Genotype-Renal Function Correlation in Type 2 Autosomal Dominant Polycystic Kidney Disease

RICCARDO MAGISTRONI,*‡‡ NING HE,* KAIRONG WANG,* ROBIN ANDREW,* ANN JOHNSON,† PATRICIA GABOW,† ELIZABETH DICKS,‡ PATRICK PARFREY,‡ ROSER TORRA,§ JOSE L. SAN-MILLAN,¶ ELIECER COTO,# MARJAN VAN DIJK,@ MARTIJN BREUNING,** DORIEN PETERS,** NADJA BOGDANOVA,†† GIULIA LIGABUE,‡‡ ALBERTO ALBERTAZZI,‡‡ NICK HATEBOER,§§ KYPROULA DEMETRIOU,¶¶ ALKIS PIERIDES,¶¶ CONSTANTINOS DELTAS,## PETER ST. GEORGE-HYSLOP,* DAVID RAVINE,§§ and YORK PEI*

**Division of Genomic Medicine, University Health Network, Toronto, Canada; †Renal Division, University of Colorado Health Sciences Center, Denver, CO; ‡Division of Nephrology, Memorial University, St. John's, Newfoundland, Canada; §Division of Nephrology, Fundacio Puigvert, Barcelona, Spain; ¶Unidad de Genetica Molecular, Hospital Ramon y Cajal, Madrid, Spain; #Instituto Reina Sofia de Investigaciones Nephrologicas, Hospital Central de Asturias, Oviedo, Spain; @Academisch Ziekenhuis and **Afdeling Anthropogenetica Rijksuniversiteit Leiden, Leiden, the Netherlands; ††Institut Fur Humangenetik, Westfalische Wilheims-Universitat, Munster, Germany; ‡‡Division of Nephrology, University of Modena and Reggio Emilia, Modena, Italy; §§Institute of Medical Genetics, University Hospital of Wales, Cardiff, UK; ¶¶Department of Nephrology, Nicosia General Hospital, Cyprus; and ##Department of Biological Sciences, University of Cyprus and Department of Molecular Genetics, Cyprus Institute of Neurology and Genetics, Cyprus.*

Abstract. Autosomal dominant polycystic kidney disease (ADPKD) is a common Mendelian disorder that affects approximately 1 in 1000 live births. Mutations of two genes, *PKD1* and *PKD2*, account for the disease in approximately 80 to 85% and 10 to 15% of the cases, respectively. Significant interfamilial and intrafamilial renal disease variability in ADPKD has been well documented. Locus heterogeneity is a major determinant for interfamilial disease variability (*i.e.*, patients from *PKD1*-linked families have a significantly earlier onset of ESRD compared with patients from *PKD2*-linked families). More recently, two studies have suggested that allelic heterogeneity might influence renal disease severity. The current study examined the genotype-renal function correlation in 461 affected individuals from 71 ADPKD families with known *PKD2* mutations. Fifty different mutations were identified in these families, spanning between exon 1 and 14 of *PKD2*. Most (94%) of these mutations were predicted to be inactivating. The renal outcomes of these patients, including the age of onset of end-stage renal disease (ESRD) and chronic renal failure (CRF; defined as creatinine clearance $\leq$ 50 ml/min, calculated using the Cockroft and Gault formula), were analyzed. Of all the affected individuals clinically assessed, 117 (25.4%) had ESRD, 47 (10.2%) died without ESRD, 65 (14.0%) had CRF, and 232 (50.3%) had neither CRF nor ESRD at the last follow-up. Female patients, compared with male patients, had a later mean age of onset of ESRD (76.0 [95% CI, 73.8 to 78.1] *versus* 68.1 [95% CI, 66.0 to 70.2] yr) and CRF (72.5 [95% CI, 70.1 to 74.9] *versus* 63.7 [95% CI, 61.4 to 66.0] yr). Linear regression and renal survival analyses revealed that the location of *PKD2* mutations did not influence the age of onset of ESRD. However, patients with splice site mutations appeared to have milder renal disease compared with patients with other mutation types ($P < 0.04$ by log rank test; adjusted for the gender effect). Considerable renal disease variability was also found among affected individuals with the same *PKD2* mutations. This variability can confound the determination of allelic effects and supports the notion that additional genetic and/or environmental factors may modulate the renal disease severity in ADPKD.

Autosomal dominant polycystic kidney disease (ADPKD [MIM 173900]) is the most common hereditary kidney disorder, with an incidence of approximately 1 in 1000 live births, and accounts for approximately 5 to 8% of end-stage renal disease (ESRD) (1,2). It is characterized by the progressive formation and enlargement of renal cysts, typically leading to chronic renal failure by late middle age. Other manifestations of this disorder, such as cyst formation in non-renal organs, cardiac valvular defects, colonic diverticulosis, and intracranial arterial aneurysms, accompany the renal disease variably. Linkage studies in ADPKD families have documented genetic heterogeneity (3,4), and at least two disease genes (*PKD1* [MIM 601313] on chromosome 16p13.3 and *PKD2* [MIM 173910] on chromosome 4q13–23) have been identified and characterized (5–7). A rare putative third disease gene (PKD3 [MIM 600666]) has been implicated by the identification of a small number of families unlinked to the known gene loci (8,9). Mutations of *PKD1* and *PKD2* account for the disease in

Received November 27, 2002. Accepted January 20, 2003.

Correspondence to Dr. York Pei, Division of Nephrology and Genomic Medicine, University Health Network, 13 EN-228, 200 Elizabeth Street, Toronto, Ontario, Canada M5G 2C4. Phone: 416-340-4257; Fax: 416-340-4999; E-mail: york.pei@uhn.on.ca

1046-6673/1405-1164
Journal of the American Society of Nephrology

DOI: 10.1097/01.ASN.0000061774.90975.25

approximately 80 to 85% and approximately 10 to 15% of Caucasian ADPKD families, respectively (1,10). Polycystins 1 and 2, the gene products of *PKD1* and *PKD2*, are transmembrane proteins that share sequence homology and are currently thought to be part of a novel signaling pathway that regulates intracellular calcium (11–13). Polycystin 1 is predicted to have a receptor-like structure and may be involved in cell-cell and/or cell-matrix interaction (12,13). In contrast, polycystin 2 shares significant homology to and can function as a cation ion channel subunit, with nonselective permeability (12–14). Both proteins have been shown to interact *in vitro* through their cytoplasmic region, with polycystin 1 likely functioning as a regulator of polycystin 2 (11–14).

Disease progression of ADPKD is highly variable, with age at onset of ESRD ranging from childhood to old age (1). Genetic locus heterogeneity is a major determinant for intrafamilial disease variability: patients from *PKD1*-linked families have a significantly earlier onset of ESRD or death when compared with patients from *PKD2*-linked families (median age: 53 [95% CI, 51.2 to 54.8] *versus* 69 [95% CI, 66.9 to 71.3] yr) (15). More recently, two studies have suggested that allelic heterogeneity in ADPKD might also influence renal disease severity. In the first study, patients with mutations in the 3' half of *PKD1* had milder renal disease than patients with mutations in the 5' half of the gene (16). In the second study, patients with mutations in the 3' half of *PKD2* had milder composite scores for renal complications (*i.e.*, presence or absence of hypertension, hematuria, renal calculi, and urinary tract infection) than patients with mutations in the 5' half of the gene (17). However, correlation of renal function with genotype data has not been assessed in the patients with *PKD2* mutations. In the current study, we report a pooled analysis of genotype-renal function correlation in 461 affected individuals from 71 ADPKD families with known *PKD2* mutations.

Materials and Methods

Study Patients

The clinical and genetic data of 461 affected members from 71 ADPKD families with known germline *PKD2* mutations were analyzed. The *PKD2* mutations in 50 families have been previously

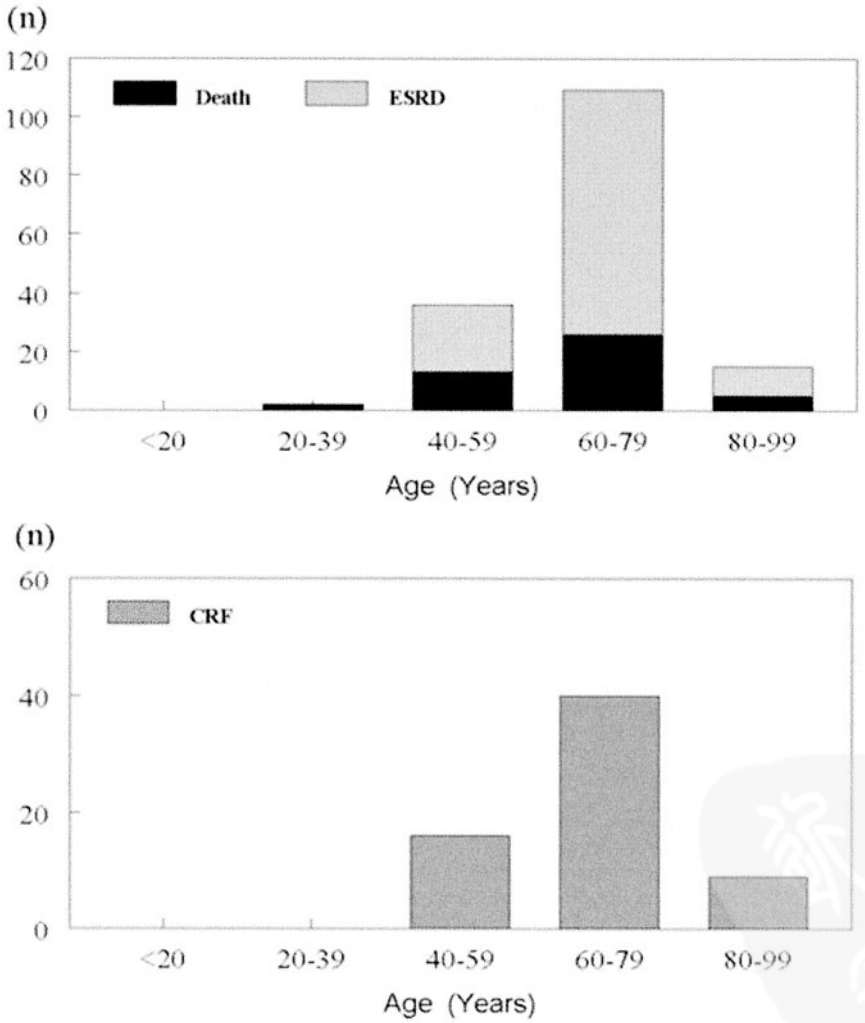

Figure 1. Frequency distribution of age of onset of end-stage renal disease (ESRD), death (without ESRD), and chronic renal failure (CRF) in the study patients. Typical of type 2 ADPKD, the mode of distribution of these events occurred at a relatively late age (age range, 60 to 79 yr). However, considerable renal disease variability (age range of onset of ESRD, 40 to 88 yr) was also evident between individual patients.

described (17–25), and the remaining mutations from 21 families are reported here for the first time. The diagnosis of ADPKD in the patients and at-risk individuals from each study family was established using well-established ultrasound-based criteria (26). Any affected individual with a concomitant renal disease (*e.g.*, diabetic nephropathy) was excluded from this study. The demographic, clinical, and laboratory data (including serum creatinine and/or creatinine clearance) were obtained from all the participants after obtaining informed consent. All the research protocols used in the study have been approved by the institutional review boards of all the participating centers.

Definitions of Primary Renal Outcome

Two renal disease outcomes were used in this study. End-stage renal disease (ESRD) is defined as severe chronic renal failure with a serum creatinine value $\geq$ 500 uM (or 5.7 mg/dl) or the requirement of renal replacement therapy (chronic dialysis or renal transplantation). Chronic renal failure (CRF) is defined as moderately severe chronic renal failure with a calculated creatinine clearance $\leq$ 50 ml/min per 1.73 m^2 by the formula of Cockroft and Gault (27). The study subjects were classified as hypertensive if their systolic or diastolic BP exceeded the 95th percentile predicted for age and gender or if they required anti-hypertensive treatment.

PKD2 Mutational Analyses

DNA isolation and mutation screening methods have been previously detailed (17–24). Single-stranded conformational polymorphism (SSCP), heteroduplex analysis (HA), or direct sequencing was employed to screen all 15 exons and their flanking intronic sequences of *PKD2* (28) for *PKD2* mutations in one definitively affected individual from each study family. Whenever possible, segregation of a specific mutation with ADPKD was tested within each family by SSCP, HA, restriction-digestion, or allele-specific oligonucleotide hybridization. All nonconservative missense changes identified were also tested to determine whether they were present in at least 100 normal chromosomes. Both strands of the PCR templates containing any variants were sequenced by the dideoxy terminator method using an ABI 373 or 377 DNA Sequencer (Applied Biosystems). *PKD2* mutations were considered 5' mutations if they were located in the first half of the open reading frame (nucleotides 1 to 1452), or 3' mutations if they were located in the second half of the open reading frame (nucleotides 1453 to 2904). Gross deletion, nonsense, and frameshift mutations were classified as truncating mutations, and missense and splice mutations were analyzed separately.

Statistical Analyses

Time from birth to ESRD (*i.e.*, renal death) was computed by the product-limit (*i.e.*, Kaplan-Meier) method of survival analysis. To assess the differences in renal survival (*i.e.*, freedom from ESRD) between specific patient groups of interest, a two-sided log-rank test was used. Additionally, time to ESRD plus death was analyzed as an additional outcome measure. The effects of the covariates (*i.e.*, gender and hypertension) on renal survival were tested using the univariate Cox proportional hazards model or the log-rank test for continuous or categorical variables, respectively (29). Linear regression analysis was also performed to assess the influence of the position of the *PKD2* mutations on the age of onset of ESRD and CRF separately. The running average of the age of onset of ESRD and CRF in Figure 2 was based on the best fitting cubic spline function of the age *versus* nucleotide relationship. For the linear regression and renal survival analyses, the results based on ESRD alone or ESRD plus death were not significantly different. Thus, only the results based on ESRD alone will be presented because this outcome measure provides a more accurate assignment of renal survival. All the analyses were performed using the SPSS version 11.0.1 (SPSS, Chicago, IL) and PRISM version 3.0 (GraphPad Software, Inc. San Diego, CA) statistical packages.

Results

Clinical Characteristics of Study Patients

We analyzed the clinical and genetic data of 461 affected individuals (44.2% male; 55.8% female) from 71 families in

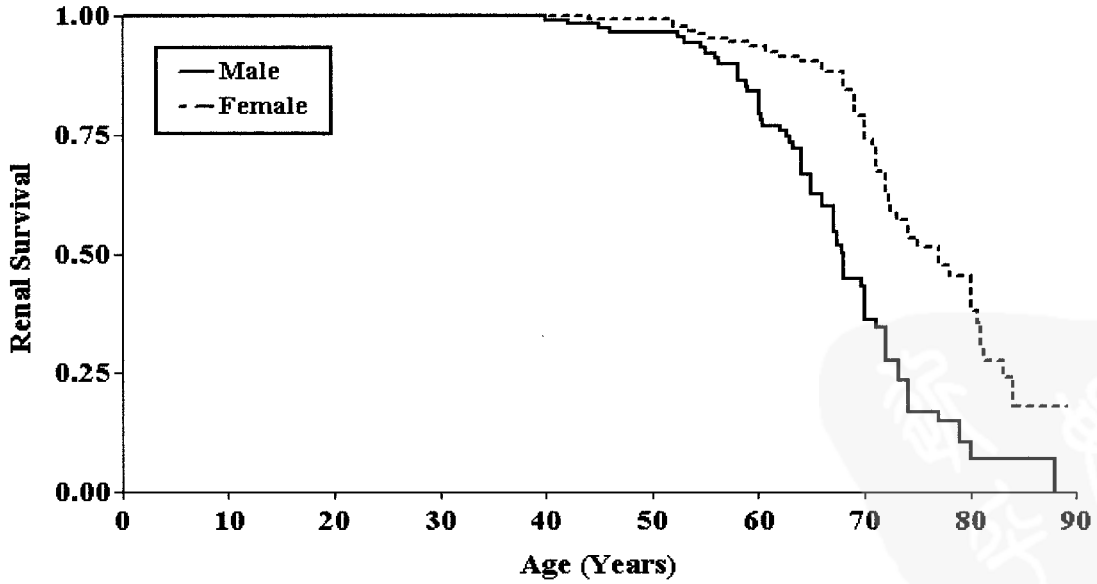

Figure 2. Renal survival (*i.e.*, absence of ESRD) analysis. The probability of renal survival differs significantly between female (n = 221) than male (n = 174) patients with *PKD2* mutations (χ^2 = 27.9; $P < 0.00005$ by log-rank test). On average, female patients developed ESRD 8 yr later than male patients with *PKD2* mutations.

Table 1. The spectrum of *PKD2* mutations in study families

Family	Exon	Nucleotide Sequence Change	Predicted Protein Change
1[g]	1 to 15	PKD2del	Gene deletion
2[a]	1	124ins52	Frameshift 42→108X
3[b]	1	125–128delC	Frameshift 43→116X
4[c]	1	InsC231	Frameshift 78→90X
5[b]	1	306–307delG	Frameshift 103→116X
6[d]	1	534–538insC	Frameshift 180→212X
7[c]	1	C556T	R186X
8[a]	1	IVS1+1G→A	Splicing defect
9[e]	2	InsC693	Frameshift 194→231X
10[c]	2	667 delG	Frameshift 222→232X
11[f]	4	857 delC	Frameshift 286→315X
12[a], 13[a]	4	C916T	R306X
14[c]	4	C958T	R320X
15[d]	4	972–973insC	Frameshift 325→340X
16[g]	4	G1066C	A356P
17[a]	4	IVS4+2T→G	Splicing defect
18[a]	4	IVS4+3del4	Splicing defect
19[a], 20[a]	4	IVS4+1→del4	Splicing defect
21[a]	5	T1158A	T386X
22[a], 23[d]	5	1194del2	Frameshift 399→407X
24[e]	5	C1213T	Q405X
25[d]	5	T1240G	W414G
26[b], 27[b], 28[c], 29[b]	5	C1249T	R417X
30[a], 31[a], 32[g], 33[g], 34[g]	5	IVS5+1G→A	Splicing defect
35[g], 36[g]	6	1335ins4	Frameshift 446→478X
37[g]	6	dup1369–1346	Frameshift 457→476X
38[d]	6	1362–1363delTT	Frameshift 455→460X
39[b], 40[c]	6	C1390T	R464X
41[d]	6	1445delT	Frameshift 482→513X
42[b]	6	1446–1149del4	Frameshift 482→513X
43[a]	7	C1609T	Q537X
44[h]	7	C1663T	Q555X
45[h]	7	1699del4T	Frameshift 567→582X
46[d]	7	IVS7-3C→G	Splicing defect
47[c]	8	C1753T	Q585X
48[a]	8	T1894C	C632R
49[a]	9	1998del4	Frameshift 666→672X
50[c]	10	2023–2024delAT	Frameshift 675→681X
51[h]	11	2151delG	Frameshift 717→735X
52[b], 53[c], 54[g]	11	2152–2159delA	Frameshift 720→736X
55[b], 56[b], 57[b]	11	2152–2159insA	Frameshift 720→724X
58[a], 59[e], 60[e]	11	C2224T	R742X
61[a]	12	IVS12-7T→A	Splicing defect
62[c]	13	C2419T	R807X
63[a]	13	G2420A	R807Q
64[i]	13	2436insT	L813X
65[c]	13	G2509T	E837X
66[g]	13	AG2511C	Frameshift 837→843X
67[g], 68[g]	14	C2533T	R845X
69[a], 70[a], 71[a]	14	C2614T	R872X

[a] Family described in this article for the first time.
[b] Family previously reported by Pei *et al.* (20).
[c] Family previously reported by Hateboer *et al.* (15).
[d] Family previously reported by Veldhuisen B *et al.* (18).
[e] Family previously reported by Demetriou *et al.* (22).
[f] Family previously reported by Aguiari *et al.* (21).
[g] Family previously reported by Torra *et al.* (24).
[h] Family previously reported by Viribay *et al.* (19).
[i] Family previously reported by Iglesias *et al.* (23).

which the germline *PKD2* mutations were characterized. Overall, 117 of these affected individuals (25.4%) had ESRD, 47 died without ESRD (10.2%), 65 (14.0%) had CRF, and 232 (50.3%) had neither CRF nor ESRD at the last follow-up. Although ruptured intracranial arterial aneurysm was the primary cause of death in at least seven affected individuals who died without ESRD, the cause of death was not available for the remaining 40 patients. BP information was available in 103 of 182 individuals with either ESRD or CRF, and hypertension was present in 68 (66%) of these 103 individuals. Figure 1 shows the frequency distribution of the study patents by their age of onset of ESRD, CRF, and death. Typical of type 2 ADPKD, the mode of distribution of these events occurred at a relatively late age (*i.e.*, age range of 60 to 79 yr). However, considerable renal disease variability (*e.g.*, age range of onset of ESRD: 40 to 88 yr) was also evident between individual patients. In general, female patients had milder renal disease than male patients (*i.e.*, the mean age of onset of ESRD and CRF was 76.0 [95% CI, 73.8 to 78.1] *versus* 68.1 [95% CI, 66.0 to 70.2] and 72.5 [95% CI, 70.1 to 74.9] *versus* 63.7 [95% CI, 61.4 to 66.0] yr, respectively). The renal survival (*i.e.*, absence of ESRD) curves also differed significantly between the two gender groups (Figure 2).

Spectrum of PKD2 Mutations

Fifty different mutations from 71 families were included in the current study (Table 1). These mutations spanned the entire *PKD2*, with the exception of exons 3 and 15, for which no mutations have been reported thus far. Among these 71 families, 27 (38.0%) had nonsense mutations, 27 (38.0%) had frameshift mutations, 12 (16.9%) had splice site mutations, 4 (5.6%) had missense mutations, and one (1.5%) had a complete deletion of *PKD2*. The four missense mutations (A356P; W414G; C632R; R807Q) included in this study all involved nonconservative amino acid changes, segregated only in the affected members of the same family and were not observed in at least 100 normal chromosomes. Several mutations recurred in apparently unrelated families. Of interest, a single nucleotide deletion or insertion of a polyadenosine tract (2152–2159delA and 2152–2159insA) on exon 11 accounted for frameshift mutations in six families. Additionally, the same splice site mutation in exon 5 (*i.e.* IVS5+1G→A) was responsible for the mutation in three Spanish and two Canadian families. The frequencies of various clinical events (*i.e.*, ESRD, CRF, and death) in the patients with different types of *PKD2* mutations are detailed in Table 2.

Genotype-Renal Function Correlation

We analyzed the correlation between the age of onset of ESRD and the nucleotide position of the *PKD2* mutation in the study patients (upper panel of Figure 3). Linear regression analysis showed a correlation coefficient (r) of 0.109 (r^2 = 0.012), and the slope of the regression line did not differ significantly from zero (slope value: 0.001 [95% CI, −0.001 to 0.004]). Similarly, we analyzed the correlation between the age of onset of CRF and the nucleotide position of the *PKD2* mutation in the study patients (upper panel of Figure 3). Linear regression analysis showed a correlation coefficient (r) of 0.036 (*i.e.*, r^2 = 0.001), and the slope did not differ significantly from zero (slope value: −0.0004 [95% CI, −0.004 to 0.003]). These results remained unchanged when the correlation was stratified by gender. At the last follow-up, 232 study patients had not developed either CRF or ESRD and a plot of their age by the position of the *PKD2* mutations is shown in the lower panel of Figure 3. Considerable renal disease variability was noted among patients with the same mutations. For example, five patients (*i.e.*, boxed in the upper panel of Figure 3) whose mutations clustered around the nucleotide position of 1000 to 1500 of the open reading frame of *PKD2*, had atypically severe renal disease (*i.e.*, ESRD before 50 yr of age). However, on close inspection they represented cases at one end of the spectrum of disease severity among patients with the same *PKD2* mutations (Figure 4). In contrast, several patients, who did not have CRF even after 70 yr of age (lower panel of Figure 3), represented cases with exceptionally mild disease.

We also analyzed the renal survival of the study patients by the types (*i.e.*, missense, truncating, and splice site) as well as location (*i.e.*, 5' or nt 1–1452 *versus* 3' or nt 1453–2904) of their *PKD2* mutations (Table 3; Figure 5). We found that neither the types (P = 0.26 by log-rank test; upper panel) nor the location (P = 0.78 by log-rank test; lower panel) of the *PKD2* mutations influence the renal survival of our patients. Gender was a significant determinant of renal survival; we therefore also repeated the survival analyses using univariate proportional-hazards models to include gender as a covariate. In the gender-adjusted analysis, we found that patients with splice site mutations appeared to have a more favorable renal survival compared with patients with other mutation types (*i.e.*,

Table 2. Distribution of patients with different clinical outcomes by *PKD2* mutation types[a]

	Gene Deletion	Nonsense Mutations	Missense Mutations	Splice Site Mutations	Frameshift Mutations	Total
Patients with ESRD	5	52	9	10	41	117
Patients who died without ESRD	0	18	1	4	24	47
Patients with CRF	0	23	2	12	28	65
Patients without CRF or ESRD	6	76	14	19	117	232
Total	11	169	26	45	210	461

[a] ESRD, end-stage renal disease; CRF, chronic renal failure.

$P = 0.046$; Figure 6). On the other hand, the 5' and 3' location of the *PKD2* mutations did not influence renal survival. Hypertension (*i.e.*, presence or absence), included as a covariate in univariate proportional-hazards models, did not change our findings.

Discussion

In this study, we have examined the genotype-renal function correlation in a large cohort of patients from families with known *PKD2* mutations. Consistent with the published literature (3,15), we found in our study patients a late age of onset of ESRD (*i.e.*, mean age of 72 yr) and a strong gender effect on renal disease severity (*i.e.*, $P < 0.00005$ by log-rank test). The mutations identified in the study families, spanning between exons 1 and 14, also covered the entire spectrum of *PKD2* mutations reported to date (25). Several of these mutations are of particular interest (Table 1). For example, the four missense mutations (A356P; W414G; C632R; R807Q) included in this study all involved nonconservative amino acid changes, segregated only in the affected members of the family, and were

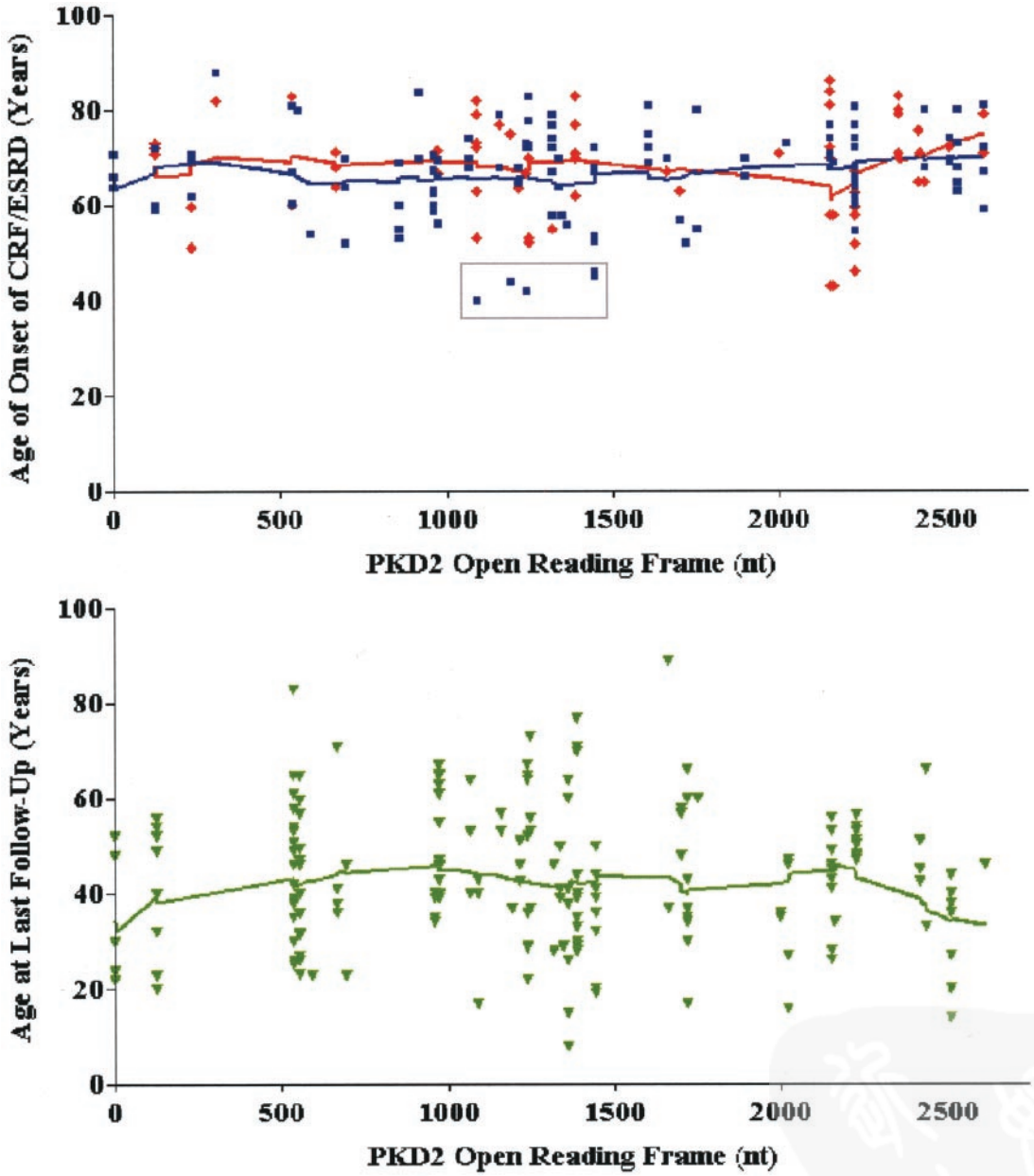

Figure 3. Linear regression analysis of renal disease severity and nucleotide position of *PKD2* mutations in the study patients. The slope of the regression line for patients with ESRD (blue square) and CRF (red diamond) did not significantly differ from zero (upper panel). Considerable disease variability was noted among patients with the same mutations. For example, five patients with four different *PKD2* mutations (boxed in the upper panel) had atypically severe disease with ESRD before 50 yr of age. However, these patients represented discordant cases among patients with the same *PKD2* mutation (see Figure 4). In contrast, several patients (green triangles in lower panel), who did not have CRF even after 70 yr of age, represented cases with exceptionally mild disease.

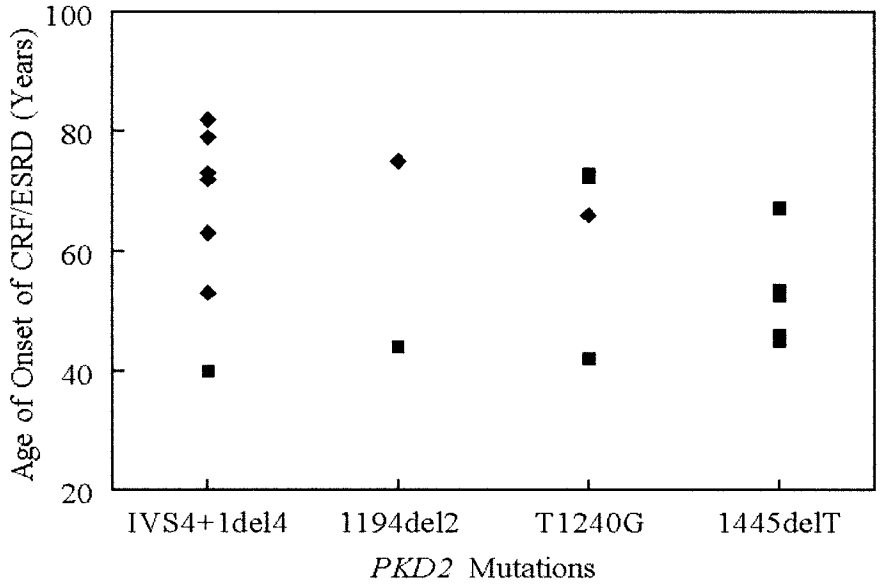

Figure 4. Significant renal disease variability among patients with the same *PKD2* mutations. Five patients (see Figure 3), whose mutations clustered around the nucleotide position of 1000 to 1500 of the open reading frame of *PKD2*, had atypically severe renal disease. However, they represented discordant cases among patients with the same *PKD2* mutations. One or more older patients with the same mutations had mild renal disease typical of type 2 ADPKD (square denotes ESRD; diamond denotes CRF).

Table 3. Time to ESRD or ESRD plus death by gender and mutation location[a]

	Mean Time (yr)	Median Time (yr)
ESRD		
overall	72.3 (70.7 to 73.9)	72.0 (70.7 to 73.3)
male	68.1 (66.0 to 70.2)	68.0 (65.4 to 70.6)
female	76.0 (73.8 to 78.1)	77.0 (71.9 to 82.1)
mutations 5′ to nt 1452	72.2 (69.8 to 74.6)	71.0 (68.7 to 73.3)
mutations 3′ to nt 1453	72.5 (70.3 to 74.6)	72.0 (69.2 to 74.8)
ESRD + death		
overall	70.7 (69.3 to 72.1)	71.0 (69.9 to 72.1)
male	67.3 (65.5 to 69.1)	67.4 (66.0 to 68.8)
female	73.9 (71.9 to 75.8)	74.0 (70.3 to 77.7)
mutations 5′ to nt 1452	70.0 (68.0 to 72.0)	70.0 (68.5 to 71.5)
mutations 3′ to nt 1453	71.5 (69.6 to 73.5)	72.0 (69.8 to 74.2)

[a] Mean and median time (95% confidence interval) from Kaplan-Meier survival analyses.

not observed in the normal population. They are also predicted to change within the mutant polycystin 2–specific amino acids that are evolutionarily conserved between different organisms (data not shown). However, structural-functional analyses have not been performed on these mutations. Nonetheless, W414G, by virtue of its location within the polycystin domain, may disrupt the dimerization of polycystin 2 with itself and with polycystin 1 (12,14). In contrast, C632R and R807Q, located in the pore region and endoplasmic reticulum retention domain, may disrupt the channel function of polycystin 2 (11,14). It is also conceivable that some of these missense changes may in fact result in aberrant splicing, as recently described in another *PKD2* mutation: nt A2657G (30). Of the recurring mutations, two involving an insertion or deletion of a polyadenosine tract (*i.e.*, 2152–2159delA and 2152–2159insA) are of interest. These mutations were observed in six apparently unrelated families from different geographical regions, suggesting that the polyadenosine tract ($p(A)_8$: nt 2152–2159) on exon 11 is a regional hot spot within *PKD2* that predisposes to both germline (Table 1) and somatic mutations (31), presumably by "slipped strand mispairing" (20).

The location but not the types of *PKD1* mutations has been recently reported to influence renal disease severity in a large cohort of patients with type 1 ADPKD (16). Specifically, patients with mutations localized to the 5' half of *PKD1* had an earlier onset of ESRD compared with patients with mutations localized to the 3' half of the gene (*i.e.*, median age of onset of ESRD: 53 *versus* 56 yr, respectively; $P = 0.025$). However,

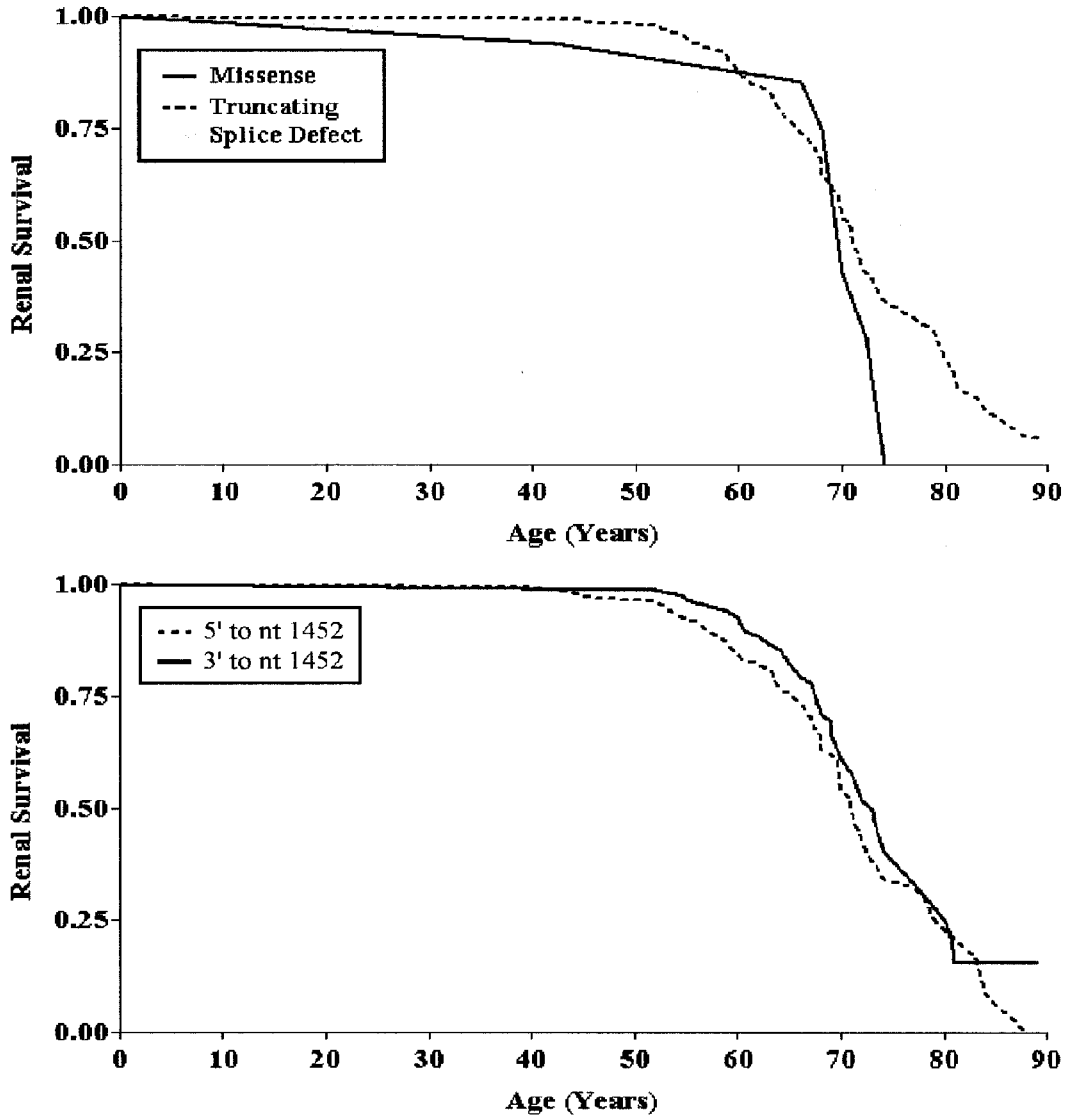

Figure 5. Renal survival (*i.e.*, absence of ESRD) of the study patients by the types (*i.e.*, missense, truncating, or splice site mutations) and location (*i.e.*, 5' half *versus* 3' half) of their *PKD2* mutations. Neither the types ($P = 0.26$ by log-rank test; upper panel) nor the location ($P = 0.78$ by log-rank test; lower panel) of the mutations appeared to influence the renal survival of the study patients.

this effect is weak compared with the locus effect between *PKD1* and *PKD2* mutations (15). Given that most *PKD1* mutations are predicted to be protein-truncating, these data have led to the suggestion that mutations in the 3'-end of this gene may result in partially functional gene products (16). In the current study, we were unable to detect a position effect in our patients by either linear regression or renal survival analyses. With a large patient sample size and adjustment for the gender effect, we believe that our analyses are robust. Most *PKD2* mutations are also predicted to truncate the C'-terminus of polycystin 2; it therefore appears that the loss of the coil-coil interaction region with polycystin 1 (*i.e.*, the most distal C'-terminal functional domain so far identified) may be sufficient to completely inactivate the mutant protein (11,12,14). Recent human (31–33) and knockout mouse (34,35) studies have suggested that a common mechanism for individual cyst formation in ADPKD results from the inactivation of *PKD1* or *PKD2* within an epithelial cell, through germline and somatic mutations. Our data are consistent with this classical two-hit model of cystogenesis in ADPKD and suggest that most *PKD2* mutations are completely inactivating.

In contrast to the lack of correlation between the mutation types and renal disease severity seen in type 1 ADPKD (16), we found that our patients with *PKD2* splice site mutations appeared to have milder renal disease compared with patients with other mutation types ($P = 0.046$ by log-rank test; Figure 6). Among the families with the splice site mutations, one family (family 61; IVS12–7T→A; Table 1) had notably mild disease, with five elderly affected individuals developing only CRF at age 70, 71, 79, 80, and 83 yr, respectively. It is conceivable that certain splice site mutations may be "leaky," so that low levels of a normal protein product can still be

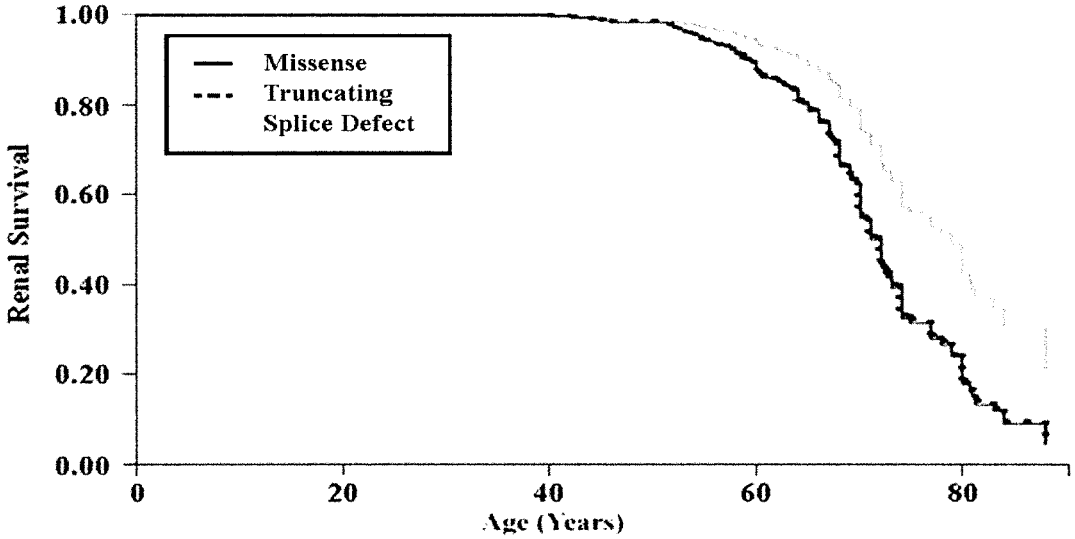

Figure 6. Gender-adjusted renal survival analysis suggests a more favorable outcome in patients with splice site mutations. Univariate Cox proportionate hazards analysis with gender included as a covariate indicates that patients with splice site mutations may have a more favorable renal survival (*i.e.*, absence of ESRD) than patients with missense and truncating mutations ($P = 0.046$).

generated by the mutant allele. Should this be the case, a threshold model for polycystin 2 within individual epithelial cells of patients with such mutations may provide an additional mechanism for the cystogenic process. Under this model, individual cells with low levels of a functional polycystin may be triggered into a cystogenic pathway by local stresses and other stochastic factors (36,37). Alternatively, it is possible that the above observation may be spurious, given the borderline statistical association and the presence of a significant "modifier effect" (see below).

The most definitive conclusion of our study is that significant renal disease variability exists among the patients with the same *PKD2* mutations (Figures 1 and 3). Within individual families, we have observed both elderly patients with very mild renal disease and younger patients with ESRD (Figure 4). This is consistent with the finding of significant renal disease variability within families with *PKD1*-linkage (38) and among patients with the same *PKD1* mutations (16). Taken together, these data suggest the existence of a modifier effect for ADPKD (17,38,39). Recent studies have shown that the phenotypic variability of a number of Mendelian disorders is in fact complex because of the existence and interaction of genetic and environmental modifiers (40). Some recent examples include cystic fibrosis (41) and Hirschsprung disease (42), in which one or more modifier loci/genes have been implicated from both animal models and patients. Indeed, several population-based studies have recently examined the polymorphic variants of the angiotensin-converting enzyme (ACE) (16,43–46) and endothelial nitric oxide synthase (eNOS) (47,48) genes as modifiers of renal disease progression in ADPKD. However, these studies are limited by their research study design and small patient sample size, and they have produced conflicting results. Future studies using a family-based research design (49) and properly-powered patient sample size will be required to dissect the individual components of this modifier effect. The identification of specific genetic and environmental modifiers will have important relevance for individual patient prognostication and mechanism-based therapy in ADPKD.

Acknowledgments

We are indebted to all the participating members of the ADPKD families. Supported by grants from Polycystic Kidney Research Foundation, Canadian Institutes of Health Research (MOP53324), and Kidney Foundation of Canada (Y.P.); Department of Health and Human Services, Public Health Service, USA, and General Clinical Research Centers Program of the Division of Research Resources, National Institutes of Health, USA (A.J. and P.G.); Cyprus Kidney Association (C.D.).

Electronic Database Information

The accession number and URL for data in this article are as follows:

Online Mendelian Inheritance of Man (OMIM): http://www.ncbi.nim.nih.gov/Omim (for PKD [MIM173900]; *PKD1* [MIM 601313]; *PKD2* [MIM 173910]; and PKD3 [MIM 600666]).

References

1. Gabow PA: Autosomal dominant polycystic kidney disease. *N Engl J Med* 329: 332–342, 1993
2. Harris PC: Autosomal dominant polycystic kidney disease: Clues to pathogenesis. *Hum Molec Genet* 8: 1861–1869, 1999
3. Parfrey PS, Bear JC, Morgan J, Cramer BC, McManamon PJ, Gault MH, Churchill DN, Singh M, Hewitt R, Somlo S, Reeders S: The diagnosis and prognosis of autosomal dominant polycystic kidney disease. *N Engl J Med.* 323: 1085–1090, 1990
4. Ravine D, Walker RG, Gibson RN, Forrest SM, Richards RI, Friend K, Sheffield LJ, Kincaid-Smith P, Danks DM: Phenotype and genotype heterogeneity in autosomal dominant polycystic kidney disease. *Lancet* 340: 1330–1333, 1992
5. 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
6. International Polycystic Kidney Disease Consortium: Polycystic kidney disease: The complete structure of the PKD1 gene and its protein. *Cell* 81: 289–298, 1995
7. Mochizuki T, Wu G, Hayashi T, Xenophontos SL, Veldhuisen B, Saris JJ, Reynolds DM, Cai Y, Gabow PA, Pierides A, Kimberling WJ, Breuning MH, Deltas CC, Peters DJ, Somlo S: PKD2, a gene for polycystic kidney disease that encodes an integral membrane protein. *Science* 272: 1339–1342, 1996
8. 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
9. Paterson A, Pei Y: A third gene for autosomal dominant polycystic kidney disease? *Kidney Int* 54: 1759–1761, 1998
10. Peters DJ, Sandkuijl LA: Genetic heterogeneity of polycystic kidney disease in Europe. *Contrib Nephrol* 97: 128–139, 1992
11. Somlo S, Ehrlich B: Human disease: Calcium signaling in polycystic kidney disease. *Curr Biol* 11: R356–R360, 2001
12. Hanaoka K, Qian F, Boletta A, Bhunia AK, Piontek K, Tsiokas L, Sukhatme VP, Guggino WB, Germino GG: Co-assembly of polycystin-1 and -2 produces unique cation-permeable currents. *Nature* 408: 990–994, 2000
13. Igarashi P, Somlo S: Genetics and pathogenesis of polycystic kidney disease. *J Am Soc Nephrol* 13: 2384–2398, 2002
14. Koulen P, Cai Y, Geng L, Maeda Y, Nishimura S, Witzgall R, Ehrlich BE, Somlo S: Polycystin-2 is an intracellular calcium release channel. *Nat Cell Biol* 4, 191–197, 2002
15. Hateboer N, v 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. European PKD 1:PKD 2 Study Group. *Lancet* 353: 103–107, 1999
16. Rossetti S, Burton S, Strmecki L, Pond G, San Millan J, Zerres K, Barratt TM, Ozen S, Torres V, Bergstralh EJ, Winearls C, Harris PC: The position of the polycystic kidney disease 1 gene mutation correlates with the severity of renal disease. *J Am Soc Nephrol* 13: 1230–7, 2002
17. Hateboer N, Veldhuisen B, Peters D, Breuning MH, San-Millan JL, Bogdanova N, Coto E, van Dijk MA, Afzal AR, Jeffery S, Saggar-Malik AK, Torra R, Dimitrakov D, Martinez I, de Castro SS, Krawczak M, Ravine D: Location of mutations within the PKD2 gene influences clinical outcome. *Kidney Int* 57: 1444–1451, 2000
18. Veldhuisen B, Saris JJ, de Haij S, Hayashi T, Reynolds DM, Mochizuki T, Elles R, Fossdal R, Bogdanova N, van Dijk MA, Coto E, Ravine D, Norby S, Verellen-Dumoulin C, Breuning MH, Somlo S, Peters DJ: A spectrum of mutations in the second gene for autosomal dominant polycystic kidney disease. *Am J Hum Genet* 61: 547–555, 1997
19. Viribay M, Hayashi T, Telleria D, Mochizuki T, Reynolds DM, Alonso R, Lens XM, Moreno F, Harris PC, Somlo S, San Millan JL: Novel stop and frameshifting mutations in the autosomal dominant polycystic kidney disease 2 gene. *Hum Genet* 101: 229–234, 1997
20. Pei Y, He N, Wang K, Kasenda M, Paterson AD, Chan G, Liang Y, Roscoe J, Brissenden J, Hefferton D, Parfrey P, Somlo S, St George-Hyslop P: A spectrum of mutations in the polycystic kidney disease-2 gene from eight Canadian kindreds. *J Am Soc Nephrol* 9: 1853–1860, 1998
21. Aguiari G, Manzati E, Penolazzi L, Micheletti F, Augello G, Vitali ED, Cappelli G, Cai Y, Reynolds D, Somlo S, Piva R, del Senno L: Mutations in autosomal dominant polycystic kidney disease 2 gene: Reduced expression of PKD2 protein in lymphoblastoid cells. *Am J Kidney Dis* 33: 880–885, 1999
22. Demetriou K, Tziakouri C, Anninou K, Eleftheriou A, Koptides M, Nicolaou A, Deltas C, Pierides A: Autosomal dominant polycystic kidney disease-type 2. Ultrasound, genetic and clinical correlations. *Nephrol Dial Transplant* 15: 205–211, 2000
23. Iglesias DM, Telleria D, Viribay M, Herrera M, Bernath VA, Kornblihtt AR, Martin RS, Millan JL: A novel frameshift mutation (2436insT) produces an immediate stop codon in the autosomal dominant polycystic kidney disease 2 gene. *Nephrol Dial Transplant* 15: 477–480, 2000
24. 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
25. Deltas CC: Mutations of the human polycystic kidney disease 2 (PKD2) gene. *Hum Mutat* 18: 13–24, 2001
26. Ravine D, Gibson RN, Walker RG, Sheffield LJ, Kincaid-Smith P, Danks DM: Evaluation of ultrasonographic diagnostic criteria for autosomal dominant polycystic kidney disease 1. *Lancet* 343: 824–827, 1994
27. Cockcroft DW, Gault MH: Prediction of creatinine clearance from serum creatinine. *Nephron* 16: 31–41, 1976
28. Hayashi T, Mochizuki T, Reynolds D, Wu Y, Cai Y, Somlo S: Characterization of the exon structure of the Polycystic Kidney Disease2 gene. *Genomics* 44: 131–136, 1997
29. Cox DR: Regression models and life-tables. *J Royal Statist Soc* 34: 187–220, 1972
30. Reynolds D, Hayashi T, Cai YQ, Veldhuisen B, Watnick T, Lens X, Mochizuki T, Qian F, Maeda Y, Li L, Fossdal R, Coto E, Wu GQ, Breuning M, Germino G, Peters D, Somlo S: Aberrant splicing in the PKD2 gene as a cause of polycystic kidney disease. *J Am Soc Nephrol* 10: 2342–2351, 1999
31. Watnick T, He N, Wang KW, Liang Y, Parfrey P, Hefferton D, St. George-Hyslop P, Germino G, Pei Y: Somatic mutations in *PKD1* in ADPKD2 tissue suggest a possible pathogenic role of trans-heterozygous mutations. *Nature Genet* 25: 143–144, 2000
32. 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
33. Koptides M, Hadjimichael C, Koupepidou P, Pierides A, Constantinou Deltas C: Germinal and somatic mutations in the PKD2 gene of renal cysts in autosomal dominant polycystic kidney disease. *Hum Mol Genet* 8: 509–513, 1999
34. Lu W, Shen X, Pavlova A, Lakkis M, Ward CJ, Pritchard L, Harris PC, Genest DR, Perez-Atayde AR, Zhou J: Comparison of *Pkd1*-targeted mutants reveals that loss of polycystin-1 causes cystogenesis and bone defects. *Hum Mol Genet* 10: 2385–2389, 2001
35. Wu GQ, D'Agati V, Cai Y, Markowitz G, Park JH, Reynolds D, Maeda Y, Le TC, Hou H, Kucherlapati R, Edelmann W, Somlo S: Somatic inactivation of *Pkd2* results in polycystic kidney disease. *Cell* 93: 177–188, 1998
36. Wilkie A: The molecular basis of genetic dominance. *J Med Genet* 31: 89–98, 1994
37. 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
38. Hateboer N, Lazarou LP, Williams AJ, Holmans P, Ravine D: Familial phenotype differences in PKD1. *Kidney Int* 56: 34–40, 1999
39. Pei Y: A "two-hit" model of cystogenesis in autosomal dominant polycystic kidney disease? *Trends Mol Med* 7: 151–156, 2001

40. Dipple KM, McCabe ER: Modifier genes convert "simple" Mendelian disorders to complex traits. *Mol Genet Metab* 71: 43–50, 2000
41. Zielenski J, Corey M, Rozmahel R, Markiewicz D, Aznarez I, Casals T, Larriba S, Mercier B, Cutting GR, Krebsova A, Macek M Jr, Langfelder-Schwind E, Marshall BC, DeCelie-Germana J, Claustres M, Palacio A, Bal J, Nowakowska A, Ferec C, Estivill X, Durie P, Tsui LC: Detection of a cystic fibrosis modifier locus for meconium ileus on human chromosome 19q13. *Nat Genet* 22: 128–129, 1999
42. Carrasquillo MM, McCallion AS, Puffenberger EG, Kashuk CS, Nouri N, Chakravarti A: Genome-wide association study and mouse model identify interaction between RET and EDNRB pathways in Hirschsprung disease. *Nat Genet* 32: 237–244, 2002
43. 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
44. Perez-Oller L, Torra R, Bandenas C, Mila M, Darnell A: Influence of the angiotensin converting enzyme polymorphism in the progression of renal failure in autosomal dominant polycystic kidney disease. *Am J Kidney Dis* 34: 273–278, 1999
45. van Dijk MA, Breuning MH, Peters DJ, Chang PC: The ACE insertion/deletion polymorphism has no influence on progression of renal function loss in autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant* 15: 836–839, 2000
46. Schiavello T, Burke V, Bogdanova N, Jasik P, Melsom S, Boudville N, Robertson K, Angelicheva D, Dworniczak B, Lemmens M, Horst J, Todorov V, Dimitrakov D, Sulowicz W, Krasniak A, Stompor T, Beilin L, Hallmayer J, Kalaydjieva L, Thomas M: Angiotensin-converting enzyme activity and the ACE Alu polymorphism in autosomal dominant polycystic kidney disease. *Nephrol Dial Transplant* 16: 2323–2327, 2001
47. 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
48. 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
49. Martin ER, Monks SA, Warren LL, Kaplan NL: A test for linkage and association in general pedigrees: the pedigree disequilibrium test. *Am J Hum Genet* 67: 146–154, 2000