The Spectrum of Systemic Involvement in Adults Presenting with Renal Lesion and Mitochondrial tRNA(Leu) Gene Mutation

BRUNO GUÉRY,* GABRIEL CHOUKROUN,‡ LAURE-HÉLÈNE NOËL,* PIERRE CLAVEL,‡ AGNÈS RÖTIG,§ SOPHIE LEBON,§ PIERRE RUSTIN,§ CHRISTINE BELLANÉ-CHANTELOT,¶ BÉATRICE MOUGENOT,# JEAN-PIERRE GRÜNFFELD,* and DOMINIQUE CHAUVEAU*

*Service de Néphrologie and INSERM U507, Hôpital Necker, Paris; ‡Service de Néphrologie, Hôpital Sud, Amiens; §Service de Néphrologie, Hôpital de Brabois, Vandoeuvre-lès-Nancy; ¶Département de Génétique Cytogénétique, Hôpital Saint-Antoine, Paris; and #Service d’Anatomie Pathologique, Hôpital Tenon, Paris.

Abstract. The A3243G mutation of the mitochondrial tRNA(Leu) gene has been recently reported in rare patients with focal and segmental glomerulosclerosis (FSGS). However, the full spectrum of systemic and kidney manifestations in adults presenting with this mutation remains poorly defined. Assessment of renal and nonrenal manifestations was performed in nine patients with A3243G mutation and prominent kidney disease diagnosed in adulthood. At first renal evaluation, median age was 35 years. Renal lesions consisted of FSGS (n = 2), tubulointerstitial nephropathy (n = 3), or bilateral enlarged cystic kidneys (n = 1). All but one patient exhibited extrarenal manifestations: deafness (8 of 9) requiring hearing aid in half the cases, diabetes mellitus (3 of 9), neuromuscular involvement (2 of 9), hypertrophic cardiomyopathy (1 of 9), and macular dystrophy (1 of 9). After a median follow-up of 5 yr, five patients progressed to end-stage renal disease between the ages of 15 and 51 years, four being successfully transplanted. Similarly, extrarenal manifestations progressed since all patients had deafness and diabetes (including three posttransplants), while half had neuromuscular, cardiac, or retinal involvement. In the adult patients with A3243G mutation and renal involvement, preexisting deafness is almost consistently found. While FSGS remains the most typical lesion, tubulointerstitial nephropathy or bilateral, enlarged cystic kidneys may also be encountered. In most cases, diabetes mellitus, macular dystrophy, hypertrophic cardiomyopathy, or neuromuscular features occur later in the course of the disease. The severity of the clinical course is heterogeneous, with end-stage renal failure being reached between the second and sixth decades. Renal transplantation may be offered to these patients, despite a high incidence of steroid-induced diabetes mellitus.

The mitochondrial respiratory chain generates energy by producing ATP through oxidative phosphorylation. It results from the complementation of protein subunits issued from the nuclear and the mitochondrial genomes. The mitochondrial DNA (mtDNA) encodes for 13 essential subunits of the respiratory chain, as well as the 22 transfer RNA (tRNA) and 2 ribosomal RNA (rRNA) genes enabling intraorganellar protein synthesis (1).

Inherited mitochondriopathies are the result of either mitochondrial or nuclear gene defects. The former is either sporadic or maternally inherited and results from rearrangements, deletions, or point mutations responsible for a wide spectrum of human diseases (2). Brain and striated muscle were first identified and likely remain the most prevalent affected tissues. Pancreas and neurosensorial organs were then recognized as potential victims of mtDNA mutations (3). Eventually renal involvement attracted attention (4,5).

The molecular mechanisms of phenotype heterogeneity are not elucidated. Heteroplasmy and a threshold effect have been raised (2,3). In affected individuals, heteroplasmy refers to the presence of mutated and normal mtDNA within cells. The proportion of heteroplasmy required for a deleterious phenotype, referred to as the threshold effect, varies among different organs according to balance between oxydative supply and demand. Additional mechanisms of cellular toxicity beyond respiratory chain may depend upon accumulation of toxic substrates, including lactate (6).

The A3243G point mutation in the leucine UUR tRNA gene is considered the most prevalent mtDNA defect, with a 1:7000 prevalence in Finnish adults (7). The mutation was first ascertained in children with MELAS syndrome (mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes) (8). However, some families predominantly express diabetes and deafness (9–10), while some individuals present with cardiomyopathy (11) or chronic progressive external ophthalmoplegia (12). Last, focal and segmental glomerulosclerosis...
(FSGS) was found in adult patients carrying the A3243G mutation (4,13–17).

The present study reviews our current experience in assessing nine patients with prominent kidney involvement along with the A3243G mtDNA mutation. One of them presented with bilateral enlarged cystic kidneys, a phenotype still unreported in mitochondrial diseases. The aim of our study was to unravel the spectrum of kidney manifestations in adulthood and to delineate the systemic involvement pointing to mitochondrial disease.

Materials and Methods
Inclusion Criteria
To delineate the phenotype in adults with mitochondrial disorder including renal involvement, we reviewed the charts of nine patients diagnosed with mitochondrial DNA A3243G mutation and prominent renal complication. For inclusion in the study, the following criteria were required: (1) proteinuria > 1 g/24 h or chronic renal failure (creatinine clearance < 80 ml/min); (2) mitochondrial DNA A3243G mutation in peripheral blood leukocytes or affected tissues; (3) no other cause of renal failure.

Clinical Evaluation
Renal involvement including age at onset, first manifestations, and course were reviewed in medical charts. Hypertension was defined by BP above 140/90 mmHg. Nephrotic syndrome was defined by proteinuria > 3 g/24 h and serum albumin < 3.0 g/dl. Hematuria was assessed on dipstick urinalysis. Creatinine clearance was calculated according to Cockcroft formula. Screening for Fanconi syndrome relied on determination of serum kalemia, bicarbonate, phosphorus, and uric acid. For light microscopy study of renal biopsies, tissue was fixed in Bouin’s solution, embedded in paraffin, and sectioned at 2 µm. The sections were stained with Masson’s trichrome, hematoxylin and eosin, periodic acid-Schiff, and silver methenamine. For immunofluorescence (IF) studies, frozen tissue specimens were stained with commercially available antisera (Dako, Denmark). Electron microscopy study was achieved by fixation in glutaraldehyde and osmium tetroxide, and embedding in Epon 812. Ultrathin sections were stained with COX activity and to delineate the systemic involvement pointing to mitochondrial disease.

Molecular Analysis of the A3243G Mutation in the tRNA LeucineUR Gene
Identification and quantification of the A3243G mutation in the mitochondrial tRNA leucine gene were performed using genomic DNA isolated from peripheral blood leukocytes, or from muscle or urine sediments. The molecular analysis used an allele-specific PCR. The common oligonucleotide and the two primers specific for the normal and mutant alleles were respectively defined at position 3077–3095 and 3243–3271 (positions are given in reference to the mitochondrial sequence, accession number J01415). Two PCR, each one with an allele-specific primer, were carried out in a 40-µl volume containing 100 ng of genomic DNA, 1× PCR buffer (Applied Biosystems), 200 µM dNTP, 75 ng of each primer, and 1 U AmpliTaq DNA polymerase (Applied Biosystems). An aliquot of each PCR product was loaded into agarose gel and stained for detection of the mutation. The remaining PCR products were purified, then stained by the PicoGreen reagent (Molecular probes), and quantified by fluorometry. Identification of the A3243G mutation and estimation of heteroplasmy were systematically monitored with control DNAs.

Results
Genetic and Biochemical Data
Nine patients belonging to six unrelated families were studied. The point mutation A3243G within mtDNA was found in blood lymphocytes in eight of nine patients. The percentage of mutant genome was quantified in six of them and ranged from 5 to 25%. In the last patient (case 9), the A3243G mutation was demonstrated on muscle biopsy, and heteroplasmy was 60%. Urine heteroplasmy was assessed in one dialysis patient (case 1) and in two renal transplant recipients (cases 3 and 4) and reached 5% in all, a figure similar to blood lymphocyte heteroplasmy.

Increased plasma lactate concentration was found in three tested patients (cases 1, 2, and 7), ranging from 2.3 to 4 mmol/L (n < 1.5).

Report of Clinical Cases
Renal and nonrenal manifestations are summarized in Tables 1 to 3. Body mass index ranged from 13.5 to 23.8 kg/m² (mean, 18.4). None of the patients exhibited hypokalemia, hypophosphatemia, or hypouricemia that would have suggested a complete Fanconi syndrome.

Case 1 (Sporadic Case).
A 50-yr-old woman was referred for polycystic kidney disease and chronic renal failure. Since age 25, she had bilateral sensorineural deafness requiring hearing aid (Table 1). Type 2 diabetes was recognized at age 46 and was initially treated with sulfonylurea. Hypertrophic cardiomyopathy was diagnosed, along with easily controlled hypertension. At referral, physical examination disclosed bilateral palpable kidneys. Fundoscopy was normal. There was no relevant family history. Creatinine clearance was 15 ml/min with full-blown nephrotic syndrome (serum albumin 2.2 g/dl) (Table 2). Abdominal CT showed large-sized kidneys with multiple cortical cysts (Figure 1) without liver cyst. A renal biopsy
was performed (Table 3). On echocardiography, interventricular septum and the posterior wall were 20 mm and 18 mm, respectively. The patient started renal replacement therapy at age 51.

**Case 2 (Familial Case).** A 41-yr-old woman was referred for end-stage renal failure and mild proteinuria. Bilateral sensorineural deafness required hearing aid since age 36. Ultrasound (US) scan showed bilateral small-sized kidneys. A diagnosis of Alport syndrome was retained since her sister also complained of deafness. Regular hemodialysis was started. Six months later, she presented transient ischemic attack. At age 43, she received a kidney graft. Three years later, she devel-

<table>
<thead>
<tr>
<th>Patient</th>
<th>Deafness</th>
<th>Neuromuscular Manifestations</th>
<th>Diabetes Mellitus</th>
<th>Macular Dystrophy</th>
<th>Cardiomyopathy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-22</td>
<td>—</td>
<td>-1</td>
<td>—</td>
<td>-1</td>
</tr>
<tr>
<td>2</td>
<td>-5</td>
<td>0</td>
<td>+5</td>
<td>+5</td>
<td>—</td>
</tr>
<tr>
<td>3</td>
<td>-6</td>
<td>-5</td>
<td>+2</td>
<td>—</td>
<td>+4</td>
</tr>
<tr>
<td>4</td>
<td>-15</td>
<td>+14</td>
<td>+12</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5</td>
<td>-3</td>
<td>—</td>
<td>+5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>6</td>
<td>-5</td>
<td>—</td>
<td>+1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>7</td>
<td>+5</td>
<td>+12</td>
<td>+12</td>
<td>+12</td>
<td>+12</td>
</tr>
<tr>
<td>8</td>
<td>-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>+6</td>
</tr>
<tr>
<td>9</td>
<td>-16</td>
<td>-1</td>
<td>-2</td>
<td>+7</td>
<td>+6</td>
</tr>
</tbody>
</table>

* —, No involvement.

**Table 2.** Renal findings in nine patients with A3243G mtDNA mutation at initial evaluation and last follow-up*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at First Renal Evaluation (yr)</th>
<th>Age at Onset of Proteinuria (yr)</th>
<th>Pu (g/d)</th>
<th>Nephrotic Syndrome</th>
<th>Ccr (ml/min)</th>
<th>Renal Pathology</th>
<th>Duration of Follow-Up (yr)</th>
<th>Outcome at Last Follow-Up (age, yr)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>47</td>
<td>3</td>
<td>+</td>
<td>15</td>
<td>FSGS + cysts</td>
<td>1</td>
<td>ESRF (51)</td>
</tr>
<tr>
<td>2</td>
<td>41</td>
<td>41</td>
<td>2</td>
<td>+</td>
<td>&lt;10</td>
<td>ND</td>
<td>6</td>
<td>ESRF (41) - KT (43)</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>32</td>
<td>3.7</td>
<td>-</td>
<td>36</td>
<td>ND</td>
<td>7</td>
<td>ESRF (36) - KT (39)</td>
</tr>
<tr>
<td>4</td>
<td>42</td>
<td>30</td>
<td>1</td>
<td>-</td>
<td>&lt;10</td>
<td>ND</td>
<td>5</td>
<td>ESRF (42) - KT (42)</td>
</tr>
<tr>
<td>5</td>
<td>33</td>
<td>27</td>
<td>1.5</td>
<td>-</td>
<td>72</td>
<td>TIN</td>
<td>2</td>
<td>Ccr: 70 ml/min (35)</td>
</tr>
<tr>
<td>6</td>
<td>35</td>
<td>34</td>
<td>1.2</td>
<td>-</td>
<td>110</td>
<td>ND</td>
<td>0</td>
<td>Ccr: 110 ml/min (35)</td>
</tr>
<tr>
<td>7</td>
<td>14</td>
<td>5</td>
<td>&gt;3</td>
<td>-</td>
<td>30</td>
<td>FSGS</td>
<td>5</td>
<td>ESRF (15) - KT (17)</td>
</tr>
<tr>
<td>8</td>
<td>24</td>
<td>24</td>
<td>1</td>
<td>-</td>
<td>69</td>
<td>TIN</td>
<td>10</td>
<td>Ccr: 56 ml/min (34)</td>
</tr>
<tr>
<td>9</td>
<td>47</td>
<td>41</td>
<td>1</td>
<td>-</td>
<td>35</td>
<td>TIN</td>
<td>4</td>
<td>Ccr: 20 ml/min (51)</td>
</tr>
</tbody>
</table>

* Pu, proteinuria; Ccr, creatinine clearance; FSGS, focal and segmental glomerulonephritis; ND, not done; TIN, tubulointerstitial nephropathy; ESRF, end-stage renal failure; KT, kidney transplantation.

**Table 3.** Renal pathology in five patients with A3243G mutation: diagnoses and semiquantitative lesion scoring*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Number of Glomeruli</th>
<th>Global Sclerosis</th>
<th>Segmental Sclerosis</th>
<th>Location of Segmental Sclerosis</th>
<th>Podocyte Hypertrophy</th>
<th>Tubulointerstitial Changes</th>
<th>Arteriolar Hyaline Thickenings</th>
<th>Arterial Fibrous Intimal Thickening</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>6 (60%)</td>
<td>3 (30%)</td>
<td>Parahilar, peripheral</td>
<td>+</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>7</td>
<td>3 (43%)</td>
<td>0 (0%)</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>4</td>
<td>0 (0%)</td>
<td>1 (25%)</td>
<td>Parahilar</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>10</td>
<td>1 (10%)</td>
<td>0 (0%)</td>
<td>—</td>
<td>+</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>4</td>
<td>3 (75%)</td>
<td>0 (0%)</td>
<td>—</td>
<td>—</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

* Kidney specimens were analyzed by light microscopy, using histologic sections at 3 to 4 microns and staining with hematoxylin and eosin, and PAS or silver. Grading of severity of interstitial or vascular fibrosis relied on the Banff 97 working classification (18).

*0, No; 1, mild; 2, moderate; 3, severe.
opposed type 2 diabetes requiring sulfonylurea. Fluorescein angiography showed a bilateral macular pattern dystrophy without diabetic retinopathy. Four years posttransplant, serum creatinine was 63 μmol/L.

**Case 3 (Familial Case).** This 32-yr-old man was referred for heavy proteinuria. He suffered from muscle fatigability and severe bilateral sensorineural deafness. His sister also had renal failure and deafness (see case 4). BP was normal. Serum creatinine was 204 μmol/L and serum albumin 4.2 g/dl. Both kidneys were small on US scan. At age 33, a hearing aid was requested. One year later, type 2 diabetes was diagnosed and treated by diet only. Ophthalmologic examination was normal. There were no anti-islet antibodies. At age 36, when renal replacement was initiated, echocardiography revealed hypertrophic cardiomyopathy (interventricular septum, 17 mm; posterior wall, 16 mm). After kidney engraftment performed at age 39, glucose control required insulin therapy.

**Case 4 (Familial Case).** The sister of patient 3 was referred for end-stage renal failure and bilateral deafness at age 42. Mild proteinuria had been recognized since age 30. By US scan, both kidneys were small. Mild hypertension was treated with enalapril. Three weeks posttransplant she developed type 2 diabetes requiring insulin therapy. Funduscopy was normal. There were no anti-islet antibodies. At age 44, she presented with transient ischemic stroke. Cerebral MRI revealed bilateral basal ganglia calcifications and cerebral white-matter abnormalities (Figure 3). Five years posttransplantation, serum creatinine was 92 μmol/L.

**Case 5 (Familial Case).** This 33-yr-old man was referred for persistent proteinuria and mild renal failure. BP was normal. Bilateral neurosensory deafness was recognized at age 24 and required a hearing aid at age 33. Type 2 diabetes was diagnosed at age 32. Fluorescein angiography was normal. Glicazide was started. Three relatives were clinically affected (for sib, see below): his mother had type 2 diabetes, hearing loss, hypertrophic cardiomyopathy, macular pattern dystrophy, and the need for dialysis at age 58; her brother also had diabetes mellitus, deafness, and stroke. In the proband, US scan revealed normal-sized kidneys, and a renal biopsy was performed.

**Case 6 (Familial Case).** The sister of patient 5 was a 35-yr-old woman referred for preeclampsia at 23 wk of gestation. Bilateral deafness required hearing aid. She had experienced four prior pregnancies terminated by unexplained miscarriage or fetal death before 25 wk of gestation. Proteinuria preceded the latter pregnancy for 1 yr. Diabetes mellitus had developed early in pregnancy, requiring insulin therapy. Serum creatinine at admission was 45 μmol/L.

**Case 7 (Sporadic Case).** This 14-yr-old girl was referred for chronic renal failure. Proteinuria was known since the age of 5. She also suffered from bilateral neurosensory hearing loss. On referral, BP was normal, creatinine clearance was 30 ml/min, and serum albumin was 3.4 g/dl with heavy proteinuria. Renal biopsy evidenced FSGS unresponsive to steroid therapy. The patient required hemodialysis 1 yr later. At age 17, early posttransplant diabetic ketoacidosis required insulin therapy. Ophthalmologic examination revealed bilateral macular dystrophy. Hypertrophic cardiomyopathy was also found. Five months later, the patient developed seizures. Magnetic resonance imaging (MRI) demonstrated left occipital stroke, bilateral basal ganglia calcifications, cerebellar atrophy, and enlargement of the ventricles. Lactate level was increased in cerebrospinal fluid. At age 19, the patient needed a hearing aid, and her serum creatinine was 93 μmol/L.

**Case 8 (Familial Case).** This 24-yr-old man with bilateral deafness was referred for Wernicke’s aphasia and seizures. His mother had type 2 diabetes. His sister was also affected (see case 9). In the proband, MRI of the brain showed left temporoparietal infarction. Type 2 diabetes was incidentally diagnosed. There were no anti-islet antibodies. Ophthalmologic examination revealed bilateral macular dystrophy. Proteinuria and mild chronic renal failure with normal-sized kidneys were also found. BP was normal. A renal biopsy was performed. Following recurrent seizures at age 30, MRI disclosed basal ganglia calcifications, with diffuse atrophy of cerebral and cerebellar cortices and enlargement of the ventricles. Electroencephalography study demonstrated sensorimotor polyneuropathy. Muscle biopsy showed a nonspecific heterogeneity of fiber size, with normal mitochondria by electron microscopy. Hypertrophic cardiomyopathy was diagnosed. At age 34, his serum creatinine was 125 μmol/L.

**Case 9 (Familial Case).** The sister of patient 8 had bilateral neurosensory deafness. Her daughter died age 9 with unexplained neurologic manifestations. At age 39, type 2 diabetes and hypertension were found. One year later, following transient ischemic stroke, MRI showed cerebral white-matter abnormalities and bilateral basal calcifications. At age 47, she was referred for chronic renal failure. Serum creatinine was 180 μmol/L. BP was 120/60 mmHg while on antihypertensive therapy. Echocardiography disclosed hypertrophic cardiomyopathy (interventricular septum thickness, 15 mm; posterior wall, 13 mm). US-scan showed normal-sized kidneys prompt-
ing renal biopsy. A few months later, the patient presented with recurrent stroke. Funduscropy showed bilateral macular dystrophy. Muscle biopsy was not suggestive of mitochondriopathy, even by electron microscopy. However a 60% rate of heteroplasmy for the A3243G mtDNA mutation was found. At age 51, creatinine clearance was 20 ml/mn.

**Histopathologic Findings**

Renal biopsy specimens were available in five patients (Table 3). By light microscopy, none exhibited diabetes-related glomerulopathy. In two patients, FSGS was the most striking lesion. In the remaining cases, severe tubulointerstitial nephropathy was obvious. Mild to severe arteriolar hyaline thickening and fibrous intimal thickening of interlobular arteries were found in all but one case. Immunofluorescence study was unremarkable. Electron microscopy examination disclosed an increased number of enlarged mitochondria with abnormal cristae in some of the podocytes, including foot processes (cases 1 and 5; Figure 2B) and/or in proximal tubular cells (cases 5 and 9; Figure 2C), but not in mesangial, and endothe-
lial cells, nor in smooth muscle cells of the renal arteries. In two cases, electron microscopy failed to disclose abnormal mitochondria. Last, no cyst was evidenced on renal biopsy in case 1. Enzyme measurement assay and functional histochemical stains failed to show COX-deficient activity within kidney homogenate or even COX-deficient cells.

Discussion

Extrarenal manifestations are efficient tools for diagnosing many renal disorders, including inherited conditions. Among the latter, mitochondrialopathies have recently emerged as protein disorders. However, the full spectrum of renal and extrarenal manifestations has yet to be reported in great detail in adulthood. As suggested in Table 1, phenotypes tended to be similar among our nine patients. All of them experienced deafness, and diabetes mellitus was consistently found. Pooling our data with previous series in adults with kidney involvement, 20 of 24 patients suffered hearing loss (4,13–17). In all but one of our patients, deafness predated renal involvement with an interval ranging from 3 to 22 yr. Distinctive features include onset at a young age, bilateral high frequencies involvement in the early stage (22), and progressive worsening with many patients requiring a prosthetic hearing aid before 35 yr of age. In addition, deafness usually developed before the onset of diabetes. Since deafness was associated with hereditary nephritis, a diagnosis of Alport syndrome was long assumed in three of four patients in Jansen’s series (4) and in two of our patients lacking renal biopsy, until a firm identification of A3243G mutation. Microscopic hematuria may help differentiate between the two disorders, because it is a constant finding in the former but is absent in the latter. In addition, hearing loss is generally stable and nonprogressive in Alport adult patients.

Diabetes mellitus was diagnosed in 12 of the 15 adult patients previously studied (4,13–17). In our series, all the patients developed type 2 diabetes between 17 and 46 yr of age. However diabetes was a late manifestation, diagnosed up to 12 yr after recognition of kidney disease. None of our patients had diabetic glomerulopathy on kidney biopsy. In addition, none was overweight. Initially, only two out of eight nonpregnant patients required insulin therapy. Positive family history of diabetes was documented in 6 of 9, and islet-cell antibodies were absent in 3 of 3 tested individuals. After recognition of diabetes, follow-up ranged from 1 to 12 yr (mean, 5 yr) and no diabetic retinopathy was detected. In a French cohort of 54 diabetic patients with A3243G-related diabetes, the prevalence of diabetic retinopathy was extremely low, reaching only 8% after 12 yr follow-up, while the corresponding figure is 30 to 75% in the common forms of type 2 diabetes. Also in this group, only 13% of cases required insulin therapy at diagnosis (23).

A distinctive feature of ophthalmologic involvement consisted of the macular dystrophy observed in four of our patients. Macular dystrophy is characterized by a linear pigmentation surrounding the macula and the optic disc. Crude prevalence of such retinal lesion reached 86% in the French series of patients with A3243G-related diabetes (24). Visual acuity is usually not affected. Recognizing macular dystrophy plays a key role in diagnosing mitochondrialopathy, and ruling out alternative diagnoses, including Alport syndrome.

Muscular involvement was much less severe compared with the extreme disability encountered in the MELAS patients. Only one of nine patients developed severe muscular atrophy. In contrast, five patients had cardiomyopathy, including one presenting with congestive heart failure and four others having symmetrical severe left ventricular hypertrophy on echocardiography. None had atrioventricular block or Wolff-Parkinson-White syndrome. Hypertrophic cardiomyopathy has been reported in mitochondrialopathy, including rare cases of A3243G mtDNA mutation (25,26).

Stroke-like episodes belong to the spectrum of MELAS. In this series, four episodes occurred in four patients younger than 45 yr of age, without sequelae. In addition, seizures developed in two patients. In young adults below 45 yr of age, the A3243G mutation accounts for 1% of all strokes, and up to 6% of occipital brain infarcts (27). Imaging studies by CT-scan or MRI mimic ischemic stroke, although they do not necessarily conform to vascular territories. Bilateral basal-ganglia calcifications are common findings on CT. Pathophysiology of vascular obstruction remains unknown, although accumulation of altered mitochondria in smooth muscle and endothelial cells of small arteries probably plays a role (28). Secondary prevention with aspirin is therefore questionable in these patients.

In childhood, a large spectrum of renal involvement has been found associated with mitochondrialopathy, occurring either as a presenting manifestation or late in the course of a
multisystemic disorder (5,29). Proximal tubular dysfunction with de Toni-Debré-Fanconi syndrome is the most frequent presentation (5). Less often, chronic tubulointerstitial nephritis (20,30–33) or isolated renal tubular acidosis (34) and even Bartter-like syndrome (35) have been reported. A minority of children presented with the A3243G mutation and heavy proteinuria related to focal and segmental glomerulosclerosis (36–39). In adulthood, the A3243G mutation is so far the unique mtDNA mutation responsible for kidney disease. In two studies of Japanese diabetic patients on regular dialysis, its prevalence reached 0.8 and 5.9%, respectively (40,41). In a French diabetic population, renal involvement of various severities was found in 28% of 54 patients with A3243G mutation after a 12-yr follow-up (22). Last, in nondiabetic patients with non-nephrotic FSGS, the A3243G mutation was found in four of seven cases, but in none of 25 nephrotic FSGS (16).

In addition to our experience in nine patients, a comprehensive description of kidney involvement is available for 15 adult cases issued from three short series (4,15–16) and three sporadic cases (13–14,17) thus totaling 24 patients (Table 4). Two additional reports lack accurate details for proper assessment (26,42). Female predominance is striking, with a 19:5 female: male ratio (79%). Renal involvement was found to occur in teenagers and young adults, leading to first evaluation at a median age of 32 yr (range, 14 to 50 yr). Of note, kidney abnormalities were the first manifestation of disease in one third of these patients. At presentation, proteinuria averaged 1.5 g/d (range, 0.07 to 5 g/d), with only two patients having nephrotic syndrome. No hematuria was found. BP fell within normal range in 66% of the cases, even at late stage of renal decline. None of these adult patients exhibited tubular dysfunction. Renal imaging at presentation was unremarkable except in one case of our series exhibiting multiple cysts and enlarged kidneys mimicking polycystic kidney disease. Apart from the four patients identified after kidney engraftment by Jansen et al., 11 (55%) of 20 had creatinine clearance above 60 ml/min at presentation, while seven had moderate or severe renal failure and two required prompt renal replacement therapy. After a mean follow-up of 5 yr (1–17), seven additional patients progressed to end-stage renal failure. Altogether, renal replacement therapy was started at a median age of 33 yr (15 to 51 yr). However two of our patients reached the sixth decade without end-stage renal failure.

Renal biopsy has been performed in 19 of 24 patients. Pathologic findings never were the first means for diagnosing mitochondrialopathy, but ruled out diabetic glomerulopathy in all cases. They fall into four categories (1) In 79% of cases, FSGS was the most striking feature (4,13–17). So far there was no evidence that light microscopy may help for distinguishing among A3243G-related and primary FSGS. By electron microscopy, podocytes exhibited accumulation of abnormal mitochondria with variations in size and shape, and disarrangement of cristae. In our experience, the abnormal finding of mitochondria in foot processes of podocytes could be pathognomonic of A3243G-related FSGS. However, some individuals lack detectable mitochondrial abnormalities at the ultrastructural level. In addition, mitochondria of mesangial and endothelial glomerular cells were never found to be altered. (2) Severe hyaline changes within cytoplasm of smooth muscle cells of afferent arterioles and small arteries have been pointed out by Moulonguet-Doleris et al. They reported on a pattern of necrotic myocyte similar to cyclosporin nephropathy (15). Such mitochondrial angiopathy was not found by Hotta et al. (16) or in our patients. (3) Three of our patients had nonspecific tubulointerstitial lesions in the absence of FSGS. Both proximal and distal tubules were atrophic. There was no interstitial cell proliferation, but mitochondrial accumulation in tubular cells was observed in two of three cases (cases 5 and 9). Similar tubulointerstitial involvement has been reported in children with multisystemic mitochondrialopathy unrelated to the A32443G mutation (20,30–33). (4) One of our patients had renal cysts mimicking polycystic kidney disease. Whether it is a chance association with unrelated cystic disorder or it expands the pathologic spectrum of A3243G-related nephropathy cannot be assessed because no microscopic cyst was available for electron microscopy or COX:SDH stains on renal biopsy. So far, microscopic cysts have been reported in a single child exhibiting glomerular cystic disease associated with another mitochondrialopathy (43).

The mechanisms accounting for kidney heterogeneity remain uncertain. Interestingly, in one of the few mouse models of mitochondrial diseases, most mice died of renal failure (44).

Table 4. Renal involvement in three prior series and three case reports of adult patients with A3243G mtDNA mutation

<table>
<thead>
<tr>
<th>Series (Ref)</th>
<th>Number of Patients</th>
<th>Age at Onset of Proteinuria (yr)</th>
<th>Nephrotic Syndrome</th>
<th>Renal Pathology</th>
<th>Number and Age (yr) of Patients on ESRF at Last Follow-Up</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jansen (4)</td>
<td>4</td>
<td>26 (17 to 35)</td>
<td>NA</td>
<td>2</td>
<td>1 (lobular GN)</td>
</tr>
<tr>
<td>Moulounguet (15)</td>
<td>4</td>
<td>20 (18 to 23)</td>
<td>NA</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Hotta (16)</td>
<td>4</td>
<td>14 (10 to 17)</td>
<td>0</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Kurogouchi (13)</td>
<td>1</td>
<td>18</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Nakamura (14)</td>
<td>1</td>
<td>23</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Hirano (17)</td>
<td>1</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Present series</td>
<td>9</td>
<td>31 (5 to 47)</td>
<td>1</td>
<td>2</td>
<td>3 (TIN)</td>
</tr>
</tbody>
</table>

* FSGS, focal and segmental glomerulosclerosis; NA, not available; GN, glomerulonephritis; TIN, tubulointerstitial nephropathy; ESRF, end-stage renal failure.
In this model, the mutant mtDNA carried a 4696-bp deletion. Although comprehensive description of kidney lesion is not available, proximal and distal tubular dilatation was a prominent feature. In human, reduced energy production in epithelial cells may easily account for impaired tubular function. In contrast, the mechanism selectively responsible for podocyte injury remains speculative. According to the implication of mitochondrial machinery in free radicals generation and apoptosis (6), podocyte death might result from dysregulation of either pathway. The generation of animal model of FSGS-related mitochondrialopathy would be a valuable tool. The role of heteroplasmy has also been hypothesized to account for tissue involvement. Blood lymphocyte heteroplasmy is unlikely to predict renal involvement. In our patients, it ranged between 5 and 25%, while in prior series the corresponding figures were 6 to 34%, 18 to 28%, and 49 to 59%, respectively (4,15–16). Kidney heteroplasmy was measured in two patients in Moulonguet’s series, reaching 55 and 66% respectively (15). However the relevance of these data are questionable, since no control was provided (A3243G patients without nephropathy). Moreover, urine sediment heteroplasmy does not provide accurate estimation of renal involvement, because we could demonstrate that the A3243G mutation is present in renal transplant patients. This is not unexpected because most urinary cells originate from the lower urinary tract.

In the patient with renal involvement, what should be the approach to diagnose A3243G-related disease? Family history is valuable when there is evidence of maternal inheritance. Extrarenal findings including concomitant deafness, diabetes with macular dystrophy, neuromuscular involvement, or hypertrophic cardiomyopathy should suggest the diagnosis. Should kidney involvement be the unique presenting manifestation, making the diagnosis is extremely difficult. Blood lactate concentration and lactate:pyruvate ratio may be increased at rest or after exercise. Depending on clinical presentation, increased lactate levels in cerebrospinal fluid or specific changes (red-ragged fibers) on muscle biopsy may also be useful. As exemplified in one of our patients, normal functional histochemical staining on kidney tissue (COX:SDH ratio) does not rule out mitochondrialopathy. Hence, in the adult patients, we would argue for performing a straightforward molecular diagnosis of the A3243G mutation in leukocytes or urine and if negative in any affected tissue. In addition, further studies should prospectively assess the prevalence of the renal-limited form of the disease, possibly focusing on non-nephrotic FSGS, or familial or steroid-resistant FSGS, or unexplained tubulointerstitial nephropathy.

How should we treat renal involvement in the A3243G patients? So far, neither preventive therapy nor a cure is available for improving clinical involvement in mitochondrial cytopathy, although anecdotal reports documented potential benefits (45). More specifically, corticosteroid therapy was ineffective on the course of proteinuria in all six patients who received this treatment (case 7 in this series; 15–16). We therefore strongly advocate against its use in the patients with A3243G-related FSGS. Nephroprotective approaches including BP control and lowering proteinuria remains mandatory.

Renal transplantation has been successfully performed in four of our patients and six previous cases (4,15). As expected, no recurrence of the disease was observed on kidney graft. It is unknown whether immunosuppressive therapy hastens the clinical course of extrarenal manifestations. However it should be highlighted that six of six nondiabetic patients developed diabetes mellitus early posttransplant. In addition, two patients presented stroke-like episodes within 3 yr (case 4 in this series; 15). Renal transplantation from related donors may deserve discussion; there is no specific contraindication for the father to be the donor. In contrast, the A3243G mutation should be definitely ruled out when considering other potential donors, including the mother or siblings.

In conclusion, the present study shows that in the adult patients with A3243G mutation, renal involvement is unlikely to be the presenting manifestation of mitochondrial cytopathy. Preexisting deafness is almost consistently found. Diabetes mellitus, macular dystrophy, hypertrophic cardiomyopathy, or neuromuscular features occur later in the course of the disease. Moreover, the renal spectrum is wider than previously thought. While FSGS remains the most typical lesion, we observed that tubulointerstitial nephropathy and bilateral enlarged cystic kidneys may be encountered. The severity of the clinical course is heterogeneous, with severe renal failure being reached at between the second and the sixth decade. Renal transplantation may be offered to these patients, although steroid therapy increases the risk of developing diabetes mellitus.

Acknowledgments

We gratefully thank Dr. Raifat Mkdassi, Dr. Hamid Netfi, and Dr. Pierre-François Westeel for providing clinical data, Dr. Carole Cordonnier, Dr. Jacqueline Champigneulle, and Dr. Marie-Claire Gubler for valuable discussions on pathologic findings.

References


Access to UpToDate on-line is available for additional clinical information at http://www.jasn.org/