MUC1 makes me miserable

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Monogenic diseases make an important contribution to the overall burden of kidney disease – accounting for well in excess of 10% of people needing renal replacement therapy. In this setting an accurate diagnosis is advantageous because it can provide prognostic information for the patient (and doctor), inform treatment decisions (sometimes including the use of specific therapies) and disclose risk of recurrence following transplantation. In addition, a genetic diagnosis can reveal the mode of transmission of the disorder, informing risk to relatives and (future) off-spring and allowing screening of relatives, facilitating early therapeutic intervention and informing decisions around live related transplantation.

Some genetic kidney disorders can be diagnosed clinically either by extra-renal or syndromic characteristics or else distinctive biochemical, imaging, or histological features that, especially in the presence of a family history, point towards an underlying diagnosis that can be confirmed by molecular analysis of the right gene or genes. However, autosomal dominant tubulointerstitial kidney disease (ADTKD) due to a *MUC1* mutation is remarkable because it usually presents without any distinctive clinical or imaging abnormalities and affected individuals, prior to the onset of renal impairment, can have no clinical, urinary or biochemical abnormalities at all. Furthermore, kidney biopsy, usually performed when there is biochemical evidence of renal impairment, is miserably non-specific, showing interstitial fibrosis and tubular atrophy, typically with evidence of vasculopathy but minimal inflammation. In this context a reliable and specific diagnostic test is especially important, both to establish the diagnosis and to be able to test for early disease in at-risk family members.

In fact, *MUC1* is a frequent cause of ADTKD but, despite the locus being identified from linkage studies as long ago as 1998[1] (shortly before *UMOD*[2]) the mystery around MUC1 kidney disease was not resolved until 2013[3]; 15 years of headaches for the many patients, doctors, and researchers who have an interest in understanding the cause of this particular kidney problem.

The *MUC1* gene encodes Mucin 1, a protein that is found on the apical surface of epithelial cells in many parts of the body. During biosynthesis it undergoes autocatalytic cleavage and the mature protein consists of an N-terminal mucin domain that contains a heavily glycosylated, 20-amino acid Variable Number Tandem Repeat (VNTR) which binds non-covalently to the smaller C-terminal transmembrane subunit that has a cytoplasmic tail which can undergo phosphorylation and mediates endocytosis, trafficking to the nucleus and recycling. Numerous biological activities have been attributed to Mucin 1, including promoting cell growth and survival and also defending against bacterial invasion[4].

The heavily glycosylated VNTR domain is encoded by a degenerate 60 base pair tandem repeat (with most alleles comprising between 20 and 125 copies) that is very rich in guanine and cytosine, making amplification and sequencing, even by advanced techniques (eg massively parallel or next generation sequencing), inefficient. This is the main reason that it was missed for 15 years: nobody could

sequence this region and if you cannot see something, you will not notice it. The first pathogenic *MUC1* mutations to be identified in patients with ADTKD were cytosine insertions occurring immediately after a 7-cytosine homopolymer within the VNTR[3]) but detecting this mutation (or other mutations within the VNTR that have subsequently been associated with ADTKD[5]) is technically demanding and so far not widely available.

The known *MUC1* mutations associated with ADTKD are so far restricted to this particular VNTR domain. All result in a +1 shift in the reading frame, which is translated to produce a new peptide, termed MUC1-fs, comprising, after a normal N-terminal peptide, the product of translation of the frame-shifted VNTR 3' of the mutation followed by a short tail because the first stop codon in this reading frame occurs beyond the VNTR. This results in MUC1-fs proteins that lack the C-terminal transmembrane domain of Mucin 1 and are of varying size depending, in each family, on how many repeats are on the allele bearing the mutation and in which repeat the mutation is situated. It is notable that kidney disease has not been linked to *MUC1* deletions or nonsense mutations in humans (despite the fact that publicly available variant databases indicate that such variants are present in approximately 1:5,000 of the population) and that kidney failure is not observed in *Muc1*-null mice[6]. Therefore, the presence of MUC1-fs protein appears to be the *sine qua non* of ADTKD-MUC1, although the mechanism by which this results in disease has not been elucidated (yet).

In this issue of the Journal, two papers demonstrate the utility of antibodies raised against the MUC1-fs protein to study this disease.

Knaup and colleagues use antibodies that specifically recognise MUC1-fs and the C-terminal (wild type) MUC1 to confirm expression of the wild-type MUC1 protein in healthy and ADTKD-MUC1 kidneys[7]. They show that mutant protein is found in tubular cells from the thick ascending limb to the collecting duct in a similar anatomical distribution to that of the wild type protein. Of note, cells in a distinct part of the thick ascending limb also express uromodulin, which is mutated in a distinct form of ADTKD, termed ADTKD-UMOD. In common with mutant uromodulin, MUC1-fs lacks the apical localisation of the wild type protein and it is possible that the overlap in anatomical and cellular localization of both mutant proteins reflects overlap in pathogenic mechanisms in ADTKD-MUC1 and ADTKD-UMOD. Knaup *et al.* go on to show that immunohistochemistry can be used to screen for and diagnose ADTKD-MUC1 in stored kidney specimens and suggest that this assay could be readily deployed in clinical laboratories. Using this approach, they were able to identify previously undiagnosed cases of ADTKD-MUC1 from a biopsy repository and they infer that the disease is not as rare as is generally recognised.

In a separate study, Zivna and colleagues established an immunohistochemical assay using cells isolated from urine[8]. They used this assay to identify MUC1-fs in patients with proven ADTKD-MUC1 and some who had tested negative for the MUC1 C-insertion mutation. This led to the identification of 5 novel VNTR mutations in 6 families with ADTKD, out of a total of 475 that were included in the study. Although not yet validated as a clinical test, this urinary cell assay offers the possibility that a sensitive and non-invasive test for the disease could become available for individuals with suspected ADTKD-MUC1, which would be especially valuable in the investigation of younger individuals in whom an invasive kidney biopsy could be avoided.

Both studies convincingly demonstrate increased ascertainment of ADTKD-MUC1 using MUC1-fs protein detection, implying that this methodology offers a way to broaden availability of testing for the disease in the clinic by identifying families most likely to benefit from more laborious molecular testing.

While the mechanisms leading to kidney damage in inherited tubulointerstitial kidney diseases remain poorly understood, these studies further implicate the MUC1-fs protein itself in the causal pathway of ADTKD-MUC1 and it remains to be determined whether there is commonality in pathogenesis with the cellular abnormalities (for example disruption of mitochondrial function or endoplasmic reticulum stress) that have been identified in other forms of autosomal dominant and mitochondrially inherited tubulointerstitial kidney diseases[9-12].

Some mysteries around MUC1 remain, but the work presented in these two papers is most welcome to alleviate some of our ignorance around "unexplained" kidney failure.

Disclosure

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