The effect of progressive glomerular disease on megalin-mediated endocytosis in the kidney

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Abstract

Background. A well-characterized dog model of the X-linked collagen disease Alport syndrome (XLAS) was used to study the effect of progressive glomerular disease on megalin-mediated endocytosis. In XLAS, altered structure and function of the glomerular basement membrane induces a progressive proteinuric nephropathy.

Methods. The investigation was performed in male XLAS dogs and age-matched normal male littermates. The urine profile and megalin-mediated endocytosis in the proximal tubule of six healthy and six XLAS dogs were examined at 2, 4, 6, 8 and 10 months of age using SDS–PAGE, immuno blotting and immunohistochemistry.

Results. Gradually increasing urinary excretion of proteins over time and a reduced content of the same proteins in proximal tubule cells were found. Besides the glomerular component of the proteinuria, a significant tubular component was found, which is due to a progressive change in the uptake of low-molecular-weight (LMW) ligands by megalin. Furthermore, the protein overload present in the lumen of the proximal tubule exceeds the reabsorption capacity of megalin and the co-receptor cubilin and results in a combined low- and high-molecular-weight (HMW) proteinuria. Also, a shift in the distribution of lysosomes was seen in the XLAS dogs suggesting changes in the lysosomal degradation pattern in response to the altered endocytosis.

Conclusions. The present study shows that the increased glomerular permeability and the subsequently altered megalin-mediated and megalin-dependent cubilin-mediated endocytosis lead to a partial LMW proteinuria and partial HMW proteinuria.

Keywords: alport syndrome; cubilin; glomerular disease; megalin; proteinuria

Introduction

Under normal conditions, the glomerular basement membrane (GBM) serves as a filtration barrier between the bloodstream and the tubular lumen of the nephrons. Plasma flowing through glomerular capillaries is filtered into the Bowman's space. The passage across the glomerular filtration barrier of all free proteins of molecular weight lower than approximately 30 kDa and radius lower than 20 Å, referred to as low-molecular-weight (LMW) proteins, is almost completely unrestricted in the healthy animal and human, thereby allowing the transfer of free LMW proteins such as β₂-microglobulin, α₁-microglobulin and retinol-binding protein (RBP) [1,2]. However, for proteins of intermediate molecular weight (IMW) such as albumin, transferrin and vitamin D binding protein (DBP), only a small fraction of each protein is filtered. Finally, the filtration of high-molecular-weight (HMW) proteins as IgG and α₂-macroglobulin is virtually non-existent. Under normal conditions, all proteins that cross the glomerular filtration barrier are excreted in negligible amounts in the urine due to very efficient reabsorption by megalin-mediated endocytosis by the proximal tubule cells.

The endocytic receptor megalin functions as a scavenger receptor for which more than 50 ligands have been identified. Megalin is a 600-kDa receptor and is a member of the Low Density Lipoprotein Receptor gene family [3–5]. Megalin is especially highly expressed in the kidney, where it is located to the apical plasma membrane and the endocytic machinery of proximal tubule cells [3,4,6]. In addition to megalin, the receptor cubilin, 460 kDa, also mediates uptake of proteins in the proximal tubule, but since cubilin does not have a transmembrane segment and thus is not able to induce internalization, the receptor is dependent on megalin to be internalized [7–11]. Furthermore, normal expression of cubilin is dependent on the transmembrane protein Amnionless (AMN) [12]. Cubilin is co-expressed with megalin in several tissues [4]. Both receptors mediate endocytosis of their own individual ligands, but they also share the uptake of a number of ligands including DBP, albumin, myoglobin, haemoglobin, immunoglobulin light chains and receptor-associated protein (RAP) [3,5].

The composition and quantity of proteins in the tubular lumen thus are determined by both glomerular filtration
and by tubular reabsorption carried out by megalin and cubulin-mediated endocytosis.

To study the effect of progressive glomerular proteinuria on receptor-mediated endocytosis, the well-characterized dog model of the X-linked collagen disease Alport syndrome (XLAS) was used [13,14]. Studies of XLAS show that structure and function of the glomerular filtration barrier is altered [15–20]. XLAS is a progressive hereditary disease caused by a mutation in the Col4a5 gene resulting in the absence of type IV collagen alpha-5 chains, leading to disruption of type IV collagen alpha-3, alpha-4 and alpha-5 chain networks in basement membranes. XLAS is characterized by progressive nephritis, with haematuria and proteinuria leading to end-stage kidney disease, as well as by deafness and ocular defects [15,21,22]. The GBM of Alport patients has been shown to thicken unevenly with focal and segmental thickening and eventually split due to the lack of an intact and well-functioning mature collagen chain network [15–20,23,24]. This is caused by immature collagen chain networks failing to reach maturity. Previous studies have shown that urinary excretion of proteins are increased in XLAS patients and XLAS dogs [21,25,26]. The goal of this study was to determine the effect on megalin-mediated endocytosis of gradually increasing glomerular filtration of proteins in a model of progressive glomerular disease.

Materials and methods

Renal tissue and urine

Serial canine renal tissue specimens and serial urine samples were obtained from six XLAS males and six age-matched normal male littersmates in a kindred of mixed-breed dogs from Navasota, TX [13,14]. Renal biopsies were obtained at 4, 6 and 8 months of age from all dogs using an ultrasound-guided needle biopsy technique [27]. XLAS males were sacrificed at 9–10 months of age when their serum creatinine concentration became >450 μmol/l. Renal specimens were obtained during a necropsy performed immediately after death. The normal males were either biopsied (four dogs) or necropsied (two dogs) at the same ages as when their XLAS male littermates were sacrificed. All tissue specimens were formalin fixed and subsequently embedded in paraffin. Additionally, small specimens of renal cortex obtained at necropsy were placed in 2.5% glutaraldehyde in Millonig fixative for 1 h, stained with saturated uranyl acetate for 10 min followed by staining with lead citrate and examined in a Leica DMR microscope equipped with a Leica DFC320 camera. Images were transferred by a Leica TFC Twain 6.1.0 program and processed using Adobe Photoshop 8.0.

Immunohistochemistry

For light microscope immunohistochemistry, 2 μm tissue paraffin sections were cut on a Leica RM 2165 microtome. Sections were heated, placed in xylene overnight, rehydrated in graded alcohols, permeabilized with 0.05% saponin (1% BSA, 0.2% gelatine, 0.05% saponin in 0.01M phosphate-buffered saline (PBS)) and blocked for endogenous peroxidase before incubation with 5% skim milk in PBS. The sections were then incubated with primary antibodies at 4°C overnight with primary antibody in PBS-T with 1% BSA. After washing in PBS-T for 10 min, the sections were incubated 1 h with Alexa Fluor®-labelled secondary antibody in PBS-T with 5% skim milk. A final wash in PBS-T and PBS, membranes were scanned on Odyssey Infrared Imaging System (LI-COR) version 1.2 to detect the fluorescent signal. Quantification of fluorescence intensity was done by measurement of integrated intensity.

Statistical analyses

Data were analysed using Microsoft Excel. Quantitative data are presented as means ± SD. For statistical comparison, the Mann–Whitney rank sum test was used.

Morphological studies

For electron microscopic morphology studies, 50 nm epon sections were stained with saturated uranyl acetate for 10 min followed by staining with lead citrate and studied in a Phillips CM100 electron microscope.

Results

Dogs

In order to verify the use of the X-linked Alport syndrome dogs as a model system of glomerular disease, biochemical
data regarding the renal function of the dogs were gathered and analysed (Table 1). These data show progressive proteinuria and deterioration of renal function among the XLAS dogs based on urine protein-to-creatinine ratio (UPC), serum creatinine and glomerular filtration rate (GFR) measurements. The normal age-matched male littersmates had normal renal functions.

Progressive proteinuria in serial urine samples from XLAS dogs

To establish the presence of proteinuria in canine XLAS, serial urine samples from XLAS dogs and controls were analysed by gel electrophoresis and subsequently stained with Coomassie blue staining reagent (Figure 1). We found that proteinuria gradually increased in the urine from the XLAS dogs during the first 6 months. At the age of 4–6 months, the XLAS dogs began exhibiting a combined low- and high-molecular-weight proteinuria. No proteins were found in the urine from the normal age-matched male littersmates.

Megalin ligands present in the urine from XLAS dogs

The high levels of LMW proteins in the urine are consistent with an impaired tubular reabsorption of LMW proteins. As megalin is the main receptor responsible for endocytic uptake of protein in the proximal tubule, the presence of megalin ligands in the urine was investigated in canine urine samples taken at 2, 4, 6, 8 and 9–10 months of age. All the LMW megalin ligands examined by immunoblotting; \( \alpha_1 \)-microglobulin, retinol-binding protein and \( \beta_2 \)-microglobulin were present in the urine from the XLAS dogs at 4 or 6 months of age and thereafter gradually increased (Figure 2). Virtually no protein was present in the urine from normal male littersmates. No proteins were found in the urine from the normal age-matched male littersmates.

Ligands exclusively for cubilin also present at high levels in the urine from XLAS dogs

In addition to megalin, cubilin also participates in the receptor-mediated uptake of proteins from the tubular lumen. To establish if cubilin was also affected by XLAS, the urinary levels of the pure cubilin ligands DBP, albumin and IgG were also found in the canine XLAS urine when the dogs were 4 months of age. Then, instead of progressively increasing the excretion level appeared to stagnate for the rest of the measuring period for albumin and IgG, though not for the ligand DBP (Figure 2 and Table 2).

<table>
<thead>
<tr>
<th>Number of dogs</th>
<th>UPC</th>
<th>Serum creatinine</th>
<th>GFR*</th>
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<tr>
<td>XLAS dogs</td>
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<tr>
<td>Age, 10 weeks</td>
<td>6</td>
<td>0.31 (±0.10)</td>
<td>48.62 (±8.84)</td>
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<tr>
<td>Age, 18 weeks</td>
<td>6</td>
<td>7.89 (±5.39)*</td>
<td>63.65 (±7.07)</td>
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<td>Age, 26 weeks</td>
<td>6</td>
<td>15.54 (±3.65)*</td>
<td>121.11 (±25.64)*</td>
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<tr>
<td>Age, 34 weeks</td>
<td>6</td>
<td>15.46 (±2.94)*</td>
<td>244.87 (±62.76)*</td>
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<tr>
<td>Age, 40 (range 37–43) weeks</td>
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<td>18.53 (±6.12)*</td>
<td>503.88 (±108.73)*</td>
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<tr>
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<td>54.81 (±7.07)</td>
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<tr>
<td>Age, 18 weeks</td>
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<td>0.39 (±0.22)</td>
<td>68.07 (±8.84)</td>
</tr>
<tr>
<td>Age, 26 weeks</td>
<td>6</td>
<td>0.26 (±0.17)</td>
<td>81.33 (±11.49)</td>
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<td>Age, 34 weeks</td>
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<td>88.4 (±5.30)</td>
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<tr>
<td>Age, 43.33 (range 42–44) weeks</td>
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<td>0.28 (±0.12)</td>
<td>97.24 (±8.84)</td>
</tr>
</tbody>
</table>

Data are means (±SD). UPC, urine protein-to-creatinine ratio (reference range, <0.5); GFR, glomerular filtration rate (ml/min/kg); Serum creatinine (μmol/l)(reference range, 40–130)*P < 0.05, XLAS versus healthy.

*GFR was not measured before week 17 of age. Furthermore, GFR was performed 1 week prior to the listed age with the exception of the last two measurements among the XLAS dogs and healthy dogs. These were performed at age 34 and 40.33 (range 36–44) for the XLAS dogs and age 34 and 43.33 (range 43–44) for the healthy dogs.

Elevated levels of the LMW and HMW megalin ligands DBP, albumin and IgG were also found in the canine XLAS urine when the dogs were 4 months of age. Then, instead of progressively increasing the excretion level appeared to stagnate for the rest of the measuring period for albumin and IgG, though not for the ligand DBP (Figure 2 and Table 2).

Fig. 1. SDS–PAGE gel showing a representative protein excretion pattern from a XLAS dog and a normal age-matched littermate. X2–X10 correspond to the serial urine samples from XLAS male dogs taken at 2, 4, 6, 8 and 10 months of age, respectively. C2–C10 correspond to the serial urine samples from normal male littersmates also taken at 2, 4, 6, 8 and 10 months of age, respectively. The amounts of urine applied were normalized for creatinine concentration. Proteins were identified by Coomassie staining. Proteinuria is detectable in XLAS males at 4 months; urine protein levels appear to plateau at 6 months of age. No protein is found in control urine samples.
transferrin and apolipoprotein A-1 were examined. Apolipoprotein A-1 is freely filtered and therefore used as the main indicator for dysfunctional cubilin-mediated endocytosis. Apolipoprotein A-1 and also transferrin were found to be present at high levels in the urine from the XLAS dogs (Figure 2 and Table 2). As shown in Figure 2, the excretion of transferrin gradually increased in the urine from the XLAS dogs with the highest excretion at 8–10 months of age, whereas the level of apolipoprotein A-1 was steady after the age of 6 months. When protein level was quantified, apolipoprotein A-1 was found to be significantly gradually increasing from 4 months of age (Table 2).

Shedding of megalin and cubilin in the XLAS urine

As an indicator of tubular alteration and changed megalin-mediated and megalin-dependent cubilin-mediated endocytosis, the levels of megalin and cubilin in the urine were investigated (Figure 2). At the age of 4 months and on-
wards, fragments of both receptors were found in the urine from the XLAS dogs, whereas virtually no receptor shedding was seen in the urine from the normal male littermates. No evident change in the expression of either megalin or cubilin was seen by immunohistochemistry performed on serial renal biopsy/necropsy specimens (data not shown).

**Altered megalin-mediated and cubilin-dependent endocytosis**

To further establish the altered receptor-mediated endocytosis of LMW and HMW proteins in canine XLAS, immunohistochemistry of megalin and cubilin ligands was performed on serial renal biopsy/necropsy specimens. Light microscopic immunohistochemistry revealed a progressive decrease in megalin- and/or megalin-dependent cubilin-mediated endocytosis in XLAS dogs of the ligands $\alpha_1$-microglobulin, RBP, $\beta_2$-microglobulin and apolipoprotein A-1 (Figure 3A–D; not all data shown). The progressive decrease in the endocytosis is reflected by a progressive decrease in vesicular labelling culminating with vesicular labelling restricted to the apical part of the cell on the last biopsy/necropsy specimen.

When we examined the combined megalin and cubilin ligand DBP, we did not see any intracellular accumulation of this protein at all in the biopsies from the XLAS dogs at 4, 6 and 8 months (data not shown). In the last biopsy/necropsy specimens, we saw brush border staining but without apical vesicular labelling. This is not consistent with the gradually increasing urinary excretion of DBP (Table 2).

Surprisingly, intracellular labelling of albumin was not found to be reduced on the first three serial biopsies (Figure 3, the second and third biopsies are not shown), but at the last biopsy/necropsy, it resembled the apical vesicular labelling seen for $\alpha_1$-microglobulin, RBP, $\beta_2$-microglobulin and apolipoprotein A-1 (Figure 3E and F).

At the age of 6–10 months, receptor-mediated endocytosis of all investigated LMW proteins in the proximal tubules of the majority of the nephrons in XLAS dogs was markedly reduced. In addition to the LMW proteins, the uptake of the IMW and HMW proteins albumin, transferrin and IgG (Figure 3G and H) also was impaired.

Of notable significance, apical accumulation of ligands was found in the cells of the proximal tubules when the XLAS dogs were 8–10 months old. At a higher magnification, the ligands seemed to be located just underneath the brush border of the cells (Figure 4).

**Altered distribution of lysosomes in proximal tubule cells**

In addition to impaired endocytosis in canine XLAS, the lysosomal degradation step in the intracellular pathway for processing reabsorbed proteins was investigated. This was accomplished by immunohistochemical labelling of lysosomal membranes with LAMP-1 to assess the distribution of lysosomes within the cells. The distribution of lysosomes was shifted from the perinuclear compartment to the apical part of the proximal tubule cells in XLAS dogs compared with normal male littermates. At the age of 8–10 months, lysosomes in the proximal tubule cells of the XLAS dogs were no longer found throughout the cell but instead were, like the accumulated protein, found only in the apical portions of the cells (Figure 5A and B). Additionally, we examined the distribution of lysosomes on epon sections of renal tissue from both XLAS and normal male littermates. Although only sparse material was available, this also showed a change in lysosomal localization within the proximal tubule of the XLAS dogs. Again, the distribution of lysosomes was shifted from the perinuclear

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**Fig. 3.** Reduced cellular content of the ligands $\alpha_1$-microglobulin (A, B), apolipoprotein A-1 (C, D), albumin (E, F) and IgG (G, H) was found in serial renal biopsies/necropsies from XLAS dogs by immunohistochemistry, indicating an impaired megalin-mediated and megalin-dependent cubilin-mediated endocytosis. The micrographs A, C, E and G are from the first biopsy taken at the age of 4 months, whereas the micrographs B, D, F and H are from the last biopsy/necropsy taken when the dogs were approximately 8 to 10 months old. There is a progressive reduced cellular content of all ligands, except albumin, which only show a reduced cellular content on the last tissue sample (necropsy). Primary antibodies used: a-$\alpha_1$-microglobulin 1:2400, a-apolipoprotein A-1 1:8000, a-albumin 1:100 000 and a-IgG(H) 1:800. HRP-conjugated secondary antibodies were used. Scale bar 50 μm.

**Fig. 4.** Apical accumulation of proteins. At the age of 8–10 months, the ligands examined seem, despite a reduced reabsorbed amount, to be accumulating in the area just underneath the brush border of the proximal tubule cells in the XLAS dog (B) compared with the normal male littermate (A). Here, the labelling for the megalin ligand RBP is shown. Primary antibody used: a-RBP 1:10000. HRP-conjugated secondary antibodies were used. Scale bar 50 μm.
compartment to the apical part of the proximal tubule cells (Figure 5C and D).

Morphology

Microscopic assessments of the biopsies from both the XLAS dogs and the normal male littermates showed a normal renal microanatomy up to 4 months of age hereafter, progressive thickness and enlargement of the glomeruli along with interstitial fibrosis were present in the XLAS dogs. Further substantial differences were seen on the necropsies at the approximate age of 9–10 months. The cortex from XLAS dogs exhibited distinctive changes of chronic tubulointerstitial nephritis with mononuclear cell inflammation, tubular atrophy, severe fibrosis and enlargement of glomeruli, contrary to the normal littermates. Not all nephrons were affected to the same extent, which is consistent with not all glomeruli being equally affected in the XLAS dogs [20,22].

The fact that the proximal tubules of all nephrons are not affected to the same degree is consistent with the finding of three morphologically distinct states of the tubular cells in relation to ligand labelling at the age of 9–10 months. First and foremost, the majority of the tubular cells showed an apical vesicular protein accumulation and brush border labelling but otherwise normal morphology. Secondly, some of the tubular cells were highly atrophic with a short cubic shape and aberrant presence of

Fig. 5. Change in lysosomal localization within proximal tubular epithelial cells. The distribution of lysosomes is shifted from the perinuclear compartment to the apical part of the proximal tubule cells in 8–10-months-old XLAS dogs compared with age-matched normal male littermates. Staining with a-lysosomal associated membrane glycoprotein 1 (LAMP-1) shows the change in lysosomal localization at the light microscopic level (A, B). Furthermore, as shown at the bottom of the figure, the localization of lysosomes (L) at electron microscopic level also seems to be shifted to the apical part of the proximal tubule cells (C, D). A and C are sections from normal male littermates and D are from XLAS male dogs. Primary antibody used: a-LAMP-1 1:1200. HRP-conjugated secondary antibody was used. Light microscopy: scale bar 50 μm. Electron microscopy: scale bar 5 μm. L, lysosomes; BB, brush border; E, endocytic vacuoles; M, mitochondria.
Fig. 6. Composition of proteinuria during progression of the glomerular disease XLAS. Under normal physiologic conditions, LMW proteins are freely filtered and almost completely reabsorbed. There is little or no filtration of IMW and HMW proteins. In early stage of XLAS, filtered IMW and HMW proteins compete with LMW proteins for reabsorption, resulting in the appearance of LMW, IMW and HMW proteins in final urine. The uptake and degradation of protein by healthy proximal tubule cells remain intact. In the advanced stage of XLAS, uptake and degradation of filtered LMW, IMW and HMW proteins by damaged proximal tubule cells are abnormal, resulting in high levels of LMW, IMW and HMW proteins in final urine despite reduced GFR.

Discussion

In the human kidney, XLAS is characterized as a glomerular disease with progressive nephritis with haematuria and proteinuria [22]. As XLAS progresses, renal function is significantly reduced, and virtually all affected males with the disease develop renal failure [15,22]. The histopathologic and ultrastructural features of XLAS include glomerular sclerosis and tubulointerstitial fibrosis [17,20–23].

In the present study, we investigated the influence of progressive glomerular disease on megalin-mediated endocytosis using a canine model of XLAS. This model recapitulates the key clinical, immunohistochemical, pathological and ultrastructural features of human XLAS renal disease [14].

The major finding in this study is that the mechanisms underlying the increased urinary excretion of proteins in canine XLAS have an advancing tubular component as well as a glomerular component (Figure 6). The tubular component is due to a progressive change in the binding and reabsorption of LMW ligands during the first 6–8 months of the disease. We hypothesize that megalin-mediated and megalin-dependent cubilin-mediated endocytosis of normally completely reabsorbed LMW proteins is decreased due to competition with the uptake of IMW and HMW proteins that are present in high amounts in the tubular lumen as a result of their increased glomerular filtration occurring in XLAS. At later stages when the dogs are 8–10 months old, the finding of apical vesicular protein accumulation in the proximal tubule cells in the biopsy/necropsy specimens suggests that general cellular dysfunction could be disrupting receptor-mediated endocytosis. Furthermore, the impaired endocytosis found on the necropsies from the dogs at the age of 9–10 months did not seem to be restricted to particular ligands but appeared to affect a broad array of ligands of varying molecular weight. In addition to the LMW proteins, the uptake of the IMW and HMW proteins albumin, transferrin and IgG also were reduced at this stage, indicating a non-specific impairment of megalin-mediated and megalin-dependent cubilin-mediated endocytosis, presumably due to the occurrence of an overall cellular dysfunction. Other signs of dysfunction are shedding of megalin and cubilin fragments in the urine and tubular atrophy. Moreover, a notable shift in the distribution of
lysosomes from the perinuclear compartment to the apical part of the proximal tubule cells was seen in the XLAS dogs, suggesting subsequent changes in the lysosomal degradation pattern in response to the altered receptor-mediated endocytosis.

Furthermore, when examining the protein composition of the urine from XLAS male dogs, we found a combined glomerular and tubular proteinuria with the presence of LMW, IMW and HMW proteins. The presence of IMW and HMW proteins is consistent with an increased glomerular permeability [1,20], whereas the presence of LMW proteins indicates reduced or damaged tubular LMW protein reabsorption [1,3,10]. As mentioned, we believe this altered protein reabsorption results from two separate events. Early in XLAS, increasing amounts of IMW and HMW proteins in the tubular lumen compete with the normally freely filtered LMW proteins for binding sites on megalin and cubilin, reducing the reabsorption of LMW proteins and resulting in excretion of these proteins in the final urine. Secondly, the tubular protein reabsorption may be altered as XLAS progresses due to toxicity of the protein overload in the lumen of the proximal tubules directly or indirectly [10,28–37]. This is consistent with the significantly reduced receptor-mediated endocytosis observed in XLAS dogs at the age of 8–10 months. In addition, the impaired endocytosis could be caused by potentially nephrotoxic proteins that are normally absent from the tubular lumen but have a high affinity for the megalin receptor. A number of previous studies have pointed to different proteins as being potentially toxic to the proximal tubule when present in high amounts, including IgG, albumin, transferrin and growth factors [32–35,38–44]. Our results suggest that alteration and/or overload of protein degradation pathways in proximal tubular epithelial cells is a feature of advanced XLAS. Dysfunction or overload in the cellular pathway of protein degradation also could explain the late-stage alteration of tubular protein reabsorption, since a shift in the distribution of lysosomes in the proximal tubule cells of XLAS dogs was found. The shift in lysosomal distribution also could be a response to reduced endocytosis of proteins with a subsequently reduced need for protein degradation by the lysosomes. The lysosomes formed might then migrate to the apical part of the cell in order to fuse with the relatively few endosomes present due to reduced megalin-mediated and megalin-dependent cubilin-mediated endocytosis. Alternatively, however, the opposite also could be the case in which the proximal tubule cells are processing the reabsorbed proteins at an increased speed and thereby apicalizing their lysosomal degradation in response to the tubular protein overload.

Our results show that the increased glomerular permeability and the subsequently impaired megalin-mediated and megalin-dependent cubilin-mediated endocytosis lead to a partial LMW proteinuria and partial HMW proteinuria. Therefore, not only the quantity but also the composition of the proteinuria can be used in monitoring the progression of glomerular diseases and the almost unavoidable progression into renal failure.

The negative influence on tubular function in glomerular diseases is often, if not always, present, and progression to end-stage renal failure is the final common pathway of various proteinuric nephropathies [23,30,32,35–37]. Whether the progression to end-stage renal failure is due to the tubular toxicity of the protein overload in the lumen of the proximal tubules or due to encroachment of a glomerular injury onto the glomerulartubular junction is not yet fully understood [45–48]. Our results based on in vivo investigations propose a possible association between protein overload and impairment of receptor-mediated endocytosis with subsequent tubular dysfunction and tubular atrophy, thereby supporting a tubular pathway for the formation of tubulointerstitial fibrosis and tubular atrophy leading to end-stage renal disease.

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Conflict of interest statement. None declared.

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