Homozygosity for a Rare *FASTKD2* Variant Resulting in an Adult Onset Autosomal Recessive Mitochondrial Podocytopathy

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Mitochondrial cytopathies can have kidney involvement in up to half of cases. Their diagnosis is challenging due to phenotypic variability, lack of noninvasive tests to assess mitochondrial dysfunction, and genetic heterogeneity. We report on a young adult male with hypertrophic cardiomyopathy (HCM) and chronic kidney disease (CKD) with subnephrotic proteinuria who presented to the emergency department with kidney failure and hypervolemia requiring dialysis. A kidney biopsy showed focal segmental and global glomerulosclerosis, extensive foot process effacement, and abnormal mitochondria in podocytes and tubular epithelial cells; the genetic workup identified a rare *FASTKD2* exon 2 variant, c.29G>C p.(Ser10Thr), in homozygosity; and functional mitochondrial assays in cultured skin fibroblasts showed reduction in FASTKD2 protein expression and moderate combined impairment in mitochondrial respiratory chain (MRC) assembly and function. This is the first report of a FASTKD2-associated cardiorenal mitochondrial cytopathy, characterized by young adult-onset proteinuric CKD and dilated HCM, in the absence of the severe neurologic manifestations described in patients with biallelic *FASTKD2* variants. We hypothesize that the increased production of reactive oxygen species associated with moderate MRC impairment could result in a smoldering podocytopathy with progressive proteinuric CKD, without overt tubulopathy or encephalomyopathy—which might be, instead, pathogenically related to adenosine triphosphate deficiency.

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Mitochondrial cytopathies are a heterogeneous group of disorders caused by defects in the mitochondrial oxidative phosphorylation system. Most cases present in the first years of life with some degree of encephalomyopathy.[1] In up to half of patients there is kidney involvement[2] consisting either of tubulopathy or glomerular disease, namely focal segmental glomerulosclerosis (FSGS).

Fas-activated serine-threonine kinase domain-containing type 2 (FASTKD2) is a protein involved in the posttranscriptional regulation of mitochondrial DNA (mtDNA) expression,[3] encoded by the nuclear DNA gene *FASTKD2*. Biallelic *FASTKD2* variants have been reported to cause combined oxidative phosphorylation deficiency-44 (COXPD44), an autosomal recessive mitochondrial disorder most typically presenting as an infantile encephalomyopathy.[4-6]

Herein, we report the detection of homozygosity for a rare *FASTKD2* single-nucleotide polymorphism (SNP) in a young adult male presenting with progressive chronic kidney disease (CKD) and subnephrotic proteinuria, in whom the kidney pathology findings and the results of in vitro functional mitochondrial studies in cultured skin-derived fibroblasts strongly supported the annotation of the patient's *FASTKD2* genotype as recessively pathogenic.

Case Report

A 37-year-old Portuguese male presented to the emergency department with severe hypertension (201/136 mm Hg), congestive heart failure, and kidney failure (serum creatinine 7.82 mg/dL), requiring urgent hemodialysis for treatment of the fluid overload. He was an active smoker (30 pack-year) and reported daily alcohol consumption of about 75 grams. The patient's family history was negative, including for parental consanguinity. His height was 1.75 m, and he weighed 75 kg. Except for severe azotemia, overt proteinuria (1 g/L), moderate anemia, and highly increased brain natriuretic peptide levels, the admission laboratory tests were unremarkable (Table S1). On ultrasound examination, the kidneys were 9.5 cm long and exhibited increased parenchymal echogenicity. The left ventricular (LV) ejection fraction estimated by echocardiography was 22%. Despite normalization of blood pressure and the improvement of heart failure with the resolution of hypervolemia, the patient remained dialysis dependent.

Two years before hospitalization, on investigation of the patient's chronic exertional dyspnea, stage 2 hypertension had been diagnosed with hypertrophic cardiomyopathy (HCM) and CKD, stage G3bA3, with baseline serum creatinine of 2.10 mg/dL and 24-hour urine protein excretion of 1.9 g (Table S1). Cardiac magnetic resonance imaging showed LV hypertrophy (LV mass, 220 g; maximal wall thickness, 16 mm) with moderate dilation (end-diastolic volume of 235 mL) and LV ejection fraction of 44%, without evidence of myocardial fibrosis or scarring. Combination therapy with 5 antihypertensive and diuretic agents was required to achieve adequate control of blood pressure, and the kidney function significantly improved in the short term (Table S1).

Unfortunately, the patient had to move abroad for work, self-ceased all prescribed medications, and was eventually lost to follow-up observation.

A kidney biopsy obtained to clarify the cause of kidney failure showed lesions of global and segmental glomerulosclerosis, with moderate tubulointerstitial fibrosis and arteriolar medial thickening (Fig 1). No immune deposits were detected either by immunofluorescence or electron microscopy (EM). Additionally, EM revealed extensive foot process effacement and the presence of large, morphologically abnormal mitochondria within podocytes and tubular epithelial cells.

Sequencing of the transfer RNA leucine 1 gene (*MT-TL1*), a mitochondrial gene associated with FSGS and a range of cardiac manifestations including HCM,[2,7] did not identify any clinically relevant variants. Subsequently, trio exome analysis revealed homozygosity for the *FASTKD2* c.29G>C p.(Ser10Thr) SNP, in exon 2—SNP database (dbSNP) identifier rs147727753[8] (US National Center for Biotechnology Information)—classified as a "variant of uncertain significance" (VUS) according to current classification standards.[9]

Western blot analysis of the patient's cultured skin fibroblasts following an approach described elsewhere[10] showed significantly decreased FASTKD2 expression and a lower mitochondrial respiratory chain (MRC) complex IV assembly as compared to complex II (Fig 2). Spectrophotometry evaluation according to established protocols[11] showed moderately reduced activity of MRC complexes I, III, and V (Fig 2).

Based on the kidney histopathological findings and the results of the mitochondrial functional studies, the final diagnosis of FASTKD2-associated mitochondrial podocytopathy was established.

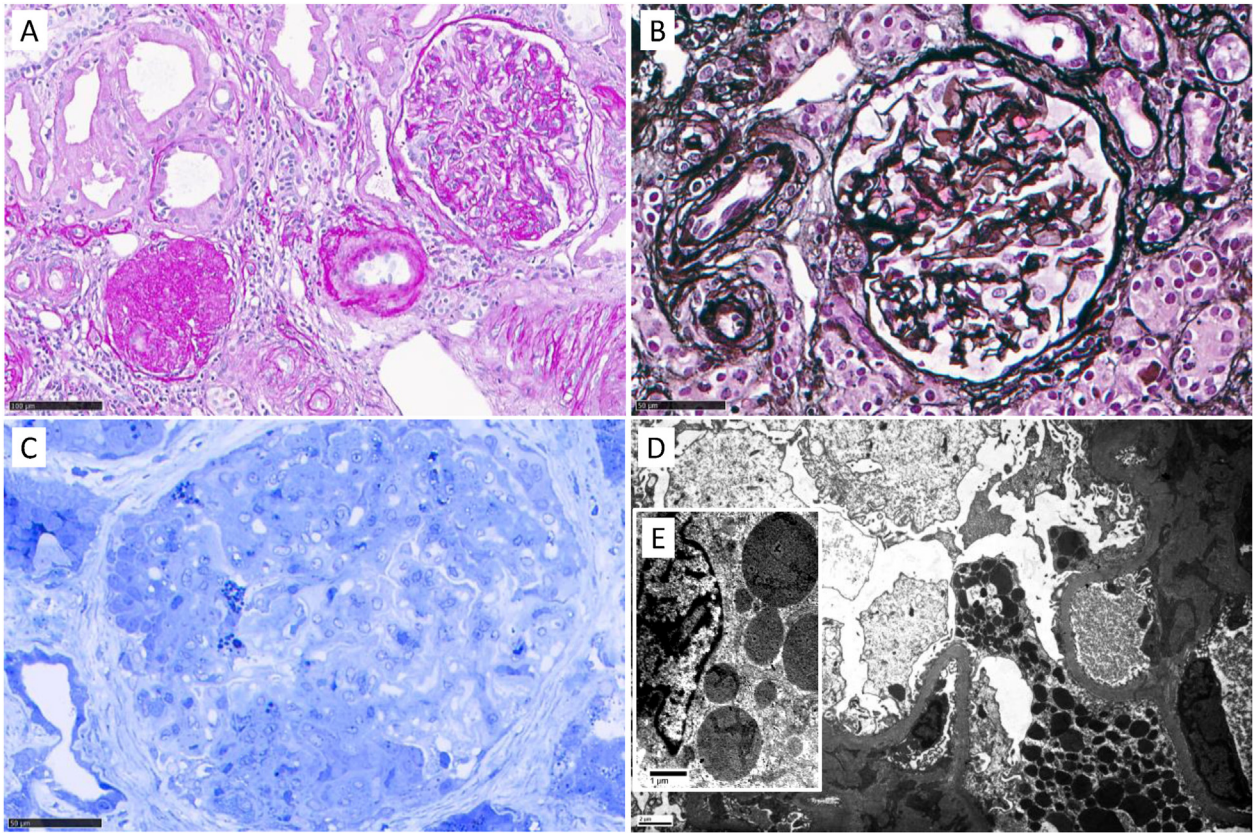

Figure 1. Kidney biopsy findings. (A) Nonspecific chronic lesions: global and segmental glomerulosclerosis, moderate tubulointerstitial fibrosis and arteriolar medial thickening (light microscopy; periodic acid Schiff stain; original magnification, ×100; scale bar = 100 μm). Overall, there were 37 glomeruli available for light microscopy review, of which 15 were globally sclerotic and 2 showed segmental glomerulosclerosis. (B) Podocytes showing prominent cytoplasmic ballooning (light microscopy; Jones silver stain; original magnification, ×400; scale bar = 50 μm). Glomerulus showing extensive segmental sclerosis (light microscopy; resin-embedded 1-μm-thick scout section for electron microscopy; toluidine blue stain; original magnification, ×400; scale bar = 50 μm). (D) On electron microscopy, the podocytes showed extensive foot process effacement and contained large numbers of pleomorphic, rounded, variable sized mitochondria, measuring up to >2 μm in greatest diameter, exhibiting no or only a few cristae (transmission electron microscopy; original magnification, ×5,000; scale bar = 2 μm). (E) Inset: Dysmorphic enlarged mitochondria were also present in tubular epithelial cells, some of them showing simplified cristae (transmission electron microscopy; original magnification ×13,700; scale bar = 1 μm).

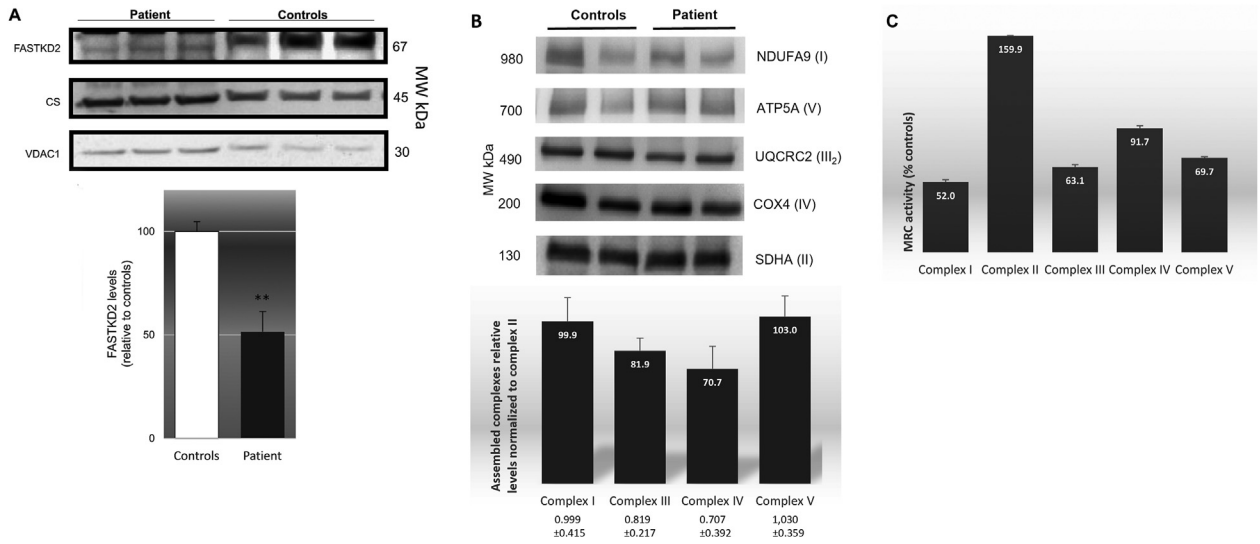

Figure 2. Mitochondrial respiratory chain assembly and functional study on fibroblasts. (A) Relative FASTKD2 expression in patient-derived fibroblasts as compared with controls. FASTKD2 protein levels were measured in fibroblast-derived mitochondria-enriched protein fractions from the patient and healthy controls and normalized to CS protein levels, using a chemiluminescent WB protocol, after protein separation by PAGE. VDAC1 was used as mitochondrial loading control. A representative blot is shown above the histogram. Quantitative data are expressed as mean ± SEM, generated from 4 independent experiments using independent sample preparations of both the patient's fibroblasts (n = 4) and 3 healthy controls (n = 6): the mean (± SEM) relative expression of FASTKD2 compared with controls was 51.6% (± 19.6%); the error bars shown in the bar plot represent the upper limit of the 95% CI for the SEM. $^{**}P = 0.005$, unpaired *t* test. (B) Relative expression of the fully assembled OXPHOS complexes of the MRC in patient-derived fibroblasts, as a percentage of complex II expression (used as internal control). Mitochondrial membrane proteins were isolated from crude mitochondrial fractions of fibroblasts and separated in gradient blue native PAGE; the 5 OXPHOS complexes were subsequently quantified by chemiluminescent WB using primary antibodies directed against the following specific subunits: NDUFA9 (NADH:ubiquinone oxidoreductase subunit A9), for complex I; ATP5A (ATP synthase F1 subunit alpha), for complex V; UQCRC2 (ubiquinol-cytochrome C reductase core protein 2), for complex III; COX4 (cytochrome C oxidase subunit 4), for complex IV; and SDHA (succinate dehydrogenase complex flavoprotein subunit A), for complex II. The numerical values and the error bars shown in the bar plot, respectively, represent the mean and the upper limit of the 95% CI for the SEM, based on 7-8 measurements; the mean ± SEM of the relative expression of complexes I, III, IV, and V is shown numerically below the histogram and did not differ significantly from controls. According to the distribution of the corresponding experimental data values, the statistical analyses were carried out using the unpaired *t* test for complexes III ($P = 0.10$), IV ($P = 0.2$), and V ($P = 0.8$), and the Mann-Whitney test for complex I ($P = 0.5$). (C) Relative activities of the MRC complexes in patient-derived fibroblasts, expressed as percentage of mean control values. Frozen-thawed fibroblasts were submitted to spectrophotometric determination of the catalytic activities of the mitochondrial complexes I-V, which were normalized to the CS activities, and compared to the mean values of activities of reference controls. The numerical values and the error bars shown in the bar plot respectively represent the mean and the upper limit of the 95% CI for the SEM, based on 2 measurements. Detailed laboratory methods are included in Item S1. The statistical analyses and artwork were performed with GraphPad Prism version 5.00 for Windows (GraphPad Software) and GNU Image Manipulation Program (GIMP), version 2.10.34 (available from: https://www.gimp.org). Abbreviations: CS, citrate synthase; MRC, mitochondrial respiratory chain; MW kDa, molecular weight in kilodaltons; OXPHOS, oxidative phosphorylation; PAGE, polyacrylamide gel electrophoresis; SEM, standard error of the mean; VDAC1, voltage-dependent anion channel 1 protein; WB, Western blot.

Discussion

To our knowledge, this is the first report of a patient with a young adult-onset FASTKD2-associated cardiorenal mitochondrial cytopathy, characterized by proteinuric CKD and dilated HCM but lacking any of the neurologic manifestations described in children diagnosed with COXPD44.[4-6] Remarkably, in neurologically affected patients, HCM has previously been documented in a 12-year-old girl,[6] and progressive CKD in a brother and sister aged 11 and 16 years, respectively.[12]

The *FASTKD2* c.29G>C p.(Ser10Thr) variant identified in homozygosity in our patient is rare in the general population and has conflicting interpretations of pathogenicity.[13] However, pathogenicity has not been ruled out, and the association with human disease requires further investigation.

The kidney biopsy was diagnostic of a podocytopathy, with the large and morphologically abnormal mitochondria observed in both podocytes and tubular epithelial cells raising suspicion of a mitochondrial cytopathy. Moreover, in vitro mitochondrial functional studies, performed in cultured skin fibroblasts from the patient, demonstrated significantly reduced FASTKD2 expression, moderate reduction of MRC complex IV assembly, and reduced activity of MRC complexes I, III, and IV. The expression of

FASTKD2 and the activity of MRC complexes were not specifically measured in kidney or heart tissue samples, and no skeletal muscle or myocardial biopsy samples were obtained for additional morphological or functional studies. However, because *FASTKD2* is a nuclear DNA gene—and thus is not influenced by heteroplasmy or variable mtDNA copy number levels—it is reasonable to assume that the functional studies in skin fibroblasts are biologically representative of the clinically affected organs, supporting the imputation of pathogenicity to the homozygous *FASTKD2* c.29G>C p.(Ser10Thr) genotype. In addition, fibroblasts derived from patients' skin biopsies have been widely used as models for studies in mitochondrial disorders.[11]

FASTKD2 encodes a transmembrane protein containing a Fas-activated serine-threonine kinase domain that localizes at the mitochondria RNA granules, where it forms a functional module with RNA-binding proteins and mitochondrial pseudouridine synthases. This module plays an important role in the pseudo uridylation of 16S ribosomal RNA, essential for mitochondrial ribosome assembly and translation of mtDNA encoded proteins.[3]

In homozygosity or compound heterozygosity, pathogenic *FASTKD2* variants cause COXPD44, an MRC disorder usually presenting in infancy or early childhood with multisystemic manifestations including global developmental delay, hypotonia, abnormal movements, and epilepsy.[4-6] However, as suggested by clinical observations and results of animal model studies,[6] FASTKD2-associated mitochondrial disease has a higher degree of clinical heterogeneity, and HCM can be part of the phenotype. Mitochondrial functional studies[6] have revealed that pathogenic *FASTKD2* variants correlate with moderate to severe reduction in the protein expression and deficiency of all MRC complexes but complex II, which is the only MRC complex encoded entirely by nuclear genes.[14]

Mitochondrial DNA changes are estimated to account for two-thirds of mitochondrial diseases with renal involvement,[2] with tubulopathy resulting mostly from mtDNA deletions and FSGS resulting from the more frequent mtDNA point variants.[1] Point variants or gross rearrangements in mtDNA genes involved in mitochondrial protein synthesis typically result in combined MRC complex defects that spare complex II.[14]

Primary coenzyme Q10 (CoQ10; ubiquinone) deficiencies are well-known causes of FSGS, but only 5 of the 9 nuclear genes encoding proteins involved in CoQ biosynthesis have so far been associated with proteinuria or nephrotic syndrome and response to oral CoQ10 supplementation.[14] It is unclear why mtDNA point variants (such as the common *MT-TL1* m.3243A>G) but not mtDNA deletions and pathogenic variants in only a subset of the nuclear genes involved in CoQ10 biosynthesis pathway are associated with podocytopathy. Podocyte bioenergetics is a disputed matter, with some studies showing a predominant role for glycolysis and others indicating dependency on mitochondrial function.[15] However, it is well-established that MRC dysfunction is a main source of excessive reactive oxygen species (ROS) production,[16] and the predominance of ROS production over ATP depletion in moderate (but not severe) CoQ10 deficiency has been proposed as an explanation for podocyte injury being a feature of only some CoQ10 biosynthesis defects.[14,17]

In our patient, the moderate combined defect in MRC activity might have led to podocyte injury through ROS, giving rise to chronic podocytopathy with proteinuria and progressive CKD, without features of ATP deficiency such as encephalomyopathy (which is the hallmark of COXPD44) or tubulopathy. The absence of formal assays of tubular function is a limitation of this case study, but the patient had no obvious glycosuria or history of kidney stones/nephrocalcinosis to suggest overt tubulopathy.

Recognition of mitochondrial kidney disease will remain challenging due to variable phenotypic expressivity, genetic heterogeneity, and lack of noninvasive tests to assess mitochondrial dysfunction. Observation of increased numbers of mitochondria, or of aberrant mitochondrial morphology on EM examination of a kidney biopsy sample, is an important clue to diagnosis.[18] Wider availability of genetic testing is changing the diagnostic rate, but appropriate patient and method selection remain challenging. Furthermore, biomolecular functional studies are frequently necessary for proper annotation of VUS and establishing the diagnosis.

In summary, we report a FASTKD2-associated adult-onset cardiorenal mitochondrial cytopathy, without central nervous system involvement, that should be considered in the differential diagnosis of cardiorenal phenotypes. Given the beneficial effect of CoQ10 supplementation reported in sibs homozygous for a deleterious *FASTKD2* variant,[12] at least for delaying CKD progression, improving clinicians' awareness to this mitochondrial cytopathy will be critical not only for proper genetic counseling but also for earlier therapeutic intervention, eventually allowing to clarify the effectiveness of CoQ10 supplementation in affected individuals.

Supplementary Material

Supplementary File (PDF)

Item S1: Supplementary methods.

Table S1: Laboratory data at baseline assessment and during hospitalization.

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