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Familial phenotype differences in PKD1[1]

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Background. Mutations within the PKD1 gene are responsible for the most common and most severe form of autosomal dominant polycystic kidney disease (ADPKD). Although it is known that there is a wide range of disease severity within PKD1 families, it is uncertain whether differences in clinical severity also occur among PKD1 families.

Methods. Ten large South Wales ADPKD families with at least 12 affected members were included in the study. From affected members, clinical information was obtained, including survival data and the presence of ADPKD-associated complications. Family members who were at risk of having inherited ADPKD but were proven to be non-affected were included as controls. Linkage and haplotype analysis were performed with highly polymorphic microsatellite markers closely linked to the PKD1 gene. Survival data were analyzed by the Kaplan–Meier method and the log rank test. Logistic regression analysis was used to test for differences in complication rates between families.

Results. Haplotype analysis revealed that each family had PKD1-linked disease with a unique disease-associated haplotype. Interfamily differences were observed in overall survival ($P = 0.0004$), renal survival ($P = 0.0001$), hypertension prevalence ($P = 0.013$), and hernia ($P = 0.048$). Individuals with hypertension had significantly worse overall ($P = 0.0085$) and renal ($P = 0.03$) survival compared with those without hypertension. No statistically significant differences in the prevalence of hypertension and hernia were observed among controls.

Conclusion. We conclude that phenotype differences exist between PKD1 families, which, on the basis of having unique disease-associated haplotypes, are likely to be associated with a heterogeneous range of underlying PKD1 mutations.

Autosomal dominant polycystic kidney disease (ADPKD) is an important cause of chronic renal failure, with approximately 77% of patients deceased or with end-stage renal disease (ESRD) at the age of 70 years [1]. Extrarenal manifestations can be divided into cystic and noncystic, with cysts occurring in liver, ovary, pancreas, spleen, and central nervous system [2]. Common complications arising from the disorder include hypertension, macrohematuria, urinary tract infection, abdominal and back pain, cardiac valve abnormalities, hernia, and subarachnoid hemorrhage from intracranial aneurysms.

There is marked variability of clinical expression in ADPKD, which is explained, in part, by genetic heterogeneity. The condition is caused by mutations in at least three different genes: PKD1 on chromosome 16p13.3 [3], PKD2 on 4q21-23 [4], and a third, thus far unidentified gene [5–7]. PKD1 is responsible for ADPKD in approximately 85% of European families [8] and is associated with poorer survival and a more frequent occurrence of complications [9–11]. Although a wide range of mutations have been reported within the PKD1 gene, they have been found in only a small proportion of families studied. The reasons for this low yield are the large size of the gene and the presence of several duplications with high homology, involving approximately two thirds of the gene at the 5′ end [3, 12–20]. As a result, not enough data have yet become available to determine whether the nature of the underlying mutation influences disease severity. In contrast, familial differences, correlating with different mutations in the same gene, have been observed in a number of other genetic diseases, including cystic fibrosis, familial polyposis coli, hypertrophic obstructive cardiomyopathy, and Marfan syndrome [21–24]. In view of the wide variety of PKD1 mutations that have been identified to date, it is possible that there is interfamily variability of PKD1 clinical expression. Until a larger proportion of mutations across all regions of the PKD1 gene can be identified, evidence to assess whether the nature of the underlying PKD1 mutation influences the disease phenotype is reliant on statistical comparison of the phenotypes found in large PKD1 families. To test the hypothesis that there are differences in clinical expression between PKD1 families, we evaluated 10 large PKD1-linked families from the same geographic location.

[1] **See Editorial, p. 344.**

Key words: kidney, polycystic kidney disease, heredity, hypertension, hernia, gene mutations.

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METHODS

Families and clinical evaluation

Large ADPKD families with 12 or more members known to be affected were selected from the South Wales ADPKD register. Within each family, information was collected from three categories of ADPKD-affected individuals and also from at-risk family members who were proven to be non-affected.

Previously diagnosed. The medical history and current renal symptoms of previously diagnosed individuals were recorded, including age at diagnosis, the factors leading to diagnosis, as well as information on the following: hypertension requiring treatment, macrohematuria, urinary tract infection, urinary tract calculi, and hernia of the anterior abdominal wall. For macrohematuria, a history of passing urine with visible blood was regarded as sufficient. Individuals were considered to have suffered from urinary tract infection if, at any stage in the past, they had been diagnosed or treated for cystitis, pyelonephritis, or cyst infection. A positive urine or blood culture was not required for the diagnosis. For urinary tract calculi, radiological evidence of stone formation (ultrasound, plain x-rays, intravenous pyelograms, CT scans) was required. Any history of hernia of the anterior abdominal wall, that is, inguinal or umbilical hernia, was sufficient for inclusion, regardless of the size or treatment modality. The age at the onset of renal failure was recorded for those on renal replacement therapy. When necessary, additional medical details were obtained from the attending doctor.

Newly diagnosed. These were individuals with an affected parent who was not previously known to have ADPKD and who was diagnosed during this study by ultrasound examination [25] or DNA-linkage tests. Like the previously diagnosed group, their medical history was recorded, together with documentation of any existing renal symptoms. Physical examination included resting supine and erect systolic and diastolic (phase V) blood pressure measurements. The World Health Organization criteria for hypertension were used, and individuals were considered to be hypertensive if the systolic blood pressure was $\geq$160 mm Hg or the diastolic blood pressure was $\geq$95 mm Hg. Abdominal ultrasound was arranged, and the presence and number of renal and hepatic cysts were recorded.

Deceased affected. Information was collected on affected individuals who had died, including their dates of birth and death, mode of and age of diagnosis, complications when known, particularly hypertension requiring treatment, and cause of death. The age at onset of end-stage renal failure was taken to be the age at which long-term replacement therapy for renal function became necessary or, in people who died of renal failure, the age at death.

Non-affected. Family members at 25 or 50% risk of having inherited ADPKD who had been found to have a normal ultrasound scan over the age of 30 or who were at a less than 1% risk of having inherited the PKD1 gene according to linkage analysis were included as controls. Survival data were recorded, as well as information about the following: hypertension requiring treatment, macrohematuria, urinary tract infection, urinary tract calculi, and hernia of the anterior abdominal wall.

The ethical committees of Morriston Hospital in Swansea and the University Hospital of Wales in Cardiff both approved this study, and DNA samples were taken after informed consent was obtained from patients and their family members.

DNA microsatellite analysis

Four families were already known to be linked to the PKD1 gene, and the methods and results of the linkage analysis have been reported elsewhere [26]. Using microsatellite polymorphisms, the remaining six families were tested for evidence of linkage to the PKD1 locus. We used the CA repeat KG8 (intragenic marker at the 3′ end of the gene) and the complex polymorphism SM6 (proximal to the gene). Haplotype analysis was performed on all families to assess for the presence of possible common founder mutations. The markers KG8, SM6, and SM7, all tightly linked to the PKD1 locus, were used to determine family haplotypes. Genomic DNA (30 ng) was polymerase chain reaction (PCR) amplified in a final volume of 12.5 μl containing, 1 × Cetus buffer II (PE Applied Biosystems, Warrington, UK), 1.5 mmol/liter $MgCl_2$, 200 μmol/liter of each dATP, dGTP, dCTP, and dTTP (Pharmacia LKB, Piscataway, NJ, USA), 3 pmol of each primer [one of primers was end labeled with 0.22 μCi of γ^{33}PdATP, 1 × kinase buffer 0.33 units kinase enzyme (Life Technologies, Ltd., Paisley, UK)] and one unit of Amplitaq Gold (Applied Biosystems). Amplification conditions were an initial denaturing step (12 min for 95°C), followed by 29 cycles of 94°C for 60 seconds, the primer specific annealing temperature (T_a) for 60 seconds and 72°C for 60 seconds. A final extension step for 60 seconds at the T_a and for 10 minutes at 72°C was performed. The T_a was 56°C for KG8 and 60°C for SM6 and 55°C for SM7. Polymerase chain reaction (PCR) products were run on a 6% denaturing polyacrylamide gel (National Diagnostics, Atlanta, GA, USA). The gel was vacuum dried and autoradiographed for approximately 18 hours.

Linkage

Linkage analyses were performed on the 10 families using CYRILLIC 2.0 (Cherwell Scientific Publishing, Ltd., Oxford, UK) and the MLINK program [27]. Family LOD scores were included in the following weighting

Table 1. Survival data of 10 South Wales PDK1 families

Family	Number of affected members	Number deceased/ESRD	Median survival (to ESRD/death)	Number ESRD	Median renal survival (to ESRD) (95% CI)
1	37	25	47.6 (44.5–50.6)	20	48.4 (43.8–52.9)
2	13	8	49.0 (43.2–54.8)	7	55.0 (44.2–65.8)
3	12	7	48.0 (39.9–56.1)	2	61.3 (30.0–92.6)
4	16	8	49.9 (44.3–55.6)	5	54.5 (45.1–63.7)
5	13	8	49.3 (47.0–51.6)	6	50.0 (48.2–51.8)
6	15	9	52.5 (47.0–58.1)	6	55.1 (49.8–60.4)
7	12	8	45.3 (35.3–55.2)	7	45.3 (35.3–55.2)
8	17	11	47.9 (40.6–55.3)	6	49.5 (47.6–51.4)
9	26	10	48.0 (46.2–49.8)	5	57.0 (47.7–66.3)
10	14	4	75.3 (54.2–76.1)	1	75.3[a]
Total	175	98	49.0 (47.4–50.6)	25	47.6 (44.5–50.6)

[a] Unable to calculate CI because of lack of endpoints

formula to estimate the likelihood of a family being linked to the PKD1 locus.

$$P(PKD1) = (\alpha_{PKD1})(10^{LodPKD1})/[(\alpha_{PKD1})(10^{LodPKD1}) + (\alpha_{PKD2}) + (1-\alpha_{PKD1} -\alpha_{PKD2})]$$

The prevalence estimates used for PKD1 (α_{PKD1}), PKD2 (α_{PKD2}), and 1-α_{PKD1} –α_{PKD2} were 0.84, 0.15, and 0.01, respectively.

Statistical methods

The product-limit method of survival analysis was used to determine survival probabilities. For overall survival, the endpoint was defined as the age at death or onset of ESRD. Affected individuals who were still alive and not in ESRD were entered as censored data. For renal survival, the endpoint was defined as the age at onset of ESRD. Individuals who died of causes other than uremia and who had not reached ESRD during their lifetime were included as censored data. Survival differences were analyzed by the log rank test. When investigating differences in frequencies of complications between the groups of affected family members, logistic regression analysis was used to adjust for the effects of age and sex, and the same method was used to investigate differences between the groups of nonaffected family members. The analyses were performed using the statistical package SPSS version 6.0.

RESULTS

Ten families, with a mean of 17.5 (12 to 37) affected members, were identified (Table 1). The 175 ADPKD affected members of these families comprised 83 men and 92 women, of whom 98 (56%) had died or had reached ESRD. Information from 88 nonaffected individuals, 46 men and 42 women, was available for analysis. The linkage to the PKD1 locus on chromosome 16p13.3 was confirmed for all 10 families, with LOD scores ranging from 1.4 to 9.1, indicating that the linkage to the PKD1 locus was more than 99% for all families (Table 2). Haplotype analysis revealed the presence of a unique disease-associated haplotype in each family. Interfamily differences were detected when comparing overall survival (log rank test, $P = 0.0004$; Fig. 1). Family 7 had the shortest survival, with a median age of 45.3 years, and family 10 had the longest, with a median age of 75.3 years. Inspection of Figure 1 suggested that family 10 may be an unusual outlier. In view of this, the family was removed from the group, and the analysis was repeated. The log rank test revealed the persistence of a significant difference of phenotype among the remaining nine PKD1 families ($P = 0.018$). For renal survival (Figure 2), the difference between families was also highly significant ($P = 0.0001$). Again, the removal of family 10 did not result in the loss of a statistically significant difference between the remaining families ($P = 0.011$). Analysis of the survival data of nonaffected family members was hampered by the small number of individuals who were proven to be nonaffected before their death, and in five families, none of the controls had reached an endpoint, therefore preventing the comparison of survival data.

Table 2. LOD scores (Z) and final probabilities of being linked to the PKD1 locus of 10 South Wales families

Family	Z	Final probability PKD1 linked
1	9.1	>0.999
2	4.1	>0.999
3	1.4	0.992
4	1.5	0.994
5	3.3	>0.999
6	2.8	>0.999
7	3.8	>0.999
8	3.0	>0.999
9	8.2	>0.999
10	1.9	0.998

Although the prevalence of hypertension in affected individuals increased with age ($\chi^2 = 28.7$, $P < 0.00001$),

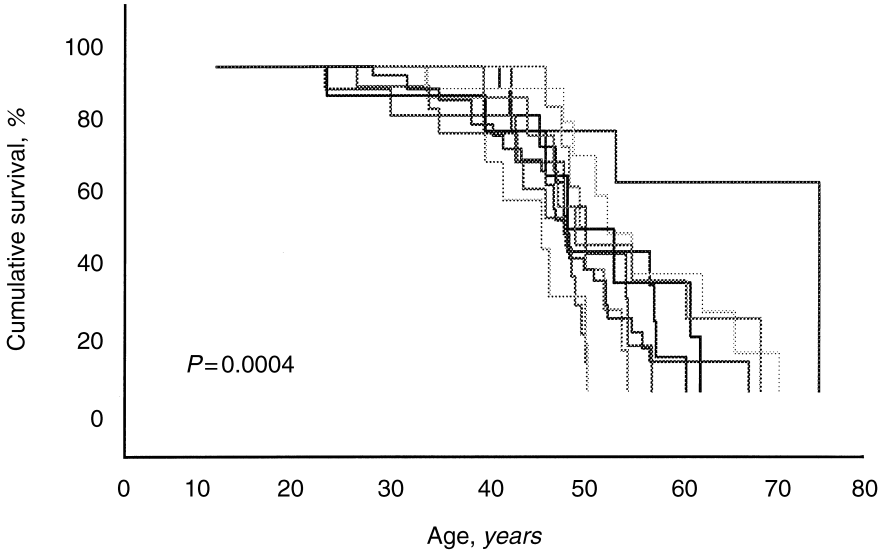

Fig. 1. Overall survival for 10 South Wales PKD1 families (endpoint defined as death or end-stage renal disease).

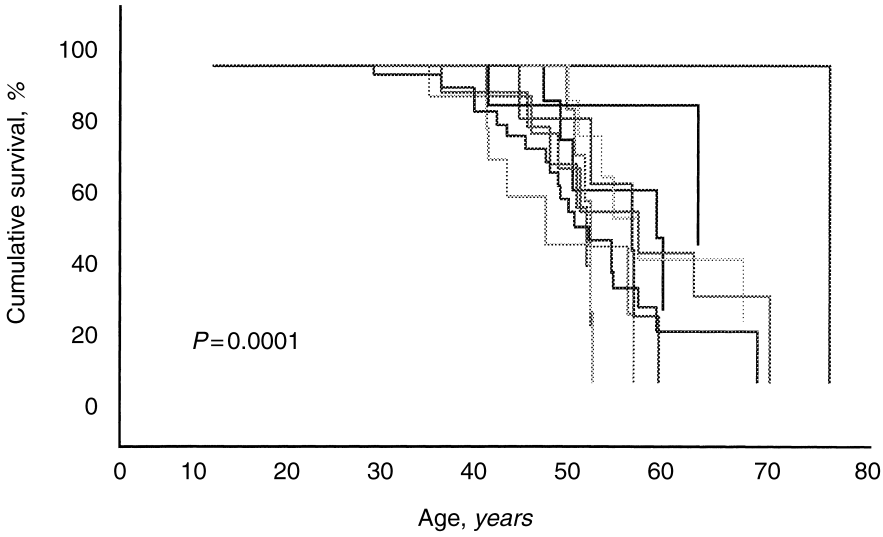

Fig. 2. Renal survival for 10 South Wales PKD1 families (endpoint defined as end-stage renal disease).

there was no significant difference between affected males and females ($\chi^2 = 0.14$, $P = 0.71$). Logistic regression analysis, correcting for age and sex, showed a significant difference in the prevalence of hypertension occurring in the 10 families ($\chi^2 = 20.82$, $P = 0.013$). Kaplan–Meier survival analysis, corrected for sex, showed that individuals with hypertension, compared with those without hypertension, had significantly worse overall survival ($P = 0.0085$), as well as renal survival ($P = 0.03$). The prevalence of macrohematuria was observed to rise with increasing age ($\chi^2 = 9.43$, $P = 0.0021$). After correcting for sex and age, the prevalence of macrohematuria did not differ significantly between PKD1 families. The prevalence of renal stones, demonstrated by radiological imaging, increased with age ($\chi^2 = 4.62$, $P = 0.032$) and was higher in women, although this did not reach statistical significance ($\chi^2 = 3.84$, $P = 0.050$). After correcting for age and sex, no significant difference was observed between families. As expected, women experienced more urinary tract infections than men ($\chi^2 = 14.92$, $P = 0.00011$). After correcting for sex and age, no significant difference between families was identified. The prevalence of hernia increased with age ($\chi^2 = 4.14$, $P = 0.042$) and was higher in men, although this gender difference did not reach statistical significance ($\chi^2 = 3.79$, $P = 0.052$). After correcting for age and sex, a significant difference between families was identified ($\chi^2 = 17.04$, $P = 0.048$). Among the nonaffected control population, the prevalence of hypertension increased with age ($\chi^2 = 16.21$, $P < 0.0005$), and no statistically significant differ-

ence was detected between the prevalence in males and females ($\chi^2 = 0.02$, $P = 0.89$). In contrast to the findings among affected individuals, no statistically significant differences in the prevalence of hypertension were found between the 10 groups of nonaffected subjects ($\chi^2 = 9.19$, $P > 0.4$). The prevalence of abdominal wall hernia increased with age in the control population ($\chi^2 = 14.97$, $P < 0.0005$) and was higher in men than in women ($\chi^2 = 5.02$, $P = 0.025$). After correcting for age and sex, no statistically significant differences were found for the prevalence of hernia among the nonaffected members of the different families ($\chi^2 = 9.96$, $P > 0.3$).

DISCUSSION

These data demonstrate for the first time, to our knowledge, that phenotype differences exist among PKD1 families. In addition to differences in overall survival and renal survival, we detected marked familial variation in the prevalence of disease-associated complications, which persisted after the correction for age and sex. Among those complications, the prevalence of hypertension varied the most between families. Significant variation in the prevalence of hernia of the anterior abdominal wall was also observed. These differences in the prevalence of hypertension and hernia were not detected between the groups of nonaffected family members, strongly suggesting that the observed differences are most likely related to the nature of the underlying PKD1 mutation, although it remains possible that other genetic influences linked to the PKD1 locus could play a role in determining disease severity.

Hypertension occurs commonly in ADPKD and frequently develops before renal function becomes impaired [28, 29]. The pathogenesis of hypertension in ADPKD is complex with cyst growth, renal handling of sodium, activation of the renin-aldosterone system, elevated plasma volume, and increased atrial natriuretic peptide and plasma endothelin levels all found to be associated with hypertension in this disorder [30]. Previous reports, as well as the data reported here, reveal that hypertension is strongly associated with a poorer survival, including renal survival [1, 31]. Aside from increasing the rate of renal decline, it is likely that the high prevalence of hypertension contributes to cardiovascular disease, the most frequent cause of death in ADPKD patients [32]. Our observation that the prevalence of hypertension varied the most supports the view that hypertension is an important determinant of overall survival, as well as of renal survival.

Inguinal and umbilical hernia are well-known complications of ADPKD [33, 34], which, along with cardiac valve defects and intracranial aneurysms, may be the result of a thus far unidentified extracellular matrix function of one or both of the polycystins. In this context, the description of a PKD1 kindred with an overlap connective tissue syndrome, reminiscent of Marfan syndrome, is of interest [35]. The observation of significant variation in the prevalence of abdominal hernia among the PKD1 families in this study could indicate that some PKD1 mutations predispose to an increased risk of connective tissue complications. Although this finding is consistent with other reports of familial clustering of aortic dissection and intracranial aneurysms [36, 37], proof of the hypothesis that the nature of the underlying PKD1 mutation influences the risk of connective tissue complications will have to await the identification of specific mutations in families with unusual connective tissue features.

Although no interfamily differences were observed in the prevalence of urinary tract infection, macrohematuria, and renal calculi, it must be pointed out that limitations to the power of interfamily comparative studies such as this prevent detection of less obvious differences, even if they existed.

Only two previous studies have compared patterns of clinical expression within and between ADPKD families [38, 39]. The study by Milutinovic et al demonstrated by means of pair analysis that the phenotypic expression of ADPKD has considerable intrafamilial variation and that the intrafamilial and interfamilial patterns of progression of disease did not differ significantly [38]. In the study by Torra et al, interfamilial variability, based on age of onset of ESRD, was observed to be higher than intrafamilial variability [39]. Neither study separated out PKD1 from PKD2 or other genotypes. Setting aside these earlier reports and the study reported here, there are no other reports comparing survival data among PKD1 families.

Although a considerable number of mutations and polymorphisms have been identified in the PKD1 gene, as well as in the PKD2 gene, it is not known to what extent the nature of the underlying mutation correlates with the human ADPKD phenotype. For PKD1, because of the complexities of screening for mutations in the duplicated region, most mutations reported to date are from the single copy region at the 3′ end of the gene. The majority of these mutations are stop or frame shifting mutations, resulting in premature truncation of the PKD1 gene product. Another type of PKD1 mutation involves large deletions, disrupting the PKD1 gene and the adjacent tuberous sclerosis type 2 gene (TSC2). These contiguous gene deletions result in tuberous sclerosis and severe, childhood onset, polycystic kidney disease [40, 41] and are currently the only clear evidence of a genotype/phenotype correlation involving PKD1. More recently, several different strategies have been applied to identifying mutations in the duplicated region of the PKD1 gene [17, 18]. However, the yield remains low, and mutations have been characterized in only a

small proportion of PKD1 families that have contributed to the various mutation reports. Consistent with this, no mutations have yet been identified among the 10 PKD1 families included in this study.

It is likely that there are a number of influences, both genetic and environmental, that modify the clinical course of PKD1, and the report of a set of twins with the same germline nonsense mutation but with very different disease severity lends support to this concept [16]. Currently, evidence of such modifying influences is limited to a report of the angiotensin-converting enzyme (ACE) insertion/deletion (I/D) polymorphism modifying PKD1 disease severity, with the DD genotype being associated with an increased risk for ESRD [42]. Although there is the possibility that other hereditary background factors may modify disease severity, it is highly unlikely that such factors would have a significant impact in large numbers of affected individuals within a single family, unless these modifying genes or polymorphisms were tightly linked to the PKD1 locus. Proof that the observed differences between families are due to underlying mutational differences will ultimately require detection of the mutations. Nevertheless, the hypothesis is attractive, particularly as very few recurrent PKD1 mutations have been reported. The identification of unique PKD1-linked haplotypes in each of our families provides additional evidence, as does our finding that the unaffected relatives of those with ADPKD in each family did not have similarly raised rates of complications. This latter finding makes it unlikely that environmental or other genetic factors, unlinked to the PKD1 locus, have contributed to the observed differences.

In conclusion, we have detected significant differences in overall and renal survival among 10 PKD1-linked families from the same geographic region, each with a unique PKD1-associated haplotype. Differences have also been observed in the prevalence of complications associated with ADPKD, particularly hypertension. The most attractive explanation for these observed differences is that the nature of the underlying PKD1 mutation influences clinical expression of disease.

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