

## Editorial

## NOS3 as a potential modifier of ADPKD phenotypic variability: Progress towards an answer

The extreme intra- and inter-familial variability in the rate of progression to end-stage renal failure (ESRF) in autosomal dominant polycystic kidney disease (ADPKD) has been recognized for many decades. However the cause of such variability remains obscure even considering the rapid progress made in understanding the molecular and cellular pathogenesis of the disease. It is likely to involve genetic, environmental and individual factors such as epigenetics in addition to the known and well-described genic and allelic effects seen in *PKD1* and *PKD2*, the genes responsible for ADPKD. Individuals with a *PKD1* mutation reach ESRF on average in their mid-50s. More prolonged renal survival is associated with mis-sense compared with loss-of-functions mutations. Mutations in *PKD2* are associated with even greater renal survival with an average age of renal failure in the 70s.<sup>1</sup> Whilst these genic and allelic effects are strong in ADPKD they do not help our understanding of the disease variability observed within single families with a presumably common causative genetic mutation. The contribution of genetic modifiers to disease variability has been assessed in several studies. In families with *PKD1* mutations, genetic variation may account for 32–42% of the variance in estimated GFR (eGFR) before ESRD and 43–78%<sup>2</sup> of the variance in age at ESRF. So far the genetic modifiers are unknown although variants in *DKK3* have been implicated.<sup>2</sup> Inconsistent results from small studies using candidate genes such as *ACE* have been disappointing. At a time when the promise of effective therapeutics may be able to be tangibly fulfilled within the medium to long term, a greater understanding of the causes of disease variability and how to predict long term outcomes is urgently required.

Endothelial dysfunction has been implicated in the pathogenesis of ADPKD and the development of hypertension even independently of other markers of renal dysfunction. Absolute or relative diminution of nitric oxide (NO) production in ADPKD, its regulation by the protein product of the *PKD1* gene, polycystin-1, have supported the possible role of NO production in contributing to ADPKD phenotypic variability. Whilst investigated and reported upon before, most studies looking at functional genetic variation in the endothelial nitric oxide synthase gene (*NOS3*) have also

been limited to small numbers of participants where other factors that are known to predict disease severity such as underlying gene mutation or renal volume have not been assessed. These studies have therefore been considerably underpowered to detect even a modest effect size. As such, the question of whether genetic influences on NO production exert a disease modifier effect in ADPKD have not been convincingly or resoundingly answered.

In recent issues two research groups have further explored the potential role for *NOS3* to act as an ADPKD modifier gene. Ramanathan *et al.*<sup>3</sup> investigated several SNP and polymorphisms in a south Indian cohort using a case control methodology which did not reveal such an association or modifier effect. Subsequently Xue *et al.*<sup>4</sup> have undertaken a systematic review and meta analysis including the previous study. The results of 11 published studies across Asian, European, North American and Middle Eastern populations, suggested that one allele in a dominant model (A allele of 4b/a polymorphism, *NOS3*) is associated with increased ESRF risk, another in a recessive model conferred the opposite effect (GG allele of Glu298Asp variant, *NOS3*). Of further interest is that the later recessive model allele appears to exert its modifier effect in a somewhat gender dependent fashion.

This finding is of interest for several reasons. First, a meta analysis of all available studies demonstrated statistically significant associations of different *NOS3* variants with either accelerated or delayed renal progression in ADPKD. *NOS3* is a biologically plausible candidate gene and this study appears to answer more convincingly than has previously been possible the question as to whether *NOS3* variants exert a phenotypic modifier effect in ADPKD.

Second, it further emphasizes that a large well-designed prospective case control study is still required to confirm these findings. Such a study is likely to be challenging. There is an absolute requirement for high quality genotype and/or phenotype data to control for known factors that influence disease severity and to provide better clinical end-points. Such end-points may be age at ESRF<sup>5</sup> or alternatively new measures such as height or age adjusted renal volume.

Third, these findings add further impetus to developing an international approach to understanding ADPKD by collecting prospective data on a large cohort of ADPKD patients and their family members. This approach has supported definitive studies in a wide range of other diseases, both monogenic and complex. Several large studies to identify modifier genes in ADPKD are already underway utilizing a

Competing interests: AM is a recipient of an RBWH Foundation Research Postgraduate Scholarship, RACP Foundation Jacquot Research Entry Scholarship, past recipient of a Churchill Fellowship and ANZSN Travelling Fellowship, and has received a travel grant from Amgen.

genome wide approach to avoid the potential pitfalls of smaller candidate gene studies. Ultimately the hope is that such studies will identify additional therapeutic targets to challenge the current paradigm of inexorable renal function decline, excess morbidity and mortality. Understanding which patients are more likely to progress to ESRF will be important in delivering new treatments judiciously and with maximal benefit. It is through such efforts to both understand and treat ADPKD that the prognosis of the next generation can be improved compared with their forbears.

In conclusion, NOS3 variants may exert a modifier effect in ADPKD and thus account for some of the phenotypic variability observed in this condition. This potential effect requires confirmation in large prospective cohort studies along with the identification of other genetic and environmental factors that can be modified to improve renal survival in ADPKD, something that has not changed in recent decades.

## AUTHORS' CONTRIBUTIONS

AM and DS have made substantial contributions to conception, been involved in drafting of the manuscript and have given final approval of the version to be published.

ANDREW MALLETT<sup>1,2</sup> and RICHARD SANDFORD<sup>3,4</sup>

<sup>1</sup>Department of Renal Medicine, Royal Brisbane and Women's Hospital, <sup>2</sup>CKD.QLD and Centre for Kidney Disease Research, School of Medicine, University of Queensland, Brisbane, Queensland, Australia,

<sup>3</sup>Academic Department of Medical Genetics, University of Cambridge School of Clinical Medicine, and <sup>4</sup>Department of Medical Genetics, Addenbrookes' Hospital, Cambridge, UK

## REFERENCES

1. Corneec-Le Gall E, Audrezet MP, Chen JM *et al.* Type of PKD1 mutation influences renal outcome in ADPKD. *J. Am. Soc. Nephrol.* 2013; **24**: 1006–13.
2. Liu M, Shi S, Senthilnathan S *et al.* Genetic variation of DKK3 may modify renal disease severity in ADPKD. *J. Am. Soc. Nephrol.* 2010; **21**: 1510–20.
3. Ramanathan G, Periyasamy S, Lakkakula BV. NOS3 tagSNPs does not modify the association between CKD progression in ADPKD. *Nephrology (Carlton)* 2014; **19**: 537–41.
4. Xue C, Zhou CC, Sun LJ *et al.* Effects of endothelial nitric oxide synthase gene on end stage renal disease progression in autosomal dominant polycystic kidney disease. *Nephrology (Carlton)* 2014; **19**: 630–7.
5. Corneec-Le Gall E, Hourmant M, Morin M *et al.* The PRO-PKD score, a new algorithm to predict renal outcome in autosomal dominant polycystic kidney disease (ADPKD). *Nephrol. Dial. Transplant.* 2014; **29** (Suppl 3): iii5–6.