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# 176th ENMC International Workshop: Diagnosis and treatment of Coenzyme $Q_{10}$ deficiency

**Shamima Rahman**[1,2,3], **Catherine F Clarke**[4], and **Michio Hirano**[5]

[1]Clinical and Molecular Genetics Unit, UCL Institute of Child Health, London, WC1N 1EH, UK

[2]MRC Centre for Neuromuscular Diseases, National Hospital for Neurology, Queen Square, London WC1N 3BG, UK

[3]Metabolic Unit, Great Ormond Street Hospital, London WC1N 3JH, UK

[4]Department of Chemistry and Biochemistry and the Molecular Biology Institute, University of California, Los Angeles, USA

[5]H. Houston Merritt Clinical Research Center, Department of Neurology, Columbia University Medical Center, New York, USA

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## 1 Introduction

An ENMC meeting was held in Naarden, the Netherlands (July 9–11th 2010) with 12 clinical and basic scientists and 3 industrial representatives from Europe (France, Belgium, Germany, Italy, Spain, Switzerland, the Netherlands and the UK) and the USA, to discuss their collective experience with the molecular underpinnings, diagnosis, and management of patients with deficiency of coenzyme $Q_{10}$ ($CoQ_{10}$).

$CoQ_{10}$, also known as ubiquinone, is a unique electron carrier [1]. It is essential for aerobic organisms because of its role in mitochondrial energy production as a chaperone of electrons from complexes I and II (and also from the electron transfer flavoproteins, ETF) to complex III of the respiratory chain. In addition, this lipophilic molecule has numerous other vital functions in cells that include: serving as antioxidant in both lipoproteins and cell membranes; connecting energy production with important cellular pathways such as the cell cycle and DNA replication and repair through its role in pyrimidine biosynthesis; modulation of apoptosis through its regulation of the transition pore; and maintenance of body temperature via its action on uncoupling proteins.

The aims of this workshop were: to define how to clinically recognise and diagnose patients with $CoQ_{10}$ deficiency; to optimize strategies to identify the underlying molecular genetic



**Address for correspondence:** Shamima Rahman, Clinical and Molecular Genetics Unit, UCL Institute of Child Health, 30 Guilford Street, London WC1N 1EH, Tel: +44(0)207 905 2608, Fax: +44(0)207 404 6191, S.Rahman@ich.ucl.ac.uk.

defects; to review basic $CoQ_{10}$ biology; and to delineate therapeutic options for this group of disorders, which represent the most readily treatable subset of mitochondrial diseases.

## 2. Meeting outcomes

### 2.1 Biosynthesis of Coenzyme Q

Primary $CoQ_{10}$ deficiencies are caused by defects of the ubiquinone biosynthesis pathway, which is a complex and incompletely understood metabolic pathway [2]. In humans, the benzoquinone ring is synthesized from tyrosine or phenylalanine, while the polyprenyl side-chain is generated from acetyl-CoA through the mevalonate pathway and the enzymatic activity of decaprenyl diphosphate synthase. After condensation of 4-hydroxybenoate (4HB) with the polyprenyl tail, the ring undergoes decarboxylation, hydroxylation and methylation modifications to produce $CoQ_{10}$. The biosynthesis of CoQ has been studied extensively in *Escherichia coli* and in several eukaryotic models including yeasts (*Saccharomyces cerevisiae* and *Schizosaccharomyces pombe*), *Caenorhabditis elegans*, plants, and animals [2–5]. An overview showing the CoQ branch of the isoprene biosynthetic pathway is shown in Figure 1. The polyprenyl tail of Q is produced by one of the non-sterol branch points emanating from a farnesyl-diphosphate precursor. The species specificity of the tail of CoQ*n* (where *n* = the number of isoprene units) is determined by the Coq1 polypeptide in yeast, and by the Dps1/Dlp1 complex in *S. pombe* and the homologous Pdss1/Pdss2 complex in animal cells. In humans, the decaprenyl diphosphate synthase heterotetramer encoded by the *PDSS1* and *PDSS2* genes is responsible for producing the decaprenyl-diphosphate tail precursor of $CoQ_{10}$.

The aromatic ring precursor in coenzyme Q biosynthesis is 4HB. In contrast to bacteria which can produce 4HB via *de novo* synthesis [6], animals rely on a dietary source of essential amino acids, and utilize tyrosine and phenylalanine to generate the aromatic ring precursor of $CoQ_{10}$. *S. cerevisiae* utilizes 4HB derived from tyrosine as the aromatic ring precursor of CoQ [4]. Recent independent and complementary studies by two groups show that para-aminobenzoic acid (pABA) also serves as an aromatic ring precursor of CoQ in *S. cerevisiae* [7, 8]. Pierrel *et al*. identified pABA as a Q biosynthetic precursor by studying the role of ferredoxin (Yah1) and ferredoxin reductase (Arh1) in Q biosynthesis [7]. They found that yeast mutants depleted in either Yah1 or Arh1 had defects in CoQ biosynthesis and accumulated a new prenylated intermediate with an amino substituent on the ring (3-hexapreny1-4-aminophenol). The discovery of this compound allowed the investigators to isolate pABA as a ring precursor in Q biosynthesis.

Marbois *et al*. [8] utilized a different approach to identify pABA as a new ring precursor in yeast Q biosynthesis. Using wild-type yeast cells, they identified a novel intermediate that was 1 Da less in mass than the previously characterized 3-hexapreny1-4-hydroxybenzoate intermediate. The mass difference and product ions suggested a ring with an amino substituent rather than a hydroxyl group. This notion was confirmed by demonstration that both $^{14}C$-labeled pABA and $^{13}C_6$-pABA were shown to be incorporated into $DMQ_6$ and $CoQ_6$ by yeast. It is not yet clear how the nitrogen substituent is removed from the ring but the process likely includes a Schiff-base mediated deimination.

The use of pABA as a ring precursor in *S. cerevisiae* opens up questions regarding the possible use of pABA as a ring precursor in animal and human cells. In general, pABA is thought to compete with 4HB at the Coq2 step, and the product, prenyl-pABA is generally considered to be a dead-end product [1]. Several other aromatic ring inhibitors of Q biosynthesis, including 4-nitrobenzoic acid, also function as competitive inhibitors of Q biosynthesis in mammalian cells [9]. It may be important to test different concentrations of

pABA and to re-evaluate with dose-response studies the role of pABA as a potential ring precursor in animal and human cells in culture.

### 2.2 Clinical recognition of coenzyme $Q_{10}$ deficiency

Clinical recognition of $CoQ_{10}$ deficiency is difficult because of extreme clinical heterogeneity, reflecting the heterogeneity of mitochondrial disease in general. However, some recognisable clinical phenotypes are emerging (Table 1). The first reported presentation of $CoQ_{10}$ deficiency was of recurrent rhabdomyolysis associated with seizures and mental retardation [10]. Six cases from 4 families have been reported in total, with age of onset ranging from <2 to 15 years. Other clinical features associated with this phenotype include proximal muscle weakness, cerebellar symptoms, migraine, ptosis and lactic acidosis, and residual muscle $CoQ_{10}$ levels are typically 4–16% of those observed in normal controls. In one patient with this encephalomyopathic phenotype plus ataxia, mutations were identified in the *ADCK3* (*CABC1*) gene encoding a kinase that is thought to regulate $CoQ_{10}$ biosynthesis [11,12] (see section 2.4).

In 2000, Rötig *et al*. reported three siblings with a new phenotype of $CoQ_{10}$ deficiency, namely a multisystem disorder of infancy with early-onset neurological disease initially manifesting as nystagmus and sensorineural hearing loss with subsequent ataxia, spasticity, dystonia, and cognitive dysfunction [13]. Severe steroid-resistant nephrotic syndrome due to glomerulosclerosis was fatal in one sibling and necessitated renal transplantation in the other two. Progressive visual failure in these children resulted from a combination of pigmentary retinopathy, optic atrophy, and cataracts. Seven additional cases of infantile-onset multisystemic disease and severe $CoQ_{10}$ deficiency have been reported with prominent encephalopathy and nephropathy as the major clinical manifestations [14–18]. Most patients had steroid-resistant nephrotic syndrome with focal segmental glomerulosclerosis on renal biopsy. Other clinical features included Leigh syndrome, progressive ataxia, stroke-like episodes and hypertrophic cardiomyopathy. Most untreated cases of infantile-onset $CoQ_{10}$ deficiency have been lethal in childhood; however, Mollet *et al*. described 14 and 22 year-old siblings with infantile onset deafness, macrocephaly, mental retardation, optic and peripheral neuropathies, obesity, cardiac valvulopathy, and livido reticularis [12]. Almost all patients reported with this phenotype have subsequently been shown to have mutations in genes encoding $CoQ_{10}$ biosynthetic enzymes (see section 2.4).

The most frequent presentation of $CoQ_{10}$ deficiency appears to be ataxia that typically begins in childhood and is often associated with seizures [19, 20]. At least 31 cases of this genetically heterogeneous condition have been reported and it is most frequently due to mutations in a kinase [12,19–21], ADCK3, that is thought to modulate CoQ biosynthesis. In addition, Quinzii and colleagues identified *APTX* mutations as a cause of secondary $CoQ_{10}$ deficiency in three siblings with ataxia [22]. Le Ber and colleagues confirmed that *APTX* mutations caused secondary $CoQ_{10}$ deficiency in muscle from five of six patients with oculomotor apraxia type 1 (A0A1) [23]. Less frequently observed phenotypes of $CoQ_{10}$ deficiency include slowly progressive Leigh syndrome (initially reported in two adult sisters with encephalopathy and severe mental retardation associated with ataxia, deafness, growth retardation and lactic acidosis) [24] and isolated myopathy [25–27]. Patients with the myopathic form have presented with exercise intolerance between 6 and 33 years, associated with proximal muscle weakness, elevated CK (up to 20 times upper limit of normal) and lactic acidosis (up to 10 times normal levels after exercise) and no evidence of disease affecting other organ systems. There is typically a dramatic response to exogenous $CoQ_{10}$. The genetic basis of $CoQ_{10}$-deficient mild Leigh syndrome remains unknown, but most cases of myopathic $CoQ_{10}$ deficiency appear to be secondary to multiple acyl-CoA dehydrogenase deficiency (MADD) [27](see section 2.4).

The considerable overlap of the $CoQ_{10}$ deficient phenotypes with other mitochondrial disorders makes clinical recognition of $CoQ_{10}$ deficiency extremely challenging. Nevertheless, it is important to be vigilant for clinical clues to diagnose patients so treatment can be initiated early in the disease. For example, it is important to suspect infantile-onset $CoQ_{10}$ deficiency when evaluating young children with nephropathy, particularly steroid-resistant nephrotic syndrome, even in the absence of encephalopathy. Neuro-imaging may provide helpful diagnostic clues, since cerebellar atrophy is frequently seen in the $CoQ_{10}$ deficiencies and is present in almost every subgroup of $CoQ_{10}$ deficiency. However, this is a relatively nonspecific finding and cerebellar involvement is often seen in other mitochondrial respiratory chain disorders such as pontocerebellar hypoplasia type 6 (caused by mutations in the *RARS2* gene which plays an essential role in mitochondrial translation), as well as in non-mitochondrial disorders such as the Joubert and Wolfram syndromes [28]. The triad of encephalopathy (seizures, mental retardation or both), myopathy with recurrent myoglobinuria, and ragged-red fibres is characteristic of the encephalomyopathic form of $CoQ_{10}$ deficiency, whilst the combination of myopathy with markedly elevated CK and histological evidence of increased lipid have been observed in the myopathic form of $CoQ_{10}$ deficiency. Although helpful, clinical features alone are insufficient to definitively diagnose $CoQ_{10}$ deficiency or to distinguish between primary and secondary $CoQ_{10}$ deficiencies. As a consequence, evaluation of patients with suspected $CoQ_{10}$ deficiency relies on biochemical and molecular genetic investigation.

### 2.3 Laboratory investigation of Coenzyme $Q_{10}$ deficiency

**Biochemical screening tests—**The best method for diagnosing $CoQ_{10}$ deficiency biochemically was debated. Initial biochemical testing should include measurement of blood (plasma or serum) lactate, although a normal lactate level does not exclude $CoQ_{10}$ deficiency. It was agreed that measurement of ubiquinone in a muscle biopsy remains the gold standard test for diagnosing $CoQ_{10}$ deficiency. $CoQ_{10}$ deficiency should be considered when muscle histology reveals ragged-red fibres and prominent lipid deposition, although these features are not a universal finding. It is important to assay NADH-cytochrome *c* reductase (a combined assay interrogating complexes I+III [CI+III]) or succinate cytochrome *c* reductase (complexes II+III [CII+III]) in frozen muscle homogenates, since these assays rely on endogenous $CoQ_{10}$ levels and thus should be decreased in $CoQ_{10}$ deficiency states even when individual respiratory chain activities are normal. However there are cases of mild $CoQ_{10}$ deficiencies where activities of CI+III, CII+III, or both have been normal, and so the consensus recommendation is to measure the concentration of $CoQ_{10}$ directly in skeletal muscle by high performance liquid chromatography (HPLC) in all cases where deficiency is suspected clinically (Figure 2).

Lack of commercially available non-physiological internal standards (IS) presents a major difficulty in the assessment of $CoQ_{10}$ status in tissues. Initial studies used coenzyme $Q_9$ as the IS of choice. However, $CoQ_9$ is present in normal human tissues as a result of dietary contamination and synthesis by intestinal microorganisms. There is therefore a need for an alternative IS that is not influenced by exogenous or endogenous ubiquinones. For this reason, the non-physiological di-propoxy-$CoQ_{10}$ IS has been synthesised, and this has enabled accurate determination of $CoQ_{10}$ levels in muscle and other tissues [29].

Although skeletal muscle is generally accepted as the tissue of choice for the determination of $CoQ_{10}$, muscle biopsy is an invasive procedure and it would be desirable to have a non-invasive screening test for $CoQ_{10}$ deficiency. Initial screening investigations in blood focused on $CoQ_{10}$ measurements in plasma. However, plasma $CoQ_{10}$ appears to be highly dependent upon the concentration of lipoproteins, which act as carriers of $CoQ_{10}$ in the circulation. Dietary intake also significantly influences plasma $CoQ_{10}$ concentrations,

contributing up to 25% of the total amount (9). Therefore, concentration of plasma $CoQ_{10}$ may not accurately reflect the level in tissues. Assessment of blood mononuclear cells (MNC) has been suggested as an alternative tissue for $CoQ_{10}$ evaluation. MNC represent a stable, easily isolated sample, which reflect changes in cellular $CoQ_{10}$ status following supplementation [29]. Other tissues which may be considered are lymphoblastoid cell lines, primary fibroblasts or myoblasts. However, some patients with genetically confirmed $CoQ_{10}$ biosynthetic defects had normal $CoQ_{10}$ levels in fibroblasts [21], so it may be necessary to test more than one tissue to confirm the diagnosis.

**Biosynthesis assays—**Assays of $CoQ_{10}$ biosynthesis in cultured skin fibroblasts can be used to confirm a defect in the $CoQ_{10}$ biosynthetic pathway. These assays utilise radiolabelled substrates for the biosynthesis of $CoQ_{10}$. Typically [$^{3}$H]mevalonate and [$^{14}$C]4HB are used in cell culture, whilst [$^{3}$H]decaprenyl-PP is used in homogenized fibroblast extracts [14, 16]. The combined use of [$^{14}$C]4HB and [$^{3}$H]decaprenyl-PP may be useful to discriminate defects upstream or downstream of the reaction catalyzed by decaprenyl diphosphate synthase. For example, *COQ2* mutant fibroblasts synthesised less radiolabelled $CoQ_{10}$ with both the [$^{14}$C]4HB and the [$^{3}$H]decaprenyl-PP substrates, whereas radiolabelled $CoQ_{10}$ production was reduced with [$^{14}$C]4HB but normal with [$^{3}$H]decaprenyl-PP in *PDSS2* mutant fibroblasts [14, 16]. Multiple steps in the $CoQ_{10}$ biosynthetic pathway cannot be distinguished using the available assays, but this may be possible in the future using tandem mass spectrometry methods to identify accumulation of abnormal metabolites. Preliminary work in this area was presented [Simon Eaton, personal communication].

## 2.4 Genetics of Coenzyme $Q_{10}$ deficiency

**Primary Coenzyme $Q_{10}$ deficiency—**Mutations in 6 genes encoding components of the $CoQ_{10}$ biosynthetic pathway have been associated with human $CoQ_{10}$ deficiency to date [12, 14–16, 21, 30, 31]. The first genetic defect was identified by homozygosity mapping; two siblings with nephropathy, one of whom also had an infantile onset encephalomyopathy, were demonstrated to have mutations in the *COQ2* gene encoding the PHB-polyprenyl transferase [16]. *COQ2* mutations have been described in six patients from 4 different families [15, 16, 32]. Phenotypes range from a fatal neonatal multisystem disorder to isolated nephrotic syndrome. Nephrotic syndrome was present in all reported patients and typically preceded the neurological manifestations. Preliminary data indicate that clinical variability depends at least in part on the residual activity of individual mutants, but environmental factors may also play an important role in determining the clinical phenotype of patients.

Rötig and co-workers used biochemical approaches to identify accumulating intermediates in cells with the infantile multisystem form of $CoQ_{10}$ deficiency, followed by sequence analysis of candidate genes [13]. This led to demonstration of transprenyltransferase deficiency in one family, which was subsequently shown to be caused by a pathogenic mutation in the *PDSS2* gene [13][Rötig, personal communication]. Transprenyltransferase, the enzyme that generates the decaprenyl side-chain of the $CoQ_{10}$ molecule, is a heterotetramer composed of two different subunits encoded by the *PDSS1* and *PDSS2* genes in humans. In a second family, homozygosity mapping was used to identify a pathogenic mutation in the *PDSS1* gene [15]. In an infant with fatal Leigh syndrome and nephrotic syndrome, compound heterozygous *PDSS2* mutations were identified by candidate gene sequencing [14]. In toto, four families with *PDSS1* or *PDSS2* mutations have now been identified, all presenting with infantile-onset multisystemic disorders [15, 33][Rötig, personal communication]. Surprisingly, *PDSS1*-mutant patients have a very different clinical presentation from patients with *PDSS2* mutations despite the fact that defects in either gene

result in transprenyltransferase deficiency. The siblings with *PDSS1* mutations had a complex clinical phenotype with deafness, macrocephaly, mental retardation, optic and peripheral neuropathies, obesity and cardiomyopathy without nephropathy and with prolonged survival. In contrast, patients with *PDSS2* mutations have infantile-onset encephalopathy and steroid-resistant nephrotic syndrome, which if untreated can be fatal. Curiously, the nephro-encephalopathy phenotype of COQ2 deficiency overlaps with that of PDSS2 deficiency and the renal involvement in both disorders is recapitulated by *PDSS2* mutant mice (see section 2.5). It is not known why patients with *PDSS1* mutations do not develop nephropathy. Possible explanations include variations of $CoQ_{10}$ levels in tissues, differences in reactive oxygen species overproduction, or variable expression levels of the two genes in different tissues.

Mutations in *ADCK3* were reported simultaneously by Lagier-Tourenne *et al*. and Mollet *et al*. who performed homozygosity mapping to identify the causative gene in families with autosomal recessive juvenile-onset cerebellar ataxia and atrophy [12, 21]. *ADCK3* mutations have been reported in 12 patients (8 families) making this gene the most frequent cause of primary $CoQ_{10}$ deficiency [12, 21, 34]. In addition to ataxia, some patients have had epilepsy, mild cognitive impairment, corticospinal tract signs, dystonia, and pes cavus. Fibroblasts from patients with *ADCK3* mutations had normal or decreased levels of $CoQ_{10}$, but muscle biopsies from four patients uniformly revealed $CoQ_{10}$ deficiency. *ADCK3 (CABC1)* encodes a putative kinase, which appears to modulate biosynthesis of $CoQ_{10}$. The homologous gene in *S. pombe* was initially postulated to encode a protein required for activity of bc1 complex, which led to the original gene name CABC1 [35–37]; however, subsequent studies from Catherine Clarke's group demonstrated the role of this protein in $CoQ_{10}$ biosynthesis [38, 39]. Studies of the yeast homologue, Coq8p, indicate that the protein is required to maintain stability of Coq3p, a ubiquinone biosynthetic protein [40].

Mutation of *COQ9* has been reported in a single patient, who presented in the neonatal period with lactic acidosis and multisystem problems, including mild left ventricular hypertrophy, renal tubulopathy and a severe seizure and movement disorder associated with cerebral and cerebellar hypoplasia/atrophy [17]. Although there was only one affected child in this family, and the parents were not known to be related, a combined homozygosity mapping and candidate gene approach was successfully used to identify a homozygous nonsense mutation in *COQ9* in this patient [30]. The precise function of COQ9 remains unknown, but the observations of an accumulated intermediate in HPLC analysis of CoQ species and reduced $CoQ_{10}$ biosynthesis using [$^{14}$C]4HB substrate in fibroblasts from the deficient patient, suggests that the COQ9 gene product is a *bona fide* $CoQ_{10}$ biosynthetic enzyme.

This year, mutations in another gene, *COQ6*, have been reported in 11 patients from 5 different kindreds, with a phenotype similar to that observed in patients with mutations in *COQ2* or *PDSS2* [31]. All had nephrotic syndrome with onset in the first years of life, associated with sensorineural hearing loss in all 9 cases tested, and 3 also had CNS involvement with seizures or ataxia.

It has not been possible to identify the underlying genetic defect in more than 50 patients with $CoQ_{10}$ deficiency despite sequencing all of the known biosynthetic genes [Quinzii, Navas, and Hirano, personal communication]. Possible explanations for this observation are that there may still be further biosynthetic enzymes, which have not yet been characterised in humans; the responsible mutations may be in regulatory sequences located distant to the genes of interest, or in as yet unknown regulatory genes; or that the $CoQ_{10}$ deficiency in these patients may be a secondary phenomenon.

**Secondary Coenzyme $Q_{10}$ deficiencies—**Mutations in the *ETFDH* gene (encoding the electron transfer flavoprotein dehydrogenase) were identified in patients who had been initially reported as having the isolated myopathy phenotype of $CoQ_{10}$ deficiency [27]. These patients presented with fluctuating proximal and axial myopathy, and exercise intolerance. Episodic encephalopathy, hepatopathy and periodic vomiting, often triggered by metabolic stress, were also observed. A few patients experienced rhabdomyolysis and some died of coma or sudden unexplained death. Triggering factors included infections, metabolic stress, pregnancy, surgery, psychological stress and drugs. Biochemical investigations showed increased CK and lactate levels, with low carnitine and a characteristic acylcarnitine profile on tandem mass spectrometry suggestive of MADD [41]. Muscle biopsies exhibited mild to severe vacuolar changes, increased lipid content, ragged-red fibres, focal or diffuse SDH deficiency, COX-negative fibres, combined CI+III and CII+III deficiencies, and decreased level of $CoQ_{10}$ in muscle (50% of normal). The mechanism of $CoQ_{10}$ deficiency in MADD remains unknown and it does not appear to be a universal feature of this disorder, since some patients have been demonstrated to have normal $CoQ_{10}$ levels [42,43].

Ataxia with oculomotor apraxia type I (AOA1) is one of a group of spinocerebellar ataxias with axonal neuropathy, but also has apraxia of eye movements [44]. Onset is typically in childhood with cerebellar symptoms followed by axonal sensorimotor neuropathy, often with cognitive impairment. The disorder is caused by recessive mutations in the *APTX* gene, which encodes a protein involved in single- and double-strand nuclear DNA break repair. In 2005 mutations in APTX were identified in one family with a cerebellar ataxic presentation of $CoQ_{10}$ deficiency and clinical improvement with $CoQ_{10}$ supplementation [22]. Low levels of $CoQ_{10}$ were subsequently found in muscle and fibroblasts of some patients with typical AOA1 [23]. However in other AOA1 patients $CoQ_{10}$ levels may be normal or even elevated. Clear genotype-phenotype correlations have yet to be established, and the mechanism of $CoQ_{10}$ deficiency in this disorder remains unknown.

Secondary $CoQ_{10}$ deficiency has also been reported in a number of patients with primary mtDNA mutations. For example, levels of $CoQ_{10}$ in muscle from 25 patients with mitochondrial encephalopathies, mostly due to mtDNA mutations, were significantly lower than controls [45] while a multicenter study of 76 patients with heterogeneous mitochondrial diseases detected $CoQ_{10}$ deficiency in 28 (37%) of which nine had pathogenic mtDNA mutations [46]. Five patients with MELAS had $CoQ_{10}$ levels of ~40–60% of control levels [Navas, personal communication]. However the reasons for $CoQ_{10}$ deficiency in these patients remain obscure, nor is the prevalence of secondary $CoQ_{10}$ deficiency known.

### 2.5 Models of Coenzyme $Q_{10}$ deficiency

**Human cell culture models—**Cell culture models have revealed insights into the pathogenesis of $CoQ_{10}$ deficiency. Initial studies in cultured fibroblasts from two siblings with infantile-onset $CoQ_{10}$ deficiency showed mild respiratory chain defects, but did not detect elevated superoxide anions, lipid peroxidation, or apoptosis-mediated cell death [47]. Lopez-Martin and colleagues showed that *COQ2* mutant fibroblasts require uridine to sustain growth and proposed that deficiency of $CoQ_{10}$ impaired de novo pyrimidine biosynthesis because of the dependence of dihydro-orotate dehydrogenase on ubiquinone [48]. In the same two cell lines plus two other cells lines from patients with genetically undefined $CoQ_{10}$ deficiency, Rodriguez-Hernandez and colleagues noted increased autophagy of mitochondria [49].

Quinzii *et al*. initially observed that *PDSS2* mutant fibroblasts have 12% residual $CoQ_{10}$ and markedly reduced ATP synthesis, but do not show increased reactive oxygen species (ROS) or oxidative stress, while *COQ2* mutant fibroblasts with 30% $CoQ_{10}$ content have mild defects of ATP synthesis and significantly increased ROS production as well as oxidation of

lipids and proteins and cell death [50]. They have extended the studies to other patient cell lines with variable degrees of $CoQ_{10}$ deficiency due to different mutations in *COQ2*, *ADCK3*, and *COQ9*, and have observed similar correlations between levels of $CoQ_{10}$ and cellular phenotype: 10–15% or >60% residual $CoQ_{10}$ levels are not associated with significant ROS production whereas 30–50% residual $CoQ_{10}$ content is associated with maximal ROS production and cell death [51]. Thus, *in vitro*, severe deficiency of $CoQ_{10}$ causes bioenergetic defects, while moderate deficiency produces ROS, oxidative damage, and cell death.

**Animal models—**The first phenotype of CoQ deficiency described in *C. elegans* was the aging or *clock* phenotype [52]. Strains harbouring mutations in the *clk-l/coq-7* gene showed a delayed aging phenotype including extension of lifespan. These animals had impaired CoQ production and accumulated demethoxy-CoQ (DMQ), an intermediate of CoQ biosynthesis indicating a primary CoQ deficiency [53]. Attempts to induce *coq* knockout (KO) strains mainly resulted in developmental arrest at early stages. The Coq-8 gene KO strain arrests late in life (Larval 3–4 [L3–L4] stages) and is therefore another useful model of primary CoQ deficiency [54]. Animal development was arrested at stages corresponding to higher expression of *coq-8* in wild-type animals. Furthermore, sterility of these animals was caused by arrest at the 8th to 10th blastocyte division, corresponding to the stage of *coq-8* expression to recycle new mitochondria. This animal model is useful not only to study development and lifespan phenotypes, but also genes involved in the regulation of the CoQ biosynthesis pathway.

In the early 1970s, a spontaneous mutant mouse, termed *kd*, was identified to have autosomal recessive kidney disease [55]. Homozygous *kd/kd* mice appear healthy for at least the first 8 weeks of life, but histological examination of the kidneys at about 12 weeks reveals a mononuclear cell infiltrate and tubular dilation. Over time the entire kidney is involved and the mice die of renal failure [56]. The mutation responsible for the *kd/kd* phenotype was identified as a V117M amino acid substitution within PLMP, a prenyltransferase-like mitochondrial protein [57]. This polypeptide was subsequently identified as PDSS2, required for synthesis of the polyprenyl-diphosphate tail precursor in coenzyme Q synthesis [58, 59]. Although young homozygous $Pdss2^{kd/kd}$ mice have a normal content of $CoQ_9$ and $CoQ_{10}$ in kidney, they fail to produce the elevated levels of $CoQ_9$ and $CoQ_{10}$ present in kidneys of wild-type mice that normally occurs at about 40 days of age. The onset of proteinuria and kidney disease ensues several weeks after the normal increase in $CoQ_9$ and $CoQ_{10}$ fails to occur [59]. Conditional knockout mice showed that proteinuria and kidney disease could be recapitulated when *Pdss2* was deleted in podocytes (*Podocin/cre*,$Pdss2^{loxP/loxP}$) but not when targeted to renal tubular epithelium, monocytes or hepatocytes [58].

### 2.6 Treatment of Coenzyme $Q_{10}$ deficiency

**Clinical observations—**In general, supplementation with oral $CoQ_{10}$ (10–30 mg/kg/day in children and 1,200–3,000 mg/day in adults) seems effective in patients with *COQ2* mutations, especially for the neurological and renal manifestations of this disorder. In contrast, poor responses to $CoQ_{10}$ supplementation have been observed in patients with *PDSS2* mutations and in the patient with a homozygous *COQ9* mutation. The reasons for the disparate responses to $CoQ_{10}$ are not known. A likely contributing factor is the poor bioavailability of $CoQ_{10}$; less than 5% of oral $CoQ_{10}$ reaches plasma in humans and rodent studies have demonstrated low uptake of CoQ by tissues with particularly little or no detectable uptake by brain except in aged rats [1, 60–62]. Thus, the blood-brain barrier appears to impair central nervous system uptake of $CoQ_{10}$. Hence, tissue involvement may influence response to $CoQ_{10}$ supplementation as illustrated by the ubiquinone-responsive

$CoQ_{10}$ deficient patients with *COQ2* mutations who manifested nephrotic syndrome and in one case had stroke-like episodes suggesting involvement of vascular structures, which is likely to be amenable to $CoQ_{10}$ supplementation [16,18, 32, 63]. In contrast brain tissue was clearly affected in the $CoQ_{10}$-refractory patients with Leigh syndrome due to *PDSS2* mutations and refractory seizures caused by *COQ9* deficiency [14, 30].

Furthermore, because CoQ is highly lipophilic, exogenously administered CoQ will be integrated into plasma and other cellular membranes before reaching the inner mitochondrial membrane. This notion is supported by cell culture studies [33] (see *In vitro* treatment of cell models of coenzyme $Q_{10}$ deficiency). Because of the poor bioavailability and delayed mitochondrial uptake of ubiquinone, early $CoQ_{10}$ supplementation is crucial for the success of the therapy. Furthermore, it appears that $CoQ_{10}$ can block the progression of the disease but cannot reverse established tissue damage. Therefore intense efforts should be directed towards a prompt diagnosis of these patients.

One study assessed the clinical outcome after $CoQ_{10}$ therapy in patients presenting with ataxia, with or without associated $CoQ_{10}$ deficiency [64]. This was an open-label prospective study in 14 patients classified into two groups according to $CoQ_{10}$ values in muscle or fibroblasts (deficient or not). Mutation analysis of four genes involved in $CoQ_{10}$ biosynthesis (*COQ2, PDSS1, PDSS2* and *ADCK3*) revealed a missense point mutation in *ADCK3* in a single patient, and the genetic basis of $CoQ_{10}$ deficiency was unknown in the remaining cases. Patients were evaluated clinically (ICARS scale, MRI and video-tape registration) and biochemically at baseline and every 6 months during a period of 2 years after $CoQ_{10}$ treatment (30 mg/Kg/day). Patients with $CoQ_{10}$ deficiency showed a statistically significant reduction of ICARS scores (Wilcoxon test: $p = 0.018$) after 2 years of $CoQ_{10}$ treatment as compared to baseline conditions. In patients without $CoQ_{10}$ deficiency, no statistically significant differences were observed in total ICARS scores after therapy, although one patient from this group showed a remarkable clinical amelioration. Biochemical diagnosis of $CoQ_{10}$ deficiency was a useful tool for the selection of patients who are good candidates for treatment, since all of them responded to therapy. However, the remarkable clinical response in one case without $CoQ_{10}$ deficiency in fibroblasts or muscle (and the previous observation that some patients with *ADCK3* mutations do not have $CoQ_{10}$ deficiency) highlights the importance of therapeutic trials of $CoQ_{10}$ in patients with ataxia, in order to identify those who are $CoQ_{10}$-responsive.

Response to treatment is also variable in patients with secondary $CoQ_{10}$ deficiency syndromes. In patients with myopathy due to *ETFDH* mutations, riboflavin supplementation was generally followed by clinical improvement and normalisation of CK and lactate levels. In a few patients, a positive effect was also shown after additional supplementation with $CoQ_{10}$. A low fat and high carbohydrate diet should be recommended and patients should avoid fasting. Most patients are symptom-free on riboflavin alone, however about 15% of patients have a severe, early onset, multisystem disease with no improvement on therapy. In three siblings with cerebellar ataxia due to a homozygous stop codon mutation in *APTX* and secondary $CoQ_{10}$ deficiency in muscle, high-dose $CoQ_{10}$ supplementation (up to 3,000 mg daily) was associated with improved ambulation in all and resolution of seizures in the affected sister [22].

***In vitro* treatment of cell models of Coenzyme $Q_{10}$ deficiency—**Identification of factors that can influence the efficacy of $CoQ_{10}$ supplementation may be useful to improve the treatment of $CoQ_{10}$ deficiency. Results in fibroblasts with mutations in *COQ9, COQ2* and *PDSS2* revealed that the prolonged pharmacokinetics of $CoQ_{10}$ to reach the mitochondrial respiratory chain critically affects restoration of energy status of human $CoQ_{10}$ deficient cells [33]. This may explain the delayed clinical response of $CoQ_{10}$

deficiency patients to oral supplementation with $CoQ_{10}$. Additionally, it was demonstrated that short tail ubiquinone analogues cannot substitute for $CoQ_{10}$ in the mitochondrial respiratory chain of human $CoQ_{10}$ deficient fibroblasts, thus revealing the importance of the decaprenyl tail. Oxidative stress and cell death can be attenuated by the administration of lipophilic and hydrophilic antioxidants. Therefore, complementary administration of antioxidants with high bioavailability may be helpful in $CoQ_{10}$ deficient patients.

**Treatment of a Coenzyme $Q_{10}$ deficient mouse model—**Supplementation with $CoQ_{10}$ (200 mg/kg/day, beginning at weaning) was shown to provide a dramatic, though partial, rescue of proteinuria and nephritis in the *Pdss*$^{2kd/kd}$ mice [59]. The supplementation did not result in elevated levels of $CoQ_{10}$ in the kidney. It was speculated that the benefit of $CoQ_{10}$ supplementation may be achieved through its action as an antioxidant or co-antioxidant in conjunction with vitamin E [3]. These results suggest that supplementation with $CoQ_{10}$ may provide substantial benefit in treatment of human $CoQ_{10}$ deficiencies with associated kidney disease, and in certain forms of human focal segmental glomerulosclerosis that mirror the renal disease in the *Pdss2*$^{kd/kd}$ mouse.

## 3. Future prospects

As a result of the workshop, an international consortium was established with the aim of optimising the diagnosis and treatment of patients with $CoQ_{10}$ deficiency. The prevalence of $CoQ_{10}$ deficiency remains unknown but, because of its novelty and relative obscurity, the consortium members suspect that this condition is underdiagnosed. The lack of a specific '$CoQ_{10}$ phenotype' hampers clinical recognition of this disorder, but it is hoped that the diagnostic algorithm presented here will help to increase awareness of and diagnostic rates for this condition. $CoQ_{10}$ deficiency should especially be considered in patients with steroid-resistant nephrotic syndrome and those with an otherwise unexplained cerebellar ataxia. At present it is recommended that $CoQ_{10}$ is measured routinely in all muscle samples from patients with suspected mitochondrial disease, and not just in those biopsies where reduced activities of complexes I+III or II+III are observed. The consortium will work to establish diagnostic criteria and standardise methods for laboratory diagnosis of $CoQ_{10}$ deficiency, including systematic evaluation of methods for measuring $CoQ_{10}$ in PMCs, lymphoblastoid cell lines and primary fibroblasts, as well as skeletal muscle. If sufficient laboratories are offering $CoQ_{10}$ measurements, an External Quality Assessment (EQA) system will be set up.

Genetic diagnosis of $CoQ_{10}$ deficiency remains challenging because of the genetic heterogeneity of this disease group. A further difficulty is that primary and secondary $CoQ_{10}$ deficiencies are often clinically indistinguishable. At present there is no clear genotype-phenotype correlation, but patients with an infantile presentation of encephalopathy and steroid-resistant nephrotic syndrome seem particularly likely to have primary $CoQ_{10}$ deficiency. Targeted sequencing of the known human $CoQ_{10}$ biosynthetic genes should be performed in this subgroup of patients, whilst patients with ataxic presentations should be screened for *ADCK3* and/or *APTX* mutations. In other patients, radioisotope methods for $CoQ_{10}$ biosynthesis assays may help to determine whether the observed $CoQ_{10}$ deficiency is primary or secondary, and the likely level of the defect. In selected cases, a homozygosity mapping or whole exome sequencing approach may be fruitful. However the former requires consanguineous families and the latter may not detect defects outside the known likely candidate genes (i.e. genes already known to be involved in $CoQ_{10}$ biosynthesis). In an attempt to reduce confusion regarding gene nomenclature of CoQ biosynthetic genes, we have successfully applied to HUGO and SGD to formally change the gene name from *CABC1* to *ADCK3*. A database of known mutation and SNPs in CoQ biosynthetic genes is also planned.

Further evidence is needed regarding optimal treatment of $CoQ_{10}$ deficiency, namely the most effective therapeutic agent ($CoQ_{10}$, a synthetic analogue, or a combination of both), dosage regimens and route of administration. Furthermore, at present it is not at all clear which of the many functions of $CoQ_{10}$ (electron transfer, antioxidant, pyrimidine biosynthesis, modulation of apoptosis or extramitochondrial roles) are most relevant to human disease in deficiency states. Understanding this will be critical to developing therapeutic advances for this group of potentially eminently treatable diseases.

## 4. Chairpersons

Shamima Rahman, UCL Institute of Child Health, London, UK

Michio Hirano, Columbia University, New York, USA

## 5. Workshop participants

Clinicians & Scientists

Catherine Clarke, UCLA, USA

Iain Hargreaves, National Hospital, London, UK

Michio Hirano, Columbia University, New York, USA

Rita Horvath, Institute of Genetic Medicine, Newcastle, UK

Luis C López, Universidad de Granada, Spain

Placido Navas, CABD, Seville, Spain

Maria del Mar O'Callaghan, Barcelona, Spain

Hélène Puccio, IGBMC, Strasbourg, France

Catarina Quinzii, Columbia University, New York, USA

Shamima Rahman, UCL Institute of Child Health, London, UK

Agnes Rötig, INSERM, Paris, France

Leonardo Salviati, University of Padova, Italy

Baziel van Engelen, Nijmegen, The Netherlands (ENMC)

Industry representatives

Nuri Gueven, Santhera, Switzerland

Peter Lambrechts, Kaneka, Belgium

Bill Shrader, Edison pharmaceuticals, USA

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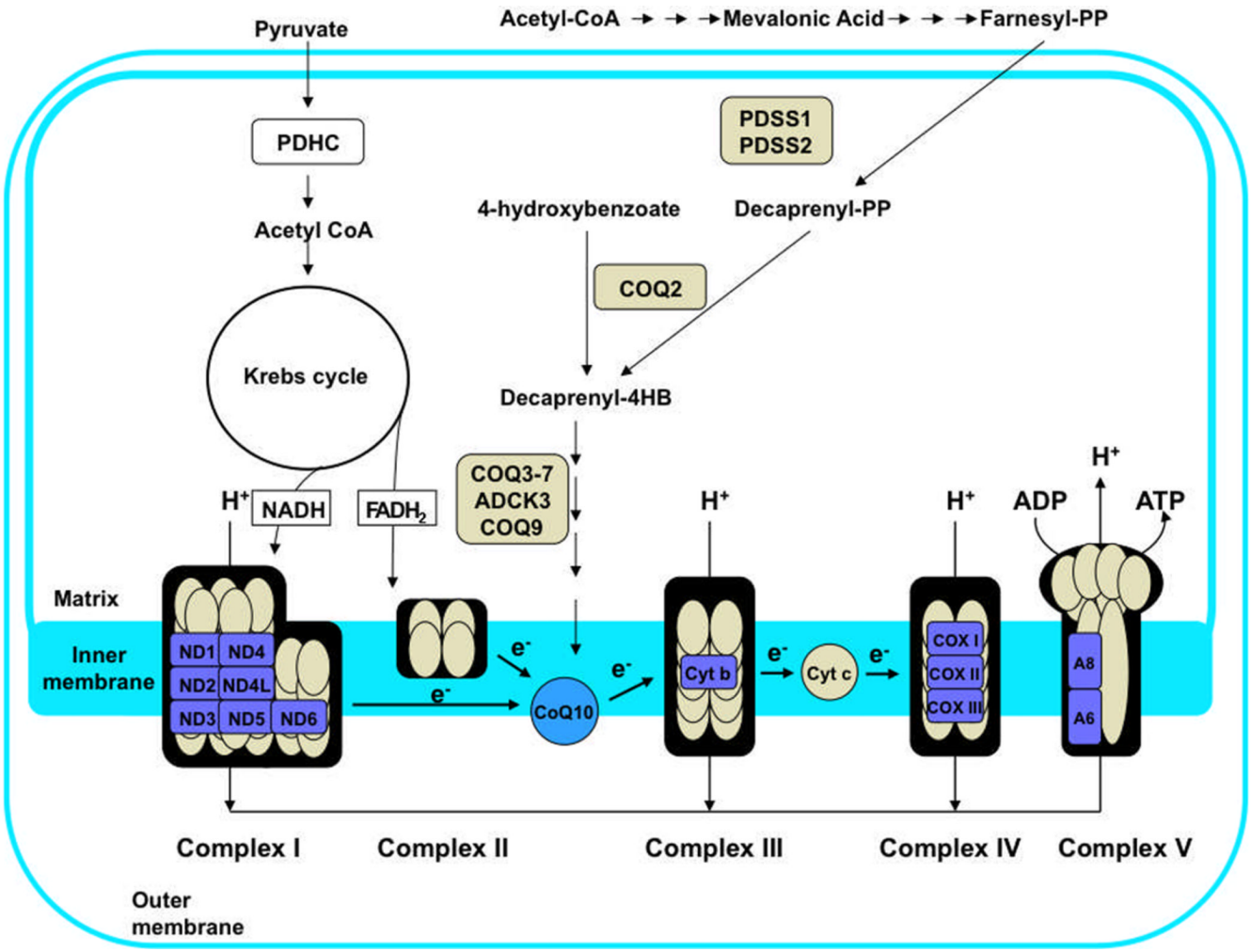


**Figure 1.**
Biosynthesis of Coenzyme $Q_{10}$ Deficiency

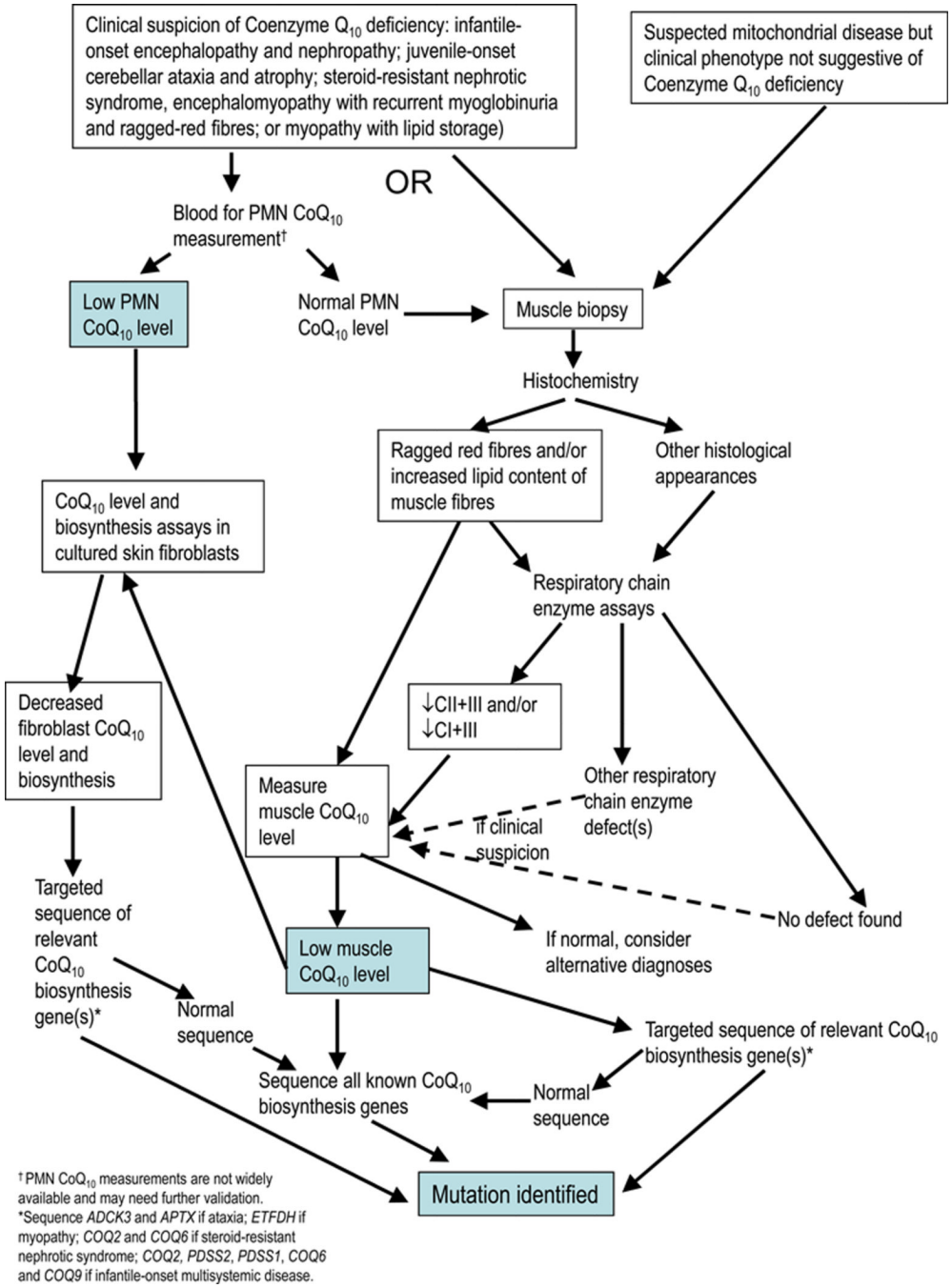


**Figure 2.**
Investigation of Suspected Coenzyme $Q_{10}$ Deficiency

## Table 1

Clinical phenotypes associated with coenzyme $Q_{10}$ deficiency

| Clinical phenotype | Number of cases reported | Associated genetic defects | References |
|---|---|---|---|
| Recurrent rhabdomyolysis with encephalopathy (seizures, mental retardation, or both) | 6 | Unknown in most cases; *ADCK3* mutations in one family | [10–12] |
| Infantile-onset multisystem disorder (mainly encephalopathy plus nephropathy) | 13 | *COQ2, PDSS1, PDSS2, COQ9, COQ6;* other unknown gene(s) | [14–16, 30, 31] |
| Steroid-resistant nephrotic syndrome (+/− sensorineural hearing loss) | 10 | *COQ2, COQ6* | [16, 18, 31, 32] |
| Ataxia | 31 | *ADCK3, APTX;* other unknown gene(s) | [12, 21, 34] |
| Leigh syndrome | 3 | *PDSS2;* other unknown gene(s) | [14, 24] |
| Myopathy | 9 | *ETFDH;* other unknown gene(s) | [26, 27] |