CoQ deficiency causes disruption of mitochondrial sulfide oxidation, a new pathomechanism associated with this syndrome

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Abstract

Coenzyme Q (CoQ) is a key component of the mitochondrial respiratory chain, but it also has several other functions in the cellular metabolism. One of them is to function as an electron carrier in the reaction catalyzed by sulfide:quinone oxidoreductase (SQR), which catalyzes the first reaction in the hydrogen sulfide oxidation pathway. Therefore, SQR may be affected by CoQ deficiency. Using human skin fibroblasts and two mouse models with primary CoQ deficiency, we demonstrate that severe CoQ deficiency causes a reduction in SQR levels and activity, which leads to an alteration of mitochondrial sulfide metabolism. In cerebrum of Coq9R239X mice, the deficit in SQR induces an increase in thiosulfate sulfurtransferase and sulfite oxidase, as well as modifications in the levels of thios. As a result, biosynthetic pathways of glutamate, serotonine, and catecholamines were altered in the cerebrum, and the blood pressure was reduced. Therefore, this study reveals the reduction in SQR activity as one of the pathomechanisms associated with CoQ deficiency syndrome.

Keywords blood pressure; COX; glutathione; mitochondrial disease; SQR

Subject Categories Genetics, Gene Therapy & Genetic Disease; Metabolism

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Introduction

Primary and secondary coenzyme Q (CoQ) deficiencies are clinically and genetically heterogeneous, with muscle, kidneys, and central nervous system being the main affected organs and systems. Because of the function of CoQ as antioxidant and as electron carrier in the mitochondrial respiratory chain, increases in oxidative stress and/or a decline in mitochondrial energy production and pyrimidine biosynthesis have been identified as pathomechanisms of the disease (Quinzii et al, 2008, 2010; Emmanuelli et al, 2012).

Besides the antioxidant and bioenergetics functions, CoQ links the mitochondrial electron transport chain to the TCA cycle by succinate dehydrogenase (EC 1.3.5.1), to β-oxidation by electron-transfer flavoprotein:ubiquinone oxidoreductase (EC 1.5.5.1), to the shuffling of reduction equivalents from the cytoplasm by glycerol-3-phosphate dehydrogenase (EC 1.1.99.5), to the synthesis of pyrimidines by dihydroorotate dehydrogenase (EC 1.3.3.1), to the metabolism of glycine by choline dehydrogenase (EC 1.1.99.1), to arginine and proline metabolism by proline dehydrogenase (EC 1.5.99.8), and to sulfide metabolism by sulfide:quinone oxidoreductase (EC 1.8.99.1; SQR). However, the relative contributions of these pathways to the overall pathophysiology of CoQ deficiency have not been evaluated so far, and only a defect in pyrimidine biosynthesis in cell culture and a decline in the steady-state levels of SQR protein in a proteomic analysis on heart and kidney of Coq9R239X mice have been reported (Lopez-Martín et al, 2007; Lohman et al, 2014).

Sulfide:quinone oxidoreductase catalyzes the first step in the mitochondrial sulfide oxidation pathway. In this reaction, H2S is oxidized...
by SQR, forming a protein-bound persulfide. Two electrons from the oxidation of H₂S are transferred via flavin adenine dinucleotide to CoQ and then to the electron transport chain. The SQR-bound persulfide is transferred to an acceptor such as glutathione (GSH) or sulfite, resulting in the generation of GSH persulfide (GSSH) or thiosulfate, respectively (Fig 1). The persulfide group from GSSH is oxidized to sulfite by a sulfur dioxygenase (EC 1.13.11.18; SDO) (also known as ETHE1 or persulfide dioxygenase). Sulfite can then either be oxidized to sulfate by sulfite oxidase (EC 1.8.3.1; SO) or converted to thiosulfate via addition of a persulfide catalyzed by the thiosulfate sulfurtransferase or rhodanese (EC 2.8.1.1; TST). The sulfane sulfur from thiosulfate can be remobilized by another sulfurtransferase called thiosulfate reductase (EC 2.8.1.3; TR) (Hildebrandt & Grieshaber, 2008; Kabil et al., 2014; Libiad et al., 2014; Di Meo et al., 2015).

How low levels of CoQ affect SQR activity has been studied in Schizosaccharomyces pombe mutant strains Δdps1 (homologue to PDSS1) and Δppt1 (homologue to COQ2). In those mutant strains, high accumulation of sulfide was reported (Uchida et al., 2000; Zhang et al., 2008). In fission yeasts, sulfide is required for the biosynthesis of both methionine and cysteine (Brzywczynski et al., 2002). This latter aminoacid is required to synthesize glutathione, an important antioxidant in mammalian cells. Curiously, CoQ-deficient fission yeasts require cysteine and glutathione to grow on minimal medium (Uchida et al., 2000).

In mammals, hydrogen sulfide is increasingly being recognized as an important signaling molecule in both nervous and cardiovascular systems. Increase in hydrogen sulfide levels induces an increase in the concentration of serotonin and a decrease in norepinephrine, aspartate, GABA, and glutamate (Skrajny et al., 1992; Roth et al., 1995). In the cardiovascular system, hydrogen sulfide induces smooth muscle relaxation and enhances vasodilation (Kabil et al., 2014). Moreover, accumulation of hydrogen sulfide due to mutations in ETHE1 has been associated with cytochrome oxidase (COX) deficiency in ethylmalonic encephalopathy (Tiranti et al., 2009).

Based on those data, CoQ deficiency could induce a decrease in SQR activity with a concomitant increase in hydrogen sulfide levels, a fact that may contribute to the pathophysiology of CoQ deficiency. Thus, in this study, we evaluate mitochondrial hydrogen sulfide metabolism in cell and mouse models of CoQ deficiency due to mutations in different CoQ biosynthetic genes (Appendix Fig S1), with the aim of elucidating the pathophysiological consequences of an alteration in this pathway.

**Results**

**Primary CoQ deficiency causes a decline in SQR activity**

As previously reported, the two mouse models of CoQ deficiency used in this study, Coq9<sup>R239X</sup> and Coq9<sup>Q95X</sup>, have different residual CoQ levels, resulting in different clinical phenotypes (Luna-Sanchez et al., 2015). While Coq9<sup>R239X</sup> mice have 10–15% of residual CoQ levels, Coq9<sup>Q95X</sup> mice exhibit a phenotype characterized by a decline in SQR activity with a concomitant increase in hydrogen sulfide levels, a fact that may contribute to the pathophysiology of CoQ deficiency.

**Figure 1.** Hydrogen sulfide oxidation pathway in mammalian mitochondria.

SQR, sulfide:quinone oxidoreductase; TR, thiosulfate reductase; SDO, sulfur dioxygenase or ETHE1; SO, sulfite oxidase or SUOX; TST, thiosulfate sulfurtransferase or rhodanese.

**H₂S → hydrogen sulfide**

**SO₃²⁻ → sulfite**

**SSO₃²⁻ → thiosulfate**

**GS⁻ → glutathione**

**GSS⁻ → glutathione persulfide**

**SO₄²⁻ → sulfate**

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levels in cerebrum and kidney, Coq9 Q95X mice have 40–50% of residual CoQ in the same tissues. In muscle, however, both mouse models show similar CoQ levels, 10–20% as compared to wild-type animals (Luna-Sánchez et al., 2015). As a result, Coq9 R239X mice develop a fatal mitochondrial encephalopathy, while Coq9 295X mice develop a late-onset mild mitochondrial myopathy (Garcia-Corzo et al., 2013; Luna-Sánchez et al., 2015). Because CoQ acts as an electron acceptor in the reaction catalyzed by SQR in the mitochondrial sulfide oxidation pathway, we first evaluated whether the deficit in CoQ could affect mitochondrial H₂S metabolism in these mouse models. Thus, we first checked the consequences of low CoQ levels on cerebral, renal, and muscular SQR of Coq9 295X mice at 3 months of age. The levels of Sqr mRNA were similar between the three experimental groups (Fig 1A–C), and only a reduction (by 25%) in kidneys of Coq9 Q95X mice was detected (Fig 1B). At the protein level, the changes were more dramatic. The levels of SQR protein were significantly reduced in cerebrum, kidneys, and muscle of Coq9 R239X mice, while Coq9 295X mice only showed reductions in the levels of SQR in muscle (Fig 1D and F). In parallel to the reduction in SQR protein levels, the activity of SQR was significantly reduced in kidneys and muscle of Coq9 R239X and Coq9 295X mice (Fig 1G and H). While in kidneys, SQR activity in Coq9 295X mice was lower than Coq9 Q95X mice (Fig 1E), SQR activity was similar in muscle of both mutant mouse strains (Fig 1F). In the cerebrum, the SQR protein was only detected in isolated mitochondria (Fig 1D) and no band was detected in tissue homogenate. This reflects the low abundance of SQR in brain (Geiger et al., 2013) (http://pax-db.org/protein/2093754/Sqrdl). Accordingly, we were not able to measure SQR activity in cerebrum.

Based on the results obtained in Coq9 mutant mice, where SQR levels and function were decreased in correlation with the residual CoQ levels, we next evaluated if this alteration was also present in levels and function were decreased in correlation with the residual CoQ levels and activity of TST, which also takes part in this pathway, in the tissues of mutant mice. An increase in TST levels was detected in cerebrum of Coq9 R239X mice (Fig 4A), while in kidneys and muscle, the differences were not statistically significant (Fig 4B and C). Nevertheless, TST activity was significantly increased in both cerebrum and kidneys of Coq9 R239X mice, while TST activity in the same tissues of Coq9 295X mice was similar to the activity in control mice (Fig 4D and E). In muscle, where the activity of TST is lower than in other two tissues, the TST activity was not altered in both mutant mouse strains (Fig 4F). Coq9 +/+ , Coq9 R239X , and Coq9 295X mice showed similarities in the levels of ETHE1 (SDO) in cerebrum, kidneys, and muscle (Fig 4G–I). The levels of SUOX (SO) were significantly higher in cerebrum of Coq9 R239X mice compared to those levels in Coq9 +/+ and Coq9 Q95X mice (Fig 4J). In kidneys and muscle, however, the levels of SUOX were similar in the three experimental groups (Fig 4K and L).

**Disruption of the mitochondrial sulfide oxidation pathway induces changes in thios levels and metabolic disturbances in the cerebrum of Coq9 R239X mice**

Because an alteration in the metabolism of hydrogen sulfide may affect the levels of thiols, we then measured the levels of sulfides, thiosulfate, sulfate, and glutathione in mouse tissues. The levels of sulfides in the cerebrum of mutant mice were similar to those levels in control mice (Fig 5A and B). In kidneys, however, the levels of sulfides in Coq9 R239X mice were higher than in Coq9 +/+ and Coq9 Q95X mice (Fig 5A and B). The levels of thiosulfate and sulfite were below the detection limit (5 μM) in all tested tissues.

The major non-protein thiol in cells is GSH, which is synthesized in the cytosol and imported into mitochondria and into other organelles, where it plays an essential role in the antioxidant defense against reactive oxygen species (ROS) (Ribas et al., 2014). The levels of total GSH in the cytosol of cerebrum, muscle, and kidney were significantly decreased in Coq9 R239X mice (Fig 6A and Appendix Fig S4A and B). However, the total GSH levels in mitochondria were normal in the three tissues of Coq9 R239X mice (Fig 6A and Appendix Fig S2A and B). The activities of the GSH-utilizing enzymes, GPx and GRd, were significantly decreased in the cytosolic fraction of cerebrum of Coq9 R239X mice compared to wild-type animals (Fig 6B), while in muscle and kidney, the differences were statistically significant for the renal GPx (Appendix Fig S2C and D). The decrease in GPx and GRd activities in cerebrum of Coq9 R239X was due to a decline in the levels of GPx and GRd (Fig 6C and D). These decreases in the glutathione enzymes did not modify the GSSG/GSH ratios in cytosol and mitochondria (Appendix Fig S3). Therefore, our results show a global depletion in the glutathione system in cerebrum of Coq9 R239X, a mouse model of mitochondrial encephalopathy with severe histopathological signs of spongiform degeneration and reactive astroglisis in the cerebrum.

The GSH depletion in the cerebrum may be due to a decrease in the levels of glutamate, one of the three amino acids components of GSH, with the parallel increase in N-acetylglutamate (Fig 6E). In addition, we found an increase in metabolites (L-tryptophan, 5-HIAA, and N-acetyltryptophan) of serotonin biosynthesis (Fig 6E) and a decrease in L-tyrosine (Fig 6E), which is essential in the biosynthesis of dopamine, norepinephrine, and epinephrine, in the cerebrum of Coq9 R239X mice.

To know whether the SQR deficiency is generally responsible for the depletion in the glutathione system, we measured GSH after Sqr silencing in Hepa1c1c7 cells. The silencing induced a significant decrease in the levels of Sqr mRNA (Appendix Fig S4A) and SQR
However, the total GSH did not change in Hepa1c1c7 cells after Sqr silencing (Appendix Fig S4C). In the mutant skin fibroblast with primary CoQ deficiency, where the SQR deficiency is chronic but less severe than in mice tissues or after Sqr silencing, the GSH levels were also similar to those levels in control fibroblasts (Appendix Fig S4D).

Figure 2. SQR levels and activity depend on CoQ levels in mice tissues.

A–H Sqr mRNA levels (A–C), SQR protein levels (D–F), and SQR activity (G, H) in cerebrum (A, D), kidneys (B, E, G), and muscle (C, F, H) of Coq9+/+, Coq9R239X, and Coq9Q95X mice. Note that SQR Western blots were performed in isolated cerebral mitochondria due to the low levels of this protein in cerebrum. In kidneys and muscle, the Western blots were performed in tissue homogenates. Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001; Coq9R239X and Coq9Q95X mice versus Coq9+/+ mice (one-way ANOVA with a Tukey’s post hoc test; n = 5–9 for each group).

Source data are available online for this figure.
H₂S supplementation in wild-type animals induces changes in neurotransmitters levels but does not alter mitochondrial sulfide oxidation pathway

The changes observed in the levels of the proteins involved in mitochondrial sulfide oxidation pathway, as well as in the levels of neurotransmitters in cerebrum of Coq₉R²³⁹X mice may be attributed to the increase in hydrogen sulfide. Thus, we treated control fibroblast and wild-type mice with the H₂S donor GYY4137. After the treatment, the levels of SQR (Fig 7A) and TST (Fig 7B) did not change in control fibroblasts. Also, TST levels were similar in kidneys (Fig 7C) and cerebrum (Fig 7D) after the treatment compared with the levels in untreated animals. Nevertheless, GYY4137 induced a decrease in the levels of L-Glu and DA and an increase in the levels of 5-HIAA in cerebrum of Coq₉⁺/⁺ mice (Fig 7E), a similar tendency observed in the cerebrum of Coq₉R²³⁹X mice (Fig 6E).

Pathophysiological consequences of reductions in CoQ levels and SQR activity

It has been proposed that hydrogen sulfide metabolism influences COX activity and regulates blood pressure (Kabil et al., 2014). COX activity was, however, normal in cerebrum, kidneys, and muscle of Coq₉R²³⁹X and Coq₉G₀⁹₅X mice (Fig 8A). COX stain did not show any signs of COX deficiency in the gastrocnemius muscle of Coq₉R²³⁹X and Coq₉G₀⁹₅X mice (Fig 8B).

The measurement of blood pressure in Coq₉R²³⁹X mice revealed a decrease in systolic and diastolic blood pressure...
Figure 4. Changes in the proteins involved in the mitochondrial sulfide oxidation pathway in response to SQR deficiency in Coq9R239X mice.

A–C TST protein levels in cerebrum (A), kidneys (B), and muscle (C) of Coq9+/+, Coq9R239X, and Coq9Q95X mice.

D–F TST activity in cerebrum (D), kidneys (E), and muscle (F) of Coq9+/+, Coq9R239X, and Coq9Q95X mice.

G–I ETEH1 (SDO) protein levels in cerebrum (G), kidneys (H), and muscle (I) of Coq9+/+, Coq9R239X, and Coq9Q95X mice.

J–L SUOX protein levels in cerebrum (J), kidneys (K), and muscle (L) of Coq9+/+, Coq9R239X, and Coq9Q95X mice.

Data information: Images in panels (C, F, and I) were obtained from the same membrane after stripping and re-blotting. Data are expressed as mean ± SD. *P < 0.05; **P < 0.01, ***P < 0.001, Coq9R239X and Coq9Q95X mice versus Coq9+/+ mice. #P < 0.05, ##P < 0.01, ###P < 0.001, Coq9R239X versus Coq9Q95X mice (one-way ANOVA with a Tukey’s post hoc test; n = 5–9 for each group).

Source data are available online for this figure.
as compared to age-matched wild-type animals (Fig 9A). These changes were not related to the heart rate because the beats per minute were similar in Coq9R239X and Coq9+/+ mice (Fig 9B).

**Discussion**

Sulfide:quinone oxidoreductase is a mitochondrial enzyme that requires CoQ as acceptor of electrons, thereby feeding electrons
Figure 6. Glutathione system and neurotransmitters biosynthesis are compromised in cerebrum of Coq9<sup>R239X</sup> mice.

A  Total GSH in cytosol and mitochondria of cerebrum of Coq9<sup>+/+</sup> and Coq9<sup>R239X</sup> mice.
B  Cytosolic GPx and GRd activities in cerebrum of Coq9<sup>+/+</sup> and Coq9<sup>R239X</sup> mice.
C, D Levels of GPx4 (C) and GRd (D) protein in cerebral homogenate of Coq9<sup>+/+</sup> and Coq9<sup>R239X</sup> mice.
E  Levels of L-glutamate (L-Glu), N-acetylglutamate (NacGlu), L-tryptophan (L-Trp), 5-HIAA, N-acetyltryptophan (NALT), L-tyrosine (L-Tyr) in cerebrum of Coq9<sup>+/+</sup> and Coq9<sup>R239X</sup> mice.
F  Biosynthetic pathway of GSH, serotonin, and catecholamine.

Data information: Data are expressed as mean ± SD. *P < 0.05; **P < 0.01; ***P < 0.001. Coq9<sup>R239X</sup> mice versus Coq9<sup>+/+</sup> mice (t-test; n = 5 for each group).

Source data are available online for this figure.
Figure 7. Supplementation with an H$_2$S donor in wild-type animals induces changes in neurotransmitters levels.

A, B SQR (A) and TST (B) protein levels in human skin fibroblasts supplemented with the H$_2$S donor GYY4137.

C, D TST protein level in kidneys (C) and cerebrum (D) of Coq9+/+ mice supplemented with the H$_2$S donor GYY4137.

E Levels of neurotransmitters in cerebrum of Coq9+/+ mice supplemented with the H$_2$S donor GYY4137.

Data information: Images in panels (A and B) were obtained from the same membrane after stripping and re-blotting. Data are expressed as mean ± SD. **$P < 0.01$; ***$P < 0.001$; Coq9+/+ mice supplemented with GYY4137 versus Coq9+/+ mice (t-test; $n = 4–6$ for each group).

Source data are available online for this figure.
into the mitochondrial electron transport chain (Hildebrandt & Grieshaber, 2008; Modis et al., 2013). Low levels of CoQ may thus affect the activity of SQR and its downstream reactions. Here, we demonstrate that severe CoQ deficiency causes a dramatic reduction in SQR levels and activity, which lead to an alteration of the mitochondrial sulfide metabolism. This pattern was observed in a mouse model of primary CoQ deficiency as well as in skin fibroblasts of patients with primary CoQ deficiency due to mutations in different CoQ biosynthetic genes. The deficit in SQR induces changes in the mitochondrial sulfide oxidation pathway with modifications in the levels of thiols. As a result, biosynthetic pathways of some neurotransmitters were altered in the cerebrum and the blood pressure was reduced. Therefore, this study reveals the reduction in SQR activity as one of the pathomechanisms associated with the CoQ deficiency syndrome.

Low levels of CoQ induce a disruption in the mitochondrial hydrogen sulfide oxidation pathway

In this work, we first investigated the tissue levels of Sqr mRNA and SQR protein, as well as the SQR activity, in two mouse models of
primary CoQ deficiency: the Coq9R239X mouse model with fatal mitochondrial encephalopathy and the Coq9Q95X mouse model with late-onset mild mitochondrial myopathy (Garcia-Corzo et al., 2013; Luna-Sanchez et al., 2015). Our results confirm that the abundance of the SQR protein is high in kidney, medium-low in muscle, and very low in cerebrum (Geiger et al., 2013; http://pax-db.org/protein/2093754/Sqrdl). However, the pattern of response to CoQ deficiency is similar in the three tissues; that is, SQR protein levels and SQR activity in cerebrum, kidneys, and muscle show a correlation between the severity of CoQ deficiency and the decrease in SQR protein levels and activity. Moreover, the deficit on SQR is not limited to mutations in the Coq9 gene because similar results were obtained in human skin fibroblasts with mutations in different CoQ biosynthetic genes, that is, PDSS2, COQ2, COQ4, and COQ9. In the mutant fibroblasts, CoQ10 depletion causes a significant impairment of SQR-driven respiration, compared with control fibroblasts (Ziosi et al., 2017). These results also point out that the deficit in SQR occurs in human primary CoQ10 deficiency. Moreover, exogenous supplementation with CoQ10 both in vitro and in vivo, in human and mouse, increased the SQR levels in the mutant cells and mice. The increase in SQR protein levels correlate with the increase in SQR-driven respiration in presence of 5 μM of CoQ10 (Ziosi et al., 2017). This confirms that the levels of SQR depend on the levels of CoQ and that exogenous CoQ10 supplementation may normalize SQR levels and activity in patients with primary CoQ10 deficiency.

As a consequence of the reduced SQR levels, TST activity was increased in cerebrum and kidneys. Interestingly, the cerebral TST activity in Coq9R239X mice was increased at a higher magnitude (2.3-fold increase) than the renal TST (1.3-fold increase), compared in both cases with TST activity in control animals. However, control cells have normal SQR activity that can metabolize hydrogen sulfide. Therefore, we cannot discard that such increases in TST enzyme in Coq9R239X mice may be a response to the high levels of hydrogen sulfide, which probably lead to an increase in protein sulfhydration (Mustafa et al., 2009; Gao et al., 2015). As a consequence, the function of proteins that can be regulated by this posttranslational modification would be affected, and the expression of enzymes potentially involved in the removal of persulfide groups, such as sulfurtransferases, might be induced. Similarly, the levels of SO were only increased in cerebrum of Coq9R239X compared to wild-type animals. Together, these data point out that the disturbances in the mitochondrial hydrogen sulfide oxidation pathway are more accentuated in the cerebrum of the encephalopathic Coq9R239X mouse model.
Cerebrum of Cogq<sup>R239X</sup> mice shows depletion in the glutathione system: a possible connection with the disruption in the mitochondrial hydrogen sulfide oxidation pathway

Thiosulfate sulfurtransferase uses GSH and GSH metabolites in its reaction. Therefore, changes in TST activity may induce alterations in GSH metabolism. Moreover, GSH requires cysteine in its biosynthesis, and this amino acid needs hydrogen sulfide for its biosynthesis in fission yeasts (Bryznychy et al., 2002). In these organisms, the addition of cysteine reduces the hydrogen sulfide production with CoQ deficiency, suggesting that hydrogen sulfide and cysteine biosynthetic pathways are coordinately regulated by feedback mechanisms (Zhang et al., 2008). While the sources of cysteine are apparently different in mammals, the therapeutic effects of NAC, a prodrug of L-cysteine utilized to increase GSH, in ETHE1 knockout mice suggest that mitochondrial sulfide oxidation pathway may exert some influence on GSH metabolism (Viscomi et al., 2010). In fact, sulfur amino acid restriction increased expression of the enzyme cystathionine γ-lyase in mouse liver, resulting in increased hydrogen sulfide production and decreased levels of GSH (Hine et al., 2015). Our results showing depletion in the glutathione system in cerebrum would validate this premise, confirming one of the cytotoxic effects of hydrogen sulfide (Truong et al., 2006). However, when we checked the direct relation between SQR and GSH systems by transient silencing of SQR mRNA in Hepa cells, we did not obtain a positive correlation. To test a long-term, but moderate, deficiency in SQR, we measured GSH levels in human skin fibroblasts with primary CoQ deficiency. However, also GSH levels were normal in those cases. Thus, the GSH depletion in the cerebrum, kidney, and muscle might be unrelated to the SQR deficiency or, if there is any relation, this one should be linked with a long-term severe SQR deficiency with increased TST activity because the latter enzyme might play a key role in GSH metabolism (Remelli et al., 2012). Alternatively, the depletion in the glutathione levels may be related to a reduction in its precursors, for example, cerebral levels of L-glutamate, an essential aminoacid for GSH biosynthesis, were decreased in Cogq<sup>R239X</sup> mice. Moreover, it is important to note that the depletion in GSH levels ran in parallel to a reduction in the levels and activity of the GSH-utilizing enzymes GPx4 and GRd and these changes may be critical for the increase in oxidative damage, neural death, and astrogliosis observed in the cerebrum of Cogq<sup>R239X</sup> mice (Seiler et al., 2008; Yoo et al., 2012; Garcia-Corzo et al., 2013).

Pathophysiological consequences of SQR deficiency

The changes in neurotransmitters biosynthesis in the cerebrum of Cogq<sup>R239X</sup> mice are not limited to the reduction in L-glutamate but also to the biosynthesis of serotonin and catecholamines. These alterations might be a consequence of the disruption in hydrogen sulfide metabolism because a dose-dependent and time-dependent decrease in glutamate levels and increase in serotonin levels have been described in the cerebrum and frontal cortex of rats chronically exposed to 20 ppm and 75 ppm of hydrogen sulfide (Skrajny et al., 1992; Roth et al., 1995). The same authors reported a decrease in the levels of norepinephrine with the exposure to 20 ppm of hydrogen sulfide and an increase in those levels with the exposure to 75 ppm of hydrogen sulfide (Skrajny et al., 1992). Norepinephrine and epinephrine levels were, however, decreased in isolated porcine iris-ciliary body exposed to increased concentrations of NaSH, which is a commonly used hydrogen sulfide donor (Kulkarni et al., 2009). The changes in the levels of L-glutamate, 5-HIAA, and dopamine observed in wild-type animals after 2 weeks of supplementation with the H<sub>2</sub>S donor GYY4173 confirm the influence of hydrogen sulfide in the levels of some neurotransmitters. Thus, the changes in the biosynthesis of amino acid neurotransmitters may contribute to the encephalopathy reported in patients and mice with fatal Cogq mutations (Duncan et al., 2009; Garcia-Corzo et al., 2013; Luna-Sánchez et al., 2015; Danhauser et al., 2016) and add new evidences about the potential role of hydrogen sulfide as endogenous neuromodulator (Eto et al., 2002a,b). Curiously, multiple system atrophy, a neurodegenerative disorder related to disruption in catecholamines metabolism, neuronal death, and astrogliosis, and clinically manifested with problems in movement and autonomic functions of the body such as blood pressure regulation, has been recently associated with mutations in COQ2 (Mutiple-System Atrophy Research Collaboration, 2013), a gene involved in CoQ biosynthesis (Ashby et al., 1992; Uchida et al., 2000; Quinzii et al., 2006).

In the cardiovascular system, hydrogen sulfide regulates smooth muscle contractility (Kabil et al., 2014). Generally, hydrogen sulfide induces smooth muscle relaxation, but under particular conditions, it may induces vasoconstriction (Hosoki et al., 1997; Kabil et al., 2014). Mice in which the Cse gene is disrupted exhibit hypertension in comparison with wild-type animals (Yang et al., 2008), although in a second and independent study, Cse KO mice were reported to be normotensive (Ishii et al., 2010). Our results showing a decrease in blood pressure in Cogq<sup>R239X</sup> mice would validate the role of hydrogen sulfide in smooth muscle relaxation. However, we cannot exclude that the decrease in blood pressure could also be the result of the severe dysfunction in the brainstem (Garcia-Corzo et al., 2013), where the cardiac and vasomotor centers are localized to regulate the autonomic functions of heart rate and blood pressure. Nevertheless, in contrast to the Ndufs4 KO mice, another mouse model of Leigh syndrome in which a low heart rate was associated with the brainstem pathology (Quintana et al., 2012), the heart rate was normal in Cogq<sup>R239X</sup> mice.

Finally, it has been also reported that hydrogen sulfide and/or its metabolites can interfere with COX activity (Kabil et al., 2014; Szabo et al., 2014). Our results did not show a decrease in COX activity in the mutant mice, as it has been reported in ethylmalonic encephalopathy due to mutations in ETHE1 (Tiranti et al., 2009). However, the increase in hydrogen sulfide in ETHE1 mice was higher than in Cogq<sup>R239X</sup> mice, compared in both cases with hydrogen sulfide levels in wild-type animals. Moreover, ETHE1 mice accumulate thiocysteine, while Cogq<sup>R239X</sup> mice do not (Tiranti et al., 2009; Di Meo et al., 2011). Therefore, hydrogen sulfide and/or thiosulfate must reach critical levels in order to produce toxic effects on COX activity.

Conclusions

Our study demonstrates that primary CoQ deficiency is associated with a disruption of the mitochondrial hydrogen sulfide oxidation pathway, which may be a new pathomechanism associated with this syndrome and may contribute to explain its clinical heterogeneity. According to that, primary CoQ deficiency may be considered the first disease associated with a defect in SQR and, together with
ETHE1 and SO deficiencies (Mudd et al., 1967; Garrett et al., 1998; Mineri et al., 2008; Tiranti et al., 2009), the third disease occurring with a defect in the mitochondrial hydrogen sulfide oxidation pathway. Moreover, this new pathomechanism should be taken into consideration for the treatment of primary CoQ deficiency and the evaluation of new experimental therapies.

**Materials and Methods**

**Mouse models and treatments**

The Coq9R239X and Coq9G052X mouse models were previously generated and characterized under mix of C57BL/6J and C57BL/6J genetic backgrounds (Garcia-Corzo et al., 2013; Luna-Sánchez et al., 2015). Coq9R239X/+ mice were crossed in order to generate Coq9+/−, Coq9R239X/+, and Coq9R239X/R239X (referred in the article to as Coq9R239X). Coq9G052X/+ mice were crossed in order to generate Coq9+/−, Coq9G052X/+, and Coq9G052X/R239X (referred in the article to as Coq9G052X). Only homozygous wild-type and mutant mice between 3 and 5 months of age were used in the study. Coq9R239X mice were treated with ubiquinol-10 (Q10H2) in the drinking water in a dose of 240 mg/kg bw/day during 2 months, as previously reported (Garcia-Corzo et al., 2014). Coq9+/− mice were treated with the H2S donor GYY4137 in the drinking water in a dose of 50 mg/kg bw/day during 2 weeks (Hine et al., 2015). Animals were genotyped and randomly assigned in experimental groups. Equal number of males and females were assigned in each experimental group, and no sex differences were observed in the results. A total number of 152 mice were used in this study, excluding breeders and heterozygous mice obtained in each experimental group. Equal number of males and females were assigned in each experimental group, and no sex differences were observed in the results. A total number of 152 mice were used in this study, excluding breeders and heterozygous mice obtained in each experimental group.

Mice were housed in the Animal Facility of the University of Granada under an SPF zone with lights on at 7:00 AM and off at 7:00 PM. Mice had unlimited access to water and rodent chow. All experiments were performed according to a protocol approved by the Institutional Animal Care and Use Committee of the University of Granada (procedures 92-CEEA-OH-2015) and were in accordance with the European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes (CETS # 123), the directive 2010/63/EU on the protection of animals used for scientific purposes and the Spanish law (R.D. 53/2013).

**Cell culture and treatments**

Primary mutant and control fibroblasts were grown in high glucose DMEM-GlutaMAX medium supplemented with 10% FBS, 1% MEM non-essential amino acids, and 1% antibiotics/antimycotic at 37°C and 5% CO2. Control and mutant fibroblasts were treated with 5 μM CoQ10 during 1 or 7 days, as previously reported (Lopez et al., 2010). Control fibroblasts were treated with different concentrations of the H2S donor GYY4137 for 5 days, as published elsewhere (Lee et al., 2011).

The Hepa1c1c7 murine hepatoma cell line was obtained from cell bank of the University of Granada and maintained in MEMx GlutaMAX with 10% FBS and 1% antibiotics/antimycotics at 37°C and 5% CO2.

**Subcellular fractionation**

Mitochondrial isolation was performed as previously described (Fernandez-Vizarra et al., 2002). Tissues were homogenized in a glass–Teflon homogenizer. Kidney was homogenized (1:4, w/v) in the homogenization medium A (0.32 M sucrose, 1 mM EDTA, 10 mM Tris–HCl, pH 7.4); cerebrum was homogenized (1:5, w/v) in the homogenization medium A plus 0.2% free fatty acids BSA, and skeletal muscle was homogenized (1:20, w/v) with Ultratexure in homogenization medium C (0.12 M KCl, 0.02 M HEPEs, 2 mM MgCl2, 1 mM EGTA, 5 mg/ml free fatty acids BSA). Kidney and cerebrum homogenates were centrifuged at 1,000 × g for 5 min at 4°C to remove nuclei and debris. Cytosol was collected from supernatants after centrifuging at 14,400 × g for 2 min at 4°C and stored at −80°C after the addition of Halt™ protease and phosphatase inhibitor cocktail (ThermoFisher). The mitochondrial pellet was washed and suspended in homogenization medium and centrifuged again for 14,400 × g for 2 min at 4°C. The final crude mitochondrial pellet was stored at −80°C. Skeletal muscle homogenate was centrifuged at 600 × g for 10 min at 4°C. The supernatant (s1) was kept on ice, and the pellet was re-suspended in 8 volumes of buffer A and centrifuged at 600 × g for 10 min at 4°C. The subsequent supernatant (s2) was combined with s1 and centrifuged at 17,000 × g for 10 min at 4°C. The cytosolic supernatant obtained was stored at −80°C after the addition of Halt™ protease and phosphatase inhibitor cocktail (ThermoFisher), and the pellet obtained was re-suspended in 10 volumes of medium A and centrifuged at 7,000 × g for 10 min at 4°C. The pellet was re-suspended in 1 volume of medium B (0.3 M sucrose, 2 mM HEPEs, 0.1 mM EGTA) and centrifuged at 3,000 × g for 10 min at 4°C.

**Quantification of CoQ10 levels in human skin fibroblasts**

After lipid extraction from homogenized cultured skin fibroblasts, CoQ10 was determined via reversed-phase HPLC coupled to electrochemical (EC) detection (Lopez et al., 2010). The results were expressed in ng CoQ10/tg protein.

**Gene expression analyses**

Total cellular RNA from frozen tissue was extracted and electrophoresed in a 1.5% agarose gel to check the RNA integrity. RNA from muscle and cerebrum samples was extracted with RNeasy Fibrous Tissue Midi kit (for muscle) and RNeasy Lipid Tissue Mini kit (for cerebrum) (Qiagen, Hilden, Germany) and treated with RNase-Free DNase (Qiagen). RNA from kidney samples was extracted with Real Total RNA Spin Plus Kit (Real). Total RNA was quantified by optical density at 260/280 nm and was used to generate cDNA with High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Amplification was performed with quantitative real-time PCR, by standard curve method, with specific Taqman probes (from Applied Biosystems) for the targeted gene mouse Sqrdl (Mm00502443_m1) and the mouse Hprr probe as a standard loading control (Mm01545399_m1) (Luna-Sanchez et al., 2015).

**Sample preparation and Western blot analysis in cells**

For Western blot analyses in cerebrum, kidney, and muscle, samples were homogenized in T-PER® buffer (Thermo Scientific)
with protease inhibitor cocktail (Pierce) at 1,100 rpm in a glass–Teflon homogenizer. Homogenates were sonicated and centrifuged at 1,000 × g for 5 min at 4°C, and the resultant supernatants were used for Western blot analysis. For Western blot analyses in cerebral mitochondria, the pellets containing the mitochondrial fraction were re-suspended in RIPA buffer with protease inhibitor cocktail. For Western blot analyses in skin fibroblasts, cells were collected, washed twice with 1× PBS, and homogenized in RIPA buffer with protease inhibitor cocktail. Homogenates were centrifuged at 14,000 × g for 15 min at 4°C, and the resultant supernatants were used for Western blot analysis. 60 μg of protein from the sample extracts was electrophoresed in 12% Mini-PROTEAN TGX™ precast gels (Bio-Rad) using the electrophoresis system mini-PROTEAN Tetra Cell (Bio-Rad). Proteins were transferred onto PVDF 0.45-μm membranes using a mini Trans-blot Cell (Bio-Rad) or Trans-blot Cell (Bio-Rad) and probed with target antibodies. Protein–antibody interactions were detected with peroxidase-conjugated horse anti-mouse, anti-rabbit, or anti-goat IgG antibodies using Amersham ECL™ Prime Western Blotting Detection Reagent (GE Healthcare, Buckinghamshire, UK). Band quantification was carried out using an Image Station 2000R (Kodak, Spain) and a Kodak 1D 3.6 software. Protein band intensity was normalized to VDAC1 (mitochondrial proteins) or GAPDH, and the data expressed in terms of percent relative to wild-type mice or control cells (Luna-Sanchez et al, 2015).

The following primary antibodies were used: anti-SQRDL (Proteintech, 17256-1-AP), anti-TST (Proteintech, 16311-1-AP), Anti-SUOX (Proteintech, 15075-1-AP), anti-ETHE1 (Sigma, HPA029029), anti-GPx4 (Abcam, ab125066), anti-GRd (Santa Cruz Biotechnology, sc-32886), anti-VDAC1 (Abcam, ab14734), and anti-GAPDH (Santa Cruz Biotechnology, sc-166574).

**Histochemical analysis of COX activity**

Muscle samples (gastrocnemius) were freed from excess connective tissue, embedded in OCT compound (Tissue-Tek), and oriented so that fibers could be cut transversely. Samples then were snap-frozen in precooled isopentane in liquid nitrogen. 8-μm-thick cryosections were placed on Superfrost microscope slides at −20°C by using a Leica CM1510S Cryostat and stained for detection of COX activity as described previously (Tanji & Bonilla, 2008; Luna-Sanchez et al, 2015). The sections were examined, and digital images were acquired using a Carl Zeiss Primo Star Optic microscope and a Magnifier AxioCam Icc3 digital camera.

**Enzymatic activities**

Sulfide:quinone oxidoreductase activity was determined in isolated mitochondria by following the enzymatic reduction rate of decylubiquinone at 275 nm upon sulfide addition (100 μM) (Hildebrandt & Grieshaber, 2008; Theissen & Martin, 2008).

Thiosulfate sulfurtransferase activity was determined in isolated mitochondria by measuring the formation of thiocyanate from cyanide and thiosulfate (Sorbo, 1955; Hildebrandt & Grieshaber, 2008).

Cytochrome oxidase activity was measured in isolated mitochondria following the reduction in cytochrome C (cyt C) at 550 nm (DiMauro et al, 1987).

Glutathione peroxidase (GPx) and reductase (GRd) activities were measured spectrophotometrically from cytosolic fractions following the NADPH oxidation for 3 min at 340 nm on a Shimadzu UV spectrophotometer (UV-1700; Duisburg, Germany) (Griffith, 1999; Lopez et al, 2006). The enzyme activities were expressed as nmol/min/mg protein. In both cases, non-enzymatic NADPH oxidation was subtracted from the overall rates.

**Measurement of sulfides, thiosulfate, and sulfite**

The concentrations of sulfite and thiosulfate were measured using the monobromobimane HPLC method (Hildebrandt & Grieshaber, 2008). Additionally, hydrogen sulfide release was measured in approximately 100 mg fresh tissue homogenate in passive lysis buffer (Promega) supplemented with 10 mM Cys and 8 mM PLP. A lead acetate hydrogen sulfide detection paper (Sigma) was placed above the liquid phase in a closed Eppendorf tube and incubated for 5 h at 37°C until lead sulfide darkening of the paper occurred (Hine et al, 2015).

**GSH measurement**

Glutathione measurements were performed in cytosol and mitochondrial fractions of mouse tissues, as well as in Hepa1c1c7 cells and human skin fibroblasts, which were cultured in Opti-MEM medium lacking FBS for 72 h in order to avoid influence of the GSH contained in the FBS.

Glutathione was measured by an established fluorometric method (Hissin & Hilf, 1976). Mitochondrial pellets were resuspended in sodium phosphate buffer (A) (100 mM sodium phosphate, 5 mM EDTA-Na2, pH 8.0). Mitochondrial and cytosolic fractions were deproteinized with ice-cold TCA 40% and centrifuged at 20,000 × g for 15 min. For GSH measurement, the supernatant was incubated with a solution of (A) and orthophthalaldehyde/ethanol (B) (1 mg/ml) for 15 min at room temperature. The fluorescence of the samples was then measured at 340 nm excitation and 420 nm emission wavelengths in a spectrofluorometer plate reader (Bio-Tek Instruments Inc., Winooski, VT, USA). For GSSG measurement, the supernatant was preincubated with N-ethylmaleimide solution (5 mg/ml) for 40 min and then alkalized with 0.1 N NaOH (C). Aliquots of these mixtures were then incubated with (B) and (C) for 15 min at room temperature. The fluorescence was then measured as before. GSH and GSSG concentrations were calculated according to standard curves prepared, and the levels of GSH and GSSG are expressed in nmol/mg protein.

**Metabolite quantification in the cerebrum**

To quantify the metabolites in the cerebrum of Coq9+/+ and Coq9R239X mice, frozen samples (−80°C) were lyophilized (Virtis-Benchtop K, Fisher Scientific, Spain) previous to sample homogenization and metabolite extraction. The extraction/homogenization method was adapted from Romisch-Margl et al (2012). Briefly, metabolites were extracted from liver and brain samples by adding 300 μl of a mixture of precooled methanol/water (8:2, v/v) to ~50 mg of lyophilized sample and extracted/homogenized in 1.5-ml microcentrifugation tubes with 1.4-mm stainless steel beads using a bead-beating homogenizer (Bullet blender blue, Next Advance,
USA) equipped with an integrated cooler unit. The tissues were homogenized three times for 30 s at 6,000 rpm. Afterward, the tubes were bath-sonicated for 1 min, incubated at 4 °C for 60 min, and centrifuged at 5,000 × g for 15 min at 4°C. Supernatants were analyzed by LC-(ESI)qTOF.

LC-HRMS analyses were performed according to Agilent METLIN/PCDL method [2] using a 1290 infinity UHPLC coupled to a 6550 ESI-QTOF (Agilent Technologies, USA) operated in positive and negative electrospray ionization mode. Briefly, metabolites were separated on Zorbax SB-Aq RR (50 × 2.1 mm., 1.8 µm) column using a continuous gradient elution.

LC-HRMS data were deconvoluted using Find by Molecular Feature algorithm from Mass Hunter Qualitative analysis software (Agilent Technologies), and detected features were aligned across samples using the Mass Profiler Professional (MPP) software (Agilent Technologies). Relative quantification of metabolites was based on peak area of each feature normalized by sample weight. Metabolite identification was performed by using METLIN/PCDL database with the ID browser extension from MPP software, which combines retention time with accurate mass matching to provide greater confidence in compound identification.

To figure out whether an increase in H₂S was responsible for the changes in neurotransmitters levels, L-glutamate, 5-HIAA, L-tyrosine, and dopamine were quantified in cerebrum of Coq9⁺/− mice and Coq9⁻/⁻ mice supplemented with GYY4137. The quantification was performed by UHPLC-CMS/MS following the method described by Santos-Falinda and colleagues (Santos-Fandila et al, 2015).

**Silencing SQR**

siRNA oligonucleotides, Silencer Select Pre-designed siRNAs (Life Technologies, Carlsbad, CA, USA), were used for the transient silencing of SQR. For SQR, the applied sequence contained the 5’-3’ sense, GCCUCAGUAAACAUCCGGCUU and antisense 3’-5’, AACCGGAUGUUUACUGACGca. This SQR-silencing siRNA (Ambion s81773) was complementary with the mRNAs belonging to RefSeq NM_001162503.1 and NM_021507.5 genes and also targeted exon 3. For negative control, non-targeting siRNAs were applied with the same chemical modifications for enhanced efficacy as in other Silencer Select siRNAs (Ambion). Silencing was conducted as described previously (Modis K, Faseb 2012). Cells (80,000 cells/well) were seeded into 6-well tissue culture plates and cultured in normal culture medium to reach 50% confluence. At this point, the growth medium was replaced with Opti-MEM medium lacking FBS and antibiotics/antimicrobics, followed by transfection with 25 pmol siRNA fragments per well at 30 nM forming complexes with 7.5 ml of Lipofectamine® RNAiMAX (Life Technologies). Control cells were transfected in parallel with non-targeting siRNA (Life Technologies).

**Blood pressure and heart rate measurements in mice**

Systolic blood pressure (SBP) and heart rate (HR) were measured in conscious, precarmed, and restrained mice by tail-cuff plethysmography (Digital Pressure Meter LE 5001, Letica S.A., Barcelona, Spain) as described previously (Gomez-Guzman et al, 2014). Briefly, mice were held in a plastic tube, and their tail was put through a rubber cuff, and the cuff was inflated with air. The pressure level at which the first pulse appeared, after blood flow had been interrupted with the inflated cuff, was designated SBP. At least fifteen determinations were made in every session, and the mean of the lowest ten values within 5 mmHg was taken as the SBP level. HR values were obtained as average of several determinations simultaneously to SBP level.

**Statistical analysis**

All statistical analyses were performed using the Prism 6 scientific software. Data are expressed as the mean ± SD of four–six experiments per group. A one-way ANOVA with a Tukey’s post hoc test was used to compare the differences between three experimental groups. Studies with two experimental groups were evaluated using unpaired Student’s t-test. A P-value of < 0.05 was considered to be statistically significant.

**Expanded View** for this article is available online.

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Author contributions
ML-S led the study, conducted the experiments related to the hydrogen sulfide metabolism, COX activity/staining, and the GSH system, analyzed the results, and wrote the manuscript. AH-G conducted most of the Western blot assays, performed the experimental procedures with the H2S donor, participated in the experiments with HeLa cells and human skin fibroblasts, and analyzed the results. JC-S conducted some Western blot assays and the experiments with HeLa cells. TMH supervised and conducted the enzymatic assays and some HPLC analysis. EB-C conducted some experiments with HeLa cells and human skin fibroblasts. AS-F quantified the neurotransmitter norepinephrine after supplementation with GYY4137. RKAS conducted some histochemistry assays. MR and JD performed the analysis of blood pressure and heart rate. HP, MS, and FD provided the human skin fibroblasts from patients with CoQ deficiency. GE and DA-C contributed to the discussion and edited the manuscript. LCL conceived the idea for the project, supervised the experiments, and edited the manuscript. All authors critically reviewed the manuscript.

Conflict of interest
The authors declare that they have no conflict of interest.

For more information
Online Mendelian Inheritance in Man (OMIM): PDSS2, COQ2, COQ4, COQ9 http://omim.org/entry/610564?search=pdds2&highlight=pdds2 http://omim.org/entry/609825?search=COQ2&highlight=coq2 http://omim.org/entry/612898?search=COQ4&highlight=coq4 http://omim.org/entry/612837?search=COQ9&highlight=coq9

International mito-patients
http://www.mitopatients.org/

The association of mitochondrial disease patients in Spain
http://www.aepmi.org/publicoIngles/index.php

The United Mitochondrial Disease Foundation
http://www.umdf.org/

References
Ashby MN, Kutsunai SY, Ackerman S, Tzagoloff A, Edwards PA (1992) COQ2 is a candidate for the structural gene encoding para-hydroxybenzoate: polyphenyltransferase. J Biol Chem 267: 4128–4136
Brywczyc J, Sienko M, Kucharska A, Paszewski A (2002) Sulphur amino acid synthesis in Schizosaccharomyces pombe represents a specific variant of sulphur metabolism in fungi. Yeast 19: 29–35
Danhauser K, Herebian D, Haack TB, Rodenburg RJ, Strom TM, Meitingen T, Klee D, Mayatepek E, Proksch H, Distelmaier F (2016) Fatal neonatal encephalopathy and lactic acidosis caused by a homozygous loss-of-function variant in COQ9. Eur J Hum Genet 24: 450–454
Di Meo I, Fagioliari C, Prelli A, Viscomi C, Zeviani M, Tiranti V (2011) Chronic exposure to sulfide causes accelerated degradation of cytochrome c oxidase in ethylmalonic encephalopathy. Antioxid Redox Signal 15: 353–362
Di Meo I, Lamperti C, Tiranti V (2015) Mitochondrial diseases caused by toxic compound accumulation: from etiopathology to therapeutic approaches. EMBO Mol Med 7: 1257–1266
DiMauro S, Servidei S, Zeviani M, DiRocco M, DeVivo DC, DiDonato S, Uziel G, Berry K, Hoganson G, Johnsen SD (1987) Cytochrome c oxidase deficiency in Leigh syndrome. Ann Neurol 22: 498–506
Duncan AJ, Bitner-Glindzicz M, Meunier B, Costello H, Hargreaves IP, Lopez LC, Hirano M, Quinzii CM, Sadowski MJ, Hardy J et al (2009) 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 84: 558–566
Emmanuelli V, Lopez LC, Berardo A, Naini A, Tadesse S, Wen B, D’Agostino E, Solomon M, DiMauro S, Quinzii C et al (2012) Heterogeneity of coenzyme Q10 deficiency: patient study and literature review. Arch Neurol 69: 978–983
Eto K, Asada T, Arima K, Makifuchi T, Kimura H (2002a) Brain hydrogen sulfide is severely decreased in Alzheimer’s disease. Biochem Biophys Res Commun 293: 1485–1488
Eto K, Ogasawara M, Umemura K, Nagai Y, Kimura H (2002b) Hydrogen sulfide is produced in response to neuronal excitation. J Neurosci 22: 3386–3391
Fernandez-Vizarra E, Lopez-Perez MJ, Enriquez JA (2002) Isolation of biogenetically competent mitochondria from mammalian tissues and cultured cells. Methods 26: 292–297
Gao XH, Krokowski D, Guan BJ, Bederman I, Majumder M, Parisien M, Diatchenko L, Kobil O, Willard B, Banerjee R et al (2015) Quantitative H2S-mediated protein sulfhydration reveals metabolic reprogramming during the integrated stress response. Elife 4: e10067
Garcia-Corzo L, Luna-Sanchez M, Doerrier C, Garcia JA, Guaras A, Acin-Perez R, Bullejos-Peregrin J, Lopez A, Escames G, Enriquez JA et al (2013) Dysfunctional Coq9 protein causes predominant encephalomyopathy associated with CoQ deficiency. Hum Mol Genet 22: 1233–1248
Garcia-Corzo L, Luna-Sanchez M, Doerrier C, Ortiz F, Escames G, Acuna-Castroviejo D, Lopez LC (2014) Ubiquinol-10 ameliorates mitochondrial encephalopathy associated with CoQ deficiency. Biochim Biophys Acta 1842: 893–901
Garrett RM, Johnson JL, Graf TN, Feigenbaum A, Rajagopalan KV (1998) Human sulfite oxidase R160Q: identification of the mutation in a sulfite oxidase-deficient patient and expression and characterization of the mutant enzyme. Proc Natl Acad Sci USA 95: 6394–6398
Geiger T, Velic A, Macek B, Lundberg E, Kampf C, Nagaraj N, Uhlen M, Cox J, Mann M (2013) Initial quantitative proteomic map of 28 mouse tissues using the SILAC mouse. Mol Cell Proteomics 12: 1709–1722
Gomez-Guzman M, Jimenez R, Romero M, Sanchez M, Zarzuelo MJ, Gomez-Guzman M, Jimenez R, Romero M, Sanchez M, Zarzuelo MJ, Gomez-Morales M, O’Valle F, Lopez-Farre AJ, Algieri F, Galvez J et al (2014) Chronic hydroxychloroquine improves endothelial dysfunction and protects kidney in a mouse model of systemic lupus erythematosus. Hypertension 64: 330–337
Griffith OW (1999) Biologic and pharmacologic regulation of mammalian glutathione synthesis. Free Radic Biol Med 27: 922–935
Hildebrandt TM, Grieshaber MK (2008) Three enzymatic activities catalyze the oxidation of sulfide to thiosulfate in mammalian and invertebrate mitochondria. FEBS J 275: 3352–3361
Hine C, Harputlugil E, Zhang Y, Ruckenstuhl C, Lee BC, Brace L, Longchamp A, Trevino-Villarreal JH, Mejia P, Ozaki CK et al (2015) Endogenous hydrogen sulfide production is essential for dietary restriction benefits. Cell 160: 132–144
Hisson PJ, Hilf R (1976) A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal Biochem 74: 214–226
Hosoki R, Matsuki N, Kimura H (1997) The possible role of hydrogen sulfide as an endogenous smooth muscle relaxant in synergy with nitric oxide. 

*Biochem Biophys Res Commun* 237: 527 – 531

Ishii I, Akahoshi N, Yamada H, Nakano S, Izumi T, Suematsu M (2010) 

Cystathionine-gamma-lyase-deficient mice require dietary cysteine to protect against acute lethal myopathy and oxidative injury. 

*J Biol Chem* 285: 26358 – 26368

Kabili O, Vitvitsky V, Banerjee R (2014) Sulfur as a signaling nutrient through hydrogen sulfide. 

*Annu Rev Nutr* 34: 171 – 205

Kulkarni KH, Monjok EM, Zeyssig R, Kouamou G, Bongmba ON, Opere CA, Njie 

Polymerase chain reaction. 

*Science* 321: 744

Lee ZW, Zhou J, Chen CS, Zhao Y, Tan CH, Li L, Moore PK, Deng LW (2011) 

The slow-releasing hydrogen sulfide donor, GYY4137, exhibits novel anti-cancer effects in vitro and in vivo. 

*PLoS One* 6: e21077

Lubiad M, Yadav PK, Vitvitsky V, Martinov M, Banerjee R (2014) Organization of the human mitochondrial hydrogen sulfide oxidation pathway. 

*J Biol Chem* 289: 30909 – 30917

Lohman DC, Forouhar F, Beebe ET, Stefely MS, Minogue CE, Ulbrich A, Stefely L, Lopez LC, Escames G, Tapias V, Utrilla P, Leon J, Acuna-Castroviejo D (2007) 

Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: its relation with mitochondrial dysfunction. 

*FEBS J* 275: 1372 – 1383

Lopez LC, Escames G, Tapias V, Utrilla P, Leon J, Acuna-Castroviejo D (2006) 

Identification of an inducible nitric oxide synthase in diaphragm mitochondria from septic mice: its relation with mitochondrial dysfunction and prevention by melatonin. 

*Int J Biochem Cell Biol* 38: 267 – 278

Lopez LC, Quinzii CM, Area E, Naini A, Rahman S, Schuelke M, Salviati L, DiMauro S, Hirano M (2010) 

Treatment of CoQ10 deficient fibroblasts with ubiquinone, CoQ analogs, and vitamin C: time- and compound-dependent effects. 

*PLoS One* 5: e11887

Lopez-Martin JM, Quinzii CM, Luna-Sanchez M, Montini G, DiMauro S, Quinzii C, Hirano M, Rodriguez-Hernandez A, Cordero MD, Sanchez-Alcazar JA et al (2007) 

Missense mutation of the COQ2 gene causes defects of bioenergetics and de novo pyrimidine synthesis. 

*Hum Mol Genet* 16: 1091 – 1097

Luna-Sanchez M, Diaz-Casado E, Barca E, Tejada MA, Montilla-Garcia A, Cobos Ej, Escames G, Acuna-Castroviejo D, Quinzii CM, Lopez LC (2015) 

The clinical heterogeneity of coenzyme Q10 deficiency results from genotypic differences in the Coq9 gene. 

*EMBO Mol Med* 7: 670 – 687

Mineri R, Rimoldi M, Burlina AB, Koskull S, Perletti C, Heese B, von Dobeln U, Lohman DC, Forouhar F, Beebe ET, Stefely MS, Minogue CE, Ulbrich A, Stefely L, Lopez LC, Escames G, Tapias V, Utrilla P, Leon J, Acuna-Castroviejo D (2007) 

Organization of the human mitochondrial hydrogen sulfide oxidation pathway. 

*J Biol Chem* 289: 30909 – 30917

Modis K, Coletta C, Erdelyi K, Papapetropoulos A, Szabo C (2013) 

Intramitochondrial hydrogen sulfide production by 3-mercaptopropionate sulfurrtransferase maintains mitochondrial electron flow and supports cellular bioenergetics. 

*FASEB J* 27: 601 – 611

Mudd SH, Irreverre F, Lastar L (1967) Sulfite oxidase deficiency in man: demonstration of the enzymatic defect. 

*Science* 156: 1599 – 1602

Multiple-System Atrophy Research Collaboration (2013) Mutations in COQ2 in familial and sporadic multiple-system atrophy. 

*Neurology* 79: 233 – 244

Mustafa AK, Cadalla MM, Sen N, Kim S, Mu W, Gazi SK, Barrow RK, Yang G, Wang R, Snyder SH (2009) H2S signals through protein S-sulfhydration. 

*Sci Signal* 2: ra72

Quintana A, Zanella S, Koch H, Kruse SE, Lee D, Ramirez JM, Palmiter RD (2012) Fatal breathing dysfunction in a mouse model of Leigh syndrome. 

*Clin Inves* 122: 2599 – 2608

Quinzii CM, Naini A, Salvati L, Trevisson E, Navas P, DiMauro S, Hirano M (2006) 

A mutation in para-hydroxybenzoate-polyprenyl transferase (COQ2) causes primary coenzyme Q10 deficiency. 

*Am J Hum Genet* 78: 345 – 349

Quinzii CM, Lopez LC, Von-Moltke J, Naini A, Krishna S, Schuelke M, Salvati L, Navas P, DiMauro S, Hirano M (2008) 

Respiratory chain dysfunction and oxidative stress correlate with severity of primary CoQ10 deficiency. 

*FASEB J* 22: 1874 – 1885

Quinzii CM, Lopez LC, Gilkerson RW, Dorado B, Coku J, Naini AB, Lagier-Tourenne C, Schuelke L, Carrozzo R et al (2010) 

Reactive oxygen species, oxidative stress, and cell death correlate with level of CoQ10 deficiency. 

*FASEB J* 24: 3733 – 3743

Remelli W, Guerrieri N, Kiodermann J, Papenbrock J, Pagani S, Forlani F (2012) 

Involvement of the Azoatabacter vinelandii rhodanase-like protein RhdA in the glutathione regeneration pathway. 

*PLoS One* 7: e45199

Ribas V, Garcia-Ruiz C, Fernandez-Checa JC (2014) Glutathione and mitochondria. 

*Front Pharmacol* 5: 151

Romisch-Margl W, Prehn C, Bogumil R, Rohring C, Suhre K, Adamski J (2012) Procedure for tissue sample preparation and metabolite extraction for high-throughput targeted metabolomics. 

*Metabolomics* 8: 133 – 142

Roth SH, Skrjany B, Reifenstein RJ (1995) Alteration of the morphology and neurochemistry of the developing mammalian nervous system by hydrogen sulphide. 

*Clin Exp Pharmacol Physiol* 22: 379 – 380

Santos-Fandila A, Vazquez E, Barranco A, Zafra-Gomez A, Navalon A, Rueda R, Ramirez M (2015) 

Identification of 17 neurotransmitters, metabolites and precursors in zebrafish through the life cycle using ultrahigh performance liquid chromatography-tandem mass spectrometry. 

*J Chromatogr B Analyt Technol Biomed Life Sci* 1001: 191 – 201

Seiler A, Schneider M, Forster H, Roth S, Wirth EK, Culumsee C, Plesnilia N, Kremmer E, Radmark O, Wurst W et al (2008) Glutathione peroxidase 4 senses and translates oxidative stress into 12/15-lipoxygenase dependent- and AIF-mediated cell death. 

*Cell Metab* 8: 237 – 248

Skrjany B, Hannah RS, Roth SH (1992) Low concentrations of hydrogen sulphide alter monoamine levels in the developing rat central nervous system. 

*Can J Physiol Pharmacol* 70: 1515 – 1518

Sorbo BH (1955) Rhodanese. 

*Methods Enzymol* 2: 334 – 337

Szabo C, Ransy C, Modis K, Andriamihaja M, Murghes B, Coletta C, Olah G, Yanagi K, Bousillaud F (2014) Regulation of mitochondrial bioenergetic function by hydrogen sulfide. 

Part I. Biochemical and physiological mechanisms. 

*Br J Pharmacol* 171: 2099 – 2122

Tanji K, Bonilla E (2008) Light microscopic methods to visualize mitochondria on tissue sections. 

*Methods* 46: 274 – 280

Theissen U, Martin W (2008) Sulfide: quinone oxidoreductase (SQR) from the lugworm Arenicola marina shows cyanide- and thioredoxin-dependent activity. 

*FEBS J* 275: 1131 – 1139

Tiranti V, Viscomi C, Hildebrandt T, Di Meo I, Mineri R, Tiveron C, Levitt MD, Parelle A, Fagioli G, Rimoldi M et al (2009) LOSS of ET1E, a mitochondrial dioxygenase, causes fatal sulfide toxicity in ethylmalonic encephalopathy. 

*Nat Med* 15: 200 – 205

Truong DH, Eghbal MA, Hindmarsh W, Roth SH, O’Brien PJ (2006) Molecular mechanisms of hydrogen sulfide toxicity. 

*Drug Metab Rev* 38: 733 – 744

Uchida N, Suzuki K, Saiki R, Kainou T, Tanaka K, Matsuda H, Kawamukai M (2000) Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for p-hydroxybenzoate-polyprenyl diphosphate transferase. 

*J Bacteriol* 182: 6993 – 6999

Viscomi C, Burlina AB, Dweikat I, Saviozio M, Lamperti C, Hildebrandt T, Tiranti V, Zeviani M (2010) Combined treatment with oral metronidazole
and N-acetylcysteine is effective in ethylmalonic encephalopathy. Nat Med 16: 869–871
Yang G, Wu L, Jiang B, Yang W, Qi J, Cao K, Meng Q, Mustafa AK, Mu W, Zhang S et al (2008) H2S as a physiologic vasorelaxant: hypertension in mice with deletion of cystathionine gamma-lyase. Science 322: 587–590
Yoo SE, Chen L, Na R, Liu Y, Rios C, Van Remmen H, Richardson A, Ran Q (2012) Gpx4 ablation in adult mice results in a lethal phenotype accompanied by neuronal loss in brain. Free Radic Biol Med 52: 1820–1827
Zhang M, Wakitani S, Hayashi K, Miki R, Kawamukai M (2008) High production of sulfide in coenzyme Q deficient fission yeast. BioFactors 32: 91–98
Ziosi M, Di Meo I, Kleiner C, Barca E, Sánchez-Quintero M, Jiang H, Qiao C, Ghezzi D, Koenig M, Rodenburg RJ et al (2017) Coenzyme Q deficiency causes impairment of the sulfide oxidation pathway. EMBO Mol Med 9: 96–111

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