Renal Pathology in Fabry Disease

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Fabry disease is an X-linked recessive lysosomal storage disease that is caused by deficient activity of the lysosomal enzyme α-galactosidase A (α-Gal A), an enzyme that cleaves terminal α-galactosyl residues. This deficiency results in progressive lysosomal accumulation of glycosphingolipid with terminal α-galactosyl residues, particularly globotriaosylceramide (Gb3). Gb3 accumulates in many cells, particularly in renal epithelial cells, endothelial cells, pericytes, vascular smooth muscle cells, cardiomyocytes, and neurons of the autonomic nervous system (1).

The genetic defect occurs in all cell types, but involvement differs greatly among different organs and cell types. This heterogeneity likely reflects different rates of sphingolipid metabolism. Thus the minimum threshold requirement for α-Gal A activity to prevent Gb3 accumulation varies across cell types due to the type and amount of substrates that are recycled by the different cells (2). Renal lesions are found in both hemizygous (male) and heterozygous (female) patients. Renal symptoms in the latter are typically milder and delayed by 2 to 3 decades, but there is considerable variability (3). The variability is likely the result of the random nature of X inactivation, resulting in considerable variability in α-Gal A activity among carriers and (at least theoretically) within one carrier individual among various tissues or regions of a single tissue.

Light Microscopy

The renal pathology of Fabry disease was first described just over 50 years ago and has been the subject of numerous case reports and series since then (4). Glomeruli on light microscopy show hypertrophic glomerular visceral epithelial cells (podocytes) distended with foamy appearing vacuoles, mesangial widening, and varying degrees of glomerular obsolescence (Figure 1, A and B). Within the glomerulus, the largest amount of lipid material is seen in podocytes, followed by the parietal epithelial, mesangial, and glomerular endothelial cells. With disease progression, glomeruli exhibit mesangial widening, in some cases segmental glomerulosclerosis, and ultimately global glomerulosclerosis.

Vacuolation is also present in the capillary endothelium and distal tubular epithelial cells, including those of Henle’s loop and the collecting duct, particularly intercalated cells (Figure 1C), and less commonly in proximal tubular epithelial cells. Vascular involvement includes deposition in capillary, arterial, and arteriolar endothelial cells, pericytes, and smooth muscle cells (Figure 1D and Figure 2B). In severe disease, there is progressive tubular atrophy, interstitial fibrosis, and varying amounts of interstitial fibrosis.

In general, making the diagnosis of Fabry’s disease on a needle biopsy is not difficult, particularly if electron microscopy is performed. There is somewhat more difficulty in determining whether the disease has progressed or stabilized over a period of time, because damage is not uniform in all glomeruli or all areas of the tubulointerstitium. Therefore, clinical research protocols that rely on histologic scores to determine outcome will require careful attention to the method of scoring renal pathology, and an adequate amount of tissue must be available.

Various special staining procedures may aid in making the diagnosis of Fabry disease on the basis of accumulation of sphingolipid. On tissue that has been subjected to routine processing, which removes some lipid components, hematoxylin and eosin or periodic acid Schiff (PAS) stains demonstrate only vacuolated cells. The storage material can be stained on routine sections with Luxol fast blue, which identifies polar lipids (5). On frozen sections, the storage material can be demonstrated with several approaches, including staining with PAS, Luxol fast blue, Oil red O (6), and Sudan black (6,7). Greater specificity can be obtained by using the lectins, Griffonia (Bandeiraea) simplicifolia-I and Ricinus communis-I, which identify α- and β-galactosyl residues (7). Furthermore, under polarized light, the storage material is birefringent and exhibits autofluorescence. Fabry deposits are best demonstrated using tissue that has been fixed in glutaraldehyde or Trump’s fixative, embedded in Epon, and stained with toluidine blue or methylene blue/azure II, yielding dark blue cytoplasmatic inclusions (Figure 1, B through D).

Immunofluorescence is generally not contributory, although some patients may have focal mesangial deposits of C3 or IgM in a nonspecific fashion (3).

Electron Microscopy

Electron microscopic studies demonstrate enlarged secondary lysosomes (myeloid or Zebra bodies) packed with lamel-
lated membrane structures (Figure 2, A and B). These inclusions can vary in appearance, from granular to lamellated, the latter being more diagnostic. The periodicity of the lamellated membrane structures when measured using routine plastic thin sections is estimated to be 4 to 5 nm (3,8), but the periodicity of their structures is 14 to 15 nm when studied by freeze fracture electron microscopy, due to better tissue preservation (8).

With progression of the disease, there is fusion of podocyte foot processes in association with advancing proteinuria, occasionally focal glomerular and tubular epithelial necrosis, and thickening of glomerular and tubular basement membranes (3). In our studies, focal duplication of glomerular basement membrane was noted (Alroy J, unpublished findings).

The presence of other, coexisting renal diseases may complicate the pathologic picture. Indeed, 2 of 21 Fabry patients who had renal tissue studied by electron microscopy in the context of a prospective therapeutic trial had membranoproliferative glomerulonephritis with subendothelial immune deposits, in one case associated with hepatitis C infection (Alroy J, unpublished findings).
Urine Cytology

Urine cytology presents a noninvasive and underutilized approach to making the diagnosis of Fabry disease or demonstrating renal involvement with Fabry disease, although the sensitivity of this approach, particularly early in the course of renal disease, is unknown. Most cells (76%) present in the urine of Fabry patients are tubular epithelial cells (9). Fabry inclusions can be identified with the PAS or Papanicolau stains.
One case report has suggested that renal 
assumption), this suggests that residual enzyme activity in 
leukocyte activity in leukocytes (14). If renal 
cells are of donor origin (12,13).

Kidney in Renal Transplantation
Renal tissue of well-functioning allografts examined 6 and 8 
mo after renal transplant has shown Fabry inclusions in the 
vascular endothelium, demonstrable only by electron micros-
ropy (7,10). This has been proposed to represent colonization of the 
allograft vasculature by host endothelial cells, a process 
that is believed to affect all renal allografts (11). Appearance of 
Fabry inclusions in glomerular or tubular cells appears more 
rarely and after a longer duration; presumably, these cells are 
relatively protected from elevated circulating levels of sphin-
golipids by normal cellular expression of α-Gal A, as these 
cells are of donor origin (12,13).

Mechanisms of Renal Injury
We have recently reported that renal Gb3 content, renal 
pathology, and renal function correlate with residual α-Gal A 
activity in leukocytes (14). If renal α-Gal A activity correlates 
with leukocyte α-Gal A activity (a reasonable but untested 
assumption), this suggests that residual enzyme activity in 
renal parenchymal cells retards progression of renal disease. 
One case report has suggested that renal α-Gal A activity was 
reduced compared with liver α-Gal A activity when each was 
expressed as a fraction of normal α-Gal A activity in that 
organ; the mechanism for such a finding is unclear (15). 
Similarly, we found that Fabry patients with conservative 
misense mutations (defined as those that do not change the 
amino acid residue class) have delayed appearance of renal 
disease compared with patients with nonconservative missense 
mutations or other mutations (those resulting in deletions, 
insertions, or premature stop codons) (14).

We propose three mechanisms that might explain the seg-
mental and global glomerulosclerosis that characterizes Fabry 
disease: microvascular disease, podocyte injury, and tubulo-
interstitial injury.

Gubler et al. (3) made the perspicacious observation that in 
older Fabry patients, those 25 to 50 yr old, the progressive 
renal pathologic changes in the glomeruli and tubulointersti-
tium may be related to ischemic change. These changes include 
glomerulosclerosis, often with wrinkled and partially collapsed 
glomerular basement membrane, tubular atrophy, interstitial 
fibrosis, and vascular thickening. These changes were gener-
ally absent or mild in patients under 25 yr of age. In particular, 
these investigators noted that the earliest and most consistent 
degenerative alteration was arterial “fibrinoid” deposits and 
suggested that these were due to necrosis of smooth muscle 
cells fatally overloaded with Gb3 deposits. Hypertension is not 
a common feature of Fabry disease, although it may occur with 
progressive renal dysfunction (14). Therefore, following 
Gubler and colleagues, we hypothesize that one mechanism of 
renal injury in Fabry disease is accumulation of Gb3 within the 
arterial vessel wall and subsequent vascular compromise. In 
this regard, the renal vasculature is similar to the coronary and 
cerebral vessels, in which large vessel deposition of Gb3 is 
associated with premature vascular disease that is responsible 
for premature death in many patients.

Toxic accumulation of Gb3 within the podocyte may con-
stitute a second important mechanism of glomerular injury. 
Podocytes are highly differentiated cells; their foot processes 
and slit-diaphragms constitute a critical portion of the glomer-
ular filtration barrier that retards the entry large molecules into 
the urinary space. These cells are postmitotic and fail to un-
dergo proliferation under most pathologic circumstances (an 
exception being the collapsing variant of focal segmental glo-
merulosclerosis), which means that they generally are not 
replaced when they are lost due to lethal injury. Kriz and 
Lemley (16) have proposed that when podocytes are lost, the 
denuded glomerular basement contacts the parietal epithelial 
cells and forms a synechia. Within the synechia, there is 
activation and proliferation of cells, especially mesangial cells, 
the entry of immune cells, including macrophages, and the 
accumulation extracellular matrix protein. This repair response 
may be driven in part by the leakage from the circulation into 
the synechia of macromolecules, including cytokines, chemo-
kines, and growth factors, via the impaired glomerular filtra-
tion barrier. Matrix expansion and subsequent collapse of the 
capillary loop appears as the focus of solidification. This 
constitutes the lesion of segmental glomerulosclerosis, which 
progresses to global glomerulosclerosis. We propose as a 
working hypothesis that Gb3 induces podocyte injury, result-
ing in focal and ultimately global glomerulosclerosis.

Deposition of Gb3 within tubular epithelial cells may lead to 
focal tubular atrophy and interstitial fibrosis. As this process 
progresses, the glomeruli upstream of more severely affected 
tubules may function poorly or not at all. Other glomeruli may 
undergo hypertrophy to compensate, and hyperfiltration in 
these glomeruli may trigger a secondary form of focal segmen-
tal glomerulosclerosis. Evidence in support of this mechanism 
would include demonstration of glomerular enlargement, par-
cularly in the early stages of glomerular and tubular injury in 
Fabry disease; these glomerular measurements have not been 
published to date.

What then are the most suitable pathologic measures to 
define the status of Fabry renal disease in a given patient and 
to predict whether therapeutic intervention will likely alter the 
future course of renal dysfunction in that patient? Eng et al. 
(17) selected the change in microvascular inclusions in inter-
stitial endothelial cells of kidney, heart, and skin as a primary 
endpoint in their recent trial of α-Gal A replacement. We feel 
that the importance of this parameter as a potential surrogate 
marker for the progression of renal dysfunction (impaired 
GFR) and other renal pathology (glomerulosclerosis, intersti-
tial fibrosis) is uncertain and needs to be tested in a longitudi-
nal study. We have used additional pathologic markers, in-
cluding mesangial expansion, glomerulosclerosis, and 
terstitial fibrosis in another recent trial of α-Gal A replace-
ment (18). Arguably, these pathologic markers are also un-
tested as suitable markers for progressive renal functional 
decline in Fabry disease. Nevertheless, similar composite in-
dices have been used to measure disease progression, such as lupus nephritis (19). Mesangial expansion correlates with impaired GFR in diabetic nephropathy (20). Interstitial fibrosis has long been recognized as correlating with GFR in most renal diseases (21). The extent of glomerulosclerosis does not correlate as well, perhaps due to the complete reabsorption of globally obsolescent glomeruli, leading to a milder pathology score than would otherwise be the case.

Conclusion

In Fabry disease, the kidney is affected in all male hemizygotes and in some female heterozygotes. Histologically, renal involvement is characterized by glycolipid deposits in glomerular cells (particularly in podocytes and also in mesangial cells and endothelial cells), tubular epithelial cells (particularly of the distal nephron), and vascular cells (endothelial cells of capillaries, veins, and arteries, and vascular smooth muscle cells). Progression of kidney disease is characterized by segmental and global glomerulosclerosis, tubular atrophy, and interstitial fibrosis. In patients with Fabry disease, renal involvement may be suspected by the appearance of proteinuria and/or renal insufficiency. The urine sediment contains cells with characteristic inclusions demonstrated by light microscopy and electron microscopy. Renal biopsy is occasionally helpful in making the diagnosis of Fabry disease when this diagnosis was not previously entertained, but in general other approaches (measurement of plasma or leukocyte α-Gal A activity, skin biopsy, examination of the urine sediment, or α-gal A gene) offer less invasive ways to establish the diagnosis. On the other hand, renal biopsy may be helpful in excluding coexisting renal diseases, and quantitative analysis of renal biopsy material in future studies may be useful in evaluating the efficacy of new treatments for Fabry disease.

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