A stable, nonsense mutation associated with a case of infantile onset polycystic kidney disease 1 (PKD1)

Belén Peral¹, Albert C. M. Ong¹, José L. San Millán¹,*, Vicki Gamble¹, Lesley Rees² and Peter C. Harris¹,*

¹MRC Molecular Haematology Unit, Institute of Molecular Medicine, John Radcliffe Hospital, Oxford OX3 9DU, UK and ²Department of Paediatric Nephrology, Royal Free Hospital, London NW3 1QG, UK

Received January 16, 1996; Revised and Accepted February 2, 1996

Autosomal dominant polycystic kidney disease (ADPKD) is the most common single gene disorder resulting in renal failure. It is generally an adult onset disease, but rarely, cases of severe childhood polycystic disease arise in ADPKD families. The clear clinical anticipation in these pedigrees has led to the suggestion that the mutation may be an unstable trinucleotide repeat. We have now identified a nonsense mutation, Tyr3818Stop, in one such family (P117) within the peat. We have now identified a nonsense mutation, that the mutation may be an unstable trinucleotide re-
cipitation has led to the suggestion disease arise in ADPKD families. The clear clinical anti-
caise, but rarely, cases of severe childhood polycystic
sults as typical adult onset disease in the father, and of grandpaternal origin. PKD1 mani-
fests as typical adult onset disease in the father, but is seen as severe disease, detected as enlarged polycys-
tic kidneys in utero, in one of a pair of dizygotic twins; the other twin has the mutation but no evidence of
cysts, consistent with an adult onset disease course. The finding of the same stable mutation associated
with very different disease severity in this family indicates that phenotypic variation in PKD1 is not due to
dynamic mutation. It seems most likely that a small number of modifying factors may radically affect the
 course of disease in PKD1; identification of such fac-
tors will have important prognostic implications in this
disorder.

INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common human genetic diseases [incidence 1:1000 (1)], and a frequent cause of end stage renal disease (ESRD). It is characterised by progressive cyst development and expansion, leading to ESRD on average at ~60 years (2), as well as by various extra renal manifestations (3). Detailed analysis has revealed that ADPKD is genetically heterogeneous and exhibits considerable phenotypic variability. At least three different loci are now known to cause ADPKD: polycystic kidney disease 1 (PKD1) in chromosome region 16p13.3 (4) [~85% of cases (5)]; PKD2 at 4q13–23 (6,7) and an, as yet, unmapped locus, PKD3 (8). A consistent difference in disease severity has been noted between PKD1 and PKD2; PKD1 patients typically have earlier onset of disease and earlier ESRD [an average age at ESRD of 56 years compared to 71.5 years for PKD2 (9)].

Genetic heterogeneity does not however explain all of the phenotypic variability in ADPKD with ample evidence of variation within the PKD1 population. An example of a family with consistently mild disease has been described (10) and other studies have suggested considerable intrafamilial variability (11,12). Fick et al. (13) argued that the intrafamilial variability showed a pattern of anticipation in the majority of families studied. An extreme example of this phenotypic variability is manifest in rare, childhood cases of ADPKD. In its most severe form, ADPKD can present as massively enlarged bilateral polycystic kidneys detected in utero (14–18). Studies of early onset cases have shown that they arise in families with normal, adult presenting disease, although a significant risk of recurrent childhood disease in sibs has been noted (15–17). A maternal predominance of the transmitting parent has also been observed (16,17), and a high proportion of apparent new mutations within these families (17). In the limited number of families where linkage analysis has been described, all childhood cases are in PKD1 pedigrees (18). The clear clinical anticipation in families containing early onset cases, plus the evidence for anticipation in typical ADPKD families, has led to the proposal that an unstable trinucleotide repeat may be the mechanism of disease (13,16).

Recently the PKD1 gene has been identified (19) and fully sequenced (20–22); analysis has, however, shown no trinucleotide repeats larger than five copies in the genomic region encoding PKD1 (20). Furthermore, the mutations so far described in typical PKD1 families are various stable, frame-shifting, nonsense or deletional changes in the PKD1 gene (23,24). The basis of disease has been determined in one group of patients with early onset PKD1, but which also manifest tuberous sclerosis. In these cases, deletions of the PKD1 and adjacent tuberous sclerosis gene, TSC2, have been characterised (25). Nevertheless, the basis of severe childhood PKD in typical ADPKD families remains unclear.

Here we describe a nonsense mutation in the PKD1 gene in a severely affected child which was also found in her clinically normal twin brother and father who has typical adult onset disease.

*To whom correspondence should be addressed
*Present address: Unidad de Genética Molecular, Hospital Ramón y Cajal, 28034 Madrid, Spain
RESULTS

Clinical details

Dizygotic twins (III1 and 2; Fig. 1) were born at 34 weeks gestation to a 26 year old mother (II2) from family P117. Enlarged bilateral polycystic kidneys were diagnosed by ultrasound in II2 at 31 weeks gestation: III1 remained normal throughout the pregnancy. At birth, both kidneys of II2 were easily palpable and renal cysts were detectable by ultrasound: on measurement at 10 months bilateral enlarged polycystic kidneys of 8 cm were observed (>95th centile). In the absence of a family history of PKD, autosomal recessive polycystic kidney disease (ARPKD) was initially diagnosed. However, this was revised when bilateral, multiple renal cysts were detected in the father (28 years) on ultrasound. He was asymptomatic and had normal renal size and function. The mother showed no evidence of PKD clinically or on ultrasound and all six siblings of the father were well; two showed no renal cysts on ultrasound at 35 and 33 years. The grandparents (I1 and 2) are well, except for mild treated hypertension in the grandmother who had normal renal function and ultrasound. The grandfather refused ultrasound examination but had normal renal function at 55 years. II2 is now 6 years of age, her kidneys continue to be enlarged (>95th centile), with multiple cysts (Fig. 2) and has been hypertensive since age 2; her renal function remains normal. III1 continued to show no renal cysts on ultrasound examination when analysed at 5 years of age.

Molecular analysis

The PKD1 transcript of II2 was analysed for mutations by SSCP and an abnormal fragment was detected with the primers NN (24). Direct sequencing showed a C to A transversion at and an abnormal fragment was detected with the primers NN TAC, to a stop codon, resulting in truncation of the protein by 485 amino acids. Genomic DNA from nine available members of P117 was amplified with the NN primers, the product of 352 bp digested with BsaAI and analysed in a 2.5% agarose gel; Figure 1 shows the result for six family members. The father (II1) and the twin brother (III1), as well as II2, have the mutation. Neither of the grandparents (I1 or II2) has this substitution, hence the mutation is a de novo event in II1, associated with the development of PKD1. Linkage analysis using four microsatellite markers from the PKD1 region (KG8, SM6, 16AC2.5 and CW2) ruled out the possibility of non-paternity and indeed showed that the affected chromosome was of grandpaternal (I1) origin (Fig. 1).

This mutation was not detected in a further screen of 240 unrelated ADPKD individuals.

DISCUSSION

There has been considerable speculation about the basis of phenotypic variation in PKD1 and, in particular, the cause of rare cases of severe childhood polycystic disease. Our analysis of one such family has revealed a nonsense mutation, Tyr3818Stop, in the father (II1) with adult onset ADPKD, the severely affected child (III2) and her clinically normal twin brother (III1). The lack of evidence of cystic disease in III1 (at 5 years) is consistent with an adult onset course of disease. The change is an adenosine mutation within the family, which is of grandpaternal origin and the position of the mutation, in the 3′ region of the gene, is similar to that seen in some other families with adult onset PKD1 (24). The inheritance of the same, stable, DNA change in individuals with such different disease manifestations indicates that the phenotypic variability observed in PKD1 is not due to a dynamic mutation within the PKD1 gene. Interestingly, a recent study of 74 German ADPKD families casts doubt on anticipation in ADPKD (12). Geberth et al. (12) found similar results to Fick et al. (13), that is, in approximately 50% of families the age at ESRD was younger in the offspring. However, they further noted in the other ~50% of pedigrees that the age at ESRD was younger in the parent and concluded that the results did not support anticipation, but were compatible with variation due to other genetic and/or environmental factors.

A number of specific events may explain childhood PKD1; however, analysis of P117 and similar families (15–17) make many of these seem unlikely. A second dominant PKD mutation could have been inherited from the unaffected parent, but no evidence of renal cysts were found on ultrasound examination of II2, or in other reported cases (16,17). A de novo change may have occurred in this allele (or a second change in the mutated allele), but this would not explain the reported recurrence risk (15–17). Tyr3818Stop is a new mutation in the parental generation and de novo changes have been noted in several other early onset families (17). Although this suggests that mosaicism may be important, this appears to be excluded in this case as the extreme differences in phenotype are seen in generation III, after the disease gene has been transmitted through a generation. The disease allele may be differentially expressed, but we saw no evidence of this, with expression detected in lymphocyte mRNA from the normal and abnormal allele of each affected individual.

Figure 1. Pedigree 117 showing the affected father II1, and dizygotic twins (III1 and III2); III2 is severely affected. The genetic haplotype generated with the microsatellites, KG8, SM6, 16AC2.5 and CW2 is represented below each individual. The shaded boxes indicate the same haplotype, light shading without the Tyr3818Stop mutation and dark shading with the mutation. The gel shows amplified genomic DNA from the family members digested with BsaAI. Affected individuals have lost a BsaAl site and therefore show the larger 352 bp fragment. Individuals III1, III1 and III2 have the mutation, but this change is not detected in either grandparent.
Figure 2. Ultrasound of the left kidney of III2 taken at 5 years of age. A single large cyst (large C) and multiple smaller cysts (small c) can be seen within the kidney. Similar cystic changes were also seen in the right kidney (not shown).

Figure 3. Direct sequencing of III2 cDNA showing a C→A transversion at position 11665nt resulting in a Tyr→Stop mutation at 3818aa. (data not shown). Differential expression in the kidney, however, cannot be excluded. The maternal predominance that has been noted in the transmitting parent of early onset cases (16,17) (although not in our pedigree) may reflect imprinting of the PKD1 gene; Bear et al. (26) have shown that PKD1 is significantly more severe if maternally inherited.

The recurrence risk of severe disease noted in the literature (15–17) suggests that inherited factors are important. It is possible that a subtle change at PKD1 may be inherited from the unaffected partner, which only manifests in association with a dominant mutation; different maternal PKD1 alleles are inherited by the twins with very different disease manifestations in this case. However, individuals with two or more affected children from different partners (15), and severely affected cousins and uncles (27,28), indicate that some of these inherited factors are transmitted from the parent with disease. If these factors are not related to the PKD1 mutation itself, we might suppose that they are modifying factors which may also be inherited from the partner. The high calculated recurrence risk for early onset disease (16) indicates that the number of such modifying factors may be small. Interestingly, recent studies in mouse models of PKD indicate that modifying factors can have a profound influence on disease severity (29). We could imagine that the uncloned ARPKD locus on chromosome 6 (30) or human equivalents of the many recessive PKD loci identified in rodents (31) could encode such modifying factors, although these probably reflect just a small proportion of all potential modifiers.

In conclusion, it is unlikely that a dynamic mutation explains the phenotypic variability in PKD1, but it is probable that a small number of modifying factors radically affect disease severity. Mapping and characterisation of these factors may identify important prognostic indicators, especially useful in families with a history of early onset disease. Furthermore, such modifying proteins may interact with the PKD1 protein, polycystin, and identify further steps of a cystic pathway.

MATERIALS AND METHODS

Mutation detection

Lymphoblast cell lines were established from members of P117 by transformation of peripheral lymphocytes with EBV. Total RNA was isolated from cell lines or blood samples using the guanidinium thiocyanate/phenol extraction method (32). cDNA was synthesised from 5 µg total RNA in 50 µl as previously described (19). cDNA was amplified with the NN primers [11534–11746 nt of PKD1; exons 40–41 (24)] using a DMSO containing buffer (33) and annealing at 62°C with 0.5 mM...
MgCl₂. The product of 213 bp was analysed by SSCP, as outlined previously (24). The RT-PCR fragment generated with the NN primers from III2 was sequenced directly using a single 5′-biotinylated primer to facilitate the preparation of single-stranded template (Dynabeads M-280 Streptavidin) (34).

Genomic analysis

To analyse the mutation in the entire family, 50 ng genomic DNA of each individual was amplified with the NN primers, as above. To analyse the mutation in the entire family, 50 ng genomic DNA is observed. Haplotype analysis was performed with the microsatellites: KG8, SM6, 16AC2.5 and CW2 (35).

ACKNOWLEDGEMENTS

We thank J. Sloane-Stanley for tissue culture, L. Rose for manuscript preparation and Professor Sir D. J. Weatherall for support. This work was funded by the Wellcome Trust, the Medical Research Council, the National Kidney Research Fund (ACMO), the Polycystic Kidney Research Foundation and Spanish Fondo de Investigaciones Sanitarias and Comunidad de Madrid (JLSM).

REFERENCES