

## Familial Nephropathy and Multiple Exostoses With *Exostosin-1 (EXT1)* Gene Mutation

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### ABSTRACT

Glomerular deposition of fibrillar collagen is a characteristic finding of genetically distinct conditions, including nail-patella syndrome and collagen type III glomerulopathy. A case of familial nephropathy in which steroid-sensitive nephrotic syndrome and glomerular deposits of fibrillar collagen are associated with multiple exostoses due to mutation of the *EXT1* gene is described. This gene encodes a glycosyltransferase required for synthesis of heparan sulfate glycosaminoglycans. There is deficiency of heparan sulfate and perlecan, together with accumulation of collagens, in the matrix of *EXT1*-associated osteochondromas. Similar glomerular basement membrane abnormalities could offer an explanation for both the renal ultrastructural changes and steroid-sensitive nephrotic syndrome.

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A 37-yr-old woman presented with the nephrotic syndrome. Previously, she had had several episodes of skin infection responding to antibiotics, impaired hearing since birth and had been diagnosed with multiple exostoses in childhood. These included symptomatic lesions in the upper medial tibiae, left humerus and radius, and neck of the right femur. At presentation, there was marked peripheral edema, and investigations revealed 24 g/d proteinuria, albumin 23 g/L, and serum creatinine 1.1 mg/dl. She was found to be hypothyroid and was commenced on thyroxine.

Importantly, there was a strong family

history of renal disease and hearing impairment, which is illustrated in Figure 1. One brother had steroid-sensitive nephrotic syndrome at the age of 4 yr but did not have a renal biopsy. Her father had acute renal failure at the age of 69 yr, for which a renal biopsy had been performed. Review of his biopsy revealed a pauci-immune focal segmental proliferative glomerulonephritis with crescents. Electron microscopy showed largely normal capillary walls but with abundant fibrillar collagen within an expanded mesangium. She also reported that two nephews had childhood nephrotic syndrome and a cousin had “kidney disease” and exostoses.

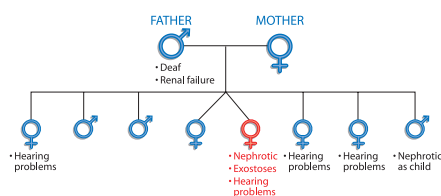
A renal biopsy was performed. Following this, the patient was treated with 60 mg/d of prednisolone, resulting in a prompt remission of her nephrotic syndrome. She suffered a relapse 3 mo later on discontinuation of steroids, which responded to reintroduction of 40 mg prednisolone. Steroids were replaced with cyclosporine after 4 mo and she re-

mained in remission with trace proteinuria until cyclosporine was stopped 3.5 yr later. Six months after this, she suffered another relapse of nephrotic syndrome that responded to 60 mg prednisolone and reintroduction of cyclosporine. After a further relapse 18 mo later and because of the development of adverse corticosteroid effects, she was treated with a 2-mo course of cyclophosphamide (2.5 mg/kg, orally). Ten years after her initial presentation, she remains in full remission and off steroids. Renal function has remained normal throughout with a current serum creatinine of 1.1 mg/dl.

To investigate the cause of her multiple exostoses she underwent sequencing of the gene encoding *exostosin-1 (EXT1)* on chromosome 8, which demonstrated a frameshift type 2 mutation 238 del A.

### RENAL BIOPSY

The renal biopsy contained 13 glomeruli, one of which was globally sclerosed. The remaining glomeruli showed only subtle changes at light microscopy (Figure 2a) with a mild increase in mesangial cellularity and matrix, and focal thickening of

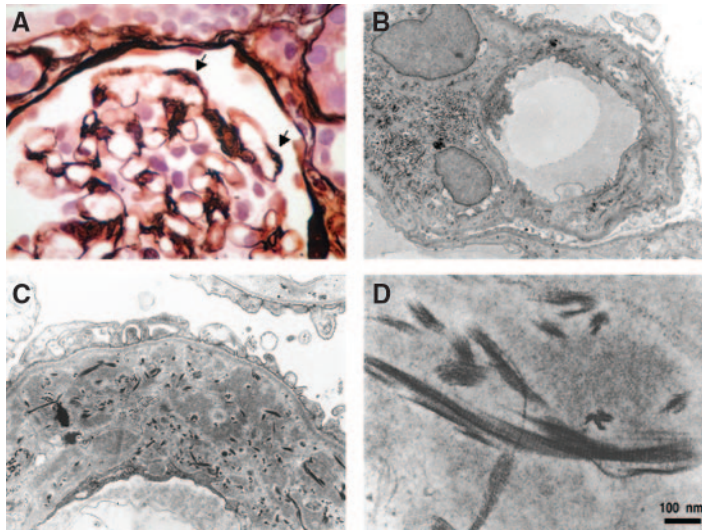


**Figure 1.** First-degree relatives of proband (in red).

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**Figure 2.** Renal biopsy: Light microscopy (a; Jones silver, original magnification  $\times 60$ ) shows increased mesangial matrix and focal thickening of capillary walls (arrows). Electron microscopy shows accumulation of fibrillar collagen in mesangium (b) and capillary walls (c). High power demonstrates the characteristic cross-striated fibrils of collagen (d).

capillary walls evident on silver stain. There was no segmental sclerosis or membrane spikes. Immunohistochemistry was negative for immunoglobulin G (IgG), IgA, IgM, C3, and C1q.

Electron microscopy showed widespread mesangial expansion by fibrillar collagen (Figure 2b). Glomerular capillary walls appeared normal in many loops, other than moderate effacement of podocyte foot processes. However, others showed expansion of capillary walls by subendothelial electron dense material within, which were abundant collagen fibrils (Figure 2, c and d). The lamina densa in these capillaries showed areas of duplication with focal mesangial cell interposition.

### Differential Diagnosis of the Renal Biopsy

Glomerular deposition of fibrillar collagen may be seen to a minor degree in many chronic glomerular diseases but is the dominant finding in the nephropathy of nail-patella syndrome and collagen type III glomerulopathy. As in this case, the light microscopic changes in nail-patella syndrome are typically mild, and glomeruli may appear normal initially. By contrast, collagen type III glomerulopathy is always associated with obvious mesangial matrix expansion and

thickening of capillary walls. At electron microscopy, in nail-patella syndrome, the glomerular basement membrane is primarily involved and expanded by fibrillar collagen, whereas in our case and in patients with collagen type III glomerulopathy, the lamina densa is preserved; fibrillar collagen accumulates in the subendothelial area and mesangium and may result in membrane duplication and mesangial cell interposition. The morphology, clinical presentation, and pathogenesis of these conditions are summarized in Table 1.

### Renal Pathophysiology

These three conditions (nail-patella syndrome, collagen type III glomerulopathy, and the current case) are linked by a common morphologic lesion, the accumulation of fibrillar collagen within glomeruli, but the mechanisms by which this develops are likely to be diverse. Our understanding of the pathophysiology of the morphologic changes and proteinuria in these patients is far from complete and requires knowledge of the underlying genetic abnormalities.

### Nail-patella Syndrome

Nail-patella syndrome results from mutations of the gene encoding *LMX1B* on chromosome 9.<sup>1</sup> *LMX1B* is a LIM-home-

**Table 1.** Comparison of this case with other conditions associated with glomerular deposition of fibrillar collagens

Condition	Gene Involved (inheritance)	Function of Gene	Glomerular Changes	Electron Microscopy	Renal Presentation
Nail-patella syndrome	<i>LMX1B</i> (autosomal dominant)	Transcription factor, expressed by podocytes	Normal or focal mild basement membrane thickening	Collagen fibrils in lamina densa of glomerular basement membrane	Persistent proteinuria $\pm$ hematuria (nephrotic syndrome unusual); chronic renal failure in 30%
Collagen type III glomerulopathy	Unknown (autosomal recessive)	Unknown, but there is systemic stimulation of type III collagen synthesis	Basement membrane thickening and mesangial matrix expansion	Mesangial and subendothelial collagen fibrils	Persistent proteinuria $\pm$ hematuria (nephrotic syndrome unusual); chronic renal failure, particularly in early-onset disease
Hereditary multiple exostoses	<i>EXT1/2</i> (autosomal dominant)	Glycosyltransferase required for heparan sulfate synthesis	Mild mesangial matrix expansion, focal mild basement membrane thickening	Mesangial and subendothelial collagen fibrils	Steroid-sensitive nephrotic syndrome

odomain transcription factor that plays a central role in limb development; hence, the skeletal abnormalities that result from mutations in this gene. It is also expressed by podocytes and regulates transcription of the genes for the  $\alpha 3$  and  $\alpha 4$  chains of type IV collagen, podocin, and CD2AP. How this relates to the development of the nephropathy, which is a variable feature of nail-patella syndrome, is uncertain.<sup>2</sup> There is, however, a link between genotype and phenotype; individuals with a mutation in the *LMX1B* homeodomain have a higher frequency of proteinuria than those with mutations in the *LIM* domains.<sup>3</sup>

### Collagen Type III Glomerulopathy

Most cases of this rare condition show an autosomal recessive pattern of inheritance.<sup>4</sup> It is a systemic disease; serum procollagen III peptide is invariably elevated, and extrarenal accumulation of type III collagen is reported.<sup>5</sup> The nature of the genetic abnormality is unknown.

### Hereditary Multiple Exostoses

The autosomal dominantly inherited condition hereditary multiple exostoses has been linked to two genes, *EXT1* on chromosome 8 and *EXT2* on chromosome 11, that account for 80% of affected individuals. The gene encoding *EXT1* (8q24.11-q24.13) encodes a 86.3-kDa endoplasmic reticulum-localized type II transmembrane glycoprotein. *EXT1* and *EXT2* form a hetero-oligomeric complex

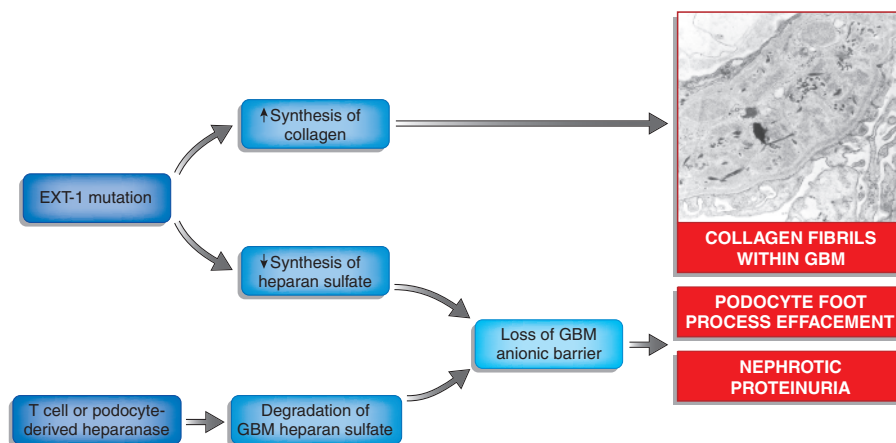
that accumulates in the Golgi apparatus and has glycosyltransferase activity that is essential for the synthesis and expression of heparan sulfate glycosaminoglycans.<sup>6</sup>

The mRNA encoding *EXT1* is expressed ubiquitously in many tissues including the kidney, although its renal protein expression has not been investigated. In patients with hereditary multiple exostoses, functional loss of *EXT1* results in exostoses (osteochondromas), but inactivation of both copies of the gene (germline mutation plus loss of the remaining wild-type allele) is not required for development of the bone lesions.<sup>7</sup> In these lesions, the cartilage matrix shows absence of heparan sulfate,<sup>8</sup> is deficient in perlecan and decorin, and contains increased amounts of collagens I and X.<sup>9</sup> Similar loss of heparan sulfate proteoglycans and increased fibrillar collagens in the glomerular capillary walls may account for the clinical presentation and biopsy findings in our patient (Figure 3).

Although the proteinuria in patients with nail-patella syndrome and collagen type III glomerulopathy is not steroid-sensitive, renal involvement in our patient manifest as steroid-sensitive nephrotic syndrome. The etiology of most cases of steroid-sensitive nephrotic syndrome is unknown. In contrast to steroid-resistant nephrotic focal segmental glomerulosclerosis, which appears to be primarily a podocyte

disease, there is growing evidence that in steroid-sensitive nephrotic syndrome, abnormalities of the glomerular basement membrane play a central role. The normal glomerular basement membrane is rich in heparan sulfate proteoglycans, conferring a negative charge barrier to macromolecules, and there is both experimental and clinical evidence that loss of this anionic barrier, because of degradation of the heparan sulfate chains by the enzyme heparanase, results in proteinuria. In experimental models, enzymatic depletion of heparan sulfate in the glomerular basement membrane results in increased permeability,<sup>10,11</sup> and injection of animals with a monoclonal antibody against glomerular basement membrane heparan sulfate induces acute selective proteinuria.<sup>12</sup> In normal glomeruli, there is minimal expression of heparanase, but a marked increase in glomerular heparanase staining is observed in the acute puromycin aminonucleoside nephrosis model of nephrotic syndrome.<sup>13</sup> Transgenic mice that overexpress heparanase in all tissues are proteinuric and show effacement of podocyte foot processes, similar to that seen in minimal change nephropathy.<sup>14</sup> Renal biopsies from patients with minimal change nephropathy show absent or markedly reduced glomerular basement membrane staining with an antibody against heparan sulfate side chains but normal staining for the agrin core protein of heparan sulfate proteoglycans.<sup>15</sup> Recently, it has been demonstrated that relapsing steroid-sensitive nephrotic syndrome in children is associated with elevated urinary heparanase activity,<sup>16</sup> implicating loss of heparan sulfate in the pathogenesis of the nephrotic syndrome in these patients. Heparanase is expressed by peripheral T lymphocytes, providing a link between the immune abnormalities and glomerular basement membrane changes observed in minimal change nephropathy.

In our patient with steroid-sensitive nephrotic syndrome and glomerular fibrillar collagen deposition, a pathogenic role for the *EXT1* mutation seems



**Figure 3.** Hypothetical pathophysiologic mechanisms in this patient and minimal change nephrotic syndrome.

highly likely, although we cannot formally exclude a contribution from other inherited abnormalities. Furthermore, an impairment of heparan sulfate synthesis cannot be the sole explanation for the nephrotic syndrome, as our patient did not develop renal symptoms until adulthood. The genetic defect and deficiency in heparan sulfate proteoglycan may, however, render the basement membrane susceptible to further, heparanase-induced loss of heparan sulfate and the development of the nephrotic syndrome.

Familial steroid-sensitive nephrotic syndrome is rare and genetic studies have not found a linkage to any gene known to be associated with the nephrotic syndrome.<sup>17,18</sup> Our kindred demonstrate a novel association between hereditary multiple exostoses and glomerular disease. While hearing impairment, a feature in this family, is seen in Langer-Giedion syndrome,<sup>19</sup> a syndrome caused by more extensive chromosomal deletions that encompass *EXT1*, there is no reported association between *EXT1* abnormalities and renal disease. Our understanding of the genetic basis for this phenotype is incomplete, but the association of a primary defect in heparan sulfate synthesis and steroid-sensitive nephrotic syndrome may have important implications for the pathogenesis of the much commoner nonfamilial minimal change nephrotic syndrome. As is often the case in medicine, an understanding of common conditions may emerge from the study of rare genetic disorders.

## DISCLOSURES

None.

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