

# Congenital urinary tract obstruction: defining markers of developmental kidney injury

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Congenital urinary tract obstruction (diagnosed antenatally by ultrasound screening) is one of the main causes of end-stage kidney disease in children. The extent of kidney injury in early gestation and the resultant abnormality in kidney development determine fetal outcome and postnatal renal function. Unfortunately, the current approach to diagnostic evaluation of the severity of injury has inherently poor diagnostic and prognostic value because it is based on the assessment of fetal tubular function from fetal urine samples rather than on estimates of the dysplastic changes in the injured developing kidney. To improve the outcome in children with congenital urinary tract obstruction, new biomarkers reflecting these structural changes are needed. Genomic and proteomic techniques that have emerged in the past decade can help identify the key genes and proteins from biological fluids, including amniotic fluid, that might reflect the extent of injury to the developing kidney.

Congenital abnormalities are detected in 1–2% of pregnancies, with involvement of the urinary tract being seen in ~20% of these cases (1). Registries and databases in Europe (2), North and Central America (3), and Australia and New Zealand (4) show that hypoplasia/dysplasia of the kidney, often associated with obstructive nephropathy, is the leading cause of end-stage kidney disease in childhood.

The outcome for patients with severe obstructive nephropathy has not improved significantly over the past two decades despite advances in antenatal diagnosis and therapeutic interventions (5,6). The currently available diagnostic tests lack sufficient clinical sensitivity and specificity because they reflect the functional changes in the immature kidney (pathophysiology) rather than the degree of structural damage and potential for postnatal deficit (pathogenesis).

In this article, we review current diagnostic and therapeutic approaches to human fetal obstructive nephropathy, discuss the advantages and disadvantages of the available tests, and outline the potential for new diagnostic approaches to this condition.

## ESTIMATES OF ABNORMAL FETAL KIDNEY STRUCTURE

Most cases of congenital urinary tract obstruction are identified during the second-trimester ultrasound screening. A distended

thick-walled bladder, hydroureters, and bilateral hydronephrosis in the fetus imply lower-urinary-tract obstruction (**Figure 1a**) (7). Increased echogenicity of the kidneys, a poorly defined corticomedullary border, and cystic parenchymal changes suggest renal dysplasia (**Figure 1b**) (8). When associated with severe oligohydramnios in early pregnancy, these changes carry a poor prognosis and are associated with a >90% perinatal mortality (9).

Studies analyzing the predictive value of antenatal ultrasound screening have yielded conflicting results. Anumba *et al.* (10) reported that echogenic and/or cystic changes of fetal kidneys have a predictive value of 59% in detecting dysplasia histologically or chronic kidney disease on postnatal follow-up. The predictive value of normal parenchyma (on prenatal ultrasound) in detecting the absence of dysplasia or normal renal function was 56%. Detailed ultrasound examination of the urinary tract, particularly when performed by an experienced operator, yields a higher accuracy of diagnosis of congenital urinary tract obstruction (11). Fetal magnetic resonance imaging can provide a more detailed assessment of the fetal urinary tract (12,13), but predictive values of magnetic resonance imaging for congenital urinary tract obstruction are difficult to ascertain, given the selective use of this method and the small numbers of published cases.

With regard to fetal kidney biopsy, there has been a poor success rate in obtaining renal tissue from a compromised kidney. This, combined with the fact that additional information is likely to be minimal, precludes wider use of this invasive procedure (14).

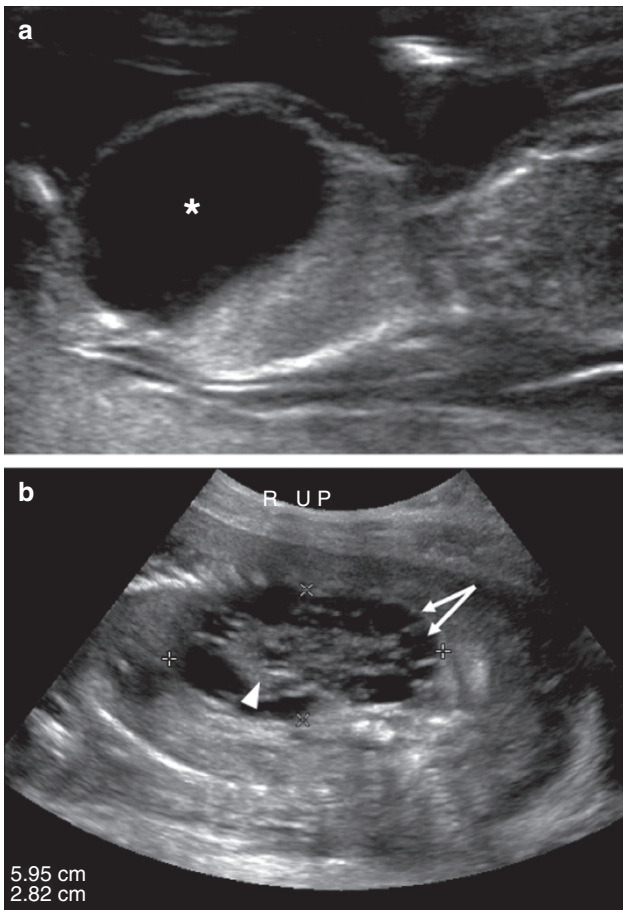
Consequently, although the currently available imaging modalities may provide an accurate diagnosis of congenital renal disorders, they are poor predictors of postnatal function, particularly as regards intermediate forms of dysplasia.

## ESTIMATES OF ABNORMAL FETAL KIDNEY FUNCTION

Measurement of fetal glomerular filtration rate (GFR) would be an ideal determinant of renal function, a good surrogate for the severity of dysplasia, and an efficient predictor of postnatal function; however, there are no normative data for this parameter in uncomplicated pregnancies (15), and it is challenging to determine GFR accurately in the fetus.  $\beta_2$ -microglobulin, the light

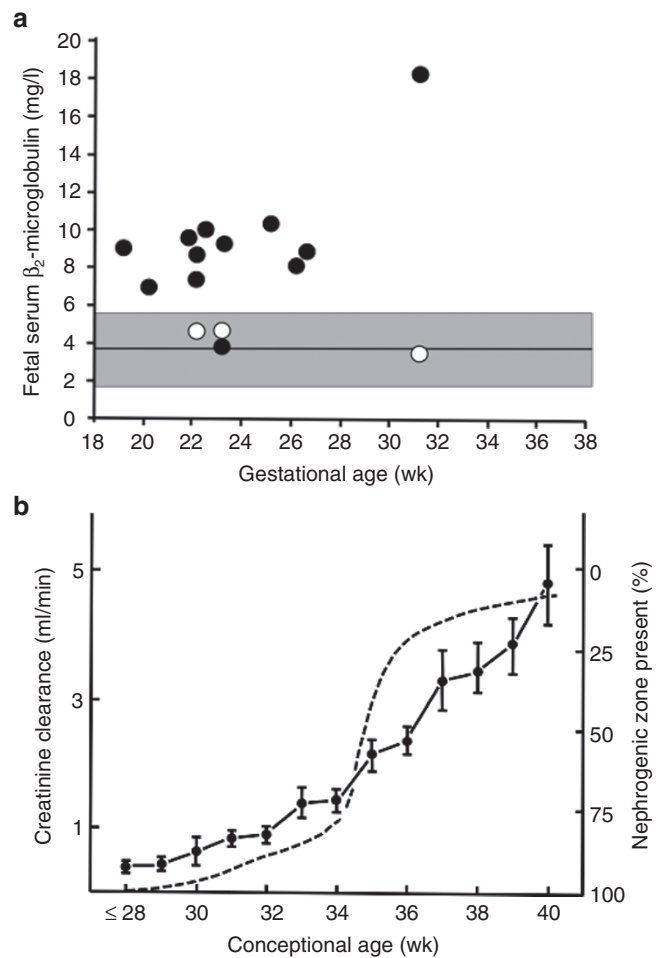
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Received 5 March 2012; accepted 6 June 2012; advance online publication 3 October 2012. doi:10.1038/pr.2012.113



**Figure 1.** Antenatal ultrasound of congenital urinary tract obstruction. (a) Ultrasound image of a 12-wk-gestation human fetus with bladder outlet obstruction caused by posterior urethral valves (PUVs), showing enlarged bladder (asterisk \*). (b) Ultrasound image of an enlarged, echogenic 21-wk-gestation kidney with multiple parenchymal cysts (arrows) and poorly defined corticomedullary border (arrowhead) in a human fetus with PUVs (courtesy of Ken Lim, British Columbia Women’s Hospital, Vancouver, Canada). R, right kidney; UP, anterior view.

chain of the class I major histocompatibility antigens, has been used as an indirect measure of fetal GFR. It is advantageous to use this antigen for GFR estimation because of its constant production by the fetus, its inability to cross the placental barrier, and its free filtration at the glomerulus (16–18). Fetuses with urinary tract obstruction had higher serum  $\beta_2$ -microglobulin levels than those without obstruction (Figure 2a, ref. 19). A fetal serum  $\beta_2$ -microglobulin cut-off value of 5.6 mg/l had a sensitivity of 80%, a specificity of 98.6%, a positive predictive value of 88.9%, and a negative predictive value of 97.1% for postnatal renal failure (16). Filtered  $\beta_2$ -microglobulin undergoes 99.9% degradation by proximal tubular cells. Levels <6 mg/l in urine are considered “normal” in fetuses with congenital urinary tract obstruction (20), whereas levels >13 mg/l, reflecting tubular damage, were invariably associated with perinatal death (21). However, the usefulness of  $\beta_2$ -microglobulin as a marker is limited because (i) there is a lack of normative data; (ii) the available studies were carried out in small numbers of patients; (iii) measurements reported in different studies were made at different



**Figure 2.** Developmental changes in fetal serum  $\beta_2$ -microglobulin and GFR, and their relationship to nephrogenesis. (a) Fetal serum  $\beta_2$ -microglobulin levels in 15 human fetuses with urinary tract obstruction as compared with the reference range (shaded area) of levels from 73 fetuses without urinary tract obstruction (filled circles: fetuses with renal damage, open circles: fetuses without renal damage). Reference range represents the mean value  $\pm$  2 SD of serum  $\beta_2$ -microglobulin, with the upper limit of 5.6 mg/l. Values above the upper limit of the reference range were recorded in 11 of the 12 fetuses with renal damage. Adapted from ref. 19, with permission from John Wiley & Sons. (b) The pattern of change in GFR, measured by validated creatinine clearance (solid line) and persistence of the nephrogenic zone in the renal cortex (dotted line) in relation to the conceptual age of the human neonate. Data obtained from samples from 205 neonates; N ranging from 7 to 26 in groups at various gestational ages. Adapted from ref. 28 with permission. GFR, glomerular filtration rate.

stages of gestation; and (iv) the studies show variable measures of outcome. In addition, fetal blood sampling carries the risks of bleeding, amniotic fluid leak, infection, and fetal death.

In addition to GFR, urinary analytes have also been used in the antenatal evaluation of kidney function and as predictors of postnatal outcome. Antenatal estimates are dynamic and are dependent on gestational age and, as with glomerular filtration, tubular function also undergoes significant changes during fetal maturation. During early development, the urinary ultrafiltrate is minimally modified by its passage through the nephron. With maturation, the tubules and collecting ducts become more efficient in reabsorption of water and electrolytes. Glick *et al.* (22)

reported findings of normal levels of fetal urinary electrolytes and correlated these values with autopsy and biopsy findings, as well as clinical outcomes, in 20 fetuses with bladder outlet obstruction. A fetal urine sodium <100 mEq/l, chloride <90 mEq/l, and urine osmolality >210 mEq/l at a mean gestational age of 23.8 wk predicted “good outcome,” reflected as the presence of nondysplastic kidneys at autopsy or biopsy, or normal renal and pulmonary function at birth. A recent comprehensive, systematic review of the literature on the accuracy of fetal urine analysis as a predictor of postnatal renal function in cases of congenital lower-urinary-tract obstruction revealed that there is currently no individual analyte or threshold with significant clinical accuracy (23). The thresholds of the most widely investigated measures (sodium, chloride, calcium, osmolality, and  $\beta_2$ -microglobulin) varied widely among the studies, and not all the studies correlated these thresholds with gestational age.

### ANTENATAL INTERVENTION

The aim of antenatal intervention in lower-urinary-tract obstruction is to relieve the obstruction in fetuses that would benefit from such shunting (24). In most cases, these therapeutic decisions, which also include decisions involving termination of pregnancy, are based on studies of fetal urine and amniotic fluid, as outlined earlier in the paper. A large meta-analysis studied the outcomes of pregnancies complicated by urinary tract obstruction and oligohydramnios in which vesico-amniotic shunting was performed. Not surprisingly, given the inherent drawbacks of the selection criteria, the analysis reports 47% perinatal survival rate after successful shunting, with 40% of the survivors developing end-stage renal disease on follow-up (5). Similarly, long-term outcome studies in these patients show a high prevalence of bladder dysfunction requiring regular catheterization or placement of a vesicostomy, as well as urinary tract infections. There was also a tendency to develop asthma and recurrent respiratory infections because of pulmonary hypoplasia (25). Therefore, urinary diversion in the fetus has not resulted in the anticipated improvement in outcomes in pregnancies complicated by severe congenital urinary tract obstruction.

### DEVELOPMENT OF GLOMERULAR AND TUBULAR FUNCTION

Clearly, the lack of accurate predictors of postnatal outcome and the difficulty in selecting fetuses that might benefit from intervention are important reasons for the poor outcomes in fetuses with congenital urinary tract obstruction. We suggest that the development of more robust clinical outcome predictors calls for a reconsideration of fetal kidney physiology.

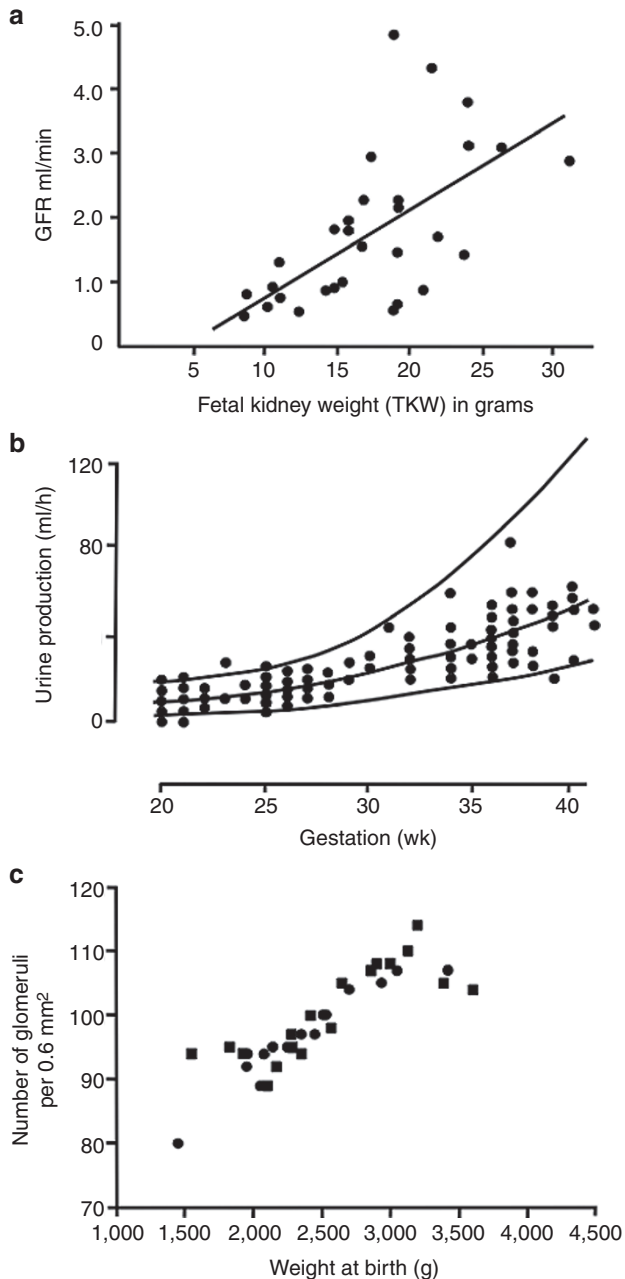
Kidney function in the fetus is not sufficient to balance all the metabolic requirements of growth, nor is it necessary, given the interposition of the placenta and maternal–fetal membranes. Nevertheless, the contribution of fetal kidneys to fluid and electrolyte balance gradually increases as gestation progresses, eventually supplanting the contribution of the placenta at the time of birth. In humans, glomerular filtration begins shortly after 9 wk of gestation, which is when the first functional glomeruli appear (26). With time, up to 34 wk of gestation, the GFR

shows a slow linear increase, reflecting the development of new nephrons. Thereafter the rate increases rapidly with the completion of nephrogenesis (27), renal blood redistribution, and the engagement of younger cortical nephrons (Figure 2b, ref. 28) (29). Fetal GFR increases in proportion to the body mass of the fetus (30). With the increase in cardiac output and growth of the renal vascular bed during gestation, renal blood flow also increases (31). The hemodynamic evolution of the fetal kidney is characterized by a shift from a low-flow, high-resistance organ, with most of the blood flow supplying the inner cortex, to a high-flow, low-resistance organ, with most of the blood flow supplying the outer cortex (32).

Although the details of fetal kidney physiology are well defined, the clinical evaluation of fetal GFR remains a challenging exercise with elusive outcomes. A number of surrogates have been proposed. Given that GFR increases with fetal weight (Figure 3a) and that urine output increases with advancing gestation (Figure 3b), fetal urinary flow rates have been used as a marker of fetal GFR (33,34). However, as in the postnatal kidney, urine output does not correlate well with absolute GFR. Glomerular number, which would also presumably reflect fetal kidney function, is also proportional to fetal weight. In fact, the increase in glomerular number, GFR, and fetal urine flow correlate closely with the increase in kidney mass, and it is therefore reasonable to assume that the main contributing factor is the addition of new nephrons (Figure 3c) (35,36). Amniotic fluid volume has also been used as a surrogate marker of GFR during the second half of pregnancy and is important in predicting outcomes. In the early fetal period, most of the amniotic fluid is produced by the amnion, placenta, and umbilical cord. Amniotic fluid volume increases from ~25 ml at 10 wk of gestation to ~400 ml at 20 wk of gestation when the fetal kidneys become the main source, although the total volume can vary substantially. By 28 wk of gestation, the amniotic fluid volume reaches a plateau of ~800 ml until term, with a slight decline post-term (37). Severe impairment of fetal kidney function, as seen in select cases of congenital urinary tract obstruction, will manifest as oligo/anhydramnios from mid-trimester onward; however the specificity of amniotic fluid volume as a marker of fetal kidney injury in general is poor (38).

Estimates of fetal kidney integrity have also been attempted on the basis of urinary analyte levels, which can be measured in either fetal urine or in amniotic fluid. In making such estimates, the effects of normal developmental regulation of tubular maturation should be properly considered and interpreted. Fetal glomerular filtrate, and ultimately fetal urine, is modified by solute reabsorption and excretion via specific transporters and channels in the tubular epithelia. The maturation rates of these transporters and channels are differentially regulated in the specific segments of the fetal nephron, and occur over the full duration of gestation and into postnatal life (39). Therefore, for the purposes of interpretation, the concentration of a particular solute in fetal urine or amniotic fluid must be compared to normative values for that same gestational age; however, such data are, for the most part, not currently

available. Fetal urinary sodium is the most commonly used predictor of postnatal kidney outcome. Reabsorption of filtered sodium increases in both proximal and distal tubules with progressing gestation and develops even earlier in the collecting duct, where the final regulation of sodium excretion is achieved (40). Consequently, under normal circumstances, the fractional excretion of sodium progressively decreases during



**Figure 3.** The relationship between fetal GFR, fetal urine production, and the number of glomeruli in the developing kidney. **(a)** Changes in GFR in fetal sheep in relation to total kidney weight (TKW) during gestation (GFR ml/min =  $0.5295 + 0.1311$  (TKW);  $r = 0.65$ ). Adapted with permission from ref. 35. **(b)** Hourly fetal urine production rate across gestation in human fetuses. Adapted with permission from ref. 33. **(c)** Relationship between birth weight and the number of glomeruli in the subcapsular cortex of human kidneys (filled squares: males; open circles: females). Reprinted and adapted with permission from ref. 36. GFR, glomerular filtration rate.

gestation (Figure 4a) (41), which means that higher urinary and amniotic fluid sodium concentrations are expected in earlier stages of gestation. No normative data are available for fetal urine and amniotic fluid concentrations of potassium, phosphate, calcium, amino acids, glucose, magnesium, and organic acids. However, the somatic growth of the fetus requires positive potassium and phosphate balance (42), and this is reflected in the lower fractional excretions of these solutes in newborns as compared to children and adults (43,44). With advancing gestational age, calcium, amino acid, and glucose excretion decrease, while magnesium and organic acid excretion increase, reflecting maturation of the relevant solute-specific transporters (45–47).

Several studies have investigated the usefulness of fetal urine  $\beta_2$ -microglobulin concentration as a predictor of postnatal kidney outcome. As in the case of other solutes, the capacity to reabsorb and degrade  $\beta_2$ -microglobulin increases as the proximal tubular epithelium matures with normal growth and development of the fetal kidney (48). As a result, fractional excretion of  $\beta_2$ -microglobulin decreases until a plateau is reached at ~34 wk of gestation (49) (Figure 4b). Therefore in interpreting fetal urine and amniotic fluid levels, particularly before 34 wk of gestation, it is important to be mindful of the normal rapid changes in excretion from week to week.

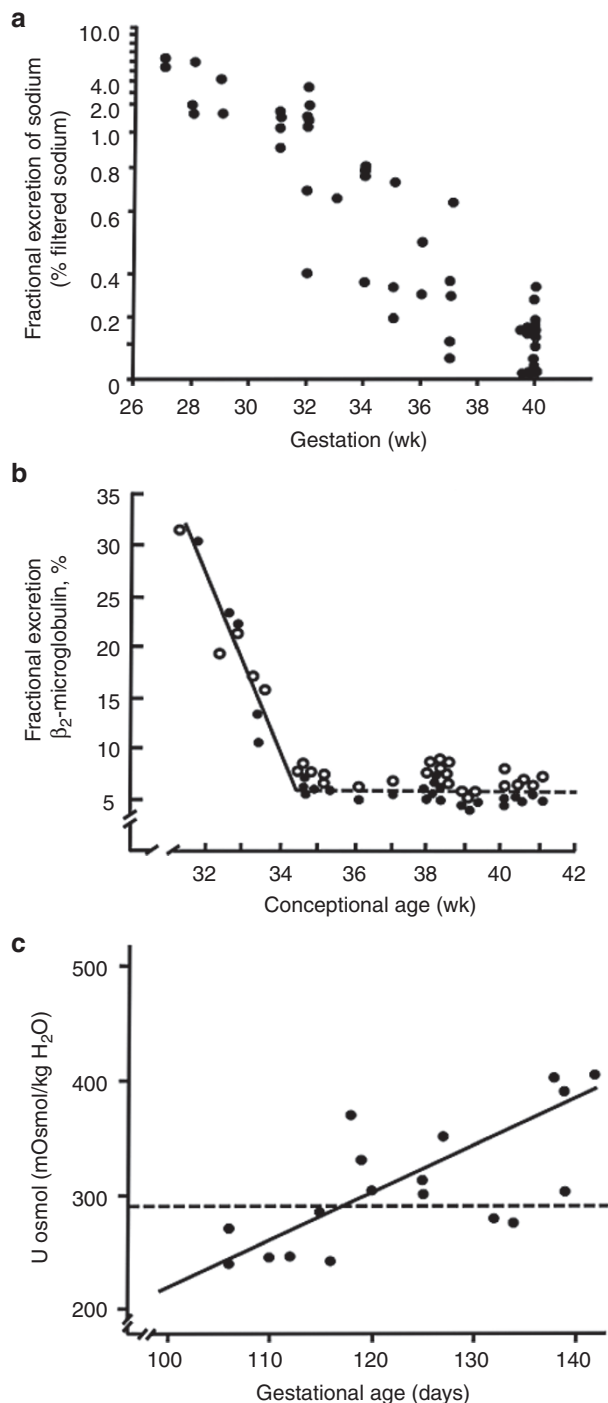
The interpretation of the osmolality of fetal urine is also complicated by the fact that the fetal kidney's ability to concentrate urine (active sodium reabsorption in the loop of Henle and recycling of urea in the distal nephron) and its ability to dilute it (collecting duct cell responsiveness to antidiuretic hormone) mature with advancing gestational age (50–52). Nonetheless, early fetal urine is hypotonic as compared to urine produced later in gestation, and the fetal kidneys are able to respond to physiologic stimuli by concentrating urine; this has been demonstrated in animal studies (Figure 4c) (50).

In summary, the interpretation of analyte concentration levels in fetal urine and amniotic fluid is a complex task. The concentration levels are determined by a number of different mechanisms that are differentially regulated and develop at different times in different segments of the fetal nephron. These include the expression of segment-specific ion transporters, whose maturation is in flux during gestation. Although there is adequate knowledge available about the normal maturation of physiology in humans, normative data for the developing fetus are understandably lacking. These pitfalls have limited the clinical usefulness of these analytes.

#### THE EFFECT OF CONGENITAL URINARY TRACT OBSTRUCTION ON FUNCTION AND FORM

The extent of kidney injury in congenital urinary tract obstruction depends on multiple factors such as the anatomical site of the obstruction, its severity and completeness, as well as its duration. Several animal models have defined the histopathology and pathogenesis of congenital urinary tract obstruction (53). These models have demonstrated that early obstruction leads to kidney dysplasia, characterized by cystic transformation of the developing nephron, abnormal glomerular





**Figure 4.** Decrease in fractional excretion of solutes and increase in the concentrating ability during fetal tubular maturation. **(a)** Fractional excretion of sodium shows changes with gestational age in the human fetus ( $r = 0.755$ ,  $P < 0.01$ ). Adapted from ref. 41 with permission from John Wiley & Sons. **(b)** Fractional excretion of  $\beta_2$ -microglobulin in 56 healthy preterm and term infants studied on day 1 (open circles) and day 4 (filled circles). Adapted and reprinted by permission from Macmillan Publishers Ltd from ref. 49. **(c)** Relationship between fetal gestational age and fetal urine osmolality (U osmol) during arginine vasopressin (AVP) infusion in ewes (term 145 d) ( $y = (4.11 \times X) - 190.1$ ,  $r = 0.70$ ,  $P < 0.001$ ) showing the relationship between fetal age and maximum U osmol (solid line). The dotted line represents mean plasma osmolality ( $291 \pm 1.45$ ) during AVP infusion. Adapted and reprinted with permission from ref. 50.

development, and medullary hypoplasia, whereas obstruction later in gestation causes hydronephrosis without dysplastic changes (54–57). The nonhuman primate model of obstructive nephropathy (58) highlights the characteristic dysplastic changes in the obstructed kidneys, characterized by a significant decrease in glomerular number and nephron endowment, glomerular cyst formation, and significant podocyte death (53). Alterations that become evident as early as during the S-shape stage of nephron development suggest abnormal nephron induction resulting from ureteric duct obstruction and injury. Tubulointerstitial changes include dilatation of the tubules and collecting ducts, marked expansion of the renal interstitium, and a striking formation of the pericyclic and periductal fibromuscular collars. Collecting-duct epithelial–mesenchymal transition and cell remodeling have also been implicated in the response to tubular injury and in the development of interstitial fibrosis (59).

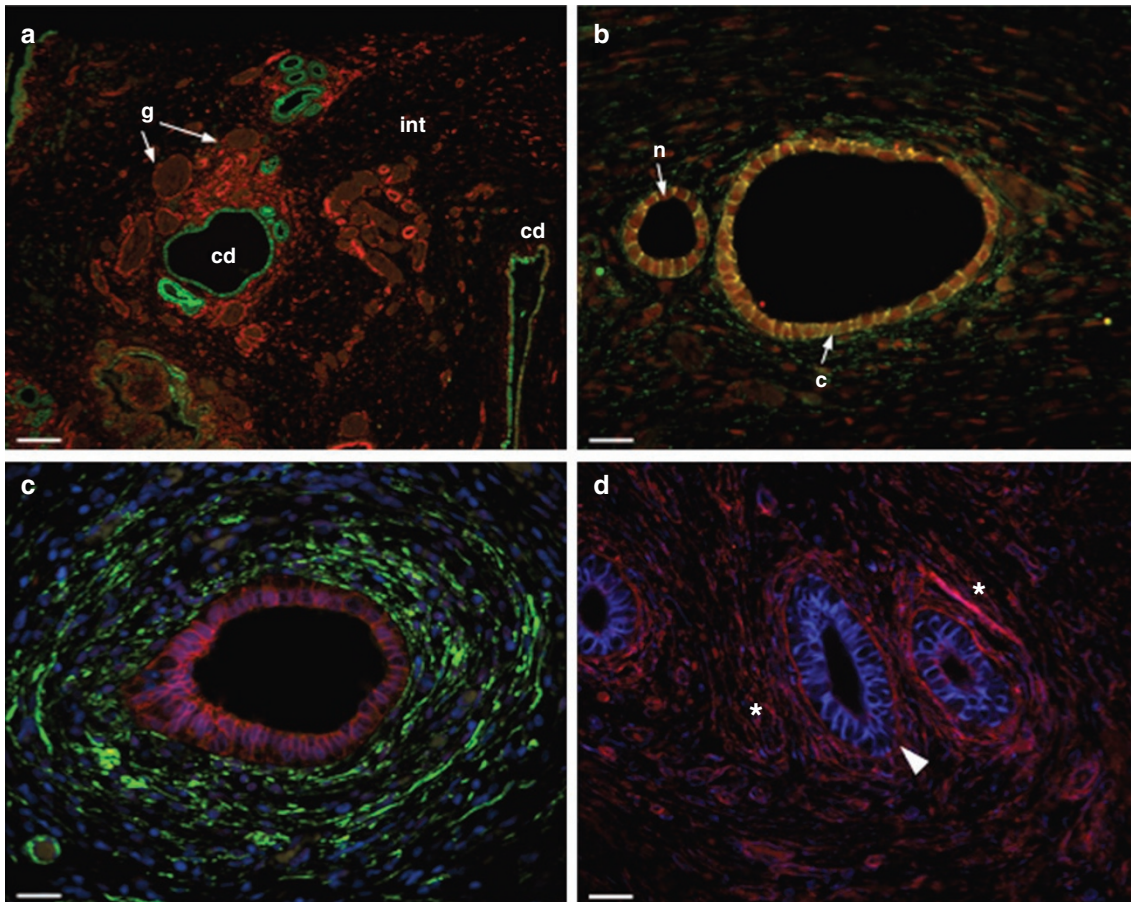
As in the fetal monkey model, in the obstructed human fetal kidney, the collecting-duct epithelium undergoes a phenotypic transition that is evident early in the process of obstruction, with a loss of epithelial proteins such as E-cadherin and a gain of mesenchymal proteins such as vimentin. These cells also display *de novo* expression of  $\alpha$ -smooth-muscle actin, associated with and proportional to the extent of periductal fibromuscular collar formation. Reduction in glomerular endowment and cystic changes of all nephron segments are also prominent (60) (Figure 5).

The significant tubulointerstitial changes in the obstructed fetal kidney (including epithelial phenotypic transition and fibrosis), as well as their effects on glomerular and tubular function, are not accurately reflected in current prenatal testing of affected fetuses. It is currently not possible to arrive at a close approximation of the number of glomeruli in the kidney at any stage of gestation, or to estimate accurately the extent of tubulointerstitial damage caused by obstruction. These are both fundamental prerequisites for determining postnatal and long-term kidney outcome. There is therefore a need to develop new surrogate markers of structural changes. Some of these putative biomarkers of congenital urinary tract obstruction are described in the next section.

#### DEVELOPMENTAL KIDNEY INJURY

Parallels can be drawn between injury of kidneys during fetal development, which we refer to here as developmental kidney injury, and injuries that occur postnatally, referred to as acute kidney injury. The morbidity and mortality associated with both types of injury remain high despite the fact that there is now a better understanding of their pathophysiology. One of the main reasons for poor outcomes in children with congenital urinary tract obstruction is the lack of methods to adequately assess the severity of kidney injury *in utero*. This is attributable, in large part, to the lack of early clinical markers of injury.

The ideal biomarker of developmental kidney injury would be one that can be obtained from easily accessible biological samples, is rapidly measurable using a standardized technique in a routine clinical laboratory, is highly sensitive and specific as



**Figure 5.** Kidney histopathology of human congenital urinary tract obstruction. Human kidneys were analyzed by immunohistochemistry as previously described (60). The mounted tissue sections were probed with the designated primary antibodies. The nuclei were stained with 4',6-diamidino-2-phenylindole (DAPI) before being mounted on slides. The analysis was performed using epifluorescent microscopy. **(a)** Decreased number of glomeruli (g), dilatation of the collecting ducts (cd), and expansion of the interstitium (int) in the cortex of an obstructed 36-wk-gestation human fetal kidney (red = vimentin; green = cytokeratin) (bar = 100  $\mu$ m). **(b)** Collecting duct epithelium of an obstructed 36-wk gestation kidney shows the dissociation of epithelial proteins, E-cadherin (red) and  $\beta$ -catenin (green), from the cellular membrane, and their translocation to the nucleus (n) and cytoplasm (c), respectively. Yellow staining at the intercellular membranes represents the colocalization of E-cadherin and  $\beta$ -catenin (bar = 25  $\mu$ m). **(c)** Formation of fibromuscular collars consisting of cells expressing  $\alpha$ -smooth-muscle actin (green) surrounding a dilated medullary cd (red = E-cadherin; blue = DAPI) in an obstructed 36-wk-gestation kidney (bar = 25  $\mu$ m). **(d)** Obstructed 36-wk gestation kidney shows cd epithelial cells with *de novo* expression of the mesenchymal protein vimentin (blue), disruption of collagen (red) in the ductal basement membrane (arrowhead), and deposition of collagen in the expanded interstitium (\*) (bar = 25  $\mu$ m).

a predictive marker, allows stratification of risk, and correlates with postnatal clinical outcomes such as mortality, the requirement for dialysis, and morbidity (61–63). The first step in the search for such a biomarker is to understand the cellular and molecular events leading to obstructive renal dysplasia. Animal studies, such as those described earlier in this paper, are invaluable in defining processes such as tubular phenotypic changes and interstitial fibrosis in the obstructed kidneys. The key proteins involved in these events, such as transforming growth factor- $\beta$  (TGF- $\beta$ ) and the epithelial and mesenchymal cell proteins reflecting cell injury and repair, can be measured in biological fluids (Table 1). Illustrative examples of potentially useful biomarkers in congenital urinary tract obstruction include components of the renin–angiotensin system (64), inflammatory markers including monocyte chemoattractant peptide-1 (65,66), and adhesion molecules such as intercellular adhesion molecule-1 (67). Several studies have confirmed the utility of

urinary TGF- $\beta$  levels as a marker of the severity of renal dysplasia in upper (68–70) as well as lower (71) urinary tract obstruction in the postnatal period. However, to date, no studies have investigated TGF- $\beta$  as a prenatal biomarker of renal dysplasia.

The question then is, can the putative biomarkers be measured in the most relevant biological specimens? Undoubtedly, fetal kidney tissue would be the best diagnostic source, and amniotic fluid the safest. It is also important to realize that each biological specimen can be divided into separate compartments containing different proteins. For example, a study of normal adult urine showed that ~48% of total protein was contained in sediments, 49% was soluble, and the remaining 3% was in exosomes (72). Urinary exosomes, which are 40–80 nm vesicles of endocytic origin, are a particularly interesting source of biomarkers that might provide unique information about the degree of injury to the cells and to the tissue releasing them. The proteome of

**Table 1.** Potential biomarkers of fetal obstructive nephropathy and their biological significance

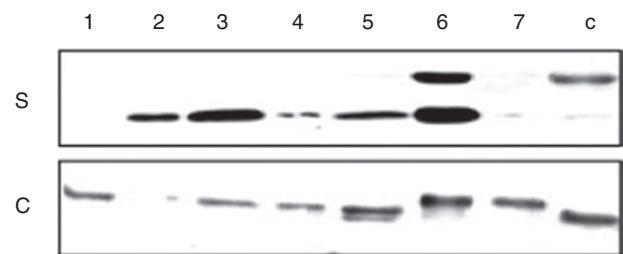
Biomarker	Site of origin	Potential biological significance
Aquaporin-2	Collecting duct (CD) principal epithelial cell	Decreased excretion reflects paucity of CD principal cells, resulting in poor ability to concentrate urine
Vacuolar-type H(+)-ATPase	CD-intercalated epithelial cell	Decreased excretion reflects paucity of CD-intercalated cells, resulting in poor ability to acidify urine
Transforming growth factor- $\beta$ 1	Fetal renal mesenchymal cells (interstitial fibroblasts, myofibroblasts, pericytes)	Increased excretion reflects development of tissue fibrosis
E-cadherin	Epithelial cells of renal tubules and CDs	Decreased excretion reflects damage to the epithelial cytoskeleton
$\beta$ -catenin	Epithelial cells of renal tubules and CDs	Increased excretion reflects increased synthesis and translocation of this cytoskeletal protein into cell cytoplasm
Transient receptor potential vanilloid 4	Epithelial cells of the distal fetal nephron	Increased excretion might reflect increased synthesis of this molecular sensor of fluid flow
L1 cell adhesion molecule	Basolateral membrane of CD epithelial cells	Increased excretion reflects translocation of this protein to the apical membrane of CD cells and <i>de novo</i> expression in epithelial cells of distal tubules and the loop of Henle

urinary exosomes has been characterized and includes hundreds of proteins specific to each type of epithelial cell in the lining of the urinary tract (73). Exosomes can also be recovered from amniotic fluid, a fact that has significant implications for the prenatal diagnosis and evaluation of many diseases, including congenital urinary tract obstruction (74). In the developing obstructed kidney, collecting-duct epithelial cell injury is an important component of the response to obstruction and is probably an early event. It is characterized by a decrease in expression levels of characteristic epithelial proteins and an increase in expression levels of mesenchymal cell proteins in the kidney (59), measurable in fetal urine and amniotic fluid. Results from urinary exosome proteomics suggest that these biomarkers may correlate with the degree of the tubulointerstitial damage. Specifically, candidate biomarkers of obstructive nephropathy derived from the injured collecting duct, including aquaporin 2, vacuolar-type H(+)-ATPase (V-ATPase), TGF- $\beta$ 1, N-cadherin, transient receptor potential vanilloid 4, E-cadherin,  $\beta$ -catenin, and L1 cell adhesion molecule can be readily measured in whole urine and urine exosomes (Figure 6).

Finally, new biomarkers will need to correlate with meaningful outcomes in long-term clinical studies once they are integrated into clinical practice. It is unlikely that any single analyte will have sufficient power to become a “gold standard” test for developmental kidney injury. It is more likely that several urine or amniotic fluid biomarkers in combination will provide the best accuracy and predictive value.

## CONCLUSIONS

The outcomes remain poor for children with congenital urinary tract obstruction. One of the main reasons for this situation is the lack of predictive prenatal tests that can accurately identify the severity of disease. The focus on the development of new biomarkers may allow the identification of key proteins that could be measured in biological fluids, thereby reflecting the extent of developmental kidney injury. Such new biomarkers will need to be evaluated in human



**Figure 6.** Western blot analysis of urine. Western blot analysis was performed on urine from children with the diagnosis of posterior urethral valves (S) and from age-matched controls with no kidney disease (C). Urine was centrifuged and the supernatant concentrated before being run on an agarose gel. Blots were probed with an antibody to TGF- $\beta$ 1 (Santa Cruz Biotechnology, Santa Cruz, CA), as previously described (75). The results demonstrate that it is possible to detect TGF- $\beta$ 1 in urine, both in subjects with posterior urethral valves (PUVs) and in controls. Numbers at the top designate the sample lanes. The final lane (c) represents urine from a positive control. TGF- $\beta$ 1, transforming growth factor- $\beta$ 1.

clinical studies with multicenter collaboration in order to enroll sufficient numbers of patients, allow long-term follow-up for clinically meaningful outcomes, and ultimately provide confidence in the level of accuracy and prediction of severity of disease.

## STATEMENT OF FINANCIAL SUPPORT

This study was supported, in part, by grants from the Kidney Foundation of Canada (to D.G.M.), Canadian Institutes of Health Research (to D.G.M.), BC Provincial Renal Agency (to P.T.), and the National Institutes of Health (to A.F.T.).

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