Clinical and molecular basis of hepatocerebral mitochondrial DNA depletion syndrome in Japan: Evaluation of outcomes after liver transplantation

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Abstract

Background Hepatocerebral mitochondrial DNA depletion syndrome (MTDPS) is a disease caused by defects in mitochondrial DNA maintenance and leads to liver failure and neurological complications during infancy. Liver transplantation (LT) remains controversial due to poor outcomes associated with extrahepatic symptoms. The purposes of this study were to clarify the current clinical and molecular features of hepatocerebral MTDPS and to evaluate the outcomes of LT in MTDPS patients in Japan.

Results We retrospectively assessed the clinical and genetic findings, as well as the clinical courses, of 23 hepatocerebral MTDPS patients from a pool of 999 patients who were diagnosed with mitochondrial diseases between 2007 and 2019. Causative genes were identified in 19 of 23 patients: MPV17 (n = 13), DGUOK (n = 3), POLG (n = 1), and MICOS13 (n = 1). Eight MPV17-deficient patients harbored c.451dupC and all three DGUOK-deficient patients harbored c.143-307_170del335. The most common initial manifestation was failure to thrive (n = 13, 56.5%). The most frequent liver symptom was cholestasis (n = 21, 91.3%). LT was performed on 12 patients, including nine MPV17-deficient and two DGUOK-deficient patients. Among the 12 transplanted patients, five, including one with mild intellectual disability, survived; while seven who had remarkable neurological symptoms before LT died. Five of the MPV17-deficient survivors had either c.149G>A or c.293C>T.

Conclusions MPV17 was the most common genetic cause of hepatocerebral MTDPS. The outcome of LT for MTDPS was not favorable, as previously reported, however, patients harboring MPV17 mutations associated with mild phenotypes such as c.149G>A or c.293C>T, and exhibiting no marked neurologic manifestations before LT, had a better prognosis after LT.

Background Mitochondrial diseases are clinically and genetically heterogeneous disorders that affect multiple organs, are characterized by impaired energy production, and can present at any age. Neurological symptoms are the most common clinical presentation and liver involvement is observed in approximately 10–20% of cases, particularly in patients that present as neonates or during early infancy.

Mitochondrial DNA depletion syndrome (MTDPS) is caused by defects in any of the proteins involved in mtDNA maintenance, leading to quantitative and qualitative defects in mtDNA, and currently has been classified as mtDNA maintenance defects. MTDPS has three clinical phenotypes; myopathic, encephalomyopathic, and hepatocerebral. Hepatocerebral MTDPS is known to cause acute liver failure in infancy and is associated with mutations in DGUOK, MPV17, POLG, SUCLG1, and TWNK.

Liver transplantation (LT) is considered as a definitive treatment option for pediatric patients with liver failure, and the survival rate for pediatric LT recipients in Japan is more than 85% at five years. Contrarily, previous studies have reported that the overall survival rate of LT performed for mitochondrial hepatopathies was only 30% due to post-LT deterioration of neurologic and extrahepatic symptoms. LT for mitochondrial diseases may be considered in patients with isolated liver disease, however, it is difficult to exclude the development or deterioration of extrahepatic manifestation before LT in a clinical setting.

In 2013, we reported the clinical and molecular characteristics of MTDPS in Japan, identifying 13 patients with hepatocerebral MTDPS. Among those patients, mutations in DGUOK were the most frequently observed
followed by *MPV17* and *POLG*. Since that earlier report, we have diagnosed ten additional patients with hepatocerebral MTDPS and have performed LT on patients with mitochondrial hepatopathies\(^8\)–\(^{10}\). The purposes of this study were to clarify the current clinical and molecular features of hepatocerebral MTDPS and to evaluate the outcomes of LT in MTDPS patients in Japan.

**Methods**

We performed a retrospective review of patients who were diagnosed with hepatocerebral MTDPS from 2007 to 2019. Patients were enzymatically and/or genetically diagnosed and diagnosis of MTDPS was confirmed by quantitative polymerase chain reaction (qPCR). This study was approved by the ethics boards of Chiba Children's Hospital and Saitama Medical University.

**Patients**

We have used biochemical and molecular genetic testing to diagnose mitochondrial diseases in Japan since 2007. A total of 999 patients were diagnosed with mitochondrial diseases, 101 (10.1%) of which were mitochondrial hepatopathies. Among these, 23 patients were diagnosed with hepatocerebral MTDPS.

**Mitochondrial respiratory chain enzyme activity**

We examined mitochondrial respiratory chain enzyme activity using liver samples as previously described\(^{11}\). Enzymatic diagnosis was confirmed according to the diagnostic criteria described by Bernier *et al*\(^{12}\).

**Quantitative polymerase chain reaction**

Nuclear DNA and mtDNA were enumerated by qPCR, according to the previously described methods\(^7\)–\(^{13}\). The mtDNA gene *ND1* was compared against a nuclear reference gene, exon 24 of *CFTR*. A relative copy number of mtDNA to nuclear DNA of <35% was defined as mtDNA depletion.

**Results**

**Clinical characteristics**

Table 1 shows the clinical characteristics of the patients. The cohort comprised 23 patients (11 male and 12 female) with hepatocerebral MTDPS from 19 non-consanguineous families; 15 of the patients have been reported earlier\(^3\)–\(^7\),\(^8\),\(^10\). Twenty patients (87%) presented with initial manifestations during infancy, and six of those developed initial symptoms during the neonatal period. The most common initial manifestation was failure to thrive, seen in 13 patients (56.5%), followed by vomiting (8/23 patients), and jaundice (4/23 patients). Mitochondrial respiratory chain enzymes were analyzed in 22 of the patients, and multiple enzyme deficiencies in liver tissues were noted in 19. All affected patients tested for mtDNA content showed significant mtDNA depletion, ranging from 0.5 to 31.7%. Causative genes were identified in 18 of the 23 hepatocerebral MTDPS patients. Liver
manifestations are shown in Table 2. The most frequently observed liver symptom was cholestasis (21/23 patients, 91.3%); meanwhile hepatomegaly, fatty liver, liver fibrosis, and liver failure were observed in 15 (68.1%), 16 (72.7%), 17 (77.2%), and 20 patients (87.0%), respectively. Furthermore, hepatocellular carcinoma (HCC) developed in two patients with MPV17 deficiency, and the level of α-fetoprotein was highly variable, ranging from 24.9 to 503,320 ng/mL.

Table 3 shows the breakdown of extrahepatic manifestations. Failure to thrive (18/21) was the most common extrahepatic involvement. Vomiting (10/22) and feeding difficulties (11/23), which developed during the neonatal period, were also frequent symptoms. Hypoglycemia and lactic acidosis were found in 15 and 16 patients, respectively. Pulmonary hypertension (PH) was observed in 5/22 patients (MPV17, four patients [Pt936, Pt1244, Pt1273, and Pt1943]; DGUOK, one patient [Pt66]). Hypothermia was also observed in one patient with DGUOK deficiency.

### Molecular investigations

We identified causative genes in 18 of the 23 patients, including mutations in MPV17 (13 patients), DGUOK (3 patients), POLG (one patient) and MICOS13 (one patient). The variants that are predicted to be pathogenic are presented in Table 4. Homozygous or compound heterozygous c.451dupC (p.L151Pfs*39) with other mutations were detected in 8 of 13 MPV17 deficient patients. The c.143-307_170del335 mutation was found in all three patients with DGUOK deficiency. Pt94 had a novel homozygous frameshift mutation, c.13_29del (p.W6Pfs*71) in MICOS13.

### Liver transplantation and prognosis

LT from a living donor was performed on 12 patients, including nine with MPV17 deficiency and two with DGUOK deficiency (Table 4). The reasons for performing LT were as follows: two patients (Pt68YB and Pt2017ES) had HCC, two patients (Pt2017EB and Pt2017YS) had multiple hepatic masses and end-stage liver disease, and the remainder of the patients had liver failure. Neurological manifestations were also observed before LT in nine patients other than Pt1702, Pt2017YS, and Pt2017ES. Furthermore, five of the 12 LT patients (41.7%) survived, four of which were MPV17-deficient patients (Pt1702, Pt2017EB, Pt2017YS, and Pt2017ES). Three of the four patients who presented with onset after six months of age (75%) survived, whereas only two of the eight patients with onset before five months (25%) survived. Moreover, Pt2017YS and Pt2017ES with MPV17 deficiency did not develop any complications following LT, whereas Pt2017EB, who had mild intellectual disability before LT, presented with mild headache after LT. Lastly, Pt1702 who received LT due to liver failure at the age of eight months had normal liver function after LT, however, developed epilepsy, mild intellectual disability, dysarthria, fine motor dysfunction, white matter lesion in magnetic resonance imaging of the brain, and psychosis after six years of age.

Following LT, three MPV17-deficient patients (Pt936, Pt1244, and Pt1273) and one DGUOK-deficient patient (Pt66) developed PH, as did one MPV17-deficient patient (Pt1943) that did not undergo LT. All five patients suffering from PH showed poor prognosis. The causes of death after LT were respiratory failure due to sepsis in Pt936, PH in Pt1244 and heart failure in Pt1273. Tissue vulnerability following LT caused a ruptured suture in Pt68EB, who
developed peritonitis and sepsis. Pt68YB died of sepsis and acute respiratory distress syndrome caused by pneumonia after LT.

Two of the 11 patients (Pt339 and Pt1589) who did not receive LT survived. Pt 339 with MPV17 deficiency survived for 11 years after the onset of MTDPS. She presented with failure to thrive since infancy, and developed liver failure accompanied by acute encephalopathy at the age of two years. The liver failure was ameliorated by medical management, however, her liver disease gradually progressed to cirrhosis causing esophageal varices, which were treated by endoscopic variceal ligation. She ultimately developed certain neurological manifestations: spastic gait, intellectual disability, and seizure. At the time of writing this report, she had spastic paralysis associated with white matter lesion, and required the use of a wheelchair.

Among eight MPV17-deficient patients who harbored a frameshift mutation (c.451dupC) in at least one allele, only one patient (Pt1702) survived. All of the patients with MPV17 mutations who survived had either c.149G>A, or c.293C>T in at least one allele. Pt339 and Pt1702, both with c.293C>T, developed neurological symptoms, including seizures, intellectual disability, and psychiatric symptoms, while sibling patients with c.149G>A did not exhibit neurological manifestations, other than mild intellectual disability and headaches for Pt2017EB.

**Discussion**

Mutations in *MPV17* were the most common genetic cause of MTDPS in the patient cohort involved in this study, followed by mutations in *DGUOK*. The prevalence of these mutations in MTDPS patients observed in our study differs from that of other studies. For example, a previous study reported that *POLG* mutations, followed by *MPV17* and *DGUOK* mutations, were the most common in European countries. Meanwhile, another showed that *MPV17* and *DGUOK* were the most, and second most, frequent genetic causes of MTDPS, respectively, although all parents of the patients in that study were consanguineous.

Whole exome sequencing identified a homozygous c.13_29del in *MICOS13*, in Pt94. This novel mutation caused reduced levels of *MICOS13* mRNA and protein in the patient's fibroblasts and was confirmed by a rescue assay using a lentivirus system. Although MICOS13 has not been reported to be directly involved in mitochondrial DNA replication, several reports support this relationship. For example, IMMT (also known as MIC60 or Mitofilin) has a critical role in MICOS assembly and mitochondrial DNA organization. IMMT directly contacts mtDNA and is involved in the D-loop architecture. Other studies showed that a defect in CHCHD10, which is also related to MICOS complex function, resulted in decreased MICOS complex organization, reduced copy number and caused instability of mtDNA.

The frameshift mutation c.451dupC was seen in 8 of 13 patients (61.5%) in our cohort with MPV17 deficiency. A homozygous c.451dupC mutation was also identified in Korean sibling patients who died from liver failure at the age of six months, however, has not reported elsewhere. Japanese patients with the homozygous c.451dupC also developed liver failure requiring LT during infancy and died within two years of LT. It is, therefore, conceivable that the c.451dupC mutation might be more frequent in East Asian populations, and homozygosity at this locus is likely associated with poor outcomes regardless of whether LT is performed. In contrast, two patients with c.293C>T and sibling patients with c.149G>A, in which homozygosity is associated with a comparatively better
prognosis, survived regardless of LT\textsuperscript{3,20}. Taken together, patients harboring c.149G>A or c.293C>T in at least one MPV17 allele of might show milder phenotypes.

LT in patients with mitochondrial diseases remains controversial due to the potential for extrahepatic manifestations. In guidelines for pediatric patients, LT for patients with mitochondrial diseases involving severe and life-threatening extrahepatic multi organ manifestations is contraindicated due to the high possibility of neurological deterioration\textsuperscript{21}. In such patients, limited data are available regarding the efficacy of LT and long-term prognosis, and outcomes are known to be heterogenous\textsuperscript{1,22-24}. It has been reported that five of 14 patients with DGUOK mutations survived for more than five years after LT without severe neurological symptoms, even though some patients presented with muscle hypotonia and psychomotor retardation before transplantation\textsuperscript{22}. In that study, all survivors harbored at least one mutation that predicted a DGUOK protein with some potential residual activity. Moreover, in our cohort, overall survival rate following LT for MTDPS was 41.7%, which was lower than that for other diseases (> 85%)\textsuperscript{5}. We also found that survival rate of LT patients with onset after six months of age (75%) was higher than that of onset before five months (25%). LT may, therefore, be more effective in patients with later onset.

Table 5 summarizes 20 patients with MPV17 mutations who received LT\textsuperscript{20,25-31}, including nine patients from our cohort. Nine of these 20 patients (45.0%) survived after LT. Patients with the homozygous c.149G>A, or compound heterozygous c.149G>A or c.293C>T, with other mutations tended to show a better prognosis after LT. Eight of the 11 deceased patients (72.7%) presented with neurological involvements before LT. Mild neurological symptoms were observed before LT in just one of the nine patients that survived, however, seven patients (77.8%) manifested with neurological abnormalities after LT. Collectively, patients harboring c.149G>A or c.293C>T in at least one allele without marked neurological manifestations might have a better prognosis after LT.

A previous study found that three out of 14 patients with DGUOK mutations developed PH after LT\textsuperscript{22}. In the current study, PH was observed in five patients including four with MPV17 deficiency and one with DGUOK deficiency. Four of these developed PH after LT. Although PH can be caused by chronic liver disease and portal hypertension, PH after LT is infrequent; PH has also been reported in patients with primary mitochondrial diseases (e.g. m.3243A>G, NFU1, BOLA3)\textsuperscript{32,33}, however, the mechanism underlying this remains unknown.

Conclusions

In conclusion, the survival rate for MTDPS patients after LT in our cohort was lower than that for other diseases, however, LT was relatively effective in patients with later onset. Our results also suggest that a better life prognosis after LT might be expected in MTDPS patients who have MPV17 mutations, such as c.149G>A or c.293C>T, that are associated with milder phenotypes and do not have marked neurological manifestations before LT.

Abbreviations

MTDPS, mitochondrial DNA depletion syndrome; LT, liver transplantation; qPCR, quantitative polymerase chain reaction; HCC, hepatocellular carcinoma; PH, pulmonary hypertension; VUS, variant of unknown significance; IUGR, intrauterine growth restriction
Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000.

Consent for publication

Written informed consent was obtained from the parents of all subjects included in the study.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that they have no conflict of interest.

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Author’s contributions

M.S., N.K., and S.K. designed the study. M.S., N.K., K.I., and A.M. drafted the manuscript. N.A., Y.S., T.E., S.U., A.I., T.F., R.I., A.F., M.K., and J.M. collected and provided the patient data. M.O.T and T.T. performed enzyme and qPCR analyses. The whole scheme was planned and supervised by Y.O., A.O., and K.M. Professional advice on the draft was given by T.F., Y.K., K.T., and K.S. K.M. critically revised the manuscript.

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Tables

Table 1. Clinical characteristics of 23 hepatocerebral MTDPS patients.
| ID      | Gene    | Sex | Age at onset | Initial manifestation(s)                                | Affected complexes | %mtDNA in Liver |
|---------|---------|-----|--------------|---------------------------------------------------------|--------------------|-----------------|
| Pt68EB  | MPV17   | M   | 3 m          | failure to thrive, hypotonia, jaundice                  | CI+III+IV (liver)  | 7.8             |
| Pt68YB  | MPV17   | M   | 8 m          | failure to thrive, jaundice                             | CI+III+IV (liver)  | 6.6             |
| Pt292   | MPV17   | F   | 1 m          | failure to thrive, vomiting                             | CI+III (liver)     | 9.8             |
| Pt339   | MPV17   | F   | 8 m          | failure to thrive                                      | CI+III (liver)     | 20.5            |
| Pt936   | MPV17   | M   | 1 m          | failure to thrive                                      | CI+III+IV (liver)  | 8.0             |
| Pt1244  | MPV17   | M   | 1 m          | failure to thrive                                      | CI+III+IV (liver)  | 1.2             |
| Pt1273  | MPV17   | F   | 1 m          | failure to thrive, vomiting                             | CI+III+IV (liver)  | 3.4             |
| Pt1702  | MPV17   | M   | neonate      | failure to thrive, vomiting                             | NA                 | NA              |
| Pt1943  | MPV17   | M   | neonate      | tachypnea, jaundice                                    | CI+III (liver)     | 0.5             |
| Pt2017EB| MPV17   | M   | 7 m          | liver failure                                           | CI+III (liver)     | 0.7             |
| Pt2017YS| MPV17   | F   | 1 y          | vomiting, lethargy                                     | normal (Fb)        | NA              |
| Pt2017ES| MPV17   | F   | 4y5m         | vomiting, lethargy                                     | CI+II+IV (liver)   | 0.6             |
| Pt2170  | MPV17   | F   | 7m           | failure to thrive, cholestasis, liver dysfunction       | CI+II+III (liver)  | 15.3            |
| Pt50YS  | DGUOK   | F   | neonate      | tachypnea, hypothermia, hypoglycemia                    | CI+III+IV (liver)  | 6.0             |
| Pt50ES  | DGUOK   | F   | 3 m          | failure to thrive, incomplete head control             | CI+III+IV (liver)  | 3.0             |
| Pt66    | DGUOK   | F   | neonate      | feeding difficulty                                     | CI+III+IV (liver)  | 2.3             |
| Pt92    | DGUOK   | M   | 1 m          | failure to thrive, jaundice                             | CI+II+III (liver)  | 18.4            |
| Pt74    | POLG    | F   | 4 m          | failure to thrive, lethargy, hypotonia, vomiting        | CI+III+IV (liver)  | 3.3             |
| Pt94    | MICOS13 | F   | 3 m          | breath holding                                          | CI (liver)         | 11.5            |
| Pt1156  | TWNK    | M   | neonate      | hypoglycemia, lactic acidosis                           | CI+IV (liver)      | 6.3             |
| Pt63    | ND      | M   | 2 m          | failure to thrive, vomiting                             | CI (liver)         | 23.7            |
| Pt148   | ND      | F   | neonate      | vomiting                                                | CI (liver)         | 31.7            |
|         |         | M   |              | elevated                                                |                    |                  |
EB, elder brother; YB, younger brother; YS, younger sister; ES, elder sister; ND, not detected; C, complex; NA, not available; Fb, fibroblast

Table 2. Liver manifestations in 23 hepatocerebral MTDPS patients.

| ID   | Gene    | Cholestasis | Hepatomegaly | Fatty liver | Fibrosis | Liver failure | Tumor         | AFP (ng/mL) |
|------|---------|-------------|--------------|-------------|----------|---------------|---------------|-------------|
| Pt68EB | MPV17   | +           | +            | +           | +        | +             | -             | NA          |
| Pt68YB | MPV17   | +           | -            | +           | +        | -             | HCC           | 24,000      |
| Pt292 | MPV17   | +           | +            | +           | -        | +             | -             | 219,980     |
| Pt339 | MPV17   | +           | -            | +           | +        | -             | -             | NA          |
| Pt936 | MPV17   | +           | +            | +           | +        | -             | 315,521       |
| Pt1244 | MPV17   | +           | +            | +           | +        | -             | -             | 503,320     |
| Pt1273 | MPV17   | +           | +            | +           | +        | -             | 93,619        |
| Pt1702 | MPV17   | +           | -            | +           | +        | -             | NA            |
| Pt1943 | MPV17   | +           | -            | +           | -        | +             | -             | NA          |
| Pt2017EB | MPV17 | +           | +            | +           | +        | Multiple hepatic nodules | 413          |
| Pt2017YS | MPV17  | +           | -            | +           | -        | +             | Multiple hepatic nodules | 3,078       |
| Pt2017ES | MPV17 | +           | -            | +           | +        | -             | HCC           | 1,332       |
| Pt2170 | MPV17   | +           | +            | +           | +        | -             | 60,500        |
| Pt50YS  | DGUOK   | +           | +            | -           | -        | +             | -             | NA          |
| Pt50ES  | DGUOK   | -           | -            | -           | -        | +             | -             | NA          |
| Pt66    | DGUOK   | +           | -            | +           | +        | -             | -             | NA          |
| Pt92    | DGUOK   | +           | +            | -           | +        | +             | >50,000       |
| Pt74    | POLG    | +           | +            | +           | +        | -             | NA            |
| Pt94    | MICOS13 | +           | +            | +           | +        | -             | NA            |
| Pt1156  | TWNK    | +           | +            | +           | +        | -             | NA            |
| Pt63    | ND      | +           | +            | -           | +        | -             | 200,000       |
| Pt148   | ND      | +           | -            | -           | +        | -             | 8,400         |
| Pt1589  | ND      | -           | NA           | NA          | NA       | -             | -             | NA          |

EB, elder brother; YB, younger brother; YS, younger sister; ES, elder sister; ND, not detected; HCC, hepatocellular carcinoma; NA, not available; AFP, α-fetoprotein

Table 3. Extrahepatic manifestations in hepatocerebral MTDPS patients (n=23).
Table 4. Body weight at birth and at follow-up.

| ID     | Gene   | Sex | Gestational age | Birth Body weight (g) | SD | Age at exam | Postnatal Body weight (kg) | SD |
|--------|--------|-----|-----------------|-----------------------|----|-------------|----------------------------|----|
| Pt68EB | MPV17  | M   | 37w0d           | 3060                  | 1  | 3m          | 4.6                        | -2.7|
| Pt68YB | MPV17  | M   | 40w0d           | 3260                  | 0.1| 8m          | 5.5                        | -3.1|
| Pt292  | MPV17  | F   | 40w5d           | 3428                  | 1  | 7m          | 4.8                        | -3.5|
| Pt339  | MPV17  | F   | NA              | NA                    | NA | 2y7m        | 10.2                       | -2  |
| Pt936  | MPV17  | M   | 38w0d           | 3240                  | 1.3| 4m          | 4.5                        | -3  |
| Pt1244 | MPV17  | M   | 40w0d           | 2909                  | -0.9| 4m          | 3.7                        | -4  |
| Pt1273 | MPV17  | F   | 39w0d           | 3010                  | 0.1| 1y          | 5                          | -4.7|
| Pt1702 | MPV17  | M   | NA              | NA                    | NA | NA          | NA                         | NA  |
| Pt1943 | MPV17  | M   | 37w5d           | 2692                  | -0.5| 6m          | 4.28                       | -4.1|
| Pt2017EB| MPV17   | M   | 38w0d           | 2950                  | 0.5| 7y          | 20.05                      | -0.7|
| Pt2017YS| MPV17    | F   | 37w6d           | 2830                  | 0.1| 4y          | 14.85                      | -0.2|
| Pt2017ES| MPV17    | F   | 38w0d           | 2728                  | 0.1| 7y          | 22.1                       | -0.1|
| Pt2170 | MPV17  | F   | 36w2d           | 2428                  | 0  | 1y1m        | 6.54                       | -2.8|
| Pt50YS | DGUOK   | F   | 40w2d           | 2750                  | -1.2| NA          | NA                         | NA  |
| Pt50ES | DGUOK   | F   | 40w0d           | 2510                  | -1.5| NA          | NA                         | NA  |
| Pt66   | DGUOK   | F   | 37w3d           | 1688                  | -3.2| 8m          | 3.2                        | -5.5|
| Pt92   | DGUOK   | M   | 40w0d           | 3120                  | -0.3| 6m          | 7.2                        | -0.9|
| Pt74   | POLG    | F   | 40w0d           | normal                | NA | 4m          | 5.6                        | -2.3|
| Pt94   | MICS13  | F   | 40w3d           | 2780                  | -0.8| NA          | NA                         | NA  |
| Pt1156 | TWNK    | M   | 37w3d           | 1992                  | -2.1| 2m          | 2.7                        | -4.2|
| Pt63   | ND      | M   | 37w0d           | 1884                  | -2.5| 7m          | 5.4                        | -3.7|
| Pt148  | ND      | F   | 38w4d           | 2254                  | -1.7| 15d         | 2.4                        | -1.5|
| Pt1589 | ND      | M   | 23w5d           | 624                   | 0  | NA          | NA                         | NA  |

|             | MPV17 (Mean±SD) | non-MPV17 (Mean±SD) | P value |
|-------------|------------------|----------------------|---------|
| Birth       | 0.3±0.7 (n=11)   | -1.7±0.9 (n=8)       | <0.01   |
| Postnatal   | -2.6±1.5 (n=12)  | -3.0±1.8 (n=6)       | 0.59    |
Table 5. Identified gene mutations in patients, liver transplantation status, and clinical outcomes.

| ID  | Gene  | Allele 1                              | Allele 2                              | LT (age)     | Outcome |
|-----|-------|---------------------------------------|---------------------------------------|--------------|---------|
| Pt68EB | MPV17 | c.451dupC : p.L151Pfs*39              | c.509C>T : p.S170F                    | + (1y5mo)    | died (1y10m) |
| Pt68YB | MPV17 | c.451dupC : p.L151Pfs*39              | c.509C>T : p.S170F                    | + (6y)       | died (6y)   |
| Pt292 | MPV17 | c.451dupC : p.L151Pfs*39              | c.148C>T : p.R50W                     | -            | died (1y2m) |
| Pt339 | MPV17 | c.293C>T : p.P98L                     | c.376-1G>A                            | -            | alive (11y) |
| Pt936 | MPV17 | c.451dupC : p.L151Pfs*39              | c.451dupC : p.L151Pfs*39             | + (4m)       | died (1y9m) |
| Pt1244| MPV17 | c.451dupC : p.L151Pfs*39              | c.451dupC : p.L151Pfs*39             | + (11m)      | died (2y6m) |
| Pt1273| MPV17 | c.451dupC : p.L151Pfs*39              | c.71-2_79del11ins4                   | + (1y)       | died (3y)   |
| Pt1702| MPV17 | c.451dupC : p.L151Pfs*39              | c.293C>T : p.P98L                     | + (8m)       | alive (23y) |
| Pt1943| MPV17 | c.451dupC : p.L151Pfs*39              | c.308_310del : p.C103del             | -            | died (10m) |
| Pt2017EB | MPV17 | c.148C>T : p.R50W                     | c.149G>A : p.R50Q                     | + (7y)       | alive (8y) |
| Pt2017YS | MPV17 | c.148C>T : p.R50W                     | c.149G>A : p.R50Q                     | -            | alive (5y) |
| Pt2017ES | MPV17 | c.148C>T : p.R50W                     | c.149G>A : p.R50Q                     | + (7y)       | alive (8y) |
| Pt2170| MPV17 | c.148C>T : p.R50W                     | c.271_273del : p.L91del              | -            | died (1y11m) |
| Pt50YS | DGUOK | c.143-307_170del335                  | c.143-307_170del335                  | -            | died (9mo) |
| Pt50ES | DGUOK | c.143-307_170del335                  | c.143-307_170del335                  | + (1y6m)    | died (1y7m) |
| Pt66  | DGUOK | c.143-307_170del335                  | c.743T>C : p.L248P                    | + (8m)       | died (1y6m) |
| Pt92  | DGUOK | c.143-307_170del335                  | c.143-307_170del335                  | -            | died (7m) |
| Pt74  | POLG  | c.3554T>C : p.I1185T                  | c.2870C>T : p.A957V                   | -            | died (8m) |
| Pt94  | MICOS13 | VUS                                   | VUS                                   | -            | died (8m) |
| Pt1156 | TWNK  | VUS                                   | VUS                                   | -            | died (7m) |
| Pt63  | ND    | -                                     | -                                     | + (9m)       | alive (16y) |
| Pt148 | ND    | -                                     | -                                     | -            | died (1m)  |
| Pt1589| ND    | -                                     | -                                     | -            | alive (6y) |

**MPV17: NM_002437, DGUOK: NM_080918, POLG: NM_002693, TWNK: NM_021830**

EB, elder brother; YB, younger brother; YS, younger sister; ES, elder sister; ND, not detected; VUS, variant of unknown significance; LT, liver transplantation

Table 6. Molecular and neurological findings as well as outcomes in 19 MPV17-deficient patients who received LT.
| Sex     | Allele 1 | Allele 2 | Age at onset | Neurological findings | LT age | Outcome        |
|---------|----------|----------|--------------|-----------------------|--------|----------------|
| Pt68EB M | c.451dupC: c.509C>T: p.L151Fs*3: p.S170F | 3 m | hypotonia + | 17 mo | died (1y10m) |
| Pt68YB M | c.451dup C: c.509C>T: p.L151Fs*3: p.S170F | 8 m | hypotonia + | 6 y  | died (6y)    |
| Pt936 M  | c.451dup C: c.451dup C: p.L151Fs*3: p.L151Fs*3 | 1 m | developmental delay, hypotonia | 4 m  | died (1y9m)  |
| Pt1244 M | c.451dup C: c.451dup C: p.L151Fs*3: p.L151Fs*3 | 1 m | developmental delay, hypotonia | 11 m | died (2y6m)  |
| Pt1273 F | c.451dup C: c.71-2_79del11ins4 | 1 m | developmental delay | 1 y  | died (3y)    |
| Pt1702 M | c.451dup C: c.293C>T: p.P.L151Fs*3: p.P98L | neonate | - | psychosis, intellectual disability, fine motor dysfunction, dysarthria | 8 m  | alive (23y) |
| Pt2017EB M | c.148C>T: p.R50W | 7 m | mild intellectual disability | 7 y  | alive (8y)   |
| Pt2017ES F | c.148C>T: p.R50W | 1 y | - | - | 7 y | alive (8y) |
| Parini 2009 M | c.149G>A: p.R50Q | 1 m | - | developmental delay, ataxia, severe motor-sensory axonal polyneuropathy | 2 y  | alive (6y)   |
| Karadimas 2006 F | c.149G>A: p.R50Q | 6 m | - | hypotonia, gross and fine motor delay, peripheral neuropathy | 9 m  | alive (12y) |
| Karadimas 2006 F | c.149G>A: p.R50Q | 1 m | hypotonia, hyporeflexia | 16 m | died (2y)   |
| Karadimas 2006 F | c.149G>A: p.R50Q | 4 m | - | peripheral neuropathy | 11 y | alive (21y) |
| Wong 2007 M | c.206G>A: p.W69* | birth | - | - | 5 m | died (6m)   |
| Navarro 2008 M | c.70+5G>A: c.70+5G>A | 2 m | hypotonia + | 1 y | died (2y)   |
| El-Hattab 2010 M | c.262A>G: p.K88E | neonate | NA | developmental delay, muscle weakness hypotonia | NA  | died (2.5y) |
| El-Hattab 2010 M | c.485C>A: p.A162D | infancy | NA | NA | NA | alive (4y) |
| Mudd 2012 M | c.22_23insCND | infancy | hypotonia, mild motor delay | 7 y  | died (9y)   |
| Uusimaa 2014 M | c.191G>A: p.P64R | 5 m | - | progressive demyelinating peripheral neuropathy | 3 y  | alive (11.5y) |
| Vilarinho 2014 F | c.148C>T: p.R50W | 5 y | - | dystonia, tremor, seizure | 9 y  | died (10y)   |