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Molecular biology and nuclear medicine in pediatric hydronephrosis

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Pediatric hydronephrosis may correspond to very different clinical situations, ranging from fully benign reversible dilatation to severe obstructive nephropathy. The genetic research is difficult, mainly because the condition is probably polygenic and the embryology of the urinary system is very complex and depends on a multifaceted interaction of genetic and environmental factors. Molecular biology has gained new insights in the complicated urinary system and in the mechanisms of obstructive nephropathy. Some mediators (tumor growth factor, tumor necrosis factor, renin angiotensin system, etc.) could be considered molecular markers of obstruction and it has been proposed to introduce them in clinical decision making, in order to make an accurate selection of patients needing surgical correction. Scintigraphy has been a standard procedure in the management of pediatric hydronephrosis for decades and has been used in many clinical studies designed to evaluate the role of selected molecular markers in clinical settings. The relationships between scintigraphic parameters and molecular mediators seems promising, in particular for the evaluation of the Reanin Angiotensin System, which plays many roles in the natural history of pediatric hydronephrosis. Angiotensin up-regulation is a turning point in many pediatric hydronephrosis and can be unveiled by captopril scintigraphy, which allows a timely diagnosis of obstruction, before irreversible parenchymal injury and loss of renal function.

KEY WORDS: Hydronephrosis - Nuclear medicine - Molecular biology - Infant - Ureteral obstruction, diagnosis.

Fetal hydronephrosis is frequently diagnosed during echographic screening, with pyelectasis being identified in 4.5% of screened population.[1] Spontaneous resolution is a frequent outcome for simple unilateral forms, even for high degree dilatation.[2] Therefore, the prognosis is significantly better when compared to more severe forms of congenital anomalies of the kidney and urinary tract, as posterior urethral valves or hypodysplasia, which are associated with a significant risk for dialysis.[3-7] Nonetheless, surgery and/or long-term follow-up are required in a substantial number of cases, up to 1.5% of screened patients,[1] that is onethird of observed cases. Since spontaneous resolution is so frequent, it is mandatory to avoid unnecessary surgical interventions, but the prevention of renal function loss secondary to chronic obstruction is also necessary.

There is no single test that allows to identify reliably which patients will need surgical correction, or to predict the future course of each patient.[8, 9] The usual multidisciplinary follow-up, based mainly on echography and diuretic scintigraphy is the best compromise, as of today, in the clinical setting, but the need for improved tests has been emphasized many times.

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Even if obstructive uropathy comprises the largest fraction of identifiable causes of renal failure in infants and children [10] no agreement has been reached on the very definition of urinary tract obstruction and the debate on indications for therapeutic interventions is still ongoing. It is largely agreed that the key point is the loss of renal function, including the developing potential of the kidney,[11, 12] but this definition remains ambiguous, even as a working concept. The revolution brought by molecular biology has been changing the understanding of hydronephrosis in the last years, through better comprehension of its genetic and molecular bases. This progress will modify its clinical approach and the use of diagnostic techniques as diuretic scintigraphy is going to change accordingly.

Genetics

The complex development of the urinary tract depends on various genetic and environmental factors, whose role is been actively explored in mice and humans. Hydronephrosis has been associated with cryptic chromosomal anomalies,[13] isolated mutations [14] or experimental knockout of a wide variety of genes.[15] Gene linkage studies performed in animal models of spontaneous hydronephrosis led to the identification of a significant locus on chromosome 6 and suggestive loci on chromosome 2, containing large number of genes for the search for possible candidates.

In a recent study Aoki *et al.*[16] showed that inhibitor of DNA binding 2 (Id2) knockout (*Id2_/_*) is frequently associated with hydronephrosis in adult mice and *Id2* haploinsufficiency frequently shows the same phenotype, whit hampered urinary flow. *Id2* is a component of the helix-loop-helix family of transcription factors that promote cellular growth and inhibit differentiation. This action could be related to the reduction in Angiotensin receptor II type 1 (Agtr1b), which has been observed by the same researchers in the uretero-pelvic junction (UPJ) of adult *Id2* mutant mice. Previous studies have shown that *Agtr1_/_* mice show morphological changes in the kidney leading to hydronephrosis, with urinary outflow obstruction.[17] In the rat, *Id2* is localized on chromosome 6 and *Agtr1b* on chromosome 2.

Occurrence of UPJ obstruction (UPJO) in more than one member of the same family further supports the hypothesis of a genetic base for hydronephrosis and some data [18] suggest a dominant inheritance pattern linked with chromosome 6p. This finding has not been confirmed in other families,[19] which is not surprising, because genetic heterogeneity has been already described in many nephropathies.

Some cases of congenital UPJO are caused by primary defects of smooth muscle differentiation and morphogenesis. A gene (*Tshz3*), belonging to a family of mammalian *Teashirt* (*Tshz*) genes coding for transcription factors, is expressed in mesenchymal cells forming the wall of embrionic ureter in mice. *Tshz3* function is required for upregulation of myocardin, a molecule involved in the transcriptional machinery that directs the expression of smooth muscle contractile proteins.[20] Mice with null mutation of *Tshz3* do not form smooth muscle cells in the proximal ureter. Homozygous mutant mice invariably have bilateral fetal hydronephrosis, whereas a subset of animals with just one mutant allele have unilateral hydronephrosis.[21] The expression data presented in the paper by Caubit *et al.* were consistent with *TSHZ2* (chromosome 20q13.2) and *TSHZ3* (19q13.11) as candidate genes for congenital UPJO.

Wnts are glycoproteins contributing to intercellular signal transduction. Wnt signaling is modified by the activity of Wnts themselves, by specific receptors (frizzleds) and inhibitors such as secreted frizzled-related proteins (sFRPs), by Wnt inhibitory factor-1 (WIF-1) and by the Dickkopf family. Wnt-4 plays a crucial role in normal renal organogenesis, particularly tubulogenesis. Renal mRNA expression patterns of selected genes involved in Wnt signaling confirm the role of Wnts, their receptors and inhibitors in spontaneous congenital obstructive uropathy in rats with spontaneous congenital obstructive uropathy.[22]

Obstructive uropathy has been associated with many other gene mutations [23] (Bmp4, Foxc1/c2, Gata2, Aqp2, Lim1, Pax2, Renin, Ret, Robo2, etc.), which disrupt the complex integration of progenitor cells during the development of the urinary tract. The comprehension of this intricate signaling network is the critical step in grasping the pathogenesis and natural history of congenital hydronephrosis in the next future.

Experimental models of molecular signaling in hydronephrosis

Many experimental models have been proposed to elucidate the pathogenesis and the natural history

of obstructive nephropathy, spacing from fetal sheep and primates to dogs, mice or rats. From the simple complete obstruction there has been an evolution towards sophisticated models, where partial reversible obstruction tries to mimic more precisely the human situation.[10] The results obtained in this field during the past years have led to the identification of some key-points in the progression from chronic obstruction to tissue damage and irreversible function loss.[8]

The damage sequence starts with the caspase-dependent apoptosis of tubular cells and with interstitial inflammation, which are accompanied and followed by progressive interstitial fibrosis and eventually by glomero-tubular injury.

The axial strain due to dilatation can trigger directly apoptosis, which is further amplified by tumor growth factor β1 (TGF-β1), tumor necrosis factor α (TNF-α), Fas and other mediators. Their action is intensified by a concomitant reduction in protective factors, as epidermal growth factor (EGF), endothelial nitric oxide synthase etc. The concomitant upregulation of many chemokines, adhesion molecules (integrins, selectins, intercellular adhesion molecule-1 [ICAM-1], vascular adhesion molecule-1 [VCAM-1]), osteopontine and of monocyte chemotactic protein-1 (MCP-1) stimulates macrophage infiltration, a well recognized trait of obstructive nephropathy. Mononuclear cells have direct harmful effects on renal tissue and contribute greatly to the creation and modulation of the biochemical network leading to tissue damage.[24]

TGF-β1, mainly modulated by the renin-angiotensin system (RAS), plays a pivotal role in promoting interstitial fibrosis, which is also directly stimulated by the RAS itself, by CTGF, PDGF and plasminogen inhibitor-1 (PAI-1). The proliferation of resident interstitial fibroblasts is accompanied by the epithelial-mesenchymal transition (EMT) of some medullary alecting duct cells. They migrate to the interstitium, under the action of TGF-β1 again, and transform themselves in fibroblast or myo-fibroblasts, expressing α-smooth muscle actin (αSMA).[25]

Fibrogenic cytokines, as TGF-β1 and TNF-α, stimulate also the proliferation and phenotypic transition of endothelial cells, pericytes and stem cells. This process may configure a depletion of the growth and repair potential of the hydronephrotic kidney in children, with long-term consequences in the adult life.

The role of oxidative stress in chronic obstruction has been actively studied and there is evidence for an increase in oxidants following ureteral obstruction,[26-28] parallelled by a reduction of endogenous antioxidants.

Histopathologic changes in hydronephrosis

Experimental models have led to the concept of obstruction nephropathy in children as a spectrum of tissue changes, from simple dilatation to tissue damage with function loss, up to severe development impairment or dysplasia. The final outcome is determined by obstruction severity and duration and by the time of its onset during fetal life: the earlier the beginning, the worst the consequences on renal development.[10]

Histologic studies of human specimens have confirmed in pediatric hydronephrosis the wide spectrum of alterations observed in animal experiments. Kidneys affected by uretero-pelvic obstruction (UPJO) may exhibit mature and completely differentiated parenchyma with or without histological lesions as a consequence of obstruction. On the other hand, it is possible to observe different degrees of dysplasia, characterized by the persistence of immature and incompletely differentiated renal tissue.[29]

Even the anomalies at the UPJ can vary from localized fibrosis to diffuse abnormalities and a whole-length ureteral involvement, indicating widespread abnormalities in the ureteral wall.[30] A marked variability has been observed also in parenchymal changes related to UPJO in man. This knowledge is almost exclusively based on renal biopsies in patients undergoing pyeloplasty, due to a diagnosis of obstruction based mainly on the usual combination of clinical and instrumental (*i.e.*, ultrasonographic and scintigraphic) data. In the majority of cases the parenchyma appears relatively well preserved and the most frequent changes have been observed at the glomerular level.

Nevertheless, subtle changes have been described, involving the proximal tubule, with a reduction in tubular mass even in the absence of overt tubulo-interstitial injuries. Rosen *et al.* found tubular alterations in one quarter of specimens and it was associated with interstitial fibrosis in half of them. The more severe the tubular lesions, the more frequent were the changes seen at glomerular level. Quite surprisingly tubulo-interstitial injury was not constantly associated with

parenchymal thinning in high degree human hydronephrosis. This is in contrast with data collected from animals affected by severe experimental hydronephrosis [31] and could be explained by the different type of obstruction: in humans it is partial, but in many experimental studies it is complete. The relationship between duration of obstruction and severity of histopathological changes in hydronephrotic children has been evaluated with conflicting results. Some authors did not confirm this association [32] when they considered only the post-natal life, but fetal life must be taken into account in such an analysis, because it represents the most critical period for kidney maturation. Chronic interstitial nephropathy has been observed in a significantly higher proportion in children presenting an earlier onset of hydronephrosis during the fetal life, with 24 weeks of gestation as a threshold.[33]

Biomarkers

Despite the progress made in understanding its pathophysiology, pediatric hydronephrosis remains a clinical challenge, because no single technique has been able to identify reliably which patients needs surgical correction and to suggest the most appropriate time for surgery.[34] The actual standard of practice relies on a combination of sonography and scintigraphy, but it is far from satisfactory. Despite watchful follow-up the condition can silently progress to a measurable loss of function in the affected kidney and the recovery is not guaranteed, even after prompt surgical correction.[33]

Considering the number of mediators involved in the onset of tissue injury during chronic obstruction, urinary biomarkers are natural candidates in this situation and many of the molecules identified in animal studies have been evaluated as potential diagnostic tools in children.

A glucosaminidase (N-acetyl-β D-Glucosaminidase, NAG) has been found increased when obstruction is present in animal models of hydronephrosis;[35] the result has been replicated in humans,[36] but there is no demonstration of its clinical value in identifying children who should undergo surgery

Experimental studies have demonstrated the relationship between urinary excretion of TGF-β1 and the following onset of kidney damage [24] but the finding could not be confirmed in a group of hydronephrotic children.

A significant increase in urinary MACP-1 levels has been detected in children with UPJO [37] and is parallelled by a reduction in urinary epithelial growth factor (EGF), confirming the loss of protective factors already observed in animals. Postoperative measurements showed an inverted trend, with lowered MACP-1 levels and increasing urinary EGF excretion, approaching the situation of normal controls. Of interest, the excretion of MACP-1 was higher in patients with recurrent urinary tract infections; therefore EGF reduction in urine seems a better indicator of the severity of obstructive nephropathy.

Some studies have addressed not only the molecular mechanisms of tissue damage in pediatric hydronephrosis, but also the molecular basis of functional derangement, as the reduction of urinary concentrating capability. Polyuria is often observed when obstruction resolves,[38, 39] *i.e.* in the first days after passing kidney stones or after surgical correction of a stenotic ureter, and depends on many complex pathways. Laboratory experiments have established that the mechanism involves different tubular segments and is finely modulated, with vasopressin and Na transporters acting as key factors in animal models.[40]

The main regulator of collecting duct water permeability has been identified as the vasopressin-sensitive water channel aquaporin-2 (AQP-2), which is translocated to the apical membrane of collecting duct cells when increased water reabsorption is needed.[41-43] Evaluation of AQP-2 in humans is difficult, because individual variability is very high and a normalcy range is difficult to define.

A down regulation of AQP-2 has been found,[44] in children operated for histologically documented unilateral UPJO, during the immediate post-operative period (1-4 days), when polyuria is usually observed in humans and in animal models. Separate urine collection confirmed a significantly higher urinary output from the operated kidney, associated with a significant reduction in sodium concentration, confirming the impaired concentrating capability in the post-obstructed kidney. The excretion of AQP-2 was similarly reduced in the operated kidney and the difference with the contralateral non affected kidney disappeared only on the 5th day after surgery. Low AQP-2 excretion was accompanied by a concomitant increase in PGE-2 urinary levels, which have been described in animals and in similar clinical settings. Increased PGE-2, possibly part of a compensatory mechanism to maintain glomerular

filtration during obstruction,[45] could have the side effect of AQP-2 trafficking reduction,[46] which leads to the postobstructive polyuria. It is worth of note that split renal function measured by [^{99m}Tc]MAG3 scintigraphy before surgery was within normal limits (46%±1.8%) in this group of patients, supporting the hypothesis of a compensatory mechanism acting in the hydronephrotic kidneys. Other researchers have confirmed the down regulation of aquaporins in pediatric hydronephrosis:[47] the expression of AQP1-4 mRNA and protein abundance is reduced in proportion with the degree of hydronephrosis graded by ultrasound in human congenital hydronephrosis.

In this and in many other studies, the research on molecular markers opens exciting perspectives for the comprehension of hydronephrosis and for a better diagnosis, even if no single marker has been able to discriminate obstructed kidneys from hydronephrosis without obstruction.

Nuclear medicine and molecular biology

Nuclear medicine has made great contributions to the understanding and the clinical management of hydronephrosis, particularly in children, but in a significant number of cases the clinical puzzle is too complex to be solved. The result of diuretic scintigraphy falls in a gray zone in many patients, despite the simultaneous assessment of split renal function and urine washout.

It must be observed that as of today no single imaging technique has been capable to solve the clinical complexity of pediatric hydronephrosis. The best practical results have been achieved combining ultrasound scan and scintigraphy, following various schedules. The need for a better diagnostic test is one of the major stimuli behind the research for different approaches, as molecular markers, but a long investigative work is needed to identify and validate the new techniques, on the first hand by confrontation with the actual standard of practice. Therefore, nuclear medicine continues to play a critical role not only in the clinical setting, but also in the realm of experimental investigation, where it has been used as a standard method to evaluate the correspondence between biological markers and parameters extracted from the analysis of scintigraphic images.

Systematic evaluation of histopatholgic changes in pediatric hydronephrosis showed an inverse relationship between the size of the proximal tubules, typically reduced in hydronephrotic kidneys, and the washout time calculated on the diuretic renogram.[30] Another interesting consideration can be made on the same paper, when reporting that extreme parenchymal thinning shows commonly well preserved parenchyma with limited tubulointerstitial injury. This finding correlates well with the clinical observation that severely dilated kidneys quite often exhibit a relatively well preserved split renal function, with percent value in the normal range.

Diuretic [^{99m}Tc]MAG-3 scintigraphy has been evaluated with regard to the expression and excretion of MCP-1, a specific chemotactic and activating factor for monocytes, in children operated for UPJO.[37] A significant direct correlation was found between MCP-1 urinary levels and increased radiopharmaceutical retention in the renal parenchyma, as expressed by the tracer mean transit time. In the same study a significant improvement was observed for split renal function after surgical correction of the stenosis. This is the goal of many diagnostic and therapeutic strategies but often a perfect correction, with normal postoperative washout, does not obtain a recovery in the function of the hydronephrotic kidney. Moreover, the patients with recurrent urinary tract infections (UTI) showed a significant reduction of the renal function in the hydronephrotic kidney when compared to subjects without history of UTI. This suggests a combined mechanism for parenchymal damage, with chronic obstruction effects amplified by infection. A third interesting relationship was observed between preoperatory levels of urinary EGF, a protective factor in the signaling network of hydpronephrotic kidneys, and functional recovery after surgery, expressed by the increase in single kidney clearance measured by scintigraphic uptake of [^{99m}Tc]MAG-3.

Chronic interstitial nephropathy has been investigated in many experimental models of obstructive hydronephrosis but it is difficult to confirm the findings of these studies in humans. Usually tissue samples are obtained from microbiopsies during surgical correction of UPJO and some researchers have investigated the relationship between diuretic [^{99m}Tc]MAG-3 scintigraphy and tissue damage in pediatric patients. As previously said the spectrum of histopathologic changes in kidneys affected by UPJO is quite wide. In the most severe forms the renal tissue is dysplastic, with persisting immature and incompletely differen-

tiated renal tissue.[29] Otherwise, the normal mature renal tissue can present various degree of histological damage, involving tubular and/or glomerular compartment, or may result only minimally affected or even normal.

Both split renal function and post-void washout in diuretic [^{99m}Tc]MAG-3 scintigraphy resulted worsened in patients operated for UPJO presenting the histopathologic pattern combining interstitial inflammation, fibrosis, tubular atrophy and glomerular sclerosis.[33] The scintigraphic parameters were significantly lower in the tissue-damage group, showing the signs of interstitial nephropathy, when compared with children operated for the same disease but without any histological sign of kidney injury.

A significant elevation of inflammation and fibrosis markers was observed only in the interstitial nephropathy group, as expected on the basis of animal experiments Tissue expression of TGF-β1, α-SMA and vimentin were significantly correlated with preoperative split renal function and washout evaluated by diuretic scintigraphy. Furthermore, a significant relationship was found between mRNA for angiotensin receptors (Agtr1 and Agtr2) and scintigraphic parameters.

The relationship between impaired post-void washout and damage markers could even suggest a chance for reconsidering the role of drainage evaluation in clinical decision making, at least in serial renographic follow-up, where worsening of washout parameters in well standardized scintigraphies could precede a drop in split renal function.

The aforementioned study confirmed the role the Renin-Angiotensin-System (RAS) in the hydronephrotic kidneys in children. It has been proposed many times that at least in some cases glomerular vasoconstriction in pediatric hydronephrosis has a compensatory significance, as in renovascular hypertension caused by renal artery stenosis. Therefore, a ACE-inhibitor, as captopril, should determine a drop in the function of the obstructed kidney, whereas in simple non-obstructed hydronephrosis the renal function would remain unchanged. One could speculate that such patients with highly activated RAS have probably a more severe obstruction and would benefit from a prompt surgical relief of obstruction, to avoid the effects of a prolonged activation of the RAS on the renal parenchyma.

This hypothesis was tested using standard captopril renography with [^{99m}Tc]DTPA in a group of 23 children with suspected pyelo-ureteral or uretero-vescical obstruction.[48]

The findings reflects probably the inhomogeneity of the patients group in this preliminary study, which included also some cases of bilateral dilatation. The split renal function remained unchanged during captopril challenge in the majority of patients. The subgroup showing a significant decrease in split renal function had a more severe impairment of washout, as expressed by diuretic half-time and it was suggested that captopril renography could indeed pick up obstructed patients, when the balance between vasoconstriction and vasodilatation is disrupted, before the onset of irreversible tissue damage. Unfortunately, the authors did not obtain the consent to perform postoperative captopril scintigraphy in the four patients who underwent surgery, since the postoperative basal study showed in all cases a good washout.

The activation of the RAS in children affected by UPJO has been confirmed in another study,[49] which considered only children affected by unilateral hydronephrosis. In this group of patients [^{99m}Tc]DTPA captopril scintigraphy resulted positive (function decrease ≥5%) in 8/25 patients; six of them were considered obstructed, on the base of standard clinical protocol, and underwent surgery. All positive patients but one had a reduced (≤40%) split renal function in basal conditions, probably representing a subgroup already with some degree of parenchymal injury determining a loss of function.

Nevertheless, the data support the hypothesis of RAS activation in obstructed hydronephrosis, even if no postoperative studies were performed, and show a high negative predictive value for captopril scintigraphy, since no patient with normal response needed surgery during the follow-up.

Different results were obtained, using [^{99m}Tc]MAG3 captopril scintigraphy, in 12 children undergoing surgical correction for unilateral ureteropelvic obstruction.[50] The preoperative [^{99m}Tc]MAG3 captopril renography showed in every case a marked worsening of the washout only in the hydronephrotic kidney, without any change in all but one of the contralateral kidneys. No significant changes were observed in the split renal function and radiotracer retention was limited to calyces and pelvis of the affected kidney. The decision to operate was based on the usual parameters (increasing dilatation on echography, reduction of split renal function, worsening of parenchymal thinning, etc.) during the standard follow-up, without

considering the results of captopril scintigraphy. The washout of obstructed units reverted to normal after surgical correction in all patients, without any significant modification during captopril challenge. These findings point to a direct action of angiotensin on pelvi-ureteral peristalsis in the hydronephrotic kidneys. Animal experiments have shown that AT receptors plays a critical role in the development of the pelvic peacemaker,[51] the group of cells where the peristaltic wave arises during excretion of the urinary bolus.[52] A mice model for congenital hydronephrosis shows that defective pelvi-ureteral peristalsis can lead to severe hydronephrosis without physical stricture of the ureteropelvic junction.[53] This intriguing model is based on calcineurin-deficient animals and calcineurin has been proposed as a specific key-factor in the development of the pelvic and ureteral cells devoted to peristalsis control. It is well known that RAS is another key factor in the building of the peristaltic machinery [51] and it has been proposed recently that this action is mediated by calcineurin.[23] These experimental findings support the clinical observation that captopril has strong effects on the washout phase, at least in some in hydronephrotic kidneys. This could be the premise for a diagnostic use of captopril scintigraphy aimed directly at the excretion impairment, which could precede not only the function loss but also the compensatory mechanisms dependent on vasoconstriction.

Conclusions

Molecular biology studies have made tremendous progress in the comprehension of hydronephrosis during the last years. This is part of a generalized effort in this research fields, where new experimental models have been created, shifting from simple ureteral obstruction to more refined setup. The surgical ligature has evolved in modeling partial reversible obstruction and now genetic manipulation produces rats and mice with a situation very close to the human congenital hydronephrosis secondary to UPJO. An important advantage of these techniques is related to the different timing of nephrogenesis. In humans it is complete by 34 weeks of gestational life, whereas in mice and rats it continues postnatally, allowing the collection of data otherwise impossible to obtain in clinical research.

Histopathology has shown that the clinical complexity of pediatric hydronephrosis mirrors the variable histopathologic patterns, ranging from normal renal tissue to extreme dysplasia.

The most striking progresses have been made in the identification of the cellular mechanisms causing renal damage in UPJO. Apoptosis, inflammation and fibrosis interact with each other, through a complex network of molecular signals, involving many categories of transmitters. Some of them, as TGF-β1, MACP-1 and EGF, have been proposed as molecular markers of obstructive disease in a perspective of clinical decision making. This approach is very promising from a theoretical point of view, but today no single marker has demonstrated the capability of pinpointing patients needing surgical treatment. Better results could be obtained by simultaneous evaluation of multiple markers: urinary proteomics holds perhaps the greatest chances in this field and some preliminary results are encouraging,[54] but researchers have many obstacles to overcome before a routine use.

The same holds true for genetics: animal studies have identified many candidate genes for UPJO, but there is still much to be done to identify mutations causing human congenital urinary tract obstruction. Despite the vast progress made in the comprehension of its evolution further investigation is necessary to translate the laboratory knowledge into improvements in diagnosis and treatment.

The better comprehension of obstructive nephropathy at cellular and subcellular level has confirmed the impossibility for imaging studies to catch the subtle changes in the first phases of obstructive nephropathy.[30, 55] Nonetheless, diuretic scintigraphy remains the most experimented technique for non-invasive assessment of split renal function and it will be difficult to find a substitute for it in the immediate future, because many problems need to be solved before the introduction of molecular markers in the diagnostic routine of pediatric hydronephrosis. Their urinary concentration is usually low and only some of them are measurable by mass spectrometry. This approach has given some promising preliminary results [54] but the majority of mediators, as growth factors and cytokines, are in the low-detectability group.[56] Moreover, when measuring has been possible, a high inter-individual variability has been observed [57] and this is further complicated by the age-related changes, secondary to renal development and maturation.

Therefore, in the next future, diuretic scintigraphy is going to play a significant role in the clinical man-

agement of pediatric hydronephrosis. Furthermore, as a tracer technique, it offers the opportunity to correlate many aspects of the research on experimental models with the functional status of patients.

Pharmacological intervention has been a mainstay for decades in diagnostic nuclear medicine, after the introduction of the furosemide stimulus as a standard technique. Today there is growing evidence suggesting that captopril could in fact represent a promising line of development, with strong bases in the most advanced outposts of the molecular research. If the preliminary results will be confirmed in larger series, the identification of RAS activation in hydronephrotic kidneys could be a strong indicator for surgery, both as a marker of compensatory vasoconstriction to maintain filtration and as a mechanism supporting the failing peristalsis of an obstructed excretory system.

This information could also have a significant prognostic value for the long-term effects of hydronephrosis, which can be, at least in complex cases, quite severe, as shown in recent studies [7] on the outcome of congenital anomalies of the kidney and urinary tract (CAKUT).

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