Autosomal dominant polycystic kidney disease: modifier genes and endothelial dysfunction

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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, about half the patients with ADPKD have end-stage renal disease (ESRD). In Europe and North America, ADPKD is responsible for 5–10% of the patients requiring renal replacement therapy [1]. ADPKD is also characterized by extrarenal manifestations (e.g. intracranial aneurysms or liver cysts) and hypertension. The latter occurs in 50–70% of patients, even before any reduction in renal function [1]. Mutations in two genes, *PKD1* and *PKD2*, are responsible for ~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 signalling pathways that regulate renal tubular cell maturation [2]. In this comment, we will discuss the role and potential implications of disease-modifying genes in ADPKD.

ADPKD is characterized by variable renal disease progression

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]. In addition, the nature and/or location of mutations within *PKD1* is associated with differences in renal disease progression [5]. Intrafamilial variability could result from a combination of environmental and genetic factors, influencing all steps leading to the disease [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 germline mutation [6]. Micro-environmental or genetic factors determining the rate of second hits could modulate cystogenesis and, thereby, affect renal disease progression. Alternatively, modifier genes may influence polycystin-mediated signal transduction pathways, cyst fluid accumulation or other mechanisms involved in the progression of ADPKD [3].

**Evidence supporting the role of modifier genes in ADPKD**

It is increasingly recognized that the phenotype of Mendelian disorders is influenced by modifier genes, i.e. inherited genetic variations distinct from the disease locus [7]. Such modifier genes have been evidenced in cystic fibrosis, familial Mediterranean fever or familial hypercholesterolaemia [8], as well as in murine models of polycystic kidney disease [9,10]. Studies in mouse models of ADPKD have demonstrated a consistent, greater than additive cystic phenotype in *trans*-heterozygous *Pkd1*+/−:*Pkd2*+/− mice vs singly heterozygous *Pkd1*+/− or *Pkd2*+/− mice [11]. These effects of trans-heterozygous mutations, which are observed in littermates and thus unlikely to be linked to a different genetic background, are best explained by a modifier effect exerted by the polycystins genes themselves [11]. Another argument for modifier genes influencing renal disease progression in ADPKD is provided by the excess variability in the age at ESRD observed among siblings or dizygotic twins vs genetically identical monozygotic twins [12; A. Persu and O. Devuyst, unpublished observations].

**Candidate modifier genes in ADPKD**

Only a few genes likely to influence the progression of ADPKD have been investigated thus far. In view of the activation of the renin–angiotensin system and early hypertension, *ACE*, the gene encoding the angiotensin-converting enzyme (ACE), appeared as an attractive candidate. A 287 bp insertion (I)/deletion (D) Alu fragment located within intron 16 of the gene is known to influence ACE plasma levels, the highest levels being associated with the DD genotype [13]. It was thus...
Hypothesized that DD patients with ADPKD might be characterized by increased angiotensin II levels and faster renal function decline. Although the latter hypothesis was supported by the study of Baboolal et al. [14], the deleterious effect of the DD genotype in ADPKD appeared to be only marginal or was not confirmed in subsequent studies [3,5]. Small sample size, population admixture and lack of consideration of genetic and environmental factors likely to modulate the effects of the ACE genotype could explain this discrepancy.

Several lines of evidence suggest that the cAMP-regulated cystic fibrosis transmembrane conductance regulator (CFTR) Cl− channel mediates fluid secretion, and possibly cyst enlargement in ADPKD [15]. It is of interest that a milder renal phenotype has been reported in ADPKD families harbouring two rare mutations in the CF gene that encodes CFTR [16]. However, a detailed study of the most frequent ΔF508 mutation and intron 8 polymorphic TN locus of CF did not show an influence on renal progression of ADPKD [17]. These data suggest that the potential protective role of some CFTR mutations in ADPKD may be related to the nature of the mutation and/or the residual expression of the mutated CFTR [17].

Endothelial dysfunction in ADPKD

Both Pkd1 and Pkd2 are expressed in the endothelium and vascular smooth muscle lining human blood vessels [18,19]. Wild-type mice express Pkd1 in the vessels [20,21], whereas knockout Pkd1 mice show oedema, localized haemorrhages [20–22] and increased microvascular permeability [20]. Furthermore, Pkd1+/− mice are characterized by an impaired acetylcholine (Ach)-induced endothelium-dependent relaxation of the aorta [22]. A similar impairment of the endothelium-dependent vasorelaxation, contrasting with an intact response to exogenous nitric oxide (NO), has also been documented in normotensive ADPKD patients [23]. These data suggest that endothelial dysfunction, secondary to an impaired release of NO, exists in ADPKD.

NO and endothelial NO synthase

NO is the molecular counterpart of the endothelium-derived relaxing factor [24]. In endothelial cells, NO is synthesized from l-arginine by the endothelial NO synthase (eNOS), a constitutively expressed enzyme that is encoded by the ENOS (NOS3) gene located on 7q36 [25]. The enzymatic activity of eNOS is regulated by intracellular Ca2+ levels, as well as by Ca2+-independent mechanisms such as the phosphatidylinositol-3-OH kinase (PI3k)/Akt pathway [26]. Once released, NO diffuses rapidly through cell membranes and relaxes smooth muscle cells through the production of guanosine 3′,5′-cyclic monophosphate (cGMP) (Figure 1A). Furthermore, NO inhibits platelet activation, regulates angiogenesis and controls microvascular permeability [24]. The influence of ENOS on hypertension, coronary vasospasm, atherosclerosis and, most importantly, progression of diabetic nephropathy [27,28] led to the hypothesis that it could be a modifier gene in ADPKD.

Modifier effect of ENOS in ADPKD

The effect of ENOS on renal disease progression in ADPKD has been assessed recently in a large series of unrelated patients from a restricted geographic area [29]. The frequent Glu298Asp (E/D298) polymorphism of exon 7 of ENOS was associated with a significant, 5 year lower age at ESRD in the whole group of ADPKD males, as well as a lower renal survival (Kaplan–Meier analysis) in males from PKD1-linked families. In contrast, no effect was found in females with ADPKD. The more critical influence of ENOS in ADPKD males may be explained by the fact that oestrogens stimulate eNOS expression and the release of NO in endothelial cells [30].

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 patients harbouring the Asp allele [29]. The functionality of this polymorphism has been confirmed independently in vitro [28]. The mechanism by which this conservative amino acid substitution leads to degradation and decreased activity of eNOS remains to be elucidated. The Glu298 of eNOS is conserved among species, and located within the oxygenase domain of eNOS [31]. An increased degradation of the Asp298 eNOS may yield a cleaved protein that could contribute to endothelial dysfunction [32]. Alternatively, the Glu298Asp polymorphism may influence the complex post-translational regulation of eNOS [33]. Thus, lower enzymatic activity and/or partial cleavage of eNOS could be responsible for increased endothelial dysfunction, and possibly increased systemic blood pressure and/or alteration of intra-renal microcirculation, in ADPKD patients harbouring the Asp298 allele (Figure 1B).

Conclusions and perspectives

Several lines of evidence from human studies and mouse models indicate that modifier genes play a role in the variable renal disease progression encountered in ADPKD. These modifiers could influence cystogenesis and cyst progression, but also factors involved in renal disease progression. The demonstration of endothelial dysfunction in ADPKD [22,23] and the effect of a frequent polymorphism of ENOS on renal disease progression [29] suggest that the endothelial release of NO may be such a factor.

These results offer several perspectives for the search for genetic modifiers and the pathophysiology and progression of ADPKD. Recently, Walker et al. were
not able to find an association between the Glu298Asp polymorphism and the age at ESRD in a cohort of 80 families with proven PKD1 mutation [34]. A first necessity is thus 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 [34,35]. Conflicting results in association studies, even restricted to PKD1 patients [4,5,29,34], may arise from inclusion of diverse ethnic groups or related patients, particularly if the latter originate from large families with unusual phenotypes [35].

Fig. 1. (A) Endothelial synthesis and biological roles of NO. NO is synthesized from L-arginine by eNOS, together with a stoichiometric production of L-citrulline. The reaction requires molecular oxygen, nicotinamide adenine dinucleotide phosphate (NADPH) and cofactors including tetrahydrobiopterin (THB₄), flavin adenine dinucleotide (FAD), flavin adenine mononucleotide (FMN) and calmodulin (CaM). Intracellular Ca²⁺ levels control the activity of eNOS by maintaining the Ca²⁺/CaM complex essential to activate the constitutive enzyme. The formation of a Ca²⁺/CaM complex explains the increase in endothelial NO production elicited by acetylcholine (Ach). Phosphorylation of eNOS by the PI3k/Akt pathway represents a Ca²⁺-independent regulatory mechanism. The polycystins (PKD1 and PKD2) are expressed in endothelial cells, where they may influence intracellular Ca²⁺ levels and/or the PI3k/Akt pathway. Once synthesized, NO diffuses rapidly through cell membranes. At nanomolar concentrations, NO reacts with the ferrous haem (Fe) in soluble guanylate cyclase (sGC) to produce the second messenger cGMP from GTP. This increase in cGMP in the vascular smooth muscle cells leads to their relaxation. At high concentrations, the biological actions of NO depend on the redox status of the tissue. The irreversible reaction of NO with the superoxide anion (O₂⁻) leads to the formation of peroxynitrite (ONOO⁻), a powerful and potentially toxic oxidant molecule. (B) Hypothesis for the modifier effect of ENOS in ADPKD. Variants of ENOS are associated with lower enzymatic activity and/or partial cleavage of eNOS. These modifications could be responsible for increased endothelial dysfunction in ADPKD patients, particularly in males, characterized by lower basal NO levels. The accelerated renal function degradation observed in the latter could be due to increased systemic blood pressure and/or alteration of intrarenal microcirculation.
Furthermore, association studies should include allele frequencies and linkage disequilibrium coefficients for several polymorphisms within the gene of interest, since a given variant may not be the causal polymorphism, but only a marker in linkage disequilibrium with the modifier locus [29,35]. Other elements that will be critical in assessing the relevance of association studies are informations on the functionality of the polymorphism and the molecular basis of its effect on the disease.

Secondly, the increasingly documented role of polycystins in the regulation of intracellular calcium levels [2] and their implication in the PI3k/Akt pathway [36] suggest that this family of proteins might indeed participate in the complex regulation of eNOS. In that respect, Pkd1 and Pkd2 knockout mice will be useful to document the endothelial dysfunction in ADPKD and investigate the putative link between the loss of polycystin function and altered eNOS expression/activity.

Finally, insights derived from genetic modifiers may lead to therapeutic opportunities. From the data summarized above, a logical approach would be to address specifically the endothelial dysfunction in ADPKD. Nitrates come first to mind, but their systemic use is limited by the redox status of the endothelin [37]. Similarly, the effect of other types of NO donors may be limited by the redox status of the tissue and the potential formation of peroxynitrite (Figure 1A). These limitations may be resolved by using drugs that stimulate eNOS expression in endothelial cells, such as statins or ACE inhibitors [38]. In any case, the possibility of pharmacomodulation by the ENOS genotype should be kept in mind.

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References

Controversial issues in the treatment of hyperkalaemia

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Keywords: β₂-adrenergic agents; cation-exchange resins; hyperkalaemia; insulin; sodium bicarbonate; treatment

Introduction

Hyperkalaemia is a frequent medical emergency that can cause life-threatening cardiac arrhythmias [1]. Its management remains controversial [2]. We shall examine the clinical evidence for therapies used to induce a shift of potassium (K⁺) into cells and the role of cation-exchange resins.

Insulin

Several clinical studies support the use of insulin for the treatment of acute hyperkalaemia in patients with end-stage renal disease (ESRD) [3–7]. Blumberg et al. [3] showed that the administration of close to 20 units of regular insulin with glucose caused the plasma potassium (Pₖ) to fall rapidly; a drop of close to 1 mM was observed at 60 min. Supraphysiological levels of insulin in plasma are required for maximal K⁺ shift. Hypoglycaemia is a frequent complication [3]. Supplementary parenteral glucose and blood glucose monitoring are essential.

Although some advocate treating non-diabetic hyperkalaemic patients with glucose without insulin, we feel that this is unwise because the high levels of insulin required might not be achieved. Also, hypertonic glucose may cause K⁺ to shift out of cells in patients with inadequate insulin reserves, leading to a rise in Pₖ [8].

β₂-adrenergic agonists

The ability of β₂-adrenergic stimulation to lower Pₖ in patients with ESRD has been demonstrated [4,6,9–14]. Allon et al. [12] treated patients on haemodialysis who had hyperkalaemia with 10 or 20 mg of nebulized albuterol or placebo on three separate occasions. The administration of albuterol caused a decrease in Pₖ within 30 min and the effect was sustained for at least 2 h. The mean maximum decrease in Pₖ was 0.6 mM with the 10 mg dose and 1.0 mM with 20 mg. Two

References


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