Progressive Loss of Renal Function Is an Age-Dependent Heritable Trait in Type 1 Autosomal Dominant Polycystic Kidney Disease

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Significant intrafamilial phenotypic variability is well documented in autosomal dominant polycystic kidney disease (ADPKD) and suggests a modifier effect. In this study, variance components analysis was performed to estimate the contribution of genetic factors for within-family renal disease variability in 406 patients from 66 type 1 ADPKD families. Overall, 39% of the study patients had ESRD at their last follow-up, and their renal survival did not differ by gender ($P = 0.35$, log-rank test). Because their frequency plot of creatinine clearance (Ccr) assumed a bimodal distribution with a marked kurtosis that was not improved by transformations, the study cohort was decomposed into two separate groups (non-ESRD [$n = 247$] and ESRD [$n = 159$]) in which the Ccr plots were normally distributed. The heritability (h^2) of Ccr and age at ESRD (age_{ESRD}) and the genetic correlations between these measures and their covariates were estimated. In patients without ESRD, a significant heritability was found for Ccr ($h^2 = 0.42$; $P = 0.0015$) after adjusting for age ($P = 0.0001$), systolic BP ($P = 0.0006$), and treatment with angiotensin-converting enzyme inhibitor/angiotensin receptor blocker ($P = 0.00001$). Birth year, gender, BMI, diastolic and mean BP, and pack-years of cigarette smoking did not significantly influence the heritability of this trait. In patients with ESRD, age_{ESRD} provides a better measure than Ccr, which was very narrowly distributed. A significant heritability was found for age_{ESRD} ($h^2 = 0.78$; $P = 0.00009$) in these latter patients. None of the above covariates influenced the heritability of this trait. It is concluded that a significant modifier gene effect influences the progression of renal disease in type 1 ADPKD.

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Autosomal dominant polycystic kidney disease (ADPKD) (MIM 173900) is the most common hereditary kidney disorder with an incidence of approximately 1/1000 live births and accounts for approximately 5 to 8% of ESRD (1,2). It is characterized by 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 nonrenal organs, cardiac valvular defects, colonic diverticulosis, and intracranial arterial aneurysms, accompany the renal disease variably. Two genes (*PKD1* [MIM 601313] and *PKD2* [MIM 173910]) have been identified and respectively account for the disease in approximately 80 to 85% and approximately 10 to 15% of families in the white population (3,4). Polycystin 1 and 2, the gene products of *PKD1* and *PKD2*, are transmembrane proteins that are thought to be components of a novel signaling pathway that regulates intracellular calcium (2,4). Polycystin 1 is predicted to have a receptor-like structure and may be involved in cell–cell and/or cell–matrix interaction (4). By contrast, polycystin 2 is thought to function as a cation ion channel subunit with nonselective permeability (4). Both proteins have been shown to interact *in vitro* through their cytoplasmic region (2,4) and transmit fluid flow–mediated mechanosensation by the primary cilium in renal epithelium (4,5). Disruption of the function of polycystin 1 or 2 may cause ADPKD owing to the inability of the tubular epithelial cells to sense mechanical cues that normally regulate tissue morphogenesis (4,5).

Disease progression of ADPKD is highly variable, with the age at onset of ESRD ranging from childhood to old age (1,2). Gene locus effect is a major determinant for interfamilial disease variability: patients from *PKD1*-linked families have a much earlier onset of ESRD than patients from *PKD2*-linked families (median age, 53 [95% confidence interval (CI), 51.2 to 54.8] vs. 69 [95% CI, 66.9 to 71.3] years) (6,7). A gender effect on renal survival (*i.e.*, absence of ESRD) favoring the female patients is also evident in type 2 but in not type 1 ADPKD (7–9). In addition, allelic heterogeneity may have a weak effect on renal disease progression in type 1 (8) but not type 2 ADPKD

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(9). However, significant intrafamilial renal disease variability has been well documented in both type 1 and 2 ADPKD (9–11). These latter findings suggest that renal disease progression in ADPKD may be modified by genetic, environmental, and stochastic factors independent of the germline PKD mutations. Nevertheless, the magnitude of the genetic effects for within-family renal disease variability in ADPKD is not currently known. In the present study, we performed variance components analysis to quantify the contribution of genetic factors for within-family renal disease variability in type 1 ADPKD. In addition, we estimated the sample size required for both candidate gene association and genome-wide linkage studies as a first step toward the mapping of genetic modifiers for type 1 ADPKD.

Materials and Methods

Study Patients

We studied 66 multiplex type 1 ADPKD families from Toronto, Newfoundland, and Denver consisting of 554 affected and 317 unaffected family members. In each family, we had detailed information on the pedigree structure over at least three generations. In addition, we genotyped these families with at least three polymorphic simple-sequence repeat markers at each of the *PKD1* and *PKD2* loci. An autosomal dominant model from a single locus with a disease allele frequency of 0.01 was assumed. Two-point linkage analysis was performed using the MLINK program of the FASTLINK package. Most of these families were found to have significant evidence for *PKD1* linkage (Table 1). In all of the smaller families, inspection of the *PKD1* and *PKD2* haplotypes showed that the disease was inconsistent with *PKD2* linkage. The assignment of disease status in the at-risk individuals was based on both genotype data and ultrasonographic findings using age-dependent criteria (12). The study cohort used for the quantitative genetic analysis consisted of 406 affected individuals in whom demographic, clinical, and laboratory data were available. Eighty-one percent (120 of 148) of the affected individuals who were excluded were born before 1930. The research protocols for the study were approved by the Institutional Review Boards of the participating centers.

Clinical Assessment

We collected in the study patients their age, gender, height, weight, systolic (SBP) and diastolic BP (DBP), and antihypertensive treatment including treatment with an angiotensin-converting enzyme inhibitor (ACEI) or angiotensin receptor blocker (ARB). In addition, we assessed their cumulative exposure (pack-years) to cigarette smoking. Serum creatinine was measured using standard Technicon methods on a SMAC II analyzer (Technicon, Tarrytown, NY) with similar reference ranges in all three centers. Two primary renal disease outcomes were used in this study. Creatinine clearance (Ccr) was calculated on the basis of age, gender, body weight, and serum creatinine using the formula of Cockroft and Gault (13) and normalized to 1.73 m^2 body surface area. Age of onset at ESRD (age_{ESRD}) was defined as the age at initiation of renal replacement therapy (chronic dialysis or renal transplantation). Any patient with concomitant renal disease (*e.g.*, diabetic nephropathy) was excluded from this study.

Statistical Analyses

Continuous variables are expressed as mean ± SD, and categorical data are expressed as proportions. Time from birth to ESRD (renal death) was computed by the product-limit method of survival analysis using the SAS statistical package v8 (Carey, NC). To assess the effects of gender on renal survival (freedom from ESRD), we used a two-sided log-rank test (14). Heritability (h^2) in this study is broadly defined as the proportion of total phenotypic variability of a trait that is attributable to genetic effects (15). We estimated the heritability of Ccr and age_{ESRD} using a pedigree-based variance components approach (15–17). This novel approach of quantitative genetic analysis allows for the integration of phenotype and genotype data from multiple affected relative pairs from large extended pedigrees and is more powerful than the traditional sibling pair approach (15,17). With this approach, a quantitative trait is assumed to have a multivariate normal distribution and the relative components of the variance are estimated using maximum-likelihood analysis. The total phenotypic variability of the study trait is partitioned into three components: (*1*) an additive genetic variance, as a result of the sum of the average effects of all of the genes that influence the trait variance; (*2*) a shared environmental variance, caused by the effects of environmental factors common to the households; and (*3*) a random environmental variance that is specific for each individual. The random environmental variance also absorbs nonadditive genetic effects, such as interactions between alleles within loci (dominance effects), interactions between alleles at different loci (epistatic effects), and effects caused by gene–environment interactions. Thus, in general, the above models provide an underestimation of the genetic contribution to the trait variance (16,17). For age_{ESRD}, we also fitted a Cox proportional hazard model and derived Martingale residuals from the Cox regression to be used as a quantitative trait in the variance components analysis. All of the affected individuals within a pedigree were connected through their common ancestors, and the phenotypes of any affected and unaffected individuals who were not analyzed in the study were coded as unknown. We also corrected for ascertainment bias by conditioning on the likelihood of observing the trait value of the proband in each family. All of the covariates screened with a $P < 0.1$ were forced in the model. The statistical genetic analyses were performed using the computer software SOLAR v2.1.2 (15).

Results

Clinical Characteristics of Study Patients

The clinical characteristics of our study patients are shown in Table 2. Of the entire patient cohort, 47% (190 of 406) were male and 68% (276 of 406) had hypertension at the last follow-up. Among the hypertensive patients, 50% (138 of 276) of them were treated with an ACEI or ARB. The mean BP at the last follow-up did not differ significantly between our male and female patients. Overall, approximately 36% (144 of 406) of our patients were (current or ex-) cigarette smokers, and there was a trend for more male smokers (two-tailed $P = 0.098$ by Fisher exact test). The cumulative dose of cigarette smoking for the entire cohort was 6.6 ± 13 pack-years and for the smokers was 18 ± 6 pack-years.

At the last follow-up, the Ccr of the study patients was 55 ± 43 ml/min per 1.73 m^2, and 39% (159 of 406) of them had ESRD. The patients who developed ESRD on average were 12 yr older than those without ESRD. The median age at onset of ESRD in the male and female patients were 54 (95% CI, 52 to 56) and 53 (95% CI, 51 to 57) years, respectively. We found that the probability of renal survival did not differ by gender in the study patients ($\chi^2 = 0.88$, $P = 0.35$ by log-rank test; Figure 1). These findings are consistent with the results of two large recent studies showing an absence of gender effect on the renal survival of patients with type 1 ADPKD (7,8). We also found that

Table 1. Genetic linkage data for the study families[a]

Family No.	Family Size		Genetic Evidence of *PKD1* Linkage	
	Affected[b]	Unaffected	LOD Score (*PKD1*)[c]	LOD Score (*PKD2*)[d]
1	6	3	1.8	<−2
2	8	3	2.1	<−2
3	6	4	1.93	<−2
4	5	5	1.5	<−2
5	5	3	1.2	−0.74
6	8	5	2.4	<−2
7	4	4	0.99	−1.06
8	4	3	0.75	−0.66
9	4	5	1.05	−1.35
10	5	4	1.15	−0.66
11	11	8	4.8	<−2
12	10	6	3.45	<−2
13	6	3	1.15	<−2
14	4	3	0.75	−1.26
15	6	3	1.2	−0.89
16	6	3	1.2	<−2
17	10	6	3.6	<−2
18	4	3	0.75	−0.90
19	6	4	1.45	<−2
20	4	3	0.75	−1.68
21	5	4	0.99	−0.23
22	5	3	0.99	−0.76
23	7	3	1.85	<−2
24	6	4	1.5	<−2
25	4	2	0.5	−1.72
26	4	3	0.75	−0.81
27	6	3	1.15	<−2
28	7	2	1.35	<−2
29	5	2	0.99	−0.49
30	4	5	0.99	<−2
31	12	4	3.3	<−2
32	5	3	0.9	−1.27
33	7	3	1.85	<−2
34	10	4	2.75	<−2
35	9	5	1.85	<−2
36	11	7	3.6	<−2
37	40[e]	8	6.6	<−2
38	6	4	1.25	<−2
39	5	3	1.93	<−2
40	9	6	3.44	<−2
41	6	3	1.79	<−2
42	6	4	1.95	<−2
43	9	3	2.17	<−2
44	6	3	1.78	<−2
45	5	3	1.15	−0.81
46	10	5	3.16	<−2
47	8	4	2.93	<−2
48	7	2	1.62	−1.29
49	6	3	1.15	−0.56
50	6	5	1.48	−0.48
51	9	6	3.43	<−2
52	6	3	1.12	−0.23
53	8	3	1.68	<−2
54	4	3	0.9	−0.49
55	4	4	0.99	−0.56
56	9	3	2.35	<−2
57	4	4	0.99	−1.37
58	5	4	1.36	−0.99
59	8	5	2.33	<−2
60	3	4	0.99	−1.27
61	11	3	3.25	<−2
62	4	3	1.15	−0.56
63	9	4	2.86	<−2
64	7	3	1.51	<−2
65	15	4	3.65	<−2
66	9	4	2.75	<−2

[a]LOD, logarithm of odds.
[b]Genotyped individuals without clinical and laboratory data were excluded from the heritability study.
[c]Maximal two-point linkage score from *PKD1* simple-sequence repeat (SSR) markers.
[d]Minimal two-point linkage score from *PKD2* SSR markers.
[e]The LOD scores presented here were calculated base on 26 affected individuals; the disease status in the remaining at-risk individuals was determined by restriction analysis of *PKD1* mutation (IVS39-25del 72bp; FS3755→3798X).

the frequency plot of Ccr values in our study patients fit a bimodal distribution, with the two modes reflecting patients with and without ESRD (Figure 2).

Heritability Estimates for Renal Disease Progression

We found a high heritability for Ccr (age- and gender-adjusted $h^2 = 0.69$, SE = 0.13; $P = 8.1 \times 10^{9}$) in our study cohort ($n = 406$). However, as we noted above, the frequency plot of Ccr deviated significantly from the expected normality (Figure 2). In an alternative approach, we derived Martingale residuals from a Cox regression model for age_{ESRD}. Although we also found a significant heritability for the Martingale residuals of age_{ESRD} (age- and gender-adjusted $h^2 = 0.38$, SE = 0.08; $P = 1.1 \times 10^{-8}$), there was marked kurtosis in this outcome as well. We attempted various transformations on these data sets, which generally improved the skewness but not kurtosis. By decomposing our study patients into two nonoverlapping groups with ($n = 159$) and without ESRD ($n = 247$), we noted that the frequency plot for Ccr was normally distributed within these patient groups (Figure 3). We therefore performed the heritability analysis separately in these patient groups.

Heritability Analysis in Patients without ESRD

We examined Ccr as a quantitative trait and performed univariate analysis to test for statistical significance of the following covariates: age, birth year, gender, BMI, SBP, DBP, mean

Table 2. Clinical characteristics of study patients[a]

	Men	Women	Both
Entire patient cohort	$n = 190$	$n = 216$	$n = 406$
Age at last follow-up (yr)	44 ± 12	42 ± 12	43 ± 12
Body mass index (kg/m^2)	27 ± 4.5	25 ± 5.6	26 ± 5.1
Mean BP (mmHg)	100 ± 11	97 ± 9.5	98 ± 10
Hypertension (%)	70	66	68
ACEI/ARB[b] (%)	52	48	50
Smoker (%)	40	32	35.5
Smoking (pack-years)	7.2 ± 14	6.0 ± 12	6.6 ± 13
Ccr at last follow-up (ml/min per 1.73 m^2)	52 ± 44	58 ± 41	55 ± 43
Patients without ESRD at last follow-up	$n = 106$	$n = 141$	$n = 247$
age at last follow-up (yr)	38 ± 13	38 ± 12	38 ± 12.6
Ccr at last follow-up (ml/min per 1.73 m^2)	85 ± 33	82 ± 29	83 ± 31
Patients with ESRD at last follow-up	$n = 84$	$n = 75$	$n = 159$
age at ESRD (yr)	50 ± 8	50 ± 10	50 ± 9
Ccr at ESRD (ml/min per 1.73 m^2)	10.5 ± 1.9	9.6 ± 2.6	10.1 ± 2.3

[a]Data expressed as mean ± SD or %. ACEI, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker.
[b]ACEI or ARB in hypertensive patients.

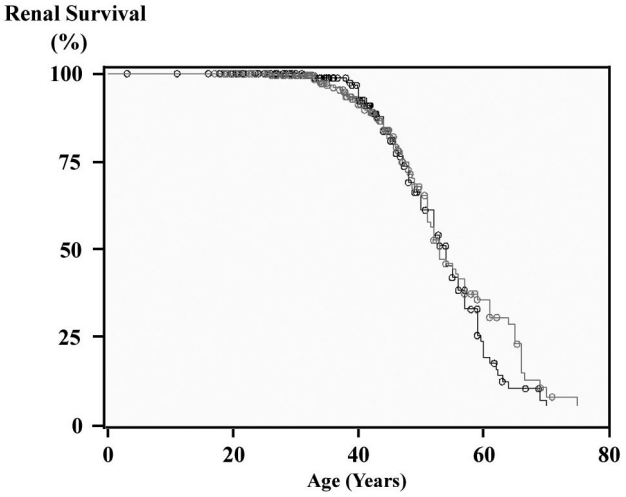

Figure 1. Renal survival (absence of ESRD) analysis of study cohort. The probability of renal survival did not differ between male (black line; $n = 190$) and female (gray line; $n = 216$) patients in our PKD1 study cohort ($\chi^2 = 0.88$; $P = 0.35$ by log-rank test). The median age at onset of ESRD in our male and female patients were 54 years (95% confidence interval [CI], 52 to 56) and 53 years (95% CI, 51 to 57), respectively. Each circle denotes a censored event.

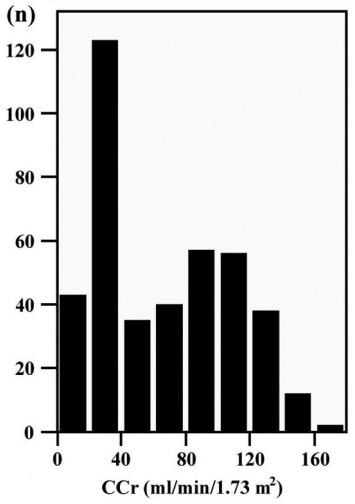

Figure 2. Frequency plot of PKD1 study patients ($n = 406$) showing a bimodal distribution of their creatinine clearance (Ccr).

BP, ACEI/ARB treatment, and pack-years of cigarette smoking. We found that age ($P = 0.0001$), SBP ($P = 0.0006$), and ACEI/ARB treatment ($P = 0.00001$) were significant covariates for this trait. Birth year, gender, BMI, DBP, mean BP, and pack-years of cigarette smoking were nonsignificant covariates for this trait. The best model that is maximized on these covariates yields a heritability estimate for Ccr of 0.42 (SE = 0.16; $P = 0.0015$). Among the significant covariates, age seemed to be most important and accounts for 41% of the variance. Age and SBP in this analysis are collinear such that when both variables were included in the same model, the effect of SBP became nonsignificant. In addition, SBP in this patient cohort is a heritable trait ($h^2 = 0.25$, SE = 0.10; $P = 0.0019$), with age being the most significant covariate accounting for 12% of the variance ($P = 1.2 \times 10^{-8}$).

Heritability Analysis in Patients with ESRD

Although the Ccr value in this patient group was also a normally distributed trait, its range (and hence variance) was distributed over a very narrow scale (Figure 3, right). In addition, the Ccr estimations at this level of renal dysfunction are imprecise. These two factors, taken together, make the heritability estimate of this trait unreliable. By contrast, the frequency

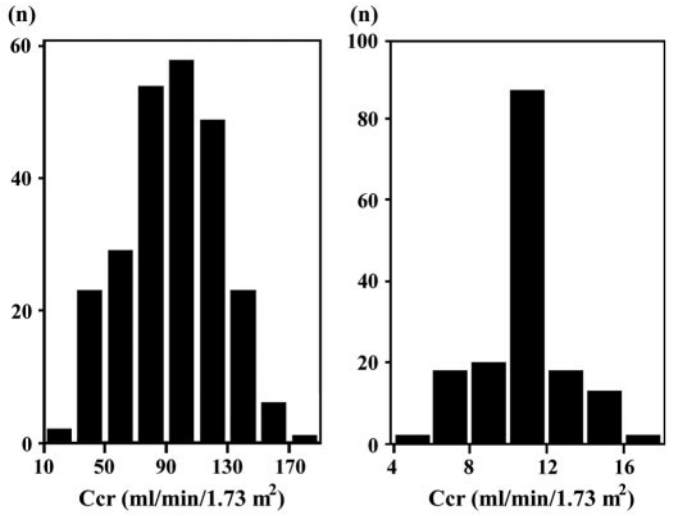

Figure 3. Frequency plots of Ccr in the PKD1 study patients with ESRD (right; $n = 159$) and without ESRD (left; $n = 247$). Both plots now assume a normal distribution. However, the Ccr values of patients with ESRD are distributed over a very narrow range (90% of the Ccr are between 6 and 14 ml/min per 1.73 m^2).

plot of age_{ESRD} is normally distributed over a wide scale (Figure 4) and therefore is a better measure for the heritability analysis. We found a high heritability for age_{ESRD} ($h^2 = 0.78$, SE = 0.24; $P = 0.00009$) in this patient group. However, none of the above covariates was found to contribute significantly to this trait.

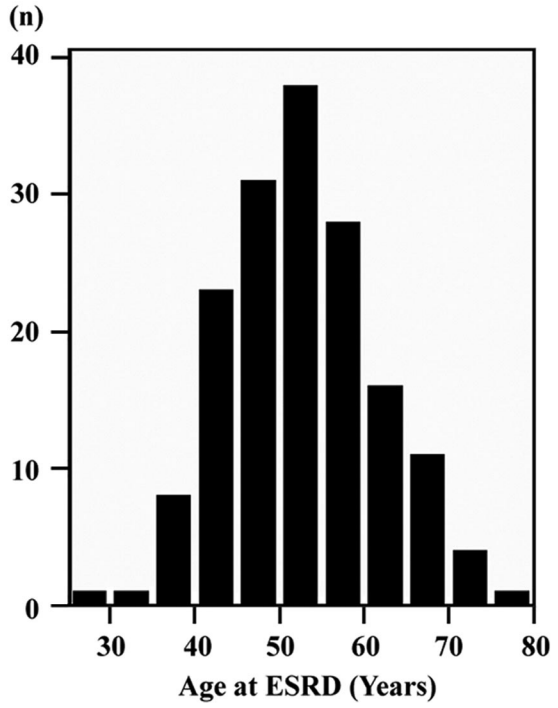

Figure 4. Frequency plot of PKD1 study patients showing a normal distribution of the age at ESRD ($n = 159$). Compared with the right panel of Figure 3, the age at ESRD displays a much wider range of variability than Ccr in the same patient group.

Discussion

Using two measures of renal disease severity (Ccr and age_{ESRD}) in patients without and with ESRD, we found heritability estimates of 42 and 78%, respectively. Thus, the most definitive conclusion of our study is that there is a significant modifier gene effect that influences the within-family renal disease severity in type 1 ADPKD. However, depending on the underlying architecture of the genetic modifiers for these traits, there are at least two possible explanations for these results. First, it is possible that the heritability estimates for Ccr and age_{ESRD} did not really differ. Rather, their apparent difference was related to the large variances of the estimates because of the modest sample size of our study. In this case, increasing the patient sample size should improve both the precision and the accuracy of these estimates. The implicit assumption of this scenario is that the same genetic modifiers play a constant role at both early and late stages of renal disease progression. Alternatively, because our patients without ESRD are on average 12 yr younger than the patients with ESRD, the difference of these heritability estimates might be real, reflecting the effects of distinct genetic modifiers at two different stages of the renal disease.

For example, it is possible that microcyst formation as a result of biallelic *PKD1* inactivation within individual renal epithelial cells (the "two-hit" model of ADPKD) may be the predominant pathogenetic event at the early stage of ADPKD, which is not associated with significant renal structural and functional alterations (18–21). By contrast, growth and expansion of macrocysts at a later stage of ADPKD can result in renal tubular compression and obstruction (21–24) and activation of genes for inflammation and apoptosis (23,25). These later events can lead to significant tubulointerstitial inflammation (21) and the formation of "atubular glomeruli" (26), mediating the loss of renal parenchyma and function.

It is interesting to note that SBP and ACEI/ARB treatment were significant covariates for the heritability estimate of Ccr but not age_{ESRD}. The high prevalence of hypertension (approximately 80%) and ACEI/ARB treatment (approximately 60%) in our cohort of patients with ESRD might have confounded the detection of a covariate effect in these qualitative clinical parameters. Alternatively, it is possible that these two clinical risk factors may not be important in the late stages of renal disease progression. Previous studies have shown that the renin-angiotensin system (RAS) is activated in the early stage of ADPKD and may modulate the development of systemic hypertension (27,28). In addition, a recent study has documented both the existence and the activation of an intrarenal RAS within the cystic tissue of patients, suggesting that angiotensin II may also modulate renal cyst growth in ADPKD (29). Taken together, these findings suggest that systemic or intrarenal activation of the RAS may modulate renal disease progression in ADPKD. Although the clinical effect of antagonism of the RAS in human ADPKD is presently inconclusive (30), our findings suggest

that genes encoding the RAS are strong biologic candidates for genetic modifiers in ADPKD.

Recent studies suggest that the variability of a complex trait disease is due to the effects of multiple genes acting in concert with environmental factors. Moreover, common modifier gene variants with modest effects contribute to the overall heritability of the disease trait (31–33). Using simulation, we performed power calculations to estimate the patient resource required for family-based association studies using a candidate gene approach (testing $\theta = 0$). For a candidate gene with a disease allele frequency of 20 to 40%, approximately 600 trios (parents and affected offspring) are sufficient to detect a dominant locus with moderately large effect (10 to 15% of the trait variance; Figure 5, top). For a recessive locus, the same sample size will be underpowered unless the disease allele frequency is in the 50% range (Figure 5, bottom). Otherwise, approximately 1000 trios are required to detect a recessive locus with similar effect size (Figure 6, bottom). To detect a modifier locus using a genome-wide linkage approach with an intermarker density of approximately 5 cM (*i.e.*, with approximately 400 microsatellite markers), at least five times the above sample sizes are required to ensure proper power. These estimates therefore suggest that the mapping of modifier genes in ADPKD will be challenging and will require international collaboration from multiple research centers. A corollary of these findings is that all of the genetic association studies in ADPKD published to date are grossly underpowered to detect a modestly strong modifier locus (35–40).

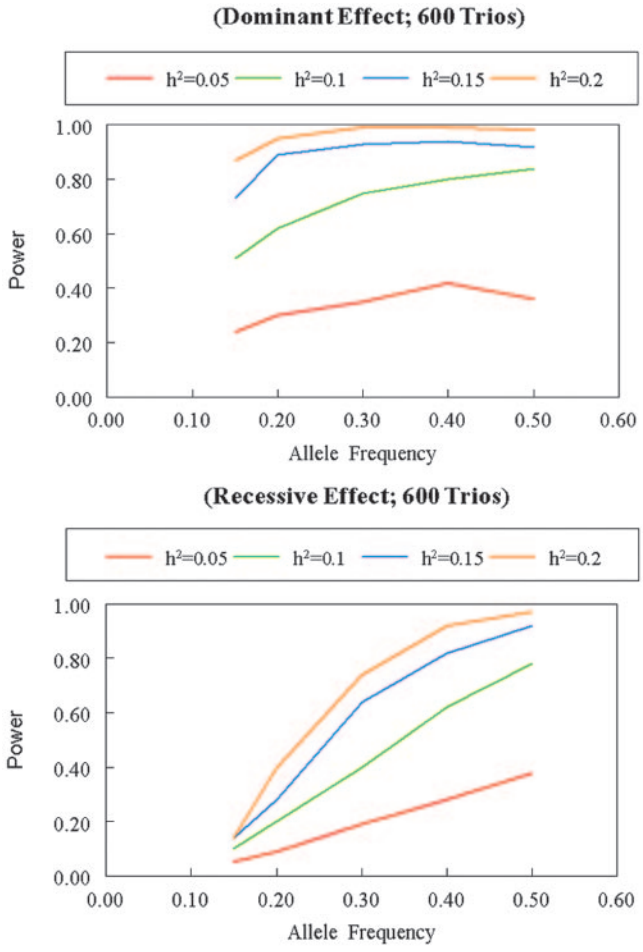

Figure 5. Power calculations for candidate gene association studies using the statistical package for family-based association tests (PBAT) (34). The data were derived from simulations of 1000 replicate samples, each containing 600 trios (two parents and one affected offspring), under random mating. A quantitative trait was simulated with a polygenic effect, a random environmental effect, and a major gene (the trait locus) effect for each individual. Heterozygosity of parental genotypes was not assumed. This method of simulation is robust for nonnormality of data. h^2 refers to the heritability of the trait accounted by the candidate gene. Two-tailed α of 0.01 was assumed.

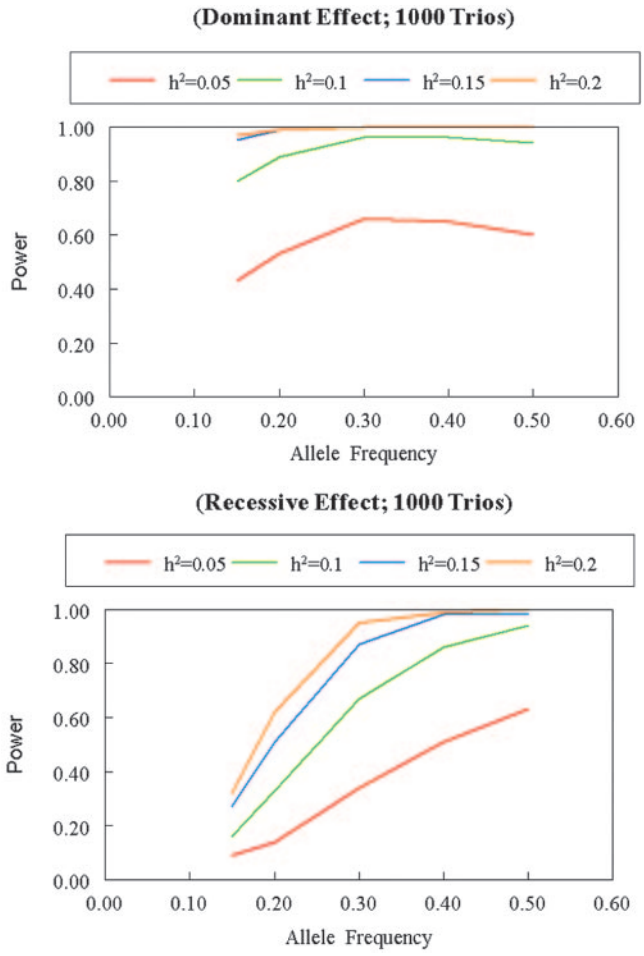

Figure 6. Power calculations for candidate gene association studies using PBAT (34). The data were derived from simulations of 1000 replicate samples, each containing 1000 trios, under random mating. A quantitative trait was simulated with a polygenic effect, a random environmental effect, and a major gene (the trait locus) effect for each individual. Heterozygosity of parental genotypes was not assumed. This method of simulation is robust for non-normality of data. h^2 refers to the heritability of the trait accounted by the candidate gene. Two-tailed α of 0.01 was assumed.

Finally, our study suggests that environmental factors may also play an important role in modifying renal disease progression in ADPKD. Recent studies suggest that somatic *PKD1* mutations within individual renal epithelial cells constitute a major mechanism for cystogenesis (18–20). Thus, the burden of somatic mutations is expected to influence the total cyst number within the kidney. In this regard, cigarette smoking may be particularly relevant among the potential environmental modifiers for ADPKD. Although we did not find that cigarette smoking was a significant covariate in our heritability analyses, our study is likely underpowered to detect such an effect given that only 35% of our patients were smokers with light to moderate exposure. Future study with a larger patient sample size and heavier exposure to cigarette smoking is needed to resolve this issue.

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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]).

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