

SHORT REPORT

Fatal neonatal encephalopathy and lactic acidosis caused by a homozygous loss-of-function variant in *COQ9*

Katharina Danhauser¹, Diran Herebian¹, Tobias B Haack^{2,3}, Richard J Rodenburg⁴, Tim M Strom^{2,3}, Thomas Meitinger^{2,3,5}, Dirk Klee⁶, Ertan Mayatepek¹, Holger Prokisch^{*,2,3,7} and Felix Distelmaier^{*,1,7}

Coenzyme Q₁₀ (CoQ₁₀) has an important role in mitochondrial energy metabolism by way of its functioning as an electron carrier in the respiratory chain. Genetic defects disrupting the endogenous biosynthesis pathway of CoQ₁₀ may lead to severe metabolic disorders with onset in early childhood. Using exome sequencing in a child with fatal neonatal lactic acidosis and encephalopathy, we identified a homozygous loss-of-function variant in *COQ9*. Functional studies in patient fibroblasts showed that the absence of the *COQ9* protein was concomitant with a strong reduction of *COQ7*, leading to a significant accumulation of the substrate of *COQ7*, 6-demethoxy ubiquinone₁₀. At the same time, the total amount of CoQ₁₀ was severely reduced, which was reflected in a significant decrease of mitochondrial respiratory chain succinate-cytochrome c oxidoreductase (complex II/III) activity. Lentiviral expression of *COQ9* restored all these parameters, confirming the causal role of the variant. Our report on the second *COQ9* patient expands the clinical spectrum associated with *COQ9* variants, indicating the importance of *COQ9* already during prenatal development. Moreover, the rescue of cellular CoQ₁₀ levels and respiratory chain complex activities by CoQ₁₀ supplementation points to the importance of an early diagnosis and immediate treatment.

European Journal of Human Genetics (2016) **24**, 450–454; doi:10.1038/ejhg.2015.133; published online 17 June 2015

INTRODUCTION

Coenzyme Q₁₀ (CoQ₁₀) is a lipid-soluble, naturally occurring substance, which functions as an essential cofactor of the mitochondrial respiratory chain.¹ It acts as an electron carrier from complex I and complex II to complex III and thereby goes through a redox cycle between the oxidized (ubiquinone) and the reduced form (ubiquinol). Moreover, CoQ₁₀ links mitochondrial fatty acid β -oxidation to the respiratory chain, participates in the biosynthesis of pyrimidines and modulates apoptosis.² In its reduced form the CoQ₁₀ molecule serves as a potent antioxidant.

CoQ₁₀ is acquired through biosynthesis or dietary intake. However, biosynthesis is the major source of CoQ₁₀, which involves the coordinated function of at least 13 genes (*PDSS1*, *PDSS2*, *COQ2*, *COQ3*, *COQ4*, *COQ5*, *COQ6*, *COQ7*, *ADCK3*, *ADCK4*, *COQ9*, *COQ10A* and *COQ10B*). To date, defects in eight of these genes were identified as a cause of primary CoQ₁₀ deficiency disorders (*PDSS1*, *PDSS2*, *COQ2*, *COQ4*, *COQ6*, *ADCK3*, *ADCK4* and *COQ9*), a clinically heterogeneous group of diseases, which frequently manifests in childhood.^{3,4} According to Quinzii *et al.*,⁵ five major clinical phenotypes can be distinguished: (1) encephalomyopathy, (2) severe infantile multisystemic disease, (3) nephropathy, (4) cerebellar ataxia and (5) isolated myopathy. Identification of CoQ₁₀ deficiency is important because CoQ₁₀ supplementation can be beneficial in certain conditions.

Until now, just a single patient with CoQ₁₀ deficiency caused by a homozygous stop variant in *COQ9* c.730C>T, p.Arg244*, leading to neonatal lactic acidosis, intractable seizures and global developmental delay, has been reported.⁶ Using exome sequencing, we identified a new variant affecting function in *COQ9* associated with neonatal encephalopathy and early death. Our report expands the clinical spectrum associated with *COQ9* variants and indicates a severe pre-/neonatal-onset phenotype. *COQ9*-deficient patient fibroblasts were characterized in detail and the functional consequences of the genetic defect are shown.

CASE REPORT

The boy reported here was the second child of healthy, first-grade consanguineous Turkish parents. His 13-year-old brother is healthy. Pregnancy was normal until the 27th gestational week when oligohydramnion was noticed. At that time the parents declined a recommended cesarean section. Finally, the child was born at 36 weeks of gestation by cesarean section due to anhydramnion. APGAR scores were 5/4/6, respectively. The neonate was small for gestational age with a birth weight of 1440 g, length of 39.5 cm and head circumference of 28.8 cm (all values below 3rd percentile). No dysmorphic stigmata were observed. During the immediate postnatal period the patient showed poor respiratory efforts, muscular hypotonia, bradycardia and

¹Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Heinrich-Heine-University Düsseldorf, Düsseldorf, Germany; ²Institute of Human Genetics, Technische Universität München, Munich, Germany; ³Institute of Human Genetics, Helmholtz Zentrum München, Neuherberg, Germany; ⁴Department of Pediatrics, Nijmegen Center for Mitochondrial Disorders, Radboud University Nijmegen Medical Center, Nijmegen, The Netherlands; ⁵Munich Cluster for Systems Neurology (SyNergy), Munich, Germany; ⁶Medical Faculty, Department of Diagnostic and Interventional Radiology, University Düsseldorf, Düsseldorf, Germany

⁷These two authors contributed equally to this work.

*Correspondence: Dr H Prokisch, Institute of Human Genetics, Technische Universität München, Trogerstr. 32, 81675 Munich, Germany. Tel: +49 89 3187 2890; E-mail: Prokisch@helmholtz-muenchen.de

or Dr F Distelmaier, Department of General Pediatrics, Neonatology and Pediatric Cardiology, University Children's Hospital, Heinrich-Heine-University Düsseldorf, Moorenstr. 5, Düsseldorf 40225, Germany. Tel: +49 211 81 17696; E-mail: Felix.Distelmaier@med.uni-duesseldorf.de

Received 27 January 2015; revised 22 April 2015; accepted 19 May 2015; published online 17 June 2015

generalized cyanosis. The child was intubated and transferred to the neonatal intensive care unit. Laboratory investigations revealed lactic acidosis (up to 22 mmol/l; norm: <1.8 mmol/l), requiring continuous buffering therapy with sodium bicarbonate. Cranial ultrasound demonstrated multiple choroid plexus cysts and symmetrical hyper-echoic signal alterations in the basal ganglia, suggesting neonatal Leigh-like syndrome (Figure 1a). During the following 4 days, lactate levels remained elevated (up to 26 mmol/l) despite intravenous glucose infusion and buffering therapy. Laboratory investigations revealed elevated blood alanine (1088 μ mol/l, norm <414 μ mol/l) and massive lactic-acid excretion in urine. On the fifth day of life, slow improvement in acidosis was noticed with minimal lactate levels of 5 mmol/l. Respiratory efforts improved and the boy was extubated with adequate spontaneous breathing on the eighth day of life. However, the child showed reduced spontaneous movements with intermittent opisthotonus and muscle stiffness in the extremities. On the tenth day of life, the child suffered from seizures and recurrent episodes of apnea and bradycardia. The boy's clinical condition further deteriorated and he died due to cardio-respiratory failure at 18 days of age. Because of a suspected mitochondrial disease, a skin biopsy was performed. Activity measurements of the oxidative phosphorylation enzymes in cultured fibroblasts showed a strongly reduced activity of respiratory chain succinate-cytochrome *c* oxidoreductase (complex II+III: 62 mU/

UCOX, norm>160), pointing to a CoQ₁₀ metabolism defect. Histological investigations of muscle and liver tissue were without specific findings.

MATERIALS AND METHODS

A detailed description of the materials and methods used for this paper can be found in the Supplementary Material.

RESULTS AND DISCUSSION

To unravel the genetic cause of the disease we performed exome sequencing as described previously.⁷ Assuming an autosomal-recessive mode of inheritance, our analyses focused on homozygous and predictively compound heterozygous nonsynonymous variants with a MAF<0.1% in 4500 control exomes. This search identified 28 genes. Only one of these genes, *COQ9* (NM_020312.2; NG_027696.1), coded for a mitochondrial protein.⁸ In addition, *COQ9* was the only gene carrying two predicted loss-of-function alleles. The identified splice site variant, c.521+1del, p.(Ser127_Arg202del), was confirmed by Sanger sequencing in the homozygous state in the index patient with the parents being heterozygous carriers. The change c.521+1del is absent from 4500 in-house control exomes and is listed only once in the heterozygous state in 111 520 alleles of the Exome Aggregation Consortium Browser (Cambridge, MA, USA;

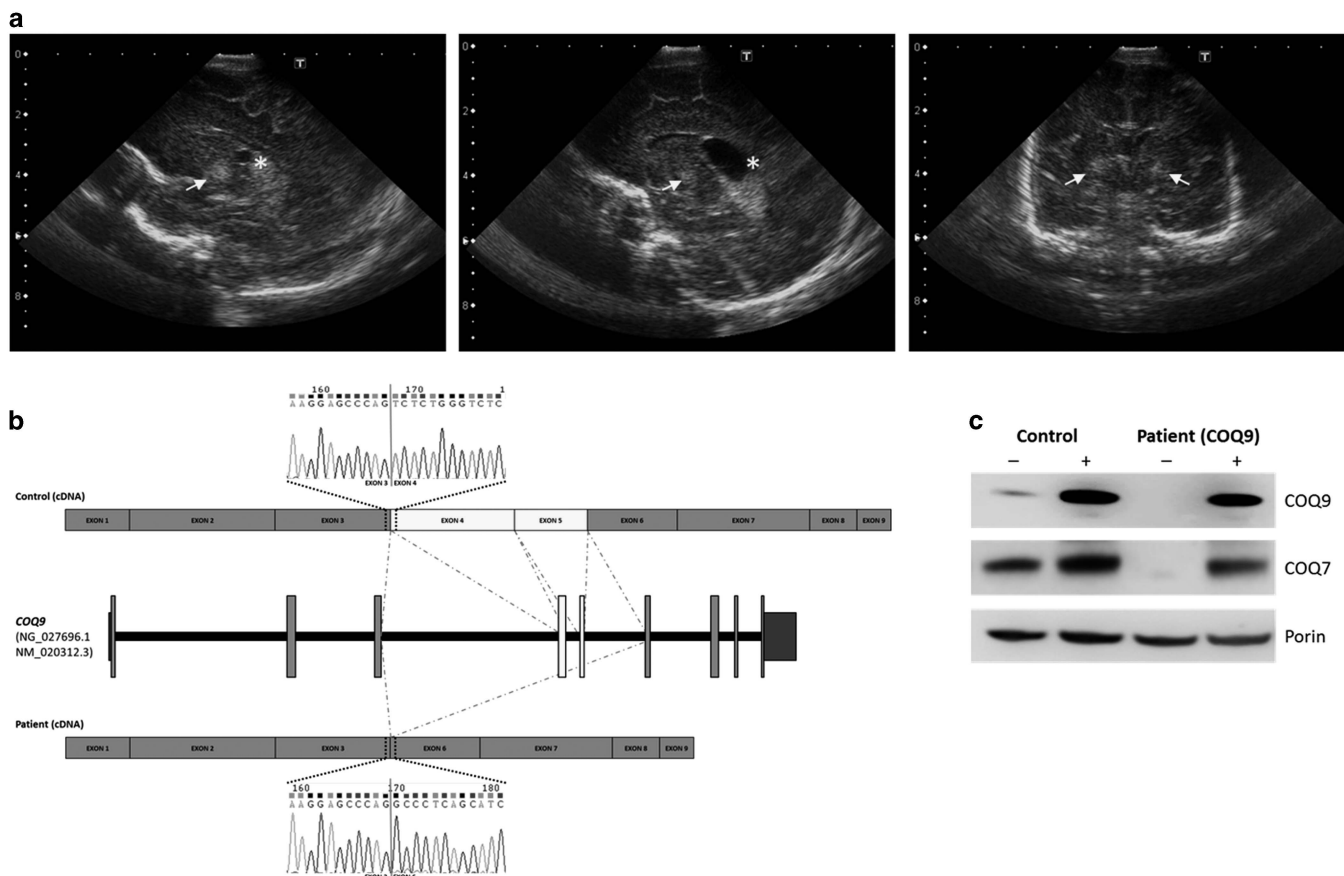
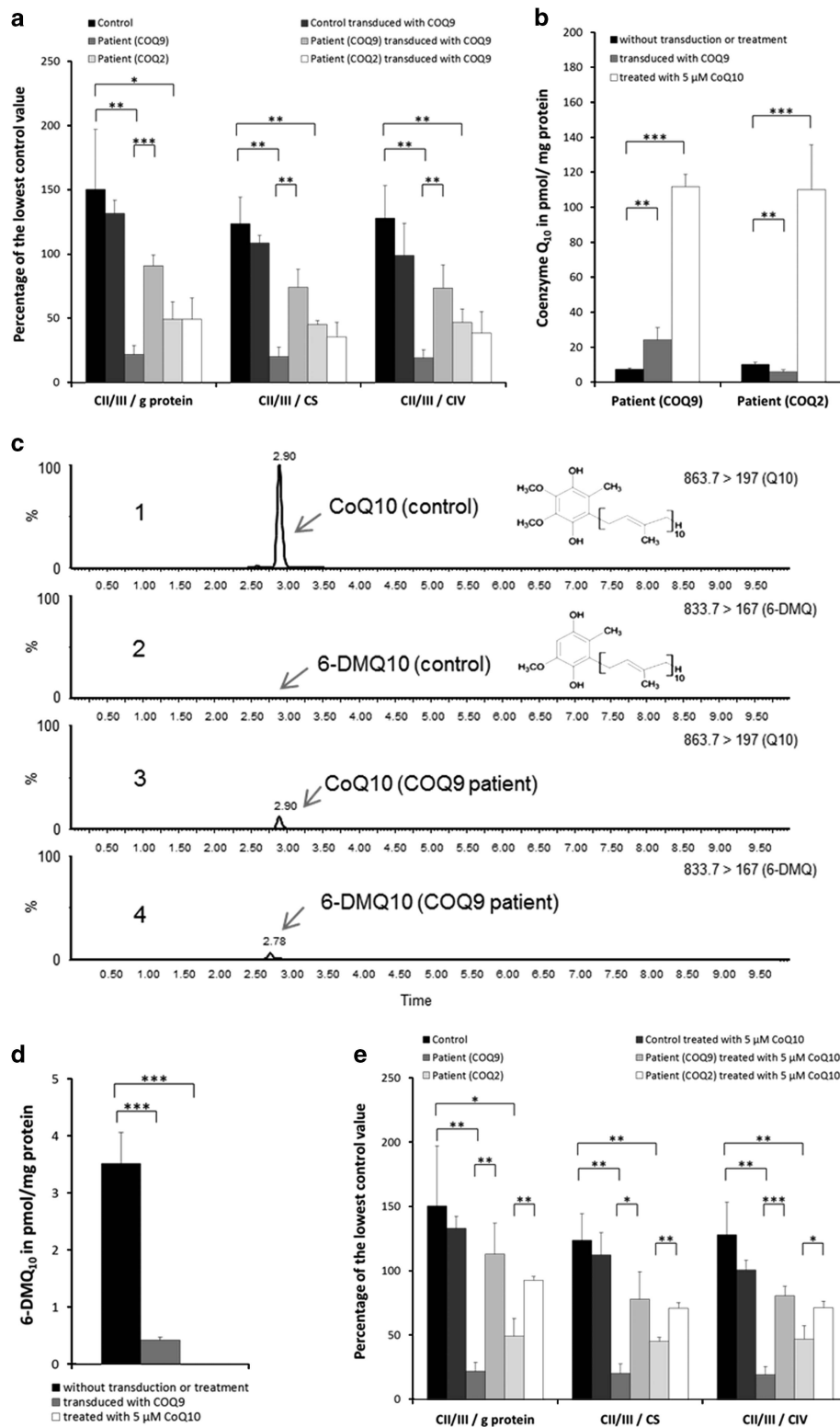


Figure 1 (a) Brain ultrasound in a newborn with a pathogenic *COQ9* variant. Left and middle panels: right and left parasagittal view, respectively, showing hyper-echoic signal within the basal ganglia (arrow) and bilateral choroid plexus cysts (asterisk). Right panel: coronal view demonstrating symmetrical hyper-echoic basal ganglia abnormalities suggesting a neonatal Leigh-like syndrome. (b) Schematic diagram showing the transcript generated in the presence of the c.521+1 deletion. Chromatogram shows that the deletion causes a skipping of exons 4 and 5 in the patient fibroblasts. (c) Western blotting on mitochondria-enriched fractions of patient and control fibroblasts (representative image). In patient cells no COQ9 is detected in the non-transduced condition (-). Signal is restored after lentiviral transduction with *COQ9* wild-type cDNA (+). In addition, a COQ7 antibody was used, demonstrating a severe reduction of COQ7 protein in patient cells. Porin (VDAC) was used as a loading marker.



<http://exac.broadinstitute.org; 12/2014>). The c.521+1 deletion is likely to significantly reduce the efficiency of the donor splice site of intron 4, with the most likely effect being skipping of exon 4 during splicing. Indeed, sequencing of the COQ9 cDNA showed a loss of exons 4 and 5 in patient fibroblasts (Figure 1b). The predicted consequence of the

variant on the COQ9 protein is a deletion of 76 amino acids, including essential parts of the lipid binding cavity.⁹

We next investigated the effect of the variant on the expression of COQ9 by immunodecoration of mitochondria-enriched proteins using a COQ9-specific antibody. Using different amounts of

Figure 2 (a) Biochemical measurement of activities of complex II/III, complex IV of the respiratory chain and citrate synthase. Patient fibroblasts show a significant decrease of complex II/III activity. After re-expression of wild-type cDNA in patient fibroblasts, a significant increase in complex II/III activity relative to protein, to citrate synthase as well as to complex IV activity is observed. The activity of complex II/III remained unchanged in control cells. In addition, lentiviral expression of wild-type *COQ9* cDNA in fibroblasts of a patient with a disease-causing homozygous variant in *COQ2* showed no effect on the activity of complex II/III, demonstrating the specificity of our complementation strategy in the *COQ9* patient. (b) Total amount of CoQ₁₀ was measured before and after lentiviral transduction as well as after treatment with CoQ₁₀. Both patient cell lines showed significantly reduced amounts of CoQ₁₀. After transduction patient fibroblasts with a variant in *COQ9* showed a significant (about threefold) increase of CoQ₁₀, whereas in control cells and in patient cells suffering from *COQ2* deficiency no significant difference could be observed. Treatment with CoQ₁₀ leads to an increase of the total amount of CoQ₁₀ in both patient cell lines. (c) MS/MS chromatograms of fibroblast lysate samples: (1) detected CoQ₁₀ in a control; (2) 6-DMQ₁₀ in a control is not present; (3) low detection of CoQ₁₀ in a *COQ9* patient; (4) detection of 6-DMQ₁₀ metabolite in a *COQ9* patient. (d) Quantification of the 6-DMQ₁₀ levels in *COQ9*-mutant fibroblasts before and after viral complementation with wild-type *COQ9*. The amount of 6-DMQ₁₀ is significantly reduced in *COQ9*-mutant fibroblasts after transduction. (e) Treatment with CoQ₁₀ in control and patient fibroblasts. Supplementation rescues complex II/III activity in fibroblasts derived from *COQ9* and *COQ2* patients whereas no differences were measured in control fibroblasts. Statistics: ****P*<0.001, ***P*<0.01 and **P*<0.05 relative to control or the indicated condition. All experimental data were obtained in at least three independent experiments.

mitochondrial proteins, we were unable to detect COQ9 in patient cells, while we always detected COQ9 in controls, indicating the degradation of the truncated protein (Figure 1c).

Next, we spectrophotometrically measured several mitochondrial enzymes using mitochondria-enriched fractions of fibroblasts. To determine respiratory chain complex II/III activity we measured the reduction of cytochrome *c*. For complex IV activity we followed the oxidation of reduced cytochrome *c*, and the activity of citrate synthase, a mitochondrial matrix enzymatic marker, was measured by following the reduction of 5,5'-dithiobis-(2-nitrobenzoic acid). In line with a defect in CoQ₁₀ biosynthesis and the defect found in fibroblasts, patient cells showed a significant decrease of complex II/III activity (Figure 2a).

To investigate whether the *COQ9* variant is causal for the observed cellular phenotypes, we expressed the functional protein using a lentiviral expression vector containing *COQ9* wild-type cDNA. Expression of *COQ9* was confirmed by western blotting (Figure 1c).¹⁰

The expression of *COQ9* in control cells with normal or reduced CoQ₁₀ levels due to *COQ2* variants had no influence on complex II/III activity (Figure 2a). However, after expression of wild-type cDNA in patient fibroblasts carrying the *COQ9* variant, we observed a significant fourfold increase in complex II/III activity.

As COQ9 is involved in the biosynthesis of CoQ₁₀, the total amount of CoQ₁₀ was measured using ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS). Both CoQ₁₀ patient cell lines showed significantly reduced amounts of CoQ₁₀, with 10 pmol/mg protein in fibroblasts with *COQ2* variants and 7.3 pmol/mg protein in fibroblasts with the *COQ9* variant (normal range 46.5–90.5 pmol/mg protein). After transduction, fibroblasts with mutant *COQ9* showed a significant (about threefold) increase of CoQ₁₀ levels, whereas the CoQ₁₀ levels remained unchanged in the *COQ2* mutant cells (Figure 2b). These findings provide evidence for a causal relation of the identified *COQ9* variant, the CoQ₁₀ deficiency and the reduced complex II/III activity.

Investigations in a mouse model and recent human studies demonstrated that COQ9 is a lipid-binding protein, which has structural homology to the TFR family (bacterial transcriptional regulators).^{9,11} However, Lohman *et al.*⁹ demonstrated that COQ9 is unlikely to act as a transcription factor and suggested that the TFR structural fold has been repurposed during evolution. Instead of catalyzing a specific reaction of the biosynthesis of CoQ₁₀ by itself, it has been speculated that COQ9 binds either CoQ₁₀ or a CoQ₁₀ precursor and forms a complex with COQ7. COQ7 catalyzes the hydroxylation of 6-demethoxy CoQ₁₀, which constitutes the pre-final step in CoQ biosynthesis.⁹ The clearly reduced amount of COQ7 in

the patient cell line, which is rescued by increasing COQ9, indicates a COQ7-stabilizing function of COQ9 (Figure 1c).

Of note, in *COQ9*-mutant mouse as well as human fibroblasts a potential intermediate of the CoQ₁₀ biosynthesis has been described.^{6,11} In mice, this metabolite was identified as 6-demethoxy ubiquinone₉. Detailed human studies are lacking so far. In order to characterize the metabolite in human cells, we performed different UPLC-ESI-MS/MS analysis techniques such as full scan, precursor ion scan or multiple reaction monitoring mode. In accordance with the studies in mice we specifically detected 6-demethoxy ubiquinone₁₀ in *COQ9* fibroblasts (Figure 2c and d). As 6-demethoxy ubiquinone₁₀ is hydroxylated by COQ7 to produce 6-hydroxy ubiquinone₁₀, the accumulation of this intermediate can be explained by the reduced amount of COQ7.⁹ As expected, the expression of *COQ9* normalized COQ7 and 6-demethoxy ubiquinone₁₀ levels (Figure 2d). The metabolite was not detected in other cell lines.

CoQ₁₀ supplementation has been reported to be beneficial in some CoQ₁₀-deficient patients. To test the treatment effect in our cell lines we supplemented them with 5 μM CoQ₁₀ for 1 week according to Lopez *et al.*¹² Treatment with CoQ₁₀ rescued complex II/III activity in *COQ9*-mutant fibroblasts significantly to ~80% of the lowest control value (Figure 2e). Nearly the same effect was observed in *COQ2*-mutant fibroblasts, whereas no differences were measured in control fibroblasts. The content of CoQ₁₀ in patient fibroblasts markedly increased up to about 11-fold after treatment, reaching control values (Figure 2b). Interestingly, in patient fibroblasts with a genetic defect in *COQ9* no 6-demethoxy ubiquinone₁₀ was detected after treatment with CoQ₁₀, which might be due to a reduced endogenous biosynthesis by sufficient exogenous supplementation with CoQ₁₀ (Figure 2d).

In conclusion, our study provides additional insights into the clinical and cellular consequences of COQ9 deficiency. The severe clinical course observed in our patient suggests a critical function of COQ9 already during prenatal development. The case, together with the description of Duncan *et al.*, places COQ9 deficiency among the most severe forms of CoQ₁₀ metabolism disorders, comparable to descriptions in patients with defects in *COQ2* or *COQ4*.^{6,13,14} However, further clinical descriptions will be required to evaluate the specific clinical spectrum of patients with *COQ9* mutations. As suggested by Duncan *et al.*,⁶ CoQ₁₀ treatment might be helpful in *COQ9*-deficient children. Accordingly, in unclear cases of neonatal/early childhood mitochondrial disease, an empirical medication with CoQ₁₀ is recommended.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

This project was supported by the BMBF-funded German Network for Mitochondrial Disorders (mitoNET #01GM1113C) and by the E-Rare project GENOMIT (01GM1207). TBH was supported by the BMBF through the Juniorverbund in der Systemmedizin 'mitOmics' (FKZ 01ZX1405C).

- 1 Laredj LN, Licitra F, Puccio HM: The molecular genetics of coenzyme Q biosynthesis in health and disease. *Biochimie* 2014; **100**: 78–87.
- 2 Bentinger M, Tekle M, Dallner G: Coenzyme Q—biosynthesis and functions. *Biochem Biophys Res Commun* 2010; **396**: 74–79.
- 3 Doimo M, Desbats MA, Cerqua C, Cassina M, Trevisson E, Salviati L: Genetics of coenzyme q10 deficiency. *Mol Syndromol* 2014; **5**: 156–162.
- 4 Emmanuele V, López LC, Berardo A *et al*: Heterogeneity of coenzyme Q10 deficiency: patient study and literature review. *Arch Neurol* 2012; **69**: 978–983.
- 5 Quinzii CM, Emmanuele V, Hirano M 2014 Clinical presentations of coenzyme q10 deficiency syndrome. *Mol Syndromol* 2014; **5**: 141–146.
- 6 Duncan AJ, Bitner-Glindzic M, Meunier B *et al*: A nonsense mutation in COQ9 causes autosomal-recessive neonatal-onset primary coenzyme Q10 deficiency: a potentially treatable form of mitochondrial disease. *Am J Hum Genet* 2009; **84**: 558–566.
- 7 Haack TB, Gorza M, Danhauser K *et al*: Phenotypic spectrum of eleven patients and five novel MTFMT mutations identified by exome sequencing and candidate gene screening. *Mol Genet Metab* 2014; **111**: 342–352.
- 8 Elstner M, Andreoli C, Klopstock T, Meitinger T, Prokisch H: The mitochondrial proteome database: MitoP2. *Methods Enzymol* 2009; **457**: 3–20.
- 9 Lohman DC, Forouhar F, Beebe ET *et al*: Mitochondrial COQ9 is a lipid-binding protein that associates with COQ7 to enable coenzyme Q biosynthesis. *Proc Natl Acad Sci USA* 2014; **111**: E4697–E4705.
- 10 Haack TB, Kopajtich R, Freisinger P *et al*: ELAC2 mutations cause a mitochondrial RNA processing defect associated with hypertrophic cardiomyopathy. *Am J Hum Genet* 2013; **93**: 211–223.
- 11 García-Corzo L, Luna-Sánchez M, Doerrier C *et al*: Dysfunctional Coq9 protein causes predominant encephalomyopathy associated with CoQ deficiency. *Hum Mol Genet* 2013; **22**: 1233–1248.
- 12 López LC, Quinzii CM, Area E *et al*: Treatment of CoQ(10) deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: time- and compound-dependent effects. *PLoS One* 2010; **5**: e11897.
- 13 Jakobs BS, van den Heuvel LP, Smeets RJ *et al*: A novel mutation in COQ2 leading to fatal infantile multisystem disease. *J Neurol Sci* 2013; **326**: 24–28.
- 14 Brea-Calvo G, Haack TB, Karall D *et al*: COQ4 mutations cause a broad spectrum of mitochondrial disorders associated with CoQ10 deficiency. *Am J Hum Genet* 2015; **96**: 309–317.

Supplementary Information accompanies this paper on *European Journal of Human Genetics* website (<http://www.nature.com/ejhg>).