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NEPHROLOGY IN BRIEF

Eclectica

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The Influence of Specific Podocin Mutations on the Age of Onset of Steroid-Resistant Nephrotic Syndrome

Twenty-eight per cent of sporadic cases of steroid-resistant nephrotic syndrome (SRNS) are caused by mutations of NPHS2, the gene encoding the podocyte protein podocin. Mutations causing autosomal recessive nephrotic syndrome in children have been detected in genes encoding the proteins nephrin (NPHS 1), podocin (NPHS 2), laminin-B-2 (LAMB 2), and phosphlipase C-e 1(PLCE 1). Dominant mutations of Wilms tumor-1gene (WT 1) also give rise to childhood nephrosis. In adults autosomal dominant nephrotic syndrome may also be caused by mutations in alpha actinin-4 (ACTN 4), and transient receptor potential cation channel, subfamily C, member 6 (TRPC6) chloride channel.

The most common mutations causing SRNS are those of the gene NPHS2 encoding the protein podocin. Eighty per cent of affected patients exhibit the morphology of focal segmental glomerulosclerosis (FSGS), are steroid-resistant, and proceed to end-stage renal failure, but have a much lower rate of disease recurrence in the renal transplant (8%) than those with "idiopathic" FSGS (33%). They present at various ages, unlike patients with the Finnish congenital nephrotic syndrome due to mutation of NPHS1 (nephrin) who invariably present in the first 3 months of life. However, a recent study by Hinkes et al. of 89 European children found NPHS2 mutations to be the most common cause of congenital and infantile nephrotic syndrome. Ninety-four per cent of these babies had truncating (nonsense or frameshift) or homozygous R138Q mutations. These findings and some suggestive data by Weber et al. prompted Hinkes et al. in collaboration with other groups to conduct a multinational study to examine the relationship of specific NPHS2 mutations to the age of onset of SRNS.

The study accumulated 430 patients with SRNS from 404 families from different countries, but there was a predominance of Central European patients. Only 23 families had more than one affected member. The patients were screened for mutations of NPHS2 by direct sequencing of all eight exons of the gene. Mutations were detected in 73 patients (18%) with SRNS. Twenty-nine of these patients (40%) had one truncating mutation (nonsense or frameshift) in combination with any other mutation (Group A), and 22 (30%) had a homozygous R138Q mutation, the "founder" NPHS2 mutation described by Boute et al. (Group B). This mutation causes podocin to be trapped in the endoplasmic reticulum, thereby disrupting the targeting of nephrin to lipid raft microdomains. Nine patients (12%) had one R138Q mutation and one missense mutation (Group C), and 13 (18%), two missense mutations other than R138Q (Group D). The study also discovered six new NPHS2 mutations.

The 51 patients in Groups A and B exhibited NS very early in life (mean onset under 1.7 years), with all but one becoming nephrotic before the age of 6 years. However, the onset of NS in patients with identical mutations could vary by several years within that time span, indicating that other factors might modify the phenotype. In contrast, the patients in Groups C and D manifested the disease at an older age (mean onset after 4.2 years). In these other patients there was no relationship between the specific mutation and the age of onset of NS. The prevalence of

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NPHS2 mutations in families having more than one affected member rose to 39%. Gender and ethnic background had no influence on the age of onset of NS in the patients with disease-causing mutations of any kind. Also, the type of mutation had no relationship to disease progression, almost all patients developing end-stage renal disease within 10 years (Kaplan Meier).

Such large scale studies enhance our understanding of SRNS in terms of genotype–phenotype relationships, but much remains to be learned, as 80% of the patients in this study had no NPHS2 mutations (J Am Soc Nephrol 2008; 19: 365 and 189).

Transtubular Potassium Gradient in the Evaluation of Hyperkalemia

Potassium secretion occurs at the late distal convoluted tubule, connecting segment, and the cortical and outer medullary collecting tubules, and depends on the K concentration gradient across the luminal membrane of the principal cell, K permeability through the membrane (K channels), sodium delivery and urine flow rate into the distal nephron, the electronegativity in the tubular lumen generated by sodium absorption, the urinary content of anions, and the availability of aldosterone and tubular responsiveness to it. The equation, transtubular potassium gradient (TTKG)=(urine K×serum Osm)/(urine Osm× serum K) has been adopted because it takes into account medullary water reabsorption, and therefore the effect of tubular water on K concentration, and it has been verified by in vivo micropuncture and microcatheterization studies in rats. For the calculation of TTKG to be valid, the urinary Na should be above 25 mEq/L, and the urine Osm equal to or greater than the serum Osm, because tubular Na delivery to the principal cell is rate limiting for K secretion, and vasopressin is necessary for that secretion.

When the TTKG is less than 6 in the presence of hyperkalemia, it usually indicates impaired renal tubular K secretion; a value under 5 in adults, under 4.1 in children, and under 4.9 in infants probably denotes inadequate aldosterone activity, whereas a value of 3 or less would reflect aldosterone absence or resistance. In the case of aldosterone deficiency or absence, the TTKG will rise to 6 or greater within 4 h of administering a physiologic dose of 9-alpha-fludrocortisone (50 mcg), but this will not happen in the case of aldosterone resistance. In some of the latter cases pharmacologic doses of 9-alpha-fludrocortisone (200 mcg) may cause a delayed rise (after 24 h) in the TTKG. In normal individuals, on the other hand, potassium loading (50 mEq) caused, in one study, the TTKG to rise over 10. Contrariwise, in hypokalemia, a TTKG greater than 2 usually indicates renal K wasting.

The TTKG has also been used to study medication-induced hyperkalemia, the most common drugs being angiotensin-converting enzyme inhibitors, spironolactone, and potassium supplements, often given together. One study compared ten patients with medication-induced hyperkalemia to normal controls and to normokalemic patients with chronic kidney disease (mean serum creatinine 1.95 mg/dl). The TTKG was 2.58+/−0.36, 6.68+/−0.55, and 5.51+/−0.87 respectively. In cyclosporine-induced hyperkalemia in patients with renal transplants the TTKG in 12 patients with mild hyperkalemia (mean 5.1 mEq/L), and mild renal insufficiency (mean serum creatinine 1.6 mg/dl) was 4.3+/−0.4, and did not increase much after pharmacologic doses of mineralocorticoid (5.6+/−0.6). Two such patients in another study had a low TTKG under 4, but had intact adrenal function as assessed by ACTH administration. Hence several mechanisms may be involved in the cyclosporin-induced hyperkalemia of renal transplant patients.

The TTKG has also been used to regulate spironolactone dosing in patients with cirrhotic ascites. When spironolactone doses were increased in 23 such patients to achieve complete blockage of aldosterone activity, i.e. TTKG under 3, 20 patients exhibited good diuresis, and only one had transients hyperkalemia of 6 mEq/L. Lastly, patients with diabetic ketoacidosis or hyperglycemic hyperosmolar syndrome may exhibit hyperkalemia despite high aldosterone levels from volume contraction and high distal tubular Na delivery from osmotic diuresis. The TTKG may initially be under 6, but it increases, and aldosterone levels decrease within 24–48 h after fluid and insulin administration. It is hypothesized that the initial tubular resistance to aldosterone is a protective mechanism, as total body K content is usually depleted (J Am Soc Nephrol 2008; 19: 424).

Comment Caution is advisable when giving spironolactone to patients with cirrhotic ascites, as many such patients are emaciated, and therefore exhibit seemingly “normal” serum creatinine, while actually having significant reductions of glomerular filtration rate (GFR).

Do Normal Glomeruli Actually Filter Nephrotic Quantities of Albumin?

W.D. Comper argues that the concept of restricted albumin filtration by charge selectivity at the glomerular capillary is not valid, that albumin is filtered like other proteins of similar hydrodynamic radius (36 Amstrongs) according to size selectivity alone, that its glomerular sieving coefficient (GSC; i.e., filtrate-to-plasma concentration ratio) is not 0.0006, but 0.04 like proteins of similar size, and that it is filtered in large quantities, then picked up very quickly in a

matter of a few seconds by the proximal tubular cells which process and return it intact to the plasma pool, and that nephrotic proteinuria results only when this tubular retrieval mechanism fails for one reason or another.

He argues that the evidence against charge selectivity is "compelling", that the anionic dextran sulfate experiments showing low GSC were not valid because its clearance was mediated by renal cell uptake and desulfation, that excess dextran sulfate (when desulfation was saturated) had the same clearance as neutral dextran, and that highly anionic, random-coil polysaccharides not degraded by renal cells had similar GSC to their uncharged counterparts. He also argues that Haraldsson et al's experiments showing low albumin GSC in the cold-perfused kidney were confounded by low-albumin perfusate and cold-induced reduction of GFR, causing a very low albumin flux across the glomerular capillary. He states furthermore that micropuncture experiments showing a low albumin GSC are confounded by a very rapid tubular retrieval pathway for albumin.

Comper asserts that his 2-photon technique establishes that the GSC for albumin is 0.04, which accords with glomerular basement membrane size selectivity. He points out that albumin pick up by the proximal tubular cells occurs within a few seconds, and that refined transmission electron microscopy demonstrates large albumin-laden vesicles along the apical and basolateral membranes of the proximal tubular cells. Toxins that inhibit proximal tubular cell transport increase albumin clearance. The absorbed albumin is returned to the plasma pool, and when this retrieval mechanism is saturated in states of overload proteinuria, another "degradation" pathway comes into play, where the tubular cells break albumin down into smaller peptides secreted into the urine.

In Rebuttal Haraldsson and Deen Present the Following Arguments The tubular protein retrieval hypothesis of Comper is not consistent with most studies of podocyte biology and glomerular function. There is indeed cellular uptake and binding of anionic dextran sulfate by glomerular cells, but the process is quickly saturable, and would therefore not influence steady state clearance data. Even the data from Comper's own laboratory show the fractional clearance of neutral horseradish peroxidase to be 2.4 times that of the anionic variety, and adding lysine or ammonium chloride to the perfusate increased the fractional clearance of the neutral form by 70% and of the anionic form by 170%, yielding a residual charge selectivity ratio of 1.6. That tubular cell transport inhibitors increase the clearance of both neutral and anionic proteins suggests that they are toxic to the glomerular filtration barrier as well, as noted by Ohlson et al. The use of lysine or ammonium chloride would therefore underestimate the glomerular charge selectivity. As for Comper's argument regarding cold-perfused kidneys, Osicka et al. noted the fractional clearance of native albumin in kidneys perfused at 37 degrees centigrade to be 0.0075 versus 0.033 for neutral albumin.

The most recent albumin GSC from Comper's laboratory is 0.034. At a GFR of 180 L/24 h, and a serum albumin of 40 g/L, the daily albumin filtration would be 240 g, an amount that defies tubular absorption according to engineering concepts applied for many years now to the design of tubular reactors. Furthermore, a plethora of experimental data do not support the assertion that albumin is absorbed intact, and 40 years ago, Maunsbach could not demonstrate any tubular reabsorption of intact autologous rat albumin. In fact, albumin is picked up by the megalin–cubilin complex controlled by a specific chloride channel, CIC-5, and there is an obligatory coupling between uptake and degradation in the lysosomes, rendering invalid Russo et al's suggestion that the megalin–cubilin complex undertakes albumin "retrieval".

There is no strong support for the notion that there is marked protein degradation in the urine, which causes urinary protein to be underestimated. Current proteomic techniques applied to patients with the Fanconi Syndrome and normal controls have not detected significant protein degradation in the urine. These same methods render doubtful the other assertion that special techniques based on the Biuret reagent and high-performance liquid chromatography are needed to detect urinary protein fragments. These latter methods are beset by serious flaws that compromise their sensitivity and specificity, and nonpeptide fragments have been mistaken for peptides.

Radiolabeled albumin data have been used to support the hypothesis of albumin retrieval. However, these methods are confounded by even very small amounts of free tracer in the urine. If 99.9% of the radioactivity is bound to albumin, the unbound 0.1% of tracer filters freely, and the albumin GSC is 0.001, there would still be ten times more free tracer than radiolabeled albumin in the urine, $(99.9\% \times 0.0001)/(0.1\% \times 1) = 10$. Such methods have also been used to produce data purportedly supporting large scale protein degradation in the urine, employing 3H counting of urine specimens fractionated by gel filtration columns that cannot discriminate between free tracer and small peptide fragments. That and the lack of evidence of urinary protein degradation when using proteomics analysis suggest strongly that the urine radioactivity data have been misinterpreted.

So how much albumin is filtered normally after all? The Fanconi Syndrome study already mentioned provides the best estimate so far of the GSC for albumin, 0.00008. At a GFR of 180 L/24 h, and a serum albumin of 40 g/L, this translates into 0.6 g protein filtered daily ($180 \times 40 \times$

0.00008). Even if the inhibition of tubular protein reabsorption in the Fanconi Syndrome is only half complete, the daily protein filtration would only be 1 g/day. Hence tubular defects may contribute to proteinuria, but glomerular barrier defects are necessary for the development of the nephrotic syndrome (over 3.5 g protein/day).

The authors reiterate that there is a sea of evidence to support the existence of a size and charge-selective glomerular barrier to protein filtration which, if compromised by disease or genetic mutations, would cause severe proteinuria and the nephrotic syndrome. There is also strong evidence for the proximal tubule megalin–cubilin complex taking up albumin, degrading it, and returning it to the plasma as amino acids, not as intact albumin. No molecular mechanism has yet been identified that would allow the tubular reabsorption of hundreds of grams of albumin daily. To ascribe the nephrotic syndrome to tubular defects, and to deny the existence of the glomerular filtration barrier is to make short shrift of a massive amount of physiologic, morphologic, and molecular biology data accumulated by an army of investigators over the last half century (J Am Soc Nephrol 2008; 19: 427 and 430).