Brief Report

Neonatal calyceal dilation and renal fibrosis resulting from loss of Adamts-1 in mouse kidney is due to a developmental dysgenesis

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Abstract

Background. A disintegrin and metalloproteinase with thrombospondin motifs 1, Adamts-1, is important for the development and function of the kidney. Mice lacking this protein present with renal lesions comprising enlarged calyces, and reduced cortex and medulla layers. Our current findings are consistent with the defect occurring due to a developmental dysgenesis.

Methods. We generated Adamts-1 null mice, and further investigated their kidney phenotype in a time course study ranging from E18.5 to 12 months of age. Immunohistochemistry was used to assess the localization of type IV collagen, TGF-β and F4/80-positive macrophages in the kidneys of Adamts-1 null mice compared to wild-type control animals. The expression of Adamts-1 mRNA was determined in metanephric kidney explants by in situ hybridization.

Results. Adamts-1 null mice have a gross kidney defect. At day 18.5 of gestation, the Adamts-1 null kidney has a normal appearance but at birth when the kidney begins to function, the defect becomes evident. During development of the kidney Adamts-1 expression was specifically detected in the developing loops of Henle, as well as in the proximal and distal convoluted tubules. Expression was not detected in the ureter, ureteric bud or its derivatives as had been previously suggested. At 6 months and 1 year of age, the Adamts-1 null mice displayed interstitial fibrosis in the cortical and medullary regions of the kidney. At 1 year of age, the Adamts-1 null mice displayed mild interstitial matrix expansion associated with increased collagen type IV expression, without apparent tubular dilatation, compared to wild-type animals.

Immunohistochemical analysis demonstrated TGF-β protein localized to infiltrating macrophages and glomeruli of Adamts-1 null mice.

Conclusions. Adamts-1 is required for the normal development of the kidney. The defect observed in its absence results from a dysgenic malformation affecting the medulla that becomes apparent at birth, once the kidneys start to function.

Keywords: Adamts-1; calyceal dilation; dysgenesis; kidney; knock-out; obstruction

Introduction

Adamts-1 (A Disintegrin and Metalloproteinase with Thrombospondin motifs) is a molecule secreted into the extracellular matrix (ECM) where it appears to be involved in remodelling of ECM components. Adamts-1 expression has been detected in a wide range of mammalian tissues, implying that the gene may be important for the development and function of a number of biological systems. Expression has been documented in the developing mouse urogenital system, with Adamts-1 transcripts detected in the kidney, and in the developing ovary and uterus [1].

In order to investigate the biological role of Adamts-1, we generated Adamts-1 null mice and have reported that null females are subfertile due to an impaired ability to ovulate [2]. Like the Adamts-1 null mice generated by Shindo et al. [3], we also find a similar kidney malformation in our mice.

This study furthers our understanding of the role of Adamts-1 in kidney structure and function. We examined the kidney structural defect in Adamts-1 null mice generated independently from those of...
Shindo et al. [3] because during our investigations, a number of differences between the kidney phenotype reported earlier and that observed in our mice became apparent. We also investigated the expression of *Adamts-1* in an *in vitro* model of the developing kidney.

**Subjects and methods**

**Generation of Adamts-1 null mice**

*Adamts-1* null mice were generated by deleting *Adamts-1* exon 2, which resulted in a frameshift and premature termination codon being introduced, with no functional protein being produced [2]. Kidneys were collected from *Adamts-1* null and wild-type mice of mixed genetic background (C57BL/6 × 129sv) at embryonic day 18.5 (E18.5), postnatal days 1 (P1) and 2 (P2), 2–3 months of age, 6 months of age and 1 year of age. Kidneys were fixed in formalin (E18.5 to P2) or Carnoy’s fixative (2 months to 1 year), sectioned and stained following standard protocols with haematoxylin and eosin (H&E), or Masson’s trichrome to detect fibrosis.

**Immunohistochemistry**

Mid-coronal slices of renal tissue from mice of 3, 6 and 12 months of age were fixed in Carnoy’s solution, embedded in paraffin wax and cut at 4μm. Sections were incubated in 20% goat serum in bovine serum albumin (BSA) made up in phosphate buffered saline (PBS; pH 7.4). Sections were incubated with a 1:50 dilution of rat anti-mouse F4/80 antibody, a marker of mature macrophages (Serotec, USA), overnight at 4°C. Localization of collagen type IV (Southern Biotechnology; 1:40 dilution) and TGF-β (Santa Cruz; 1:200 dilution) was demonstrated following overnight incubation at 4°C. After rinsing in PBS, sections were incubated with a 1:4 dilution of hydrogen peroxide to eliminate endogenous peroxidase activity. Either biotinylated goat anti-rat (F4/80, collagen IV) or swine anti-rabbit (TGF-β) secondary antibodies diluted 1:200 were used. Following a series of PBS washes sections were incubated with ABC kit (Vector Laboratories), washed and incubated with diaminobenzidine (Sigma-Aldrich, USA) for signal detection. Sections were counterstained with haematoxylin and Scott’s tap water.

**Metanephric explant culture and RNA in situ hybridization**

E11.5 metanephric kidneys were harvested from naturally mated outbred female mice after sacrifice by cervical dislocation. E11.5 metanephric kidneys were dissected using an Olympus CH30 dissection microscope and collected into ice-cold Leibovitz’s L15 media (Invitrogen). These explants were cultured as previously described [4]. Briefly, explanted metanephroi were grown for 3 days on Costar Transwell polycarbonate inserts (Corning) with a membrane pore size of 3.0μm at 37°C with 5% CO2 in MEM media (Invitrogen) supplemented with 10% fetal calf serum and 20 mM glutamine. After three days, explants were fixed at 4°C overnight in 4% paraformaldehyde. The *Adamts-1* transcript was amplified by PCR, using oligonucleotides Adam-ex4F (5’-CGCACTGATGCTAAACGACTGTG) and Adam-ko3probeR (5’-ATTTTGATGCTTTTCTGCGATCGAG). The product obtained was then cloned into pBluescript II KS (Stratagene), and T7 and T3 promoters were used to produce digoxygenin-labelled RNA sense and antisense probes as described previously [4]. Photographs were taken using Kodak Elite Ektachrome 320T film and a Leica MZ8 stereomicroscope/MPS48 photomicrographic system.

**Results**

All *Adamts-1* null mice examined displayed a kidney defect, characterised by shrinkage of both cortex and medulla and an enlarged calyceal space. The kidney defect was apparent on the first day after birth (Figure 1). No scarring or fibrosis was visible in the kidney, pelvis or uretero-pelvic junction of *Adamts-1* null mice at the time of appearance of the defect [postnatal day 1 (P1); Figure 2A and B] or at 2 months of age (data not shown). Mild fibrosis was first detected in the kidneys of 6-month-old *Adamts-1* null mice, while none was visible in wild-type mice (Figure 2C), but was not associated with increased F4/80-positive macrophage infiltration compared to wild-type mice (Figure 3G and H). Fibrosis was present in the kidneys of 1-year-old *Adamts-1* null mice, both around the glomeruli and in the pelvis and ureter (Figure 2D). By 1 year of age, the *Adamts-1* null mice displayed

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**Fig. 1.** Appearance of the kidney defect in newborn *Adamts-1* null pups. (A) Kidney sections of E18.5 wild-type and *Adamts-1* null mice. (B) Kidney sections of postnatal day 1 (P1) wild-type and *Adamts-1* null mice. Magnification 5×.
modest interstitial matrix expansion as evidenced 
by accumulation of collagen type IV expression in the 
peri-tubular and peri-glomerular interstitium, com-
pared to wild-type animals (Figure 3A–C). Collagen 
type IV was also localized to glomeruli of the 
Adamts-1 knockout mice that was not observed in wild-type 
control animals. Immunohistochemical studies showed 
increased protein expression of TGF-β localize
to infiltrating macrophages in the cortical interstitium 
and the visceral and parietal epithelium of glomeruli from 
Adamts-1 null mice (Figure 3D–F).

An analysis of the site of expression of Adamts-1 
during kidney development was performed using 
cultured explanted metanephroi as previously described 
[4]. This revealed a striking pattern of expression 
with Adamts-1 exclusively expressed in the elongating 
tubules of the maturing nephrons (Figure 4A).

Expression commenced after the formation of the 
comma-shaped bodies (Figure 4C) and was present 
in the proximal convoluted tubules and distal con-
voluted tubules (Figure 4B), but most obviously in 
the loops of Henle (Figure 4D), which balloon out 
from each S-shaped body as the nephrons mature.
In contrast to previous suggestions [3], there was 
no Adamts-1 expression in the ureter or ureteric bud, 
suggesting that Adamts-1 will not be expressed in the 
collecting ducts (Figure 4).

Fig. 2. Fibrosis in Adamts-1 null mice. (A) Kidney sections of P1 wild-type and Adamts-1 null mice. (B) Magnified regions indicated on (A) showing the uretero-pelvic region. (C) Kidney sections of 6-month-old wild-type and Adamts-1 null mice. (D) Kidney sections of one-year-old wild-type and Adamts-1 null mice. Masson’s trichrome staining: the collagen accumulation is evidenced by the blue staining. Magnification: A, 5×; B, 10×; C, D, 5×. P, pelvis; M, medulla; C, cortex.

Fig. 3. Immunohistochemistry of F4/80-positive macrophages, collagen type IV and TGF-β protein expression in the kidneys of 1 year-old Adamts-1 null mice and wild-type control animals. (A) Type IV collagen protein expression in wild-type control mice (arrows; magnification ×250). (B) Accumulation of type IV collagen protein in the peritubular interstitium of Adamts-1 null mice (arrows; magnification 250×). (C) Type IV collagen was increased in expression in glomeruli from 1-year-old Adamts-1 null mice, compared to wild-type control animals (arrows; magnification 250×). (D) TGF-β protein was localized to the visceral and parietal epithelium of glomeruli from Adamts-1 null mice (arrows; magnification 250×). (E) TGF-β protein was localized to macrophages in the tubulointerstitium of Adamts-1 null mice (arrows; magnification 150×). (F) Lack of TGF-β staining in the glomeruli and tubulointerstitium of wild-type control animals (magnification 250×). (G) F4/80-positive macrophages were observed in the cortical interstitium of wild-type control mice (arrows; magnification 250×). (H) Numbers of F4/80-positive interstitial macrophages were not found to be increased in Adamts-1 null mice compared to control animals (arrows; magnification 250×).
We observed no tubular dilatation, a downstream effect of obstructive nephropathy, in the kidneys of Adamts-1 null mice at any age. However, mild interstitial matrix expansion associated with accumulation of type IV collagen in the interstitium and glomeruli of 1-year-old Adamts-1 null mice was demonstrated. At this time-point, the glomeruli and interstitial macrophages displayed increased TGF-β protein expression, compared to age-matched wild-type animals. From these observations, it is unlikely that fibrosis participates in the establishment of the kidney defect, but rather occurs as a consequence of the structural abnormalities already present earlier on in the kidney.

The kidney phenotype observed in our mice was not consistent with a lack of Adamts-1 causing fibrosis in the uretero pelvic junction, and since the report of Shindo et al. [3] had concluded that Adamts-1 was normally expressed in this region and its absence explained the appearance of the resultant fibrotic lesion, we determined the expression pattern of Adamts-1 in the developing kidney. A distinct pattern of expression was found in the elongating tubules growing out from each S-shaped body as the nephrons form. This was most prominent in the regions forming the loops of Henle. No Adamts-1 expression could be detected by RNA in situ hybridization around the junction with the collecting ducts, suggesting that Adamts-1 is in fact not required for the structure and function of these areas. Notably, consistent with our results, no Adamts-1 expression was detected by RNA in situ hybridization in the epithelium of the ureteric bud-derived collecting ducts by another group [1].

The exact role of Adamts-1 in kidney development remains to be determined but since Adamts-1 is a secreted disintegrase, it is possible that it plays a role in the modelling of the medulla and pelvis by allowing the loops of Henle to correctly descend into this region of the kidney. A lack of this activity may explain the reduction in the size of the medulla. This hypothesis has some support in that two other members of the Adamts family, Adamts-20 and gon-1, have been shown to be essential for remodelling of extracellular matrix components to allow cells to migrate correctly. Expression of Adamts-20 by cells just ahead of migrating pigment cells is necessary for melanoblasts to colonize the coat in the trunk near the hindlimbs, the most rapidly growing part of the mouse embryo. Also, mutation of gon-1, the orthologue of Adamts-20 in Caenorhabditis elegans, causes a failure of the leader cells of the gonadal arms to migrate [reviewed in 6]. A disturbance in the normal migratory processes of the loops of Henle may also

**Discussion**

Here we report that a lack of Adamts-1 in the kidney causes a defect most probably due to developmental dysgenesis rather than to a fibrotic lesion in the utero-pelvic junction as previously reported [3]. The kidney malformation in our Adamts-1 null mice was identified on the day of birth, contradicting the report by Shindo et al. [3], who stated that the defect was evident 4 weeks after birth. Both the medulla and cortex of the kidneys of Adamts-1 null mice generated in this study were significantly reduced in size, consistent with the previous report [3]. We found that 45% of the Adamts-1 null pups die in the neonatal period [2], but those that survive, even to 1 year of age, seem to have no adverse effect due to their kidney malformation.

We found no evidence of fibrosis in the kidneys of Adamts-1 null mice generated in this study at the time of appearance of the defect, nor at 2 months of age, while Shindo et al. [3] reported the occurrence of interstitial fibrosis in the collecting ducts and ureters of their four-week-old Adamts-1 null mice. The fibrosis was proposed to cause a blockage at the uretero-pelvic junction, and hence lead to the calyceal enlargement observed. Although the knockout mice were impaired in their ability to concentrate urine, no severe renal failure was reported in these mice [5].

Online Supplementary Figure A.

![Figure A](image-url)  
**Fig. 4.** Expression of Adamts-1 in developing kidney explants as assessed by RNA in situ hybridization. (A) E11.5 mouse embryonic kidney explant after 4 days of culture at low magnification. Adamts-1 expression is seen in the developing nephrons at the time of S-shaped body formation. (B) Magnified region indicated on (A) showing Adamts-1 expression in the developing proximal convoluted tubules (pct), loops of Henle (loh) and distal convoluted tubules (dct) of the developing nephrons. (C) Early Adamts-1 expression in a comma-shaped body as it begins to elongate. (D) Adamts-1 expression in developing loops of Henle (loh). Scale bar: 200 mM for (A) and 50 mM for (B–D). This figure is available in colour as online Supplementary Figure A.
explain the apparent lack of phenotype in our Adamts-1 null kidneys until after birth. While nephron endowment ceases close to birth, the medullary interstitial field remains extensive through the first few weeks after birth and it is during this time that final remodelling and tubular maturation occurs, until the final kidney is a compact structure with very few renal medullary interstitial cells.

Although our Adamts-1 null mice display a similar type of kidney defect to that reported earlier [3], there are a number of inconsistencies. The major discrepancy is the finding that the kidney malformation observed in our mice is a direct result of a developmental dysgenesis, rather than an event secondary to fibrotic obstruction of the utero-pelvic junction. The differences between the two studies may reflect the different strategies used to disrupt the Adamts-1 gene. In our study we removed exon 2, leaving a single 34-bp loxP site in intronic sequence, whereas in the other report, exons 2–4 were replaced by a neomycin-cassette, which was not removed prior to generating the mice. We demonstrated the absence of Adamts-1 protein [2]; however, Shindo et al. failed to show a similar loss of protein expression, even though they had an anti-Adamts-1 antibody.

In conclusion, the kidney defect observed in our Adamts-1 null mice most likely stems from a cause other than a blockage of the ureter since we did not observe fibrotic lesions in the ureter nor the severe progressive disease that would be associated with obstructive nephropathy. While the precise cause has not been delineated in this study, it is evident that Adamts-1 is essential for the structural integrity of the medulla and pelvic region, and it may be that it is an inherent dysgenesis of the fine structure in this region that is causative of the distension and fibrosis observed in the kidneys of Adamts-1 null mice.

Conflict of interest statement. None declared.

References

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