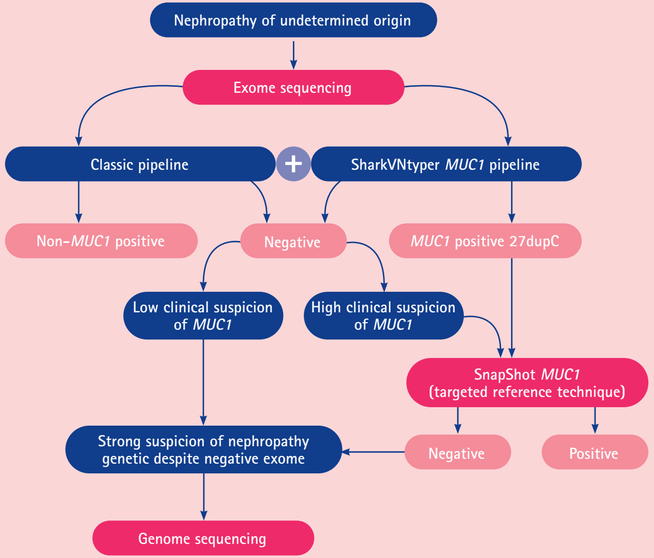
Proposed decision tree for detecting a pathogenic variant in *MUC1*



References

1 Devuyst O, Olinger E, Weber S, *et al.* Autosomal dominant tubulointerstitial kidney disease. Nat Rev Dis Primers. 2019;5(1):60. Published 2019 Sep 5. doi:10.1038/s41572-019-0109-9

2 Olinger E, Hofmann P, Kidd K, *et al.* Clinical and genetic spectra of autosomal dominant tubulointerstitial kidney disease due to mutations in UMOD and *MUC1*. Kidney Int. 2020;98(3):717-731.

3 Kirby A, Gnirke A, Jaffe DB, *et al.* Mutations causing medullary cystic kidney disease type 1 lie in a large VNTR in *MUC1* missed by massively parallel sequencing. Nat Genet. 2013;45(3):299-303. doi:10.1038/ng.2543

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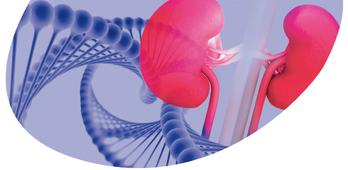
Genetic investigation of tubulointerstitial nephropathy:
a technical challenge for *MUC1* !

GENETIC INVESTIGATION OF TUBULOINTERSTITIAL NEPHROPATHY: A TECHNICAL CHALLENGE FOR *MUC1*



Autosomal dominant tubulointerstitial nephropathy (ADTKD) is one of the most common monogenic kidney diseases, second only to autosomal dominant polycystic kidney disease, accounting for around 5% of monogenic disorders causing chronic kidney disease. ADTKD is caused by mutations in various genes, including *MUC1*.

It is estimated that ADTKD-MUC1 autosomal dominant tubulointerstitial nephropathy affects one to four individuals per million population[1]; however, this is probably an underestimate, as diagnosis is difficult. In fact, the disease is very rarely investigated because it is so technically complex!
A few laboratories in Europe, including Eurofins Biomnis, offer research into 27dupC (the most frequent variation of MUC1) using a targeted approach (Snapshot).
The exome is generally considered unsuitable for studying this gene, but the Eurofins Biomnis laboratory has developed a specific pipeline that can detect 27dupC with high sensitivity.

Autosomal dominant tubulointerstitial nephropathy, due to a heterozygous variation in the *MUC1* gene, is characterised by the onset of impaired renal function and salt loss in adulthood, leading to chronic renal failure and culminating in end-stage renal disease, with a median onset in the fifth decade. There is a high degree of variability, even within families. Renal biopsy shows tubulointerstitial nephropathy, sometimes with renal cyst formation at the corticomedullary junction, although cysts are not pathognomonic of the disease and are not an essential diagnostic criterion.
More variable clinical features may be present, such as anaemia, hypertension, hyperuricaemia and gout. The features are non-specific and there is significant inter- and intra-familial variability, as well as incomplete penetrance, which can hamper clinical diagnosis (summarised by Stavrou et al., 2002, Wolf et al., 2004; reviewed by Devuyst et al., 2019). (Source OMIM).
We provide information on the features of the conventional technique and the exome for the most frequent *MUC1* variation and present here two «*MUC1*» diagnoses highlighted by exome sequencing

Conventional technique

The *MUC1* gene has the unique feature of having a nucleotide sequence of 60 nucleotides (20 amino acids) repeated a variable number of times (20 to 120): the VNTR. The pathogenic variations in the *MUC1* gene reported in the literature are cytosine duplications, as well as other frameshift variations found in the VNTR of this gene. These variations lead to toxic accumulation of the protein, which is misfolded in the cytoplasm and generates a proteinopathy.

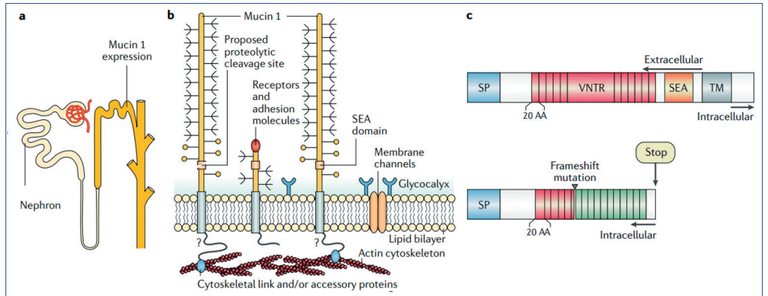


Figure 1: Function of uromodulin, after Devuyset *et al*, 2019, Nat Rev Dis Primers1.

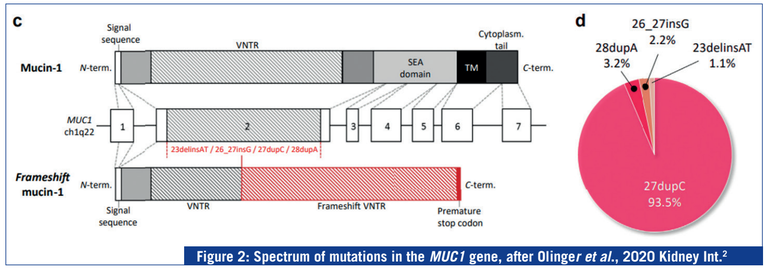


Figure 2: Spectrum of mutations in the *MUC1* gene, after Olinger *et al*., 2020 Kidney Int.[2]

Pathogenic variation can occur in any VNTR, so nomenclature is difficult. For ease of understanding, the variations are named in relation to the nucleotide number of the VNTR. The most common variation, 93.5% of the variations reported in VNTRs, is 27dupC, i.e. duplication of a cytosine at position 27 in any VNTR.

Adapted from Kirby *et al.* 2013. Nat Genet.[3],
27dupC detection is now available from Eurofins Biomnis.

This detection technique may be requested in cases of autosomal dominant tubulointerstitial nephropathy, after a negative exome result, or as 1st line treatment in the case of a very strong clinical indication (family history).

MUC1 and the exome

27dupC exome: SharkVNTyper pipeline

The Eurofins Biomnis laboratory has adapted the VNtyper® panel tool (Saei et al.) to the exome and combined it with Shark® to detect 27dupC in the *MUC1* gene.

After a validation stage involving comparison with the reference technique mentioned above, the pipeline was launched on a routine basis. Since October 2023, SharkVNtyper has been used on the exome data from every sample from a patient with kidney disease. If a variant is detected, it is confirmed by Snapshot PCR.

This exome-based bioinformatics approach does not have 100% sensitivity, so a negative result does not rule out the presence of a 27dupC MUC1, in which case the reference technique must be used.

The routine use of this approach has made it possible to diagnose 27dupC MUC1 cases that were not specifically suspected during the initial clinical evaluation, and for which the targeted snapshot technique would not have been requested. This is a real step forward in reducing diagnostic error!

Variations detectable in the exome via the classic pipeline

Certain *MUC1* variations, close to exons, are detectable via the classic exome pipeline.
Here are two clinical cases to illustrate this situation:

Example of patient file 1

Clinical information

71-year-old woman with chronic renal failure Stage IV, tubulointerstitial nephropathy; family history, including mother affected.

Variation identified:

NM_001204285.2, c.293_296dup, p.(Ser100HisfsTer122)

In this case, the duplication is present upstream of the first VNTR and creates a frameshift in the first VNTR, making this variation pathogenic. This variation was thus detected using NGS techniques.

Example of patient file 2

Clinical information

47-year-old woman, stage IV CKD, tubulointerstitial nephropathy. Family history: mother, two maternal uncles, maternal grandfather, maternal cousins (kidney transplant recipients).

Variation identified:

NM_001204285.2, c.401dup, p.(Ala135SerfsTer86)

Here, the cytosine duplication occurs in the first VNTR, which was still aligned and therefore detectable using the NGS technique.