Clinical and Molecular Characteristics of Mitochondrial DNA Depletion Syndrome Associated with Neonatal Cholestasis and Liver Failure

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Objective To determine the frequency of mitochondrial DNA depletion syndrome (MDS) in infants with cholestasis and liver failure and to further clarify the clinical, biochemical, radiologic, histopathologic, and molecular features associated with MDS due to deoxyguanosine kinase (DGUOK) and *MPV17* gene mutations.

Study design We studied 20 infants with suspected hepatocerebral MDS referred to our tertiary care center between 2007 and 2013. Genomic DNA was isolated from blood leukocytes, liver, and/or skeletal muscle samples by standard methods. Mitochondrial DNA copy number relative to nuclear DNA levels was determined in muscle and/or liver DNA using real-time quantitative polymerase chain reaction and compared with age-matched controls. Nuclear candidate genes, including *polymerase* γ, *MPV17*, and *DGUOK* were sequenced using standard analyses.

Results We identified pathogenic *MPV17* and *DGUOK* mutations in 11 infants (6 females) representing 2.5% of the 450 cases of infantile cholestasis and 22% of the 50 cases of infantile liver failure referred to our center during the study period. All of the 11 patients manifested cholestasis that was followed by a rapidly progressive liver failure and death before 2 years of life. Mitochondrial DNA depletion was demonstrated in liver or muscle for 8 out of the 11 cases where tissue was available. Seven patients had mutations in the *MPV17* gene (3 novel mutations), 4 patients had *DGUOK* mutations (of which 2 were novel mutations).

Conclusion Mutations in the *MPV17* and *DGUOK* genes are present in a significant percentage of infants with liver failure and are associated with poor prognosis. *(J Pediatr 2014;164:553-9)*.

Mitochondria are the main source of the high-energy phosphate molecule adenosine triphosphate (ATP), which is essential for all active intracellular processes. The liver is highly dependent on ATP for biosynthetic and detoxifying properties. Therefore, disorders of mitochondrial function commonly cause liver dysfunction. Mitochondria contain a separate genome, mitochondrial DNA (mtDNA), which is distinct from that of the nucleus.[1] The respiratory chain peptide components are encoded by both nuclear and mtDNA genes. Nuclear genes encode more than 70 respiratory chain subunits and an array of enzymes and cofactors required to replicate and maintain mtDNA,[2,3] including DNA polymerase γ, thymidine kinase 2, and deoxyguanosine kinase (DGUOK).

Mitochondrial DNA depletion syndrome (MDS) is clinically a heterogeneous group of disorders characterized by a severe reduction of mtDNA content and insufficient synthesis of respiratory chain complexes I, III, IV, and V in different tissues.[4,5] Liver, heart, skeletal muscle, and brain are among the most energy-dependent tissues of the body and, therefore, they are vulnerable to mtDNA depletion. The clinical phenotypes of MDS are, therefore, highly variable, leading to 3 main clinical presentations: myopathic, encephalomyopathic, and hepatocerebral. The hepatocerebral form is associated with mutations in the *DGUOK* gene,[6] the polymerase γ *1* gene,[7] the *MPV17* gene,[8] or more rarely, the Twinkle helicase gene.[9]

Mutations in *DGUOK* and *MPV17* have been identified in an increasing number of patients.[10-15] Given the common underlying pathology of mtDNA depletion and the clinical manifestations overlapping both *DGUOK* and *MPV17* groups, the majority of patients have developed liver disease within a few months after birth, with rapid deterioration, and some patients have shown relatively slow progression of liver disease or neurologic regression. However, the number of reported patients with *MPV17* mutations is still small, and the clinical courses according to the mutations or the genotype–phenotype correlation remain unclear. The aims of our study were to determine the frequency of MDS as a cause of infantile cholestasis and liver failure and to further clarify the clinical, biochemical,

ATP	Adenosine triphosphate
DGUOK	Deoxyguanosine kinase
MDS	Mitochondrial DNA depletion syndrome
MRI	Magnetic resonance imaging
mtDNA	Mitochondrial DNA

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radiologic, histopathologic, and molecular features associated with MDS due to *DGUOK* and *MPV17* gene mutations. We report 11 cases from 9 families with *MPV17* and *DGUOK* mutations. Included in our series are 3 newly-identified mutations in the *MPV17* gene and 2 novel mutations in *DGUOK* gene. The correlation between the type of mutation in *MPV17* and *DGUOK* and the phenotypic presentation of MDS is also discussed.

Methods

At King Fahad Medical City, since 2007, we have adopted a strategy for careful evaluation for mitochondrial hepatopathy in infants presenting with cholestasis and neurologic manifestations (hypotonia, seizure, nystagmus), elevated plasma lactate (>2.2 mmol/L), hypoglycemia (plasma glucose <3 mmol/L), abnormal finding on brain magnetic resonance imaging (MRI), or liver failure (international normalized ratio ≥2, unresponsive to vitamin K injection). All infants presenting to our center with cholestasis and/or liver failure undergo extensive workup to exclude infectious, structural, metabolic, endocrine, infiltrative, and familial causes. Clinical samples (blood, liver, and, occasionally, muscle tissue) on patients with suspected mitochondrial hepatic disease were referred to the Newcastle Mitochondrial National Specialized Commissioning Team Diagnostic Laboratory, Wellcome Trust Centre for Mitochondrial Research, Newcastle University, United Kingdom, for mitochondrial investigations. The study has been approved by institutional review board, and the procedures are in accordance with the Declaration of Helsinki.

During the study period (2007-2013), we investigated 20 infants with suspected hepatocerebral MDS who had been referred to King Fahad Medical City and King Abdulaziz Medical City, Riyadh, Saudi Arabia. All 20 infants underwent clinical assessment, histologic, biochemical, radiologic, and/or molecular genetic analyses. This study was approved and performed under the ethical guidelines issued by each institution for clinical studies, with written informed consent obtained for all subjects.

Histopathologic Studies

Liver specimens were obtained, within 2 weeks of presentation, through a percutaneous ultrasound guided liver biopsy after correction of coagulopathy. The liver specimens were fixed in 10% buffered formaldehyde, paraffin-embedded, and stained with hematoxylin and eosin, Masson trichrome stain for fibrous tissue and Perls' method for iron, reticulin, and periodic acid-Schiff diastase. A muscle specimen was obtained through open biopsy and was subjected to a histochemical study using the following methods: nicotinamide adenine dinucleotide-tetrazolium reductase, ATPase at both pH 9.4 and 4.3, cytochrome *c* oxidase. The liver and muscle samples were immediately frozen in liquid nitrogen after collection, stored at −80°C, and shipped on dry ice. All liver histopathology slides were reviewed by a single pediatric hepatopathologist who was blind to clinical data and diagnosis.

Molecular Genetic Investigations

Total genomic DNA was isolated from blood leukocytes, skeletal muscle, and liver samples by standard methods. MtDNA copy number relative to nuclear DNA levels in muscle and/or liver DNA was estimated and compared with age-matched normal controls[16,17] by real-time quantitative polymerase chain reaction as described previously.[18] MtDNA copy number <30% compared with age-matched controls was classed as mtDNA depletion, 30%-50% as borderline low, and >50% as normal. The entire coding and flanking intronic regions of the *MPV17* and *DGUOK* genes were amplified by polymerase chain reaction from genomic DNA and sequenced by fluorescent dideoxy sequencing (Big Dye Terminator v. 3.1 kit; Applied Biosystems, Foster City, California) and capillary electrophoresis (ABI 3130XL; Life Technologies, Warrington, United Kingdom). Results were compared with Genbank reference sequences (NM_002437.4 and NM_080918, respectively), and variants described in accordance Human Genome Variation Society nomenclature guidelines. Where available, parental blood DNA samples were analyzed for the familial mutation(s) by sequencing of the appropriate exon.

Results

We identified pathogenic *MPV17* and *DGUOK* mutations in 11 out of 20 infants with suspected hepatocerebral MDS, representing 2.5% of the 450 cases of infantile cholestasis referred to our center during the study period. The remaining 9 infants who were negative for mitochondrial disease workup presented with liver failure, the cause of which remains undetermined in spite of extensive investigations. Hepatocerebral MDS was present in 22% (11/50) of infantile liver failure cases. The remaining causes of infantile liver failure included galactosemia (n = 13), tyrosinemia (n = 3), neonatal hemochromatosis (n = 5), hemophagocytic lymphohistiocytosis (n = 4), bile acid synthesis disorder (n = 2), cortisol deficiency (n = 1), and idiopathic (n = 11).

Clinical and Laboratory Characteristics

Table I summarizes the clinical and laboratory findings in the 11 patients: 7 patients with *MPV17* mutations (patients 1-7) and 4 patients with *DGUOK* mutations (patients 8-11) were identified; patients 1, 3, and 5 have recently been published in part[19] although further clinical, histopathologic, and neuroradiologic data are presented here. All patients were full-term babies with normal birth weight except 2 infants with *DGUOK* mutation who had low birth weights (1 kg in patient 8 and 2 kg in patient 10). Microcephaly (<3 percentile for age and sex) was present in all infants except patient 1 and patient 9. All patients presented, at variable times during infancy with jaundice, failure to thrive, and hypotonia. However, the onset of initial symptoms occurred within the first 2 months of life in all patients. Mild hepatomegaly was present in all patients except patients 6 and 10, and mild splenomegaly was present in patients 4 and 11. All 11 infants showed neurologic abnormality as evidenced by hypotonia, developmental delay in all patients,