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Genetic causes of mitochondrial DNA depletion in humans

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Abstract

Mitochondrial DNA (mtDNA) depletion is characterized by a profound reduction of mtDNA copy number. The maintenance of mtDNA copy number requires several nuclear-encoded factors involved in replication and in dNTP supply. In the past decade mutations in several of these factors have been reported in a growing number of syndromes. This article reviews the current knowledge of genes causing mitochondrial DNA depletion syndromes.

Key words

Mitochondria, mtDNA depletion, respiratory chain

Introduction

Mutations in either mitochondrial DNA (mtDNA) or nuclear genes that encode proteins involved in various functions, including mtDNA maintenance may cause mitochondrial disease. Pathological alterations of mtDNA fall into three major classes: point mutations, deletion-duplications and disordered mtDNA content, that is, copy number. Reduction of mtDNA copy number to less than 30% of normal mtDNA content is defined as mtDNA depletion, which causes a heterogeneous group of severe and usually lethal diseases in infancy and childhood. While genetic causes are the focus of this review, mtDNA depletion can also be drug-induced [1]. Mitochondrial DNA depletion syndrome (MDS) was first described as congenital myopathy or hepatopathy [2]. Since then, many patients have been reported with different clinical presentations such as hepatocerebral, myopathic and encephalomyopathic forms. In these patients, there is a marked (usually tissue specific) deficiency in mtDNA level. MtDNA encodes proteins of respiratory chain (RC) complexes I, III, IV and V. Hence, mtDNA depletion results in deficiencies of multiple RC complexes, while nuclear encoded components such as complex II are expressed normally. Nevertheless, the precise RC defect may vary considerably, and an isolated deficiency in a single complex may be apparent. The recurrence of the disease in some families and the consanguinity of parents in others demonstrate that MDS is inherited in an autosomal recessive pattern, with a primary nuclear gene defect causing secondary mtDNA loss. Several gene mutations are known to result in mtDNA depletion and most of them are involved directly or indirectly in mtDNA replication or in replenishing the mitochondrial deoxyribonucleoside triphosphate (dNTP) pool for mtDNA synthesis.

Maintenance and integrity of the mitochondrial genome depend on several poorly understood factors. mtDNA is packaged into protein-DNA complexes called nucleoids. Nucleoids in cultured mammalian cells contain 5-10 mtDNA molecules [3] [4] and appear to be tethered to the inner mitochondrial membrane. The protein composition of the nucleoid is still controversial, nevertheless it is now well established that the nucleoid contains the mtDNA replisome. Crucial proteins or enzymes involved in either mtDNA replication (mtDNA polymerase γ , mtSSB, Twinkle helicase) or transcription (TFAM) are components of the nucleoid [3]. Theoretically, defects in any of the proteins involved in mtDNA replication might affect mtDNA copy number. MtDNA replication is also highly dependent on mitochondrial dNTPs supply, and mutations in several genes involved in mitochondrial dNTP synthesis may therefore cause MDS.

Incidence of mtDNA depletion

MtDNA depletion is a rare cause of mitochondrial disease, which is itself rare. There are no published prevalence studies of MDS. Based on the Necker Hospital series of patients, roughly 8% of pediatric patients with respiratory chain deficiency present with mtDNA depletion. Patients with *POLG1* mutations comprise the largest single group of individuals with MDS, and in Oxford this is one of the commonest distinct types of paediatric mitochondrial myopathy. The prevalence of Alpers-Huttenlocher syndrome, the major *POLG1* phenotype, is held to be around 1 in 100,000 [5].

MDS and abnormal mitochondrial nucleotide salvage

MtDNA replication occurs throughout the whole cell cycle, depending on specific intramitochondrial 2'-deoxyribonucleoside 5'-triphosphate (dNTP) pools [6]. Because mtDNA turns

over in post mitotic cells [7], it is clear that mtDNA is able to replicate in the absence of normal cell cycling, but it may not be completely autonomous [8]. A constant supply of dNTP is therefore required for mtDNA replication. The synthesis of dNTPs for mtDNA replication is not completely elucidated. There is no *de novo* dNTP synthesis in mitochondria. Nucleotides are either imported from the cytosol or result from the mitochondrial salvage pathway. Cytosolic dNTPs are predominantly synthesized *de novo* by the ribonucleotide reductase (RNR). RNR is S-phase specific and therefore the cytosolic *de novo* synthesis cannot provide all required dNTPs for mtDNA replication in quiescent cells and non dividing tissues. dNTPs are also synthesized via the cytosolic salvage pathway (Fig. 1). These dNTPs are imported into the mitochondria by specific transporters [9, 10].

The mitochondrial salvage pathway (Fig. 1) involves specific mitochondrial enzymes. Firstly deoxyribonucleosides are phosphorylated by deoxyribonucleoside kinases (dNK). This is followed by two further phosphorylation steps, firstly involving nucleotide monophosphate kinase (NMPK) and secondly nucleotide diphosphate kinase (NDPK). The two major dNKs are deoxyguanosine kinase (dGK) and thymidine kinase (TK), encoded by *DGUOK* and *TK2* genes respectively. In non replicating tissues, cytosolic deoxyribonucleotides levels are low and mitochondrial dNTP salvage is the major source of nucleotides. Mutations in the mitochondrial dNTP salvage pathway have been reported in different forms of MDS.

DGUOK mutations in hepatocerebral form of MDS

Mitochondrial DNA depletion of liver is the most common form of MDS, patients falling into two major phenotypes, the so-called hepatocerebral form and Alpers-Huttenlocher syndrome (see below).

Homozygosity mapping in three different consanguineous families of Druze origin identified chromosome 2p13 as the first hepatocerebral MDS disease locus. Candidate genes were sequenced and *DGUOK* was identified as the disease causing gene [11]. Since then, over 50 patients have been reported with *DGUOK* mutations (Table 1). The clinical presentation of the patients was relatively similar. Onset of the disease is in early infancy with neonatal or delayed forms (1 to 24 months). The initial symptoms are liver dysfunction, feeding difficulties, hypotonia and/or hypoglycemia. All patients manifested hyperlactatemia. Characteristically, such patients develop microvesicular steatosis in liver, with hepatocellular insufficiency progressing to liver failure. Patients usually die before reaching two years old [12]. Diagnosing such patients may be difficult unless liver is available for analysis, and such patients are frequently too sick to biopsy [13]. Enzymological measurement of the liver RC revealed combined deficiency of mtDNA-encoded complexes. The mtDNA content is usually reduced to less than 10% of normal copy number in liver of the patients and may be reduced in fibroblasts [14], whereas muscle mtDNA content is usually within the normal range.

TK2 mutations in myopathic form of MDS

TK2 was also an obvious candidate gene for MDS because its function is similar to that of *DGUOK*. Analysis of this gene revealed two missense mutations in four patients with isolated myopathy and muscle mtDNA depletion [15]. The mutation was shown to be pathogenic because TK2 activity was decreased in muscle mitochondria (Table 1). *TK2* mutations were then described in additional myopathic patients [16] but the phenotypic spectrum of *TK2* mutations turned out to be more complex than a simple myopathy. Firstly, patients with lower motor neuron disease reminiscent of

spinal muscular atrophy and encephalopathy were found to have TK2 mutations [17]. Very recently, Finnish *TK2* patients were also reported, presenting with seizures, cardiomyopathy or dystrophic changes in muscle [18]. Secondly, depletion of mtDNA at presentation may become less apparent as the disease progresses, despite clinical and biochemical deterioration [16]. The mechanism behind the increase in mtDNA copy number in this and other patients with a similar molecular progression [14, 19] remains unexplained.

Succinyl CoA synthase deficiency

Succinyl CoA synthase (SCS) is a mitochondrial matrix enzyme that catalyzes the reversible synthesis of succinate and ATP (or GTP) from succinyl-CoA and ADP in the tricarboxylic acid (TCA) cycle. This enzyme is composed of an α -subunit (SCS-G) and a β -subunit (SCS-A), encoded by the *SUCLG1* and *SUCLA2* genes respectively. By homozygosity mapping in a small consanguineous pedigree with autosomal recessive encephalomyopathy and mtDNA depletion, Elpeleg's group identified a homozygous deletion of exon 6 and part of exon 7 of *SUCLA2* gene [20]. The patients presented psychomotor retardation, muscle hypotonia, hearing impairment and seizures (Table 1). Brain MRI suggested Leigh syndrome. A deficiency of multiple RC complexes was observed in muscle and the mtDNA content was reduced to 32% of the mean value. Following this initial report, *SUCLA2* mutations were found in several patients from the remote Faroe Islands presenting encephalomyopathy, muscle mtDNA depletion, and methylmalonic aciduria [21]. The high incidence of this disorder in the Faroe Islands (1/1700) is due to a founder effect, with a carrier frequency of 1 in 33. *SUCLA2* mutations were then also reported in Italian patients with methylmalonic aciduria [22]. Mutations in *SUCLG1* encoding the SCS α -subunit were also reported in patients with fatal infantile lactic acidosis, multiple RC deficiency and a decreased amount of mtDNA (15% of normal) [23]. The mutation, a 2 bp deletion, resulted in a frame shift and a premature stop codon. A useful diagnostic clue in both of these subtypes is a mildly elevated urinary methylmalonic acid.

The pathogenesis of this disorder is poorly understood. It is not clear how the enzyme deficiency results in mtDNA depletion and RC deficiency. Under some conditions SCS-A copurifies with the nucleoside diphosphate kinase (NDPK) that catalyzes the last step of mitochondrial dNTP salvage [24]. Thus it could be that SCS deficiency, due to *SUCLA2* or *SUCLG1* mutations, disrupts an association between SCS and NDPK, resulting in an unbalanced mitochondrial dNTP pool and eventually mtDNA depletion. Moreover, the mtDNA depletion is frequently mild in muscle. The modesty of this depletion, along with the methylmalonic aciduria, suggests that SCS deficiency may also impair at least one collateral pathway that plays a role in the development of the disease.

MDS and cytosolic deoxynucleotide metabolism

The mitochondrial dNTP pool is highly dependent on cytosolic dNTPs and an unbalanced cytosolic dNTP pool can be genotoxic for mtDNA.

The first reported example of mitochondrial disease related to defective cytosolic dNTP pool is mitochondrial neurogastrointestinal encephalomyopathy (MNGIE). MNGIE is characterized clinically by ptosis, progressive external ophthalmoplegia (PEO), gastrointestinal dysmotility, peripheral neuropathy, myopathy, leukoencephalopathy and lactic acidosis. Patients may present with some or

all of these features, quite frequently as a progressive generalized mixed motor and sensory neuropathy [25]. In most cases, the onset of symptomatic disease is between the second to fifth decades, but many patients will always have been underweight. Patients present with decreased RC activities in muscle and multiple mtDNA deletion or mtDNA depletion, or both (Table 1). The disease is autosomal recessive and by linkage analysis and sequencing candidate genes, Hirano's group demonstrated that it was caused by mutations in the thymidine phosphorylase (*TYMP*) gene [26]. Thymidine phosphorylase is a catabolic enzyme that degrades thymidine and deoxyuridine and participates in the regulation of thymidine phosphate pools (Fig. 1). Interestingly thymidine phosphorylase is a cytosolic enzyme and is not directly involved in mitochondrial dNTP pool regulation. Because MNGIE patients have elevated circulating and urinary thymidine and uridine levels [27], many investigators suggest that thymidine toxicity is the cause of the phenotype. Nevertheless, there is evidence that most of the filtered thymidine is likely to be reabsorbed by the kidney which should therefore worsen the phenotype [28]. Furthermore, renal dialysis was not helpful, suggesting that the problem might be imbalance in mitochondrial dNTP pools [28] [29] rather than thymidine excess [14]. The study of intramitochondrial dNTP levels in cells from patients with MNGIE directly is technically difficult as crudely purified preparations of mitochondria are substantially contaminated with nuclear or cytoplasmic dTTP. Nevertheless, it is clear that nucleotide imbalance may be mutagenic, because as little as 4 hours' treatment of HeLA cells with thymidine supplements induced an increase of mitochondrial dTTP and dGTP pools, and multiple mtDNA deletions were apparent after 8 months of culture [30]. Finally, the thymidine phosphorylase (*TYMP*) and uridine phosphorylase (*UP*) double knockout (*TYMP*^{-/-}*UP*^{-/-}) mice manifested increased thymidine and deoxyuridine in various tissues and altered mitochondrial dNTP pools [31]. They also displayed mtDNA depletion and respiratory chain dysfunction in brain recapitulating some features of patients with MNGIE.

Ribonucleotide reductase (*RNR*) is a cytosolic enzyme that catalyses dNDP synthesis from NDP [32]. This is the rate limiting step for nuclear DNA synthesis. This ribonucleotide reductase consists of one homodimer of the large subunit R1 and one homodimer of the small subunit R2. The large subunit R1 is constitutively expressed whereas R2 gene is transcribed only during S-phase and R2 protein is destroyed in late mitosis. Thus, this R1-R2 complex is essential for dNDP synthesis for nuclear DNA replication during S-phase. However, dNTP synthesis is essential during other phases of the cell cycle for both DNA repair (after DNA damage) and for mtDNA replication. Another small subunit (p53R2 encoded by *RRM2B* gene) was identified in 2000 that is 80% identical with R2 and whose expression is cell-cycle independent [33]. *RRM2B* is a target of the p53 transcription factor which is induced by DNA damage, the R1-p53R2 complex possibly enabling dNDP synthesis for DNA repair after DNA damage. It is now clear that p53R2 plays an additional role in supplying dNTPs for mtDNA replication [34] as genetic mapping in a small North-African pedigree with profound muscle mtDNA depletion (1-2% of normal mtDNA content) demonstrated that *RRM2B* was the disease gene [35]. The first patients in whom *RRM2B* mutations were identified presented early with severe trunk hypotonia and lactic acidosis, as well as proximal tubulopathy (Table 1). Less severe clinical presentations were later reported [36]. This shows that p53R2 plays a crucial role in dNTP synthesis for mtDNA replication, especially in non-proliferating tissues such as muscle and brain.

MDS and mtDNA replication

mtDNA is replicated by the DNA polymerase γ (pol γ A), encoded by the *POLG1* gene and its accessory subunit (pol γ B), encoded by the *POLG2* gene, and replication factors such as the mitochondrial single strand DNA binding protein (mtSSB) and the Twinkle helicase (encoded by *PEO1*). Diseases resulting from mutations in *POLG* and *PEO1* genes are associated with diverse disorders such as progressive external ophthalmoplegia (PEO) [37], SANDO syndrome (Sensory ataxia, neuropathy, dysarthria, ophthalmoparesis), MIRAS (mitochondrial-associated recessive ataxia syndrome) [38], IOSCA (infantile onset spinocerebellar ataxia), Alpers-Huttenlocher syndrome [39] and MDS. Moreover, diseases associated with mutations of these two genes can be inherited as autosomal dominant [37] or recessive traits [38]. Patients present either qualitative (multiple deletions) [37] or quantitative (depletion) mtDNA abnormalities [39].

Alpers-Huttenlocher syndrome is characterized by psychomotor retardation, intractable epilepsy, and liver failure in infants and young children. Nevertheless, patients may present with isolated neurological or liver disease, most commonly convulsions. The clinical picture overlaps with hepatocerebral syndrome [39-41]. Intractable epilepsy and/or movement disorders are a major feature of Alpers-Huttenlocher syndrome, continuous seizure activity (*epilepsia partialis continua*) being a frequent terminal event [42]. The outcome is universally poor, with progressive neurologic deterioration characterized by spasticity, myoclonus, cortical blindness, and dementia. Patients with Alpers-Huttenlocher syndrome usually, but not always, exhibit RC deficiency and mtDNA depletion in liver, but both may be normal in skeletal muscle [43]. Patients with the most severe phenotypes, manifesting as mtDNA depletion in liver and muscle as well as mosaic mtDNA depletion in fibroblasts, have mutations in a catalytic domain in both *POLG* alleles. These mutations may involve either the polymerase or exonuclease domain or both [42]. More than 120 *POLG* mutations have been reported to date (<http://tools.niehs.nih.gov/polg/>) and many of these mutations may also cause PEO. The two most common *POLG* mutations found in Alpers-Huttenlocher syndrome are A467T and W748S, and patients can be homozygotes or compound heterozygotes combined with other mutations (Table 1).

Mutations in *PEO1* can cause dominant disorders such as pure PEO associated with multiple mtDNA deletions or recessive disorders including IOSCA syndrome or hepatocerebral form of MDS (Table 1). IOSCA is a severe autosomal recessively inherited neurodegenerative disorder characterized by progressive atrophy of the cerebellum, brain stem and spinal cord and sensory axonal neuropathy. This syndrome has been reported in Finnish patients and by genetic mapping a recessive homozygous Y508C founder mutation in *PEO1* gene has been found [44]. Surprisingly, RC and mtDNA copy number were normal in muscle of the patients while autosomal dominant *PEO1* gene mutations result in multiple RC deficiency and multiple mtDNA deletions in muscle. However, it has been recently shown that IOSCA syndrome is associated with brain-specific mtDNA depletion and complex I deficiency in tissue samples taken at autopsy [45].

MPV17 mutations and hepatocerebral form of MDS

The hepatocerebral form of MDS is associated with not only *DGUOK* or *POLG* mutations, but also with mutations in the *MPV17* gene (Table 1). These mutations have been identified by genetic mapping in several unrelated consanguineous families of Italian, Canadian or North-African origin [46].

The patients presented with severe hypoglycemic episodes, jaundice and elevated levels of lactate and hepatic enzymes in blood. Most of the patients died of liver failure during the first year of life. Patients who survived then developed neurological symptoms and multiple brain lesions. All probands had profound mtDNA depletion (5-15% of normal mtDNA content) in liver associated with defects of mtDNA-related respiratory chain complexes. *MPV17* mutations are also associated with Navajo neurohepatopathy, an autosomal recessive multisystem disorder prevalent in the Navajo population of the southwestern United States [47]. Patients present with liver disease, severe sensory and motor neuropathy, corneal anesthesia and scarring, cerebral leukoencephalopathy, failure to thrive, and recurrent metabolic acidosis with intercurrent illness. They all carry the same R50Q founding mutation.

MPV17 encodes a protein of yet unknown function and its role in the pathogenesis of mtDNA depletion is still unclear. In contrast to previous reports suggesting that *Mpv17* isoforms are localized in peroxisomes [48] it has recently been shown that *Mpv17* is actually a mitochondrial protein bound to the inner mitochondrial membrane [46].

Tissue specificity

Despite the ubiquitous expression of their gene products, the mtDNA depletion arising from these mutant genes is frequently restricted to specific tissues. For instance, the main tissue where depletion is apparent is liver in patients with *DGUOK*, *POLG*, *MPV17* or *PEO1* mutations, and muscle in patients with *TK2* or *RRM2B* mutations. Nevertheless, the expression of these genes is different in various organs and tissues as well as the expression of other genes involved in the affected metabolic pathway. The tissue specific regulation of these genes, especially those involved in mitochondrial dNTP pools allows some times to understand the tissue specificity of MDS.

TK2 deficiency results in muscle mtDNA depletion whereas other tissues usually display normal mtDNA copy number. Several factors could contribute to this tissue specificity. Firstly, the *de novo* cytosolic dNTP synthesis by RNR is down regulated in non-dividing tissues. Hence, the mitochondrial dNTP pool is strictly dependent on the mitochondrial salvage pathway. *TK2* deficiency should be therefore highly detrimental in these tissues whereas dividing tissues, such as liver, show normal mtDNA copy number. However, brain and heart are also non-dividing tissues and some patients also manifest cardiomyopathy and encephalopathy. A second reason is that the mitochondrial dNTP pools are different among different organs, at least in rat [49] and in mouse [10] and muscle dTTP content being very low compared to dATP, dCTP and dGTP. Thus a decreased dTTP synthesis due to *TK2* deficiency can be much more detrimental in muscle tissue than in other organs. Finally, basal *TK2* activity is very low in human muscle tissue compared to liver or heart and low *TK2/COX* and *TK2/mtDNA* ratios are observed in muscle [50]. This could also contribute to the vulnerability of skeletal muscle to *TK2* deficiency.

The *RRM2B* gene is ubiquitously expressed in humans, with high expression in skeletal muscle. In contrast, transcripts encoding R2 are undetectable in heart, brain and muscle, implying that these tissues rely entirely on p53R2 for dNTPs synthesis, whereas a low but significant amount of R2 is detected in kidney [51]. Why *RRM2B* mutations resulted only in severe neurological and/or renal involvement remains an open question. Other mechanisms, including post-translational modifications

of p53R2 or R2 or post-transcriptional modifications of their respective mRNAs, could help explain the tissue-specific phenotype of *p53R2* mutations.

The tissue specificity associated with *DGUOK*, *MPV17*, *POLG*, *PEO1*, *SUCLA2* or *SUCLG1* mutations is still not understood.

Animal models

Several mouse models with deficiencies in genes able to cause human mtDNA depletion are now available and will constitute tools for improving both the knowledge of the control of mtDNA copy number and for identifying clues for treatment.

Both *TK2* knock-in (H126N) and *TK2*^{-/-} knockout mice have been generated [52, 53]. They were both normal at birth but rapidly developed progressive weakness or growth retardation and died before 4 weeks of age. They also manifested mtDNA depletion. The knock-in mouse displayed unbalanced dNTP pools.

The *RRM2B*^{-/-} mouse developed normally until they were weaned but from then on had growth retardation and early mortality. They manifested multiorgan failure and died of renal failure [54]. Even though the role of p53R2 is to supply dNTP for DNA repair, there was no evidence of any malignancy. However, a severe mtDNA depletion was detected in muscle and kidney confirming the role of p53R2 in dNTP supply for mtDNA synthesis [35].

The *MPV17*^{-/-} mouse was generated by random insertion of a retroviral construct in the genome of mouse embryonic stem cells [55]. This mouse had severe mtDNA depletion restricted to liver but moderate decrease of RC activities. Surprisingly the animals did not develop overt hepatic failure at any age, but did develop grey hair early in adulthood and focal segmental glomerulosclerosis with massive proteinuria and cochlear lesions [56].

Whereas these various mouse models show clear alteration either of the mitochondrial dNTP pools or decreased mtDNA copy number, they do not always display RC deficiency suggesting compensatory mechanisms. This compensation may occur at the level of mtDNA transcription and possibly translation as shown for the *MPV17*^{-/-} mouse [56].

Mice with targeted deletion of both thymidine phosphorylase and uridine phosphorylase genes develop leukoencephalopathy and myopathy, but not the gastrointestinal features that are a hallmark of MNGIE [31].

Therapeutic approaches

For the majority of patients with MDS, there is as yet no treatment other than supportive care, even though this group of disorders are potentially the most treatable of mitochondrial diseases [57]. Liver transplantation is contra-indicated in Alpers-Huttenlocher syndrome because of the inevitable brain involvement that may not be apparent until later. Furthermore, 6 out of 9 infants who underwent liver transplantation for hepatocerebral syndrome due to *DGUOK* mutations died [13]. Very few *DGUOK* patients responded well to liver transplantation suggesting that which may be a therapeutic option in selected cases [17].

This contrasts with the options available for patients with thymidine phosphorylase deficiency (MNGIE) in whom there is nucleotide imbalance with systemic accumulation of its substrates, thymidine (dThd) and deoxyuridine (dUrd). While reducing the concentrations of the allegedly toxic

nucleosides to normal or nearly normal levels by dialysis was ineffective, enzyme replacement by infusion of platelets from healthy donors to patients with MNGIE reduced plasma dThd and dUrd levels [58]. Concomitantly, reduced intensity allogeneic stem cell transplantation has been performed in two patients that partially restored buffy coat thymidine phosphorylase activity and lowered plasma nucleosides in one of them [59]. While it is not yet certain that such patients improve clinically, this is nevertheless extremely encouraging, bone marrow transplant being the first potential cure for a mitochondrial disease.

Figure Legends

Figure 1. dNTP synthesis for mtDNA synthesis.

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Table 1. Nuclear gene mutations responsible of mtDNA depletion

Gene	Protein	Depleted tissue	Clinical presentation	Pathway
<i>TK2</i>	TK2	Muscle	Myopathy	mitochondrial dNTP synthesis
<i>DGUOK</i>	dGK	Liver	Hepatocerebral form	
<i>SUCLA2</i>	SCS	Muscle	Encephalomyopathy	
<i>SUCLG1</i>	SCS	Muscle and Liver	Fatal infantile lactic acidosis	
<i>POLG1</i>	POLG	Liver	Alpers-Huttenlocher syndrome, Hepatocerebral form	mt DNA replication
<i>PEO1</i>	Twinkle	Muscle	PEO	cytosolic dNTP synthesis
<i>RRM2B</i>	p53R2 Thymidine phosphorylase	Muscle	Encephalopathy	
<i>TYMP</i>	Thymidine phosphorylase	Muscle	MNGIE	
<i>MPV17</i>	MPV17	Liver	Hepatocerebral form	Unknown

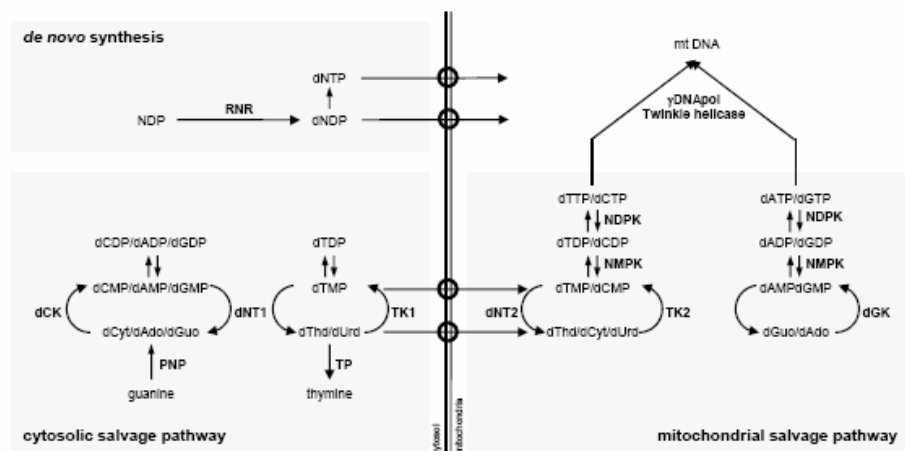


Figure 1