase defect, whereas enzymatic activities of the respiratory chain in muscle and fibroblasts were normal. These findings led to a therapeutic trial with ketogenic diet. The diet was stopped after a few months as no clinical response was observed. At 5 months of age, he had severely delayed motor development, hardly any head control, severe truncal hypotonia and intermittent episodes of opisthotonus. There was no reaction to visual or auditory stimuli. Despite gastric tube feeding, the child was severely underweight (−2.8 SD), of short stature (−3.4 SD), and microcephalic (−4.9 SD).

Laboratory investigation revealed persistently elevated lactate (2.4–6.0 mmol/L), mildly elevated CK and repeatedly normal acylcarnitines. Repeated electroencephalograms did not show epileptic discharges. Brain MRI at age 17 days showed normal myelinisation but symmetrical punctiform hyperintensities in the centrum semiovale. MR spectroscopy of basal ganglia showed elevated lactate. Ophthalmological examination suggested bilateral optic atrophy, and acoustic evoked potentials confirmed severe sensorineural deafness. At the age of 11 months, the child was found dead in his bed. Autopsy revealed morphological and histological findings of subacute necrotizing encephalopathy (Fig. 3E) and massive left ventricular hypertrophy.

Patient #68552 (F3, II:6, c.[476A>G];[476A>G], p.[Gln159Arg];[Gln159Arg]), a girl, is the sixth child of first cousins of Pakistani origin. Shortly after birth, the infant was found hypotonic with poor feeding and with high lactate (5.1 mmol/L). She was extremely irritable and had episodes of stiffness but electroencephalography (EEG) was normal. Even so, the baby was started on antiepileptic drug therapy as well as on baclofen. She was fed continuously by nasogastric tube. She showed no developmental progress nor developed any meaningful interaction with her environment and her irritability worsened episodically. Palliative care was instituted and she died aged 2 years and 4 months.

Brain MRI showed symmetrical white matter changes with a periventricular focus and extension into the subcortical areas of the frontal and parietal lobes. The thalami as well as the caudate and lentiform nuclei appeared normal. MR spectroscopy of basal ganglia showed no obvious lactate peak. Neonatal adrenoleukodystrophy, biotinidase deficiency, Krabbe disease, GM1 gangliosidosis and metachromatic leukodystrophy were excluded biochemically. There were no significant abnormalities of acylcarnitines, organic acids, glycosaminoglycans, oligosaccharides or amino acids (except a raised alanine in keeping with lactic acidosis). Invasive investigations such as muscle biopsy were refused.

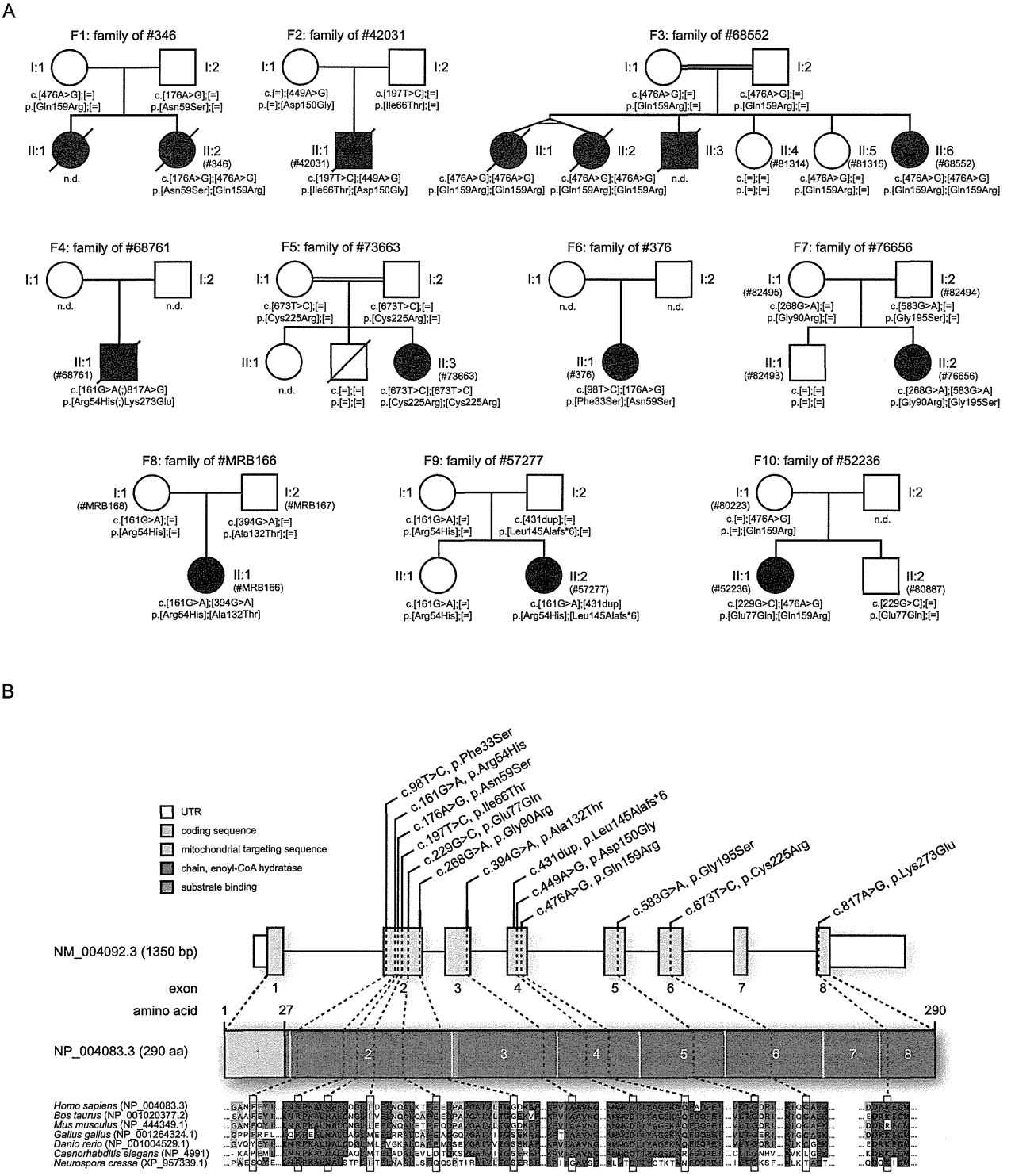

Figure 2. Pedigrees of investigated families and short-chain enoyl-CoA hydratase (*ECHS1*) structure and conservation of identified mutations. (A) Pedigrees of 10 families with mutations in *ECHS1*. Mutation status of affected (closed symbols) and unaffected (open symbols) family members. (B) Gene structure of *ECHS1* with known protein domains of the gene product and localization and conservation of amino acid residues affected by mutations. Intronic regions are not drawn to scale.

Table 1. Genetic and clinical findings in patients with *ECHS1* mutations.

ID	Sex	*ECHS1* mutations cDNA (NM_004092.3) protein (NP_004083.3)	Biochemical investigations		Clinical and biochemical features											
			Analysis	Result	AO	Course	Neuroimaging (MRI, MRS)	Hearing loss	Optic atrophy	Devel. delay	Epilepsy	Dystonia	Cardio-myopathy	Elevated lactate	2-methyl-2,3-dihydroxybutyrate	Others
F1, II:2 #346	F	c.[176A>G]; [476A>G] p.[Asn59Ser]; [Gln159Arg]	RCCI-V[2]	CI mildly↓	Birth	Died at age 4 m	At age 8 d: white matter changes, brain atrophy (Fig. 3A), more prominent until age 58 d	Yes	n.k.	n.k.	Yes	n.k.	HCM	Yes	n.d.	Unrelated parents, 1 older sister died at age 1 m
F2, II:1 #42031	M	c.[197T>C]; [449A>G] p.[Ile66Thr]; [Asp150Gly]	RCCI-V[+1] PDHc[+1] Substrate oxidation[1]	Normal Normal Pyruvate↓	Birth	Died at age 11 m	At age 17 d: normal myelinisation, symmetrical punctiform hyperintensities in centrum semiovale MRS: lactate ↑	Yes	Yes	Yes	Yes	Yes	HCM	Yes	229-fold	Autopsy revealed subacute necrotizing encephalopathy (Fig. 3E) and massive left ventricular hypertrophy.
F3, II:6 #68552	F	c.[476A>G]; [476A>G] p.[Gln159Arg]; [Gln159Arg]	n.d.	n.d.	Birth	Died at age 2.3 y	Symmetrical white matter changes	n.k.	n.k.	Yes	Yes	Yes	n.k.	Yes	n.d.	Consanguineous parents, 3 older siblings died before age 2 y, RCCI defect in muscle in tow of them
F4, II:1 #68761	M	c.[161G>A(;) 817A>G] p.[Arg54His(;) Lys273Glu]	RCCI-V ATP production	Normal Decreased	Birth	Died at age 7.5 y	At age 4 y: extensive brain atrophy MRS: normal lactate	n.k.	n.k.	Yes	Yes	Yes	No	n.k.	n.d.	Died in the course of a pulmonary infection
F5, II:3 #73663	F	c.[673T>C]; [673T>C] p.[Cys225Arg]; [Cys225Arg]	RCCI-V PDHc Substrate oxidation	Normal Normal Normal	Birth	Alive at age 2 y	Delayed myelination, T_2-hyperintense periphere white matter lesions, liquorisointense lesions in	n.k.	n.k.	Yes	Yes	No	HCM	Yes	39-fold	Consanguineous parents, a brother of this girl died at age 4 m

(*Continued*)

Table 1. Continued.

ID	Sex	*ECHS1* mutations cDNA (NM_004092.3) protein (NP_004083.3)	Biochemical investigations		Clinical and biochemical features										
			Analysis	Result	AO	Course	Neuroimaging (MRI, MRS)	Hearing loss	Optic atrophy	Devel. delay	Epilepsy	Dystonia	Cardio-myopathy	Elevated lactate	2-methyl-2,3-dihydroxybutyrate	Others
							putamen and *pallidum*									
F6, II:1 #376	F	c.[98T>C]; [176A>G] p.[Phe33Ser]; [Asn59Ser]	RCCI-IV	CIV mildly↓	Birth	Alive at ag e 3 y	Symmetrical bilateral signal abnormalities in basal ganglia (Fig. 3B)	Yes	n.k.	Yes	Yes	n.k.	DCM	Yes	n.d.	n.a.
F7, II:2 #76656	F	c.[268G>A]; [583G>A] p.[Gly90Arg]; [Gly195Ser]	RCCI-IV[1] PDHc[1]	Normal Normal	2 y	Alive at age 5 y	At age 2 y: no atrophy, but signal hyperintensities of *putamen, globus pallidus, nucleus caudatus* and periventicular white matter MRS: lactate ↑	Yes	No	Yes	No	Yes	n.k.	n.d.	sixfold	n.a.
F8, II:1 #MRB166	F	c.[161G>A]; [394G>A] p.[Arg54His]; [Ala132Thr]	n.d.	n.d.	1 y	Alive at age 8 y	n.a.	Yes	No	Yes	No	No	n.k.	Yes	n.d.	Gastroschisis, truncal ataxia, muscular hypotonia, increased muscle tonus, cochlear implant
F9, II:2 #57277	F	c.[161G>A]; [431dup] p.[Arg54His]; [Leu145Alafs*6]	RCCI-IV Pyruvate oxidation	Normal Normal	Birth	Alive at age 16 y	At age 1.5 y: increased T2-signal intensity in *putamen* and *globus pallidus* which became more prominent until age 2.2 years (Fig. 3C) MRS: lactate ↑	Yes	Yes	Yes	No	Yes	No	Yes	n.d.	Communicates through a voice computer at age 16 y

(*Continued*)

Table 1. Continued.

ID	Sex	*ECHS1* mutations cDNA (NM_004092.3) protein (NP_004083.3)	Biochemical investigations		Clinical and biochemical features											
			Analysis	Result	AO	Course	Neuroimaging (MRI, MRS)	Hearing loss	Optic atrophy	Devel. delay	Epilepsy	Dystonia	Cardio-myopathy	Elevated lactate	2-methyl-2,3-dihydroxybutyrate	Others
F10, II:1 #52236	F	c.[229G>C]; [476A>G] p.[Glu77Gln]; [Gln159Arg]	RCCI-IV	Normal	11 m	Alive at age 31 y.	At age 15 y: no atrophy, but signal hyperintensities in *nucleus caudatus* and *putamen* (Fig. 3D) MRS: normal lactate	Yes	Yes	Yes	Yes	Yes	No	Yes	Normal	Spastic tetraparesis, confined to wheelchair from age 9.5 y

ECHS1, short-chain enoyl-CoA hydratase; MRI, magnetic resonance imaging; AO, age of onset; m, months; y, years; n.a. not applicable; HCM, hypertrophic cardiomyopathy; DCM, dilated cardiomyopathy; n.d. not determined; n.k., not known, Mitochondrial respiratory chain complexes (RCC) in muscle: I, NADH:CoQ oxidoreductase; II, succinate dehydrogenase; II + III, succinate:cytochrome c reductase; IV, cytochrome c oxidase (COX).
Enzyme activities were determined in muscle biopsies if not a stated otherwise ([1]investigated in fibroblast cell lines; [2]investigated in liver) and normalized to citrate synthase (CS).

Molecular genetic screening demonstrated a maternally transmitted m.1555A>G *MTRNR1* mtDNA mutation that is characteristically associated with aminoglycoside-induced hearing loss. This was not felt to be responsible for the patient's condition.

Three older siblings of this patient had congenital lactic acidosis and died between the ages of 1 and 2 years. The first two affected children were identical twin girls and the third affected child was a boy. The history is identical in all three children. They were all born at term via normal vaginal delivery. There was no history of birth asphyxia and they were apparently well soon after birth. They fed well initially but became symptomatic aged between 1 and 2 days with generalized tonic-clonic seizures. Subsequently, they had poor feeding and severe developmental delay. The seizures were recurrent but well controlled with anticonvulsants. They were tube-fed from the first few days of life as they were unable to suck and swallow effectively. They had severe developmental delay from the outset and showed little evidence of development during infancy. They always had a poor head control, poor eye contact, and a poor smile. They were never able to reach out and their hearing was possibly impaired. There was no history of dystonia and they were said to be very hypertonic. There were no breathing problems reported. They apparently had no renal tubular acidosis but they required some treatment with bicarbonate for metabolic acidosis (lactate levels of ~5.0 mmol/L). There was no history of cardiac or liver involvement. The twin girls died aged 2 years and the boy died aged 1 year. In the twin girls, a diagnosis of complex I deficiency was established on muscle biopsy respiratory chain enzyme analysis. Testing of *ECHS1* performed on newborn screening bloodspots of the twin older siblings (F3, II:1 and II:2) confirmed that they were also homozygous for the c.476A>G, p.Gln159Arg variant. The children were treated with sodium bicarbonate, anticonvulsants, and a mitochondrial vitamin cocktail as well as nasogastric tube feeding. The family has two other healthy girls, now teenagers.

Patient #68761 (F4, II:1, c.[161G>A(;)817A>G] p.[Arg54His(;)Lys273Glu]), a boy, is the first child to healthy unrelated parents from The Netherlands. After a normal pregnancy, he was born at gestational age 39 + 1 weeks by Cesarean section on maternal indication with a birth weight of 3990 g. Apgar scores were 8 after 1 min and 9 after 5 min. Mild generalized muscular hypotonia was observed upon birth which was accompanied by feeding problems until the age of 5 months. Thereafter, his clinical condition declined and he developed severe encephalopathy with hardly any spontaneous movements of the head and trunk, swallowing problems, and episodes of inconsolable crying. He depended on

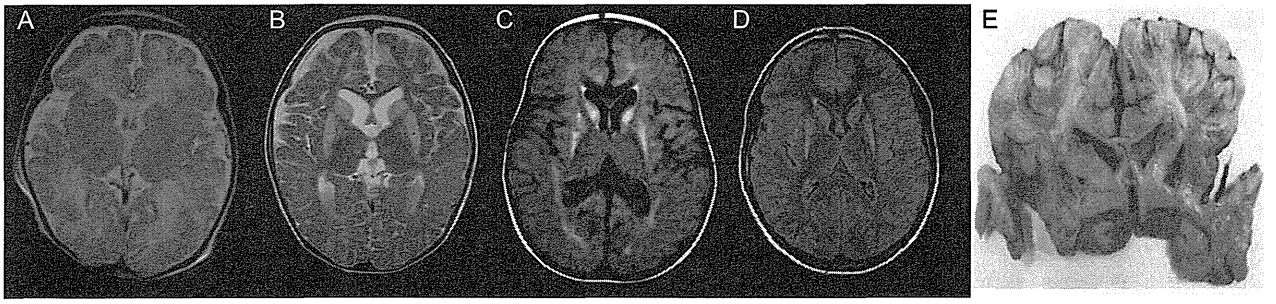

Figure 3. Spectrum of brain MRI and autopsy changes in ECHS1 patients. (A) MRI (T2) at age 8 days in individual F1, II:2 showing widespread diffuse white matter changes and brain atrophy. (B) MRI (T2) at age 8 months in individual F6, II:1 (#376) showing brain atrophy and bilateral symmetric signal hyperintensity in *caput nucleus caudatus* and *putamen*. (C) MRI (FLAIR) at age 2.2 years in individual F9, II:2 (#57277) showing increased signal in *putamen, globus pallidus* and *caput nucleus caudatus*. (D) MRI (FLAIR) at age 15 years in individual F10, II:1 (#52236) showing bilateral symmetric signal hyperintensity in *caput nucleus caudatus* and *putamen*. (E) Autopsy at age 11 months in individual F2, II:1 (#42031) showing necrotizing encephalopathy of the caudate and lenticular nuclei. MRI, magnetic resonance imaging; ECHS1, short-chain enoyl-CoA hydratase.

tube feeding and suffered from epilepsy with hypsarrhythmia and multifocal epileptic activity in the EEG at the age of 1 and 3 years.

On physical examination, he was unable to make contact or follow objects, showed virtually no spontaneous movements, but only dystonic movements of arms and legs. Over the course of the disease axial hypotonia and hypertonia of the limbs persisted, dystonic movements diminished, and he did not make any developmental progress. Laboratory tests showed chronic iron deficiency leading to anemia. Physical examination at the age of 6 years showed microcephaly, scoliosis, and muscular hypotonia. The child died at the age of 7.5 years due to respiratory insufficiency in the course of a pulmonary infection.

Brain MRI at the age of 1 year showed atrophy of caudate nuclei, corpus callosum, mesencephalon, and pons. These changes were progressive at age 4 years showing extensive white and gray matter brain atrophy, mainly in frontal and temporal lobes bilaterally with subsequent widening of the subarachnoid space and of ventricular system. Brain MR spectroscopy showed an overall decrease in NAA being most pronounced in the basal ganglia but no elevation of lactate.

Cardiac ultrasound at the ages of 1 and 4 years showed no structural or functional abnormalities. Biochemical analysis of a skeletal muscle specimen showed normal citrate synthase (CS)-adjusted activities of respiratory chain complexes I–V but a decreased overall ATP production. Pathogenic mutations of the mitochondrial DNA were excluded by Sanger sequencing of DNA from muscle.

Patient #73663 (F5, II:3, c.[673T>C];[673T>C], p.[Cys225Arg];[Cys225Arg]), a girl, was born at term as the third child of consanguineous parents after normal pregnancy and spontaneous vaginal delivery. On day 5, she was admitted to hospital due to rapid loss of body weight (24% below birth weight) and severe metabolic acidosis (pH 6.86). Lactate, alanine, and ketone bodies were also strongly increased leading to the suspicion of an inherited disorder of mitochondrial energy metabolism. Following this neonatal decompensation she showed a severe global development delay, severe generalized spastic tetraparesis, myoclonic epilepsy, and HCM. At age 16 months, she had a cardiac arrest following a diagnostic muscle and skin biopsy. She survived after 45 min of cardiopulmonary resuscitation but several complications followed this event (ARDS, sepsis, aspiration pneumonia, acute renal failure, and acute hepatic failure). At age 2.3 years she does not show active movement of arms and legs and is not able to sit, stand or walk. She neither speaks nor fixes or follows persons and objects. She reacts to voices and noise.

Cranial MRI performed at age 13 months showed delayed myelination, a thin corpus callosum, and T2 signal abnormalities in the periventricular white matter. Lesions in *putamen* and *globus pallidus* were also found. MR spectroscopy of gray and white matter was normal. Metabolic work-up revealed elevated serum lactate (up to 8.5 mmol/L), moderately elevated plasma alanine (up to 630 μmol/L), slightly elevated ethylmalonic acid in urine (60 mmol/mol creatinine), and intermittently low plasma ketone bodies in preprandial state. Hyperuricemia was found. Acylcarnitine profiling was normal. Radiometric and single enzyme analysis of OXPHOS in frozen muscle tissue did not confirm the suspected diagnosis of a mitochondrial disorder.

A brother of this girl has died at age 4 months during an acute decompensation similar to that described above. He also had severe developmental delay, elevated lactate and metabolic acidosis. Similar to his younger sister, liver,

