Variable renal disease progression in autosomal dominant polycystic kidney disease: A role for nitric oxide?

Olivier Devuyst

Department of Nephrology, Catholic University of Louvain Medical School, Brussels - Belgium

ABSTRACT: Autosomal dominant polycystic kidney disease (ADPKD) is characterized by a variable renal disease progression, which is primarily due to genetic heterogeneity (PKD1 vs. PKD2). Evidence obtained in murine models and studies of variability in siblings and twins suggest that modifier genes influence renal disease progression in ADPKD. These modifier loci could affect cystogenesis and/or cyst progression, but also more general factors, i.e. endothelial dysfunction. The demonstration of endothelial dysfunction in Pkd1$^{+/-}$ mice and ADPKD patients, and the effect of the frequent Glu298Asp polymorphism of ENOS on renal disease progression in ADPKD suggest that an impaired release of nitric oxide (NO) by endothelial cells can accelerate renal function degradation. These results also suggest that polycystins can participate in the regulation of endothelial NO synthase (eNOS) and that addressing endothelial dysfunction in ADPKD can offer a new perspective to slow renal disease progression.

Key words: *Nitric oxide, Endothelium, Modifying genes, Polycystic kidney disease*

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is characterized by the development of multiple cysts in both kidneys, causing progressive renal failure. By the age of 60 years, approximately 50% of ADPKD patients have end-stage renal disease (ESRD). In Europe and North-America, ADPKD is responsible for 5-10% of patients requiring renal replacement therapy (1). ADPKD is also characterized by extra-renal manifestations (e.g. intracranial aneurysms or liver cysts) and hypertension. Mutations in two genes, PKD1 and PKD2, are responsible for approximately 85% and 15% of ADPKD cases, respectively. The proteins encoded by PKD1 (polycystin-1) and PKD2 (polycystin-2) interact in the plasma membrane to participate in signaling pathways that regulate renal tubular cell growth and maturation (2). In this brief review, we discuss the role and potential implications of disease-modifying genes in ADPKD.

VARIABLE RENAL DISEASE PROGRESSION IN ADPKD

ADPKD is characterized by a substantial variability in renal disease progression, primarily assessed by the age at ESRD (3). Interfamilial variability is best explained by genetic heterogeneity. Indeed, PKD2 is clinically milder than PKD1 disease, as witnessed by a later age at ESRD and a lower prevalence of hypertension (4). The nature and/or location of mutations within PKD1 can also be associated with differences in renal disease progression (5). Intrafamilial variability could result from a combination of environmental and genetic factors (3). Experimental evidence suggests that cyst formation in ADPKD epithelia is triggered by a "second hit", i.e. the occurrence of a somatic mutation in the allele unaffected by the germ line mutation (6). Micro-environmental or genetic factors determining the rate of second hit could modulate cystogenesis and, thereby, affect renal disease progression. Alternatively, modifier genes can influence polycystins-mediated signal transduction pathways, cyst fluid accumulation or other mechanisms involved in the progression of ADPKD (3).

MODIFIER GENES IN ADPKD: ARGUMENTS AND CANDIDATES

It is increasingly recognized that the phenotype of Mendelian disorders is influenced by modifier genes,

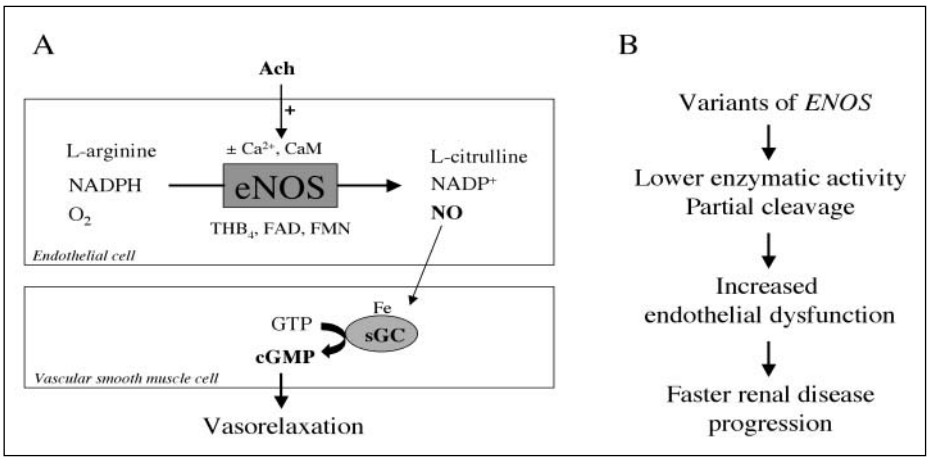

Fig. 1 - Endothelial nitric oxide synthase and its potential role in ADPKD.
(A) NO is synthesized from L-arginine by the endothelial NO synthase (eNOS), together with a stoechiometric production of L-citrulline. The reaction requires molecular oxygen, nicotinamide adenine dinucleotide phosphate (NADPH) and co-factors including tetrahydrobiopterin (THB_4), flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN) and calmodulin (CaM). Intracellular Ca^{2+} levels control the activity of eNOS by maintaining the Ca^{2+}/CaM complex essential to activate the constitutive enzyme. The formation of a Ca^{2+}/CaM complex explains the increase in endothelial NO production elicited by acetylcholine (Ach). NO diffuses rapidly through cell membranes. At nanomolar concentrations, NO reacts with the ferrous heme (Fe) in soluble guanylate cyclase (sGC) to produce the second messenger guanosine 3',5'-cyclic monophosphate (cGMP) from guanosine triphosphate (GTP). This increase in cGMP in vascular smooth muscle cells leads to their relaxation.
(B) Variants of ENOS are associated with lower enzymatic activity and/or partial cleavage of eNOS. These modifications could increase the endothelial dysfunction that is associated with ADPKD, via an increase in systemic blood pressure and/or an alteration of intra-renal microcirculation. The deleterious effect could be particularly important in males because they are characterized by lower basal NO levels.

i.e. inherited genetic variations distinct from the disease locus (7). Such modifier loci have been found in murine models of polycystic kidney disease (8, 9). Recent studies in knockout mouse models of ADPKD suggest that the cystic phenotype resulting from a somatic mutation in a given Pkd gene is modified by the expression level of the other ("trans") Pkd gene (10). The excess variability in the age at ESRD observed among ADPKD siblings in comparison with genetically identical monozygous ADPKD twins ((11), and unpublished observations) is another argument for modifier genes influencing renal disease progression in ADPKD.

Due to the activation of the renin-angiotensin system and early hypertension, the gene encoding the angiotensin-converting enzyme (ACE), appeared as an attractive candidate modifier in ADPKD. A 287-bp insertion (I) /deletion (D) fragment located within intron 16 of the gene is known to influence ACE plasma levels, the highest levels being associated with the DD genotype (12). Therefore, it was suggested that DD patients with ADPKD might be characterized by increased angiotensin II and accelerated renal function decline. Although the latter hypothesis was supported by the study of Baboolal et al (13), the deleterious effect of the DD genotype in ADPKD appeared to be only marginal or unconfirmed in subsequent studies (3, 5).

Some evidence suggests that the cAMP-regulated CFTR Cl^- channel mediates fluid secretion, and possibly cyst enlargement in ADPKD (14). Also of interest, a milder renal phenotype has been reported in ADPKD families harboring two rare mutations in the CF gene that encodes CFTR (15). However, a detailed study of the most frequent DF508 mutation and intron 8 polymorphic TN locus of CF did not show an influence on renal progression of ADPKD (16). These data suggest that the potential modifier role of the CF gene in ADPKD could be related to the nature of the mutation and/or the residual expression of the mutated CFTR (16).

NITRIC OXIDE AND ENDOTHELIAL DYSFUNCTION IN ADPKD

Nitric oxide (NO) is the molecular counterpart of the endothelium-derived relaxing factor (17). NO is synthesized from L-arginine by three NO synthase (NOS) isoforms, the neuronal NOS (nNOS), the inducible (iNOS), and the endothelial NOS (eNOS). The NOS isoforms share approximately 50% homology and are encoded by different genes (18). In particular, eNOS is encoded by ENOS (NOS3), a 26 exons-gene located on 7q36 (19). The enzymatic activity of the constitutively expressed eNOS is controlled by intracellular Ca^{2+} levels and other co-factors. After being released in endothelial cells, NO diffuses rapidly through cell membranes

and relaxes neighboring vascular smooth muscle cells through the production of guanosine 3',5'-cyclic monophosphate (cGMP) (Fig. 1A). In addition, NO inhibits platelet activation, regulates angiogenesis and controls microvascular permeability (17, 18).

Both PKD1 and PKD2 are expressed in the endothelium and the vascular smooth muscle lining human blood vessels (20, 21). Wild-type mice express Pkd1 in the blood vessels (22, 23), whereas knockout Pkd1 mice show edema, localized hemorrhages (22-24) and increased microvascular permeability (22). Furthermore, $Pkd1^{+/-}$ mice present an impaired acetylcholine (Ach)-induced endothelium-dependent relaxation of the aorta, and lower urinary excretion of NO metabolites (24). A similar impairment of the endothelium-dependent vasorelaxation, contrasting with an intact response to exogenous NO, has also been documented in normotensive ADPKD patients (25). These data suggest that endothelial dysfunction, secondary to an impaired release of NO, exists in ADPKD. Therefore, ENOS could be a modifier gene in ADPKD due to its influence on several conditions associated with endothelial dysfunction including hypertension, coronary vasospasm, atherosclerosis and progression of diabetic nephropathy (26).

Modifier effect of *ENOS* in ADPKD

The effect of ENOS on renal disease progression in ADPKD has recently been assessed in a series of 173 unrelated patients from Belgium and the north of France (27). The frequent Glu298Asp polymorphism of the exon 7 of ENOS was associated with a significant 5-year lower age at ESRD in the whole group of ADPKD males, and a lower renal survival (Kaplan-Meier analysis) in male patients from PKD1-linked families. In contrast, no deleterious effect of the ENOS polymorphism was found in female ADPKD patients, which could be related to the ability of estrogens to stimulate eNOS expression and the release of NO in endothelial cells (28).

A molecular basis for the effect of the Glu298Asp polymorphism was provided by the demonstration of decreased enzymatic activity and modified expression of eNOS in renal arteries from male ADPKD patients harboring the Asp allele (27). The mechanism by which this conservative amino acid substitution leads to degradation and decreased activity of eNOS remains to be clarified. The Glu298 of eNOS is conserved among species, and located within the oxygenase domain of eNOS (29). An increased degradation of the Asp 298 eNOS can yield a cleaved protein that could contribute to endothelial dysfunction (27). Alternatively, the Glu298Asp polymorphism can influence the complex post-translational regulation of eNOS (30). Therefore, lower enzymatic activity and/or partial cleavage of eNOS could be responsible for increased endothelial dysfunction, and accelerated renal function degradation in ADPKD patients harboring the Asp 298 allele (Fig. 1B).

Conclusions

Evidence indicates that modifier genes play a role in the variable renal disease progression that characterizes ADPKD. The modifier loci could influence cystogenesis and cyst progression, but also more general factors, i.e. the release of NO by endothelial cells. The demonstration of endothelial dysfunction in $Pkd1^{+/-}$ mice (24) and ADPKD patients (25), and the effect of a frequent polymorphism of ENOS on renal disease progression in ADPKD (27) offer both experimental and clinical perspectives. First, Pkd1 and Pkd2 knockout mice could be used to document the endothelial dysfunction in ADPKD and to investigate the putative link between the loss of polycystins function and altered eNOS expression/activity. The increasingly documented role of polycystins in the regulation of intracellular calcium levels (2) suggests that this family of proteins could participate in the complex regulation of eNOS. Secondly, the association of ENOS polymorphisms with renal disease progression in ADPKD should be investigated in populations with different genetic backgrounds. This is particularly true if the Glu298Asp polymorphism is not the causal polymorphism, but only a marker in linkage disequilibrium with the modifier locus. Finally, these results suggest that addressing the endothelial dysfunction in ADPKD by giving NO donors or drugs stimulating eNOS expression can offer a new perspective to slow renal disease progression.

Recently, Walker et al were not able to find an association between the Glu298Asp polymorphism of ENOS and the age at ESRD in a cohort of 80 families with proven PKD1 mutation (1). Conflicting results in association studies may originate from inclusion of diverse ethnic groups or related patients from large families (2). It is thus important to investigate the potential effect of ENOS polymorphisms (or any candidate modifier) on renal disease progression in larger, well-defined and genetically homogeneous ADPKD subpopulations.

Acknowledgements

The author wishes to thank Drs. J-L. Balligand, D. Chauveau, O. Feron, J-P. Grünfeld, A.G. Herman, A. Persu and Y. Pirson for fruitful discussions and collaboration.

The author's work was supported by the Communauté Française de Belgique; the Société de Néphrologie; the Belgian agencies FNRS and FRSM; and the Action de Recherches Concertées 00/05-260.

Address for correspondence:
Olivier Devuyst, M.D., Ph.D
Division of Nephrology, UCL Medical School
10 Avenue Hippocrate
B-1200 Brussels, Belgium
devuyst@nefr.ucl.ac.be

REFERENCES

1. Walker D, Consugar M, Slezak J, Rossetti S, Torres VE, Winearls CG, Harris PC. The ENOS polymorphism is not associated with severity of renal disease in polycystic kidney disease 1. Am J Kidney Dis 2003; 41: 90-4.
2. Devuyst O, Persu A, Stoenoiu MS, Lens X, Chauveau D, Pirson Y. ENOS polymorphism and renal disease progression in autosomal dominant polycystic kidney disease. Am J Kidney Dis 2003; 41: 1124-5.
3. Peters DJ, Breuning MH. Autosomal dominant polycystic kidney disease: modification of disease progression. Lancet 2001; 358: 1439-44.
4. Hateboer N, van Dijk MA, Bogdanova N, Coto E, Saggar-Malik AK, San Millan JL, Torra R, Breuning M, Ravine D. Comparison of phenotypes of polycystic kidney disease types 1 and 2. Lancet 1999; 353: 103-7.
5. Rossetti S, Burton S, Strmecki L, Pond GR, San Millan JL, Zerres K, Barratt TM, Ozen S, Torres VE, Bergstralh EJ, Winearls CG, Harris PC. The Position of the polycystic kidney disease 1 (PKD1) gene mutation correlates with the severity of renal disease. J Am Soc Nephrol 2002; 13: 1230-7.
6. Pei Y. A "two-hit" model of cystogenesis in autosomal dominant polycystic kidney disease? Trends Mol Med 2001; 7: 151-6.
7. Dipple KM, McCabe ER. Modifier genes convert "simple" Mendelian disorders to complex traits. Mol Genet Metab 2000; 71: 43-50.
8. Woo DD, Nguyen DK, Khatibi N, Olsen P. Genetic identification of two major modifier loci of polycystic kidney disease progression in pcy mice. J Clin Invest 1997; 100: 1934-40.
9. Kuida S, Beier DR. Genetic localization of interacting modifiers affecting severity in a murine model of polycystic kidney disease. Genome Res 2000; 10: 49-54.
10. Wu G, Tian X, Nishimura S, Markowitz GS, D'Agati V, Park JH, Yao L, Li L, Geng L, Zhao H, Edelmann W, Somlo S. Trans-heterozygous Pkd1 and Pkd2 mutations modify expression of polycystic kidney disease. Hum Mol Genet 2002; 11: 1845-54.
11. Levy M, Duyme M, Serbelloni P, Conte F, Sessa A, Grünfeld J-P. Is progression of renal involvement similar in twins with ADPKD? Contrib Nephrol 1995; 115: 65-71.
12. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990; 86: 1343-6.
13. Baboolal K, Ravine D, Daniels J, Williams N, Holmans P, Coles GA, Williams JD. Association of the angiotensin I converting enzyme gene deletion polymorphism with early onset of ESRF in PKD1 adult polycystic kidney disease. Kidney Int 1997; 52: 607-13.
14. Sullivan LP, Wallace DP, Grantham JJ. Epithelial transport in polycystic kidney disease. Physiol Rev 1998; 78: 1165-91.
15. O'Sullivan DA, Torres VE, Gabow PA, Thibodeau SN, King BF, Bergstralh EJ. Cystic fibrosis and the phenotypic expression of autosomal dominant polycystic kidney disease. Am J Kidney Dis 1998; 32: 976-83.
16. Persu A, Devuyst O, Lannoy N, Materne R, Brosnahan G, Gabow PA, Pirson Y, Verellen-Dumoulin C. CF gene and cystic fibrosis transmembrane conductance regulator expression in autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2000; 11: 2285-96.
17. Moncada S, Higgs A. The L-arginine-nitric oxide pathway. N Engl J Med 1993; 329: 2002-12.
18. Kone BC. Nitric oxide in renal health and disease. Am J Kidney Dis 1997; 30: 311-33.
19. Marsden PA, Heng HH, Scherer SW, Stewart RJ, Hall AV, Shi XM, Tsui LC, Schappert KT. Structure and chromosomal localization of the human constitutive endothelial nitric oxide synthase gene. J Biol Chem 1993; 268: 17478-88.
20. Torres VE, Cai Y, Chen X, Wu GQ, Geng L, Cleghorn KA, Johnson CM, Somlo S. Vascular expression of polycystin-2. J Am Soc Nephrol 2001; 12: 1-9.
21. Chauvet V, Qian F, Boute N, Cai Y, Phakdeekitacharoen B, Onuchic LF, Attie-Bitach T, Guicharnaud L, Devuyst O, Germino GG, Gubler MC. Expression of PKD1 and PKD2 transcripts and proteins in human embryo and during normal kidney development. Am J Pathol 2002; 160: 973-83.
22. Kim K, Drummond I, Ibraghimov-Beskrovnaya O, Klinger K, Arnaout MA. Polycystin 1 is required for the structural integrity of blood vessels. Proc Natl Acad Sci U S A 2000; 97: 1731-6.
23. Boulter C, Mulroy S, Webb S, Fleming S, Brindle K, Sandford R. Cardiovascular, skeletal, and renal defects in mice with a targeted disruption of the Pkd1 gene. Proc Natl Acad Sci U S A 2001; 98: 12174-9.
24. Muto S, Aiba A, Saito Y, Nakao K, Nakamura K, Tomita K, Kitamura T, Kurabayashi M, Nagai R, Higashihara E, Harris PC, Katsuki M, Horie S. Pioglitazone improves the phenotype and molecular defects of a targeted Pkd1 mutant. Hum Mol Genet 2002; 11: 1731-42.
25. Wang D, Iversen J, Strandgaard S. Endothelium-dependent relaxation of small resistance vessels is impaired in patients with autosomal dominant polycystic kidney disease. J Am Soc Nephrol 2000; 11: 1371-6.
26. Zanchi A, Moczulski DK, Hanna LS, Wantman M, Warram JH, Krolewski AS. Risk of advanced diabetic nephropathy in type 1 diabetes is associated with endothelial nitric oxide synthase gene polymorphism. Kidney Int 2000; 57: 405-13.
27. Persu A, Stoenoiu MS, Messiaen T, Davila S, Robino C, El-Khattabi O, Mourad M, Horie S, Feron O, Balligand JL, Wattiez R, Pirson Y, Chauveau D, Lens XM, Devuyst O. Modifier effect of ENOS in autosomal dominant polycystic kidney disease. Hum Mol Genet 2002; 11: 229-41.
28. Mendelsohn ME, Karas RH. The protective effects of estrogen on the cardiovascular system. N Engl J Med 1999; 340: 1801-11.
29. Fischmann TO, Hruza A, Niu XD, Fossetta JD, Lunn CA, Dolphin E, Prongay AJ, Reichert P, Lundell DJ, Narula SK, Weber PC. Structural characterization of nitric oxide synthase isoforms reveals striking active-site conservation. Nat Struct Biol 1999; 6: 233-42.
30. Fairchild TA, Fulton D, Fontana JT, Gratton JP, McCabe TJ, Sessa WC. Acidic hydrolysis as a mechanism for the cleavage of the Glu(298)-->Asp variant of human endothelial nitric-oxide synthase. J Biol Chem 2001; 276: 26674-9.