Whole-genome sequencing for CAKUT in an island population

Esther Zammit,[1,2] Alex E. Felice,[1,2] Valerie Said Conti[3]

[1]Department of Physiology & Biochemistry, Faculty of Medicine & Surgery, University of Malta; [2]The Malta BioBank, Centre for Molecular Medicine and Biobanking, University of Malta; [3]Department of Child and Adolescent Health, Mater Dei Hospital, Malta.

Background

Congenital anomalies of the kidney and urinary tract (CAKUT) are a broad spectrum of structural malformations of varying severity and renal outcome.[1] They occur in 3-6 per 1,000 live births either as an isolated characteristic, in combination or as part of a multi-organ malformation syndrome. Dialysis and kidney transplantation have been life-saving to patients with the most severe forms of CAKUT. However, the disease burden is high.

In recent years, several studies have been undertaken with the aim to uncover the underlying cause of the disease. Familial cases[2] and mouse models[3] of CAKUT suggest that genetics plays an important role in the development of renal malformations. A number of genes have been implicated with various modes of inheritance, but in most patients the disease-causing mutation remains to be discovered.

Here, we report on our progress in elucidating the genetics of CAKUT in cases of Maltese origin using whole-genome sequencing (WGS) in 10 unrelated patients.

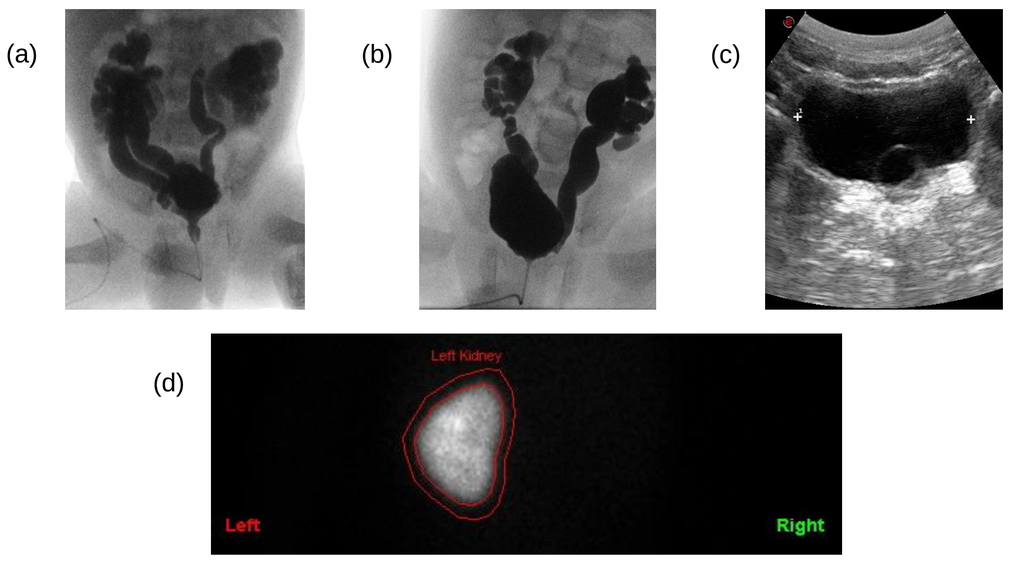

Figure 1 | Renal anomalies encountered in the clinic **(a)** bilateral duplex kidneys and vesicoureteral reflux (VUR) with posterior urethral valves (PUVs) **(b)** bilateral high-grade VUR **(c)** left ureterocele **(d)** solitary left kidney.

Methods

84 paediatric patients with CAKUT and their families were enrolled in the study following written informed consent.

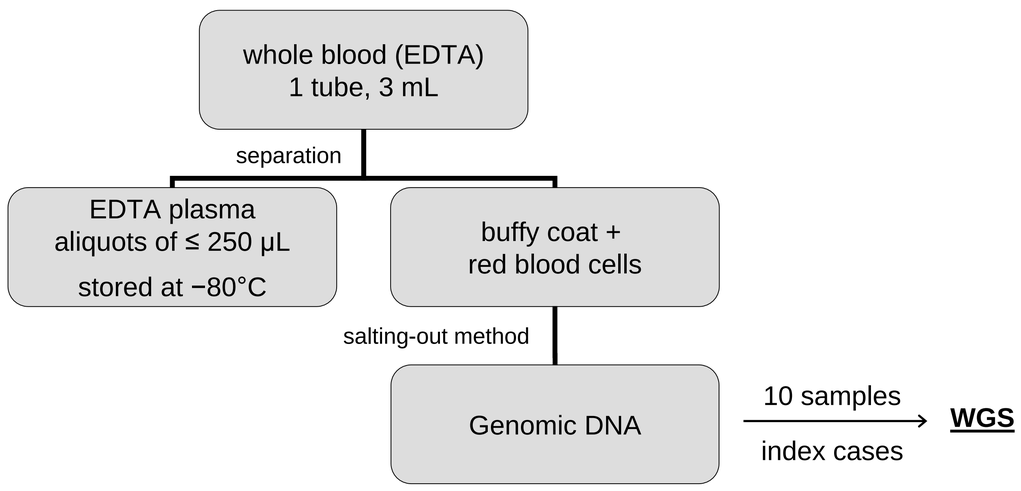

Figure 2 | Workflow for blood sample processing.

WGS was carried out at Complete Genomics using the DNA nanoarray platform.[4] Sequencing coverage was 110x. Reads were aligned with the GRCh37/hg19 human genome reference.

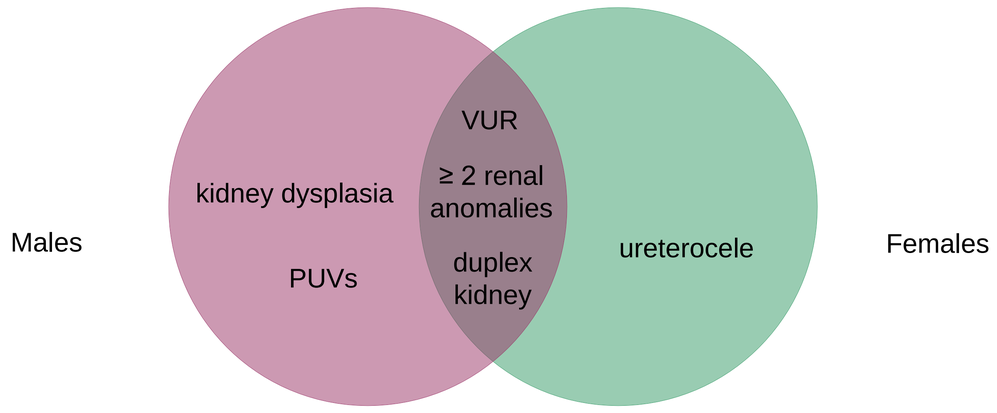

Figure 3 | Characteristics of the 10 affected individuals under study.

The coding regions of 96 genes implicated in non-syndromic CAKUT and known to play a role in kidney development, including HNF1B, PAX2, ROBO2, EYA1, RET and BMP4, are being studied at present. Synonymous variants were excluded. PolyPhen-2 and SIFT are being used to predict the pathogenicity of novel variants identified by WGS.

Efforts are underway to bring the current preanalytical handling of the biological samples for use in kidney disease research to meet the requirements of the recently published CEN/Technical Specifications for pre-examination processes. This is being done in close collaboration with the Quality working group of the biobanking research infrastructure, BBMRI-ERIC.

Results

- Established a renal collection of rare disease at the Malta BioBank.

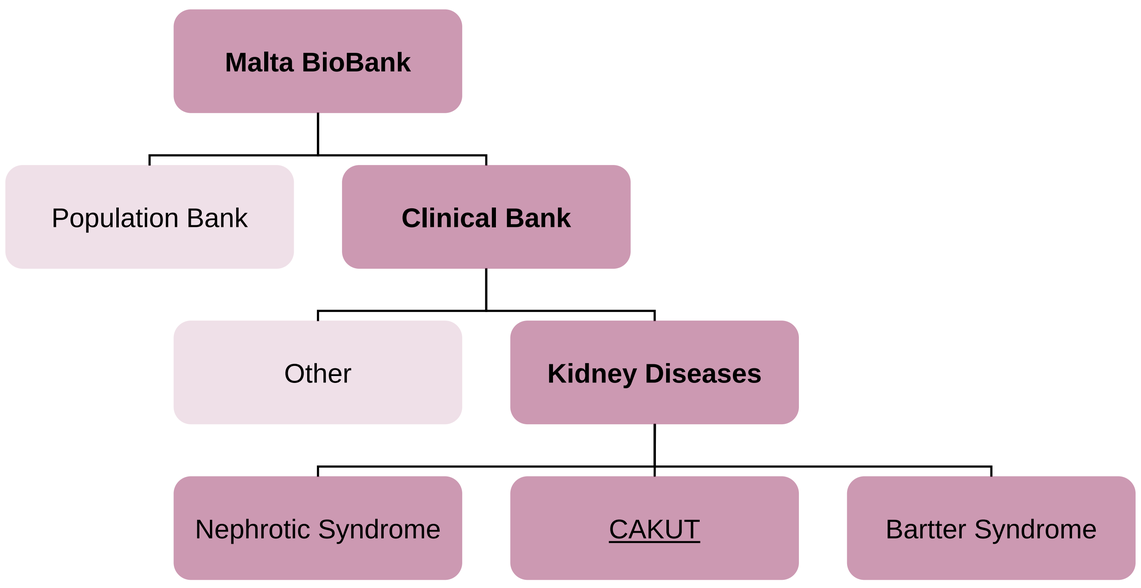

Figure 4 | Flowchart showing the current kidney disease sample collections.

- 10 WGS datasets of CAKUT patients of Maltese origin are being explored.

Conclusion

CAKUT is a rare disease. By definition it affects a small proportion of a population, which makes existing knowledge about the pathophysiology of the disease limited and hence its clinical management more challenging. An international collaboration will yield better results. Data from the Maltese registry will be added to the pool with the aim to better elucidate the genetic background of CAKUT and direct early identification and treatment of patients who are at risk of progressing to kidney failure.

References

1 Sanna-Cherchi, S., Ravani, P., Corbani, V., Parodi, S., Haupt, R., Piaggio, G., … Ghiggeri, G. M. (2009). Renal outcome in patients with congenital anomalies of the kidney and urinary tract. *Kidney Int.*, *76*, 528-533.

2 Bulum, B., Özçakar, Z. B., Üstüner, E., Düşünceli, E., Kavaz, A., Duman, D., … Yalçinkaya, F. (2013). High frequency of kidney and urinary tract anomalies in asymptomatic first-degree relatives of patients with CAKUT. *Pediatr Nephrol*, *28*, 2143-2147.

3 Miyazaki, Y., Oshima, K., Fogo, A., Hogan, B. L., & Ichikawa, I. (2000). Bone morphogenetic protein 4 regulates the budding site and elongation of the mouse ureter. *J. Clin. Invest.*, *105*, 863-873.

4 Drmanac, R., Sparks, A. B., Callow, M. J., Halpern, A. L., Burns, N. L., Kermani, B. G., … Reid, C. A. (2010). Human genome sequencing using unchained base reads on self-assembling DNA nanoarrays. *Science*, *327*, 78-81.

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