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MPV17-associated hepatocerebral mitochondrial DNA depletion syndrome: New patients and novel mutations

Ayman W. El-Hattab [a], Fang-Yuan Li [a], Eric Schmitt [a], Shulin Zhang [a], William J. Craigen [a,b], Lee-Jun C. Wong [a,*]

[a] Department of Molecular and Human Genetics, Baylor College of Medicine, 1 Baylor plaza, Houston, TX 77030, USA
[b] Department of Pediatrics, Baylor College of Medicine, 1 Baylor plaza, Houston, TX 77030, USA

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ABSTRACT

Mitochondrial DNA depletion syndromes are autosomal recessive diseases characterized by a severe decrease in mitochondrial DNA content leading to dysfunction of the affected organ. They are phenotypically heterogeneous and classified as myopathic, encephalomyopathic, or hepatocerebral. The latter group has been associated with mutations in *TWINKLE, POLG1*, *DGUOK* genes and recently with mutations in the *MPV17* gene. *MPV17* encodes a mitochondrial inner membrane protein and plays an as yet poorly understood role in mitochondrial DNA maintenance. Mutations in the *MPV17* gene have been reported in patients who came to medical attention during infancy with liver failure, hypoglycemia, failure-to-thrive and neurological symptoms. In addition, a homozygous p.R50Q mutation has been identified in patients with Navajo neurohepatopathy. To date, 13 different mutations in 21 patients have been reported. We report eight new patients with seven novel mutations, including four missense mutations (c.262A>G (p.K88E), c.280G>C (p.G94R), c.293C>T (p.P98L), and c.485C>A (p.A162D)), one in-frame deletion (c.271_273del3 (p.L91del)), one splice site substitution (c.186+2T>C), and one insertion (c.22_23insC). The p.R50Q mutation, which occurs in a CpG dinucleotide, is the most common *MPV17* mutation and, to date, has only been found in the homozygous state. Clinically, patients homozygous for p.R50Q or compound heterozygous for the p.G94R and p.P98L mutations have a better prognosis, with all the other mutations associated with early death if not treated by liver transplantation. Localizing the mutations within the predicted MPV17 protein structure reveals clustering of mutations in the region of the putative protein kinase C phosphorylation site.

Introduction

Mitochondrial diseases are a clinically heterogeneous group of disorders that result from mitochondrial respiratory chain dysfunction that can be caused by defects in mitochondrial DNA (mtDNA) or nuclear genes involved in mtDNA biogenesis and maintenance [1]. Mendelian disorders due to defects in nuclear genes can be associated with large-scale rearrangements of mtDNA (mtDNA deletion syndromes) or with reduction in the mtDNA copy number (mtDNA depletion syndromes, MDS) [2].

MDS are autosomal recessive disorders that primarily affect infants or children and are characterized by a severe decrease of mtDNA content leading to organ dysfunction that is likely due to insufficient synthesis of respiratory chain components [3,4]. MDS are phenotypically heterogeneous and may affect either a specific organ or a combination of organs, including muscle, liver, brain, and kidney. Clinically, MDS are usually classified as one of three forms: a myopathic form (OMIM #609560) associated with mutations in the thymidine kinase 2 (*TK2*) gene [5] or the p53-induced ribonucleotide reductase B subunit (*RRM2B*) gene [6,7], an encephalomyopathic form (OMIM #612073) associated with mutations in the ATP-dependant succinyl CoA synthase gene (*SUCLA2*) [8] or the GTP-dependant succinyl CoA synthase gene (*SUCLG1*) [9], and a hepatocerebral form (OMIM #251880) associated with mutations in either the Twinkle (*PEO1*) gene [10], the polymerase-γA (*POLG1*) gene [11], the deoxyguanosine kinase (*DGUOK*) gene [12], or, more recently, with mutations in the *MPV17* gene [3].

Mutations in the *MPV17* gene were initially identified in patients who presented in the first year of life with liver failure, hypoglycemia, failure-to-thrive and neurological symptoms [3,13]. Subsequently, Karadimas and colleagues [14] reported a homozygous p.R50Q mutation in six patients with Navajo neurohepatopathy (NNH), an autosomal recessive multisystem disorder prevalent in the Native American Navajo population that manifests as liver disease, sensory and motor neuropathy, corneal anesthesia

* Corresponding author. Address: Department of Molecular and Human Genetics, Baylor College of Medicine, 1 Baylor Plaza, NAB 2015, Houston, TX 77030, USA. Fax: +1 713 798 8937.
E-mail address: ljwong@bcm.edu (L.-J.C. Wong).

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and scarring, cerebral leukoencephalopathy, failure-to-thrive, and metabolic acidosis [15,16]. Recently, additional mutations in the *MPV17* gene have also been reported [4,17–19]. To date, 13 different mutations in 21 patients have been observed. In this report we describe eight new patients with seven novel mutations. We also summarize and compare the clinical manifestations and the biochemical and molecular results for these and the 21 previously reported patients.

Materials and methods

Molecular studies

The DNA was extracted from peripheral blood, cultured skin fibroblasts, muscle, or liver tissue using Gentra DNA isolation kits according to manufacturer's instructions (Gentra Systems Inc., Minneapolis, MN). The mtDNA copy number in various tissues was determined by the real-time quantitative polymerase chain reaction (RT-qPCR) using primers specific for the mitochondrial transfer $RNA^{Leu\ (uur)}$ gene and nuclear single-copy gene, β-2-microglobulin [20]. The RT-qPCR was performed in duplicate for each reaction. MtDNA depletion was diagnosed by comparing patient's mtDNA copy number with tissue and age matched controls.

Sequence-specific oligonucleotide primers linked to M13 universal primers were designed to amplify all coding exons and the flanking intronic 50 nucleotides of the *MPV17* gene. PCR products were purified on ExcelaPure 96-well ultrafiltration PCR purification plates (Edge Bio-Systems, Gaithersburg, MD). Sequencing reactions were performed with the BigDye Terminator cycle sequencing kit (version 3.1), purified with Performa DTR 96-well V3 short plates (Edge Bio-Systems), and analyzed on an ABI3730XL automated DNA sequencer with Sequencing Analysis Software version 5.1 (Applied Biosystems). DNA sequences were aligned with the *MPV17* reference sequence (GenBank accession No. NT_022184.14) using Mutation Surveyor version 2.6.1 (SoftGenetics®, State College, PA). Due to the lack of a functional assay for the mutant proteins, the pathogenicity of novel mutants was determined by clinical phenotype, amino acid conservation, and prediction algorithms, including SIFT and PolyPhen (http://sift.jcvi.org; http://genetics.bwh.harvard.edu/pph/).

Respiratory chain enzyme analysis

Spectrophotometric analysis of the respiratory chain complexes was performed on muscle samples. The muscle samples were immediately frozen in liquid nitrogen after collection, stored at −80 °C, and shipped on dry ice. The electron transport chain enzymes were assayed at 30 °C with a temperature-controlled spectrophotometer (Ultraspec 6300 Pro, Biochrom, Ltd., Cambridge, England). Each assay was performed in duplicate. The activities of nicotinamide adenine dinucleotide (NADH):ferricyanide (FeCN) reductase (complex I), succinate dehydrogenase (SDH; complex II), rotenone sensitive NADH:cytochrome c reductase (complex I + III), succinate:cytochrome c reductase (complex II + III), and cytochrome c oxidase (complex IV) were measured with appropriate electron acceptors and donors [21].

The increase or decrease in the absorbance of cytochrome c at 550 nm was measured for complex I + III, complex II + III, or complex IV. The activity of NADH:FeCN reductase was measured by the oxidation of NADH at 340 nm. For SDH, the reduction of 2,6-dichloroindophenol at 600 nm was measured. Citrate synthase (CS) was used as a marker for the mitochondrial content. Enzyme activities are expressed with respect to both the total protein and CS activity.

Results

Patients

Eight patients with *MPV17*-associated hepatocerebral MDS whose blood and tissue samples were sent to Mitochondrial Diagnostic Laboratory at Baylor College of Medicine were analyzed biochemically and molecularly. All the patients developed liver failure in infancy, except patient 8, who is of Navajo heritage and presented with liver dysfunction. Patient 7 had liver cirrhosis and developed hepatocellular carcinoma. Common neurological findings were developmental delay, hypotonia, muscle weakness, and peripheral neuropathy. Failure-to-thrive, hypoglycemia, and lactic acidosis were also common findings. Electron transport chain (ETC.) activities in muscle tissue were measured in two patients. Patient 2 has low complex I and complexes II + III activities and patient 3 has low complex I and complex IV activities. MtDNA depletion was confirmed by mtDNA copy number assessments, which ranged between 25% and 76% in muscle, 28% and 70% in blood, and 6% and 21% in liver (Table 1).

MPV17 gene mutations

In the eight reported patients, ten different mutations were identified, among which seven are novel (Tables 1 and 2). Patient 1 is homozygous for a novel c.262A>G (p.K88E) mutation. Lysine at position 88 is evolutionarily conserved from zebra fish to human and is conserved as the basic amino acid arginine in yeast. It is located near the putative protein kinase C phosphorylation site. The SIFT and PolyPhen algorithms predict p.K88E to be deleterious. Patient 2 is compound heterozygous for two novel missense mutations; c.280G>C (p.G94R) and c.293C>T (p.P98L). Glycine at position 94 shows evolutionary conservation from zebra fish to human, and PolyPhen predicts p.G94R to be deleterious. Additionally, c.280 is the first nucleotide of exon 5; therefore, this mutation may affect splicing. Proline at position 98 is conserved from yeast to human, and PolyPhen predicts p.P98L to be deleterious. Patient 3 is compound heterozygous for an in-frame deletion c.263_265del3 (p.K88del) that was reported previously [17] and for a non-coding variant at the 5′UTR, c.-22_-16del7, the significance of which is unknown. Patient 4 harbors two novel heterozygous mutations; an in-frame deletion mutation c.271_273del3 (p.L91del), resulting in deletion of the leucine residue at amino acid position 91 and a missense mutation, c.485C>A (p.A162D). Alanine at position 162 is conserved from yeast to human, except in *Drosophila* where serine is the amino acid residue; PolyPhen predicts p.A162D to be deleterious. Patient 5 is homozygous for a 1.57 kb deletion spanning the last exon (exon 8). This deletion was reported previously [4]. Patient 6 is compound heterozygous for two novel mutations; c.271_273del3 (the mutation found in patient 4), and c.186+2T>C, which is located at the invariant splice donor site. The c.186+2T>C alteration is predicted to abolish the splicing donor site of exon 3 (http://www.cbs.dtu.dk/services/NetGene2/ and http://www.fruitfly.org/seq_tools/other.html), and is thus classified as a deleterious mutation [22]. Patient 7 is heterozygous for a novel c.22_23insC frame-shift mutation. Analysis of the mRNA by reverse transcriptase PCR followed by cDNA sequencing showed skipping of exon 3. While a second mutation was not found, no normal cDNA species was detected in the blood specimen, consistent with the presence of a second unidentified null mutation. Patient 8 is homozygous for the c.149G>A (p.R50Q) mutation that was previously reported [14,3].

Table 1
The clinical manifestations of *MPV17*-associate hepatocerebral MDS and the *MPV17* mutations in the eight reported patients (1–8) and the previously reported cases. The novel mutations are shown in italics.

Patient (gender)	Ethnicity	Age of onset	Outcome (cause of death)	Clinical manifestations					mtDNA copy number	ETC. (Complexes I, II, III, & IV activities)	Mutations		Reference
				Hepatic	Neurological	Growth	Metabolic	Others			Allele 1	Allele 2	
Patient 1 (M)	Hispanic	Neonate	Liver transplant Death at 2.5 y (multi-organ failure)	Cholestasis Liver failure	Developmental delay, muscle weakness & white matter loss on brain MRI	FTT	Lactic acidosis	Hypo-parathyroidism Tubulopathy	Blood 48%		*c.262A>G p.K88E*	*c.262A>G p.K88E*	This report
Patient 2 (F)	Caucasian	Infancy	Alive at 2.5 y	Cholestasis Liver failure	Developmental delay, hypotonia & muscle weakness	FTT	Lactic acidosis	Tubulopathy GERD Diarrhea Pancreatitis	Blood 28%	Muscle: I 38%, I + III 51% II 56%, II + III 27% IV 69%	*c.280G>C p.G94R*	*c.293C>T p.P98L*	This report
Patient 3 (M)	Hispanic	4 m	Death at 15 m (liver failure)	Hepatomegaly Liver failure	Developmental delay, hypotonia & peripheral neuropathy	FTT	None	GERD	Blood 46% Muscle 25% Liver 21%	Muscle: I 43%, I + III 118% II 76%, II + III 61% IV 46%	c.-22_-16del7	c.263_265del3 p.K88del	This report
Patient 4 (M)	Caucasian	Infancy	Liver transplant Alive at 4 y	Liver Failure	Hypotonia	FTT	Lactic acidosis	GERD Diarrhea Cyclic vomiting	Blood 35% Muscle 76% Liver 19%		*c.485C>A p.A162D*	*c.271_273del3 p.L91del*	This report
Patient 5 (M)	Caucasian	5 m	Death at 11 m (liver failure)	Hepatomegaly Liver failure	Developmental delay, hypotonia, muscle weakness, encephalopathy & leukodystrophy on brain MRI	FTT	Hypoglycemia Lactic acidosis		Blood 43% Liver 6%		1.57 kb deletion spanning exon8	1.57 kb deletion spanning exon8	This report
Patient 6 (M)	Caucasian	2 m	Death at 6 m (liver failure)	Liver failure	Developmental delay, hypotonia, microcephaly & abnormal brain MRI	FTT	Hypoglycemia Lactic acidosis		Blood 39% Liver 6%		*c.186 + 2T>C*	*c.271_273del3 p.L91del*	This report
Patient 7 (M)	Caucasian	Infancy	Liver transplant Alive at 9 y	Liver failure Cirrhosis HCC	Hypotonia	FTT	None		Liver 14%		*c.22_23insC*	A second mutation was not detected	This report
Patient 8 (M)	Navajo	5 m	Alive at 9 m	Liver dysfunction	No neurological manifestations	Normal	None		Blood 70%		c.149G>A p.R50Q	c.149G>A p.R50Q	This report
Case 1 (M)[a]	Middle Eastern	2 m	Death at 5 m (liver failure)	Hepatomegaly Liver failure	Seizure & ischemic cerebrovascular infarction	FTT	Hypoglycemia Lactic acidosis		Muscle 13%	Muscle: I + III 29%, II + III 118%, IV 25%	c.206G>A p.W69X	c.206G>A p.W69X	Wong et al. [17]
Case 2 (M)[a]	Middle Eastern	Birth	Liver transplant Death at 6 m (sepsis)	Hepatomegaly Liver failure	No neurological manifestations	FTT	Lactic acidosis		Liver 4% Blood 50%	Liver: I + III 8%, II + III 38%, IV 12%	c.206G>A p.W69X	c.206G>A p.W69X	Wong et al. [17]
Case 3 (F)	Mexico	8 m	Death at 19 m (liver failure)	Hepatomegaly Liver failure	Hypotonia & encephalopathy	FTT	Lactic acidosis		Liver 5%	Liver: I + III 8%, IV 12%	c.148C>T p.R50W	c.148C>T p.R50W	Wong et al. [17]

Case 4 (M)	Caucasian	1 m	Death at 3 m (liver failure)	Hepatomegaly Liver failure	No neurological manifestations	FTT	Hypoglycemia Lactic acidosis		Liver 3% Muscle 8%	Liver: I + III 19%, II + III 48%, IV 31% Muscle: I + III 78%, II + III 67%, IV 19%	c.263_265del3 p.K88del	c.234_242del9 p.G79_T81del	Wong et al. [17]
Case 1 (M)[b]	Japanese	3 m	Liver transplant Death at 22 m (sepsis)	Cholestasis Hepatomegaly Liver failure Cirrhosis	Hypotonia	FTT	None		Liver 8%	Liver: I 0%, II 27%, III 4%, IV 14%	c.451insC	c.509C>T p.S170F	Kaji et al. [19]
Case 1 (M)[b]	Japanese	8 m	Alive at 47 m	Cholestasis Liver dysfunction	Hypotonia	FTT	Lactic acidosis		Liver 7%	Liver: I 6%, II 36%, III 9%, IV 21%	c.451insC	c.509C>T p.S170F	Kaji et al. [19]
Patient (M)	NA	2 m	Liver transplant Death at 22 m (multi-organ failure)	Liver failure Cirrhosis	Developmental delay, hypotonia, MSPN, seizure, encephalopathy & leukodystrophy on brain MRI	FTT	Hypoglycemia Lactic acidosis	Tubulopathy GI dysmotility Hypopara-thyroidism Pigmentary retinopathy	Liver 20% Muscle 20%	Liver: I + III 28%, II + III 45% II 19%, IV 24%	c.70+5G>A	c.70+5G>A	Navarro et al. [18]
Case 1 (F)[c]	Iraqi	2 m	Death at 11 m (liver failure)	Hepatomegaly Liver failure	Dystonic movement & microcephaly	FTT	Hypoglycemia Lactic acidosis	Nephrolethiasis	Liver 30%		c.359G>A p.W120X	c.359G>A p.W120X	Spinazzola et al. [4]
Case 2 (F)[c]	Iraqi	2 m	Death at 5 m (liver failure)	Hepatomegaly Liver failure	Dystrophy & hypotonia	FTT	Lactic acidosis				c.359G>A p.W120X	c.359G>A p.W120X	Spinazzola et al. [4]
Case 3 (F)	NA	Neonate	Death at 9 m (liver failure)	Hepatomegaly Liver failure	Hypotonia, nystagmus, subdural hematoma & periventricular leukomalacia on brain MRI	FTT	Hypoglycemia		Liver 16%	Liver: Decreased I and IV	c.70G>T p.G24 W	1.57 kb deletion spanning exon8	Spinazzola et al. [4]
Patient 1 (M)[d]	Navajo	Infancy	Death at 16 y (liver failure)	Cirrhosis Liver failure	Developmental delay & MSPN	FTT	Hypoglycemia Metabolic acidosis				p.R50Q	p.R50Q	Karadimas et al. [14]
Patient 2 (F)[d]	Navajo	Infancy	Death at 20 y (liver failure)	Cirrhosis Liver failure	Developmental delay, MSPN, corneal anesthesia & ulcers & leukoencephalopathy on brain MRI	FTT	Hypoglycemia Metabolic acidosis				p.R50Q	p.R50Q	Karadimas et al. [14]
Patient 3 (M)	Navajo	Infancy	Death at 15 y (liver failure)	Cirrhosis Liver failure	Developmental delay, MSPN, corneal anesthesia & ulcers & leukoencephalopathy on brain MRI	FTT	Metabolic acidosis				p.R50Q	p.R50Q	Karadimas et al. [14]
Patient 4 (F)	Navajo	Infancy	Liver transplant Living at 12 y	Hepatomegaly Liver failure	Developmental delay, hypotonia & MSPN	FTT	Metabolic acidosis				p.R50Q	p.R50Q	Karadimas et al. [14]
Patient 5 (F)	Navajo	1 m	Liver transplant Death at 2 y (sepsis)	Hepatomegaly Liver failure Cirrhosis	Hypotonia, muscle wasting & leukoencephalopathy on brain MRI	FTT	Metabolic acidosis		Liver 11%	Liver: Decreased I, III, and IV	p.R50Q	p.R50Q	Karadimas et al. [14]
Patient 6 (M)	Navajo	Infancy	Liver transplant Living at 21 y	Cirrhosis HCC	Peripheral neuropathy & white matter lesions on brain MRI	FTT	Hypoglycemia		Liver 18%		p.R50Q	p.R50Q	Karadimas et al. [14]

(*continued on next page*)

Table 1 (*continued*)

Patient (gender)	Ethnicity	Age of onset	Outcome (cause of death)	Clinical manifestations					mtDNA copy number	ETC. (Complexes I, II, III, & IV activities)	Mutations		Reference
				Hepatic	Neurological	Growth	Metabolic	Others			Allele 1	Allele 2	
1-1 (M)[e]	Southern Italy	1 m	Death at 7 m (liver failure)	Hepatomegaly Liver failure	Hypotonia	Normal at 2 m	Hypoglycemia Lactic acidosis	Neutropenia	Liver 3%	Liver: I 0.5%, II 68% III 27%, IV 25%	c.149G>A p.R50Q	c.149G>A p.R50Q	Spinazzola et al. [3] & Parini et al. [13]
1-2 (M)[e]	Southern Italy	1 m	Liver transplant Living at 6 y	Liver failure	Ataxia & MSPN	Normal at 1y	Hypoglycemia		Liver 11%	Liver: I 40%, II 124% III 66%, IV 54%	c.149G>A p.R50Q	c.149G>A p.R50Q	Spinazzola et al. [3] & Parini et al. [13]
1-4 (F)[e]	Southern Italy	5 m	Living at 13 y	Liver dysfunction	MR, MSPN, corneal scarring, muscle hypotrophy, myoclonus, ataxia & white matter lesions on brain MRI	FTT	Hypoglycemia Lactic acidosis	Scoliosis	Liver 22%	Liver: I 48%, II 133% III 60%, IV 80%	c.149G>A p.R50Q	c.149G>A p.R50Q	Spinazzola et al. [3] & Parini et al. [13]
2-4 (M)	Morocco	Infancy	Death in infancy (liver failure)	Liver failure	NA	NA	NA		Liver ~5%	Liver: I ~15%, IV ~45%	c.498C>A p.N166 K	c.498C>A p.N166 K	Spinazzola et al. [3]
3-1 (F)	Canada	Infancy	Death in infancy (liver failure)	Liver failure	NA	NA	NA		Liver ~10%	Liver: I ~25%, IV ~5%	c.148C>T p.R50 W	c.116–141del25	Spinazzola et al. [3]

ETC: electron transport chain activity, y: year(s), m: month(s), NA: not reported, FTT: failure-to-thrive, GERD: gastro-esophageal reflux disease, MSPN: motor and sensory peripheral neuropathy, HCC: Hepatocellular carcinoma, MR: mental retardation.

[a,b,c,d,] and [e] indicate members of the same family. ETC is represented as percentage of the normal control.

Table 2
The frequencies of the *MPV17* gene mutations.

Mutation type	Previously reported mutations	Frequency[a]		Novel mutations	Frequency[a]	
		Homozygous	Heterozygous		Homozygous	Heterozygous
Missense	c.149G>A p.R50Q	7	0	c.262A>G p.K88E	1	0
	c.148C>T p.R50 W	1	1	c.280G>C p.G94R	0	1
	c.498C>A p.N166 K	1	0	c.293C>T p.P98L	0	1
	c.70G>T p.G24 W	0	1	c.485C>A p.A162D	0	1
	c.509C>T p.S170F	0	1			
Nonsense	c.359G>A p.W120X	1	0			
	c.206G>A p.W69X	1	0			
In-frame deletion	c.263_265del3 p.K88del	0	2	c.271_273del3 p.L91del	0	2
	c.234_242del9 p.G79_T81del	0	1			
Large deletion	1.57 kb deletion spanning exon8	1	1			
Frame shift deletions	c.116–141del25	0	1			
Splicing site	c.70+5G>A	1	0	c.186+2T>C	0	1
Insertion	c.451insC	0	1	c.22_23insC	0	1
Total	13			7		

[a] Number of unrelated families.

Discussion

Clinical phenotype of MPV17-associated hepatocerebral MDS

Mitochondrial respiratory chain disorders of all types are estimated to affect approximately 1 in 20,000 children under 16 years of age [23]. About 10% of children with respiratory chain defects have liver dysfunction, with the onset of liver disease in the neonatal period in about half of cases [24]. Given the clinical heterogeneity and the difficulties with establishing the diagnosis, the prevalence of primary mitochondrial hepatopathies is likely to be underestimated [25].

In *MPV17*-associated hepatocerebral MDS, liver involvement appears early in the course of the disease, in contrast to the other multisystem mitochondrial disorders with prominent neuromuscular involvement in which liver complications are more commonly a late feature [17]. On the other hand, unlike other MDS [6,11,26–28], neurological involvement in *MPV17*-associated hepatocerebral MDS is generally mild at the onset of disease [13].

Prior to this report, a total of 21 patients with *MPV17*-associated hepatocerebral MDS had been described, and here we report eight new patients. All of these patients came to medical attention in infancy with liver dysfunction that progressed to liver failure. They either died or had liver transplantation performed, with the exception of patients 2 and 8, one of the patients reported by Spinazzola and colleagues [3], and one of the patients reported by Kaji et al. [19] who are living without liver transplantation at ages of 2.5 y, 8 m, 13 y, and 47 m, respectively. Patient 7 and one of the patients reported by Karadimas and colleagues [14] developed liver cirrhosis and hepatocellular carcinoma. Most of the patients exhibited neurological manifestations, including developmental delay, hypotonia, motor and sensory peripheral neuropathy, and leukodystrophy. Not surprisingly, failure-to-thrive is one of the common manifestations, although some patients have normal growth, especially early in the course of the disease [13]. Lactic acidosis and hypoglycemia are also common features. Occasional manifestations include hypoparathyroidism, renal tubulopathy, and gastrointestinal dysmotility manifested as gastro-esophageal reflux (GER), cyclic vomiting, and diarrhea. Consistent with the predominant liver involvement in *MPV17*-associated hepatocerebral MDS, mtDNA copy number is lower in liver when compared to muscle, with the percentage of control values being 3–30% in liver and 8–76% in muscle. MtDNA copy number in blood are 28–70% which is relatively higher than other tissues, indicating that blood mtDNA copy numbers are not a reliable indicator of mtDNA depletion. ETC. activity assays showed that complex I or I + III are the most affected components. Similar to the findings of mtDNA content, the liver ETC. activities are reduced more than that of muscle tissue (Table 1).

The treatment of liver disease in mitochondrial hepatopathies remains unsatisfactory. Present treatment involves the use of various vitamins, cofactors, and respiratory substrates, none of which have proven to be effective [4]. Liver transplantation in mitochondrial hepatopathy is controversial, largely because of the multisystemic nature of this disorder [4]. Liver transplantation was performed in 10 patients (7 previously reported cases and 3 of our reported patients); 5 patients died after the transplant with multi-organ failure or sepsis [3,13,14,17,19]. Out of the five patients who survived following liver transplantation, three carry the homozygous p.R50Q mutation.

Molecular aspects

The *MPV17* gene maps to chromosome 2p23.3 and contains eight exons (Fig. 1). The MPV17 protein, which is an inner mitochondrial membrane protein, is predicted to contain 4 transmembrane (TM) segments (http://pir.uniprot.org) (Fig. 2). A putative protein kinase C phosphorylation site is predicted to be in the region between TM2 and TM3 [17]. The function of the MPV17 protein and its role in the pathogenesis MDS are still unknown. However, it has been suggested that it plays a role in controlling mtDNA maintenance and oxidative phosphorylation activity in mammals and yeast [3]. The *SYM1* gene, the ortholog of *MPV17* gene in *Saccharomyces cerevisiae*, has a suggested role in the cellular response to metabolic stress [29] and in maintaining mtDNA integrity and stability [3]. In mouse fibroblasts, the absence of MPV17 was found to reduce the production of reactive oxygen species [30].

*Mpv*17 deficient mice have been reported to develop focal segmental glomerulosclerosis with massive proteinuria and renal failure, age-dependent hearing loss, and early graying of their coat [31–34]. MtDNA depletion was demonstrated in renal and liver tissues. In spite of severe mtDNA depletion, only a moderate decrease in respiratory chain enzymatic activities and mild cytoarchitectural alterations were observed in liver; neither cirrhosis nor liver failure occurred at any age [3,34].

By aligning the missense and in-frame deletion mutations to the predicted MPV17 protein structure we have observed clustering of mutations in the region of the putative protein kinase C phosphorylation site. Whether this reflects some important functional aspect for this domain of the protein or simply sites of common mutations remains unknown. The p.R50Q mutation is located

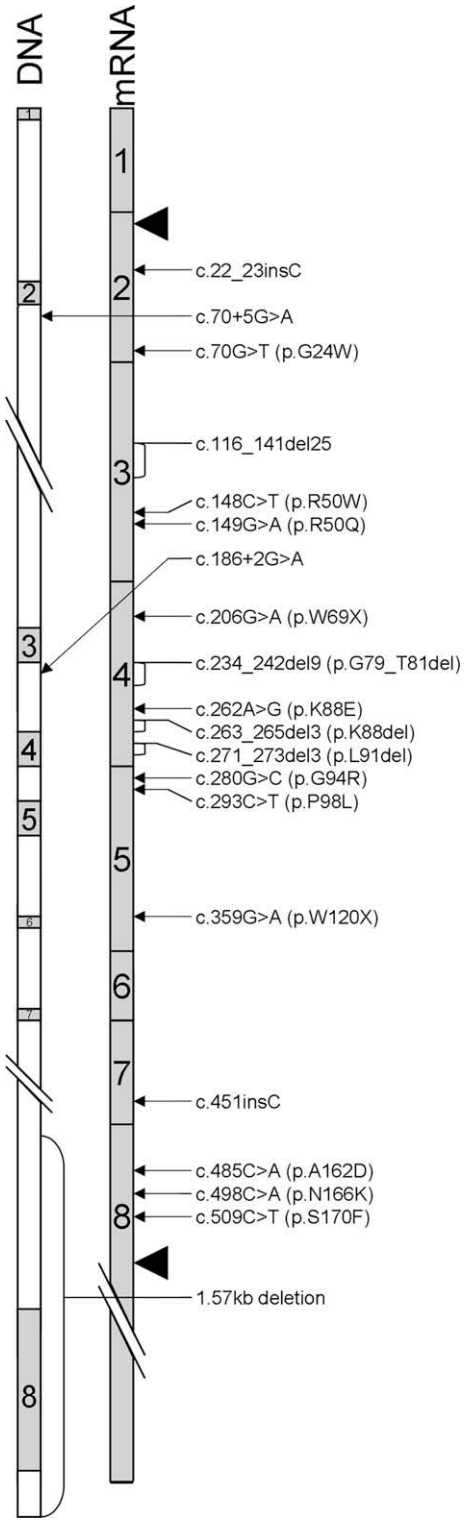

Fig. 1. The *MPV17* gene maps to 2p23.3 (27,385,864-27,399,473) spanning 13610 bp. The left vertical line which is labeled as DNA represents the *MPV17* gene's 8 exons; exon1 (coordinates: 1–51, length: 51 bp), exon 2 (581–655, 75 bp), exon 3 (9993–10109, 116 bp), exon 4 (10331–10423, 93 bp), exon 5 (10514–10609, 96 bp), exon 6 (10856–10888, 33 bp), exon 7 (11151–11203, 53 bp), and exon 8 (13121–13610, 490 bp). The right vertical line represents the mRNA. Note that the starting codon (upper arrow head) is located in exon 2 (exon 1 is non-coding) and the larger part of exon 8 is non-coding as the stop codon (lower arrow head) is located after 67 nucleotides from the beginning of this exon. The coding sequence is 531 nucleotides (NCBI Reference Sequences, Build 36.3, GenBank, NT_022184.14) including: exon 2 (1–70), exon3 (71–186), exon4 (187–279), exon 5 (280–375), exon 6 (376–408), exon 7 (409–461), and exon 8 (462–531). The *MPV17* genes mutations are demonstrated.

between TM1 and TM2. Since homozygosity for p.R50Q causes a less severe phenotype, this suggests that that this substitution better preserves function or that this region is less vital than other regions of the protein.

The p.R50Q (c.149G>A) mutation occurs at a CpG dinucleotides and has been observed in both Navajo and Italian patients. It has been estimated that up to 25% of all disease-causing human mutations occur at CpG sites [35]. This fact may explain the observation that p.R50Q is the most common mutation identified in the *MPV17* gene. However, the infrequent missense mutations c.148C>T (p.R50W) and c.293C>T (p.P98L) are also located at CpG dinucleotides.

Genotype–phenotype correlation

To date, the p.R50Q mutation has only been reported in the homozygous state. In contrast to most *MPV17* gene mutations that are associated with death in infancy or early childhood, the p.R50Q mutation is associated with longer survival, suggesting that this is a hypomorphic mutation. One homozygous patient whose mtDNA copy number was very low (3%) died at the age of 7 m, another homozygous patient who is still living at 13 y without a liver transplantation has a mtDNA copy number quantitation of 22% [3,13], suggesting that the degree of mtDNA depletion correlates with the outcome for patients with p.R50Q mutations. The missense mutations p.R50W (homozygous and compound heterozygous state), p.N166K (homozygous), p.G24W (compound heterozygous), p.K88E (homozygous), and p.A162D (compound heterozygous) all have been associated with a lower mtDNA copy number and death in infancy or early childhood if not treated by liver transplantation. However, compound heterozygosity for the p.G94R and p.P98L mutations in patient 2, who is living at 2.5 years of age without liver transplantation, may indicate a better prognosis (Table 1).

The nonsense mutations p.W120X (homozygous) and p.W69X (homozygous), the in-frame deletions c.263_265del3 (compound heterozygous), c.234_242del9 (compound heterozygous), and c.271_273del3 (compound heterozygous), the frame shift deletion c.116-141del25 (compound heterozygous), the 1.57 kb deletion spanning exon 8 (homozygous and compound heterozygous), the splice site mutations c.70+5G>A (homozygous) and c.186+2T>C (heterozygous), and the insertion mutation c.22_23insC (heterozygous) all have been associated with death in infancy or early childhood if not treated by liver transplantation (Table 1).

Recently, Kaji and colleagues have reported two siblings found to be compound heterozygous for the missense mutation c.509C>T (p.S170F) and the frame shift insertion c.451insC. The older sibling developed liver failure and died at the age of 22 m, while the younger is surviving at 47 m without development of an overt liver failure. Therefore, it was suggested that the clinical course could be influenced by viral infections and dietary and pharmacological managements.

The p.R50Q mutations have been identified in 7 families. The c.263_265del3 (p.K88del), the c.271_273del3 (p.L91del), the c.148C>T (p.R50W), and the 1.57 kb deletion spanning the last exon, each has been observed in two families. All other mutations are private mutations (Table 2).

Conclusion

MPV17-associated hepatocerebral MDS presents in infancy with liver dysfunction that progresses to liver failure and neurological manifestations. The homozygous p.R50Q mutation, which occurs in a CpG dinucleotide, is the most common mutation and is generally associated with a better prognosis. In addition, compound heterozygosity for the p.G94R and p.P98L mutations may have a better

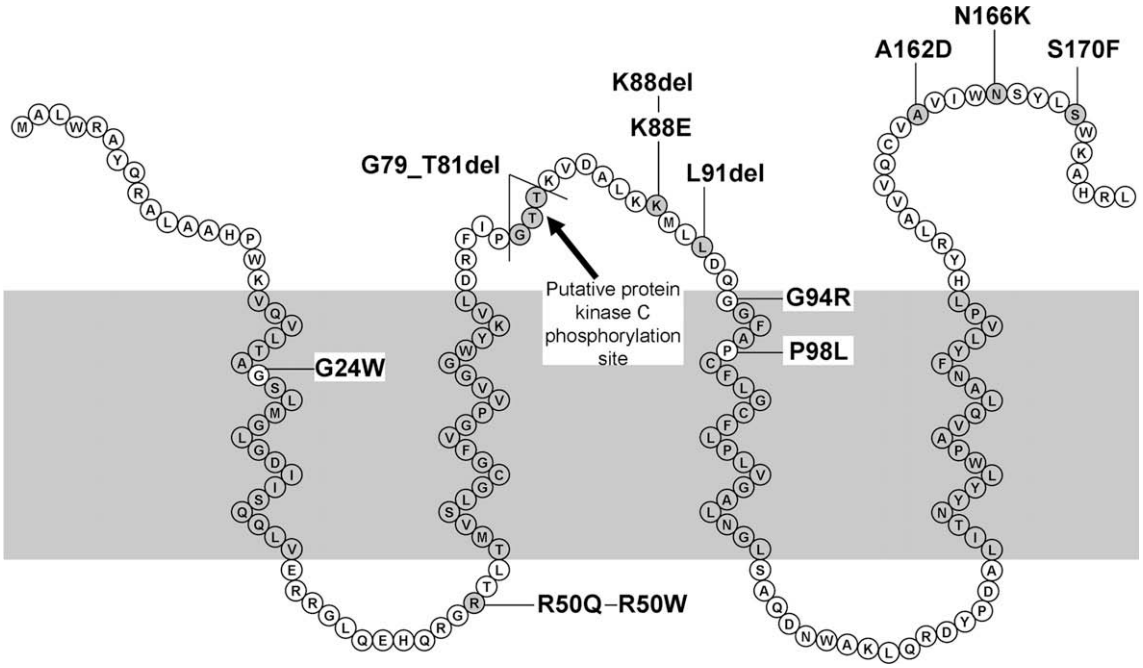

Fig. 2. The MPV17 protein, composed of 176 amino acids, is predicted to contain 4 transmembrane (TM) spans: TM1 from amino acid 18–38, TM2 from 53–73, TM3 from 94–114, and TM4 from 131–151, with short flanking hydrophilic intermembrane and matrix regions (http://pir.uniprot.org). A putative protein kinase C phosphorylation site predicted to be in the region between TM2 and TM3 (Wong et al., 2007). The missense mutations and in-frame deletions are demonstrated.

prognosis. Other mutations are associated with early death if not treated by liver transplantation. Liver transplantation resulted in death due to multi-organ failure or sepsis in about half of the cases, and p.R50Q homozygotes had better post-transplant outcomes. A clustering of mutations in the region of the putative protein kinase C phosphorylation site of the MPV 17 protein was observed.

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