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Further phenotypic heterogeneity of CoQ10 deficiency associated with Steroid Resistant Nephrotic Syndrome and novel *COQ2* and *COQ6* variants

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Genes involved in the coenzyme Q_{10} biosynthetic pathway (*COQ2*, *COQ6*, *PDSS1*, *PDSS2* and *ADCK4*) are mutated in about 1% of Steroid Resistant Nephrotic Syndrome (SRNS) cases and are often associated with neurological symptoms [1; 2]. Here we describe novel phenotypes associated with pathogenic mutations in two genes of the CoQ_{10} pathway, *COQ2* and *COQ6*, in three patients with SRNS. One novel homozygous *COQ2* variant, p.Gly390Ala (p.Gly340Ala, according to KU877220 GenBank sequence [2]) was identified by NGS (*Supplementary Information*) in two cousins (Patients P1 and P2; Figure 1A; Tables S1 and S2) with SRNS associated with focal segmental glomerulosclerosis (FSGS) lesions and podocyte foot process effacement on renal biopsy (Figure 1B, panels 1-4; Table S1). The pathogenicity of this variant is supported by its absence in public SNP database; the segregation with the disease (Figure S1, panel 1); *in silico* predictions (Figure 1C, Table S2); the reduced rate of respiratory growth and CoQ levels of yeast expressing this allele (Figure 1D); the presence of numerous dysmorphic mitochondria on renal biopsy (Figure 1B, panels 5 and 6). Remarkably, both patients harbouring *COQ2* change developed SRNS in adolescence with rapid progression to end stage renal disease (ESRD) and had only mild neurological symptoms (Table S1). Of note, both cousins received a successful kidney transplant without recurrence of proteinuria and started CoQ_{10} treatment immediately after the genetic diagnosis. They are currently asymptomatic without any neurological symptoms after a 2-year follow-up. Other authors reported patients with inherited *COQ2* changes presented with isolated renal symptoms [2], however, to date, the reported age of onset of SRNS in patients carrying *COQ2* variants was before the age of 2.5 years [2]. To our knowledge, this is the first report describing *COQ2* variants in patients with adolescent-onset SRNS and with a clinical spectrum resembling another CoQ10-glomerulopathy caused by mutations in *ADCK4* [3]. This finding recapitulates what has been observed in the Pdss2 (kd/kd) mice [2] and can be explained by the relatively mild effect of the p.Gly390Ala (p.Gly340Ala) allele, as documented by yeast studies (Figure 1D).

Mutational screening of patient P3 (Figure 1E; Table S1) revealed a novel homozygous missense change in *COQ6* gene: p.Pro261Leu (Figure 1F; Table S2). The functional effect of this variant is supported by its low MAF in the European population (1:16683, Table S2); the segregation with the disease (Figure S1, panel 2); *in silico* predictions (Figure 1G; Table S2) and the inability of *COQ6* p.Pro261Leu allele to rescue the growth defect of the deleted yeast (Figure 1H). Few patients with *COQ6* changes (12) have been reported so far, they showed SRNS with onset at a median age of 1.2 years and sensorineural deafness (7/12) but some had also encephalopathic features [4]. Our patient developed SRNS at 8 months with progressive deterioration of renal function and ESRD at 20 months. Of note, he did not present deafness or encephalopathic features. He is now on peritoneal dialysis treatment, however CoQ10 treatment was started to prevent neurological symptoms. Interestingly, there was no history of schwannomatosis in the patient's family, and unless further evidence supporting the link between heterozygous *COQ6* mutations and schwannomatosis becomes available, we do not recommend mutational screening of *COQ6* gene for Schwannomas carriers [5]. The most frequent glomerular lesion associated with *COQ6* changes is FSGS [4]. Remarkably, the analysis of renal biopsy of our patient revealed membranoproliferative glomerulonephritis (MPGN) and C3 deposits (Figure 1F) usually associated with variants and risk haplotypes of complement-pathway genes, whose analysis did not reveal any variants with MAF<0.01 (*data not shown*). In summary, this study shows that we should analyze *COQ2* and *COQ6* genes also in patients with adolescent-onset of SRNS and without neurological symptoms, to avoid unnecessary immunosuppressive therapies and provide timely and effective treatment with CoQ10.

Ethical Statement

Written informed consent was obtained from each patient and/or their parents. The study was performed according to the guidelines of the Declaration of Helsinki and the local Ethics Committees of University Hospitals of Foggia and Bari (Italy).

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FIGURE LEGEND

Figure 1. (A; E): Family 1; Family 2 **(B; F):** Light microscopy, immunofluorescence and EM images **(C; G):** Evolutionary conservation of *COQ2* Gly390 (KU877220: p.Gly340) and *COQ6* Pro261. **(D; H):** W303ΔCOQ2 and BY4741ΔCOQ6 yeasts were transformed with the low copy pCM189 plasmid expressing either wild type human *COQ2* and *COQ6,* the empty vectors, or human *COQ2* p.Gly390Ala (Gly340Ala) and *COQ6* p.Pro261Leu alleles, respectively, and grown in plates containing glycerol as sole carbon source (YPGly) for 3 and 5 days, respectively. The same strains were grown in liquid medium containing 2% galactose for 3 or 4 days, respectively, and Complex II+III (C.II+III) and Citrate Synthase (CS) activities were assayed on purified mitochondria. ND=not detectable.