Frontiers in Nephrology

Genotype–Phenotype Correlations in Autosomal Dominant and Autosomal Recessive Polycystic Kidney Disease

Sandro Rossetti and Peter C. Harris

Division of Nephrology and Hypertension, Mayo Clinic College of Medicine, Rochester, Minnesota

The phenotypes that are associated with the common forms of polycystic kidney disease (PKD)—autosomal dominant (ADPKD) and autosomal recessive (ARPKD)—are highly variable in penetrance. This is in terms of severity of renal disease, which can range from neonatal death to adequate function into old age, characteristics of the liver disease, and other extrarenal manifestations in ADPKD. Influences of the germline mutation are at the genic and allelic levels, but intrafamilial variability indicates that genetic background and environmental factors are also key. In ADPKD, the gene involved, *PKD1* or *PKD2*, is a major factor, with ESRD occurring 20 yr later in PKD2. Mutation position may also be significant, especially in terms of the likelihood of vascular events, with 5′ mutations most detrimental. Variance component analysis in ADPKD populations indicates that genetic modifiers are important, but few such factors (beyond co-inheritance of a *TSC2* mutation) have been identified. Hormonal influences, especially associated with more severe liver disease in female individuals, indicate a role for nongenetic factors. In ARPKD, the combination of mutations is critical to the phenotypic outcome. Patients with two truncating mutations have a lethal phenotype, whereas the presence of at least one missense change can be compatible with life, indicating that many missense changes are hypomorphic alleles that generate partially functional protein. Clues from animal models and other forms of PKD highlight potential modifiers. The information that is now available on both genes is of considerable prognostic value with the prospects from the ongoing genetic revolution that additional risk factors will be revealed.

J Am Soc Nephrol 18: 1374–1380, 2007. doi: 10.1681/ASN.2007010125

Positional cloning approaches have successfully identified the cause in most common Mendelian diseases in the past 20 yr or so. However, it is clear that this is only the first step to understanding how the disease manifests in an individual patient. The rule, rather than the exception, even in "simple" genetic diseases is of wide phenotypic variability, in terms of both the array of clinical phenotypes and the severity of disease (1,2). Better understanding of the factors that underlie this variability are clearly of prognostic value but may also shed light on pathogenesis. This phenotypic variability is due to heterogeneity at the gene (genic) and mutation (allelic) levels, but considerable intrafamilial variance indicates that genetic background (modifying factors) and the environment also impinge in a major way on how diseases present and progress. In reality, the clinical manifestations of Mendelian diseases are analogous to a complex trait (1). In recent years, molecular analysis of disease populations and detailed studies of families have revealed the relative contribution of the factors described in a variety of monogenic diseases. Breakthroughs in understanding the molecular basis of the common forms of polycystic kidney disease (PKD) in humans—autosomal dominant (ADPKD) and autosomal recessive (ARPKD)—mean that genotype–phenotype correlations are now possible in these disorders (Figure 1). These findings are already of prognostic value and are providing clues to pathogenesis.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. Peter C. Harris, Division of Nephrology, Mayo Clinic, 200 First Street, SW, Rochester, MN 55905. Phone: 507-266-20541; Fax: 507-2266-29315; E-mail: harris.peter@mayo.edu

Phenotypic Variability

The severity of the renal disease in ADPKD is highly variable, ranging from rare *in utero* cases with massively enlarged cystic kidneys (3,4), through more typical presentations with ESRD in the sixth decade, to cases with adequate kidney function into old age (5,6). Extrarenal cystic manifestations are common, with hepatic cysts the most clinically relevant. Approximately 75% of patients develop liver cysts by the seventh decade, but a small minority of mainly women develop massive polycystic liver disease (PLD) that requires surgical resection (7). The most important noncystic disease association is intracranial aneurysms that occur in approximately 8% of patients with ADPKD with evidence of familial clustering (8).

The presentation of ARPKD also shows a wide clinical spectrum, with the majority having significant renal disease, ranging from massively enlarged and cystic kidneys *in utero*, causing neonatal death in approximately 30% of cases, to neonatal survivors with a significant renal phenotype that may result in ESRD (9–11). At the other extreme are cases with minimal kidney involvement in which complications of congenital hepatic fibrosis, Caroli disease, and extrahepatic biliary disease

ISSN: 1046-6673/1805-1374

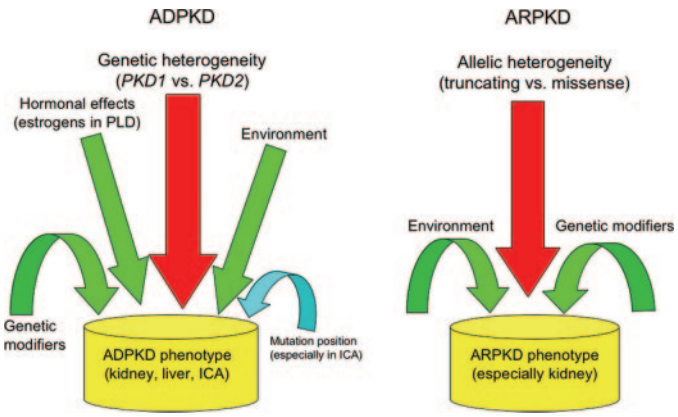

Figure 1. Diagram showing the relative contributions of various factors to the resulting phenotypes in autosomal dominant (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). Strong factors are shown in red, moderate in green, and lesser effects in blue.

are the major clinical phenotype (12,13). The biliary disease seems to be a relatively constant feature that manifests as patients age, whereas the kidney disease is an early and highly variable symptom, with kidney size often decreasing over time in the patient (14,15).

Molecular Diagnostics

ADPKD is genetically heterogeneous, with two genes identified: *PKD1* (16p13.3) and *PKD2* (4q21) (16–18). *PKD1* is the major locus, accounting for approximately 85% of families (19,20). Further genetic heterogeneity has been suggested by unlinked families (21,22), but no further genes have been identified, and, indeed, there is doubt about the existence of a *PKD3* (23–25). *PKD1* has 46 exons and encodes a large protein, polycystin-1 (4303 amino acids) (16). Exons 1 to 33 lie in a complex genomic region that is reiterated approximately six times further proximally on chromosome 16 (26,27). Similarity between *PKD1* and these pseudogenes means that locus-specific amplification methods are required to analyze *PKD1* (28). *PKD2* has 15 exons and encodes polycystin-2 (968 amino acids) (18,29).

A high level of allelic heterogeneity is found for both genes, with a total of 270 different mutations reported for *PKD1* and 73 for *PKD2* (up to 2003; Human Gene Mutation Database [http://www.hgmd.org]). More complete information in the ADPKD Mutation Database (http://pkdb.mayo.edu) describes 298 *PKD1* and 106 *PKD2* mutations. The vast majority of mutations are unique to a single family. For *PKD1*, 200 (67%) mutations are definitely pathogenic (nonsense, frameshifting, or splicing), and 98 (33%) are missense or other in-frame events. For *PKD2*, a larger proportion of mutations are truncating, 97 (91.5%), and only nine (8.5%) are in-frame. In a recent screen of 202 well-characterized probands with ADPKD (the Consortium of Radiologic Imaging Study of PKD [CRISP] population), comprehensive mutation analysis of both genes identified a probable mutation in almost 90% of cases (Rosetti *et al.*, submitted). This study involved a systematic algorithm for scoring the likely pathogenicity of missense and other atypical changes. Although these methods are far from perfect for mutation prediction, they do show the prospects for molecular diagnostics in ADPKD. Although gene-based diagnostics are not necessary in every patient with ADPKD (renal imaging is a reliable diagnostic tool in most), it can be helpful in childhood cases with unknown etiology and critical for young living-related donors for whom imaging data are less reliable. It is likely to become more important as therapies are developed.

There is little evidence of genetic heterogeneity in typical ARPKD cases. The disease gene, *PKHD1* (6p21), has 67 exons and encodes the large protein fibrocystin (4074 amino acids) (30,31). As in ADPKD, many different *PKHD1* mutations cause ARPKD. To date, 305 different mutations are listed in the *ARPKD/PKHD1* Mutation Database (http://www.humgen.rwth-aachen.de), accounting for more than 700 mutant alleles. In this case, only approximately 40% are predicted to truncate the protein, with approximately 60% missense. Several studies have used detailing algorithms to assess the pathogenicity of these changes to aid their use for diagnostics (12,32,33). Approximately one third of *PKHD1* mutations are unique to a single family. Some ancestral mutations are common in particular populations, and one mutation, T36M, of Northern European origin, accounts for approximately 17% of mutant alleles (34,35). Molecular diagnostics for ARPKD is important for prenatal testing, including preimplantation genetic diagnostics, and for establishing a firm diagnosis (36).

Genic Influences on Phenotype in ADPKD

PKD1 is a more severe disease than PKD2, with earlier diagnosis, a higher incidence of hypertension and hematuria, and ESRD occurring on average 20 yr earlier (54.3 *versus* 74 yr) (37,38). All published, early-onset ADPKD cases for which the gene is known are *PKD1* linked (39,40). Severe PLD is associated with PKD2 as well as PKD1, and the relative frequency of intracranial aneurysms (ICA) is approximately equal in the two gene types (41,42). PKD2 is a more severe disease in men (average age at ESRD 68.1 in men; 76.0 in women), but no gender difference at time to ESRD was found in recently studied PKD1 populations (37,43,44). However, analysis of magnetic resonance–derived kidney and cyst volumes in the CRISP population indicated that the relative and absolute rates of cystic growth were faster in men than in women (20). These data are consistent with an older study that suggested that male gender is associated with a poorer outcome in PKD1-linked families (6).

One family with bilineal inheritance of PKD1 and PKD2 showed that co-inheritance of a mutation in both genes is not lethal, but it did seem to be associated with more severe disease than that found with either disease alone in the family (24). Recent analysis of the CRISP population found that age-corrected PKD1 kidneys were two thirds larger than in PKD2, consistent with larger renal volume being associated with more severe disease (20,45). Analysis of the reason for the difference showed that the rate of cystic expansion was not significantly different by gene type but that PKD1 kidneys had more cysts compared with age-matched PKD2 organs. These data suggest that the rate of cyst development, especially early in the dis-

ease, is important in dictating disease severity. More early cysts in PKD1 is consistent with a two-hit model of cystogenesis (46), because *PKD1* is a larger mutational target. The process of cystogenesis, therefore, consists of a gene-related cyst initiation step and a gene-independent cyst expansion phase (20).

Allelic Influences on Phenotype

In ARPKD, the specific combination of mutations seems to dictate significantly the phenotypic outcome. When patients are defined as severe (died by the neonatal period) or moderate (survived the neonatal period), a striking finding, first proposed by Bergmann (47) and now confirmed in other studies (32,48,49), is that all patients with two mutations that are predicted to truncate fibrocystin have the lethal phenotype. As a result, missense changes are more common in those with the moderate phenotype, which have either two missense or a missense and a truncating change (49). These data indicate that a proportion of missense mutations are hypomorphic alleles that generate some partially functional fibrocystin. Alternatively spliced products, which have been suggested to be common in *PKHD1* (31), may also be a factor in the significance of some missense changes, although it is striking that regardless of the position of truncating mutations, they are associated with lethality when found with a second truncating event. The population with predominant liver disease does not show a unique mutation profile but a combination of truncating and missense or two missense, as seen in the moderate group (12). These data suggest that the minimal kidney disease that is seen in these cases is due to the presence of one or two hypomorphic missense alleles that effect the renal phenotype in a major way. Preliminary analysis of the significance of specific missense alleles has been made by analysis of the phenotype in cases which they occur with a truncating mutation or are homozygous (11). Variability between cases with the same mutation combination also emphasizes that other genetic and environmental factors influence the phenotype.

Allelic effects seem to be less evident in ADPKD. In a large study of 461 affected patients with PKD2 from 71 families, there was no clear correlation between mutation type or mutation position and the severity of kidney disease (43). Intrafamilial variability was, however, highlighted with rare cases with ESRD before 50 yr in families with otherwise more typical PKD2. In PKD1, no correlation between mutation type and disease has been found, indicating that missense changes likely inactivate the allele, similar to truncating changes. One correlation that has been described was between the position of the mutation, 5′ or 3′ to the median mutation position, whereby patients with 5′ changes reached ESRD 3 yr earlier (53 *versus* 56 yr) (44). Patients with 5′ mutations also seem to be more prone to ICA, and this was especially clear in ones with rupture before 40 yr and in families with multiple cases with ICA or other vascular events (42). The reason for this effect is not known but could be associated with cleavage of the protein at the GPS domain (3048 amino acids) into N and C terminal products or possible dominant negative effects (50).

Genetic Modifying Effect

Considerable intrafamilial phenotypic variability has been described in ADPKD. Geberth *et al.* (51) showed in parent–offspring pairs that ESRD could occur up to 26.3 yr earlier or 27.2 yr later in the offspring, illustrating considerable variability that is not accounted for by genic or allelic effects. A comparison of monozygotic twins and siblings showed greater variance in time to ESRD in siblings, supporting a role for genetic background (52). Extreme divergent phenotypes were seen in one pair of dizygotic twins, one with early-onset disease and the other with a more typical presentation (53). Two studies have analyzed intrafamilial variability in large populations (315 patients in 83 pedigrees or 406 patients in 66 pedigrees) of families with ADPKD by variance component analysis (54,55). One found that inherited differences in genetic background were estimated to account for 18 to 59% of the phenotypic variance in PKD1 disease markers in patients before ESRD when phenotypes such as renal volume, proteinuria, and serum creatinine were analyzed and 43% in the subsequent progression to ESRD (55). In the other study, analysis of patients without ESRD ($n = 247$) or with ESRD ($n = 159$) produced estimates of heritability of 42 or 78%, respectively (54). On the basis of these figures, the authors indicated that genetic modifiers account for a significant part of the variability that is seen in PKD1 and PKD2 populations and, given a large enough population, that it may be possible to identify such factors (54,56).

Several candidate gene association studies have been carried out in ADPKD to identify genetic modifiers, but consistent with studies in many other diseases, positive results have usually not been reproducible (57). Thirteen studies tested the I/D polymorphism of the angiotensin-1–converting enzyme gene (*ACE*) and, although initial studies found the DD phenotype associated with more severe disease (58), a recent meta-analysis found no significant association (59). Mixed or negative results have also been obtained with the *ENOS* gene and other components of the renin-angiotensin system (58,60,61).

A number of patients who had ADPKD and cystic fibrosis were analyzed. Three patients had milder disease compared with siblings with ADPKD alone, suggestive of a role for cystic fibrosis transmembrane conductance regulator (CFTR) in cyst fluid secretion in ADPKD (62,63), a role that is supported by other experimental data (64). However, one other study found little difference, and the total number of cases is small (65). One example in which co-inheritance of a mutant gene does influence the severity of PKD is in cases with contiguous deletions that disrupt the *PKD1* and adjacent tuberous sclerosis gene, *TSC2* (66). These patients typically have severe, early-onset PKD, plus TSC phenotypes. The TSC2 protein, tuberin, may act in similar downstream pathways to polycystin-1, suggesting synergistic effects (67), but recently a more direct mode of action was suggested with the two proteins interacting in a complex (68).

Whereas the majority of families with ARPKD show a relatively concordant phenotype, in approximately 20%, a striking intrafamilial phenotypic variability is observed (11). This can manifest as neonatal death and survival in the same family or

as a striking difference in the severity of kidney or liver disease. No association studies have been described in human ARPKD (the population is small), but other studies have highlighted potential modifiers. Genetic modifiers have been mapped in mouse models of recessive PKD, and in one case, the *Kif12* gene was identified as a possible modifier in the *cpk* mouse (69). Another possible modifying factor is the maturity onset diabetes of the young-5 (MODY5)-associated protein HNF1β that regulates *PKHD1* expression by binding to its proximal promoter (70).

Environmental Influences

It seems certain that nongenetic factors, including environmental exposures, significantly influence the severity of renal disease and other extrarenal manifestations in PKD. There is good evidence from the preponderance of PLD in women and that the disease is exacerbated by multiple pregnancies and other estrogen exposure that hormonal factors are important in the development of this disease (71). The faster growth of kidney cysts in male individuals with ADPKD and also earlier ESRD in male individuals with PKD2 suggest that male hormonal factors may be important in renal cystic disease (20,43). Caffeine exposure has been considered a risk factor in PKD, and evidence that caffeine can increase the production of cAMP in cyst-derived cells, which stimulate proliferation and fluid secretion (72), provides some justification for avoiding caffeine-containing beverages. Smoking has been shown to be a risk factor for more rapid progression in patients with renal disease, including ADPKD, with increased risk for chronic renal failure and ESRD, especially in men (73). This risk may be due to the known vascular effects and higher BP that are associated with smoking. Proteinuria and risk for chronic kidney disease and ESRD are associated with obesity, especially in men. Dietary changes, such as low-protein intake or flax seed oil, some of which have shown promise in animal studies, have been negative or not tested in human ADPKD (74).

Conclusions

It is now possible to start dissecting the relative importance of various genetic and environment factors to the presentation and progression of PKD. In ADPKD, the gene involved is a major predictor of severity, with genetic background and environmental factors moderately involved and allelic effects probably less important (Figure 1). In ARPKD, the combination of alleles is a major factor in disease severity. While candidate association studies have been disappointing for finding genetic modifiers in human PKD, recent development of high-resolution single-nucleotide polymorphism arrays and mapping the haplotype block structure of the human genome make genome-wide association studies to find modifiers now a reality. Equally important are the larger populations that now are being assembled for observational and clinical trials of ADPKD that will provide the clinically well-characterized groups that are required for these studies.

Disclosures

None.

References

1. Weatherall DJ: Phenotype-genotype relationships in monogenic disease: Lessons from the thalassaemias. *Nat Rev Genet* 2: 245–255, 2001
2. Cutting GR: Modifier genetics: Cystic fibrosis. *Annu Rev Genomics Hum Genet* 6: 237–260, 2005
3. Zerres K, Rudnik-Schoneborn S, Deget F; German Working Group on Paediatric Nephrology: Childhood onset autosomal dominant polycystic kidney disease in sibs: Clinical picture and recurrence risk. *J Med Genet* 30: 583–588, 1993
4. Fick GM, Johnson AM, Strain JD, Kimberling WJ, Kumar S, Manco-Johnson ML, Duley IT, Gabow PA: Characteristics of very early onset autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 3: 1863–1870, 1993
5. Torra R, Badenas C, Perez-Oller L, Luis J, Millan S, Nicolau C, Oppenheimer F, Mila M, Darnell A: Increased prevalence of polycystic kidney disease type 2 among elderly polycystic patients. *Am J Kidney Dis* 36: 728–734, 2000
6. Gabow PA, Johnson AM, Kaehny WD, Kimberling WJ, Lezotte DC, Duley IT, Jones RH: Factors affecting the progression of renal disease in autosomal-dominant polycystic kidney disease. *Kidney Int* 41: 1311–1319, 1992
7. Torres VE: Polycystic liver disease. In: *Polycystic Kidney Disease*, edited by Watson ML, Torres VE, Oxford, Oxford University Press, 1996, pp 500–529
8. Pirson Y, Chauveau D, Torres V: Management of cerebral aneurysms in autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 13: 269–276, 2002
9. MacRae Dell KM, Avner ED: Autosomal recessive polycystic kidney disease. GeneReviews; Genetic Disease Online Reviews at GeneTests-GeneClinics, Seattle, University of Washington, 2006. Available at: http://www.genetest.org
10. Guay-Woodford LM, Desmond RA: Autosomal recessive polycystic kidney disease: The clinical experience in North America. *Pediatrics* 111: 1072–1080, 2003
11. Bergmann C, Senderek J, Windelen E, Kupper F, Middeldorf I, Schneider F, Dornia C, Rudnik-Schoneborn S, Konrad M, Schmitt CP, Seeman T, Neuhaus TJ, Vester U, Kirfel J, Buttner R, Zerres K: Clinical consequences of *PKHD1* mutations in 164 patients with autosomal-recessive polycystic kidney disease (ARPKD). *Kidney Int* 67: 829–848, 2005
12. Adeva M, El-Youssef M, Rossetti S, Kamath PS, Kubly V, Consugar M, Milliner DS, King BF, Torres VE, Harris PC: Clinical and molecular characterization defines a broadened spectrum of autosomal recessive polycystic kidney disease(ADPKD). *Medicine* 85: 1–21, 2006
13. Goilav B, Norton KI, Satlin LM, Guay-Woodford L, Chen F, Magid MS, Emre S, Shneider BL: Predominant extrahepatic biliary disease in autosomal recessive polycystic kidney disease: A new association. *Pediatr Transplant* 10: 294–298, 2006
14. Blickman JG, Bramson RT, Herrin JT: Autosomal recessive polycystic kidney disease: Long-term sonographic findings in patients surviving the neonatal period. *AJR Am J Roentgenol* 164: 1247–1250, 1995
15. Fonck C, Chauveau D, Gagnadoux MF, Pirson Y, Grunfeld JP: Autosomal recessive polycystic kidney disease in adulthood. *Nephrol Dial Transplant* 16: 1648–1652, 2001
16. Hughes J, Ward CJ, Peral B, Aspinwall R, Clark K, San Millan JL, Gamble V, Harris PC: The polycystic kidney

disease 1 (*PKD1*) gene encodes a novel protein with multiple cell recognition domains. *Nat Genet* 10: 151–160, 1995
17. International Polycystic Kidney Disease Consortium: Polycystic kidney disease: The complete structure of the *PKD1* gene and its protein. *Cell* 81: 289–298, 1995
18. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhusien B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJM, Somlo S: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272: 1339–1342, 1996
19. Peters DJM, Sandkuijl LA: Genetic heterogeneity of polycystic kidney disease in Europe. *Contrib Nephrol* 97: 128–139, 1992
20. Harris PC, Bae K, Rossetti S, Torres VE, Grantham JJ, Chapman A, Guay-Woodford L, King BF, Wetzel LH, Baumgarten D, Kenney PJ, Consugar M, Klahr S, Bennett WM, Meyers CM, Zhang Q, Thompson PA, Zhu F, Miller JP: Cyst number but not the rate of cystic growth is associated with the mutated gene in ADPKD. *J Am Soc Nephrol* 17: 3013–3019, 2006
21. Daoust MC, Reynolds DM, Bichet DG, Somlo S: Evidence for a third genetic locus for autosomal dominant polycystic kidney disease. *Genomics* 25: 733–736, 1995
22. de Almeida S, de Almeida E, Peters D, Pinto JR, Tavora I, Lavinha J, Breuning M, Prata MM: Autosomal dominant polycystic kidney disease: Evidence for the existence of a third locus in a Portuguese family. *Hum Genet* 96: 83–88, 1995
23. Paterson AD, Pei Y: PKD3-to be or not to be? [Letter]. *Nephrol Dial Transplant* 14: 2965–2966, 1999
24. Pei Y, Paterson AD, Wang KR, He N, Hefferton D, Watnick T, Germino GG, Parfrey P, Somlo S, St George-Hyslop P: Bilineal disease and trans-heterozygotes in autosomal dominant polycystic kidney disease. *Am J Hum Genet* 68: 355–363, 2001
25. Consugar M, Rossetti S, Anderson S, DeAlmedia E, Cunningham JM, Torres VE, Harris PC: *PKD3* revisited with improved *PKD1* and *PKD2* haplotyping and mutation screening [Abstract]. *J Am Soc Nephrol* 16: 358A, 2005
26. European Polycystic Kidney Disease Consortium: The polycystic kidney disease 1 gene encodes a 14 kb transcript and lies within a duplicated region on chromosome 16. *Cell* 77: 881–894, 1994
27. Martin J, Han C, Gordon LA, Terry A, Prabhakar S, She X, Xie G, Hellsten U, Chan YM, Altherr M, Couronne O, Aerts A, Bajorek E, Black S, Blumer H, Branscomb E, Brown NC, Bruno WJ, Buckingham JM, Callen DF, Campbell CS, Campbell ML, Campbell EW, Caoile C, Challacombe JF, Chasteen LA, Chertkov O, Chi HC, Christensen M, Clark LM, Cohn JD, Denys M, Detter JC, Dickson M, Dimitrijevic-Bussod M, Escobar J, Fawcett JJ, Flowers D, Fotopulos D, Glavina T, Gomez M, Gonzales E, Goodstein D, Goodwin LA, Grady DL, Grigoriev I, Groza M, Hammon N, Hawkins T, Haydu L, Hildebrand CE, Huang W, Israni S, Jett J, Jewett PB, Kadner K, Kimball H, Kobayashi A, Krawczyk MC, Leyba T, Longmire JL, Lopez F, Lou Y, Lowry S, Ludeman T, Manohar CF, Mark GA, McMurray KL, Meincke LJ, Morgan J, Moyzis RK, Mundt MO, Munk AC, Nandkeshwar RD, Pitluck S, Pollard M, Predki P, Parson-Quintana B, Ramirez L, Rash S, Retterer J, Ricke DO, Robinson DL, Rodriguez A, Salamov A, Saunders EH, Scott D, Shough T, Stallings RL, Stalvey M, Sutherland RD, Tapia R, Tesmer JG, Thayer N, Thompson LS, Tice H, Torney DC, Tran-Gyamfi M, Tsai M, Ulanovsky LE, Ustaszewska A, Vo N, White PS, Williams AL, Wills PL, Wu JR, Wu K, Yang J, Dejong P, Bruce D, Doggett NA, Deaven L, Schmutz J, Grimwood J, Richardson P, Rokhsar DS, Eichler EE, Gilna P, Lucas SM, Myers RM, Rubin EM, Pennacchio LA: The sequence and analysis of duplication-rich human chromosome 16. *Nature* 432: 988–994, 2004
28. Rossetti S, Chauveau D, Walker D, Saggar-Malik A, Winearls CG, Torres VE, Harris PC: A complete mutation screen of the ADPKD genes by DHPLC. *Kidney Int* 61: 1588–1599, 2002
29. Hayashi T, Mochizuki T, Reynolds DM, Wu G, Cai Y, Somlo S: Characterization of the exon structure of the polycystic kidney disease 2 gene (PKD2). *Genomics* 44: 131–136, 1997
30. Ward CJ, Hogan MC, Rossetti S, Walker D, Sneddon T, Wang X, Kubly V, Cunningham JM, Bacallao R, Ishibashi M, Milliner DS, Torres VE, Harris PC: The gene mutated in autosomal recessive polycystic kidney disease encodes a large, receptor-like protein. *Nat Genet* 30: 259–269, 2002
31. Onuchic LF, Furu L, Nagasawa Y, Hou X, Eggermann T, Ren Z, Bergmann C, Senderek J, Esquivel E, Zeltner R, Rudnik-Schoneborn S, Mrug M, Sweeney W, Avner ED, Zerres K, Guay-Woodford LM, Somlo S, Germino GG: PKHD1, the polycystic kidney and hepatic disease 1 gene, encodes a novel large protein containing multiple immunoglobulin-like plexin-transcription-factor domains and parallel beta-helix 1 repeats. *Am J Hum Genet* 70: 1305–1317, 2002
32. Sharp AM, Messiaen LM, Page G, Antignac C, Gubler MC, Onuchic LF, Somlo S, Germino GG, Guay-Woodford LM: Comprehensive genomic analysis of *PKHD1* mutations in ARPKD cohorts. *J Med Genet* 42: 336–349, 2005
33. Losekoot M, Haarloo C, Ruivenkamp C, White SJ, Breuning MH, Peters DJ: Analysis of missense variants in the PKHD1-gene in patients with autosomal recessive polycystic kidney disease (ARPKD). *Hum Genet* 188: 185–206, 2005
34. Consugar MB, Anderson SA, Rossetti S, Pankratz VS, Ward CJ, Torra R, Coto E, El-Youssef HM, Kantarci S, Utsch B, Hildebrandt F, Sweeney WE, Avner ED, Torres VE, Cunningham JM, Harris PC: Haplotype analysis improves molecular diagnostics of autosomal recessive polycystic kidney disease. *Am J Kidney Dis* 45: 77–87, 2005
35. Bergmann C, Senderek J, Kupper F, Schneider F, Dornia C, Windelen E, Eggermann T, Rudnik-Schoneborn S, Kirfel J, Furu L, Onuchic LF, Rossetti S, Harris PC, Somlo S, Guay-Woodford L, Germino GG, Moser M, Buttner R, Zerres K: PKHD1 mutations in autosomal recessive polycystic kidney disease (ARPKD). *Hum Mutat* 23: 453–463, 2004
36. Zerres K, Senderek J, Rudnik-Schoneborn S, Eggermann T, Kunze J, Mononen T, Kaariainen H, Kirfel J, Moser M, Buettner R, Bergmann C: New options for prenatal diagnosis in autosomal recessive polycystic kidney disease by mutation analysis of the *PKHD1* gene. *Clin Genet* 66: 53–57, 2004
37. 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* 353: 103–107, 1999
38. Torra R, Badenas C, Darnell A, Nicolau C, Volpini V,

Revert L, Estivill X: Linkage, clinical features, and prognosis of autosomal dominant polycystic kidney disease types 1 and 2. *J Am Soc Nephrol* 7: 2142–2151, 1996
39. Michaud J, Russo P, Grignon A, Dallaire L, Bichet D, Rosenblatt D, Lamothe E, Lambert M: Autosomal dominant polycystic kidney disease in the fetus. *Am J Med Genet* 51: 240–246, 1994
40. Rossetti S, Strmecki L, Gamble V, Burton S, Sneddon V, Peral B, Roy S, Bakkaloglu A, Komel R, Winearls CG, Harris PC: Mutation analysis of the entire PKD1 gene: Genetic and diagnostic implications. *Am J Hum Genet* 68: 46–63, 2001
41. Bozza A, Aguiari G, Scapoli C, Scalia P, Perini L, De Paoli Vitali E, del Senno L: Autosomal dominant polycystic kidney disease linked to PKD2 locus in a family with severe extrarenal manifestations. *Am J Nephrol* 17: 458–461, 1997
42. Rossetti S, Chauveau D, Kubly V, Slezak J, Saggar-Malik A, Pei Y, Ong AC, Stewart F, Watson ML, Bergstralh EJ, Winearls CG, Torres VE, Harris PC: Association of mutation position in polycystic kidney disease 1 (PKD1) gene and development of a vascular phenotype. *Lancet* 361: 2196–2201, 2003
43. Magistroni R, He N, Wang K, Andrew R, Johnson A, Gabow P, Dicks E, Parfrey P, Torra R, San-Millan JL, Coto E, Van Dijk M, Breuning M, Peters D, Bogdanova N, Ligabue G, Albertazzi A, Hateboer N, Demetriou K, Pierides A, Deltas C, St George-Hyslop P, Ravine D, Pei Y: Genotype-renal function correlation in type 2 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 14: 1164–1174, 2003
44. 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* 13: 1230–1237, 2002
45. Grantham JJ, Torres VE, Chapman AB, Guay-Woodford LM, Bae KT, King BF Jr, Wetzel LH, Baumgarten DA, Kenney PJ, Harris PC, Klahr S, Bennett WM, Hirschman GN, Meyers CM, Zhang X, Zhu F, Miller JP: Volume progression in polycystic kidney disease. *N Engl J Med* 354: 2122–2130, 2006
46. Qian F, Watnick TJ, Onuchic LF, Germino GG: The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type 1. *Cell* 87: 979–987, 1996
47. Bergmann C, Senderek J, Sedlacek B, Pegiazoglou I, Puglia P, Eggermann T, Rudnik-Schneborn S, Furu L, Onuchic LF, De Baca M, Germino GG, Guay-Woodford L, Somlo S, Moser M, Buttner R, Zerres K: Spectrum of mutations in the gene for autosomal recessive polycystic kidney disease (ARPKD/PKHD1). *J Am Soc Nephrol* 14: 76–89, 2003
48. Rossetti S, Torra R, Coto E, Consugar M, Kubly V, Malaga S, Narvarro M, El-Youssef M, Torres V, Harris PC: A complete mutation screen of *PKHD1* in autosomal recessive polycystic kidney pedigrees. *Kidney Int* 64: 391–403, 2003
49. Furu L, Onuchic LF, Gharavi AG, Hou X, Esquivel EL, Nagasawa Y, Bergmann C, Senderek J, Avner E, Zerres K, Germino GG, Guay-Woodford LM, Somlo S: Milder presentation of recessive polycystic kidney disease requires presence of amino acid substitution mutations. *J Am Soc Nephrol* 14: 2004–2014, 2003
50. Qian F, Boletta A, Bhunia AK, Xu H, Liu L, Ahrabi AK, Watnick TJ, Zhou F, Germino GG: Cleavage of polycystin-1 requires the receptor for egg jelly domain and is disrupted by human autosomal-dominant polycystic kidney disease 1- associated mutations. *Proc Natl Acad Sci U S A* 99: 16981–16986, 2002
51. Geberth S, Ritz E, Zeier M, Stier E: Anticipation of age at renal death in autosomal dominant polycystic kidney disease (ADPKD)? *Nephrol Dial Transplant* 10: 1603–1606, 1995
52. Peral B, Ong ACM, San Millan JL, Gamble V, Rees L, Harris PC: A stable, nonsense mutation associated with a case of infantile onset polycystic kidney disease 1 (PKD1). *Hum Mol Genet* 5: 539–542, 1996
53. Peral B, Gamble V, San Millan JL, Strong C, Sloane-Stanley J, Moreno F, Harris PC: Splicing mutations of the polycystic kidney disease 1 (*PKD1*) gene induced by intronic deletion. *Hum Mol Genet* 4: 569–574, 1995
54. Paterson AD, Magistroni R, He N, Wang K, Johnson A, Fain PR, Dicks E, Parfrey P, St George-Hyslop P, Pei Y: Progressive loss of renal function is an age-dependent heritable trait in type 1 autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 16: 755–762, 2005
55. Fain PR, McFann KK, Taylor MR, Tison M, Johnson AM, Reed B, Schrier RW: Modifier genes play a significant role in the phenotypic expression of PKD1. *Kidney Int* 67: 1256–1267, 2005
56. Pei Y: Nature and nurture on phenotypic variability of autosomal dominant polycystic kidney disease. *Kidney Int* 67: 1630–1631, 2005
57. Hirschhorn JN, Lohmueller K, Byrne E, Hirschhorn K: A comprehensive review of genetic association studies. *Genet Med* 4: 45–61, 2002
58. 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* 52: 607–613, 1997
59. Pereira TV, Nunes AC, Rudnicki M, Magistroni R, Albertazzi A, Pereira AC, Krieger JE: Influence of ACE I/D gene polymorphism in the progression of renal failure in autosomal dominant polycystic kidney disease: A meta-analysis. *Nephrol Dial Transplant* 21: 3155–3163, 2006
60. 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* 11: 229–241, 2002
61. 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* 41: 90–94, 2003
62. O'Sullivan DA, Torres VE, Gabow PA, Thibodwau SN, King BF, Bergstralh EJ: Cystic fibrosis and the phenotype expression of autosomal dominant polycystic kidney disease. *Am J Kidney Dis* 32: 976–983, 1998
63. Xu N, Glockner JF, Rossetti S, Babovich-Vuksanovic D, Harris PC, Torres VE: Autosomal dominant polycystic kidney disease coexisting with cystic fibrosis. *J Nephrol* 19: 529–534, 2006
64. Ikeda M, Fong P, Cheng J, Boletta A, Qian F, Zhang XM,

Cai H, Germino GG, Guggino WB: A regulatory role of polycystin-1 on cystic fibrosis transmembrane conductance regulator plasma membrane expression. *Cell Physiol Biochem* 18: 9–20, 2006

65. 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* 11: 2285–2296, 2000
66. Sampson JR, Maheshwar MM, Aspinwall R, Thompson P, Cheadle JP, Ravine D, Roy S, Haan E, Bernstein J, Harris PC: Renal cystic disease in tuberous sclerosis: Role of the polycystic kidney disease 1 gene. *Am J Hum Genet* 61: 843–851, 1997
67. Harris PC, Torres VE: Understanding pathogenic mechanisms in polycystic kidney disease provides clues for therapy. *Curr Opin Nephrol Hypertens* 15: 456–463, 2006
68. Shillingford JM, Murcia NS, Larson CH, Low SH, Hedgepeth R, Brown N, Flask CA, Novick AC, Goldfarb DA, Kramer-Zucker A, Walz G, Piontek KB, Germino GG, Weimbs T: The mTOR pathway is regulated by polycystin-1, and its inhibition reverses renal cystogenesis in polycystic kidney disease. *Proc Natl Acad Sci U S A* 103: 5466–5471, 2006
69. Mrug M, Li R, Cui X, Schoeb TR, Churchill GA, Guay-Woodford LM: Kinesin family member 12 is a candidate polycystic kidney disease modifier in the *cpk* mouse. *J Am Soc Nephrol* 16: 905–916, 2005
70. Hiesberger T, Bai Y, Shao X, McNally BT, Sinclair AM, Tian X, Somlo S, Igarashi P: Mutation of hepatocyte nuclear factor-1b inhibits *Pkhd1* gene expression and produces renal cysts in mice. *J Clin Invest* 113: 814–825, 2004
71. Sherstha R, McKinley C, Russ P, Scherzinger A, Bronner T, Showalter R, Everson GT: Postmenopausal estrogen therapy selectively stimulates hepatic enlargement in women with autosomal dominant polycystic kidney disease. *Hepatology* 26: 1282–1286, 1997
72. Belibi FA, Wallace DP, Yamaguchi T, Christensen M, Reif G, Grantham JJ: The effect of caffeine on renal epithelial cells from patients with autosomal dominant polycystic kidney disease. *J Am Soc Nephrol* 13: 2723–2729, 2002
73. Orth SR, Stockmann A, Conradt C, Ritz E, Ferro M, Kreusser W, Piccoli G, Rambausek M, Roccatello D, Schafer K, Sieberth HG, Wanner C, Watschinger B, Zucchelli P: Smoking as a risk factor for end-stage renal failure in men with primary renal disease. *Kidney Int* 54: 926–931, 1998
74. Klahr S, Breyer JA, Beck GJ, Dennis VW, Hartman JA, Roth D, Steinman TI, Wang S-R, Yamamoto ME: Dietary protein restriction, blood pressure control, and the progression of polycystic kidney disease. Modification of Diet in Renal Disease Study Group. *J Am Soc Nephrol* 5: 2037–2047, 1995