ABSTRACT

Renal Fanconi syndrome (RFS) refers to the generalized dysfunction of the proximal tubule (PT) (Kleta R. Fanconi or not Fanconi? Lowe syndrome revisited. Clin J Am Soc Nephrol 2008; 3: 1244–1245). In its isolated form, RFS only affects the PT, but not the other nephron segments. The study of isolated RFS can thus provide specific insights into the function of the PT. In a recent paper, Klootwijk et al. investigated one such form of isolated RFS and revealed the underlying molecular basis (Klootwijk ED, Reichold M, Helip-Wooley A et al. Mistargeting of peroxisomal EHHADH and inherited renal Fanconi’s syndrome. N Engl J Med 2014; 370: 129–138). The affected family had been described previously, demonstrating the typical features of RFS, such as low-molecular weight proteinuria, aminoaciduria, glycosuria and phosphaturia with consequent rickets; yet, importantly, patients had no evidence of impaired glomerular filtration (Tolaymat A, Sakarcan A, Neiberger R. Idiopathic Fanconi syndrome in a family. Part I. Clinical aspects. J Am Soc Nephrol 1992; 2: 1310–1317). Inheritance was consistent with an autosomal dominant mode. Klootwijk et al. discovered a surprising explanation: a heterozygous missense mutation causing partial mistargeting of the peroxisomal enzyme EHHADH to the mitochondria. Notably, disease causing was not the absence of the enzyme in the peroxisome, but its interference with mitochondrial function. The discovery of this novel disease mechanism not only confirmed the importance of mitochondrial function for PT transport, but also demonstrated the critical dependence of PT on fatty acid metabolism for energy generation.

Keywords: acute kidney injury, EHHADH, proximal tubule, renal Fanconi syndrome

PROXIMAL TUBULE: FUNCTION AND PHYSIOLOGY

In an average adult, the kidneys (glomeruli) generate ~150 L of filtrate per day, of which ~99% is reabsorbed by the tubules, yielding a urine volume of 1–2 L (Figure 1) [1]. The vast majority of the glomerular filtrate is reabsorbed by the proximal tubule (PT), the ‘workhorse’ of tubular segments. Insight into the contributions of the individual nephron segments has been mainly provided by micropuncture experiments, where micropipettes are inserted into different segments of the nephron to measure flow and obtain samples for analysis. This technique has an important limitation, however, in that only those segments that are close to the surface of the kidney are accessible for puncture. Thus, the last accessible site in PT is the end of the proximal convolute and the next accessible site downstream is the beginning of the distal convolute [2]. In between these two sites is the straight descending PT (the pars recta or S3 segment) and the loop of Henle, and it is impossible to accurately determine the individual contributions of these segments. However, the function of all nephron segments including those not accessible by the micropuncture technique has been studied using the technique of isolated tubule perfusion [3].

In any case, the PT not only reabsorbs the vast majority of filtrate in terms of salt and water, but is also the sole site of reabsorption for many valuable solutes, such as amino- and organic acids, filtered proteins, glucose and phosphate. In quantitative terms, without a functional PT, we would lose ~120 L of water per day, plus solutes, including ~18 mol of sodium (the equivalent of roughly 1 kg of cooking salt) and >100 g of sugar. In addition, all the filtered amino acids would be lost, deficiency of which can be associated with specific diseases [4], as well as all filtered phosphate, resulting in hypophosphataemic rickets. In short, the PT is important for our survival! In addition,
it becomes evident from those numbers that Renal Fanconi syndrome (RFS) refers to an impairment of PT function, rather than a complete absence of function.

The enormous reabsorptive capacity is provided by a whole orchestra of specialized apical, basolateral and paracellular permeabilities, and driven by the electrochemical gradient generated by the basolateral $\text{Na}^+\text{-K}^+$-ATPase (see Figure 2). Not surprisingly, the PT has a high energy demand to keep the transport going, which may explain why it is also the nephron segment most susceptible to ischaemic damage [5]. Understanding PT function thus will not only elucidate the pathophysiology of the rare RFS, but may also provide insights into the more common problem of acute kidney injury [6].

**RFS: THE HISTORY**

Rickets and albuminuria secondary to kidney disease was first described in 1881 but was attributed to be a ‘disorder of adolescence’ [7]. It was the Swiss paediatrician Guido Fanconi, who first described the concept that defective renal proximal tubule reabsorption of solutes might contribute to ‘non-nephrotic glycosuric dwarfishing with hypophosphataemic rickets in early childhood’ [8]. Fanconi’s first case presented at the age of 3 months with rickets and recurrent fevers. She had glycosuria and albuminuria and progressed to terminal renal failure by 5 years of age and subsequently died. At autopsy, the renal tubule cells appeared filled with crystals, which were thought to be cystine. In subsequent reports, Debré, de Toni and Fanconi all described series of children with rickets, glycosuria and albuminuria [9–11]. In acknowledgment of this pioneering work, RFS is also referred to as Fanconi-Debré-de Toni syndrome, but in clinical practice most people use the shorter name. The presentation, course and outcome of the children described in these early descriptions varied markedly and this concurs with our clinical experience now. It reflects that RFS is not a uniform entity, but a diagnosis of proximal tubular dysfunction, which can be due to a variety of different causes. RFS can be isolated, such as in the family investigated by Klootwijk et al., or in the context of multiorgan disorders, such as nephropathic cystinosis or mitochondrial cytopathies; it can be congenital or acquired, transient or permanent, associated with progression to end-stage kidney disease (ESKD) or with stable glomerular filtration [12–14]. Moreover, it can differ in the extent and severity of tubular dysfunction. Severe and generalized PT dysfunction is seen in nephropathic cystinosis, whilst some patients with e.g. Dent disease or Lowe syndrome have wasting of low-molecular weight proteins and calcium, yet without clinically significant disturbance of phosphate and bicarbonate transport [15]. Indeed, there

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**FIGURE 1:** Genetic renal tubular diseases caused by failure of sodium reabsorption in individual nephron segments.

**FIGURE 2:** Simplified schematic of renal proximal tubular cell with key transepithelial transport systems (note, $\text{Cl}^-$ and $\text{HCO}_3^-$ transport systems have been omitted).
is some debate at what point proximal tubular dysfunction can be called RFS [16]. There seems to be agreement that several individual PT functions have to be compromised and isolated renal tubular aminoacidurias (e.g. cystinuria or Hartnup disorder) would not be labelled as renal Fanconi syndrome. Dissecting the various pathways involved in these individual diseases of course contributes to our understanding of PT function.

**RFS: THE INHERITANCE**

There are several genetic forms of RFS, the majority of which are associated with multisystem disorders (Table 1). In general, the disease mechanism for these disorders can be categorized as either (i) accumulation of a toxic metabolite (e.g. cystinosis, tyrosinaemia, galactosaemia, Fanconi–Bickel, congenital fructose intolerance and Wilson’s disease), (ii) disruption of energy provision (e.g. mitochondrial cytopathies) or (iii) disruption of endocytosis and intracellular transport (e.g. Lowe syndrome, Dent disease and ARC syndrome).

Recently, RFS was described in some patients with MODY1 (‘maturity-onset diabetes of the young’ type 1) [17]. Interestingly, this renal phenotype was seen only in patients with a specific heterozygous mutation, R76W in HNF4A, whereas those patients with other mutations in HNF4A suffered from pancreatic beta cell dysfunction only. Since HNF4A is a transcription factor, this finding suggests a specific effect of the R76W mutation on transcription of certain genes in the kidney, but which ones exactly are affected is unknown.

Of special interest are, of course, the forms of isolated RFS, as these may provide the most specific insight into PT function. Three genetic forms have been described (Table 1): Fanconi renotubular syndrome (FRTS) types 1–3. The first one was published already in 2001, including linkage to a locus on chromosome 15, yet identification of the underlying gene is yet awaited [18]. Of note, FRTS1 is also associated with progressive chronic kidney disease (CKD). Thus, identification of the underlying problem may also elucidate a mechanism of CKD progression. Other families with a similar phenotype of RFS and progressive CKD inherited in an autosomal dominant fashion had been described previously [19–21], suggesting that this may be a slightly more common form of these otherwise very rare diseases.

FRTS2 has so far been described only in two siblings. An initial clinical description was provided in 1988 [22], revealing a phenotype dominated by phosphate wasting and rickets despite elevated levels of 1,25 OH vitamin D with associated disturbances of some, but not all PT transport pathways, as e.g. renal tubular acidosis was not present, raising again the question of what constitutes RFS [16]. In 2010, an underlying mutation was reported in the form of a homozygous in-frame 21 bp duplication in SLC34A1, encoding the phosphate transporter NaPi-IIa [23]. *In vitro* studies demonstrated significant loss-of-function of the mutant transporter. The finding of SLC34A1 mutations in RFS has been surprising, given that mutations in SLC34A3, encoding another renal phosphate transporter NaPi-IIc, cause hereditary hypophosphataemic rickets with hypercalciuria [24]. No further patients with RFS and recessive mutations in SLC34A1 have so far been reported. The genetic finding, however, is consistent with the clinical observation of phosphate wasting and rickets being the key clinical abnormalities in these two siblings and that the phenotype could be ameliorated by phosphate supplementation. The underlying mechanism may be intracellular phosphate depletion with consequent insufficient ATP generation. Abnormalities in an unrelated proximal transport pathway has been observed in mice with hypophosphataemic rickets [25] and glycosuria is indeed a common observation in patients with hypophosphataemic rickets in our own clinical practice. However, other indicators of PT dysfunction, such as low-molecular weight proteinuria are normal in these patients (unpublished own observations).

FRTS3 is the form of isolated RFS that was investigated by our laboratory and reported by Klootwijk et al. [26]. We...
studied the five generation family described by Tolaymat et al. [27]. Reassessment of the clinical phenotype confirmed the original observations made by Tolaymat et al. Moreover, it demonstrated normal age-appropriate GFR in all affected family members, including the, by then, 74-year-old matriarch. This alone provided valuable insights: the lifelong loss of water and solutes, including about 1 g/day of filtered proteins does not appear to be a critical risk factor for the development of CKD.

With respect to revealing the underlying genetic cause we first performed linkage analysis, which identified a single locus on chromosome 3. Sequencing of all known genes within this locus revealed a heterozygous missense mutation in a gene called EHHADH that segregated with the disease. EHHADH encodes a bifunctional enzyme involved in peroxisomal fatty acid metabolism (also called L-PBE for L-peroxisomai bifunctional enzyme). The identification of EHHADH as the underlying gene was initially surprising: the patients did not have a clinical phenotype consistent with a peroxisomal disorder, which typically comprises severe CNS involvement [28]. Moreover, the function of Ehhaad in mice appears to be at least partially redundant, as knock-out mice do not have an apparent abnormal phenotype, except for some changes in lipid metabolites after fasting [29]. They only develop a relevant clinical phenotype, when the related enzyme D-PBE is also deleted [30, 31].

How does a heterozygote missense mutation in a gene cause disease, when we know that complete loss-of-function can easily be tolerated (at least in mice)? The solution to this conundrum came closer with the realization that the identified mutation (c.7G>A, p.E3K) introduced a de novo N-terminal mitochondrial targeting motif and indeed, subsequent cell-biological studies confirmed a mitochondrial localization of the mutated enzyme. Could the mutant enzyme perhaps impair the function of the erroneously targeted organelle, the mitochondria? To address this question, we performed respirometry in proximal tubular cells expressing either wild type or mutant EHHADH, which indeed demonstrated a significantly decreased capacity for oxidative phosphorylation in the mutant cells compared with wild type, compatible with a defect in ATP production. Therefore, we concluded that mistargeting of EHHADH led to a dominant negative effect on mitochondrial function, presumably by interference with the mitochondrial trifunctional enzyme. This enzyme is a multi-meric protein involved in mitochondrial fatty acid oxidation and has a high degree of homology to EHHADH, so that erroneous assembly of mutant mislocalised EHHADH into this enzyme may occur. Subsequent co-immunoprecipitation experiments confirmed an association of mutant but not wild type EHHADH with two subunits of the trifunctional enzyme, HADHA and HADHB (Table 2).

This dominant negative effect of mistargeting constitutes a previously unrecognized disease mechanism. Mistargeting of a peroxisomal enzyme to mitochondria had been described as a disease mechanism with a specific variant in AGXT, causing primary hyperoxaluria [32]. However, in this recessive disorder, it is the absence of peroxisomal AGXT activity that is disease causing rather than interference with mitochondrial function, as seen in FRTS3.

### Table 2. Key points

| FRTS3 is caused by a mutation in EHHADH | Discovery of a novel disease mechanism: a dominant negative effect from intracellular mistargeting of the mutated enzyme |
| LIFELONG dysfunction of PT including the loss of ~1 g/day of filtered proteins alone does not cause chronic kidney disease |
| The proximal tubule is dependent on fatty acid metabolism for energy generation |

A key question, however, remained: why did the affected patients develop an isolated renal phenotype, when the enzyme is also expressed in other tissues? Should not a generalized mitochondrial phenotype be present?

### Energy Politics of the Proximal Tubule

The explanation for this conundrum may have several components. One relates to the magnitude of the observed impairment in mitochondrial function: the capacity for oxidative phosphorylation was reduced by ~25%; while this was significant, it may be disease-causing (i.e. relevant) only in those tissues with a high metabolic demand, such as the PT. Yet a probably much more important component is a unique feature of PT fuel consumption: the PT does not utilize glucose, but is dependent on fatty acid metabolism [33]! This may reflect an evolutionary development to maximize glucose provision to the brain, in that all filtered glucose is returned into the bloodstream. However, it makes the PT uniquely susceptible to disturbances in fatty acid metabolism, consistent with the phenotype of FRTS3.

### Future Research Questions

The discovery of new disease genes and mechanisms often generate more questions than answers. Whilst the clinical and genetic data are clear, the precise nature of the mechanism requires further investigations:

Is there biochemical evidence for disturbed fatty acid metabolism? Obtaining these data from the patients would likely be difficult, as plasma levels reflect the overall metabolism, rather than the specific metabolites generated in PT. However, the question could be addressed in the cell culture system developed in our laboratory. Alternatively, cell lines could be established from proximal tubular cells shed in the urine, as described in other disorders [34]. PT cells as model to study these questions need to be differentiated as only then metabolism and transport processes reflect the situation of the PT in vivo. This is not the case for many PT cell lines, especially not if they are not differentiated.

Given the insight provided by the investigation of FRTS3, a key question is, of course, the nature of the disease mechanism in another form of RFS, FRTS1. What is the underlying gene and how does it cause RFS and CKD?

Last, but not least, what lessons from the study of rare disease can be translated to benefit the treatment of common disease? FRTS3 has clearly highlighted the importance of fatty acid metabolism for PT function. Could this be used...
therapeutically, e.g. in the prevention or treatment of acute kidney injury (AKI)? There is some evidence that it might: increased levels of certain free fatty acids have been associated with increased graft survival in renal transplant patients [35]. The fatty acid binding protein L-FABP, functions of which include the promotion of fatty acid metabolism, has been identified not only as a biomarker, but also as a protective factor in AKI (reviewed in [36]).

Thus, the journey of discovery is ongoing!

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CONFLICT OF INTEREST STATEMENT

None to declare. The review presented in this paper has not been published previously in whole or part.

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