ADCK4-Associated Glomerulopathy Causes Adolescence-Onset FSGS

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ABSTRACT

Hereditary defects of coenzyme Q10 biosynthesis cause steroid-resistant nephrotic syndrome (SRNS) as part of multiorgan involvement but may also contribute to isolated SRNS. Here, we report 26 patients from 12 families with recessive mutations in ADCK4. Mutation detection rate was 1.9% among 534 consecutively screened cases. Patients with ADCK4 mutations showed a largely renal-limited phenotype, with three subjects exhibiting occasional seizures, one subject exhibiting mild mental retardation, and one subject exhibiting retinitis pigmentosa. ADCK4 nephropathy presented during adolescence (median age, 14.1 years) with nephrotic-range proteinuria in 44% of patients and advanced CKD in 46% of patients at time of diagnosis. Renal biopsy specimens uniformly showed FSGS. Whereas 47% and 36% of patients with mutations in WT1 and NPHS2, respectively, progressed to ESRD before 10 years of age, ESRD occurred almost exclusively in the second decade of life in ADCK4 nephropathy. However, CKD progressed much faster during adolescence in ADCK4 than in WT1 and NPHS2 nephropathy, resulting in similar cumulative ESRD rates (>85% for each disorder) in the third decade of life. In conclusion, ADCK4-related glomerulopathy is an important novel differential diagnosis in adolescents with SRNS/FSGS and/or CKD of unknown origin.


Mitochondrial cytopathies are clinically and genetically heterogeneous disorders. Although most mitochondrial pathies involve multiple organ systems and often present with prominent neurologic and myopathic features in childhood, a few exhibit organ-selective phenotypes. In the kidney, mitochondriopathies typically cause proximal tubulopathy; however, glomerular dysfunction has been reported with mitochondrial DNA mutations in the tRNA{leu}{sup+} gene and coenzyme Q biosynthesis defects. 2–5

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Coenzyme Q (ubiquinone; CoQ₁₀) is a component of the mitochondrial respiratory chain, a potent lipophilic antioxidant, and a cofactor for mitochondrial dehydrogenases and in pyrimidine nucleoside biosynthesis. CoQ₁₀ is synthesized ubiquitously through a multienzyme complex at the inner mitochondrial membrane. Mutations in several genes encoding enzymes of the CoQ₁₀ biosynthetic pathway (COQ2, COQ6, and PDSS2) are associated with a glomerular phenotype. These have been collectively termed CoQ₁₀ glomerulopathies. Recently, recessive mutations in ADCK4 (AarF Domain Containing Kinase-4) have been added to this list as a novel cause of steroid-resistant nephrotic syndrome (SRNS). ADCK4 interacts with components of the CoQ₁₀ biosynthesis pathway, and patients with ADCK4 mutations have reduced cellular CoQ₁₀ content. The selective glomerular phenotype of patients with ADCK4 mutations may be the result of relative enrichment of ADCK4 and lacking expression of the related protein ADCK3 in podocytes, whereas ADCK3 expression exceeds that of ADCK4 in most other body tissues.

We identified a new patient cohort with ADCK4 glomerulopathy among 534 consecutive SRNS cases. ADCK4 mutations were found in ten patients (1.9%) from mostly consanguineous families. In five families the mutation was found in further affected siblings (Supplemental Figure 1: Families III–VII). Two additional families comprising nine affected subjects in whom ADCK4 mutations were identified by genome-wide linkage analysis or exome sequencing (Supplemental Figure 1: Families I, II), yielding a total cohort of 26 patients. Mutational analysis revealed four novel sequence variants and three previously reported homozygous mutations, namely c.645delT, c.1199_1200dupA, and c.532C>T; p.(Arg178Trp) substitution. The novel c.1339dupG variant was found in four apparently unrelated Kurdish families originating from a region in southeast Turkey, suggesting a founder effect. Bioinformatic information on the novel variants is given in Table 1 and the online supplement.

### Table 1. Summary of bioinformatic analyses of the detected novel sequence variants

<table>
<thead>
<tr>
<th>Novel variant</th>
<th>Residue change</th>
<th>Protein domain</th>
<th>MAF</th>
<th>Conservation</th>
<th>Human Splicing Finder 3.0</th>
<th>Mutation Taster prediction</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.293T&gt;G</td>
<td>p.(Leu98Arg)</td>
<td>transmembrane (helical)</td>
<td>0 (not reported)</td>
<td>highly conserved within conserved region</td>
<td>mutation in early exonic positions potentially breaking ESE site</td>
<td>102 1.0 probably damaging</td>
</tr>
<tr>
<td>c.929C&gt;T</td>
<td>p.(Pro310Leu)</td>
<td>kinase (ABC1 subdomain)</td>
<td>0 (not reported)</td>
<td>highly conserved</td>
<td>mutation in late exonic positions potentially breaking ESE site</td>
<td>98 1.0 probably damaging</td>
</tr>
<tr>
<td>c.1493_1494CC</td>
<td>p.(Ala498Glu)</td>
<td></td>
<td>0 (not reported)</td>
<td>low conservation</td>
<td>potential activation of potential acceptor site of ESE site</td>
<td>93 0.173 benign</td>
</tr>
<tr>
<td>c.1339dupG</td>
<td>p.(Glu447Glyfs10)</td>
<td></td>
<td>1:10,000</td>
<td>0.3%b</td>
<td>highly conserved</td>
<td>disease causing</td>
</tr>
</tbody>
</table>

ESE, exonic splicing enhancer; ESS, exonic splicing silencer; NA, not applicable. MA, not available. aMAF, minor allele frequency; estimation based on data of 2577 individual genomes cataloged by the 1000 Genomes Project; 6503 samples collected at NHLBI Exome Sequencing Project and data from 60,706 individuals aggregated by the Exome Aggregation Consortium (ExAC; http://exac.broadinstitute.org; accessed January 31, 2015). bIn-house allele frequency database representative for Turkish population (collection of 373 individual genomes; accessed October 22, 2014).


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The phenotypic profile is summarized in Table 2. The disease first manifested in adolescence, typically with mild to moderate proteinuria with no or mild edema. However, advanced CKD was present in almost half of patients at time of diagnosis and progression to ESRD occurred in 22 of the 26 patients within a median of 9 (interquartile range 0–44) months from diagnosis. Hematuria was present at time of diagnosis in 25% of patients, including one case with a chief complaint of macroscopic hematuria accompanied by only trace proteinuria. FSGS was diagnosed in all biopsies, including two differentiated as collapsing and tip lesion subtypes.

Signs and symptoms compatible with neurologic dysfunction were reported in six patients (Supplemental Table 1). Mild mental retardation and agoraphobia were each present in one case and two siblings had primary nocturnal enuresis. Three patients developed electroencephalogram-confirmed seizures, including two while on dialysis. One subject was eventually diagnosed with hypertension-related reversible posterior encephalopathy whereas the other two require continued anticonvulsant therapy. One patient presented with retinitis pigmentosa. No histories of hearing problems, cardiomyopathy, muscle weakness, optical nerve atrophy, or hematologic or endocrinologic abnormalities were reported in any patient. Serum lactate was episodically elevated in 4 of 11 patients tested, and transient creatine kinase elevation was noted in two patients during episodes of AKI.

The clinical phenotype of ADCK4-related glomerulopathy was compared with the phenotypes of the two most common genetic podocytopathies, i.e., NPHS2- and WT1-associated nephropathies (Figure 1, Table 2). Patients with ADCK4-related glomerulopathy were significantly older at time of diagnosis, with no cases manifesting before 5 years of age, and they presented with less severe proteinuria and less edema than WT1- or NPHS2-associated disease. Hypertension was less common than in WT1 nephropathy. FSGS was the histopathologic diagnosis in all biopsied ADCK4 cases, whereas diagnoses other than FSGS were commonly observed at time of diagnosis in NPHS2 (Mesangio proliferative GN, Minimal change GN) and WT1 nephropathy (diffuse mesangial sclerosis). In ADCK4 patients, advanced CKD at time of diagnosis was more prevalent than in NPHS2. Of patients with ADCK4 disease, 38.5% presented with CKD5, compared with 15.6% of WT1 and 2.9% of NPHS2 cases (P<0.001). Whereas 47% of WT1 and 36% of NPHS2 patients progressed to ESRD before reaching 10 years of age, ESRD occurred almost exclusively in the second decade of life in ADCK4 nephropathy (Figure 1). However, CKD progression was much faster during adolescence in ADCK4 than in WT1 and NPHS2 nephropathy, resulting in similar cumulative ESRD rates (>85%) for the three genetic forms of SRNS in the third decade of life. Neurologic deficits were more frequent in ADCK4 disease. Renal and urinary tract malformations occurred almost exclusively in WT1. Other congenital anomalies, mostly heart structural defects, were anecdotally reported in all groups.

Table 2. Comparison of clinical characteristics at time of diagnosis and prospective kidney survival of patients with ADCK4-related SRNS versus patients with NPHS2- and WT1-related glomerulopathy from the PodoNet Registry.

<table>
<thead>
<tr>
<th>ADCK4 SRNS</th>
<th>NPHS2 SRNS</th>
<th>WT1 SRNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>26</td>
<td>140</td>
</tr>
<tr>
<td>Age at first reported manifestation, years</td>
<td>14.1 (10.8–17.0)</td>
<td>3.4 (1.1–6.6)</td>
</tr>
<tr>
<td>Asymptomatic, incidental diagnosis</td>
<td>26.9%</td>
<td>22.9%</td>
</tr>
<tr>
<td>Edema (none/mild/moderate/severe)</td>
<td>54/42/4/0%</td>
<td>48/17/16/19%</td>
</tr>
<tr>
<td>Proteinuria (subnephrotic/nephrotic range)</td>
<td>57.1/43.9%</td>
<td>14.9/85.1%</td>
</tr>
<tr>
<td>Hematuria</td>
<td>25.0%</td>
<td>44.0%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>30.8%</td>
<td>15.7%</td>
</tr>
<tr>
<td>CKD stage 3–5</td>
<td>46.1%</td>
<td>13.6%</td>
</tr>
<tr>
<td>including RTT:</td>
<td>26.9%</td>
<td>2.9%</td>
</tr>
<tr>
<td>Age at start of RRT, years</td>
<td>16.1 (13.7–18.0)</td>
<td>12.9 (7.6–19.4)</td>
</tr>
</tbody>
</table>

Histopathological diagnosis

<table>
<thead>
<tr>
<th>ADCK4 SRNS</th>
<th>NPHS2 SRNS</th>
<th>WT1 SRNS</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSGS/global glomerulosclerosis</td>
<td>61.5%</td>
<td>49.3%</td>
</tr>
<tr>
<td>Diffuse mesangial sclerosis</td>
<td>0%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Mesangio proliferative GN</td>
<td>0%</td>
<td>12.9%</td>
</tr>
<tr>
<td>Minimal change GN</td>
<td>0%</td>
<td>10.7%</td>
</tr>
<tr>
<td>Other</td>
<td>0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>No data/ not performed</td>
<td>38.5%</td>
<td>21.4%</td>
</tr>
<tr>
<td>Neurologic abnormalities (seizures, NI, behavioral problems)</td>
<td>24.0%</td>
<td>5.0%</td>
</tr>
<tr>
<td>Congenital organ abnormalities</td>
<td>CANT</td>
<td>0%</td>
</tr>
</tbody>
</table>

Data are given as median (interquartile range) or percentage. Sixty-one of the patients with WT1 mutations were previously described by Lipska et al. (2014)*

*Percentages given are relative to all observation with information on a specific variable.

bP<0.05.
cP<0.01.
dP<0.001.
In patients with mutations in PDSS2, COQ2, and COQ6, the other mitochon-
drialopathy genes associated with SRNS, renal symp-
toms usually occur as part of a multisys-
temic disease complex encompassing progressive encephalopathy, ataxia, sei-
zure, mental retardation, deafness, reti-
nopathy, hypertrophic cardiomyopathy, and generalized myopathy.1–5,10 By con-
trast, ADCK4 disease typically manifests as an isolated nephropathy with only
occasional extrarenal symptomatology. Combining our cohort with five pre-
viously published cases with available data on extrarenal involvement,6 we
oversee 30 patients from 17 families with detailed phenotypic information.
Among these, 15 patients (50%) never showed any extrarenal system involve-
ment. Three patients presented seizures (thereof two while on dialysis), and two
patients each had mild mental retardation and behavioral problems. Two cases
of goiter and single cases of retinitis pig-
mentosa and a lupus-like syndrome were
reported. Occasionally observed tran-
sient mild elevations of lactate and
creatine kinase during AKI are of ques-
tionable specificity and relevance.
Hence, within the wide spectrum of
mitochondrial disorders ADCK4 muta-
tions lead to the most selective glomer-
ular involvement, possibly related to
preferential enrichment of ADCK4 in
podocytes. The cytosolic as well as mito-
chondrial localization of ADCK4 pro-
tein in podocytes has led to speculation
that ADCK4 may exert additional func-
tions other than CoQ10 biosynthesis.6
Notwithstanding the preferential renal
phenotype, patients diagnosed with
ADCK4 nephropathy should undergo
systematic and repeated screening for
subclinical extrarenal symptoms.
Our systematic screening of more
than 500 prospective SRNS cases suggests
that ADCK4 nephropathy may be the
third most common hereditary cause of
SRNS, with a detection rate of one in 50
patients compared with one in eight for
NPHS28 and one in 18 for WT1.9 The
renal phenotype of ADCK4 disease is
characterized by an insidious onset at
adolescence with mild to moderate pro-
teinuria and absence of relevant edema
in the majority of cases. As a conse-
quence of the oligosymptomatic early
course, advanced CKD is often present
at the time of diagnosis. The compari-
on of renal survival of patients with
ADCK4, WT1, and NPHS2 glomerulo-
apathies respectively demonstrates the
usual evolution of renal function in
the mitochondriopathy, with almost all
patients progressing to ESRD between
12 and 23 years of age. Hence, at the
current stage of knowledge SRNS pro-
gressing toward ESRD in the first de-
cade of life almost rules out ADCK4
disease, whereas ADCK4 nephropathy
is an important differential diagnosis to
consider in cases of adolescent-onset
multidrug-resistant proteinuria with
FSGS on biopsy. In this group, where
genetic causes are found in less than
10% of cases by conventional screen-
ing,11,12 mutations in ADCK4 may be
as common as those in NPHS2 and
WT1. Of course, the experience derived
from the first two disease cohorts com-
prising patients from 20 families with
15 different mutations is still limited.
It remains to be seen whether the suggested detection rate will be confirmed and whether the uniform disease pattern will diversify with the identification of more patients and mutations.

The CoQ₁₀ glomerulopathies represent the first hereditary forms of SRNS for which a causative molecular therapy is potentially available. Oral CoQ₁₀ supplementation may reverse proteinuria and stabilize kidney function if applied early in the disease course. The commonly late diagnosis of ADCK₄ disease so far has precluded efficient therapy in most affected patients. This situation may change in the near future with earlier diagnosis thanks to increased awareness of the disease entity, inclusion of the mitochondrial genes in routinely performed next generation sequencing (NGS) panel screening, and proteinuria screening of asymptomatic siblings of affected patients as accomplished in two children in this report. Both subjects indeed demonstrated a significant decrease of proteinuria on CoQ₁₀ supplementation, raising hopes that timely treatment may preserve podocyte and kidney function in children with ADCK₄ nephropathy.

Based on the preliminary evidence presented here, we propose to perform ADCK₄ sequencing, ideally as part of NGS panel screening, in all patients with adolescent-onset proteinuric kidney disease in whom autoimmune etiologies have been ruled out on clinical and biochemical grounds. Genetic screening should be prioritized over kidney biopsy, particularly in cases of familial disease occurrence or parental consanguinity.

In conclusion, ADCK₄ glomerulopathy is a novel cause of adolescent-onset SRNS caused by defective CoQ₁₀ biosynthesis in podocytes. This recessive Mendelian disease may present with signs and symptoms of systemic mitochondrial dysfunction, but more often manifests as isolated FSGS. Despite the late clinical manifestation, rapid progression to end-stage renal disease is common. Early diagnosis will help to identify children at early disease stages who are eligible for oral CoQ₁₀ supplementation.

**CONCISE METHODS**

ADCK₄ screening was performed in 534 consecutive SRNS patients from the PodoNet Registry and in-house biobanks at Necker Hospital in Paris, France, the Hacettepe University Nephrogenetics Laboratory, Ankara, Turkey, and the Molecular Genetics Unit at Bioscientia, Ingelheim, Germany. Clinical information was available for 349 patients, including 233 unrelated patients negative for mutations in the first-line SRNS-associated genes (NPHS2, exons 8–9 of WT1) and 116 not previously tested individuals.

The PodoNet, Necker, and Bioscientia cohorts underwent high-throughput sequencing using custom-designed multi-gene NGS panels for FSGS and related glomerulopathies. Sequencing was performed using the MiSeq/HiSeq platform (Illumina, San Diego, CA). All findings were verified by Sanger sequencing, which was also used to test eligible family members.

Comparator cohorts with SRNS related to mutations in NPHS2 (n=140) or WT1 (n=66) were extracted from the PodoNet Registry. Whole-genome linkage analysis using 250K single nucleotide polymorphism array (Affymetrix, Santa Clara, CA) followed by homozygosity mapping using VIGENOS software was performed in two index families (Supplemental Figure 1: Families I and II). Illumina TruSeq Exome Enrichment Kit was used for paired-end whole exome sequencing performed on an Illumina HiSeq 2000 sequencing system (Illumina, San Diego, CA).

Detailed clinical information on renal and extrarenal symptoms was obtained on all ADCK₄ patients by way of a standardized questionnaire. The patient-level data are given in Supplemental Table 1. Statistical analyses were performed using the STATISTICA 9.1 (StatSoft; Tulsa, OK) data analysis software system.

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**DISCLOSURES**

None.

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