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REVIEW ARTICLE

# Congenital ureteropelvic junction obstruction: human disease and animal models

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## Summary

Ureteropelvic junction (UPJ) obstruction is the most frequently observed cause of obstructive nephropathy in children. Neonatal and foetal animal models have been developed that mimic closely what is observed in human disease. The purpose of this review is to discuss how obstructive nephropathy alters kidney histology and function and describe the molecular mechanisms involved in the progression of the lesions, including inflammation, proliferation/apoptosis, renin–angiotensin system activation and fibrosis, based on both human and animal data. Also we propose that during obstructive nephropathy, hydrodynamic modifications are early inducers of the tubular lesions, which are potentially at the origin of the pathology. Finally, an important observation in animal models is that relief of obstruction during kidney development has important effects on renal function later in adult life. A major short-coming is the absence of data on the impact of UPJ obstruction on long-term adult renal function to elucidate whether these animal data are also valid in humans.

**Keywords**
fibrosis, mechanical stress, neonatal models

## Introduction

Congenital obstructive nephropathy is the main cause of end stage renal disease (ESRD) in children (Benfield *et al.* 2003). This contrasts sharply with adult ESRD which for the greater part originates from ageing and type II diabetes. Hydronephrosis, defined clinically by an enlargement of the kidney as a result of urine accumulation in the renal pelvis or calyces, is a consequence of obstructive nephropathy. The most frequently found cause of hydronephrosis is ureteropelvic junction (UPJ) obstruction with an estimated incidence of 1 in 1000–1500 (Chang *et al.* 2004). UPJ obstruction is mostly considered as a functional obstruction originating from abnormalities in the smooth muscle of the pelvis and ureter (Mendelsohn 2004). Physical obstruction of the ureter, e.g. compression of the UPJ by crossing vessels, is also observed but whether the vessel alone causes obstruction or whether there is also a functional component is still debated (Yiee *et al.* 2010). Although surgery in UPJ obstruction is efficient in protecting against short term detectable renal lesions, increasing support obtained in both experimental- and human-studies is available suggesting that UPJ obstruction induces permanent modifications of the renal parenchyma.

Other causes of congenital obstructive nephropathy include disorders of the urethra such as posterior valves or disorders of the bladder such as trigonal cysts (Roth *et al.* 2002). However, these are all rare diseases. Therefore, we will focus in this review on unilateral UPJ obstruction as it is the most frequently encountered obstructive nephropathy in children and therefore the majority of the insight of human obstructive nephropathy comes from this pathology. In addition, excellent neonatal rodent and foetal models for partial and complete UPJ obstruction have been developed (Peters 2001; Thornbill *et al.* 2005). We have attempted to combine all the available knowledge obtained in human UPJ obstruction, animal models and *in vitro* studies with the purpose of identifying concepts that hold both in the models

and in human pathology and to support future experiments aiming at the better understanding of obstructive nephropathy and drug testing.

As the response of the adult kidney to obstruction clearly differs from the response of the foetal kidney (Chevalier *et al.* 2009), all work described in this review concerns responses to foetal or neonatal obstruction. Any reference to adult obstruction is clearly indicated and foetal/neonatal obstruction is the default situation.

## Causes UPJ obstruction

### *Causes of human UPJ obstruction*

Effective urine transport depends on formation of proper connections and functioning between the kidney and the ureter. In UPJ obstruction attention has been drawn on the development of smooth muscle cells that line the pelvis and the ureter and conduct peristaltic waves to expel urine (Chang *et al.* 2004; Lye *et al.* 2010). Failure in development of the renal pelvis or impaired smooth muscle differentiation can, indeed, lead to functional obstruction and hydronephrosis in experimental models (Miyazaki *et al.* 1998). Also, analysis of tissue obtained from the ureteropelvic junction of patients which have undergone pyeloplasty (i.e. surgical reconstruction or revision of the renal pelvis to drain and decompress the kidney) have shown that the defective urinary tracts present pathological changes in both smooth muscle rearrangement and pyeloureteral innervation (Zhang *et al.* 2000). An interesting hypothesis has recently been proposed to explain the role of smooth muscle cells in kidney maturation during late human gestation. *In utero*, the head of the embryo usually rests in the mother's pelvis (Lye *et al.* 2010). In this position urine must work against gravity and when peristalsis fails, this will lead to functional obstruction of the maturing kidney.

Although the molecular mechanisms underlying this smooth muscle cell maldevelopment are still largely unknown this seems, to date, to be the most probable cause of UPJ obstruction (Mendelsohn 2004). As this review focuses on the renal consequences of UPJ obstruction, we will not discuss the (genetic) details of failure of the normal smooth muscle development in the ureter and in the pelvis. For more information in this field readers should refer to other excellent reviews (Mendelsohn 2004; Lye *et al.* 2010).

### *Animal models of UPJ obstruction*

Different animal models of UPJ obstruction exist, either spontaneous or specifically developed, that mimic human UPJ obstruction, each with specific merits and drawbacks. Specific rodent strains have been identified that present reproducible lesions of spontaneous congenital hydronephrosis (Peters 2001). Although the underlying causes responsible for this spontaneous UPJ obstruction phenotype remain poorly understood, these models are interesting in that they are naturally occurring and reflect the human pathology more rationally. However, the often observed infertility or low reproduction status of these strains limits the use of such models (Peters 2001).

Unilateral ureteral obstruction (UUO) is a well-known surgical model to study tubulointerstitial fibrosis in adult rodents (Bascands & Schanstra 2005). However, the effects of obstruction in the mature adult kidney differ widely of what happens in the developing kidney. Indeed, in the latter, the obstruction can interfere with kidney morphogenesis, growth and maturation. For this reason, different models of prenatal obstruction have been developed in many species (Peters 1997). Compared to the congenital models, they offer the advantages of controlling the onset, the duration and the severity of the pathology. In sheep, primate and guinea pig, in which renal nephrogenesis, like in humans, is completed before birth but where renal maturation continues after birth, UUO must be performed during foetal life (Chevalier *et al.* 2009). However intrauterine surgery is a complex procedure. An alternative to this approach is the use of the opossum, which is a marsupial with extrauterine foetal development. The surgery can thus be performed in the mother's pouch (Peters 1997; Chevalier *et al.* 2009). The other alternative is to study the effect of UUO in the neonate mouse, rat, rabbit or pig. In mice and rats, only 10% of the nephrogenesis is completed at birth, corresponding to the midtrimester in the human foetus and continues 10 days after birth followed by a period of renal maturation. The advantage of rodent models is the potential use of knockout or transgenic animals to study the role of a specific molecule in the pathophysiology of obstruction and the relatively easy access to these animals to increase statistical power. In rabbits and pigs, as in mice and rats, nephrogenesis is incomplete at birth and continues for 2 and 3 weeks after birth, respectively. The advantage in pigs is that the kidney anatomy is close to the human anatomy. As opposed to the unipapillary structure of the rodent kidney, pigs have multipapillary kidneys and predominantly short-looped nephrons similar to humans (Eskild-Jensen *et al.* 2007). Although very useful, the remaining question of these postnatal models is whether the neonatal kidney reacts differently to the obstruction compared to the foetal kidney. Indeed, the demand on renal function is much higher in neonates than in foetuses where placenta is partly involved in foetal blood dialysis (Peters 1997).

Another issue is the technique employed to perform the obstruction. Basically, there are two major ways to study the effect of obstruction in foetuses and neonates: complete or partial obstruction of the ureter. Complete obstruction is most often performed by a simple suture ligation of the ureter. This model mimics severe UPJ obstruction and evolves rapidly towards hydronephrosis and loss of renal parenchyma (1–2 weeks after UUO) (Chevalier *et al.* 2009). But, since in the clinical situation most of the UPJ cases present partial rather than complete obstruction, partial UUO models have been developed. One technique consists in wrapping the ureter into the underlying psoas muscle which allows the obstruction to evolve with the growing animal but leads to

variable results. Another method consists in placing a stainless steel wire of known diameter parallel to the ureter, followed by ligature of both the ureter and the stainless steel wire. Subsequent withdrawing of the wire results in a well calibrated partial obstruction. Not only this technique is more reproducible than the psoas muscle approach but also, by choosing the different diameters of the stainless steel wire, the severity of the obstruction can be controlled (Chevalier *et al.* 2009).

In conclusion, a number of animal models have been used to understand the pathophysiology of UPJ obstruction. Clearly none of the individual models is perfect, but combining data obtained in the different species and by different techniques will help to better characterize the mechanisms involved in this pathology.

## Renal consequences of UPJ obstruction

### *Histological alterations*

The spectrum of renal abnormalities varies greatly in UPJ obstruction. In human renal biopsies, one observes all lesions found in renal disease including subtle changes such as modified proximal or tubular size, chronic tubulointerstitial injury, glomerulosclerosis, fibrosis, aberration of nephron development and in severe cases (<1%) renal dysplasia (Rosen *et al.* 2008).

Studies have attempted to link the histological state of renal biopsy to the function of the obstructed kidney. However, no clear correlation between differential renal function and the gross histological status of the kidney was observed (Elder *et al.* 1995; Stock *et al.* 1995; Han *et al.* 1998; Zhang *et al.* 2000). The only observation that seems to hold is that severe UPJ obstruction (differential function of <30%) is associated with a high degree of parenchymal damage (Zhang *et al.* 2000). Also renal histology in humans was found not to be related to the duration of obstruction (Han *et al.* 1998). This absence of a clear correlation between renal function and gross renal histology might be the cause of the observed high variability in the outcome of UPJ obstruction and the persisting difficulties in the clinical assessment of UPJ obstruction (Csaicsich *et al.* 2004). However, detailed analysis of kidney histology in UPJ obstruction showed that subtle histological changes might be associated to impaired function of the obstructed kidney (Huang *et al.* 2006). It was observed that in biopsies with little tubulointerstitial changes, the sizes of both proximal and distal tubules were significantly larger in UPJ obstruction patients with significant functional obstruction (Huang *et al.* 2006).

In contrast to these observations in humans, alterations of renal parenchyma have been intensively studied in animal models of UPJ obstruction (Figure 1). Although these lesions can differ depending on the species or the severity of the pathology, a general observation is that the earlier obstruction occurs during *in utero* nephrogenesis, the more severe the associated histopathological changes (Matsell & Tarantal 2002). Alterations affect renal growth, nephron number, glomerular and tubulointerstitial histology, as well as the contralateral (i.e. non-obstructed) kidney.

*Renal growth*. Partial and complete UUO in newborn rodents is characterized by an enlarged pelvic diameter and increased kidney volume (Shi *et al.* 2004; Thornhill *et al.* 2005; Topcu *et al.* 2007; Guerin *et al.* 2008). UUO severely affects kidney growth, as shown by decreased renal mass, protein or DNA content and parenchymal atrophy (Chevalier *et al.* 1996; Chung & Chevalier 1996; Wen *et al.* 2002; Shi *et al.* 2004; Thornhill *et al.* 2005; Topcu *et al.* 2007; Guerin *et al.* 2008). UUO also inhibits nephrogenesis thereby reducing the number of functional nephrons (Josephson 1983; Chevalier *et al.* 1999; Wen *et al.* 2002; Thornhill *et al.* 2005; Burt *et al.* 2007). Many of these effects are also observed in neonatal pig model (Eskild-Jensen *et al.* 2002). In addition, and in contrast to human, variable partial UUO in the neonatal rat shows that these lesions increase with the severity of the UUO (Thornhill *et al.* 2005).

*Glomerular changes*. In the neonatal model, partial or complete UUO results in a decrease in the number of glomeruli, not only by halting nephrogenesis but also by destruction of previously formed glomeruli via apoptosis (Cachat *et al.* 2003; Eskild-Jensen *et al.* 2007) or phenotypic transformation of glomerular cells into mesenchymal cells (Thornhill *et al.* 2005; Chevalier 2008). Glomerular maturation, evaluated by the number of capillary loops and the phenotype of podocytes, is delayed (Chevalier *et al.* 1999a, 2000a; Wen *et al.* 2002; Cachat *et al.* 2003; Burt *et al.* 2007; Chen *et al.* 2007) and vascular tuft area is reduced (Chevalier *et al.* 1999a; Burt *et al.* 2007). Complete UUO increases the number of renin secreting cells (Norwood *et al.* 1994) and maintains foetal renin distribution along the afferent arteriole rather confining renin expression to the juxtaglomerular apparatus (el-Dahr *et al.* 1991; Chung & Chevalier 1996). In oppossum pups and in foetal lambs, a similar UUO-induced decrease in glomerular number is observed (Liapis *et al.* 2000; Mure *et al.* 2006a), associated to podocyte foot processes fusion (Fenghua *et al.* 2009) and cystic-like changes such as dilatation of Bowman's space and collapsed capillary loops (Liapis *et al.* 2000; Mure *et al.* 2006a; Fenghua *et al.* 2009).

*Tubulointerstitial injury*. Tubular damage is an important characteristic of UPJ obstruction, which is observed following obstruction in neonatal rodents, foetal sheep and opossum pups. They include tubular apoptosis (Chevalier *et al.* 1999a, 2000a; Liapis *et al.* 2000; Cachat *et al.* 2003; Thornhill *et al.* 2005; Lange-Sperandio *et al.* 2006, 2007; Yoo *et al.* 2006a; Burt *et al.* 2007; Eskild-Jensen *et al.* 2007a), tubular dilatation (Steinhardt *et al.* 1995; Chevalier *et al.* 1999a; Liapis *et al.* 2000, 2001; Cachat *et al.* 2003), tubular atrophy (Chevalier *et al.* 1999a, 2000a; Fern *et al.* 1999; Lange-Sperandio *et al.* 2002, 2006, 2007; Thornhill *et al.*

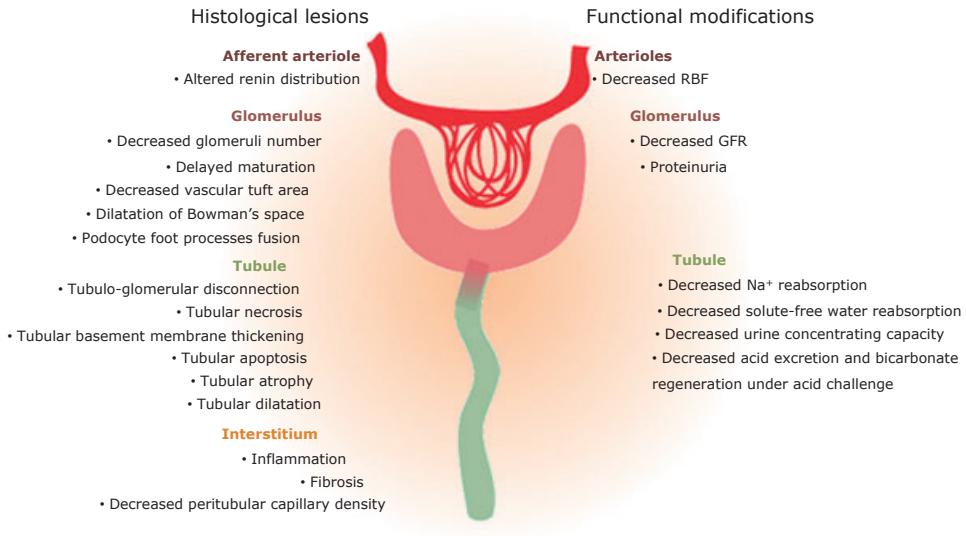


**Figure 1** Histological lesions and functional modifications induced by obstruction.

2005), tubular basement membrane thickening (Cachat *et al.* 2003) and necrosis (Cachat *et al.* 2003). Progressive apoptosis/necrosis of tubules may promote glomerulotubular disconnection leading to non functional nephrons (Thornhill *et al.* 2005). Interestingly, each tubular segment responds differently to complete UUO: apoptosis and dilatation are most consistently observed in collecting ducts and distal tubules, the tubular segments adjacent to the obstructed pelvis, whereas basement membrane thickening and necrosis predominate in the proximal tubule (Cachat *et al.* 2003). However, no overall changes in either proximal- or distal-tubule length are found in the obstructed kidney of newborn pigs, explained by a combination of a reduction in length in damaged tubules and increased length in compensating nephrons (Eskild-Jensen *et al.* 2002, 2007a). Finally, effect of UUO on tubular proliferation is not clear because both increase (Cachat *et al.* 2003; Thornhill *et al.* 2005) and decrease (Chevalier *et al.* 1999a, 2000a) in proliferation were observed in the obstructed kidney.

Partial and complete UUO also modify the peritubular space of the obstructed kidney. The main interstitial modifications are enhanced inflammation (Chevalier *et al.* 1996; Lange-Sperandio *et al.* 2002, 2006, 2007; Burt *et al.* 2007; Chen *et al.* 2007; Guerin *et al.* 2008), tubulointerstitial fibrosis (Steinhardt *et al.* 1995; Chevalier *et al.* 1999a,b, 2000a; Fern *et al.* 1999; Liapis *et al.* 2000, 2001; Lange-Sperandio *et al.* 2002, 2006, 2007; Cachat *et al.* 2003; Kiley *et al.* 2005; Mure *et al.* 2006a; Chen *et al.* 2007; Eskild-Jensen *et al.* 2007b; Guerin *et al.* 2008) and decreased peritubular capillary density. The latter has been shown to be particularly prominent in the medulla during complete UUO (Burt *et al.* 2007).

Tubular and interstitial changes are closely correlated. The number of apoptotic tubular cells was found to parallel fibrosis (Cachat *et al.* 2003; Guerin *et al.* 2008) and macrophage infiltration (Lange-Sperandio *et al.* 2002; Guerin *et al.* 2008). Interestingly, while alterations are rapidly induced following complete UUO in foetal sheep, inflammatory cells are not visible. However, a marked inflammatory infiltrate, associated with increased fibrosis, is observed if foetal obstruction continues after the birth (Mure *et al.* 2006a). Consequently, the inflammatory response has probably not a decisive role in the occurrence of renal alterations during foetal life (Mure *et al.* 2006a) and UUO induced-inflammation in newborn rodents may be influenced by the renal functional demand imposed by extrauterine life (Matsell & Tarantal 2002).

*The contralateral kidney.* In response to partial and complete UUO, the non-obstructed contralateral kidney develops compensating hypertrophy (Norwood *et al.* 1994; Chevalier 1996; Chevalier *et al.* 1996, 1999a,b; Fern *et al.* 1999; Malik *et al.* 2001; Chung & Wen *et al.* 2002; Cachat *et al.* 2003; Shi *et al.* 2004b; Topcu *et al.* 2007), the adaptative growth-rate increasing with the severity and duration of obstruction (Yoo *et al.* 2006a). Neither the number of glomeruli (Chevalier *et al.* 1999a; Cachat *et al.* 2003; Thornhill *et al.* 2005; Burt *et al.* 2007) nor their maturation index are modified (Chevalier *et al.* 2000a; Cachat *et al.* 2003). However, the mean glomerular area is higher than in the control kidney (Chevalier *et al.* 1999a; Yoo *et al.* 2006a) and renin expression is inhibited (Norwood *et al.* 1994; Chevalier *et al.* 1996) while the number and the localization of renin cells are normal (Norwood *et al.* 1994; Chevalier *et al.* 1999a). No particular modification is described in tubules excepted proliferation which is sometimes observed (Cachat *et al.* 2003) and the number of peritubular capillaries is not changed (Burt *et al.* 2007).

In conclusion, nearly all histological alterations found in humans in response to UPJ obstruction are found in the prenatal and neonatal animal UUO models suggesting that the

observations in animal models have fair chance to be valid in human UPJ obstruction.

### *Functional modifications*

It is well established that UPJ obstruction induces mild to severe impairment of kidney function, including glomerular filtration and tubular exchanges of water and solutes. Studies in humans are lacking but how these mechanisms are modified has been extensively studied in animal models of obstruction (Figure 1). Changes in the obstructed kidney, in the contralateral kidney and in the whole animal are variable, depending on species, the duration and the severity of obstruction, but also depend on the diet and the hydration status.

*Renal blood flow.* Limited data are available on the response of renal blood flow (RBF) to foetal/neonatal UUO. In the neonatal rat, 4–24 weeks of partial UUO induces a progressive reduction in RBF in the obstructed kidney (Shi *et al.* 2004b; Topcu *et al.* 2007; Wen *et al.* 2009). In foetal lamb, chronic complete UUO also decreases RBF, this effect being less severe when UUO is partial (Nguyen & Kogan 1998; Mure *et al.* 2006b). This diminution in RBF leads most probably to ischemia, thus contributing in part to the observed lesions of renal tissue.

*Glomerular filtration.* In general, neonatal UUO results in decreased glomerular filtration rate (GFR) in the obstructed kidney as seen in rats (Josephson 1983; Shi *et al.* 2004a,b; Thornhill *et al.* 2005; Topcu *et al.* 2007) and pigs (Eskild-Jensen *et al.* 2000, 2001). When partial UUO persists, the fall in GFR becomes more pronounced in the rat (Shi *et al.* 2004a) whereas it is attenuated in the pig model (Eskild-Jensen *et al.* 2000, 2001). In the non-obstructed contralateral kidney, GFR may either increase (Josephson 1983; Eskild-Jensen *et al.* 2001, 2007b) or remain unchanged (Shi *et al.* 2004a; Thornhill *et al.* 2005; Topcu *et al.* 2007) and the compensatory response of contralateral kidney depends also on the duration of obstruction (Eskild-Jensen *et al.* 2001). In parallel proteinuria develops in the obstructed kidney and contralateral kidney following partial or complete UUO, respectively (Thornhill *et al.* 2005). Finally, total GFR in obstructed animals can reach (Eskild-Jensen *et al.* 2001; Wang *et al.* 2009) or not (Shi *et al.* 2004a; Topcu *et al.* 2007) the same level as GFR in the sham operated animals.

*Tubular function.* Modification of tubular function in response to UUO in neonatal rats was extensively studied and showed profound effects of UUO on the tubular handling of water and solutes. Partial UUO is clearly associated with a defect in sodium reabsorption since the fractional excretion of sodium is increased in the obstructed kidney. As a result, and in the absence of a compensatory change in the non-obstructed kidney, animals display natriuria in spite of a decrease in the filtered sodium load (Shi *et al.* 2004a,b; Eskild-Jensen *et al.* 2007a; Topcu *et al.* 2007). In addition, the solute-free water reabsorption ($T^cH_2O$) is markedly decreased in the obstructed kidney from partially obstructed rats, indicating that the ability of these kidneys to reabsorb water in collecting ducts is reduced. $T^cH_2O$ does not seem to increase in the contralateral kidney and finally, urine osmolarity decreases, showing an impaired urinary concentrating capacity in UUO animals (Shi *et al.* 2004a,b; Topcu *et al.* 2007). These effects can be explained by looking at the UUO-induced changes in transporters expression and activity. Neonatal complete or partial UUO induces a reduction in expression and distribution of major sodium transporters and water channels including Na,K-ATPase, NHE1, NHE3 and aquaporins (AQP1, AQP2, AQP3, AQP7), associated to a down-regulation of the vasopressin 2 receptor (Table 1) (Silverstein *et al.* 2003a; Shi *et al.* 2004a; Manucha *et al.* 2007; Topcu *et al.* 2007; Wang *et al.* 2009). This decrease of transporters expression could be regulated by dynamin, a key component of the endocytic machinery involved in internalization of several transporters, as it was found frequently stimulated in UUO (Table 1) (Silverstein *et al.* 2003a; Padovano *et al.* 2009; Moeller *et al.* 2010; Ramstrom *et al.* 2010). In parallel to the regulation of transporter expression, it seems also that the activity of the transporters can be modified. Indeed, the mechano-sensitive gap-junction protein connexin 30 participates in ATP release into the tubular fluid and this inhibits salt and water reabsorption by the transporters (Sipos *et al.* 2009). Interestingly, connexin 30 expression is increased in the obstructed kidney (Table 1) (Silverstein *et al.* 2003b). Altogether, these results provide the molecular mechanism for the observed modified urine osmolality in neonatal UUO animals.

*Uremia, hydromineral and acido-basic status.* Plasma urea levels are identical in neonatal UUO and control rats (Shi *et al.* 2004a), indicating that the UUO-induced filtration defect is either not severe enough to induce hyperuremia or counterbalanced by a defect in urea reabsorption. Moreover, in spite of the reduced ability to concentrate urine, animals with neonatal UUO have normal natremia and osmolality (Shi *et al.* 2004a,b; Topcu *et al.* 2007) and are probably also not dehydrated because arterial blood pressure is either unchanged (el-Dahr *et al.* 1991; Fern *et al.* 1999; Eskild-Jensen *et al.* 2007a) or increased (Topcu *et al.* 2007), but not decreased.

Partial UUO induces a transient increase in the expression of transporters involved in the acid/base balance such as NHE3, NBC1, Pendrin and Na,K-ATPase after 7 weeks of obstruction, as a compensatory mechanism to maintain systemic acid-base balance (Table 1) (Wang *et al.* 2009). However, at later stages, this compensatory mechanism is failing and the expression of the transporters is dramatically decreased. Interestingly in these animals, the acid–base equilibrium is not modified as no changes in blood pH, bicarbonates and $CO_2$ pressure were observed (Wang *et al.* 2009). The consequences of the transporter down-regulation are observed only when animals are subjected to acid loading. During acid challenge, UUO rats fail to regulate acid

**Table 1** Literature data about tubular transport and glomerular function-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which activity or expression has been significantly modified compared to sham-animals (animal model) or control cells (*in vitro*)

| | | Animal model | | | *In vitro* | | |
|---|---|---|---|---|---|---|---|
| Gene symbol | Gene name | PUUO | CUUO | Ref | Stretch | Fluid flow | Ref. |
| AQP1 | Aquaporin 1 | Down (24w) | | Shi *et al.* (2004a) | | | |
| AQP2 | Aquaporin 2 | Down (10w) | | Topcu *et al.* (2007) | | | |
| AQP3 | Aquaporin 3 | Down (24w) | | Shi et al. (2004a) | | | |
| AQP7 | Aquaporin 7 | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| ATP1a1 | Na+/K+ ATPase | Down (7w to 24w) | | Shi et al. (2004a), Topcu *et al.* (2007), Wang *et al.* (2009) | | | |
| Avpr2 | Vasopressin V2 receptor | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| Calb1 | Calbindin D28 | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| Dnm1 | Dynamin | | Up (2w) | Silverstein *et al.* (2003a) | | | |
| Gjb6 | Connexin 30 | | Up (2w) | Silverstein *et al.* (2003b) | | | |
| Itga3 | Integrin, alpha 3 | | | | Down | | Dessapt *et al.* (2009) |
| Itgb1 | Integrin, beta 1 | | | | Down | | Dessapt *et al.* (2009) |
| Nphs1 | Nephrin; nephrosis 1 homolog | | | | Down | | Miceli *et al.* (2010) |
| Scnn1a | ENaC; epithelial Na+ channel | | | | | Up* | Satlin *et al.* (2001) |
| Slc12a3 | Thiazide-sensitive sodium-chloride cotransporter | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| Slc20a1 | Sodium-dependent phosphate transporter 1 | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| Slc21a4 | OAT-K1; kidney-specific anion transporter | | Down (2w) | Silverstein *et al.* (2003a) | | | |
| Slc26a4 | Pendrin; sodium-independent chloride/iodide transporter | Up (7w) | | Wang *et al.* (2009) | | | |
| Slc4a4 | NBC1; anion exchanger, member 4 | Up (7w) Down (14w) | | Wang *et al.* (2009) | | | |
| Slc4a7 | NBCn1; sodium bicarbonate cotransporter, member 7 | Down (14w) | | Wang *et al.* (2009) | | | |
| Slc9a1 | NHE1; sodium/hydrogen exchanger, member 1 | | Down (2w) | Manucha *et al.* (2007) | | | |
| Slc9a3 | NHE3; sodium/hydrogen exchanger, member 3 | Up (7w) | Down (2w) | Silverstein *et al.* (2003a), Wang *et al.* (2009) | | | |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; PUUO: partial UUO; CUUO: complete UUO; *activity; Ref.: references.

excretion and bicarbonate regeneration and develop metabolic acidosis whereas control rats maintain normal blood pH (Wang *et al.* 2009).

Finally, it is interesting to point out that other proteins or transporters involved in the hydromineral balance such as the sodium dependent phosphate transporter, the thiazide sensitive sodium chloride transporter, the kidney specific anion transporter OAT-K1 and calbindin D28, implicated in calcium transport regulation, are also down-regulated during experimental neonatal UUO (Table 1) (Silverstein *et al.* 2003a).

### *Renal recovery after UPJ obstruction release*

Although it has been observed that even delayed pyeloplasty leads to recovery of short term renal function (Chertin *et al.* 2006), what is the consequence of the UPJ-induced renal lesions later in life? One recent study reports that surgically corrected UPJ obstruction leads to improved renal function compared to preoperative function in patients followed just after puberty (Chertin *et al.* 2009). Unfortunately in this study the control group without pyeloplasty is missing. Moreover, since long-term studies in humans are not available, the consequence of UPJ on adult renal function is not known.

Once again, the use of experimental animal models has shed light on the renal consequences of UUO after surgical release. Studies in neonatal rodents have shown that after 5 days of complete UUO and although primary stimulus for injury was removed, structural lesions persist 1 month after release: (i) glomerular number was still low (Chevalier *et al.* 1999a), indicating that the loss of nephrons is definitive; (ii) renin expression was not confined to the juxtaglomerular apparatus thus conserving its immature expression pattern (Chevalier *et al.* 1999a); (iii) tubular deterioration was attenuated, but not reversed, since tubular atrophy with

significant apoptotic lesions was still observed (Chevalier *et al.* 1999a,b); (iv) fibrotic lesions remained; and (v) cytokine/growth factor expression did not returned to basal levels (Chevalier *et al.* 1999a,b). An exception is in neonatal mice, where the process of glomerulotubular disconnection is arrested by release of obstruction (Thornhill *et al.* 2007).

While most of the initial UUO lesions did not disappear 1 month after release of the obstruction, glomerular function was strongly improved with a normalized GFR (Chevalier *et al.* 1999a). However, tubular function was still perturbed. Indeed, urine flow rate and sodium excretion were increased in the post-obstructed kidney with normal GFR, indicating that reduced reabsorption of water and sodium persisted (Chevalier *et al.* 1999a). This suggests that modified renal transporter expression is not corrected after the release. Surprisingly, although glomerular function 1 month after release was improved, this amelioration was only transient as both GFR and tubular reabsorption were profoundly impaired in the postobstructed kidney 3 months or 1 year after release (Chevalier *et al.* 2000b, 2002). The contralateral kidney maintained a stable GFR, but hypertrophy, glomerulosclerosis, tubular atrophy, inflammation and interstitial fibrosis were observed. In addition the contralateral kidney produced proteinuric urine (Chevalier *et al.* 2000b). Thus there was progressive loss of renal function due to glomerular and tubulointerstitial damage at the level of remnant nephrons (Chevalier *et al.* 2000b). Partial UUO led to a less severe phenotype since 6 months after 1 week-partial UUO, neither GFR nor sodium and solute-free water reabsorption are decreased in the postobstructed kidney (Shi *et al.* 2004b).

As seen above for 'the earlier the obstruction the worse the outcome', also 'the earlier the release the better the outcome' holds. Experiments where partial or complete UUO were induced during nephrogenesis and released at different time-points showed that early release of neonatal obstruction provided a better protection of renal structure and function than late release (Chevalier *et al.* 1999b; Shi *et al.* 2004b). Moreover, when renal outcome was analysed 1 month after temporary complete UUO performed either during nephrogenesis or during maturation in neonatal rats, it appears that maturing kidneys are more sensitive to obstructive injury immediately following the completion of nephrogenesis than during ongoing nephrogenesis (Chevalier *et al.* 2002).

Overall, in humans the follow-up on UPJ obstruction patients, either surgically corrected or spontaneously resolving UPJ obstruction shows that renal function is unaffected upto at least shortly after puberty in the majority of the UPJ obstruction population. However the gross and fine renal histology in the human UPJ obstruction kidney and animal experiments suggest that renal lesions are present at the moment of severe obstruction that potentially have important effects on adult renal function as it has been shown in animals (Chevalier *et al.* 2000b). Whether these animal data are also valid in human UPJ obstruction remains to be studied.

## Hydrodynamic modifications as potentially primary inducer of lesions

As tubular cells are first in line during the early phases of neonatal UUO, what are the different types of stress encountered by these cells? The literature points out to two potential candidates that can be involved in the primary induction of the renal lesions: tubular ischemia resulting from renal hypoperfusion and pressure-induced compression or stretching of tubular cells. However, these hypotheses mainly originate from knowledge obtained from the adult UUO model. In this section, we will discuss whether these hypotheses are still valid in neonatal UUO and we propose a new candidate-process involved in the early phases of UUO-induced lesions.

### *Tubular ischemia induced by renal hypoperfusion*

The first candidate, often proposed to be an early inducer of renal lesions following obstruction, is tubular ischemia. In adult animals, UUO induces a rapid increase in renal blood flow (RBF) caused by local prostaglandin E2/NO generation, which decreases the resistance of afferent arterioles. However, this increase is transient as only 2 h after UUO RBF declines due to activation of the renin–angiotensin system (RAS) and/or vasoconstrictor thromboxane. This decreased RBF is associated with a prompt reduction in medullar tissue oxygen tension (Wilson 1980; Nguyen & Kogan 1998; Le Normand *et al.* 2005; Quinlan *et al.* 2008; Jensen *et al.* 2009).

In contrast, UUO in the foetal lamb leads to a delayed (1 week after UUO) and less dramatic fall in RBF than in adult UUO (Nguyen & Kogan 1998). Thus ischemia and related insults like hypoxia, nutrient depletion and waste accumulation are indeed potentially involved in the pathophysiology of both adult and foetal/neonatal obstruction. However, due to differences in timing, it seems less relevant to consider ischemia as a primary and early inducer of renal lesions in foetal than in adult UUO.

### *Tubular compression and stretch*

In adult animals, UUO induces profound and rapid elevation of intrapelvic pressure during the first hours following UUO, which is immediately transmitted to renal tubules. Indeed, micropuncture experiments indicate that during the first 2 h following complete UUO, hydrostatic pressure is increased in the proximal tubule (Gottschalk & Mylle 1956; Dal Canton *et al.* 1977). Elevated intra-tubular pressure may have two consequences on the tubular cell. Either the tubular cell is exposed to compression, due to increased transmural pressure or renal tubule is subjected to pressure-induced deformation, leading to increased stretch of the cells (Quinlan *et al.* 2008). This increased pressure is only transient. Indeed, the combination of pelvic dilatation, reduction of RBF, reduction of glomerular filtration and continuous urine drainage by lymphatic and venous circulation, allows pelvic

pressure to rapidly decline until it reaches baseline levels (Wilson 1980; Nguyen & Kogan 1998; Quinlan *et al.* 2008). This is accompanied by normalisation of intra-tubular pressure (Gottschalk & Mylle 1956; Dal Canton *et al.* 1979; Wilson 1980). Thus, although transitory, it is often considered that stretch and compression of the tubular cells are primary inducer of UUO-induced tubular lesions in adults.

Complete UUO in foetal lamb also induces elevated pressure as measured in the ureteral segment above the ligature. Interestingly, this increased pressure is not transient like in adults, but persists even 10 days after obstruction (Nguyen & Kogan 1998). This suggests that compression of tubular cells and stretch may also be considered as good candidates for primary inducers of UUO-induced lesions. Different tubular segments potentially respond differently to these mechanical stimuli. The low compliance of the proximal tubule (Cortell *et al.* 1973) and the lack of tubular proximal dilatation in the newborn mouse after chronic complete UUO (Cachat *et al.* 2003) suggest that proximal cells are resistant to stretch. In contrast, distal tubules and collecting ducts have shown to be highly compliant (Cortell *et al.* 1973) and dilate without marked cellular proliferation after foetal/neonatal UUO (Liapis *et al.* 2000; Cachat *et al.* 2003) resulting potentially in tubular stretching. Thus, increased transmural pressure and mechanical stretch may be considered as primary inducers of lesions in foetal/neonatal model of UUO for proximal and distal parts of the renal tubule, respectively.

### *Modification of urinary shear stress*

As discussed above, modifications of intra-tubular hydrodynamic forces induce compression and stretch of epithelial cells. However, it has been recently suggested that another candidate can lead to renal lesion following urinary flow modifications (Essig *et al.* 2001; Rohatgi & Flores 2010). Indeed, renal tubules are continually subjected to fluid shear stress corresponding to the frictional forces exerted by flowing urine on the tubular wall. The intensity of shear stress depends on fluid viscosity, fluid flow rate (i.e. glomerular filtration rate) and tubular diameter (i.e. level of tubular dilatation).

Little is known about early changes of GFR during foetal and neonatal obstruction. Indeed, although it is known that neonatal or foetal chronic UUO induces GFR fall (Josephson 1983; Eskild-Jensen *et al.* 2000, 2001; Shi *et al.* 2004a,b; Thornhill *et al.* 2005; Topcu *et al.* 2007), variations of GFR during the first phases of the pathology were not studied. However in adults, GFR is shortly maintained in the first hours of obstruction, most probably since the increase in intra-tubular pressure is compensated by increased RBF and glomerular capillary pressure (Dal Canton *et al.* 1977). Then, a marked decrease of GFR occurs, due to reduction of both glomerular capillary pressure and RBF induced by vasoconstriction of afferent arterioles (Gottschalk & Mylle 1956; Dal Canton *et al.* 1979). Altogether these results suggest that the potential reduction of GFR, as seen in adults, associated to the tubular dilatation can lead to UUO-induced modifications of urinary shear stress and that shear stress may therefore participate to pathophysiology of obstructive nephropathy.

In conclusion, hydrodynamic fluid changes can potentially lead to processes including tubular compression, stretch and urinary shear stress that contribute to the early induction and progression of obstructive nephropathy (Figure 2). These issues have been studied on appropriate *in vitro* models and will be discussed within the following section. In contrast, because ischaemia seems to be involved in the later phases of neonatal and foetal UUO, the *in vitro* effects of ischaemia on tubular cells will not be reported. For this the reader is referred to other manuscripts (Heyman *et al.* 2008; Tanaka & Nangaku 2010).

## Molecular mechanisms: lessons from human studies and experimental models

The majority of the research efforts in human UPJ obstruction have focused on inflammatory and profibrotic responses as these were clearly identified events in UPJ obstruction (Elder *et al.* 1995; Zhang *et al.* 2000). Although data is scarce, it is confirming the general pathological mechanisms observed in renal pathology associated to renal fibrosis including inflammation, activation of the RAS, profibrotic cytokine induction, modification of tubular enzyme activity and extracellular matrix accumulation. This information has been largely complemented and confirmed by data obtained in a variety of animal models of obstruction. Moreover, the effects of intra-tubular hydrodynamic forces modifications have been studied *in vitro*. Some limits of these experiments exist. Indeed, pressure studies are limited and the effect of stretch has mainly been investigated on podocytes and proximal tubular cells with only a few studies describing the effects of stretch on distal tubule cells. Moreover, in all these studies, cells are not derived from foetal/neonatal animals. However, these *in vitro* experiments can give some clues to understand the molecular mechanisms involved in the progression of obstructive nephropathy.

### *Inflammation*

Chronic neonatal obstructive nephropathy is associated with massive interstitial macrophage infiltration. The recruitment of inflammatory cells is mediated in part by increased secretion of chemokines by stressed tubular cells. The chemokine CCL2 (MCP-1) is a powerful chemotactic agent and is involved in monocyte activation (Leonard & Yoshimura 1990). Patients with UPJ obstruction had fourfold higher CCL2 concentrations in their urine than healthy controls. This was correlated with a significant increase in MCP-1 expression on renal biopsies as shown by *in situ* hybridization (Table 2) (Grandaliano *et al.* 2000). Four months after pyeloplasty, urinary MCP-1 concentrations decreased close to levels of control individuals. In experi-

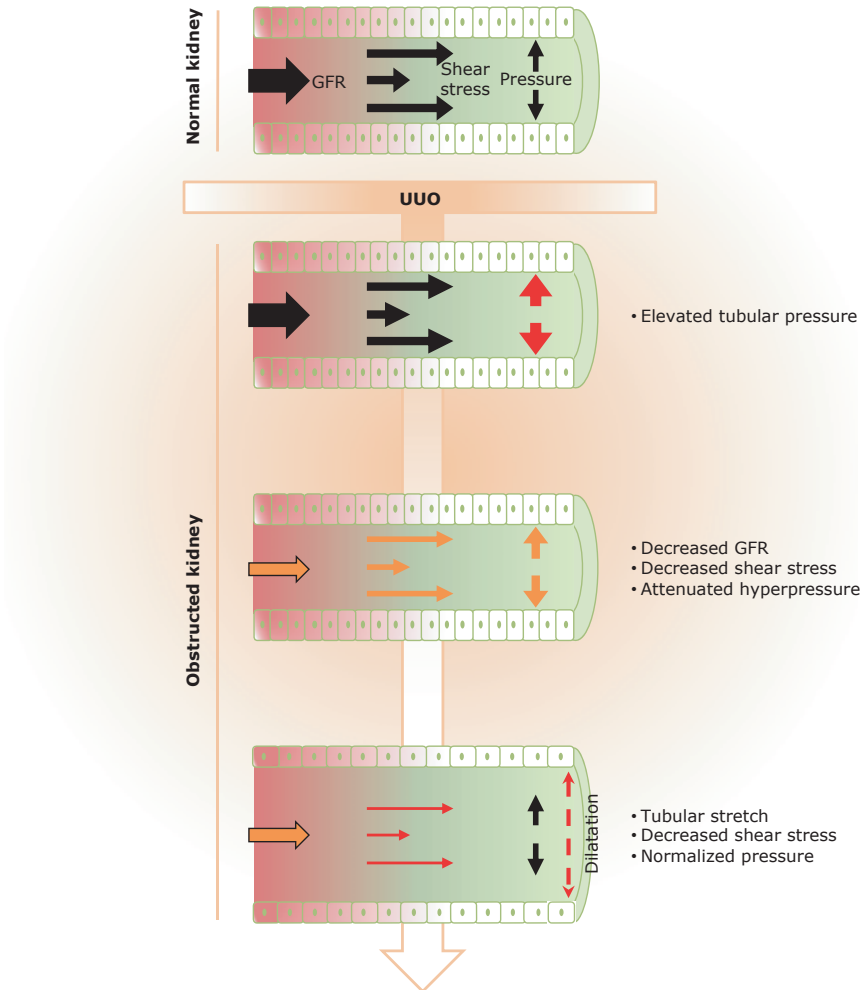


**Figure 2** Renal tubular exposure to hydrodynamic modifications following obstruction. Following intra-pelvic urine accumulation, UUO induces rapid elevation of hydrostatic pressure into the renal tubular lumina, leading to stretch and compression of the tubular cells and thus to tubular dilatation. As GFR declines and tubular dilatation increases, hyperpressure progressively returns to basal levels. However in the mean time, fluid shear stress, which depends on both GFR and tubular diameter, is strongly reduced. Thus modifications of renal hydrodynamics can induce mechanical stimuli such as pressure, shear stress and stretch. These three factors are potential insults for tubular cells and can be involved in the primary induction of the UUO-induced renal lesions.

mental models, complete neonatal obstruction also induced up-regulation of CCL2 together with other chemokines including CCL3 (MIP-1α), CCL4 (MIP-1β) and CCL5 (RANTES) (Table 2) (Silverstein *et al.* 2003a; Lange-Sperandio *et al.* 2007). Macrophage infiltration is also induced by up-regulation of adhesion molecules on endothelial and inflammatory cells. It has been shown that neonatal UUO in mice induced renal expression of intercellular adhesion molecule-1 (ICAM-1), receptor for advanced glycation end-products (RAGE) and junction adhesion molecule-C (JAM-C) adhesion molecules (Table 2) (Lange-Sperandio *et al.* 2006). Moreover, Mac-1 (αMβ2, CD11b/CD18) β2 integrin knockout, LFA-1 (αLβ2, CD11a/CD18) β2 integrin knockout, L-selectin knockout or L-, P-, and E-selectin triple knockout mice are protected from inflammation induced by neonatal UUO (Table 2) (Lange-Sperandio *et al.* 2002, 2006). To our knowledge, no data are available on adhesion molecules during UPJ in children. Finally, obstruction is also characterized by induced expression of transcription factors known to be involved in inflammation, such as interferon regulatory factor-1 (IRF-1) and nuclear factor-κB (NF-κB) (Table 2) (Silverstein *et al.* 2003a; Topcu *et al.* 2007) and of members of the pro-inflammatory tumour necrosis factor superfamily. In children, expression of the cytokine tumour necrosis factor-α (TNFα) is increased in urine during UPJ obstruction (Valles *et al.* 2003) whereas in neonatal rats, mRNA expression of Fas and Fas ligand is induced in the obstructed kidney compared to the sham (Silverstein *et al.* 2003a) (Table 2).

Effects of mechanical stretch on inflammatory mediators have been studied *in vitro*, mainly in pulmonary epithelial cells but not in tubular epithelial cells. Nevertheless, the fact that stretch modifies adhesion molecules expression, such as

**Table 2** Literature data about inflammation-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*)

| Gene symbol | Gene name | Human | Ref. | Animal model | | Ref. | *In vitro* | Ref. |
|---|---|---|---|---|---|---|---|---|
| | | | | PUUO | CUUO | | Stretch | |
| Ager | RAGE; receptor for advanced glycosylation end products | | | | Up (1d) | Lange-Sperandio *et al.* (2006) | | |
| Ccl2 | Chemokine (C-C motif) ligand 2; MCP-1 | Up (u, t) | Grandaliano *et al.* (2000) | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Ccl3 | Chemokine (C-C motif) ligand 3; MIP-1α | | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | |
| Ccl4 | Chemokine (C-C motif) ligand 4; MIP-1β | | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | |
| Ccl5 | Chemokine (C-C motif) ligand 5; RANTES | | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | |
| Cd14 | Monocyte differentiation antigen CD14 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Fas | TNF receptor superfamily, member 6 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Faslg | Fas ligand | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Icam1 | Intercellular adhesion molecule 1 | | | | Up (5d) | Lange-Sperandio *et al.* (2006) | Up | Hu *et al.* (2008) |
| IL8 | Interleukin 8; CXCL8 | | | | | | Up | Vlahakis *et al.* (1999), Yamamoto *et al.* (2001), Oudin and Pugin (2002) |
| Irf1 | Interferon regulatory factor 1 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Jam3 | JAM-C; junction adhesion molecule 3 | | | | Up (5d, 2w) | Lange-Sperandio *et al.* (2006) | | |
| Nfkb1 | NF-κB | | | Up (10w) | | Topcu *et al.* (2007) | | |
| Tnf | Tumor necrosis factor; TNF-α | Up (u) | Valles *et al.* (2003) | | | | Up | Dixon *et al.* (2008) |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; PUUO: partial UUO; CUUO: complete UUO; Ref.: references.

ICAM-1 (Hu *et al.* 2008) and cytokine production, such as TNFα (Dixon *et al.* 2008) and interleukin-8 (Vlahakis *et al.* 1999; Yamamoto *et al.* 2001; Oudin & Pugin 2002) (Table 2) in other epithelial cell types suggests that it may also trigger inflammatory signalling in renal tubules and thus participate to progression of obstructive nephropathy.

### *Fibrosis*

In almost all form of renal diseases, chronic inflammation is associated with the development of tubulointerstitial lesions of fibrosis. Fibrosis is defined by increased extracellular matrix (ECM) accumulation, due to both enhanced synthesis and decreased degradation of matrix proteins such as collagens and fibronectin. Myofibroblasts are the major extracellular matrix (ECM) producing cells. They play an important role in the fibrotic process and their renal accumulation in UPJ obstruction was evidenced by increased interstitial α-smooth muscle actin and vimentin staining (Murer *et al.* 2006). In the kidney, myofibroblasts are supposed classically to arise from the proliferation and activation of resident fibroblasts, but also from a process known as epithelial to mesenchymal transition (EMT). During EMT, tubular epithelial cells loose progressively their epithelial phenotype, detach from the surrounding cells, digest the basal membrane and migrate into the interstitium where they acquire mesenchymal properties (Liu 2010). Well-described *in vitro*, the existence of EMT *in vivo* and its real participation to the fibrotic process is still under debate. However, some characteristic features of EMT have been found during experimental UUO (Table 3): increased expression of essential signalling proteins such as Snail1, Snail2/-Slug and beta-catenin (Lange-Sperandio *et al.* 2007); decreased expression of E-Cadherin, an epithelial protein involved in cell-cell adhesion (Lange-Sperandio *et al.* 2007); increased expression of MMP2 (matrix metalloprotease 2) and MMP9 (matrix metalloprotease 9), two enzymes involved in the degradation of the tubular basal membrane (Mure *et al.* 2006a); and increased expression of mesenchymal markers such as desmin, calponin, vimentin and α-smooth muscle actin (Silverstein *et al.* 2003a; Lange-Sperandio *et al.* 2007). Some markers have also been observed in renal tubular cells submitted to mechanical stretch (Sato *et al.* 2003).

**Table 3** Literature data about fibrosis-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*)

| Gene symbol | Gene name | Human | Ref. | Animal model | | *In vitro* | | |
|---|---|---|---|---|---|---|---|---|
| | | | | CUUO | Ref. | Stretch | Shear stress | Ref. |
| Acta2 | α-SMA; actin, alpha, vascular smooth muscle | Up (t) | Murer *et al.* (2006) | Up (5d) | Lange-Sperandio *et al.* (2007) | | | |
| Cdh1 | Cadherin 1; E-cadherin | | | Down (5d) | Lange-Sperandio *et al.* (2007) | | | |
| Cnn1 | Calponin 1 | | | Up (2w) | Silverstein *et al.* (2003a) | | | |
| Col5a2 | Collagen, type V, alpha 2 (**fragments**) | Up (u) | Decramer *et al.* (2006) | | | | | |
| Col9a3 | Collagen, type IX, alpha 3 (**fragments**) | Up (u) | Decramer *et al.* (2006) | | | | | |
| Ctnnb1 | β-Catenin; catenin (cadherin associated protein), beta 1 | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | | |
| Dcn | Decorin | | | Up (2w) | Silverstein *et al.* (2003a) | | | |
| Des | Desmin | | | Up (2w) | Silverstein *et al.* (2003a) | | | |
| Egr1 | Early growth response 1; Krox24 | | | Up (2w) | Silverstein *et al.* (2003a) | | | |
| Mmp1 | Matrix metalloproteinase 1 | | | Up (6w) | Mure *et al.* (2006a) | | | |
| Mmp2 | Matrix metallopeptidase 2 | | | Up (6w) | Mure et al. (2006a) | | | |
| Mmp9 | Matrix metallopeptidase 9 | | | Up (6w) | Mure et al. (2006a) | | | |
| Pdgfa | PDGF-A | | | Up (10d, 20d) | Liapis *et al.* (2000) | | | |
| Plat | tPA; plasminogen activator, tissue | | | | | | Up | Essig *et al.* (2001) |
| Plau | uPA; plasminogen activator, urokinase | | | | | | Up | Essig *et al.* (2001) |
| Snai1 | Snail1; snail homolog 1(Drosophila) | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | | |
| Snai2 | Snail2; Slug; snail Homolog 2 (Drosophila) | | | Up (5d) | Lange-Sperandio *et al.* (2007) | | | |
| Tgfb1 | TGFβ; transforming growth factor, beta 1 | Up (u, t) | Furness *et al.* (1999), El-Sherbiny *et al.* (2002), Murer *et al.* (2006), Yang *et al.* (2006), Taha *et al.* (2007a) | Up (5d to 4w) | Chung and Chevalier (1996), Yang *et al.* (2001), Silverstein *et al.* (2003a), Lange-Sperandio *et al.* (2007) | Up | | Miyajima *et al.* (2000a,b), Dessapt *et al.* (2009) |
| Tgfbr1 | TGFβRI; transforming growth factor, beta receptor I | | | Up (10d) | Yang *et al.* (2001) | Up | | Miyajima *et al.* (2000a,b), Durvasula *et al.* (2004), Dessapt *et al.* (2009), |
| Tgfbr2 | TGFβRII; transforming growth factor, beta receptor II | | | Up (10d) | Yang *et al.* (2001) | Up | | Miyajima *et al.* (2000a,b), Durvasula *et al.* (2004), Dessapt *et al.* (2009) |
| Timp1 | Tissue inhibitor of metallopeptidase 1 | | | Up (6w) | Mure et al. (2006a) | | | |
| Timp2 | Tissue inhibitor of metalloproteinase 2 | | | Up (6w) | Mure et al. (2006a) | | | |
| Vim | Vimentin | Up (t) | Murer *et al.* (2006) | Up (5d) | Lange-Sperandio *et al.* (2007) | | | |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; CUUO: complete UUO; Ref.: references.

 

Renal collagen accumulation, as a consequence of myofibroblast appearance, has been frequently observed during human UPJ obstruction, but only using classical histology (H&E and Masson-trichrome) therefore lacking the identification of the specific ECM components (Elder *et al.* 1995; Zhang *et al.* 2000). We have observed using urinary proteome analysis increased secretion of collagen Vα and IXα3 fragments (Decramer *et al.* 2006) in UPJ obstruction patients but the origin, renal or urinary tract, of these fragments is currently unknown. Collagen accumulation is also described during experimental UUO (Chevalier *et al.* 2009) and experiments of UUO in the foetal lamb have shown increased expression of tissue inhibitors of metalloproteases (TIMP-1 and TIMP-2) (Table 3), which inhibit ECM degradation (Mure *et al.* 2006a). Interestingly, exposing cultured proximal cells to shear stress modifies both expression and activity of tPA (tissue type-plasminogen activator) and uPA (urokinase), two proteases strongly involved in ECM remodelling suggesting that modification of shear stress during obstruction could be involved in the development of fibrosis (Table 3) (Essig *et al.* 2001).

Renal fibrosis is regulated by different cytokines and growth factor. Among them, transforming growth factor beta (TGFβ) is the most potent pro-fibrogenic cytokine involved in renal diseases and is clearly associated to human and experimental obstruction. A number of studies showed that urinary TGFβ levels are increased in human UPJ obstruction (Table 3) (Furness *et al.* 1999; El-Sherbiny *et al.* 2002; Taha *et al.* 2007a). In addition, urinary TGFβ concentrations from UPJ obstruction patients were 2–4-fold higher in the pelvis than in the bladder (Furness *et al.* 1999; El-Sherbiny *et al.* 2002), suggesting that the urinary TGFβ found in the bladder is mainly coming from the obstructed kidney. This is further corroborated in renal biopsy studies where increased TGFβ expression was observed in UPJ obstruction (Murer *et al.* 2006; Yang *et al.* 2006). A decline of TGFβ urinary levels is detected after surgery, but the fact that TGFβ urinary levels decrease more slowly that urinary MCP-1 concentrations (Taha *et al.* 2007b) suggests that the inflammatory response resolves more rapidly than the fibrotic response. In experimental UUO, both TGFβ and TGFβ receptors (TGFβRI and TGFβRII, Table 3) are up-regulated in the obstructed kidney compared to sham (Chung & Chevalier 1996; Yang *et al.* 2001; Silverstein *et al.* 2003a; Lange-Sperandio *et al.* 2007). Decorin is an endogenous inhibitor of TGFβ expression and activity (Mogyorosi & Ziyadeh 1999; Wu *et al.* 2007). It is assumed that increased expression of decorin associated with increased TGFβ expression may provide a regulatory mechanism to limit the TGFβ induced injury (Diamond *et al.* 1997; Mogyorosi & Ziyadeh 1999). Very interestingly, it has been shown that during complete UUO in neonatal rats, the up-regulation of TGFβ mRNA parallels decorin expression (Table 3) (Silverstein *et al.* 2003a) and even after release of UUO, as in humans, overexpression of TGFβ-1 persists (Chevalier *et al.* 1999a). *In vitro* experiments on podocytes or proximal cells indicate that under certain conditions, mechanical stretch stimulates TGFβ signalling with increased TGFβ and TGFβ receptors expression and TGFβ secretion (Table 3) (Miyajima *et al.* 2000a,b; Durvasula *et al.* 2004; Dessapt *et al.* 2009). Moreover in non-renal cells, decorin mRNA expression is modified by mechanical stretch suggesting that it may also be the case in renal tubular cells (Table 3) (Ludwig *et al.* 2004; Ozaki *et al.* 2005). Altogether, these data suggest that TGFβ is a major mediator of renal injury (i.e. fibrosis) during UUO.

### *Nitric oxide and oxidative stress*

Nitric oxide produced by the various forms of nitric oxide (NO) synthase (NOS) has been shown to be involved in the evolution of experimental obstructive nephropathy. Increased NOS activity was also observed in humans with UPJ obstruction originating from both endothelial NOS (eNOS) and inducible NOS (iNOS) (Table 4) (Miyajima *et al.* 2000b; Valles *et al.* 2003, 2007). NOS activity is involved in a number of important processes in the kidney including glomerular haemodynamics and sodium and water excretion (Kone & Baylis 1997) that are strongly modified in the different phases of UPJ obstruction (see above). Modification of NOS activity has also been studied during experimental UUO in neonates (Table 4). Very interestingly, NO expression differs depending on the severity (i.e. complete *vs.* partial) and on the duration of the obstruction. For example, increased renal NO was associated with iNOS up-regulation 1 week after complete UUO in the neonatal rat (Manucha & Valles 2008), whereas both the NO level and eNOS and iNOS expression were reduced at a later stage (2 weeks) (Silverstein *et al.* 2003a; Manucha & Valles 2008). Decreased NO levels were associated with decreased anti-apoptotic Bcl2 expression and increased pro-apoptotic caspase 3 activity leading to the conclusion that NO is protective during complete neonatal UUO. In mice, while the protective and anti-apoptotic role of iNOS activity has been also demonstrated during complete obstruction (Yoo *et al.* 2010), it has been shown that NO has opposite effects during partial obstruction and seems to aggravate renal morphological alterations. After 1 week, partially obstructed iNOS knockout mice showed reduced renal pelvic dilatation and preserved renal papilla compared to wild-type mice although they displayed similar tubular apoptosis (Yoo *et al.* 2010). This discrepancy between complete or partial obstruction could be explained by the differential action of iNOS and NO on renal parenchyma (anti-apoptotic) and on the ureteropelvic junction (inhibition of smooth muscle contraction). While inhibition on ureteral contractile activity in complete UUO is without effect on urine retention, it can modify the response to partial UUO. This is exemplified by the fact that, in partial neonatal obstruction, iNOS knockout mice showed less hydronephrosis compared to wild-type mice because pelvic urine drainage is increased (Yoo *et al.* 2010).

*In vitro* experiments indicate that renal NO pathway is controlled by mechanical forces. Indeed, increased luminal

**Table 4** Literature data about NO and oxidative stress-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which activity or expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*).

| | | | | Animal model | | *In vitro* | | | |
|---|---|---|---|---|---|---|---|---|---|
| Gene symbol | Gene name | Human | Ref. | CUUO | Ref. | Stretch | Shear stress | Pressure | Ref. |
| Cat | Catalase | | | | | Down | | | Ricardo *et al.* (1997) |
| Nos2 | iNOS; nitric oxide synthase 2, inducible | Up* (t) | Valles *et al.* (2003) | Up (5d)<br>Down (2w) | Manucha and Valles (2008) | Up* / =* | | Up* | Miyajima *et al.* (2000a), Hegarty *et al.* (2002), Broadbelt *et al.* (2007) |
| Nos3 | eNOS; nitric oxide synthase, inducible | Up* (t) | Valles *et al.* (2003), Valles *et al.* (2007) | Down (2w) | Silverstein *et al.* (2003a) | | | | |
| Nox1 | NADPH oxidase | | | Down* (5d)<br>Up* (2w) | Manucha and Valles (2008) | | | | |
| Sod1 | SOD; superoxide dismutase | | | Down* (2w) | Manucha and Valles (2008) | | | | |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; CUUO: complete UUO; *activity; Ref.: references.

flow stimulates NO release and induces eNOS activation and translocation to apical membrane in the rat microperfused ascending limb (Ortiz *et al.* 2004). However, in order to determinate if this effect is caused by stretch, transmural pressure or shear stress modifications, experiments have been carried out studying individual change of each parameter. First, *in vitro* application of elevated hydrostatic pressure (Broadbelt *et al.* 2007) or stretch (Miyajima *et al.* 2000b; Hegarty *et al.* 2002) to proximal cells increases iNOS expression and NO production (Table 4). Interestingly, the effect of stretch seems to be species-dependent as the NO increase is induced in rat but not in human cells (Miyajima *et al.* 2000b; Hegarty *et al.* 2002). Moreover, NO donors attenuate both stretch-induced apoptosis and proliferation inhibition whereas NO inhibitors amplify them. These *in vitro* results confirm that NO exerts a protective effect for the severely obstructed stretched kidney (Miyajima *et al.* 2001; Hegarty *et al.* 2002). Second, increased shear stress has been shown to increase NO production in medullary collecting duct cells (Cai *et al.* 2000). This latter result is more difficult to directly transpose to the *in vivo* UUO situation where the shear stress is not increased but decreased due to GFR decline and tubular dilatation (see above). However it suggests that tubular NO production could be also sensitive to urinary shear stress variations.

Another consequence of chronic UUO in newborns is the increase of oxidative stress. *In vitro*, cellular stretch increases superoxide production and down-regulates catalase mRNA levels (Table 4) (Ricardo *et al.* 1997; Garvin & Hong 2008). *In vivo*, 2 weeks of complete UUO in neonatal rats induced NADPH oxidase activity and decreased superoxide dismutase activity (Table 4) (Manucha & Valles 2008). The role of NADPH oxidase-mediated ROS generation has been extensively studied *in vivo* and *in vitro*. Studies have shown that it can promote apoptosis or proliferation of renal cells, such as mesangial cells, podocytes and tubular cells (Jiang 2009). Moreover, NADPH oxidase mediates proximal tubular cell death induced by angiotensin II (Ang II) (Jiang 2009). Consistently with these results up-regulation of NADPH oxidase activity paralleled the increase of apoptosis during experimental UUO supporting the deleterious role of oxidative stress during neonatal obstructive nephropathy (Manucha & Valles 2008).

### *Apoptosis and proliferation*

During obstruction, tubular stretch, inflammation and oxidative stress induce a severe apoptotic response of both tubular and interstitial cells. Indeed urinary tubular enzymes potentially released by those cells including *N*-acetyl-β-D-glucosaminidase (Carr *et al.* 1994), alkaline phosphatase and γ-glutamyl transferase (Shokeir & Taha 2009) have been found increased in human UPJ obstruction (Table 5). The role of other intracellular mediators of apoptosis or cell survival in obstruction has been investigated in animal models. In rats, mice and opossums complete and partial UUO stimulated expression and activity of pro-apoptotic molecules

**Table 5** Literature data about apoptosis and proliferation-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which activity or expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*)

| | | | | Animal model | | | *In vitro* | |
|---|---|---|---|---|---|---|---|---|
| **Gene symbol** | **Gene name** | **Human** | **Ref.** | **PUUO** | **CUUO** | **Ref.** | **Stretch** | **Ref.** |
| Alpl | Alkaline phosphatase | Up* (u) | Shokeir and Taha (2009) | | | | | |
| Anxa5 | Annexin V | | | | | | Up | Dessapt *et al.* (2009) |
| Bad | BCL2-associated agonist of cell death | | | | Up* (7d) | Kiley *et al.* (2003) | Up* | Kiley *et al.* (2003) |
| Bcl2 | B-cell CLL/lymphoma 2 | | | | Down (2w) | Steinhardt *et al.* (1995), Manucha *et al.* (2007), Manucha and Valles (2008), | | |
| Casp3 | Caspase 3 | | | Up (2w, 4w) | Up* (2w, 4w) | Manucha *et al.* (2007), Guerin *et al.* (2008), Manucha and Valles (2008) | Up* | Dessapt *et al.* (2009) |
| Clu | Clusterin | | | | Up (2w) | Chevalier *et al.* (1996), Silverstein *et al.* (2003a) | | |
| Cycs | Cytochrome C | | | | | | Up | Kiley *et al.* (2003) |
| Dcn | Decorin | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Fas | TNF receptor superfamily, member 6 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Faslg | Fas ligand | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Ggt1 | g-Glutamyl transferase | Up* (u) | Shokeir and Taha (2009) | | | | | |
| Hexa/ Hexb | N-acetyl-β-D-glucosaminidase; Hexoaminidase | Up* (u) | Carr *et al.* (1994), Shokeir and Taha (2009) | | | | | |
| Hspa4 | HSP70; heat shock protein 4 | | | | Up (5d, 2w) | Manucha and Valles (2008) | | |
| Hspb1 | HSP27; heat shock protein 1 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| JunD | jun D proto-oncogene | | | | Up (2w) | Silverstein *et al.* (2003a) | | |
| Nfkb1 | NF-κB | | | Up (10w) | | Topcu *et al.* (2007) | | |
| Slc9a1 | NHE1; Sodium/hydrogen exchanger, member 1 | | | | Down (2w) | Manucha *et al.* (2007) | | |
| Sparc | Secreted protein, acidic, cysteine-rich (osteonectin) | | | | | | Up | Durvasula and Shankland (2005) |
| Tp53 | p53; tumor protein p53 | | | | Up (2w) | Silverstein *et al.* (2003a) | | |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; PUUO: partial UUO; CUUO: complete UUO; *: activity; Ref.: references.

such as Bad, Caspase 3, Fas, Fas ligand and p53, together with down-regulation of anti-apoptotic Bcl2 and heat shock protein 70 (HSP70) (Table 5) (Steinhardt *et al.* 1995; Kiley *et al.* 2003; Silverstein *et al.* 2003a; Manucha *et al.* 2007; Guerin *et al.* 2008; Manucha & Valles 2008). Ceramide, an endogenous lipid also considered as an intracellular mediator of cell death (Saba *et al.* 1996) was induced during neonatal UUO and paralleled apoptosis (Malik *et al.* 2001). Moreover, clusterin, which is a small heat shock protein-like involved in pro-apoptotic mechanisms induced by oxidative stress (Chevalier *et al.* 1996, 1999a; Silverstein *et al.* 2003a; Trougakos & Gonos 2006), is induced during experimental UUO (Table 5). Interestingly, clusterin over-expression persists even after the release of obstruction (Chevalier *et al.* 1999a). Some other molecules that are modified during UUO have been described to be involved in the regulation of apoptosis (Table 5): in addition to its inhibitory effect on TGFβ activity, decorin has been found to induce apoptosis on mesangial cells *in vitro* (Wu *et al.* 2008); heat shock protein 27 (HSP27) is a crucial chaperone protein activated in response of renal epithelial cellular stress to limit apoptosis (de Graauw *et al.* 2005); JunD, one of the components of the AP-1 transcription factor complex has been shown to promote cell survival in epithelial renal cells *in vitro* (Silverstein *et al.* 2003a; Lu *et al.* 2009); the sodium/proton exchanger-1 (NHE1) is involved in renal sodium transport but is also a caspase substrate and its proteolytic cleavage promotes progression towards apoptosis (Manucha *et al.* 2007; Schelling & Abu Jawdeh 2008).

The apoptotic response to mechanical stretch has been extensively studied *in vitro*. Stretch increases apoptosis or susceptibility to apoptosis in murine, human and canine epithelial renal cells (Miyajima *et al.* 2000a, 2001; Nguyen *et al.* 2000; Hegarty *et al.* 2002; Cachat *et al.* 2003; Kiley *et al.* 2003, 2005; Durvasula *et al.* 2004; Dessapt *et al.* 2009). This effect is associated with elevated markers of early apoptosis such as dephosphorylated BAD, released mitochondrial cytochrome C, caspase-3 activity and annexin V (Table 5) (Kiley *et al.* 2003; Dessapt *et al.* 2009). In addition, the sodium/proton exchanger NHE is also sensitive to mechanical stress, as described in cardiomyocytes but not yet in renal cells (Yamazaki *et al.* 1998). Underlying mechanisms seem to be dependent on the cell type since stretch-induced apoptosis is mediated by TGFβ in rat proximal cells (Miyajima *et al.* 2000a) whereas it is driven by type 1 angiotensin II receptor in mouse podocytes (Durvasula *et al.* 2004). In addition stretch dependent apoptosis is greater in the collecting duct than in proximal tubules (Cachat *et al.* 2003). This result can explain why in neonatal experimental UUO, apoptosis is more severe in collecting ducts than in proximal tubules (Cachat *et al.* 2003). Stretch not only promotes apoptosis, it also decreases proliferation as demonstrated in cultured mouse podocytes and HK-2 or MDCK cells (Hegarty *et al.* 2002, 2003; Petermann *et al.* 2002, 2005). In mice, this anti-proliferative effect is mediated through the reduction of cyclins levels and their partner CDKs associated to increment in CDK-inhibitors expression (Petermann *et al.* 2002). It is also associated to increased SPARC (secreted protein acidic and rich in cystein) levels (Table 5), a protein which *in vivo* diminishes proliferative capacity of various tissues via inhibition of mitogenic growth factors (Durvasula & Shankland 2005).

### *The renin–angiotensin system and associated partners*

Activation of the renin-angiotensin system (RAS) has been observed in many renal diseases. Also UPJ obstruction does not escape this fate. In mice and rats, renal expression of

**Table 6** Literature data about renin–angiotensin system (and associated)-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which activity or expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*)

| Gene symbol | Gene name | Human | Ref. | Animal model | | *In vitro* | |
|---|---|---|---|---|---|---|---|
| | | | | CUUO | Ref. | Stretch | Ref. |
| Agtr1 | Angiotensin II receptor, type 1 | = (t) | Murer *et al.* (2006), Valles *et al.* (2007) | Down (1d) Up (4w) | Yoo *et al.* (1997) | Up | Durvasula *et al.* (2004) Kolb *et al.* (2004) |
| Agtr2 | Angiotensin II receptor, type 2 | = (t) | Murer *et al.* (2006), Valles *et al.* (2007) | Down (1d; 4w) | Yoo *et al.* (1997) | = | Durvasula *et al.* (2004) |
| Bdkrb2 | Bradykinin receptor B2 | | | Up (4w) | Chen *et al.* (2007) | | |
| Gja4 | Connexin 37 | | | Up (2w) | Silverstein *et al.* (2003b) | | |
| Gja5 | Connexin 40 | | | Up (2w) | Silverstein *et al.* (2003b) | | |
| Pcsk1n | proSAAS; proprotein convertase 1 inhibitor | Down (u) | Decramer *et al.* (2006) | | | | |
| Ren | Renin | Up* (p) | Bajpai *et al.* (2007) | Up (2w, 4w) | el-Dahr *et al.* (1991), Chevalier *et al.* (1996), Chung and Chevalier (1996), Yoo *et al.* (1997), Silverstein *et al.* (2003a) | Up | Ricardo *et al.* (2000) |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; CUUO: complete UUO; *activity; Ref.: references.

renin is up-regulated in neonatal models of complete UUO compared to the sham operated kidney (Table 6) (el-Dahr *et al.* 1991; Chevalier *et al.* 1996; Chung & Chevalier 1996; Yoo *et al.* 1997; Silverstein *et al.* 2003a) and this is correlated with increased angiotensin II (AngII) content in the obstructed kidney (Yoo *et al.* 1997). In children, plasma renin activity was observed to gradually increase with the time of UPJ obstruction and showed to precede parameters of actual renal injury including split renal function and glomerular filtration rate. In addition plasma renin activity was reduced postoperatively (Bajpai *et al.* 2007). We have observed decreased urinary excretion of proprotein convertase subtilisin/kexin type 1 inhibitor (proSAAS) in UPJ-children that can be linked to the observed increase in renin activity (Table 6) (Decramer *et al.* 2007). proSAAS can inhibit prohormone convertase-1 (PC1) activity (Basak *et al.* 2001) and PC1 was shown to efficiently convert prorenin into renin (Benjannet *et al.* 1992). We therefore speculate that the decline of proSAAS levels in UPJ-obstruction lower PC1 inhibition and thus increase processing of renin from prorenin leading to increased activation of the RAS. Another interesting finding is the effect of UUO on connexins 37 and 40 expression. Connexins 37 and 40 are two GAP junction proteins, which are expressed in the renin-secreting cells of the juxtaglomerular apparatus and control in part the tubulo-glomerular feedback (Takenaka *et al.* 2008; Just *et al.* 2009). Intra-renal infusion of blocking peptides for Connexin 37 and 40 increased plasma renin activity and AngII levels (Takenaka *et al.* 2008). On the another hand, it has been shown that mRNA expression of connexin 37 and 40 is increased during obstruction in neonatal rats (Table 6) (Silverstein *et al.* 2003b). Taken together, these results seem controversial although one can speculate that the increased renin expression during UUO can induce a positive feedback on connexin expression to limit the hyperreninemia.

One major difference between animal models and human findings is the effect of obstruction on angiotensin receptors (Table 6). In rats with neonatal UUO, renal expression of type 1 and type 2 angiotensin receptors (AT1-R and AT2-R respectively) was decreased after 1 day of obstruction. However after 4 weeks AT1-R was overexpressed whereas AT2-R was still down-regulated (Yoo *et al.* 1997). In human, renal angiotensin receptor expression is not modified by severe UPJ obstruction (Murer *et al.* 2006; Valles *et al.* 2007). Nevertheless, caveolin-1, which is colocalized with the AT1-R in renal tubular cells, was induced in UPJ obstruction both in renal biopsies and urine (Table 6) (Valles *et al.* 2007) suggesting that, although expression is not modified, AT1-R activation is increased. Altogether, these data are in favour of activation of the RAS in UPJ obstruction.

*In vitro* data show that renal RAS is activated by mechanical stretch. This has been shown for podocytes or proximal tubule cells where AngII production, renin mRNA and AT1-R, but not AT2-R, expression are increased in response to stretch (Table 6) (Ricardo *et al.* 2000; Durvasula *et al.* 2004; Miceli *et al.* 2010). In addition, part of the stretch-induced cellular effects including apoptosis, down-regulation of nephrin (see below 'tubular transport and glomerular function') or stimulation of osteopontin expression (see below 'other cytokines and growth factors') are mediated by the AT1-R (Diamond *et al.* 1998; Ricardo *et al.* 2000; Durvasula *et al.* 2004; Miceli *et al.* 2010). Interestingly, Caveolin-1 is also a stretch sensitive protein, as demonstrated in non-renal murine cells, where translocation of caveolin-1 from caveolar to non-caveolar sites within the plasma membrane or from the plasma membrane to cytoplasm can be observed in response to stretch (Kawabe *et al.* 2004; Wang *et al.* 2010). As stretch, shear stress alters the RAS. Indeed, application of fluid shear stress on proximal cells results in relocation of AT1-R out of apical recycling endosomes into the apical surface membrane (Kolb *et al.* 2004). Thus, tubular mechanical forces can account for modifications of the RAS status in the obstructed kidney.

In adults, blocking the RAS is a well-admitted target to block the progression of renal diseases. AngII is involved in many, if not all, pathological mechanisms of renal fibrosis. It participates in the inflammatory process by stimulating expression of adhesion molecules such as VCAM-1 and ICAM-1, expression of chemokines such as CCL2 and CCL5 (Mezzano *et al.* 2001; Ruster & Wolf 2006; Wynn 2008). It is involved in oxidative stress by stimulating NADPH oxidase activity and the production of reactive oxygen species (Mezzano *et al.* 2001; Ruster & Wolf 2006; Wynn 2008). It is also involved in EMT and fibroblast activation (Mezzano *et al.* 2001; Ruster & Wolf 2006; Wynn 2008). Finally, AngII has been shown to induce collagen synthesis and TIMP-1 expression (Mezzano *et al.* 2001; Ruster & Wolf 2006; Wynn 2008). Most of the effects of AngII are mediated by the modulation of cytokine and growth factor expression such as TGFα (Transforming Growth Factor-α) and TGFβ (Mezzano *et al.* 2001; Ruster & Wolf 2006; Wynn 2008). It is interesting to point out that most of these mechanisms and molecules are also induced in the neonatal model of UUO. However, targeting the RAS in infants is no longer considered to be a valuable therapeutic strategy. Blocking the RAS during renal development in human or rodents induces severe damage to the kidney and worsens the renal lesions induced by obstruction. Administration of angiotensin converting enzyme (ACE) inhibitors or AT1-R antagonists to pregnant women lead to severe renal malformations in the foetus (Sekine *et al.* 2009). Moreover, a polymorphism into AT2-R gene involved in efficient splicing of the mRNA has been shown to be associated with UPJ in two human cohorts (Nishimura *et al.* 1999). In mouse, AT1-R, AT2-R and angiotensinogen gene knockout led to renal malformations (Sekine *et al.* 2009). In piglets with partial UUO, AT1-R blockade by candesartan prevents interstitial and glomerular apoptosis but neither fibrosis nor tubular dysfunction (Eskild-Jensen *et al.* 2007a,b). In rats, losartan, another AT1-R antagonist, aggravates lesions of partial UUO when administered during the first 10 days of life, which corresponds to the period of nephrogenesis. However, losartan was without effect when

administered 10 days after birth, which corresponds to the renal maturation period (Coleman *et al.* 2007). Inhibition of AT2-R was without effect at any time (Coleman *et al.* 2007). Other studies have shown that enalapril, an ACE inhibitor, induced functional and histological renal alterations when administered during nephrogenesis (Guron *et al.* 1999), but did not exert additional deleterious effect in partial UUO (Chen *et al.* 2007). Conversely, administration during the maturation period had no effect in control rats (Guron *et al.* 1999) but worsened renal lesions induced by partial UUO (Chen *et al.* 2007). These results seem controversial. However it is important to keep in mind that ACE inhibition or AT1-R blockade is not equivalent. ACE activity not only generates AngII but also other AngII related peptides such as Ang1-7, which exerts its biological effect through AT1-R independent mechanisms (Ruster & Wolf 2006). Moreover AngII can be generated by other serine proteases than ACE, such as chymase, which is not affected by ACE inhibition (Ruster & Wolf 2006).

In conclusion, the RAS is induced during obstruction and seems to be related to most of the deleterious mechanisms involved in this pathology. However, the role of RAS during kidney development is also crucial and cannot be targeted easily. One alternative could be to target the RAS-associated kinin-kallikrein system (KKS). KKS is composed by two receptors, the B1 and the B2 receptors and by their respective ligands, des-arg9bradykinin and bradykinin (Leeb-Lundberg *et al.* 2005). KKS is linked to the RAS through the ACE since ACE is not only involved into the conversion of AngI in AngII but also in the degradation of bradykinin (Leeb-Lundberg *et al.* 2005).

The B2 receptor has been shown to exert a renal protective anti-fibrotic effect in different adult rodents models by promoting extracellular matrix degradation (Schanstra *et al.* 2002; Okada *et al.* 2004; Seccia *et al.* 2006) and its expression was increased during UUO in rat neonates (Table 6) (Chen *et al.* 2007). One can thus speculate that exogenous administration of B2 receptor ligand during neonatal obstructive nephropathy can be an interesting therapeutic strategy. However the protective effects of ACE inhibitors in adults are known to be mediated, at least in part, by increased bradykinin expression or B2 receptor activation (Okada *et al.* 2004; Seccia *et al.* 2006). Therefore the severe renal alterations in rodents and humans during the neonatal or the foetal period upon ACE inhibition might also be, at least partially, mediated by bradykinin or its B2 receptor.

On the another hand, we have recently demonstrated that kinin B1 receptor blockade significantly improves both renal inflammation and fibrosis in two models of renal disease (Klein *et al.* 2009, 2010). Contrarily to the B2 receptor, which is constitutively expressed, the B1 receptor is not detectable under physiological conditions but over-expressed at the site of injury during inflammation (Leeb-Lundberg *et al.* 2005). Moreover, B1 receptor knockout mice showed no renal alterations (Pesquero *et al.* 2000). All these arguments support the hypothesis that targeting the B1 receptor during UUO in neonates should have limited impact on non-inflamed contralateral kidney and on renal development and thus be potentially beneficial in obstructive nephropathy. Further studies need to investigate whether this therapeutic strategy is a valuable approach during congenital obstructive nephropathy.

### *Tubular transport and glomerular podocytes*

As described above, UUO induces severe impairment of kidney function including profound modification of glomerular filtration and tubular function. Little information about the regulation of renal transporters by mechanical stimuli is available. Nevertheless, it appears that NHE and ENac, the apical sodium transporters in proximal tubule and collecting duct respectively, are activated by fluid flow (Table 1) (Preisig 1992; Satlin *et al.* 2001). If the changes result from hydrostatic pressure, membrane stretch or shear stress are however conflicting (Satlin *et al.* 2001; Carattino *et al.* 2004). Besides, fluid flow augments apical and basal release of nucleotides which subsequently activates purinergic receptors in isolated perfused renal tubules (Jensen *et al.* 2007). This effect is also observed in polarized MDCK cells subjected to transepithelial pressure changes (Praetorius *et al.* 2005). Altogether, these results suggest that mechanical aggression could modify salt and water reabsorption in the obstructed kidney (Sipos *et al.* 2009).

To our knowledge, the effect of UUO on the function of glomerular podocytes has never been investigated *in vivo*, neither in human nor in animals. However, numerous stretching experiments have been performed *in vitro* using podocytes. Indeed, these cells are probably subjected to stretch *in vivo* during obstruction. First, when elevated hydrostatic pelvis pressure due to urine accumulation is transmitted to Bowman's capsule, it could generate podocyte deformation. Moreover, when hydrostatic pressure increases in the glomerular capillary, as a result of afferent arteriole vasodilatation, it induces capillary distention, which may be transmitted to podocytes, leading to their stretching. As mentioned above, stretched-podocytes show activation of RAS and TGFβ axis, stimulation of apoptosis and inhibition of cellular proliferation. In addition to these effects, stretch induces morphologic alterations such as podocyte hypertrophy (Petermann *et al.* 2005) and stress fibers reorganization (Endlich *et al.* 2001). It down-regulates α3β1 integrin (Table 1), which allows podocyte ancrage to glomerular basement membrane *in vivo*, thus leading to reduced cellular adhesion (Dessapt *et al.* 2009). Finally, stretch reduces expression of nephrin in podocyte foot process (Table 1), a key protein of the slit diaphragm, thereby undermining the integrity of the filtration barrier. This effect is dependent of angiotensin-AT1-R system (Miceli *et al.* 2010) and could be mediated by nephrin internalization. Indeed it was recently demonstrated that nephrin trafficking depends on dynamin (Qin *et al.* 2009), a protein which is up-regulated in the obstructed kidney after neonatal complete UUO (Silverstein *et al.* 2003a). Altogether, these results suggest that stretch injury may induce podocyte dysfunction, thus leading to

proteinuria and ultimately glomerulosclerosis, as observed in adult rats having undergone UUO during nephogenesis (Chevalier *et al.* 2000b). To test this hypothesis, further studies on podocyte function and in particular on nephrin status should be undertaken in human biopsies and animal models of UPJ obstruction.

### *Other cytokines and growth factors*

*Epidermal growth factor family.* Members of the epidermal growth factor (EGF) family are suggested to be mediators of normal tubulogenesis and tubular regeneration (Zeng *et al.* 2009). In children studies on the urinary EGF secretion during UPJ obstruction have been contradictory. UPJ obstruction was found without effect on urinary EGF excretion (Yang *et al.* 2006) while others have observed reduced urinary EGF expression (Grandaliano *et al.* 2000). However additional evidence on renal biopsies tends to support the decreased renal EGF expression (Table 7) (Grandaliano *et al.* 2000; Yang *et al.* 2006).

Results in animal models are also complicated. In neonatal rat subjected to complete UUO, a marked suppression of EGF is observed in the obstructed kidney (Chung & Chevalier 1996) whereas TGFα (Transforming Growth Factor-α), another ligand of the EGF family, and the EGF-receptor Erb1 are up-regulated (Nguyen *et al.* 1999a). Moreover, in rat neonatal partial UUO, although up-regulation of the TGFα and the EGF receptor was still observed, no significant effect was shown in EGF and heparin-binding EGF (HB-EGF, a ligand for EGF-receptor) expression, after 24 weeks of obstruction (Table 7) (Bor *et al.* 2006).

Both EGF and TGFα are known to be involved in kidney development and alteration in their expression can thus be associated with modified nephrogenesis or renal maturation (Carev *et al.* 2008; Zeng *et al.* 2009). But many *in vivo* and *in vitro* observations indicate that EGF is mainly involved in modulation of apoptosis during obstructive nephropathy. Interestingly, EGF can be a protector or a deleterious factor depending on the disease severity and the species. Indeed, EGF administration attenuates UUO-induced renal injury by increasing tubular proliferation and reducing apoptosis, tubular dilation, tubular atrophy and interstitial fibrosis in rat neonates (Chevalier *et al.* 1998; Wen *et al.* 2009). Moreover, while expression of EGF is not normalized after release of UUO (Chevalier *et al.* 1999a), EGF treatment improves recovery following relief (Chevalier *et al.* 1999a,c). In parallel, treatment with EGF decreases stretch-induced apoptosis in rat proximal tubular cell *in vitro* (Nguyen *et al.* 2000; Kiley *et al.* 2003). Conversely, EGF administration during neonatal mouse obstruction does not correct UUO-induced apoptosis *in vivo* (Kiley *et al.* 2005) and aggravates stretch-induced apoptosis in mouse proximal tubular cell *in vitro* (Kiley *et al.* 2005). In addition, obstructed kidney of neonatal mice lacking EGF or with diminished EGF receptor activity elicit less apoptosis than wild-type mice (Kiley *et al.* 2005). It was proposed that constitutive Src activity in mouse is the underlying cause of EGF receptor dysregulation

**Table 7** Literature data about cytokine and growth factor-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which activity or expression has been significantly modified compared to healthy control (human), sham-animals (animal model) or control cells (*in vitro*)

| | | | | Animal model | | | *In vitro* | | |
|---|---|---|---|---|---|---|---|---|---|
| **Gene symbol** | **Gene name** | **Human** | **Ref.** | **PUUO** | **CUUO** | **Ref.** | **Stretch** | **Pressure** | **Ref.** |
| Egf | Epidermal growth factor | = / Down (u, t) | Grandaliano *et al.* (2000), Yang *et al.* (2006), Taha et al. (2007a) | | Down (2w, 4w) | Chung and Chevalier (1996) | | | |
| Egfr | Epidermal growth factor receptor; Erbb1 | | | | Up (5d to 4w) | Nguyen *et al.* (1999a) | | Up* | (Broadbelt *et al.* (2009) |
| Tgfa | TGFα; transforming growth factor α | | | | Up (5d to 4w) | Nguyen et al. (1999a) | | | |
| Vegfa | Vascular endothelial growth factor A | | | Up/Down (2w, 4w) | Down (2w, 4w) | Burt *et al.* (2007), Fenghua *et al.* (2009) | | | |
| Edn1 | Endothelin 1 | Up (u) | Taha et al. (2007a) | | | | Up | | Diamond *et al.* (1998), Endlich *et al.* (2002) |
| Spp1 | Osteopontin; secreted phosphoprotein 1 | | | | | | | | |

u: urine; t: tissue; p: plasma; w: weeks of obstruction; d: days of obstruction; CUUO: complete UUO; *activity; Ref.: references.

and susceptibility to EGF-induced cell death (Kiley & Chevalier 2007).

Because of these striking species differences, it is hard to speculate whether EGF administration could be benificial or deleterious in humans. However it is interesting to point out that application of elevated pressure activates the EGF receptor in human proximal tubular cells *in vitro* (Table 7) and consecutively leads to iNOS and NO expression which has been shown to attenuate experimental obstructive renal injury (see above) (Broadbelt *et al.* 2009).

*Vascular endothelial growth factor.* Vascular endothelial growth factor (VEGF) is a pleiotropic cytokine known to be involved in many processes in kidney physiology and pathology such as glomerular capillary development and permeability regulation (Robert & Abrahamson 2001; Schrijvers *et al.* 2004), growth and proliferation of glomerular and peritubular capillary endothelial cells (Schrijvers *et al.* 2004) or inhibition of apoptosis in tubular epithelial cells (Villegas *et al.* 2005). It has been often proposed that during renal diseases increased VEGF levels can prevent the loss of peritubular capillaries, thus reducing hypoxia and subsequent development of renal fibrosis. However, a number of studies have shown that VEGF can be either deleterious or beneficial, depending on the form of renal disease (Schrijvers *et al.* 2004). In neonates, relationships between experimental UUO and VEGF are not fully understood. While complete UUO induced a complete loss of renal VEGF expression in foetal lambs and neonatal rats (Burt *et al.* 2007; Fenghua *et al.* 2009), partial UUO in neonatal rats led to strong inter-individual heterogeneity, with VEGF expression varying between down- to up-regulation (Table 7) (Burt *et al.* 2007). Moreover, exogenous VEGF administration tended to aggravate the UUO-induced loss of peritubular capillaries (Burt *et al.* 2007). All these results suggest a complex role of VEGF on the developing kidney during obstructive nephropathy, depending on the severity of the disease and probably on the global pathological context.

*Insulin-like growth factor.* Different studies have shown that insulin-like growth factor 1 (IGF-1) could play a protective role in the pathology of obstructive nephropathy. As stated above, mechanical stretch of proximal cells induced Bad-mediated cell death *in vitro* (Kiley *et al.* 2003). Interestingly, treatment with IGF-1 during stretch strongly decreased apoptosis by restoring bad phosphorylation level (Kiley *et al.* 2003). Altough IGF-1 and IGF-1 receptors expression is not modified during neonatal UUO in rats exogenous IGF-1 administration significantly decreased the UUO-induced apoptosis as well as tubular atrophy and interstitial fibrosis (Chevalier *et al.* 2000a). Similarly, while increasing inflammation, IGF-1 administration protected against the loss of renal architecture and decreased interstitial fibrosis during UUO in opossum pups (Steinhardt *et al.* 1995). Studies measuring the effect of IGF-1 administration on UUO-induced loss of renal function are absent. However these results suggest that IGF-1 may be a potential therapeutic target in the pathology of obstructive nephropathy.

*Endothelin-1.* Endothelin-1 (ET-1) is a potent vasoconstrictor that has been implicated in the tissue damage and dysfunction associated with UUO (Josephson & Hemsen 1994; Feldman *et al.* 2000) and UPJ obstruction (Taha *et al.* 2007a). It has been shown that urinary ET-1 levels were 4-fold higher in UPJ obstruction patients than in healthy controls, patients with vesicoureteral reflux and patients with renal stones (Table 7). Surgery decreased urinary ET-1 levels that slowly declined over 1 year (Taha *et al.* 2007a). This shows that, as observed for urinary TGFβ, that relief of obstruction does not rapidly decrease the concentrations of molecules that are potentially involved in disease progression.

*Osteopontin.* Osteopontin is a secreted glycosylated phosphoprotein, which has been shown to be up-regulated in a number of experimental models of renal disease and in human nephropathy (Xie *et al.* 2001). Osteopontin seems to have two opposite roles in the kidney. On one hand, osteopontin has been shown to promote macrophage chemotaxis and renal fibrosis (Xie *et al.* 2001; Tian *et al.* 2006). On another hand, osteopontin is strongly involved in renal protection by inhibiting oxidative stress, decreasing interstitial and tubular cell apoptosis and participating in the regeneration of tubular cells (Xie *et al.* 2001). Many factors have been shown to be involved in increased osteopontin expression, such as TNF-α, PDGF, TGFβ and EGF and it has been shown that osteopontin could modulate AngII-induced renal injury (Xie *et al.* 2001; Wolak *et al.*2009). Following mechanical stretch, rat proximal cells and mouse podocytes exhibit an increase in osteopontin mRNA levels, which is normalized following pretreatment with an AT1-R antagonist (Table 7) (Diamond *et al.* 1998; Endlich *et al.* 2002). Experimental UUO in osteopontin knockout neonatal mice showed that osteopontin blockade exerted a deleterious role on tubular and interstitial apoptosis (Yoo *et al.* 2006b). However this result was counterbalanced by an attenuation of fibrotic lesions in osteopontin knockout compared to WT mice, although no significant effect was shown on macrophage accumulation (Yoo *et al.* 2006b). In this study, the effect of osteopontin blockade on functional parameters has not been investigated. Future experiments should explore whether the protective (i.e. anti-apoptotic) or deleterious (i.e. pro-fibrotic) role of osteopontin dominates on kidney function during obstructive nephropathy.

### *Renal development*

Finally, as stated previously, the major difference between adult and neonatal UUO models is the impairment of immature kidney development. Very interestingly, some studies have shown that UUO induced up-regulation of some genes involved in kidney embryogenesis, such as decorin and lumi-

**Table 8** Literature data about renal development-related genes and proteins that have been altered during obstructive nephropathy. We have reported here only molecules of which expression has been significantly modified compared to sham-animals (animal model)

| Gene symbol | Gene name | Animal model | |
|---|---|---|---|
| | | CUUO | Ref. |
| Dcn | Decorin | Up (2w) | Silverstein *et al.* (2003a) |
| Lum | Lumican | Up (2w) | Silverstein *et al.* (2003a) |
| Pax2 | Paired box 2 | Up (10w) | Fenghua *et al.* (2009) |
| Tp53 | p53; tumor protein p53 | Up (2w) | Silverstein *et al.* (2003a) |
| Wnt4 | Wingless-related MMTV integration site 4 | Up (4w) | Nguyen et al. (1999a) |

w: weeks of obstruction; d: days of obstruction; CUUO: complete UUO; Ref.: references.

can (Silverstein *et al.* 2003a) involved in connective tissue organization during organ differentiation (Wilda *et al.* 2000), p53 (Silverstein *et al.* 2003a) which regulates metanephric development (Saifudeen *et al.* 2009) or Pax-2 (Fenghua *et al.* 2009) and Wnt4 (Nguyen *et al.* 1999b), two molecules involved in the mesenchymal to epithelial transition of the condensing mesenchyme (Table 8) (Lindoso *et al.* 2009).

## Conclusion

Summarizing, review of the literature both on human UPJ obstruction and the neonatal- and foetal-obstruction models strongly suggest that those models closely mimic human pathology. This observation therefore justifies the use of these models to test the effects of pharmacological interventions on evolution of the nephropathy associated to ureteropelvic junction obstruction. The molecular mechanisms involved in the pathogenesis of obstructive nephropathy follow closely what is observed in a number of nephropathies including inflammation, proliferation/apoptosis, growth factor induction, RAS activation and fibrosis. A major exception of obstructive nephropathy is the important fall in expression of many transporters. Also we have pointed in this review to the potential inducers of aggression of the tubular cell that is first in-line in obstructive nephropathy. Therapeutics aiming the blockade of this early tubular aggression might have a good future. Finally one important question remains for the long-term effects of obstructive nephropathy in humans. Does UPJ-obstruction induce permanent lesions, as suggested by the animal models? Longer follow-up studies in humans should shed light on this important remaining question.

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