Mitochondrial DNA Mutations in Focal Segmental Glomerulosclerosis Lesions

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Abstract. Glomerular epithelial cells are primary pathogenic sites in focal segmental glomerulosclerosis (FGS) lesions. Glomerular epithelial cells are regarded as terminally differentiated cells that do not proliferate. These characteristics are also noted for neurons and muscular cells, which are major sites of mitochondrial DNA (mtDNA) mutation accumulation. Screening for mtDNA mutations was performed with renal biopsy specimens from patients with primary FGS and patients with IgA nephropathy (as subjects with secondary FGS and as control subjects). mtDNA extracted from kidney biopsy specimens was amplified with appropriate primer pairs for study of the mtDNA point mutations 3243A→G, 3271T→C, 8344A→G, and 8993T→G/C, as well as the common deletion (a 4977-bp deletion spanning mtDNA nucleotide pairs 8469 to 13447). *In situ* amplification of both total mtDNA and the common deletion was also performed. Two patients with FGS demonstrated the 3243A→G point mutation; 12 patients with FGS and seven patients with IgA nephropathy accompanied by glomerulosclerotic lesions exhibited the common deletion in their kidney tissue. No patient demonstrated the mtDNA mutations 3271T→C, 8344A→G, or 8993T→G/C. The degree of heteroplasmy for the 3243A→G point mutation was >85%; however, the heteroplasmy for the common deletion was <1%. As determined with *in situ* PCR, normal mtDNA was mainly distributed in the tubular epithelium and mtDNA with the common deletion was mainly distributed among glomerular epithelial cells. In conclusion, it is suggested that mtDNA mutations are distributed in glomerular epithelial cells among some patients with primary FGS or secondary FGS with IgA nephropathy. These mutations may be related to glomerular epithelial cell damage.

Focal segmental glomerulosclerosis (FGS) lesions represent the final common pathway for nephron degeneration in many forms of chronic progressive renal failure (1). The initial pathologic changes in FGS are thought to occur in glomerular epithelial cells (1,2). Renal epithelial cell damage attributable to mitochondrial dysfunction in congenital nephrotic syndrome was recently reported (3,4). Furthermore, glomerular involvement of an A→G transition at mitochondrial DNA (mtDNA) position 3243 in the gene for tRNA[Leu] yielded minor glomerular abnormalities (5) or FGS (6–9).

mtDNA is a 16,569-bp closed circular duplex and is the only extranuclear DNA in the human species. mtDNA encodes 13 respiratory chain polypeptides, two rRNA, and 22 tRNA (10,11). mtDNA is prone to oxidative damage (12), because it lacks histone-like coverage and is very close to the inner mitochondrial membrane, which is the major intracellular source of reactive oxygen species (13–15). Furthermore, accumulation of age-dependent mtDNA mutations has been reported (15,16); in particular, a 4977-bp deletion spanning mtDNA nucleotide pairs 8469 to 13447 (the common deletion) has frequently been reported (17–19). Age-dependent accumulation of an A→G transition at mtDNA position 3243 has also been reported (20–22). Blood cells often exhibit low levels of mutated mtDNA, with mutations such as the common deletion and the 3243A→G transition (23–25). In general, glomerular epithelial cells are regarded as terminally differentiated cells that do not proliferate (26–28). These characteristics are also noted for neurons and muscular cells, which are regarded as major sites for the accumulation of mtDNA mutations. In this study, to clarify the relationship between FGS lesions and mtDNA mutations, we performed mtDNA mutation screening of kidney biopsy specimens and studied the *in situ* accumulation of mtDNA mutations in glomerular epithelial cells.

Materials and Methods
Kidney Samples and DNA Isolation
Renal tissues from patients with primary FGS or IgA nephropathy were obtained in kidney biopsies performed at the University of

Tsukuba or one of its affiliated hospitals. The patients were assigned to one of three groups, *i.e.*, (*1*) primary FGS (16 patients), (*2*) familial FGS (seven patients with maternally inherited renal diseases and/or neurologic diseases), or (*3*) IgA nephropathy (16 patients). The characteristics of the patients are presented in Table 1. All patients were accepted with written informed consent. The indications for renal biopsy were as follows: for 11 patients with primary FGS and one patient with IgA nephropathy, nephrotic syndrome; for the other patients, close examination of chance proteinuria and/or hematuria. The kidney biopsy specimens were processed for light microscopy, immunofluorescence assays, and electron microscopy by using routine methods. Total DNA was extracted from paraffin-embedded samples by using DEXPAT (Takara Biomedicals Inc., Tokyo, Japan), following the recommendations of the manufacturer.

Qualitative PCR

Regions of the mitochondrial genome between positions 3130 and 3301, positions 8257 and 8386, and positions 8837 and 9017 were amplified by PCR with a GeneAmp PCR 9600 system (Perkin-Elmer, Norwalk, CT). PCR amplifications were performed in 50-μl volumes with 200 μM levels of each dNTP, 0.5 μM levels of each primer, 1 U of Ex Taq polymerase (Takara Biomedicals), PCR reaction buffer (Takara Biomedicals), and 200 ng of each DNA template. The primer sets used in this study are indicated in Table 2. The amplification conditions included 35 cycles of denaturation at 94°C for 30 s, annealing at 55 to 57°C for 30 s, and extension at 72°C for 1 min, with an initial 3-min denaturation step at 94°C. The amplified fragments were digested with restriction endonucleases (*Apa*I, *Afl*II, *Bgl*I, or *Hpa*II; Takara Biomedicals). The digested DNA fragments were separated on 8% polyacrylamide gels, and the gels were stained with SYBR Green I nucleic acid stain (Takara Biomedicals). For detection of the common deletion (a 4977-bp deletion between positions 8482 and 13459), the common deletion primer set (Table 2) was used. The amplification conditions included 30 cycles of denaturation at 94°C for 30 s and annealing at 55°C for 30 s, using Ampri Wax PCR Gem 50 (Perkin-Elmer) for hot-start PCR, according to the instructions provided by the manufacturer.

Quantitative PCR

The frequency of the A→G mutation at nucleotide position 3243 of mtDNA was determined by colony-directed PCR and restriction fragment length polymorphism analysis of subcloned PCR products from >40 randomly selected colonies in each specimen. Subcloning of the PCR products was performed by using an Original TA cloning kit (Invitrogen, San Diego, CA), according to the instructions provided by the manufacturer, as follows. Fresh PCR products were ligated into pCR2.1 vector, transformed into One Shot cells, and incubated overnight at 37°C. More than 40 growth colonies were selected, and

colony-directed PCR and restriction fragment length polymorphism analyses were performed.

The frequency of the common deletion was determined in triplicate by using a Perkin-Elmer 7700 TaqMan PCR system. The forward primer for the mitochondrial common deletion was 5'-CCCCCATACTCCTTACACTA-3' (positions 8406 to 8425), and the reverse primer was 5'-TGCGGTTTCGATGATGTGGT-3' (positions 13533 to 13514). The probe was FAM-CCTACCTCCCTCACCAT-TGGCAGCCTAG-TAMRA (positions 8467 to 8482 and13459 to 13471). For total mtDNA, the primers were 5'-GACGAGCTAC-CTAAGAACAG-3' (forward; positions 1913 to 1932), 5'-GAGGGT-TCTGTGGGCAAATT-3' (reverse; positions 2070 to 2051), and FAM-CGACAAACCTACCGAGCCTGG-TAMRA (probe; positions 1989 to 2009). The amplification conditions included 50 cycles of denaturation at 95°C for 15 s and annealing at 53°C for 60 s, with an initial 10-min denaturation step at 95°C. The deletion levels, expressed as percentages of both the common deletion and the total mtDNA level, were calculated from linear-range amplification plots of the sequence detector v1.6 (Perkin-Elmer). Extrapolation of the plots to the zero-amplification cycle yielded the relative amounts of mtDNA before the PCR amplification.

In Situ PCR

In situ PCR was performed as described previously (29), with adjustments as follows. Paraffin-embedded, 5-μm, kidney biopsy sections were used. The slides were deparaffinized and rehydrated by using standard protocols. The slides were then transferred into phosphate-buffered saline (PBS) (pH 7.4) and briefly equilibrated. The tissue sections were permeabilized by a 10-min treatment with proteinase K (10 μg/ml) in 100 mM Tris-HCl (pH 7.5), 5 mM ethylenediaminetetraacetate, in a humidified chamber, and were boiled in citrate buffer (pH 6.0) with microwave exposure. The slides were transferred into cold PBS for at least 5 min and were refixed with 4% paraformaldehyde in PBS. The slides were removed from the PBS and carefully spot-dried around the tissue sections with Kimwipes. PCR cocktail (50-μl reaction volume with final concentrations of 0.5 μM forward and reverse primers and 0.2 μM TaqMan probe, in 25 μl of TaqMan Universal PCR Master Mix; Perkin-Elmer) was applied to the slide, and a Takara slide seal (Takara Biomedicals) for *in situ* PCR under hot-start conditions was applied, to seal the reaction mixture over the tissue. The PCR profile consisted of an initial 2-min denaturation step at 50°C and 10 min at 95°C, typically followed by 28 to 40 cycles of denaturation at 94°C for 15 s and annealing at 53°C for 1 min, in a thermal cycler (PTC-100-16MS; MJ Research, Watertown,glomerulosclMA). *In situ* amplification of both total mtDNA and the common deletion region was observed with confocal laser microscopy (TCS SP2; Leica, Wetzlar, Germany). In control experiments, PCR cocktails omitting both forward and reverse primers were used for each *in situ* PCR amplification.

Table 1. Characteristics of the patients[a]

Classification	n	Median Age (Range) (yr)	Male (%)	Nephrotic-Range Proteinuria (%)	Median Serum Creatinine Concentration (Range) (mg/dl)
Primary FGS	17	31 (14 to 84)	76.5	70.6	1.1 (0.6 to 8.5)
Familial FGS	7	32 (11 to 58)	42.9	14.3	0.9 (0.4 to 2.4)
IgA nephropathy	16	46 (13 to 73)	56.3	12.5	0.9 (0.5 to 1.7)

[a] FGS, focal segmental glomerulosclerosis.

Table 2. Primers used in this study

Mutation	Sequence	Amplification Products (bp)	Endonuclease	Mutant Fragments (bp)
3243A→G	5′-AGGACAAGAGAAATAAGGCC-3′ 5′-CACGTTGGGGCCTTTGCGTA-3′	294	*Apa*I	184 + 112
3271T→C	5′-AGGACAAGAGAAATAAAGGC-3′ 5′-TAAGAAGAGGAATTGAACCTCTGACCTTAAA-3′	170	*Afl*II	140 + 30
8344A→G	5′-AGCCCACTGTAAAGCTAACT-3′ 5′-TTGGGGCATTTCACTGTAAAGCCGTGTTGGT-3′	167	*Bgl*I	134 + 30
8993T→G/C	5′-GGCCATCCCCTTATGAGCGG-3′ 5′-ATGTTAGGCGGTTAGGCGTAC-3′	180	*Hpa*II	157 + 23
Common deletion	5′-ACTACCACCTACCTCCCTCACC-3′ 5′-GCTTGTCAGGGAGGTAGCGATG-3′			160

Statistical Analyses

Data were expressed as median values and ranges, because of the asymmetrical distribution of the data. The Mann-Whitney U test was used for comparisons. A P value of <0.05 was considered statistically significant.

Results

Screening for mtDNA Point Mutations

Of the patients with FGS, two patients with familial FGS demonstrated mtDNA 3243A→G point mutations (Figure 1). Both patients exhibited sensory hearing loss. One patient had a family history of hearing loss (his mother), and his mother demonstrated the same point mutation in her peripheral lymphocyte DNA sample. The other patient had a family history of end-stage renal disease (her mother, who died 8 yr earlier) and hearing loss (her mother and younger sister). Figure 2 presents the light-microscopic findings for a renal biopsy specimen from a patient with the 3243A→G point mutation. Typical segmental sclerotic changes and hyaline lesions were observed near the vascular pole. No vascular lesions were observed in the glomeruli. Cystic tubular dilations, tubular degeneration, and interstitial fibrosis were obvious in a serial renal biopsy specimen obtained from this patient 3 yr later. Figure 3 presents the electron-microscopic findings for a patient with the 3243A→G point mutation. Abnormally developed mitochondria were observed in the glomerular epithelium. Quantitative evaluation of wild-type and mutant mtDNA demonstrated that the proportions of mutant mtDNA were 88% for one patient and 85% for the other patient in kidney biopsy samples and 80 and 56%, respectively, in peripheral lymphocyte DNA samples. No patient demonstrated mtDNA 3271T→C, 8344A→G, or 8993T→G/C point mutations in the analyzed kidney biopsy samples (Table 3).
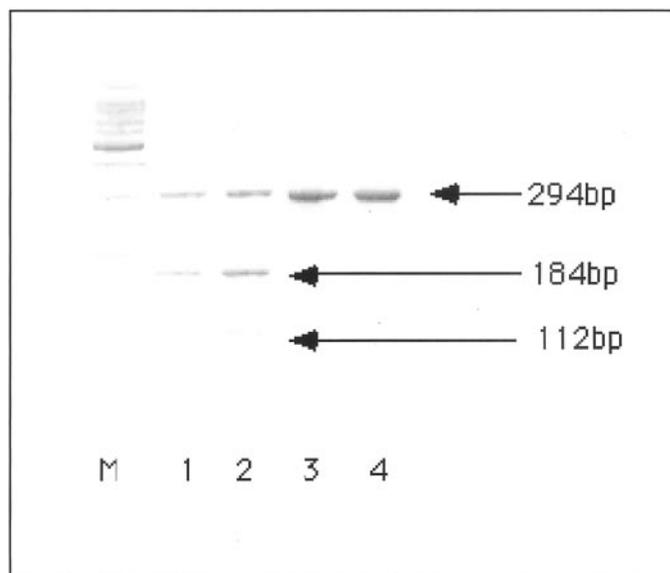

Figure 1. Mitochondrial DNA (mtDNA) fragments from patients with the 3243A→G point mutation and a normal control subject, amplified by PCR and digested with *Apa*I. M, 100-bp ladder; lanes 1 and 2, patients; lanes 3 and 4, normal control subject. The 184- and 112-bp products were observed for the patients; only a 294-bp product was observed for the other patients.
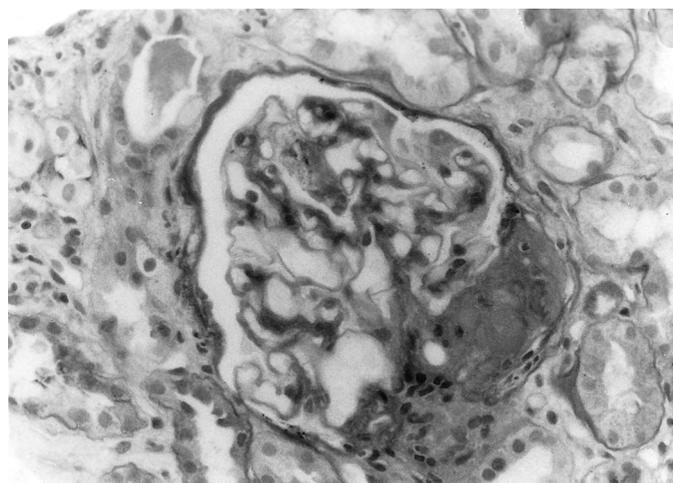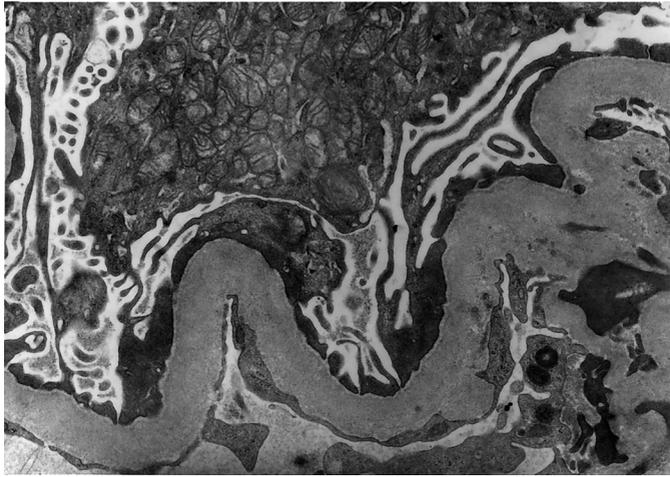

Figure 2. Light-microscopic findings for a patient with the 3243A→G mutation. Focal and segmental hyalinosis and sclerosis at the vascular pole were observed. Periodic acid-Schiff stain. Magnification, ×400.

Figure 3. Electron-microscopic findings for a patient with the 3243A→G mutation. Large and small mitochondria with abnormally developed cristae were observed in the cytoplasm of the glomerular epithelium.glomerulosclMagnification, ×10,000.

Screening for the mtDNA Common Deletion

The mtDNA common deletion was observed for nine patients with primary FGS, three patients with familial FGS, and seven patients with IgA nephropathy (Table 3). Figure 4 presents the amplification plots for total mtDNA and the common deletion, with real-time PCR. The degree of heteroplasmy for the common deletion was <1% for all patients (Table 3). Age-dependent accumulation of the common deletion was not observed in this study, and the common deletion was not related to renal function or proteinuria. The common deletion was not related to glomerular alterations in primary or familial FGS; however, the total numbers of segmental sclerotic glomeruli and glomeruli with adhesion of the capillary tuft to Bowman's capsule were significantly increased in IgA nephropathy with the common deletion (Table 4). Figure 5 presents the electron-microscopic findings for a patient with primary FGS and the common deletion. Accumulated dysmorphic mitochondria, including abnormally developed mitochondria, were observed in the glomerular epithelium. However, the other patients did not demonstrate epithelial mitochondrial abnormalities in extensive electron-microscopic examinations.

In Situ *Detection of the Common Deletion*

Figure 6 presents the *in situ* PCR results for total mtDNA. Most of the mtDNA signals were observed in the cytoplasm of the renal tubular epithelium. Figure 7 presents the *in situ* PCR results for the common deletion. The common deletion was observed mainly in the glomerular epithelium. In both experiments, neither mtDNA nor common deletion signals were detected when assays were performed with a PCR mixture omitting both forward and reverse primers (data not shown).

Discussion

FGS is morphologically characterized by segmental areas of sclerosis in some glomeruli. FGS lesions represent a heterogeneous category that includes several types of glomerular le-

sions and several clinical syndromes or manifestations. FGS associated with mtDNA mutations was recently reported (6–9). In general, glomerular epithelial cells are regarded as terminally differentiated cells that do not proliferate (26–28). These characteristics are also noted for neurons and muscular cells, which are regarded as major sites for the accumulation of mtDNA mutations. In our screening of mtDNA mutations in renal biopsy specimens, two patients with a mtDNA 3243A→G point mutation and 19 patients with a common deletion were identified.

A mtDNA 3243A→G point mutation was originally noted for patients with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (10). However, several studies have suggested that this mutation is associated with diabetes mellitus and/or deafness without neurologic involvement (7–9). Our patients with the mtDNA 3243A→G point mutation exhibited maternally inherited sensory hearing loss or renal disease. Several forms of familial FGS have been reported, including those involving mutations in glomerular epithelial cytoskeleton components such as podocin (30) and α-actinin 4 (31) or structural components of the slit diaphragm such as nephrin (32). The mtDNA 3243A→G point mutation is regarded as another form of familial FGS. This mutation has been observed in approximately 0.6 to 1.5% of patients with type 2 diabetes mellitus (25,33) and in approximately 16.3/ 100,000 individuals in the general adult population (34). Guillausseau *et al.* (35) reported that 28% of patients with type 2 diabetes mellitus and the mtDNA 3243A→G point mutation exhibited kidney disease, and renal histologic analyses for three patients who underwent renal biopsies demonstrated FGS. Therefore, the mtDNA 3243A→G point mutation may be the most frequent etiologic mutation in familial FGS.

The pathogenesis of FGS lesions with the mtDNA 3243A→G point mutation exhibits some discrepancies. Mochizuki *et al.* (7) and Doleris *et al.* (8) reported that vascular smooth muscle cell injury attributable to mitochondrial damage led to arteriolar hyaline lesions, which abolished the autoregulatory mechanism for glomerular pressure; subsequent renal hemodynamic alterations might occur, resulting in FGS lesions. In contrast, the study by Hotta *et al.* (9) and our study demonstrated that abnormal mitochondria accumulated in glomerular epithelial cells, which led to glomerular epithelial dysfunction, resulting in FGS lesions. More than 85% of mtDNA in analyzed kidney samples exhibited this point mutation. Although the distribution of mtDNA is abundant in the renal tubular epithelium, early renal manifestations of the mtDNA 3243A→G point mutation involve glomerulosclerotic changes. In general, most mitochondrial diseases exhibit a delayed onset and a progressive course. The phenotypic expressions of these diseases are affected by both the predisposing mutation and an age-related factor, which causes a decline in mitochondrial function (36). Although the characteristics of glomerular epithelial cells may cause an accumulation of mutated mtDNA during mtDNA replication, aging and continuous oxidative stress also damage not only the glomeruli but also tubular tissues.

In screening for the common deletion, this mutation was

Table 3. Mitochondrial DNA mutations in kidney biopsy specimens

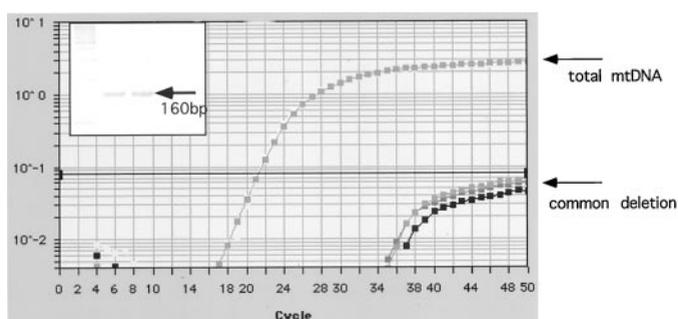| Classification	No. of Patients					Proportion of Common Deletion (%)
	3243A→G	3271T→C	8344A→G	8993T→G/C	Common Deletion	
Primary FGS	0	0	0	0	10/17 (58.8%)	1.57E−03 (2.05E−09 to 8.52E−02)
Familial FGS	2/7(28.6%)	0	0	0	3/7 (42.9 %)	1.69E−05 (8.18E−06 to 2.57E−05)
IgA nephropathy	0	0	0	0	7/16 (43.8%)	6.80E−04 (5.15E−06 to 7.10E−02)

Figure 4. PCR amplification of the common deletion (inset) and TaqMan PCR amplification plots of total mtDNA and the common deletion. A 160-bp product was observed. M, 100-bp ladder; lane 1, patient 1; lane 2, patient 2; lane 3, patient 15; lane 4, normal control sample. Total mtDNA amplification was observed after 17 cycles, and common deletion amplification was observed after 35 cycles.

detected for nine patients with primary FGS, three patients with familial FGS, and seven patients with IgA nephropathy; the level of the mutation was extremely low. The common deletion was thus observed not only in primary FGS but also in other glomerular diseases, including IgA nephropathy. Indeed, we also detected the common deletion in renal biopsy samples from patients with diabetic glomerulosclerosis (37). However, Simonetti *et al.* (18) reported that the common deletion was not observed in mtDNA from a kidney sample from an aged normal subject, and Liu *et al.* (20) reported that the common deletion was not observed in mtDNA from normal kidney samples from <37-yr-old subjects and the maximal mutation rate among normal aged subjects was 0.001% in a kidney sample from a 76-yr-old subject. Although the level of this mutation might be extremely low, most of the patients in this study with the common deletion were thought to exhibit abnormal conditions. Furthermore, Hayashi *et al.* (38) reported that >60% accumulation of the common deletion mutation was needed for progressive inhibition of mitochondrial translation and reduction of cytochrome *c* oxidase activity. Therefore, it may be difficult for a pathologic change with this level of deletion mutation to manifest itself. Bhat *et al.* (19) reported that, among patients with unilateral peripheral arterial disease,

muscles from hemodynamically affected limbs exhibited greater proportions of the common deletion mutation, because of oxidative stress. The common deletion detected in this study might be the consequence of oxidative stress during the glomerular disease process. However, *in situ* distribution of the common deletion in glomerular epithelial cells was detected among our subjects. It is speculated that a few glomerular epithelial cells might exhibit accumulation of the common deletion in >60% of mtDNA in the cytoplasm, resulting in functional damage to the cells. Furthermore, a mtDNA deletion mutant mouse model has been produced (39). These mutant mice, which accumulated >80% deletion mutations in their kidney mtDNA, experienced severe renal diseases and died within 6 mo because of renal failure. The kidney is also the most likely target and accumulation organ for the mtDNA deletion mutant in this mouse model (39). Furthermore, patients with IgA nephropathy and the common deletion exhibited significantly more segmental lesions, including adhesions, than did patients without the common deletion. There were no significant relationships among other parameters, and other patient groups demonstrated no relationships with segmental lesions. Adhesion of the capillary tuft to Bowman's capsule is regarded as an initial change of FGS lesions (1). Most of the renal cells that accumulate the common deletion might continue to degenerate during the disease process. Once FGS lesions are established, glomerular epithelial cells that accumulate the common deletion might be lost. In the brains of patients with Alzheimer's disease, mtDNA common deletion levels were observed to be very low, in contrast to the presence of high levels of 8'-hydroxy-2'-deoxyguanosine, a marker of oxidative DNA damage (40). Continuous oxidative stress to renal cells during the FGS disease process might hinder the mtDNA replication apparatus (40). Solin *et al.* (4) observed a 30% decrease in mtDNA contents, compared with normal control samples, in congenital nephrotic syndrome of the Finnish type. This might have resulted in the lack of a relationship between the accumulation of apparently lower levels of the mtDNA common deletion in our patients with FGS and aging. We used renal biopsy samples, which yielded very limited amounts of tissue for mtDNA analyses; the proportions of renal cortex and medulla, and thus the number of glomeruli, might

Table 4. Clinical and histologic features of common deletion-positive or -negative patients

Classification	Age (Range) (yr)	Serum Creatinine Concentration (mg/dl)	Proteinuria (g/d)	Global Sclerosis (%)[a]	Segmental Sclerosis (%)[a]	Segmental Lesions (%)[a,b]
Primary FGS						
common deletion-positive	26.5 (14 to 75)	1.1 (0.7 to 8.5)	4.9 (0.6 to 21.8)	5.6 (0 to 23.1)	12.7 (3.8 to 50.0)	25.4 (3.8 to 100.0)
common deletion-negative	36.5 (19 to 84)	1.2 (0.6 to 2.4)	2.3 (1.0 to 7.0)	3.1 (0.0 to 35.7)	29.9 (4.0 to 75.0)	35.5 (4.0 to 75.0)
Familial FGS						
common deletion-positive	24 (23 to 58)	1.2 (0.7 to 2.4)	2.4 (1.3 to 6.3)	28.6 (11.1 to 73.7)	14.3 (5.3 to 44.4)	14.3 (5.3 to 88.9)
common deletion-negative	32 (11 to 45)	0.9 (0.4 to 1.4)	2.2 (1.0 to 3.0)	36.4 (0.0 to 60.0)	25.0 (22.7 to 40.0)	25.0 (22.7 to 40.0)
IgA nephropathy						
common deletion-positive	50.5 (19 to 73)	0.9 (0.6 to 1.1)	2.3 (0.3 to 4.0)	13.2 (11.5)	1.6 (0.0 to 23.0)	27.9 (17.0 to 100.0)[c]
common deletion-negative	35.5 (13 to 71)	0.9 (0.5 to 1.7)	1.4 (0.8 to 4.0)	2.2 (0.0 to 21.1)	0.0 (0.0 to 13.0)	17.7 (0.0 to 30.4)

[a] Number of affected glomeruli/number of total glomeruli in the section.
[b] Number of glomeruli containing segmental sclerotic lesions and/or adhesions.
[c] $P < 0.05$, compared with common deletion-negative patients.
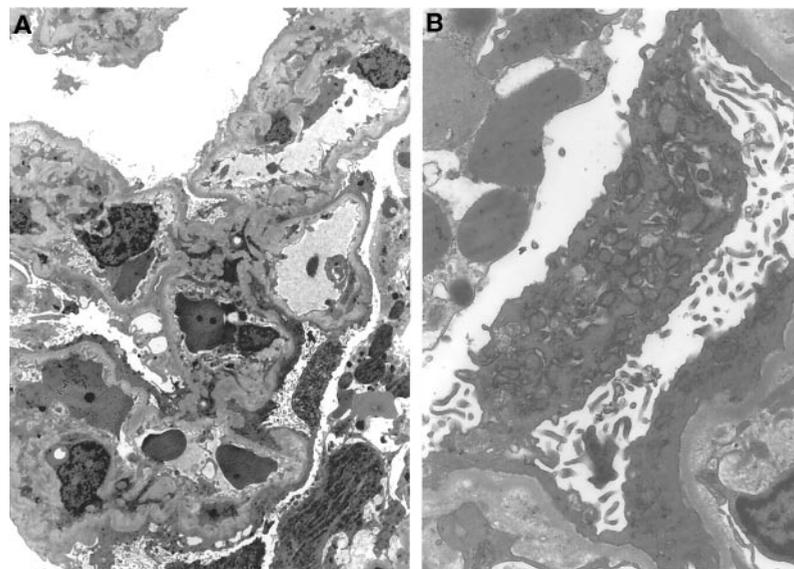
Figure 5. Electron-microscopic findings for a patient with primary focal segmental glomerulosclerosis. (A) Extensive foot process fusion was observed. (B) A higher-magnification view of the abnormal cytoplasm of a glomerular epithelial cell revealed a markedly increased number of mitochondria, of varied size and shape. Some of the mitochondria exhibited abnormally developed cristae.glomerulosclMagnification, ×2000 in A; ×10,000 in B.

be different for each sample. This could be another explanation for our difficulty in evaluating the level of mtDNA heteroplasmy in this study.

Mitochondria play a major role in apoptosis (41). Mitochondria-mediated apoptosis has been observed in rat hypertensive nephrosclerosis (42). Further studies are needed to clarify the role of mitochondria in hereditary renal diseases, as well as in aging and progressive renal diseases, and especially to determine whether distribution of the common deletion in the glo-

merular epithelium is the consequence of stress to the cells or the cause of glomerular epithelial damage attributable to mitochondrial dysfunction and glomerulosclerosis.
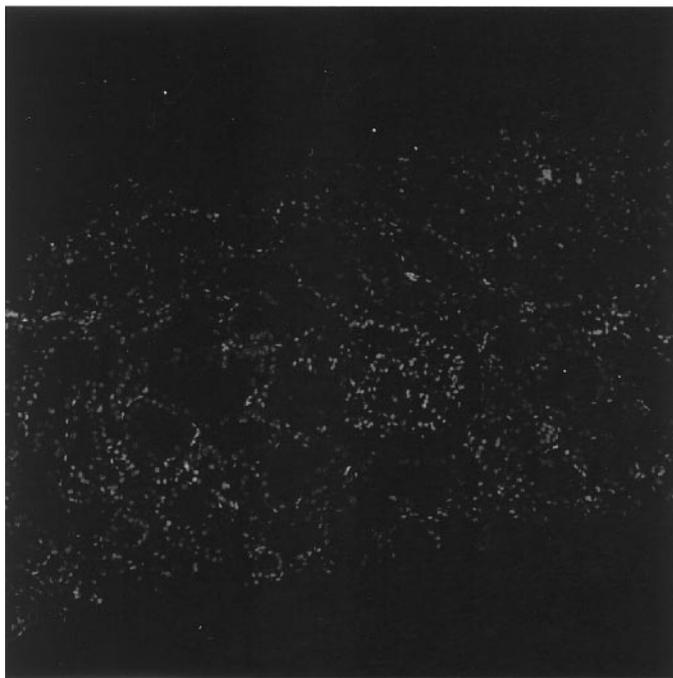
Figure 6. In situ PCR for normal mtDNA. Most of the mtDNA signal was observed in the tubular epithelium. Magnification, ×100.
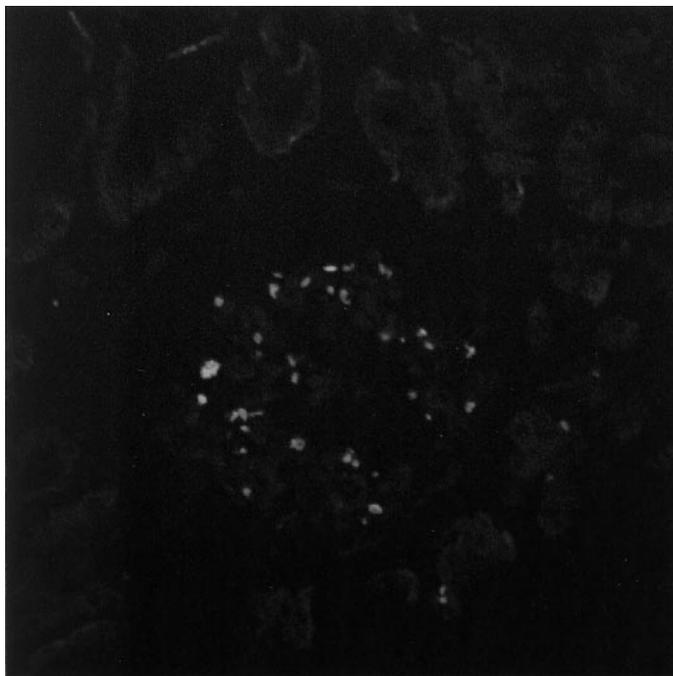

Figure 7. In situ PCR for the common deletion. The mtDNA common deletion was observed mainly in the glomerular epithelium. Occasional signals were observed in the tubular epithelium and interstitium. Magnification, ×200.

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