Survival transcriptome in the coenzyme Q_{10} deficiency syndrome is acquired by epigenetic modifications: a modelling study for human coenzyme Q_{10} deficiencies

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ABSTRACT

Objective: Coenzyme Q_{10} (CoQ_{10}) deficiency syndrome is a rare condition that causes mitochondrial dysfunction and includes a variety of clinical presentations as encephalomyopathy, ataxia and renal failure. First, we sought to set up what all have in common, and then investigate why CoQ_{10} supplementation reverses the bioenergetics alterations in cultured cells but not all the cellular phenotypes.

Design Modelling study: This work models the transcriptome of human CoQ_{10} deficiency syndrome in primary fibroblast from patients and study the genetic response to CoQ_{10} treatment in these cells.

Setting: Four hospitals and medical centres from Spain, Italy and the USA, and two research laboratories from Spain and the USA.

Participants: Primary cells were collected from patients in the above centres.

Measurements: We characterised by microarray analysis the expression profile of fibroblasts from seven CoQ_{10}-deficient patients (three had primary deficiency and four had a secondary form) and aged-matched controls, before and after CoQ_{10} supplementation. Results were validated by Q-RT-PCR. The profile of DNA (CpG) methylation was evaluated for a subset of gene with displayed altered expression.

Results: CoQ_{10}-deficient fibroblasts (independently from the aetiology) showed a common transcriptomic profile that promotes cell survival by activating cell cycle and growth, cell stress responses and inhibiting cell death and immune responses. Energy production was supported mainly by glycolysis while CoQ_{10} supplementation restored oxidative phosphorylation. Expression of genes involved in cell death pathways was partially restored by treatment, while genes involved in differentiation, cell cycle and growth were not affected. Stably demethylated genes were unaffected by treatment whereas we observed restored gene expression in either non-demethylated genes or those with an unchanged methylation pattern.

ARTICLE SUMMARY

Article focus

- To analyse the common gene expression profile in primary cell cultures of dermal fibroblasts from patients suffering any of the clinical presentation of the human syndrome of coenzyme Q_{10} (CoQ_{10}) deficiency (primary or secondary CoQ_{10} deficiency).
- To determine why CoQ_{10} treatment, the current therapy for all forms of CoQ_{10} deficiency, restored respiration but not all the cellular phenotypes.
- To investigate the stable genetic cause responsible for the survival adaptation to mitochondrial dysfunction owing to CoQ_{10} deficiency.

Key messages

- The mitochondrial dysfunction owing to CoQ_{10} deficiency induces a stable survival adaptation of somatic cells in patients at early or postnatal development by epigenetic modifications of chromatin. Deficient cells unable to maintain this survival state during differentiation would die contributing to the pathological phenotype.
- Supplementation with CoQ_{10} restores respiration through enhanced sugar rather than lipid metabolism; partially restores stress response, immunity, cell death and apoptotic pathways; and does not affect cell cycle, cell growth, and differentiation and development pathways.
- Survival transcriptome in the CoQ_{10} deficiency syndrome is acquired by epigenetic modifications of DNA: DNA-demethylated genes corresponded to unaffected genes by CoQ_{10} treatment, whereas those with unchanged DNA-methylation pattern corresponded to genes with responsive expression to CoQ_{10} supplementation. These results would approach to explain the incomplete recovery of clinical symptoms after CoQ_{10} treatment, at least in some patients.
Survival transcriptome in coenzyme Q<sub>10</sub> deficiency syndrome

**ARTICLE SUMMARY**

**Strengths and limitations of this study**
- Human CoQ<sub>10</sub> deficiencies are considered rare diseases with low prevalence, which limits the sample size.
- The genetic heterogeneity of this disease is owing to mutations in any of the 11 genes directly involved in the synthesis of CoQ<sub>10</sub>, inside mitochondria, or other mutations altering somehow the mitochondria and its metabolism, affecting their inner CoQ<sub>10</sub> synthesis as a side effect, will course with CoQ<sub>10</sub> deficiency.
- Among this genetic heterogeneity, all cells showed a common transcriptomic profile that justified their pathological phenotype, responded equally to CoQ<sub>10</sub> treatment and presented the same DNA methylation pattern.

**Conclusions:** CoQ<sub>10</sub> deficiency induces a specific transcriptomic profile that promotes cell survival, which is only partially rescued by CoQ<sub>10</sub> supplementation.

**INTRODUCTION**

Coenzyme Q<sub>10</sub> (CoQ<sub>10</sub>) is a small electron carrier which is an essential cofactor for several mitochondrial biochemical pathways such as oxidative phosphorylation, β-oxidation and pyrimidine nucleotide biosynthesis. CoQ<sub>10</sub> biosynthesis depends on a multienzyme complex that involves at least 11 proteins encoded by COQ genes. Mutations in any of these genes cause primary CoQ<sub>10</sub> deficiencies, which are clinically heterogeneous mitochondrial diseases. Clinical presentations include encephalomyopathy with lipid storage myopathy and myoglobinuria, ataxia and cerebellar atrophy, severe infantile encephalomyopathy with renal failure, isolated myopathy, and nephrotic syndrome. Secondary CoQ<sub>10</sub> deficiency has also been associated with diverse mitochondrial diseases. In all of these conditions, CoQ<sub>10</sub> supplementation partially improves symptoms and usually induces a return to normal growth and respiration in CoQ<sub>10</sub>-deficient fibroblasts. Adaptation of somatic cells to CoQ<sub>10</sub> deficiency may affect both onset and course of the disease. We document common transcriptomic profile alterations in somatic cells of CoQ<sub>10</sub>-deficient patients, their response to CoQ<sub>10</sub> supplementation, and the relationship with the DNA methylation status of specific genes.

**MATERIALS AND METHODS**

**Cells**

Primary skin fibroblasts from CoQ<sub>10</sub>-deficient patients and from aged-matched controls, at similar culture passage, were cultured at 37°C using Dulbecco’s Modified Eagle Medium (DMEM) 1 g/l glucose, L-glutamine and pyruvate (Invitrogen, Prat de Llobregat, Barcelona) supplemented with an antibiotic/antimycotic solution (Sigma Chemical Co, St Louis, Missouri) and 20% fetal bovine serum (FBS, Linus). When required, CoQ<sub>10</sub> prediluted in FBS was added to the plates at a final concentration of 30 µM (CoQ<sub>10</sub> Synthetic Minimum 98%, high-performance liquid chromatography, Sigma). We studied five patients with primary CoQ<sub>10</sub> deficiency; two siblings harboured a homozygous p.Y297C mutation in the COQ2 gene, other with a pathogenic mutation (c.483G>C) in the COQ4 gene (this paper), and another one with haploinsufficiency of COQ4. Patients with secondary CoQ<sub>10</sub> deficiency included: a mitochondrial encephalopathy, lactic acidosis and stroke-like episodes patient harboring the m.3243A>G in the mitochondrial tRNALeu(UUR) with 43% heteroplasmy level, a patient with mtDNA depletion syndrome, and a third patient with ataxia of unknown origin. Table 1 summarises the clinical phenotype and biochemical studies of these patients.

**Transcriptome analysis**

RNA extraction, probe synthesis and hybridisation with two independent expression arrays (GeneChip Human Genome U133 Plus 2.0 and GeneChip Human Gene 1.0 ST, Affymetrix) were used as described. Gene expression was validated by the MyiQ Single Color Real Time PCR Detection System (BioRad). See supplementary methods for full description.

Data had been deposited with the NCBI-GEO database, at http://www.ncbi.nlm.nih.gov/geo/, accession number GSE33941 (this SuperSeries is composed of two subset Series, see online supplementary table S7 for an explanation). Statistical analyses were performed comparing each signal of patient’s fibroblasts RNA with the corresponding signal of control RNA by two different approaches. The main statistical analysis for both GeneChip Human Genome U133 Plus 2.0 Array and GeneChip Human Gene 1.0 ST Array was achieved as previously described, which selects the most significant genes commonly and equally regulated in all samples using very stringent parameters. In a few special cases, other unselected but regulated genes were studied because of their role in specific processes and pathways. They were equally described in table 2. The second statistical analysis approach for the Gene Ontology (GO) study was performed as previously described and analyses the most altered biological processes and pathways using a lower stringency analysis, which permits to select the hundred most altered GOs in different functional categories (see online supplementary table S4) and the hundred more distorted pathways (see online supplementary table S5) that had been regulated in CoQ<sub>10</sub>-deficient cells. GO regulated in both independent analysis of primary and secondary CoQ<sub>10</sub> deficiencies (see online supplementary table 3), and those regulated by CoQ<sub>10</sub> supplementation (see online supplementary table S9) were studied using the GORILLA software (Gene Ontology enrichment analysis and visualisation tool), at http://cbl-gorilla.cs.technion.

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| Patient/cells* | Clinical phenotype | Biochemical studies (% with respect to mean reference values) | Effect of CoQ₁₀ supplementation† | Reference as cited in the text | Array and epigenetic code |
|---------------|-------------------|---------------------------------------------------------------|----------------------------------|-------------------------------|--------------------------|
| Human dermal skin fibroblast | Healthy volunteers | *Reference values* | *Reference values* | 12 | #2 #HDF #control |
| 12-year-old girl | Ataxia and cerebellar atrophy | ▶ 17% CoQ₁₀ in muscle | ▶ Improvement of neurological assessment | ▶ No biochemical studies performed | 4 |
| | Secondary CoQ₁₀ deficiency | ▶ 31% mt-RC complex I+III (muscle) | | | |
| | | ▶ 46% mt-RC complex II+III (muscle) | | | |
| | | ▶ 22% CoQ₁₀ in fibroblast | | | |
| | | ▶ 24% CoQ₁₀ biosynthesis rate | | | |
| | | ▶ ROS production (three fold) | | | |
| 33-month-old boy (his sister below) | Corticosteroid-resistant nephropathy | ▶ 23% CoQ₁₀ in muscle | ▶ Improvement of neurological assessment but not the renal dysfunction | 5 | #3 |
| | Progressive encephalomyopathy | ▶ 19% mt-RC complex I+III (muscle) | ▶ Recovery of cell growth | 17 | 12 case 3 |
| | COQ2 gene mutation (c.890A>G) | ▶ 32% mt-RC complex II+III (muscle) | ▶ Improvement of 35% complex II+III (cells) | | |
| | | ▶ 17% CoQ₁₀ in fibroblast | | | |
| | | ▶ 10% CoQ₁₀ biosynthesis rate | | | |
| | | ▶ 57% mt-RC complex II+III (cells) | | | |
| 9-month-old girl (her brother above) | Corticosteroid-resistant nephropathy | ▶ 29% CoQ₁₀ in fibroblast | ▶ Improvement of 25% complex II+III (cells) | 17 | #5 |
| | COQ2 gene mutation (c.890A>G) | ▶ 15% CoQ₁₀ biosynthesis rate | ▶ Recovery of cell growth | 12 case 4 | |
| | | ▶ 60% mt-RC complex II+III (cells) | | | |
| | | | | | |
| Boy | MELAS (A3243G mutation) | ▶ 58% CoQ₁₀ in fibroblast | ▶ Recovery of mt-RC | 8 | #4 #MEL+Q |
| | Secondary CoQ₁₀ deficiency | ▶ 35% mt-RC complex I (cells) | ▶ Recovery of ATP production | | |
| | | ▶ 41% mt-RC complex II+III (cells) | ▶ No ROS production | | |
| | | ▶ 12% mt-RC complex IV (cells) | | | |
| | | ▶ 60% mt-ΔΨ | | | |
| | | ▶ 70% mitochondrial mass | | | |
| | | ▶ ROS production (>2-fold) | | | |
| | | ▶ Defective autophagosome elimination | | | |

Continued
| Patient/cells* | Clinical phenotype | Biochemical studies (% with respect to mean reference values) | Effect of CoQ₁₀ supplementation† | Reference as cited in the text | Array and epigenetic code |
|---------------|--------------------|-------------------------------------------------------------|----------------------------------|--------------------------------|--------------------------|
| 10-day-old boy | ▶ mtDNA depletion syndrome  
▶ Neonatal encephalopathy  
▶ Secondary CoQ₁₀ deficiency | ▶ 20% CoQ₁₀ in muscle  
▶ 32% mt-RC complex I+III (muscle)  
▶ 19% mt-RC complex II+III (muscle)  
▶ 15% CoQ₁₀ in fibroblast  
▶ 85% mt-RC complex II+III (cells) | ▶ Improvement of 41% complex II+III (cells)  
▶ Recovery of cell growth | ³⁴ | #ELO  
#ELO+Q |
| 3-year-old boy | ▶ Dysomorphic features  
▶ Ventricular septal defect and weakness  
▶ Hypotonia and hyporeactivity  
▶ Moderate mental retardation  
▶ COQ4 gene deletion  
▶ Primary CoQ₁₀ deficiency  
▶ COQ4 gene mutation (c.483G>C)  
▶ Rhabdomyolysis  
▶ Primary CoQ₁₀ deficiency  
▶ Ataxia  
▶ Secondary CoQ₁₀ deficiency | ▶ 40% CoQ₁₀ in fibroblast  
▶ 44% CoQ₁₀ biosynthesis rate  
▶ 64% mt-RC complex I+III (cells)  
▶ 58% mt-RC complex II+III (cells) | ▶ Improvement in muscle tone and strength  
▶ He began to speak and walk | ¹⁸ | #GIO |
| Girl | ▶ COQ4 gene mutation (c.483G>C)  
▶ Rhabdomyolysis  
▶ Primary CoQ₁₀ deficiency  
▶ Ataxia  
▶ Secondary CoQ₁₀ deficiency | ▶ 18% CoQ₁₀ in fibroblast | ▶ Recovery of both complex I+III activity and growth of fibroblasts | This paper | #SIL+Q#epi |
| Girl | ▶ COQ4 gene mutation (c.483G>C)  
▶ Rhabdomyolysis  
▶ Primary CoQ₁₀ deficiency  
▶ Ataxia  
▶ Secondary CoQ₁₀ deficiency | ▶ 38% CoQ₁₀ in fibroblast | ▶ Improvement of ATP synthesis | ¹² case 1 | #SOF+Q#epi |

*Cultured at 37°C using DMEM 1 g/l glucose, l-glutamine, pyruvate (Invitrogen) plus antibiotic/antimycotic solution (Sigma) and 20% fetal bovine serum (FBS, Linus).
†CoQ₁₀ prediluted in FBS was added to the plates at a final concentration of 30 µM (coenzyme Q₁₀, Synthetic Minimum 98%, high-performance liquid chromatography, Sigma).
CoQ₁₀, Coenzyme Q₁₀, MELAS, mitochondrial encephalopathy, lactic acidosis and stroke-like episodes; mtDNA, mitochondrial DNA; mt-RC, mitochondrial respiratory chain; ROS, reactive oxygen species.
| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|--------------|------------|-----|-----|--------|-----------|---------|
| C7orf55      | Chromosome 7 open reading frame 55 | −2.1 | nc  | −       | −         | −       |
| BRP44        | Brain protein 44 | 2.0  | 2.3 | U      | 8.0       | −2-fold |
| C10orf58     | Chromosome 10 open reading frame 58 | −19.5 | −1.6 | pR     |           |         |
| NADH mobilisation |                  |      |     |        |           |         |
| CYB561       | Cytochrome b561 | −1.3 | nc  | O      |           |         |
| CYB5A        | Cytochrome b5-A | −1.4 | −1.5 | U      |           |         |
| CYB5R1       | Cytochrome b5 reductase 1 | −1.3 | nc  | U      |           |         |
| CYB5R2       | Cytochrome b5 reductase 2 | −1.4 | −1.9 | U      |           |         |
| CYB5R3       | Cytochrome b5 reductase 3 | −1.4 | −1.6 | R      |           |         |
| CYB5R4       | Cytochrome b5 reductase 4 | −1.3 | −1.6 | R      |           |         |
| Lipid metabolism |                |      |     |        |           |         |
| FDFT1        | Farnesyl-diphosphate farnesyltransferase 1 | −2.3 | −1.5 | U      | −4.3      | +2-fold |
| ID1I         | Isopentenyl-diphosphate δ isomerase 1 | −2.1 | nc  | U      |           |         |
| CH25H        | Cholesterol 25-hydroxylase | −10.8 | −3.2 | O      | −1.3      | −3-fold |
| RSAD2        | Radical S-adenosyl methionine domain containing 2 | −6.8 | 1.4 | pR     |           |         |
| INSIG1       | Insulin-induced gene 1 | −2.6 | 1.7 | O      |           |         |
| LDLR         | Low density lipoprotein receptor | −3.0 | −1.8 | pR     |           |         |
| SQLE         | Squalene epoxidase | −2.5 | nc  | U      |           |         |
| SCD          | Steroyl-coenzyme A desaturase (δ-9-desaturase) | −3.3 | nc  | U      |           |         |
| Insulin metabolism |                 |      |     |        |           |         |
| CPE          | Carboxypeptidase E | 10.0 | 2.5 | pR     |           |         |
| PAPPA        | Pregnancy-associated plasma protein A, pappalysin | 2.5  | 1.7 | R      | 4.8       | −5-fold |
| PCSK2        | Proprotein convertase subtilisin/kexin type 2 | −75.5 | −4.3 | O      |           |         |
| Other metabolism |               |      |     |        |           |         |
| SCIN         | Scinderin | −5.4 | −1.4 | O      |           |         |
| PYGL         | Phosphorylase, glycogen; liver | −2.5 | −1.6 | R      |           |         |
| SLC40A1      | Solute carrier family 40 (iron-regulated transporter) | 7.6  | 2.9 | R      |           |         |
| QPRT         | Quinolinate phosphoribosyltransferase | −3.4 | nc  | R      |           |         |
| ATP8B1       | ATPase, class I, type 8B and member 1 | 2.4  | nc  | pR     |           |         |
| Cell cycle |                |      |     |        |           |         |
| POSTN        | Periostin, osteoblast specific factor | 73.8 | 153.9 | U      | 238.2     | −20%    |
| VEGFA        | Vascular endothelial growth factor A | 2.9  | nc  | −      |           |         |
| SEMA5A       | Semaphorin 5A, receptor for cell growth | 3.6  | 1.6 | pR     |           |         |
| AEBP1        | AE binding protein 1 | 66.1 | nc  | R      |           |         |
| CSRPR2       | Cysteine and glycine-rich protein 2 | 5.3  | 1.5 | R      |           |         |
| DOK5         | Docking protein 5 | 6.5  | 1.6 | U      |           |         |
| MID1         | Midline 1 (Opitz/BBB syndrome) | 3.9  | 4.4 | U      |           |         |
| CHURC1       | Churchill domain containing 1 | 3.5  | nc  | −      |           |         |
| CREG1        | Repressor 1 of E1A-stimulated genes | 3.0  | 1.3 | R      |           |         |
| RUNX1        | Runt-related transcription factor 1 (aml1 oncogene) | 1.9  | 1.6 | −      |           |         |
| BHLHB5       | Basic helix-loop-helix domain containing; class B, 5 | −6.1 | −1.4 | −      |           |         |
| IFITM1       | Interferon induced transmembrane protein 1 (9–27) | −3.8 | −3.7 | O      |           |         |
| EDN1         | Endothelin 1 | −3.0 | nc  | U      |           |         |
| MATN2        | Matrin 2 | −9.2 | nc  | U      |           |         |
| MCAM         | Melanoma cell adhesion molecule | −6.7 | −3.0 | R      | −10.9     | +10%    |
| MKX          | Mohawk homeobox | −4.5 | −1.5 | −      |           |         |
| PSG6         | Pregnancy specific β-1-glycoprotein 6 | 2.6  | nc  | −      |           |         |
| DCN          | Decorin | 2.0  | −1.6 | −      |           |         |
| PKP4         | Plakophilin 4 | 2.0  | 1.4 | U      |           |         |
| EFEMP1       | EGF-containing fibulin-like extracellular matrix protein 1 | 13.2 | 2.2 | pR     |           |         |
| VCAN         | Versican | 2.8  | 2.7 | −      | 4.6       | +10%    |
| SMARCA1      | Component of SWI/SNF chromatin complex, member A1 | −1.3 | nc  | pR     |           |         |
| SMARCA4      | Component of SWI/SNF chromatin complex, member A4 | −1.9 | nc  | pR     |           |         |
| CDK6         | Cyclin-dependent kinase 6, overexpressed in tumour | 1.4  | 2.9 | U      |           |         |
| CDKN1A       | P21, inhibitor of CDK | −9.2 | −2.1 | U      |           |         |
| CDKN1C       | P57, inhibitor of CDK | −2.6 | −1.3 | R      |           |         |
| CDKN3        | Inhibitor of CDK, overexpressed in cancer cells | 1.9  | 2.7 | U      |           |         |

Continued
| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| CD31        | Cell surface antigen | −1.8 | −1.5 | R     |           |        |
| RB1         | Retinoblastoma protein | −1.4 | nc  | R     |           |        |
| E2F7        | E2F transcription factor 7 | 3.6  | nc  | U     |           |        |
| E2F8        | E2F transcription factor 8 | 2.2  | nc  | U     |           |        |
| FST         | Follistatin | 2.6  | 1.4  | O     |           |        |

**Development and differentiation**

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| BDNF        | Brain-derived neurotrophic factor | −2.9 | nc  | pR    |           |        |
| GRP         | Gastrin-releasing peptide | −263.6 | nc  |  |           |        |
| NTTNG1      | Nettin G1  | −8.3 | 1.8  | U     |           |        |
| PTN         | Pleiotrophin (neurite-growth-promoting factor1) | −2.7 | nc  | R     |           |        |
| FOXQ1       | Forkhead box Q1 | −6.5 | nc  | −     |           |        |
| HOXA11      | Homeobox A11 | −4.3 | −2.4 | U     |           |        |
| HOXC9       | Homeobox C9 | −4.8 | −2.0 | U     |           |        |
| LHX9        | Lim homeobox 9 | −93.0 | −1.5 | U     |           |        |
| SP110       | SP110 nuclear body protein | −2.5 | nc  | pR    |           |        |
| P2RY5       | Purinergic receptor P2Y; G-protein coupled, 5 | −4.4 | −1.3 | pR    |           |        |
| TSPAN10     | Tetraspanin 10 | −10.1 | nc  | −     |           |        |
| EPSTI1      | Epithelial stromal interaction 1 | −5.2 | −1.4 | R     |           |        |
| TSHZ1       | Teashirt zinc finger homeobox 1 | −2.8 | nc  | R     |           |        |
| KRT34       | Keratin 34 | −5.3 | −7.6 | R     | −5.7     | −60%   |
| TPM1        | Tropomyosin 1 (α) | −1.8 | 1.7  | −     |           |        |
| FOXP1       | Forkhead box P1 | 2.3  | nc  | −     |           |        |
| LMCD1       | Lim and cysteine-rich domains 1 | 3.8  | nc  | U     |           |        |

**Cell resistance to stress**

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| CYP1B1      | Cytochrome P450, family 1B and polypeptide 1 | 4.5  | 1.5  | −5-fold |           |        |
| MGC87042    | Similar to six epithelial antigen of prostate | 12.2 | −   | R     |           |        |
| TMEM49      | Transmembrane protein 49/microRNA 21 | 1.9  | nc  | −     |           |        |
| RAD23B      | RAD23 homologue B (Saccharomycyes cerevisiae) | 2.2  | nc  | R     |           |        |
| TXNIP       | Thioredoxin-interacting protein | 2.0  | −4.9 | −     |           |        |
| SGK1        | Serum/glucocorticoid regulated kinase 1 | 3.4  | 1.5  | −     |           |        |
| SOCS3       | Suppressor of cytokine signalling 3 | −3.6 | nc  | R     |           |        |
| ROU         | Ras homologue gene family. member U | −8.3 | nc  | O     |           |        |

**Apoptosis**

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| AIM1        | Absent in melanoma 1 | −4.5 | −1.4 | O     |           |        |
| APCDD1      | Adenomatosis polyposis coli down-regulated 1 | −6.4 | −1.8 | O     |           |        |
| MAGED1      | Melanoma antigen family D, 1 | −1.7 | nc  | U     |           |        |
| MAGED4/4B   | Melanoma antigen family D, 4/4B | −5.0 | −1.6 | U     |           |        |
| RAC2        | Small GTP-binding protein Rac2 (rho family) | −2.3 | −1.3 | U     |           |        |
| TRIM55      | Tripartite motif-containing 55 | −11.7 | −1.6 | U     |           |        |
| IFI6        | Interferon, α-inducible protein 6 | −4.9 | −1.3 | R     |           |        |
| XAF1        | XIAP associated factor-1 | −3.0 | −1.5 | R     |           |        |
| TNFRSF10D   | Tumour necrosis factor receptor superfamily 10D | 2.4  | 2.6  | U     | 15.1     | +20%   |
| SFRP1       | Secreted frizzled-related protein 1 | 8.7  | 2.5  | U     | 11.8     | −2-fold |

**Signalling**

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| ARL4C       | ADP-ribosylation factor-like 4C | 3.8  | 1.6  | pR    |           |        |
| USP53       | Ubiquitin specific peptidase 53 | 4.2  | 1.7  | −     |           |        |
| GABBR2      | γ-aminobutyric acid B receptor, 2 | 13.8 | 2.0  | U     |           |        |
| CNGA3       | Cyclic nucleotide gated channel α-3 | −67.3 | nc  | −     |           |        |
| GNG2        | G-protein, γ-2 | −4.2 | 1.4  | pR    |           |        |
| HERC6       | Hect domain and RLD 6 | −7.4 | −1.4 | R     |           |        |
| MLPH        | Melanophilin | −8.5 | −1.9 | R     |           |        |
| NCK2        | NCK adaptor protein 2 | −1.7 | nc  | −     |           |        |
| PARP14      | Poly (ADP-ribose) polymerase family, member 14 | −3.1 | −1.5 | −     |           |        |

**Immunity**

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|-------------|------------|-----|-----|--------|-----------|--------|
| CDC42SE2    | CDC42 small effector 2 | −2.8 | nc  | −     |           |        |
| LY6K        | Lymphocyte antigen 6 complex, locus K | −4.7 | 1.4  | −     |           |        |
| GALNAC4S-6ST| B cell RAG associated protein | −17.3 | −2.5 | O     |           |        |

Continued
ac.21 Full description of statistical analysis be found in the supplementary material.

**Epigenetic analysis**

DNA (CpG) methylation analysis was performed using a base-specific cleavage reaction with bisulfite combined with mass spectrometric analysis (MassCLEAVE). For the statistical analysis, the CpGs’ methylation degree for each gene was analysed with the MultiExperiment Viewer software developed by Saeed.22 See supplementary methods for full description.

**RESULTS**

**Transcriptome analysis**

We studied skin fibroblasts from four patients with primary CoQ10 deficiency and three patients with secondary CoQ10 deficiency (table 1). We analysed the transcriptomic profiles and compared them with those of cells from age-matched control individuals, and evaluated the modifications induced by supplementation with 30 μM CoQ10 for 1 week to allow recovery of ATP levels.8 16 17 A very stringent analysis selected the most significant genes displaying a common and equally altered expression in all samples (summarised in table 2 and shown with full details in online supplementary table S1). Other genes unselected by this analysis, but still abnormally expressed were also included in the study because of their role in specific processes and pathways, such as NADH mobilisation, cell cycle and immunity (see online supplementary table S1) and energetic metabolism (see online supplementary table S2). GO classification of these genes showed similar profiles when comparing independently primary-deficient and secondary-deficient fibroblasts (see online supplementary table S3). A lower stringency analysis showing the most altered biological processes and pathways selected 100 most altered GO in different functional categories (see online supplementary table S4) and 100 more distorted pathways (see online supplementary table S5) in CoQ10-deficient cells. See supplementary data for description of statistical analyses.

CoQ10 treatment modified the specific transcriptomic profile displayed by CoQ10-deficient fibroblasts (see

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**Table 2** Continued

| Gene symbol* | Gene title | FC† | FC‡ | CoQ10§ | Q-RT-PCR¶ | CoQ10** |
|--------------|------------|-----|-----|--------|-----------|---------|
| TNFSF4       | Tumour necrosis factor superfamily, member 4 | −5.9 | nc | –      | –         | –       |
| TRIM14       | Tripartite motif-containing 14 | −4.5 | nc | –      | –         | –       |
| BTN3(A2/A3)  | Butyrophilin 3 (A2/A3) | −2.0 | −1.3 | R      | –         | –       |
| IFI27        | Interferon, α-inducible protein 27 | −9.8 | nc | O      | –         | –       |
| IFI44        | Interferon-induced protein 44 | −3.3 | −2.3 | R      | –         | –       |
| IFI44L       | Interferon-induced protein 44-like | −15.0 | −1.9 | R      | –         | –       |
| IFIT1        | Interferon-induced protein (tetratricopeptide repeats 1) | −5.3 | nc | –      | –         | –       |
| IFIT3        | Interferon-induced protein (tetratricopeptide repeats 3) | −3.5 | −1.7 | R      | –         | –       |
| GBP1         | Guanylate binding protein 1, interferon-inducible | −2.7 | –   | –      | –         | –       |
| ISG15        | ISG15 ubiquitin-like modifier | −6.4 | nc | R      | –         | –       |
| MX1          | Myxovirus resistance 1 | −7.4 | −1.8 | pR     | –         | –       |
| MX2          | Myxovirus resistance 2 | −6.1 | −3.0 | pR     | –         | –       |
| OAS1         | 2′,5′-oligoadenylate synthetase 1, 40/46 kDa | −5.1 | −4.9 | R      | –         | –       |
| OAS2         | 2′,5′-oligoadenylate synthetase 2, 69/71 kDa | −6.2 | −1.6 | R      | –         | –       |
| OAS3         | 2′,5′-oligoadenylate synthetase 3, 100 kDa | −3.6 | −1.3 | R      | –         | –       |
| OASL         | 2′,5′-oligoadenylate synthetase-like | −3.1 | −2.6 | R      | –         | –       |
| PSMB9        | Proteasome subunit, β-type, 9 | −1.8 | nc | U      | –         | –       |

*In italic letter, biomarkers used in several types of cancer as described by Yoo and collaborators.28 See the text for more information.
†Full change (FC) in the comparative analysis ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Values represent the FC (mean) for each gene corresponding to different patient samples (SAM analysis; R=1.5; false discovery rate (FDR)=0%). In parenthesis, FC of non-significant genes by the statistical threshold used, which were selected owing to their role in specific processes and pathways (see the text for full details). In the case of different probes selected for one gene, values represent the mean of FC for each probe (see online supplementary table S1 for full details).
‡FC in the comparative analysis ran with Affymetrix Gene Chip Human Gene 1.0 ST Array. In parenthesis, FC of non-significant genes by the statistical threshold used. Genes with no change (nc).
§Effect of coenzyme Q10 (CoQ10) supplementation on gene expression in CoQ10 deficiency: unaffected genes by CoQ10 treatment (U); genes non-affected by CoQ10 supplementation (–). See the text and online supplementary table S8 for full details.
¶FC in gene expression analysed by quantitative real time PCR (Q-RT-PCR). See supplementary material and table S11 for primer sequence.
**Effect of CoQ10 supplementation on mRNA levels analysed by Q-RT-PCR. Positive values, increase on gene expression; negative values, decrease on gene expression.

AE binding protein 1, adipocyte enhancer binding protein 1; amyl oncogene, acute myeloid leukaemia 1 oncogene; EGF-containing fibulin-like extracellular matrix protein 1, elongation factor G-containing fibulin-like extracellular matrix protein 1; small GTP-binding protein Rac2 (rho family), small guanosine triphosphate-binding protein Rac2 (rho family); SP110 nuclear body protein, specificity protein-110 nuclear body protein.
Survival transcriptome in coenzyme $Q_{10}$ deficiency syndrome

We classified genes into five groups according to the consequence of CoQ$_{10}$ treatment on gene expression (see online supplementary table S8 for a graphical view). About 54% of probes with altered expression were unaffected by CoQ$_{10}$ supplementation. Only 36% of probes showed partial or complete normalisation of expression and 2% showed inverse regulation (figure 1). Approximately 5% of probes were specifically altered after treatment in both deficient and non-deficient cells and 3% showed small or non-specific changes (these were not considered for further analysis). After statistical analysis, we obtained 70 altered GO with a significant p value ($<0.001$) and an enrichment value that represents the most altered GO within each group (see online supplementary table S9).

Data have been deposited with the NCBI-GEO database, at http://www.ncbi.nlm.nih.gov/geo/, accession number GSE33941 (see online supplementary table S10 for an explanation). The functional description of each gene was updated from the GeneCard of The Human Gene Compendium (Weizmann Institute of Science), http://www.genecards.org/. See supplementary data for a full description of genes, biological process and pathways regulated in CoQ$_{10}$ deficiency.

CoQ$_{10}$-deficient fibroblasts readapt the energetic metabolism and CoQ$_{10}$-treatment restores

In CoQ$_{10}$-deficient fibroblasts, mitochondrial functions, including respiratory chain and tricarboxylic acid (TCA) cycle, were repressed, whereas 9 of 10 steps in glycolysis and pyruvate metabolism were activated, including lactate and pyruvate dehydrogenases (see online supplementary tables S2 and S4). Accordingly, genes involved in the negative regulation of glycolysis were downregulated, whereas those involved in its activation were upregulated (see online supplementary table S2). Furthermore, genes involved in cytosolic NADH oxidation (cytochrome $b_6$ and several oxidoreductases) were slightly repressed (table 2). The expression of genes involved in cholesterol and fatty acid metabolism was downregulated (table 2), as well all the GO related with lipid metabolism (see online supplementary table S5).

CoQ$_{10}$ supplementation normalised the expression (either partially or completely) of genes involved in the glycolytic pathway and activated the expression of repressed respiratory chain genes, whereas the TCA cycle remained unaffected (see online supplementary table S2). Most of the repressed enzymes of lipid metabolism and fatty acid β-oxidation remained downregulated (table 2), whereas several other pathways, such as monocarboxylic acid transport and the insulin response, were normalised (see online supplementary table S9). These results are in agreement with the recovery of aerobic metabolism observed in CoQ$_{10}$-deficient fibroblasts after CoQ$_{10}$ supplementation. 

CoQ$_{10}$ deficiency induces specific adaptations of cells to promote survival

The major novel finding of transcriptome profiling in CoQ$_{10}$-deficient fibroblasts was the altered expression of genes concerned with cell cycle and development and with resistance to stress and cell death (table 2). This suggests both a remodelling of differentiation and growth maintenance and an increase of cell survival mechanisms. Specifically, genes involved in cell cycle activation and maintenance were upregulated, and genes involved in cell cycle regulation increased or decreased their expression depending of their activating or repressing roles. This proliferative response was also enhanced by the repression of cellular attachment factors and by the activation of extracellular matrix proteins that reduce cell attachment and favour cell division. In parallel, GO clusters favouring cell cycle and cell division were activated, and those inhibiting cell growth were repressed (see online supplementary table S4). The differentiation of these cells was compromised because many required factors, transducers, antigens and structural proteins appeared downregulated, whereas repressors of differentiation during development were overexpressed (table 2). See supplementary material for a full description of genes, biological processes and related pathways.

Figure 1 Cluster of genes differentially expressed in coenzyme $Q_{10}$ (CoQ$_{10}$)-deficiency and after CoQ$_{10}$ supplementation. Four arrays of two representative fibroblasts from patients with Q deficiency were plotted with two arrays of control fibroblasts and nine arrays of five patient’s fibroblasts with CoQ$_{10}$ deficiency treated with 30 µM CoQ$_{10}$. Activated genes were coloured in red and repressed ones in green. Between parentheses—group classification of genes after CoQ$_{10}$ supplementation (see online supplementary Table S3).
Cell cycle activation was supported by the upregulation of CDK6 (table 2), a cyclin-dependent kinase that induces entry into the S-phase, and by a robust repression (more than ninefold) of p21/CDKN1A, an inhibitor of cyclin-dependent kinase that blocks cell cycle at the G1/S check point to stimulate cell differentiation. Moreover, subsequent pathways inactivated by p21 were enhanced in CoQ10-deficient cells (see online supplementary table S5), as well as both transcription factors E2F7 and E2F8 (table 2), which push the progression of the cell cycle, activate cell survival and inhibit apoptosis.24

Cell survival in CoQ10-deficient cells was improved by the induction of DNA-repairing mechanisms, and by the establishment of pathways that regulate Jun kinases and activate NAD(P)H-CoQ oxidoreductase, which are involved in stress responses (table 2 and see online supplementary table S4). Components of apoptosis and cell death pathways were systematically repressed (table 2), including tumour suppressor genes, antigens, intracellular mediators and effectors of cell death. Also, cell surface receptors and modulators that inhibit apoptosis were greatly activated.

Interestingly, CoQ10 treatment did not alter the newly acquired resistance to cell death in CoQ10-deficient fibroblasts, kept cell growth activated, and allowed a higher degree of differentiation (tables 2 and see online supplementary table S8). However, genes controlling stress resistance pathways and cortical cytoskeleton were completely restored, as indicated by the shifts in gene expression listed in table 2. However, treated fibroblasts kept the DNA repair mechanism activated.

Signalling-related genes and pathways were differentially affected by CoQ10 deficiency, but most of immunity-related genes showed a general downregulation (table 2). Pathways and biological processes involved in immunity regulation were restored by CoQ10 supplementation (table 2 and see online supplementary table S5).

**Stable DNA methylation profile is responsible for the specific gene expression profile in CoQ10 deficiency**

CoQ10 supplementation modified the expression of 43% of genes that were abnormally expressed in CoQ10-deficient fibroblasts (see online supplementary table S8). In the majority of these cases, expression levels were restored to those of control fibroblasts (20%), but few showed inverse regulation (2%) and others were specifically altered after CoQ10 treatment in both deficient and non-deficient cells (5%). The remaining 16% corresponded to partially restored genes, which slightly alter their expression level without changing the CoQ10-deficient pattern. These genes along with the unaffected (54%) constitute 72% of regulated genes in CoQ10 deficiency, which were not significantly altered after CoQ10 supplementation.

To explain this differential response to respiratory dysfunction, we analysed the DNA-methylation profile of 20 among the most altered genes listed in table 2. These genes encompass the main biological processes and pathways affected by CoQ10 deficiency (table 3). Upregulated genes, which were unaffected by CoQ10 supplementation, had less-defined DNA methylation sites in their promoter regions.

Genes with partial restoration of their expression after CoQ10 supplementation showed precise methylation and demethylation profiles that may explain their altered expression during CoQ10 deficiency. The methylation degree of these genes changed after treatment, and may be responsible for the modulation of expression (table 3). The patterns of methylation of activated and repressed genes in CoQ10 deficiency that could be normalised by CoQ10 supplementation, were either unaffected or only slightly affected by the treatment, and we did not detect new methylation sites after CoQ10 supplementation.

However, a few genes showed significant differences in the methylation degree after the treatment, which correspond to the partially restored genes that maintain the specific expression pattern of untreated CoQ10 deficient cells at a lower level.

Finally, reviewing the biological processes and molecular functions of regulated genes in CoQ10 deficiency, the main adaptation for cell survival activated genes by DNA demethylation, which increased the expression of genes involved in cell cycle activation, apoptosis inhibition, and cell stress resistance, meanwhile the undifferentiated state could be owing to gene repression by DNA methylation, which decreased the expression of genes involved in cell differentiation. CoQ10 treatment did not alter the methylation degree of these genes and subsequently the expression level was maintained.

**DISCUSSION**

**CoQ10-deficient fibroblasts readapt the energetic metabolism and CoQ10-treatment restores**

CoQ10 is an essential component of the mitochondrial respiratory chain,1 therefore dysfunctional mitochondria are a common finding in both primary CoQ10 deficiencies3-7 and secondary forms.8-15 Although each form presents a specific clinical phenotype, all these conditions display a substantial reduction of cellular CoQ10 content and deficit in the mitochondrial enzymatic activities of respiratory chain (table 1). Accordingly to these results, we have shown here that fibroblasts from patients with CoQ10 deficiency have reorganised their genetic resources to cope with this mitochondrial dysfunction. Consistent with the role of CoQ10 in bioenergetics, the lack of CoQ10 would force the cell to support it mainly by glycolysis, whereas both mitochondrial lipid metabolism and respiratory chain were repressed (see online supplementary tables S2 and S4). These findings, together with the mild repression of cytosolic enzymes that oxidise NADH (cytochrome b6 and its oxidoreductases listed in table 2) could indicate that NADH is
Table 3  Epigenetic modifications in CoQ10 deficiency owing to DNA (CpG) methylation/demethylation

| Gene symbol | FC* | Q-effect† | CpGs‡ | Demethylations in CoQ10 deficiency | Methylation in CoQ10 deficiency |
|-------------|-----|-----------|-------|-----------------------------------|---------------------------------|
|             |     |           |       | Degree (C/P)¶ | CpGs’ location** | Degree (C/P)¶ | CpGs’ location** |
| POSTN       | 73.8 U | 2 (16 fold) | 50%/3% | Close together (P) | 0 | – | – |
| GABBR2      | 13.8 U | 5 (40%) | 47%/37% | Close together (P) | 14 (6-fold) | 10%/22% | Close together (P) | –15% |
| VCAN        | 2.8 U | 58 (P,E,I) | 12%/7% | Scattered groups | 3 (90%) | 9%/17% | Dispersed (I) | – |
| TNFRSF10D   | 2.4 U | 59 (P,E,I) | 60%/25% | Scattered groups (P) | 0 | – | – |
| FOXP1       | 2.3 U | 85 (I) | 11 (2-fold) | 57%/32% | Scattered groups | 2 (3-fold) | 7%/19% | Close together | – |
| END1        | –3.0 U | 25 (P) | 0 | – | – | 0 | – | – |
| PARP14      | –3.1 U | 29 (P) | 0 | – | – | 3 (3-fold) | 5%/19% | Dispersed | – |
| CPE         | 11.6 pR | 26 (P,I) | 3 (4-fold) | 20%/6% | Dispersed | 0 | – | – |
| ARL4C       | 4.2 pR | 63 (P,E,I) | 5 (90%) | 52%/28% | Scattered groups | 9 (60%) | 16%/28% | Scattered groups | +7% |
| HOXA11      | –4.3 pR | 17 (P) | 0 | – | – | 8 (4-fold) | 5%/20% | Close together (P) | –3-fold |
| AEBP1       | 66.1 R | 80 (P,E,I) | 8 (25%) | 76%/61% | Scattered groups | 8 (3-fold) | 10%/27% | Widely dispersed | – |
| CYP1B1      | 4.7 R | 24 (P) | 0 | – | – | 0 | – | – |
| CHURC1      | 3.5 R | 20 (P,E,I) | 1 (50%) | 11%/7% | (I) | 0 | – | – |
| PYGL        | –2.5 R | 84 (P,E,I) | 8 (2-fold) | 12%/6% | Close together (E) | 1 (3-fold) | 4%/13% | (P) | – |
| XAF1        | –3.0 R | 25 (P,E,I) | 0 | – | – | 0 | – | – |
| EPSTI1      | –5.9 R | 34 (P,E) | 0 | – | – | 7 (2-fold) | 27%/37% | Scattered groups | – |
| MCAM        | –7.7 R | 74 (P,E,I) | 0 | – | – | 0 | – | – |
| MLPH        | –8.5 R | 8 (P) | 0 | – | – | 0 | – | – |
| PCSK2       | –94.3 O | 32 (P) | 0 | – | – | 0 | – | – |
| GRP         | –263.6 O | 73 (P,E,I) | 20 (two fold) | 53%/35% | Scattered groups | 6 (50%) | 28%/35% | Close together (P) | – |

*Full change (FC) in coenzyme Q_{10} (CoQ_{10}) deficiency (patient samples (SAM) analysis; R=1.5; false discovery rate (FDR)=0%) ran with Affymetrix GeneChip Human Genome U133 Plus 2.0 Array. Full details are shown in table 2.
†Effect of CoQ_{10} supplementation on gene expression in CoQ_{10} deficiency (for more information see online supplementary table S8): unaffected genes by CoQ_{10} treatment (U), genes that restored the expression either partial (pR) or completely (R) and genes with opposite regulation after CoQ_{10} supplementation than in CoQ_{10} deficiency (O).
‡Number of CpG islands analysed. In parenthesis, gene location of CpG islands: promoter (P), first exon (E) and first intron (I).
§Significant methylated CpGs for each gene in control and CoQ_{10}-deficient fibroblast. Significance determined by t test (p<0.01). In parenthesis, fold change in methylation degree (small changes, in %). Non-significant changes in methylation (–). Methylation degree (mean of significant CpGs). Values represent the % of CpG’s methylation of both control (C) and patient deficient in CoQ_{10} (P).
¶Methylation degree (mean of significant CpGs). In parenthesis, folding degree of observed changes.
**Location of significant CpGs. In parenthesis, gene location: promoter (P), first exon (E) and first intron (I).
††Significant changes in CpG methylation owing to CoQ_{10} supplementation in CoQ_{10} deficiency. Positive values, an increase in the methylation degree and negative values, demethylations. Significance determined by t test (p<0.01) between CoQ_{10}-supplemented fibroblasts and untreated CoQ_{10}-deficient fibroblasts. Non-significant changes in methylation (–).
mainly used for biosynthetic purposes rather than for energy production.

Supplementation with CoQ10, the current therapy for all forms of CoQ10 deficiency, restored respiration through enhanced sugar utilisation, but did not stimulate lipid metabolism. The expression of genes involved in the glycolytic pathway was partially or completely normalised after CoQ10 treatment, whereas the repressed genes involved in the respiratory chain were activated. The TCA cycle remained unaffected (see online supplementary table S2). These results are in agreement with the recovery of aerobic metabolism observed in CoQ10-deficient fibroblasts after CoQ10 supplementation.8, 16, 17

CoQ10 deficiency induces a stable survival adaptation of cells

CoQ10-deficient fibroblasts adapted several physiological processes to acquire a cellular-resistance state for survival under the conditions of mitochondrial dysfunction induced by CoQ10 deficiency. The new genetic pattern increases cell survival by activating cell cycle and growth, maintaining an undifferentiated phenotype, upregulating stress-induced proteins and inhibiting apoptosis and cell death pathways. These results recapitulate a survival network that can be observed in nutritional stress such as when cells are grown in galactose-enriched media.25

The survival adaptation shown by CoQ10-deficient cells included a global resistance mechanism that is observed also during the initial phase of tumorigenesis. In fact, the CoQ10-deficient expression profile was very similar to that described during myeloid cell transformation26 and breast tumours.27 Moreover, some of the regulated genes in CoQ10 deficiency (listed in table 2 as italicised letter) are used as biomarkers in several types of cancer,28 like KRT34, the cell cycle-related POSTN, MCAM, EFEMP1 and VCAN, and the apoptotic and cell resistance-related CYP1B1, XAF1 and TNFRSF10D. Although these biomarkers behaved in CoQ10 deficiency, the responsive to CoQ10 deficiency-enhanced DNA demethylation of genes that regulate cell cycle activation, apoptosis inhibition and cell stress resistance as part of an adaptation survival mechanism. Comparable results were observed in several models of epigenetic regulation by demethylation (see online supplementary table S5), whereas DNA methylation inhibits activation of genes related to tumorigenesis and apoptosis.30

Pathways unaffected by CoQ10 treatment corresponded to stably demethylated genes, whereas those that responded to CoQ10 supplementation were controlled by genes with unchanged methylation patterns.

We did not find changes in the methylation degree of all genes affected by CoQ10 deficiency, suggesting that other modalities of gene regulation are responsible, including epigenetic mechanisms such as histone modifications by methylation and acetylation, or even DNA methylation in CpG islands other than those studied here. Interestingly, it has been reported that CoQ10 regulates lipid metabolism in mice liver without any effect on the DNA methylation profile,31 indicating that supplemented CoQ10 by itself may not alter the DNA methylation pattern that cells acquired during the survival adaptation to CoQ10 deficiency.

Mechanisms unaffected by therapy corresponded to stably DNA demethylated genes, which were responsible for the acquisition of the undifferentiated state for survival and resistance that cells obtain during the adaptation to CoQ10 deficiency, whereas the responsive to CoQ10 supplementation were controlled by genes with unchanged methylation patterns and correspond mainly to metabolic genes and those related with the restoration of mitochondrial function.

We propose that these epigenetic changes may be established as early as during the fetal life32 in order to cope with CoQ10 deficiency; these cells then maintain this adaptive response throughout their life. We speculate that cells unable to maintain this survival mechanism during differentiation would die contributing to the pathological phenotype.

A stable DNA methylation profile is responsible of specific gene expression in CoQ10 deficiency

The cellular adaptation to CoQ10 deficiency-enhanced DNA demethylation of genes that regulate cell cycle activation, apoptosis inhibition and cell stress resistance as part of an adaptation survival mechanism. Comparable results were observed in several models of epigenetic regulation by demethylation (see online supplementary table S5), whereas DNA methylation inhibits activation of genes related to tumorigenesis and apoptosis.30

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We propose that these epigenetic changes may be established as early as during the fetal life32 in order to cope with CoQ10 deficiency; these cells then maintain this adaptive response throughout their life. We speculate that cells unable to maintain this survival mechanism during differentiation would die contributing to the pathological phenotype.

Our model has some limits: we treated cells only for 1 week and in principle we cannot rule out that prolonged exposure to CoQ10 could restore also some of the other unaffected pathways. Alternatively, incomplete recovery of the gene expression profiles could be explained by the fact that exogenous CoQ10 can rescue the bioenergetic defect, but not all other functions of CoQ10 in these cells, as it has been observed in other organisms.33

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Survival transcriptome in coenzyme Q_{10} deficiency syndrome

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Survival transcriptome in the coenzyme Q₁₀ deficiency syndrome is acquired by epigenetic modifications: a modelling study for human coenzyme Q₁₀ deficiencies

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