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Incidence of primary mitochondrial disease in children presenting with acute liver failure under 2 years of age

Patrick **McKiernan**[1,4], Sarah **Ball**[2], Saikat **Santra**[2], Katherine **Foster**[3], Carl **Fratter**[5], Joanna **Poulton**[6], Kate Craig[7], Robert **McFarland**[7], Shamima **Rahman**[8], Iain **Hargreaves**[8], Girish **Gupte**[1,4], Khalid **Sharif**[1], Robert W. **Taylor**[7].

[1]Liver Unit, [2]Clinical IMD and [3]Radiology, Birmingham Children's Hospital, Birmingham ; [4]University of Birmingham, Birmingham; [5]Oxford Medical Genetics Laboratories, Oxford University Hospitals NHS Foundation Trust; [6]Nuffield Department of Obstetrics and Gynaecology, University of Oxford; [7]Wellcome Trust Centre for Mitochondrial Research and Highly Specialised Rare Mitochondrial Disease Service, Newcastle University; [8]Genetics and Genomic Medicine, UCL Institute of Child Health, London, United Kingdom.

Correspondence to:

Dr PJ Mc Kiernan, Professor of Pediatrics

University of Pitttsburgh, Director of Hepatology, Division of Gastroenterology/Hepatology/Nutrition, Children's Hospital of Pittsburgh of UPMC

4401 Penn Avenue

Pittsburgh, PA 15224

Email: patrick.mckiernan@chp.edu

Phone: +1412-692-5326, Fax:+1 412-692-7355

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Abstract

Mitochondrial liver disease (MLD), and in particular mitochondrial DNA (mtDNA) depletion syndrome (MDS) is an important cause of acute liver failure (ALF) in infancy. Early and accurate diagnosis is important since liver transplantation (LT) is often contraindicated. It is unclear which methods are the best to diagnose MLD in the setting of ALF.

Objective. To determine the incidence of MLD in children under two with ALF and the utility of routine investigations to detect MLD.

Methods. Thirty-nine consecutive infants with ALF were admitted to a single unit from 2009-11. All were extensively investigated using an established protocol. Genes implicated in MDS were sequenced in all cases and tissue mtDNA copy number measured where available.

Results. Five infants (17%) had genetically proven MLD: *DGUOK* (n=2), *POLG* (n=2) and *MPV17* (1). Four of these died whilst one recovered. Two had normal muscle mtDNA copy number and 3 had normal muscle respiratory chain enzymes. An additional 8 children had low hepatic mtDNA copy number but pathogenic mutations were not detected. One of these developed fatal multisystemic disease following LT while five who survived remain well without evidence of multisystemic disease up to 6 years later. Magnetic resonance spectroscopy did not distinguish between those with and without MLD.

Conclusions.

Low liver mtDNA copy number may be a secondary phenomenon in ALF.

Screening for mtDNA maintenance gene mutations may be the most efficient way to confirm MLD in ALF in the first two years of life.

Key words: Acute liver failure, Mitochondrial disease, Respiratory chain deficiency, Mitochondrial DNA depletion syndrome, Liver transplantation

What is known

- Mitochondrial liver disease is an important cause of infantile liver failure
- The most effective way to diagnose mitochondrial liver disease in the setting of liver failure is unclear

What is new

- Low hepatic mitochondrial DNA copy number may be a consequence of liver disease rather than a cause of liver disease.
- Screening for known mutations causing mitochondrial liver disease may be the best diagnostic strategy.

Introduction

Acute liver failure (ALF) in infancy is a rare and devastating disease which has a very poor outcome without liver transplantation (LT). In approximately 20% of cases, infantile liver failure is caused by genetic mitochondrial liver disease (MLD) (1-3) with the commonest single entity being mitochondrial DNA (mtDNA) depletion syndrome (MDS). Mitochondria contain multiple copies of mtDNA. MDS is caused by mutations in nuclear genes involved in mtDNA replication or in the maintenance of the deoxynucleotide pools required for *de novo* mtDNA replication, resulting in a quantitative loss of mtDNA copy number (4). Pathogenic mutations causing hepatocerebral MDS have been described in a number of genes to date with the commonest reported being; - *DGUOK(5)*, *POLG(6)*, *MPV17(7)* and *PEO1* (encoding the Twinkle helicase)(8).

Normal mitochondrial function is contingent upon the expression of many other nuclear genes which encode constituent proteins of the respiratory chain, proteins needed for assembly of the respiratory chain or for translation of mtDNA-encoded proteins(9)**.** Mutations in these genes can also cause MLD and in particular mutations in *TRMU*, which encodes an enzyme essential for post-transcriptional modification of mitochondrial tRNAs, can cause infantile ALF. Such cases are particularly important to recognise as there is a significant chance of spontaneous recovery with supportive treatment. (10;11)

Definitive diagnosis of most nuclear encoded mitochondrial disorders is best established by recognizing two pathogenic mutations in known disease causing genes. However, in the absence of an informative family history this is time consuming. In the absence of a genetic diagnosis, laboratory diagnosis requires demonstrating abnormal respiratory chain function and/or loss of mtDNA copy number in clinically-relevant tissue(s).(12) The most commonly sampled tissue is muscle as it is easily accessible with well-established normal ranges(12), although in multisystemic presentations of mitochondrial disease, muscle respiratory chain

activities and mtDNA levels may be normal and muscle biopsy will *a priori* fail to detect isolated hepatic disease.(6) Consequently liver biopsy is often necessary, if feasible. However abnormalities of respiratory chain function or of mtDNA copy number in damaged liver tissue may not be due to genetic mitochondrial disease but may be a secondary change due to liver disease of other causes.(13)

The aim of this study was to to determine the incidence of genetic mitochondrial disease in a group of children presenting with ALF aged less than two years and to determine the utility of routine investigations to detect mitochondrial disease.

This study was registered as an audit of clinical practice at Birmingham Children's Hospital NHS Trust. Children were investigated and managed according to an established in house clinical protocol (see Supplemental Digital Content 1, Protocol, *http://links.lww.com/MPG/A753*) and the study was registered as an audit of clinical practice at Birmingham Children's Hospital NHS Trust.

Methods

Methods are available online as Supplemental Digital Content 2 (*http://links.lww.com/MPG/A754*).

Results

A total of 39 infants (20 female, 19 male) presented with ALF during the study period. Ethnicity was white (30), Asian (5) and black (4). Four were born prematurely and median birth weight was 2.7 kg (range 1.8-4.1). Median age at presentation was 17 days (1-689). There had been no affected siblings in these families previous to the study. There were two sets of siblings included, one being twins. Three children were from consanguineous families. Overall 10 infants died without LT during the acute illness and 18 survived without LT

(Table 1). Eleven underwent LT of whom three died in the early post-operative period. There were 2 later deaths following transplantation; one from progressive multisystemic disease after 3 months and one from vascular complications 1 year later. One child with ornithine transcarbamylase (OTC) deficiency underwent elective LT 1 year after presentation because of metabolic instability and remains well 3 years later. One child (Subject 37) had recurrent episodes of ALF and he was subsequently shown to have mutations in NBAS (Neuroblastoma Amplified Sequence) causing recurrent acute liver failure syndrome (RALF).(16)

The results of diagnostic investigations are summarised in Table 1. The largest aetiological group was infection, accounting for 12 cases, including proven HSV in 8 cases, enterovirus in 3 and adenovirus in one. Four had inborn errors of metabolism; galactosaemia (2), OTC (1), RALF (1); four had neonatal haemochromatosis phenotype and 12 cases were indeterminate despite an extensive diagnostic work up.

Five children (13%) were found to have genetically confirmed MLD all of whom had MDS. All were born at full term after normal pregnancies. Three were born to consanguineous parents. Median age at presentation was 110 days (9 days to 23 months). The genetic causes were mutations in *DGUOK* (2), *POLG* (2) and *MPV17* (1).

Four of the five children with genetic MLD showed rapid deterioration and died within 3 weeks of presentation. One child who was homozygous for a p.(Leu304Arg) mutation in *POLG* presented at 18 months old, recovered with supportive treatment only and remains well without evidence of liver disease 6 years later.

In addition, there was one unexplained case (subject 24) with some features of genetic MLD. This was a female infant who became jaundiced and unwell on the first day of life. She developed progressive encephalopathy and coagulopathy with peak INR of 3.5. Muscle biopsy showed steatosis and mtDNA depletion studies were borderline in both muscle (49%)

and liver (39%). Cranial MRI showed features of cerebral oedema only. She underwent LT aged 23 days. She made an initial smooth recovery but when aged three months developed evidence of cardiomyopathy and died of progressive systemic disease two months after LT. No evidence of a genetic cause of MLD was found.

Clinical and laboratory features of the infants with genetically proven MLD compared to those with other causes of ALF are summarised in Table 2. Children with MLD tended to have lower birth weight and presented later but these differences were not significant. Similarly, there were no significant differences in the presenting laboratory values between the 2 groups. Although the median plasma lactate levels were similar between the groups, all infants with MLD had abnormal lactate values while these were initially normal in 9/34 without MLD.

Results of tissue studies and radiology are listed in Table 1 and summarised in Table 3. Liver histology was available in 21 cases. The dominant lesion was hepatocyte necrosis in 13 cases and this was accompanied by microvesicular steatosis in three cases. Including these three cases, significant microvesicular steatosis was present in eight cases overall. Three who had genetically-confirmed MLD had liver histology available and all showed microvesicular steatosis. The remaining four biopsies showed established fibrosis/cirrhosis (3) and unexplained macrophage storage material respectively.

Liver mtDNA copy number results were available in 17 cases, two of whom had genetically proven MLD due to *DGUOK*. These two children had low (15%) and borderline (37%) liver mtDNA copy number. In 15 children without MLD, seven had normal mtDNA copy number in liver and eight had low levels of mtDNA; depletion (4) and borderline depletion (4). The causes of ALF in these eight children with decreased mtDNA copy number without genetically proven MLD were: indeterminate in six and one each of neonatal hemochromatosis and enterovirus infection. Two of these children died, two recovered

without LT and four underwent successful LT. One child, referred to earlier, underwent successful LT but died from apparent multisystemic disease 2 months later. None of the five survivors have shown evidence of multisystemic disease after up to six years follow up. Muscle biopsies were available in 12 cases. None showed specific changes suggestive of mitochondrial involvement such as ragged-red fibres. Increased intrafibre lipid was found in four of five children with MLD who underwent muscle biopsy but was only found in one of seven children without MLD. This latter child was the one who died of a multisystemic disease post LT. Muscle mtDNA copy number data were available in 11 cases, four of whom had MLD. Two children with MLD had low mtDNA copy number; one of these had complex IV deficiency in muscle tissue and one had normal enzyme activities. Two children with MLD had normal mtDNA copy number, and both also had normal respiratory chain activity. Six of seven children without MLD had normal mtDNA copy number and, in the 4 cases where these were measured, they also had normal respiratory chain enzyme activities. One had an ambiguous muscle mtDNA copy number (47%).

A total of 15 children underwent cranial magnetic resonance imaging (MRI) with diffusion-weighted imaging and 10 had magnetic resonance spectroscopy (MRS). All five children with MLD had MRI imaging which in one case (who had *POLG* mutation) showed symmetrical posterior midbrain changes similar to those reported in mitochondrial disease.(4) Three showed cerebral oedema which had a cytotoxic or demyelination pattern in two cases and a vasogenic pattern in one. Two children had an initial normal MRI, but in one case repeat MRI showed progression to vasogenic cerebral oedema. Ten children without MLD had an MRI which was normal in three and showed cerebral oedema in seven, appearing cytotoxic in two and vasogenic in five. Five children with MLD had MRS which showed a lactate peak in three. Five children without MDS had MRS which showed a lactate peak in two.

Discussion

Infantile ALF is a serious disorder with a variety of potential causes. A structured, rapid approach to diagnostic investigations in tandem with identifying and treating correctable disorders is necessary. We have confirmed that MLD is an important cause of infantile ALF and that genetically confirmed MDS is the commonest entity in this group. The outlook for affected infants is very poor and early recognition is important in order to minimise unnecessary invasive investigations, to prevent inappropriate LT and to facilitate family counselling. Ideally, diagnostic investigations should be available within days of presentation. The definitive method to diagnose MLD is by detection of two pathogenic mutations in recognised genes, hence some attempt at targeted mutation detection should be initiated at the time of initial presentation. This could later be reassessed if other diagnostic information becomes available.

In the absence of pathogenic disease-causing mutations, the diagnosis of MLD depends on tissue studies. It has been a *sine qua non* in the investigation of suspected mitochondrial disease that an affected tissue should be studied. Our findings cast doubt on this approach in the setting of ALF. We have found that reduced mtDNA copy number in affected liver tissue is not synonymous with genetically proven MDS. In three of the eight cases reported here plausible alternative causes of ALF were found. In the five unexplained cases we cannot definitely exclude mitochondrial disease as undetected genetic disorders may yet be present. However, for at least four of these cases, primary mitochondrial disease seems unlikely; no pathogenic mutations have been detected and no other evidence to support progressive mitochondrial disease has appeared even after prolonged follow up. One of these children, who developed a multisystemic disease post LT, did have some features of systemic mitochondrial disease but no genetic cause was detected.

There have been few studies examining the accuracy of low hepatic mtDNA copy number to diagnosis MLD where the primary presentation is with clinical liver disease. In end stage liver disease some studies have shown that low mtDNA copy number appeared to be specific for MDS(17), but in another study 10/45 unselected cases undergoing LT had low copy number.(18) Low ntDNA copy number has also been reported in Mauriac syndrome where the clinical findings are often reversible (19). In ALF low copy number appears to be very common irrespective of aetiology. Helbling et al(17) found low mtDNA copy number in 29 of 44 patients with ALF and all 3 cases reported by Lane et al(18) had decreased number. Decreased copy number were found even where a plausible non-mitochondrial cause of ALF existed. In contrast, Al-Hussaini and colleagues(1) found hepatic mtDNA copy number to be specific for MDS, but only 4 children where liver disease did not have a mitochondrial cause were studied.

Overall these reports are consistent with our findings and suggest that liver disease, and especially ALF, may cause a secondary lowering of mtDNA copy number as a consequence of the primary disease. We cannot exclude that as yet undetected mutations in other genes underlie these examples of mtDNA depletions. An important part of this study however is the length of subsequent follow up which makes late sequela of unrecognized disease less likely. We also cannot comment as to whether the low mtDNA copy number contribute to the pathogenesis of ALF in these cases. What we can say is that clinical management decisions, including whether to proceed with transplantation, should not be influenced by hepatic mtDNA copy number in the absence of proven mutations.

Rapid detection of pathogenic mutations in candidate genes remains the ideal method for diagnosis of MLD. The commonest causes of MLD are recessively-inherited mutations in

DGUOK, *POLG*, *MPV17*, *PEO1* and *TRMU*. (1;7;20) Certainly, screening for mutations in these genes should be initiated at presentation with infantile ALF. The prioritisation of genes to screen will depend on local experience and available facilities, while recognising that this approach will only recognise a proportion of defects.

Up to 1300 nuclear genes encode mitochondrial related proteins and the basis of many defects remain unknown(20). It is to be hoped that next generation screening (NGS) techniques, including custom captures of specific nuclear-mitochondrial genes or whole exome (WES) or whole genome sequencing (WGS), will transform this situation. For example, it is now possible to sequence the entire mitochondrial genome and all coding exons of the nuclear genes encoding mitochondrial proteins. Initial experience using this approach for children with suspected mitochondrial disease achieved a firm diagnosis in 24% of cases and a probable cause in a further 30%.(21) The major future challenge will be to ensure NGS results can be made available in a clinically relevant timescale i.e. within days if possible, and certainly within a fortnight, although this will vary according to local practice and laboratory diagnostic algorithms.

Even establishing a molecular diagnosis does not absolutely establish prognosis. While 4 of the 5 cases showed rapid progression and death from systemic disease, one child with recessive *POLG* mutations recovered spontaneously; interestingly, she was homozygous for the p.(Leu304Arg) mutation that is usually associated with a late-onset *POLG* phenotype of sensory ataxic neuropathy with dysarthria and ophthalmoplegia rather than liver disease.(22) This mutation has been reported to cause ALF in compound with a second (p.(Ala467Thr)) heterozygous *POLG* mutation(23) which supports the observation of Tzoulis *et al* that compound heterozygosity often has a worse prognosis than homozygous *POLG*

mutations.(24) Recent work has suggested that the pattern of mtDNA when visualized by fluorescence microscopy in cultured fibroblasts may also provide further prognostic information.(14) Spontaneous recovery from ALF has been previously recognised in at least one other child with *POLG* mutations(25) and emphasizes that, while LT is inappropriate in this group, these patients should not be denied appropriate supportive treatment.

Recognising and defining CNS involvement in MLD is crucial in order to guide prognosis and management. In ALF from other causes CNS involvement with encephalopathy is common and is generally reversible following successful LT. However in MLD such involvement may be a contraindication to LT. MRI abnormalities are common, but not invariable, in MLD and range from widespread generalised white matter changes to cortical atrophy to specific involvement of deeper brain structures.(1;4;26) These latter appear to be more specific for MLD but were found in only one of our cases. We found generalised abnormalities were common in ALF irrespective of cause and that there was a similar distribution between cytotoxic and vasogenic cerebral oedema whether or not liver failure was due to MLD. Similarly, MRS detection of a lactate peak did not provide useful discrimination between mitochondrial and non-mitochondrial causes. We did confirm that MRI changes may develop and evolve very quickly and that serial evaluation may be necessary. However, in this group of ill infants MRI only helped the decision on appropriateness of LT in a small proportion of cases.

In conclusion, we have shown that MLD is an important cause of infantile ALF and that mutation detection is the most robust diagnostic method.

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Table 1. Investigations and clinical outcome of 39 infants with acute liver failure admitted to Birmingham Children's Hospital from 2009-11

Subject No.	Diagnosis	Age at presentation (days)	Initial serum lactate (mmol/l)	Muscle Histology	Muscle respiratory chain activity	Muscle mtDNA copy number (%)	Liver Histology	Liver mtDNA copy number (%)	Cranial MRI	MR spectroscopy	Mutations detected	Outcome
1	MDS (*DGUOK)*	9	7.2	Increased lipid	Decrease in complex IV	45.0	Micro and macrovesicular steatosis	37.0	Vasogenic pattern cerebral oedema	Lactate peak	p.[Glu44Lys];[Glu44Lys]	Died.
2	MDS (*DGUOK)*	16	25.0	Increased lipid	Normal	50.0	Hepatocellular necrosis and microvesicular steatosis	15.0	Vasogenic pattern cerebral oedema	Lactate peak	p.[Arg39X];[Arg39X]	Died.
3	MDS (*MPV17)*	110	9.9	Increased lipid	Decrease in complexes I-IV	ND	ND	ND	Normal	Normal	p.[Gln44X];[Gln44X]	Died.
4	MDS (*POLG)*	477	6.0	Normal	Normal	55.0	ND	ND	Cytotoxic pattern cerebral oedema	Lactate peak	p.[Thr914Pro];[Ala467Thr]	Died.
5	MDS (*POLG)*	503	3.7	Increased lipid	Normal	107.0	Micro and macrovesicular steatosis	ND	post mid brain lesion in keeping with mitochondrial disease	Normal	p.[Leu304Arg];[Leu304Arg]	Recovered. Remains well 6 years later.
6	HSV	8	8.5	ND	ND	ND	Haemorrhagic necrosis	83	ND	ND	None.	Died following LT
7	HSV	11	16.0	ND	ND	ND	Haemorrhagic necrosis	ND	ND	ND	None.	Died
8	HSV	8		ND	ND	ND	ND	ND	ND	ND	None.	Recovered
9	HSV	8		ND	ND	ND	ND	ND	ND	ND	None.	Recovered
10	HSV	11		ND	ND	ND	ND	ND	ND	ND	None.	Recovered
11	HSV	11		ND	ND	ND	ND	ND	ND	ND	None.	Died following LT
12	HSV	21	6.4	ND	ND	ND	ND	ND	ND	ND	None.	Died

13	HSV	17	4.7	ND	ND	ND	ND	ND	Vasogenic pattern cerebral oedema	ND	None.	Recovered
14	Enterovirus	6	22.4	ND	ND	ND	ND	ND	ND	ND	None.	Died
15	Enterovirus	12		Normal	Normal	54.0	Haemorrhagic necrosis	34.0	ND	ND	None.	Died following LT
16	Enterovirus	9	9.6	ND	ND	ND	ND	ND	Vasogenic pattern cerebral oedema	Lactate peak	None.	Died
17	Adenovirus	112		ND	Normal	ND	ND	ND	Cytotoxic pattern cerebral oedema	ND	None.	Recovered
18	Indeterminate	498	5.6	ND	ND	ND	Panacinar necrosis	29.0	ND	ND	None.	Successful LT
19	Indeterminate	228	3.3	ND	ND	ND	Microvesicular steatosis	25.0	ND	ND	None.	Recovered
20	Indeterminate	52	3.8	ND	ND	ND	Severe fibrosis and microvesicular steatosis	40.0	ND	ND	None.	Recovered.
21	Indeterminate	349	4.7	ND	ND	ND	Panacinar necrosis and macrovesicular steatosis	42.0	ND	ND	None.	Successful LT
22	Indeterminate	260	1.2	ND	ND	ND	ND	ND	ND	ND	None.	Died
23	Indeterminate	15	4.3	Normal	Normal	127.0	Heaptocyte necrosis and macrophage storage material.	91.0	Normal	ND	None.	Died
24	Indeterminate	1	3.0	Increased lipid	ND	47.0	Panacinar necrosis	39.0	Vasogenic pattern cerebral oedema	ND	None.	Underwent LT but died of progressive multisystemic illness 2 months later.
25	Indeterminate	689	3.8	ND	ND	ND	Hepatocellular necrosis and microvesicular steatosis	60	ND	ND	None.	Recovered
26	Indeterminate	644	2.7	ND	ND	ND	Panacinar necrosis	92	ND	ND	None.	Recovered

27	Indeterminate	1	2.1	Normal	Normal	83.0	ND	ND	ND	ND	None.	Recovered
28	Indeterminate	37		Normal	Normal	81.0	Moderate fibrosis.	ND	Normal	ND	None.	Recovered
29	Indeterminate	216	4.4	ND	ND	ND	Panacinar necrosis	25	Cytotoxic pattern cerebral oedema	Normal	None.	Successful LT but died 1 year later from vascular complications
30	NH	8	4.7	ND	ND	ND	ND	ND	ND	ND	None.	Recovered.
31	NH	24	3.2	Normal	ND	ND	Panacinar necrosis	65.0	ND	ND	None.	Successful LT
32	NH	21	0.7	ND	ND	ND	Established cirrhosis	54.0	ND	ND	None.	Successful LT
33	NH	13	1.0	Normal	Normal	88.0	Panacinar necrosis	26.0	Cytotoxic pattern cerebral oedema	Normal	None.	Successful LT
34	OTC deficiency	599	2.5	ND	ND	ND	ND	ND	ND	ND	None.	Recovered. Underwent elective LT age 3.
35	Galactosemia	7		ND	ND	ND	ND	ND	ND	ND	None.	Recovered
36	Galactosemia	9		ND	ND	ND	ND	ND	ND	ND	None.	Recovered
37	RALF	539	5.8	ND	Normal	80	Microvesicular steatosis	127	Normal	Normal	None.	Recovered and had subsequent recurrent bouts of liver failure.
38	HLH	6	4.3	ND	ND	ND	ND	ND	Vasogenic pattern cerebral oedema	Lactate peak	None.	Died.
39	Chemotherapy	121	6.6	ND	ND	ND	Macro and microvesicular steatosis	ND	ND	ND	None.	Recovered

HSV, herpes simplex virus infection; NH, Neonatal haemochromatosis phenotype; OTC, Ornithine transcarbamylase deficiency; RALF, Recurrent acute liver failure and HLH, Hemophagocytic Lymphohistiocytosis, MRI, Magnetic Resonance imaging; MRS, Magnetic Resonance spectroscopy; LT, Liver transplantation; ND, Not done.

Table 2. Clinical and laboratory features in infant with and without genetically proven mitochondrial liver disease (MLD) as a cause of liver failure.

	Genetically proven MLD (n=5)	Other causes of ALF (n=34)	P value
Birth weight (kg)	2.6 (2.3 – 2.9)	2.8 (1.8 – 4.1)	0.76
Age at presentation (days)	110 (9 – 503)	16 (1 – 689)	0.33
Prothrombin time (seconds)	26 (23 – 41)	34 (18 – 120)	0.5
Serum bilirubin (mol/l)	113 (34 – 335)	146 (5– 492)	0.79
ALT (IU/l)	113 (42 – 1875)	940 (19 – 6000)	0.11
Lactate (mmol/L)	7.2 (3.7 – 25)	4.3 (0.7 – 22.4)	0.11

Table 3. Results of tissue studies and radiology where undertaken in infants with and without genetically proven mitochondrial liver disease (MLD).

	Genetically proven MLD (n=5)	Other causes of ALF (n=34)
Liver mtDNA depletion	2/2	8/15
Muscle mtDNA depletion	2/4	1/7
Abnormal muscle respiratory chain enzymes	2/5	0/4
Muscle steatosis	4/5	1/7
Hepatic steatosis	3/3	5/18
MRS lactate peak	3/5	2/5