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GENOTYPE-PHENOTYPE CORRELATION IN MITOCHONDRIAL DEPLETION SYNDROME DUE TO DGUOK DEFICIENCY

Dissertação apresentada à Faculdade de Medicina da Universidade de Coimbra, para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Medicina, realizada sob a orientação científica da Professora Doutora Manuela Grazina (Faculdade de Medicina da Universidade de Coimbra).

Sara Duarte, 2014

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RESUMO

O défice da desoxiguanosina cinase (dGUOK), causado por mutações no gene *DGUOK*, leva ao desenvolvimento da forma hepatocerebral da Síndrome de depleção de DNA mitocondrial (MDS), ligadas a uma redução drástica no número de cópias do genoma mitocondrial (mtDNA), associada a elevada mortalidade e sem estratégias terapêuticas disponíveis, na criança.

Apesar de se terem vindo a identificar novas mutações no gene *DGUOK* ao longo dos últimos anos, uma correlação genótipo-fenótipo precisa e exequível, nunca foi estabelecida. Um padrão reprodutível de associação entre o tipo e localização das mutações genéticas no gene *DGUOK* e a clínica apresentada pelo doente facultaria aos clínicos a capacidade de estimar, num estádio neonatal, ou mesmo pré-natal, o diagnóstico e a gravidade da doença, provendo os clínicos com ferramentas mais racionais para aconselhamento genético adequado às famílias e aos futuros pais.

Procedeu-se a uma busca extensiva na base de dados de referências MEDLINE para procurar todas as mutações no gene *DGUOK*, publicadas até à data. Estas alterações foram analisadas quanto à sua localização no gene e o seu impacto na função e estrutura da proteína, assim como nas manifestações clínicas apresentadas pelos doentes e foi efetuado um estudo da correlação genótipo-fenótipo.

Verificou-se a inexistência de uma correlação genótipo-fenótipo precisa e clara em doentes com MDS causado por mutações no gene *DGUOK*, revelando a existência de mecanismos mais complexos e ainda desconhecidos, que estarão subjacentes ao início e à progressão da doença.

GENOTYPE-PHENOTYPE CORRELATION IN MITOCHONDRIAL DEPLETION SYNDROME DUE TO DGUOK DEFICIENCY

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LIST OF ABBREVIATIONS

ADP	Adenosine diphosphate
ALT	Alanine transaminase
AST	Aspartate transaminase
ATP	Adenosine triphosphate
c.	Coding DNA sequence
cDNA	Complementary DNA
C10orf2	Chromosome 10 open reading frame 2
dCK	Deoxycytidine kinase
DGUOK/ dGK	Deoxyguanosine kinase
DGUOK	Gene coding for dGK
DNA	Deoxyribonucleic acid
dNDP	Deoxyribonucleotide diphosphate
dNMP	Deoxyribonucleotide monophosphate
dNTP	Deoxinucleotides triphosphate
dup	Duplication
GGT	Gamma-glutamyl transpeptidase
GDP	Guanosine diphosphate
GTP	Guanosine triphosphate
MDS	Mitochondrial DNA Depletion Syndromes
MpV17	MpV17 Mitochondrial Inner Membrane Protein

MPV17	Gene coding for MpV17 Mitochondrial Inner Membrane Protein
MRC	Mitochondrial Respiratory Chain
mtDNA	mitochondrial DNA
p.	protein sequence
POLG	Polymerase (DNA directed), gamma
POLG	Gene coding for Polymerase (DNA directed), gamma
RNA	Ribonucleic acid
RRM2B	Ribonucleotide Reductase M2 B (TP53 Inducible)
SUCLG1	Succinate-CoA Ligase, Alpha Subunit
SIFT	Sorting Intolerant From Tolerant
SUCLA2	Succinate-CoA Ligase, ADP-Forming, Beta Subunit
TK1	Thymidine kinase 1
TK2	Thymidine kinase 2
TYMP	Thymidine Phosphorylase
5'-dRP	5'-deoxyribose-5-phosphate

ABSTRACT

Deoxyguanosine kinase (coded by DGUOK) deficiency, caused by *DGUOK gene* mutations, leads to development of the hepatocerebral form of mitochondrial DNA depletion syndrome (MDS), linked to mitochondrial genome (mtDNA) copy number severe decline, associated to high mortality and absence of available therapeutic strategies in infants.

In spite of the continuous identification of new *DGUOK* mutations over the years, an accurate and feasible genotype-phenotype correlation in *DGUOK* patients is lacking. A reproducible pattern of association between the type and localization of *DGUOK* gene mutation and its effect on patients´ clinic would ground physicians with the capacity to foresee, at a neonatal or even pre-natal stage, the disease diagnostics and severity, providing the clinicians, therefore, with more rationale tools for adequate genetic counselling in families and future parents.

An extensive search on MEDLINE database was performed in order to search for all published *DGUOK* gene mutations to date. These alterations were analysed for their localization in the gene and their impact on protein structure and function and the clinical manifestations and a genotype-phenotype correlation study has been carried out.

It was evident the absence of a clear and accurate genotype-phenotype correlation in patients with MDS caused by *DGUOK* mutations, unmasking, likely, more complex and unknown mechanisms underlying disease onset and progression.

Keywords: Deoxyguanosine Kinase, *DGUOK* gene mutations, mitochondrial DNA depletion syndrome, MDS, hepatocerebral form, hepatic form.

INTRODUCTION

Aerobic organisms require energy for their metabolic activities. In eukaryotes, the main source of energy comes from ATP, the end-product of oxidative phosphorylation, a cascade of electron-transfer reactions taking place at the inner mitochondrial membrane, through the mitochondrial respiratory chain (MRC) proteins and transporters [1].

Only a minority of the MRC complexes subunits are encoded by mitochondrial DNA (mtDNA), and thus most of the key catalytic components of these electron carriers are nuclear encoded [2]. The mtDNA encodes for a total of 37 genes, 13 of which correspond to subunits of the MRC complexes I (n=7), III (n=1) and IV (n=3) and complex V - ATP synthase (n=2). The remaining 24 genes encode for ribosomal RNA (n=2) and transfer RNA (n=22), required for mtDNA encoded MRC subunits synthesis. Conversely, all the proteins involved in mtDNA biogenesis, replication and maintenance are encoded in the nuclear genome. At least approximately 1,500 nuclear genes are known to be involved in these processes [3].

Defects in either genomes, nuclear or mitochondrial, can cause MRC disorders. In the past two decades a group of autosomal recessive, phenotypically heterogeneous disorders, with Mendelian transmission [4], have been extensively reported in association with the same feature, a significant reduction in mtDNA copy number, which is known as mtDNA depletion.

MITOCHONDRIAL DEPLETION SYNDROMES

Mitochondrial depletion syndrome (MDS) comprise a group of autosomal recessive disorders characterized by a severe reduction of mtDNA content (<30%), caused by mutations

in the nuclear-encoded proteins involved in mtDNA synthesis and maintenance [4]. These proteins include POLG, C10orf2, SUCLG1, SUCLA2, TYMP, RRM2B, MPV17, DGUOK and TK2 [2, 5]. No mtDNA point mutations or rearrangements (deletions, insertions) in MDS patients have been reported to date.

Tissue-specific mtDNA depletion is a particularity of MDS disorders. Although both *DGUOK* and *TK2* genes encode enzymes belonging to the nucleoside salvage pathway in the mitochondria, mutations in the first result in marked mtDNA depletion in the liver and, in most cases, in the brain [6, 7], while TK2 deficiency is characterized by a severe mtDNA reduction in skeletal muscles [8, 9]. Since mtDNA encodes for a only 13 MRC subunits, the activity of all complexes but the pan-nuclear encoded complex II, are markedly decreased in MDS affected tissues [6].

MDS frequently manifest soon after birth [10]. In fact, the mtDNA amount almost doubles, within the first year of life, suggesting a higher demand on mtDNA synthesis, related to the aerobic respiration and high rate of tissue grow, after birth [11].

Resulting from its molecular heterogeneity, clinical characteristics of MDS patients vary within a high range, but can be divided in three presenting categories: myophathic, hepatocerebral and encephalomyophatic [2, 12].

MITOCHONDRIAL NUCLEOTIDE METABOLISM AND mtDNA REPLICATION

Mitochondrial DNA replication seems to occur independently of cell-division cycle, although some studies have argued that this is not always the case [13, 14]. Additionally, if both nuclear and mitochondrial genomes encoded proteins are needed for functional mitochondria, it is not logical to think that both mtDNA replication and cell cycle are

independent from each other. An elementary replication complex formed by the mitochondrial helicase Twinkle, the mitochondrial single stranded DNA-binding protein and the DNA polymerase gamma, are responsible for mtDNA replication and repair. Concerning repair, enzyme activities of DNA polymerase, 3´-5´ Exonuclease and 5´ dRP lyase [15, 16] play an essential role, in addition to DNA synthesis [17].

Previous studies have demonstrated that mtDNA replication is completed in approximately 1 to 2 hours [18] and an adequate and balanced pool of deoxinucleotides triphosphate (dNTP) in the mitochondrion matrix is required for this process to be efficient.

Evidence is lacking supporting *de novo* synthesis of dexynucleotides in mitochondria, so they probably must be imported from the cytosol. There are two major DNA precursor metabolic routes. The first consists on the salvage of extracellular nucleotides that cross the plasmatic membrane via a membrane nucleoside transporter. Once inside the cell, cytosolic kinases add inorganic phosphate to the 5´position of nucleosides, trapping them in the cell. Cytosolic deoxycytidine kinase (dCK) has substrate specificity for deoxyadenosine and deoxyguanosine, as well as for deoxycytidine, while TK1 catalyzes the phosphorylation of thymidine to deoxythymidine monophosphate. Monophosphate nucleosides are then converted into di- and triphosphate nucleosides by nucleoside mono- and diphosphate kinases, respectively [19, 20].

The second and major route for dNTP synthesis is catalyzed by a cytoplasmic ribonucleotide reductase, which reduces ribonucleotide diphosphate into deoxyribonucleotide diphosphate (dNDP) [21]. The dNDPs are then converted to dNTPs by a nucleoside diphosphate kinase.

Due to mitochondrial inner membrane impermeability to charged molecules, cytosolic deoxiribonucletides, deoxyribonucleotide monophosphate (dNMP), dNDP and dNTP are actively transported into the mitochondria through specialized mitochondrial deoxynucleotide

carriers. In yeast, an ATP/ADP exchanger, the GTP/GDP carrier Ggc1p and deoxynucleotide carrier Rim2p were described [22, 23]. By homology, SLC25A33 and SLC25A36 were suggested as mitochondrial deoxynucleotide transporters in humans [24]; however, only the first was confirmed and named as PNC1 [25, 26]. More recently, Drim2 was functionally characterized in *Drosophila melanogaster* [27].

The salvage pathway of deoxynucleotides synthesis inside the mitochondria is assured by two mitochondrial kinases: DGUOK, having substrate specificity for purine deoxynucleotides [28], and TK2, which phosphorylates pyrimidine deoxynucleotides [29]. Deoxinucleotide diphosphates are finally converted into dNTPs through the activity of mitochondrial nucleotide-diphosphate kinases.

Of important note, a computational study demonstrated that supply of dNTPs, by the mitochondrial salvage pathway, cover only one third of dNTPs needed for mtDNA replication, and therefore, an external source of deoxynucleotides would be necessary [18]. Further investigation is required to demonstrate which other sources account for a balanced dNTP pool in the mitochondria.

THE MITOCHONDRIAL SALVAGE ENZYME DGUOK

Mutations affecting genes coding for mitochondrial deoxynucleotide metabolism enzymes, such as TK2 and DGUOK, are known causes of mitochondrial depletion syndromes. Up to date, over 40 mutations in *DGUOK* gene associated with MDS have been described.

Human *DGUOK* nuclear gene is located in the short arm of chromosome 2 (2p13.1) and it was first identified by Johansson and colleagues [28] through *dCK* cDNA homology studies. Since then, two different isoforms for *DGUOK* have been described.

DGUOK is ubiquitously expressed in all tissues, with higher levels in muscle, liver, brain and lymphoid tissues [28, 30]. It harbours a mitochondrial translocation signal, guiding the protein into the organelle, in which it exerts its function. Post-translocation cleavage at a putative mitochondrial peptidase cleavage site processes the enzyme into its mature form [28, 30]. Enzymatic activity studies revealed that DGUOK phosphorylates deoxy-guanosine, deoxy-inosine and deoxy-adenosine units in the mitochondrion [28]. Complementarily, phosphorylation of purine deoxy-nucleotides in the cytoplasm is carried out by dCK enzyme.

DGUOK protein is composed by 277 aminoacids. Several studies have accounted to identify, in the protein, the motifs and sites important for its structure and enzymatic function. A mitochondrial translocation signal has been allocated to the first 39 aminoacids [28]. Furthermore, the phosphate donor (ATP) binds to aminoacids 45 to 53 and 206 to 208, as inferred from electronic annotation (Source: UniProt). In turn, deoxynucleotides interact with the isolated aminoacids 70, 100, 111, 118, 142, 147, 151 and 211 in the enzymatic pocket. Finally, the glutamic acid at position 141 (NP_550438), functions as a proton acceptor.

CLINICAL FEATURES IN PATIENTS WTH *DGUOK* DEFICIENCY

DGUOK deficiency presents as an infantile onset MDS with a very heterogeneous clinical phenotype and is transmitted as an autosomal recessive trait or, more frequently, as extensively reported, as a compound heterozygous disorder [4].

The more severe phenotype associated to *DGUOK* deficiency is the hepatocerebral and affects mostly neonates; patients usually die before 2 years of age [31-33]. The hepatic form is clinically milder and is characterized by acute liver failure without neurological impairment, associated to longer life expectancy (infancy or even adulthood) [32, 34-37].

Hepatocerebral form

Patients with the hepatocerebral form of MDS present with nystagmus, hypotonia and psychomotor retardation, commonly before 8 months of age. Associated with these neurological symptoms, patients invariably develop progressive liver failure [31, 33, 35, 38-43]. Elevated hepatic alanine (ALT) and aspartate aminotransferases (AST) as well as gamma-glutamyl transpeptidase (GGT) are, in most of the cases, a warning for cytolysis and cholestasis. Over half of the patients present with hepatomegaly, jaundice, coagulopathy and/ or hypoglycaemia, and a few are diagnosed with cirrhosis. Blood sampling commonly shows lactic acidemia, hiperaminoacidemia (mostly tyrosinemia), low levels of glucose and increased levels of bilirrubin. Most patients present very high levels of ferritin and alpha fetoprotein and differential diagnosis with neonatal hemochromatosis must be considered [7, 34, 38, 39, 41-47].

Disease frequently evolves from progressive cholestasis and cytolysis to fatal liver failure. Concomitantly, patients typically develop neurological features, commonly accompanied by failure to thrive. Other systemic manifestations, such as renal tubulopathy, cardiomiopathy or malabsorption, related to pancreatic insufficiency, may be observed [38, 43]. The presence of neurologic manifestations precludes liver transplantation [35].

Hepatic form

Hepatic form presents with isolated liver disease [32, 34-37, 47]. Liver failure clinical features are similar to those presented by the hepatocerebral patients.

DGUOK MUTATIONS

An extensive MEDLINE search, using the key words "*DGUOK"*, "*dGK"*, "*DGUOK mutation"*, "*mitochondrial depletion syndrome"* and "*hepatocerebral"* was carried out to allocate all case reports on *DGUOK* mutations/deficiency published to date. Reported mutations in the *DGUOK* gene found through this search are gathered in Tables 1 and 2.

DGUOK related MDS patients carry either homozygous or compound heterozygous mutations. Missense mutations are, by far, the most common type of *DGUOK* gene variant in patients, followed by nonsense and deletion mutations (tables 1 and 2). Gene mutations altering splice sites or insertions have also been described as pathological (tables 1 and 2). The most frequent mutation found in patients is a GATT duplication between nucleotides 763 and 766 in exon 6, resulting in a frameshift and a premature stop codon, translating into a truncated protein of 255 aminoacids [7, 36, 37, 39, 43, 48].

It is our aim to establish a correlation between the type, as well as the site, of the mutation in the gene and consequent alteration in the protein chain and phenotype (hepatocerebral or hepatic) observed. No stringent genotype-phenotype correlation seems to exist. Although one should expect that severe phenotypes would correspond to mutations in structurally and functionally conserved regions, hepatocerebral as well as hepatic phenotypes are observed in patients with mutations in substrate binding sites or signal peptides in the DGUOK protein chain. In addition, although some patients with isolated hepatic failure harbour homozygous mutations that changes aminoacids thought to be expendable for substrate binding (p.S107P, p.Y191C, p.D255Y), there are two hepatic patients carrying mutations in the coding gene for ATP nucleotide binding site 46 and in the translation initiating AUG codon, respectively (Table 1). Strikingly, this last variant, in which the initiation methionine is replaced by a threonine (c.2T>C), has also been described in a hepatocerebral patient (Table 1). Such observations confirm the non existence of a pattern for

disease severity prediction. A contribution of other unknown factors, genetic or not, for the observed disease phenotype, is possible.

Patients carrying missense variants, either in homozygosity or compound heterozygozity, present with the hepatic form, associating this type of mutation to a milder phenotype. On the other hand, patients harbouring duplications, deletions, insertions or splice site mutations invariably develop neurological features (Tables 1 and 2). One hypothesis for this trend is that the latest types of mutations cause dramatic changes to the protein chain, such as alteration of splicing site or frameshift with premature termination codon, which determines its non functionality. Filosto and colleagues reported the homozygous c.763_766dupGATT mutation in a patient who died at 31 months of age from liver failure and gastrointestinal complications [48]. The patient developed neurological symptoms, namely unsteady gait and limb ataxia [48]. In contrast to others opinion [49], this should be considered a hepatocerebral patient.

Compound heterozygous mutations are slightly more frequent in isolated hepatic form of MDS than in the hepatocerebral form (Table 2). Interestingly, for all *DGUOK* compound heterozygous mutations in hepatic patients reported to date, both or at least one of the allelic alterations have never been associated to a severe hepatocerebral phenotype. It is relevant to notice that, in four out of the seven heterozygous patients with hepatic phenotype (Table 2), one of the allelic variants has been described, when occurring in both alleles, in association to hepatic and cerebral symptoms. For the other three compound heterozygous patients, none of the mutations have so far been described to be causative of disease in homozygosity. These observations may indicate that, for missense compound heterozygous mutations, the presence of a non deleterious mutation (e.g. not associated, to date, with hepatocerebral form in homozygous patients) limits the phenotype to the liver, precluding the development of neurological features. Accordingly, a patient carrying the compound heterozygous mutation

p.[Y191C];[K51Q] has been diagnosed with hepatocerebral form of MDS [7]. Since homozygous p.Y191C has been described as hepatic form of MDS, and has never been found in hepatocerebral patients, one could anticipate that p.K51Q in homozigosity should cause a hepatocerebral phenotype.

Siblings harbouring the compound heterozygous mutation p.[L250S];[M1?] have been identified [34]. Both mutations have been described to generate a hepatocerebral phenotype in homozygosity. Hence, both siblings would be expected to develop neurological features. However, one of the siblings showed only liver symptoms and was alive at the time of the report (5 months old), while the other sibling died at 9 months of life. It is plausible to consider that there is a time lag for the neurological symptoms to appear in MDS patients and that such features had not developed in both children at the time of the report or death, respectively. Also, intrafamiliar variability (expressivity, for example) may determine the disease severity [34].

Freisinger and his team reported a monoallelic heterozygous patient presenting with a hepatocerebral phenotype (Table 2) whose DNA sequencing disclosed a missense mutation at codon 170, resulting in a glutamine to arginine substitution. In spite of the neurological symptoms, the patient was alive (3 months of age) at the time of the report [34]. Previous studies have detected the p.Q170R variant in approximately 2% of control individuals [50] and SIFT analysis evaluates this gene modification as non deleterious as it has been reported in the dbSNP database (http://www.ncbi.nlm.nih.gov/ projects/SNP/) [50], supporting that Q170R variant is a polymorphism rather than a pathogenic mutation. Nevertheless, the same type of analysis on *PolyPhen-2* suggests that Q170R variant affects the structure and function of DGUOK [50]. Thus, the reason why this patient developed a hepatocerebral phenotype remains unclear. A second mutation or even exonic deletion causing the disease cannot be disregarded [34].

DNA sequencing is widely used for clinical diagnosis of human mutations, but often does not detect intragenic deletions or duplications. The approach by using oligonucletide array-based comparative genomic hybridization, a targeted library of genes involved in mitochondria biogenesis and function, has been recently demonstrated to be particularly helpful as a complementary technique for the identification of whole gene deletions or large intragenic deletions, in MDS and mtDNA related disorders [51, 52].

Table 1. Homozygous mutations in the *DGUOK* gene associated with hepatocerebral and hepatic forms of MDS.

Mutation	***Location Exon/ Intron***	***Effect***	***Clinical Phenotype***	***Survival***	***Reference***
c.3G>A	1	p.M1?	Hepatocerebral	18 mo	[39]
c.2T>C	1	p.M1?	Hepatocerebral Hepatic	3 mo 3 mo	[31] [32]
c.34C>T	1	p.R12*	Hepatocerebral	42 d	[38]
c.130G>A	1	p.E44K	Hepatocerebral	4 mo	[53]
c.137A>G	1	p.N46S	Hepatic	Alive	[32]
c.143-309_170del335	Intron 1	p.A48fs*90	Hepatocerebral	12 mo	[54]
c.155C>T	2	p.S52F	Hepatocerebral	Alive	[34]
c.223T>A	2	p.W75R	Hepatocerebral	8-11mo	[55]
c.235C>T	2	p.Q79*	Hepatocerebral	4-5 mo	[33]
c.255delA	2	p.A86Pfs*13	Hepatocerebral	≤ 12 mo	[6]
c.313C>T	3	p.R105*	Hepatocerebral	5 mo	[40] [32]
c.318G>A	3	p.S107*	Hepatocerebral	3 mo	[33]
c.319T>C	3	p.S107P	Hepatic	Alive	[7]

				(3.5 yo)	
c.352C>T	3	p.R118C	Hepatocerebral	9 mo	[56]
c.444-62C>A	Intron 3	p. D147_R148ins20	Hepatocerebral	14-18 mo	[44]
c.493G>A	4	p.E165K	Hepatocerebral	11 mo	[53]
c.494A>T	4	p.E165V	Hepatocerebral	2.5 mo	[39]
c.533G>A	4	p.W178*	Hepatocerebral	12-15 mo¤	[7, 46]
c.572A>G	4	p.Y191C	Hepatic	44 d	[47]
c.592-4_592-3delTT	Intron 4	p.Q197_L237del	Hepatocerebral	6 mo	[42]
c.609_610delGT	5	p.Y204Pfs*11	Hepatocerebral	6 mo	[37]
c.617G>A	5	p.R206K	Hepatocerebral	12 mo	[55]
c.677A>G	5	p.H226R	Hepatocerebral	1-8 mo	[45, 57]
c.707+3_6delTAAG	Intron 5	p.(T235fs*9)	Hepatocerebral		[53]
c.707+417_834+3416del	Intron 5	p. K236_L277del	Hepatocerebral	6.5 mo	[41]
c.ins721_724TGAT	6	p.N253*	Hepatocerebral	4 mo	[32]
c.749T>C	6	p.L250S	Hepatocerebral	24 mo	[31, 45, 58, 59]¤

				or alive	[32]
c.763G>T	6	p.D255Y	Hepatic ¤	Alive	[60]
c.763_766dupGATT	6	p.F256*	Hepatocerebral Hepatic	1-31 mo	[43] [61] [36] [57] [39] [37] [48] [55]
c.765_769insTGAT	6	p.F256*	Hepatocerebral	4 mo	[31]

¤ liver transplantation; d, days; mo, months; yo, years old.
NOTE: *alive* refers to patient alive at the time of the report publication.

Table 2. Heterozygous mutations in the *DGUOK* gene associated with hepatocerebral and hepatic forms of MDS.

Mutation	***Location Exon/ Intron***	***Effect***	***Autosomal Inheritance***	***Clinical phenotype***	***Survival***	***Reference***
c.1A>G c.3G>A	1 1	p.M1? p.M1?	Compound heterozygous	Hepatic	Alive[¤]	[34]
c.143-309_170del335 c.743T>C	Intron 1 6	p.A48fs*90 p.L248P	Compound heterozygous	Hepatocerebral	12 mo	[54]
c.155C>T c.681_684delGTTT	2 5	p.S52F p.E228Gfs*14	Compound heterozygous	Hepatocerebral	13 mo	[34]
c.749T>C c.2T>C[§]	6 1	p.L250S p.M1?	Compound heterozygous	Hepatocerebral Hepatic	9 mo Alive	[34]
c.509A→G	4	p.Q170R	Heterozygous	Hepatocerebral	Alive	[34]
c.137A>G c.797T>G	1 6	p.N46S p.L266R	Compound heterozygous	Hepatic	Alive	[36]
c.4G>T c.142+1G>A c.591G>A	1 Intron 1 4	p.A2S p.0 p.Q197=	Compound heterozygous	Hepatocerebral	3 mo	[36]
c.3G>A c.813_814insTTT	1 7	p.M1? p.N271_T272insF	Compound heterozygous	Hepatocerebral	6.5 mo	[39]

c.425G>A c.679G>A	3 5	p.R142K p.E227K	Compound heterozygous	Hepatic	Alive	[37]
c.632A>G c.797T>G	5 6	p.E211G p.L266R	Compound heterozygous	Hepatic	9 mo	[31]
c.494A>T c.797T>G	4 6	p.E165V p.L266R	Compound heterozygous	Hepatic	18 mo	[31] [32]
c.81delC c.763_766dupGATT	1 6	p.S28Pfs*28 p.F256*	Compound heterozygous	Hepatocerebral	6 mo	[7]
c.487_490insGACA c.195G>A	4 2	p.R142Vfs*5 p.W65*	Compound heterozygous	Hepatocerebral	7-12 mo	[7]
c.605_606delGA c.591G>A	5 4	p.R202Tfs*13 p.Q197=	Compound heterozygous	Hepatocerebral	23 mo	[7]
c.572A>G c.151A>C	4 2	p.Y191C p.K51Q	Compound heterozygous	Hepatocerebral	8 mo	[7]
c.137A>G c.352C>T	1 3	p.N46S p.R118C	Compound heterozygous	Hepatic	Alive	[7]
c.592-4_592-3delTT c.677A>G	Intron 4 5	p.Q197_L237del p.H226R	Compound heterozygous	Hepatocerebral	8 mo	[7]
c.605_606delGA c.444-11C>G	5 Intron 3	p.R202Tfs*13 p.0	Compound heterozygous	Hepatocerebral	7 mo	[7]

c.679G>A c.444_591del	5 4	p.E227K p.R148_V198del	Compound heterozygous	Hepatocerebral	Alive (4 mo)	[62]
c.591G>A c.424_425delAG	4 3	p.Q197= p.R142Vfs*5	Compound heterozygous	Hepatocerebral	Alive	[32]
c.256-2A>C c.749T>C	Intron 2 6	p.(K85_S91fs*36) p.L250S	Compound heterozygous	Hepatocerebral	10 mo	[32]
c. 494A>T c.737C>G	4 6	p.E165V p.P246R	Compound heterozygous	Hepatic	Alive	[32]

* Hepatopathy comprises liver enlargement, cholestasis, hepatic cytolysis and liver failure. Central nervous systems cardinal symptoms are hypotonia, nystagmus and/or psychomotor retardation, ¤ liver transplantation, § mutation detected in two siblings, ξ cerebral symptoms (nistagmus) only develops after liver transplantation, ¤ previously identified [34] but as benign compound heterozygous. mo, months.
NOTE: *alive* refers to patient alive at the time of the report publication.

ADULT PATIENTS CARRYING *DGUOK* MUTATIONS

For many years, *DGUOK* deficiency was thought to cause exclusively hepatic and cerebral disease in children. However, two recent reports showed that mutations in the DGUOK genes can lead to a myopathic form of MDS in adulthood [50, 63].

Patients were diagnosed with myopathy at ages varying from 20 to 69 years old. They had chronic muscular fatigue, myalgia, dysphagia, dysphonia, ptosis and/or progressive external ophthalmoplegia. Liver symptoms were not observed. Muscle biopsies revealed significant decrease of mtDNA load (although not as severe as the depletion degree observed in pediatric patients) and some presented ragged red fibers. Found mutations were all in compound heterozygosity (table 3) and, as observed for hepatic patients, both allelic variants were of missense type with one of them being pathogenic, as computationally predicted or reported in patients [32, 34, 36]. The reason why these patients did not develop a neonatal hepatocerebral or hepatic phenotype is unknown. However it is conceivable that some of the DGUOK activity is preserved in these patients, capable of sustaining demanding nucleotides synthesis in early life. Since mtDNA replication rate decreases with age, even low levels of DGUOK activity may be sufficient to prevent liver and cerebral disease. Muscle involvement in these patients may be due to prolonged exposure of muscle fibres to low DGUOK activity, resulting in myopathy in adulthood.

Myopathic phenotype in DGUOK patients highlights the complex and multi-systemic range mutations this gene can have and the importance of screening patients with mitochondrial disease for all genes known to be involved in mitochondria biosynthesis and function.

Table 3. Mutations in the *DGUOK* gene associated with the myopathic form of MDS.

Mutation	*Effect*	*Autosomal Inheritance*	*Age of diagnosis*	*Reference*
c.81_82insCC c.4G>T	p.P28Sfs*57p.A2S p.A2S	Compound heterozygous	21 yo	[63]
c.605_606delGA c.462T>A	p.R202Yfs*12 p.N154K	Compound heterozygous	58 yo	[50]
c.130G>A c.462T>A	p.E44K p.N154K	Compound heterozygous	46 yo	[50]
c.186C>A c.509A>G	p.Y62X p.Q170R	Compound heterozygous	69 yo	[50]
c.605_606delGA c.137A>G	p.R202Yfs*12 p.N46S	Compound heterozygous	20 yo	[50]
c.444-11 C>G§ c.509>G	p.0 p.Q170R	Compound heterozygous	40 yo	[50]
c.444-11 C>G§ c.509A>G	p.0 p.Q170R	Compound heterozygous	44 yo	[50]

yo, years old. § siblings

CONCLUSION

Given the complexity of DGUOK associated mitochondrial disorders, it is difficult to establish a strict relation between the genotype and phenotype, which would allow the prediction of the severity and course of the disease in individual patients.

It is clear that missense mutations are associated to a milder phenotype and that compound heterozygous have better chance of developing childhood non fatal isolated hepatic or even myopathic, adult onset, disease.

Homozygous mutations are, in most cases, associated with a worse prognosis.

The capability to predict the clinical phenotype at a prenatal stage would be of great value for genetic counselling.

Thus, further studies are required to understand the pathogenesis of the mutations underlying MDS disorders.

MDS spectrum diseases, associated with *DGUOK* deficiency, are clinically very heterogeneous and since the enzyme is ubiquitously expressed; theoretically, all tissues can be affected. Tissues in which it is expressed at higher levels, such as liver and brain [28], are frequently severely affected. However, as damage from low enzymatic activity accumulates and mtDNA depletion aggravates, other organs may be involved, such as skeletal muscle and heart. Therefore, it is important to screen for *DGUOK* mutations both in infants and in adults suffering from mitochondrial disease, particularly if the liver is affected.

MATERIALS AND METHODS

A comprehensive search on MEDLINE (via PubMed) database of references for peer-reviewed literature of *DGUOK*-deficiency-associated-MDS was performed using the following keywords: "*DGUOK", "dGK",* "*DGUOK mutation"*, "*mitochondrial depletion syndrome"* and "*hepatocerebral"*. No language or date of publication restrictions were applied.

All the articles considered to be relevant were analysed for the methodological accuracy in the molecular study of *DGUOK* mutations.

A systematic summary of the findings from the different studies, in the form of easy-to-consult tables was elaborated, where a genotype-phenotype correlation is established for each published mutation in *DGUOK* gene. Data withdrawal was double-checked by four collaborators and disagreements discussed between them and the review authors.

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