J Mol Med (1998) 76:303–309

REVIEW

Klaus Zerres · Sabine Rudnik-Schöneborn
Carsten Steinkamm · Jutta Becker · Gabi Mücher

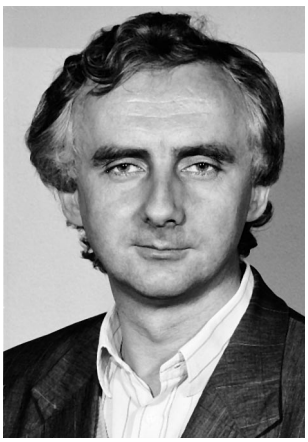
Autosomal recessive polycystic kidney disease

Received: 23 April 1997 / Accepted: 12 August 1997

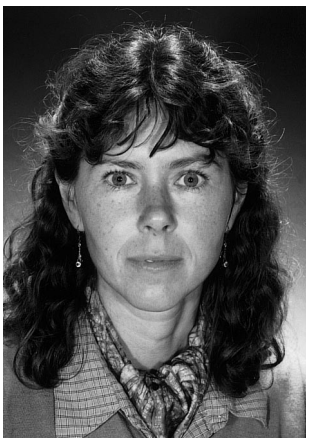
Abstract Autosomal recessive polycystic kidney disease (ARPKD) is a rare inherited disorder which usually becomes clinically manifest in early childhood, although the spectrum of ARPKD is much more variable than generally known. Presentation of ARPKD at later ages and survival into adulthood have been observed in many cases. The responsible gene has been mapped to chromosome 6p. Thus there is no evidence of genetic heterogeneity. The most important indication for DNA diagnosis is the prenatal diagnosis in families with at least one affected child. The critical region has been narrowed with the use of recombinant families of about 4 cM. Several possible candidate genes have been excluded.

Key words Autosomal recessive polycystic kidney disease · Linkage study · Genetics

Abbreviations *ARPKD* Autosomal recessive polycystic kidney disease · *ADPKD* Autosomal dominant polycystic kidney disease · *CHF* Congenital hepatic fibrosis · *GST2* Gluthathione S-transferase · *STS* Sequence-tagged sites



KLAUS ZERRES M. D.
is Professor of Clinical Genetics at the Institute for Human Genetics at the University of Bonn, Germany. His major research interests include: Clinical and genetic studies of cystic kidneys and spinal muscular atrophies. He is member of the International ARPKD consortium



GABI MÜCHER Ph. D.
is a molecular biologist at the Institute for Human Genetics at the University of Bonn, Germany. Her scientific interests concentrate on autosomal recessive polycystic kidney disease. She is member of the International ARPKD consortium.

K. Zerres (✉) · S. Rudnik-Schöneborn · C. Steinkamm · J. Becker
G. Mücher
Institut für Humangenetik, Universität Bonn, Wilhelmstrasse 31,
D-53111 Bonn, Germany

Communicated by: Hannsjörg W. Seyberth and Klaus Zerres

Clinical features and course

The most common initial features of autosomal recessive polycystic kidney disease (ARPKD) are palpable kidneys, enlarged liver, respiratory failure, hypertension, and urinary tract infections. The diagnosis can usually be made in early childhood. Some cases have been suspected or diagnosed by fetal ultrasound. Hypertension requiring drug treatment was found in 61%–70% in four studies and 100% in another study (review see [1]). Urinary tract infections occur in 30%–43% of patients. Growth retardation was observed in 25% in our study and was correlated with impaired renal function [1]. Clinical and ultrasound signs of hepatic fibrosis were detected in 29%–61% of cases. Mortality during the first year of life vary in the various studies [1–4]; 9%–24% of those diagnosed by departments of pediatric nephrology die. Respiratory difficulties, probably resulting from enlarged kidneys (particularly diaphragmatic elevation and hypoplasia of the lungs), cause death usually within hours after birth. The prognosis of those who survive the first months of life is much better. With prolonged survival renal failure and hepatic involvement become life threatening. Clinically, portal hypertension due to hepatic fibrosis often predominates. These children sometimes present with gastrointestinal bleeding from varicouse veins or hepatomegaly due to congenital hepatic fibrosis. Liver function itself, however, is usually normal.

Pathoanatomical features

Renal involvement is invariably bilateral and mainly symmetric (Figs. 1, 2). The cut surface demonstrates the cortical extension of fusiform or cylindrical spaces arranged radially throughout the renal parenchyma from medulla to cortex. ARPKD is invariably associated with a generalized portal and interlobular fibrosis of the liver accompanied by biliary duct hyperplasia and small distal portal vein branches.

Changing phenotype with prolonged survival

The occasionally prolonged survival of persons with ARPKD (up to the sixth decade) [5] indicates that the quantitative extent of cyst formation is variable, as Blyth and Ockenden [6] have noted. The most common manifestation of ARPKD is the perinatal form, with enlarged kidneys in the neonate. The extent of hepatic fibrosis increases with prolonged survival in cases with milder renal changes. There are cases with onset in early childhood showing severe liver involvement, as well as cases with onset of clinical symptoms in adulthood without symptoms of portal hypertension. With respect to the differential diagnosis one must bear in mind that the morphological picture of collecting duct ectasia looses its uniformity with increasing age. The cysts become nonuniform, and larger cysts begin to compress the renal pelvis. Changes in the pathoanatomical and radiological picture have been observed which resemble those of autosomal dominant polycystic kidney disease.

Differential diagnosis

The most important differential diagnosis is autosomal dominant polycystic kidney disease (ADPKD), which is sometimes indistinguishable from ARPKD. In these cases the demonstration of renal cysts in one parent allows the definite diagnosis of ADPKD. We follow the opinion of Ogborn [7]: “Perhaps the single most useful investigation in the evaluation of a child with early onset of cystic renal disease is ultrasound of the parents.... Thus a negative ultrasound of both parents reduces the probabilility of a diagnosis of ADPKD to the level of a spontaneous mutation.” Meckel syndrome and kidney involvement in Bardet-Biedl syndrome are important differential diagnoses, but these normally show larger cysts.

Renal cystic changes are frequently found in patients with congenital hepatic fibrosis. This coincidence can be explained by cases of mild forms of ARPKD being interpreted as congenital hepatic fibrosis. In this view, most (if not all) cases with classical autosomal recessive congenital hepatic fibrosis without further malformations should be regarded as mild manifestations of ARPKD with only slight renal involvement. Not all microscopical alterations that lead to the diagnosis of congenital hepat-

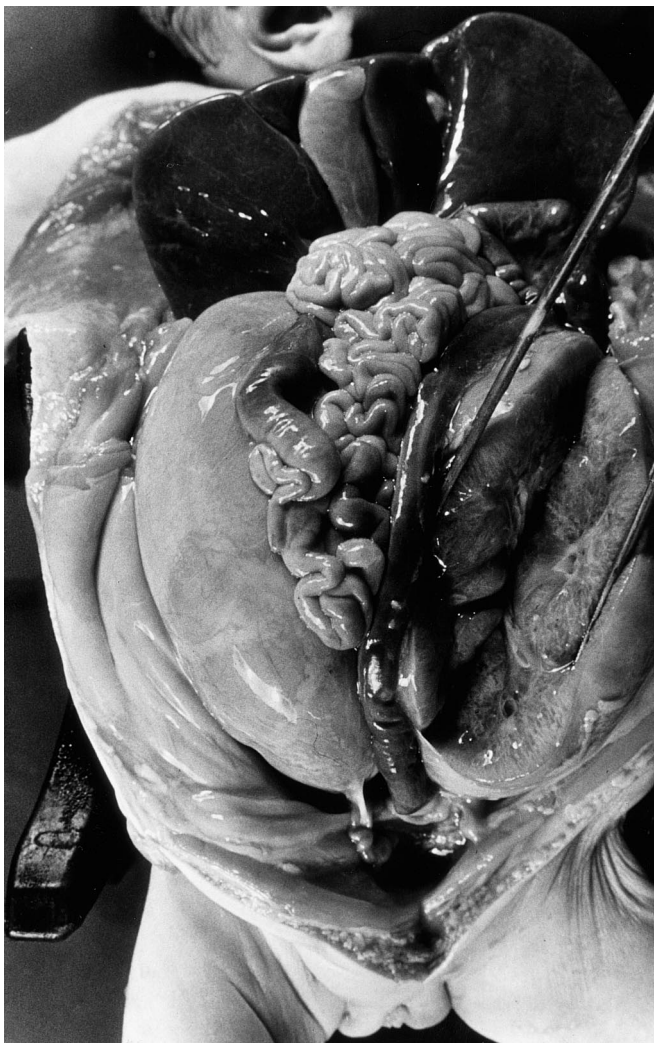


Fig. 1 Newborn with massively enlarged polycystic kidneys representing a severe manifestation of ARPKD. (From [20])

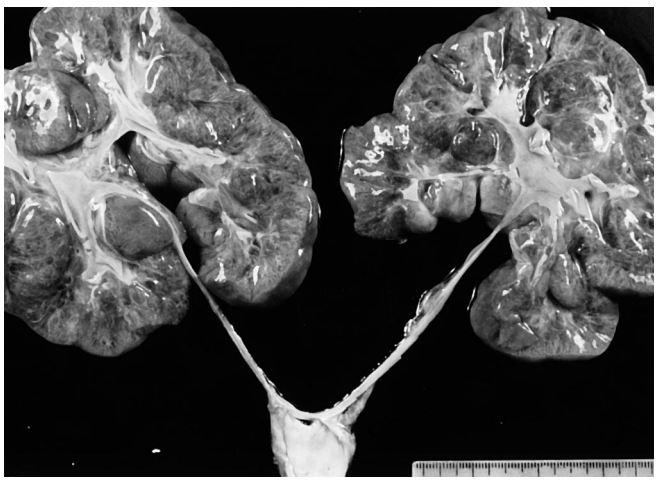


Fig. 2 Due to the uniform slight enlargement of collection ducts (up to 0.2 cm) no single cyst can be seen in the cut section giving the kidney a “spongy” appearance. (From [20])

ic fibrosis (CHF) are manifestations of ARPKD since similar changes can be found in other conditions such as Meckel syndrome, Jeune syndrome, various short rib-polydactyly syndromes, and Ivemark syndrome (see [8]). As has been recently documented, CHF has also been observed in rare cases with ADPKD [9]. Family history of ADPKD leads to the definite diagnosis of ADPKD in these cases. The existence of CHF in various diseases indicates a heterogeneous etiology, and it remains unclear whether liver involvement is identical in the different conditions. Since hepatic pathology is of special significance for the classification of cystic kidney diseases, the liver should always be examined. Gross cystic dilatation of the intrahepatic biliary tree is usually called Caroli disease. The frequent association with ARPKD is well established. Presumably ARPKD and Caroli disease are overlapping syndromes in which an abnormal development on different levels of the biliary tree due to the same pathogenetic mechanism results in two different spectra or stages of a single disease [10].

When should the diagnosis of ARPKD be made in adults?

Establishing the positive diagnosis of ARPKD in patients with polycystic kidneys is sometimes difficult. The most important finding is a negative family history and a normal ultrasound in parents (who should be older than 30 years) of patients with polycystic kidney disease. A patient's negative family history of cystic kidneys alone, however, does not confirm the diagnosis because of the possibility of illegitimate paternity or of a spontaneous mutation of ADPKD. The diagnosis is reliable in cases with negative family history and symptoms of portal hypertension. Parental consanguinity and normal parental ultrasound are also important arguments for the diagnosis of ARPKD. In all the other cases only a liver biopsy ensures the diagnosis. This is required, for example, in genetic counseling and as a obligatory prerequiste for prenatal diagnosis in families with ARPKD. Even then, however, an autosomal dominant form cannot be completely excluded since we know that CHF is found in rare cases of ADPKD [9]. The theoretical possibility of a germ cell line mosaicism must also be considered. An exact diagnosis will be possible in the future when the mutation on the DNA level can be demonstrated directly.

Course of ARPKD in siblings

In a recent study on the clinical course of 42 children from 20 sibships with ARPKD we investigated the intra- and interfamilial variability in terms of age at diagnosis, administration of antihypertensive therapy, liver affection, and renal function [11]. According to the classification of Blyth and Ockenden [6] who defined grades of severity, 12 patients were assigned to the perinatal, 9 to the neonatal, 13 to the infantile, and 8 to the juvenile subtype of ARPKD. In 11 of 20 families different subtypes among affected siblings were observed. In 7 families affected sibs belonged to related subtypes while major intrafamilial differences were observed in 4 families only. The defined subtypes therefore cannot distinguish genetic entities of ARPKD. With respect to the severity of ARPKD, there is a wide spectrum of phenotypic manifestations ranging from stillbirths to mildly affected adults, whereas intrafamilial variability of the clinical picture is generally small. Age of death, however, showed gross variation in 8 sibships.

Incidence

The exact incidence of ARPKD is unknown. Figures range from 1:6000–1:14|000 [12] to 1:55|000 [13]. We have made a rough estimate that the incidence is about 1:40|000 [8]. For practical purposes, however, a heterozygosity frequency of about 1:70 according to an incidence of 1:20|000 should be used for genetic counseling until reliable figures are available. Because of the special genetic situation in Finland the figure published by Kääriäinen et al. [14] of 1:1000 is perhaps not representative for other countries.

Genetics

In 1935 Marquardt [15] first postulated that, "In surviving individuals, cystic kidneys are inherited dominantly. In nonviable individuals cystic kidneys are recessive." The extended classification of Blyth and Ockenden [6] requires genetic interpretation. The authors favored four different gene loci as the most plausible explanation. However, a thorough review of the cases reported by Blyth and Ockenden [6] makes such a rigid classification doubtful. Multiple allelism with only a few different alleles is likely to account for the great variability in different families, particularly with regard to a possible compound heterozygosity. In addition, this would explain the relatively high intrafamilial correspondence of manifestation.

Mapping the ARPKD gene to chromosome 6p

We began in 1988 to collect ARPKD families and to perform linkage studies. We excluded linkage with markers on both ADPKD loci: chromosome 16p [16] and chromosome 4q [17]. In the cpk (congenital polycystic kidney) mouse with cystic kidneys the responsible gene has been mapped, but homologous regions in humans have been made unlikely as a possibile location for ARPKD in humans. Linkage has also been excluded with the human homologue of the mouse Tg737 gene. The Tg737 is a transgenic mouse with cysts caused by the insertional mutation and disruption of a gene on mouse chromosome 14.

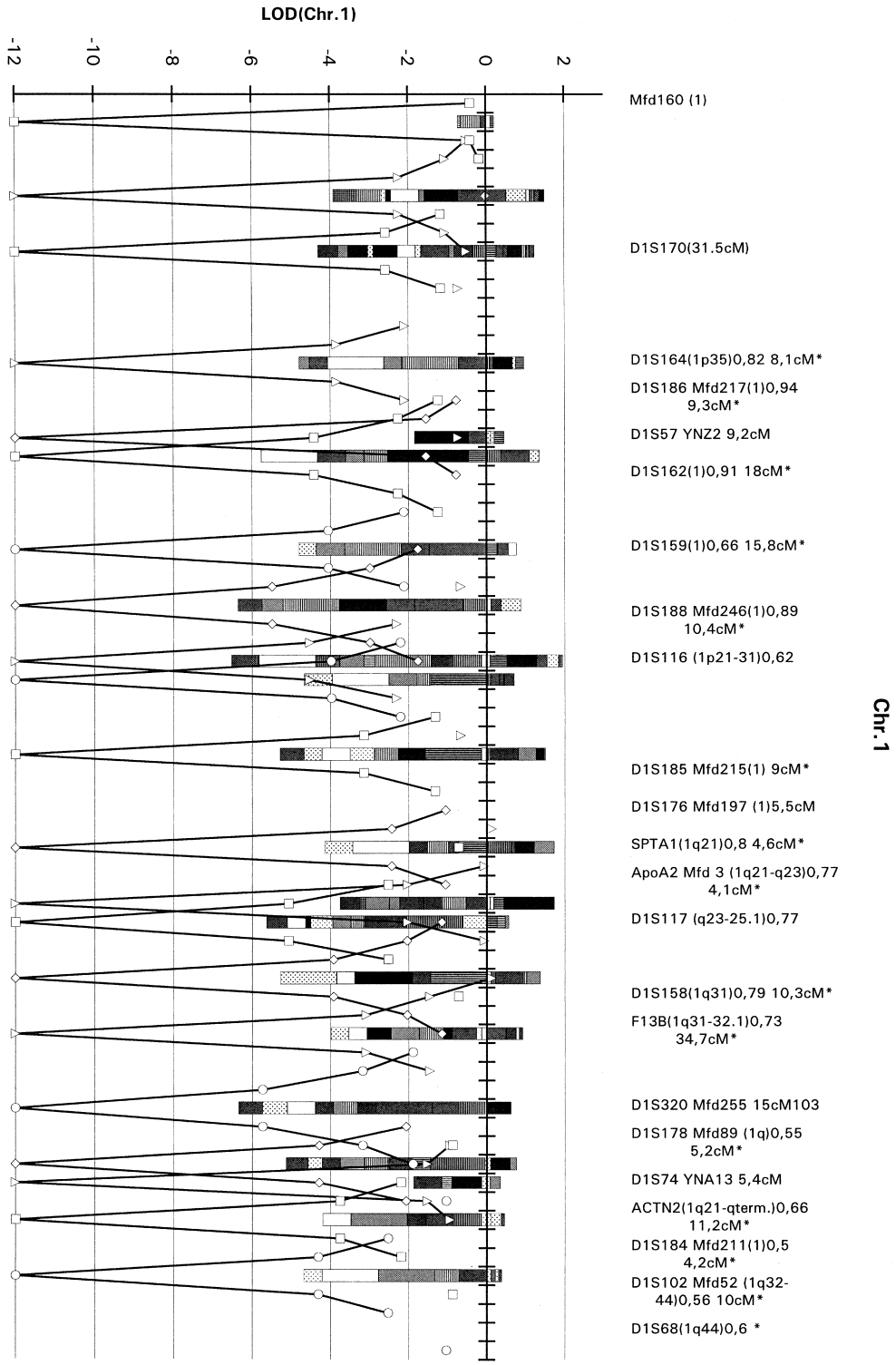


Fig. 3 Results of linkage analysis with markers on chromosome 1 by use of the LODVIEW program. *x-axis*, a map of chromosome 1, divided into intervals of 5 cM of sex-averaged genetic distance. In the position of each marker LOD scores Z (Θ=0.05) are shown as histograms, with segments representing the LOD score of each family. *Segments above the zero line*: positive LOD scores; *segments below the zero line*: negative LOD scores. Regions of exclusion can easily be recognized at the Z (Θ)≤–2 level. The histograms allow a direct evaluation of families with positive and those with negative LOD scores

Parallel to the exclusion of several candidate genes such as Na/K-ATPases, collagene genes, epidermal growth factor, and epidermal growth factor receptor, we have performed a “genome scan” by random use of microsatellite markers. These markers were provided by the German microsatellite consortium (Dr. A. Reis, Berlin). LOD scores were calculated with the LINKAGE package version 5.1 [18], and the excluded chromosomal regions were analyzed simultaneously with the LODVIEW program [19]. An example of exclusion of linkage of the ARPKD genes with markers on chromosome 1, visualized with the LODVIEW program is shown in Fig. 3. This approach has been chosen because of the possibility of genetic heterogeneity. A subset of families with linkage to a certain region would have been easily recognized with adjacent markers, while other families would

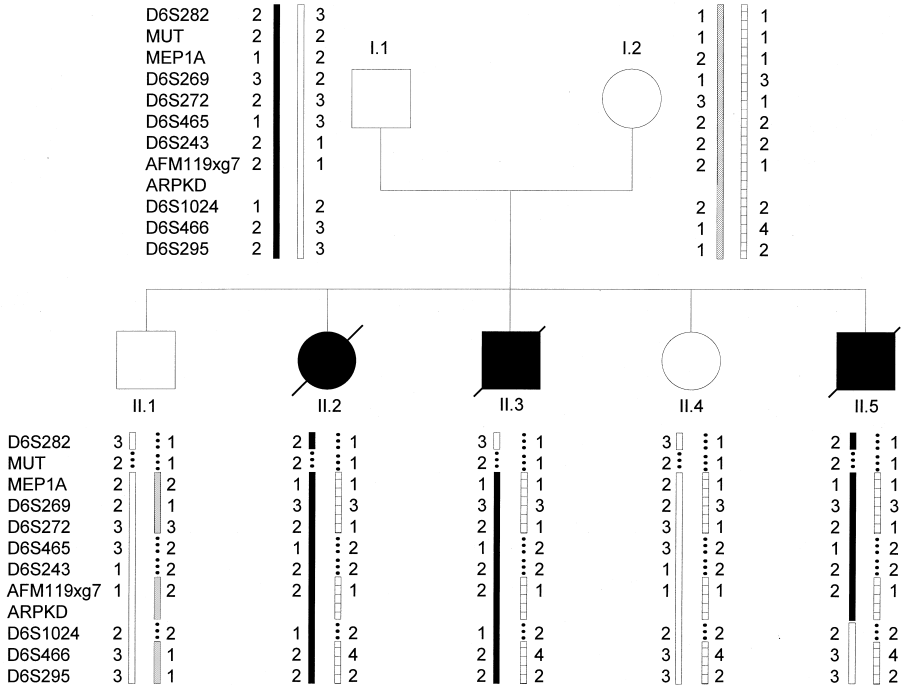


Fig. 4 ARPKD pedigree (family 68) with key recombination narrowing the critical region on chromosome 6p. The reombination occurring in child II.3 places the ARPKD locus centromeric to D6S282, and the recombination in II.5 locates the ARPKD gene telomeric to D6S1024 [24]

not show positive LOD scores with these markers. After exclusion of at least 2971 cM, representing 76% of the entire genome, we were able to localize the ARPKD gene to the chromosomal region 6p21-cen on the basis of 16 ARPKD families [20]. There was no evidence for genetic heterogeneity among different clinical phenotypes. The highest LOD score of 7.42 was obtained with D6S272 at Θ=0.00 [20]. In a more extensive study of severe early onset ARPKD cases (perinatal type) a total LOD score of 4.58 (Θ=0.01) was obtained in a collection of 21 American and European families, thus confirming 6p linkage in severe cases [21].

Narrowing the region with recombinant families

With the inclusion of additional families we performed a linkage analysis of 90 families. One key recombinant family is shown in Fig. 4 (family 68). The results of haplotype analysis in families with recombinations in the ARPKD region is summarized in Fig. 5. The flanking markers are D6S269 (telomeric) and D6S466 (centromeric). A published recombination between the ARPKD gene and D6S272 in a family in the United States defines D6S272 as a distal flanking marker. The genetic distance between the flanking markers D6S272 and D6S466 is about 4 cM (Fig. 5).

An integrated genetic and physical map

Recently the International ARPKD Consortium [22] described a YAC contig that spans about 5 cM defined by the markers D6S1253–D6S295. Forty-three sequence-tagged sites (STS) were mapped within this interval, including 20 novel STSs. A minimal set of two YACs spans the segment D6S465–D6S466, which contains the ARPKD gene locus. Estimates of their sizes suggest that the critical region is smaller than 3.1 Mb. Twenty-eight STSs map to this interval, yielding an average STS density of less than 1/150 kb.

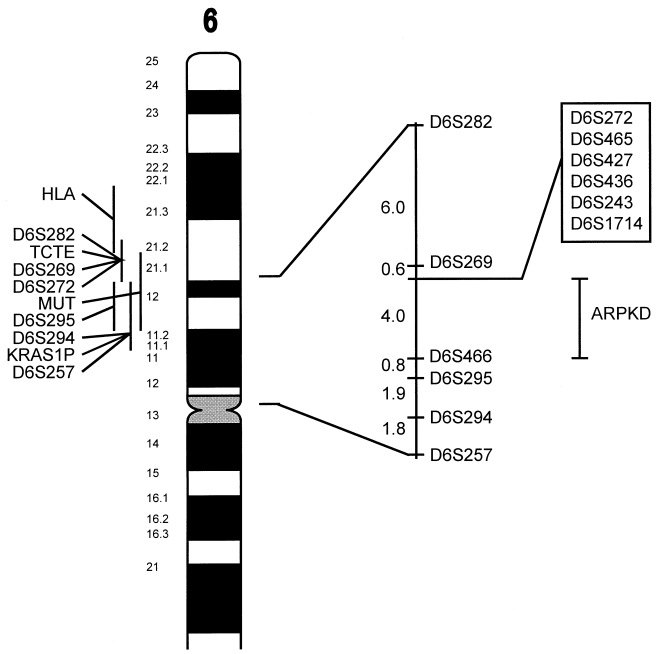


Fig. 5 Ideogram of human chromosome 6p, with an expanded genetic map of the analyzed region including the position of locus ARPKD. Distances are those published by Dib et al. [27]

Linkage disequilibrium

One important approach towards the identification of a mapped disease gene is the analysis of a possible linkage disequilibrium. Linkage disequilibrium can be expected only in cases with a very short distance between the analyzed markers or disease genes. The detection of linkage disequilibrum is less likely in a population of varying ethnic backgrounds. Because the families analyzed could be traced back to 15 different countries, there was only a small probability of detecting an existing linkage disequilibrium. The statistical analysis of the flanking markers (two-tailed Fisher's exact test) revealed a linkage disequilibrium in the subpopulation of families predominantly of German origin for alleles of D6S272 only (allele 2: P=0.024; allele 3: P=0.046). In the complete material these results were not significant. Nevertheless, the data indicate that the disease gene is closer to D6S272 than to the other flanking marker D6S466, for which no linkage disequilibrium was detected. This agrees with the observation that only one recombination was found with D6S272, but seven with D6S466 in about 200 ARPKD families.

Exclusion of candidate genes

The chromosomal region 6p12-p21.1 is very gene rich. Several genes map to this region. Among others the following genes have been excluded as the ARPKD gene:

Alpha subunit of the metalloendopeptidase meprin

Meprins are kidney and intestinal proteases encoded by two distinct genes, *MEP1A* and *MEP1B*. *MEP1A* has previously been mapped to human chromosome 6p21.2-p21.1 and proposed as a possible candidate gene for ARPKD [23, 24]. With definition of an intragenic *Hha*I restriction fragment length polymorphism amplified by PCR for linkage analysis [25], MEP1A could be excluded as the ARPKD gene because of a recombination event in one ARPKD family [24].

Gluthathione S-transferase

Gluthathione S-transferase (GST2) is involved in the metabolism of xenobiotics and carcinogens and in the binding and possible transport of some endogenous anionic compounds such as bilirubin. Although it is expressed in kidneys, it was not considered a strong candidate gene for ARPKD. There are multiple GST2 genes and pseudogenes in the 6p12 region [26]. In the nucleotid sequence of the genes we found a polymorphic trinucleotide-repeat, which we used for typing of all our ARPKD-families. The presence of two recombinations, excluded GST2 as the ARPKD gene.

Methylmalonyl coenzyme A mutase

Methylmalonyl coenzyme A mutase (MUT) is involved in the degradation of branched chain amino acids. It has been mapped to 6p21.2-p12 [27]. Although MUT does not seem to be a strong ARPKD candidate, we excluded MUT by use of an intragenic PCR restriction fragment length polymorphism. The two-allele polymorphism was informative in 20 ARPKD families. We found one recombination which excluded MUT as the ARPKD gene. Analyzing the haplotypes of 7 families who had different recombinations in the region, it was possible to map MUT distal to the D6S272/D6S243/D6S465/D6S119 cluster.

Other genes have recently been mapped to the ARPKD region. The exclusion without intragenic polymorphisms available, however, is much more difficult.

No evidence of genetic heterogeneity

So far there is no evidence of genetic heterogeneity in ARPKD. Only one family has not been able to be classified yet, as the patient and his unaffected sibling showed identical haplotypes with chromosome 6p markers. The pathoanatomic picture is compatible with ARPKD, but it cannot be regarded as absolutely typical in terms of a nonuniform dilatation of collecting ducts. Because of ambiguous histopathological findings it is difficult to regard this family as a "nonlinked" case. For diagnostic purposes the risk of a non-6p linked ARPKD is extremely low in clinically typical cases, less than 1%.

Prenatal diagnosis

Prenatal diagnosis of ARPKD by prenatal ultrasound alone is not reliable. Increased echogenicity and renal enlargement are the main ultrasound signs of ARPKD. Oligohydramnios is characteristic but not always present. In some cases enlarged kidneys are not detected before the second half of pregnancy. This has been demonstrated in six patients observed personally and many reports in literature [28]. Prenatal diagnosis by ultrasound is even more uncertain in cases of mild involvement and only a small proportion of dilated nephrons. Reports of increased trehalase activity in ARPKD [29] have not been confirmed in other studies.

Reliable prenatal diagnosis is possible by means of closely linked DNA markers. We have performed segregation analysis using information for prenatal diagnosis in 67 families. Except one, all were informative for flanking markers. In 15 families the affected children had died long before DNA studies were possible, and therefore paraffin embedded tissue only was available. Prenatal prediction in 19 families showed the following results: unaffected homozygous fetus (n=4), unaffected homozygous or heterozygous (n=1), unaffected heterozygous (n=9), and affected homozygous (n=2). In three cases a recombination between informative flanking

markers had occurred, and it was not possible to decide whether the fetus was homozygous affected or heterozygous. Necropsy confirmed the diagnosis of ARPKD in two aborted fetuses, one as early as 14 weeks gestation [30, 31]. The above evidence for linkage disequilibrium of ARPKD and D6S272 indicated a high fetal risk.

Prerequisite for prenatal diagnosis of ARPKD is a correct diagnosis in the affected child and the ultrasound exclusion of cystic kidneys in both parents. The most important differential diagnosis is early onset of ADPKD, which, however, can easily be diagnosed by the demonstration of an affected parent.

Acknowledgements Our studies were supported by the Bundesministerium für Forschung und Technologie and the Deutsche Forschungsgemeinschaft. We thank Tobias Grambow for technical assistance and Michael Knapp for calculating LOD scores, linkage disequilbrium, and multipoint data.

References

1. Zerres K, Rudnik-Schöneborn S, Deget F et al. (1996) Clinical course of 115 children with autosomal recessive polycystic kidney disease. Acta Paediatr 85:437–445
2. Kaplan BS, Fay J, Shah V, Dillon MJ, Barratt TM (1989) Autosomal recessive polycystic kidney disease. Pediatr Nephrol 3:43–49
3. Gagnadoux MF, Habib R, Levy M, Brunelle F, Broyer M (1989) Cystic renal diseases in children. Adv Nephrol 18:33–58
4. Cole BR, Conley SB, Stapleton FB (1987) Polycystic kidney disease in the first year of life. J Pediat 111:693–699
5. Neumann HPH, Zerres K, Fischer CL et al (1988) Late manifestation of autosomal-recessive polycystic kidney disease in two sisters. Am J Nephrol 8:194–197
6. Blyth H, Ockenden BG (1971) Polycystic disease of kidneys and liver presenting in childhood. J Med Genet 8:257–284
7. Ogborn MR (1994) Polycystic kidney disease – a truly pediatric problem. Pediatr Nephrol 8:762–767
8. Zerres K, Völpel MC, Weiß H (1984) Cystic kidneys. Genetics, pathologic anatomy, clinical picture, and prenatal diagnosis. Hum Genet 68:104–135
9. Cobben JM, Breuning MH, Schoots C, TenKate LP, Zerres K (1990) Congenital hepatic fibrosis in autosomal dominant polycystic kidney disease. Kidney Int 38:880–885
10. Nakanuma Y, Terada T, Ohta G, Kurachi M, Matsubara F (1981) Caroli's disease in congenital hepatic fibrosis and infantile polycystic disease. Liver 2:346–354
11. Deget F, Rudnik-Schöneborn S, Zerres K (1995) Course of autosomal recessive polycystic kidney disease (ARPKD) in siblings. A clinical comparison of 20 sibships. Clin Genet 47:248–253
12. Bosniak MA, Ambos MA (1975) Polycystic kidney disease. Semin Roentgenol 10:133–143
13. Potter EL (1972) Normal and abnormal development of the kidney. Year Book, Chicago
14. Kääriäinen H (1987) Polycystic kidney disease in children: A genetic and epidemiolgogical study of 82 Finnish patients. J Med Genet 24:474–481
15. Marquardt W (1935). Cystennieren, Cystenleber und Cystenpancreas bei zwei Geschwistern. Thesis, University of Tübingen
16. Wirth B, Zerres K, Fischbach M et al (1987) Autosomal recessive and dominant polycystic kidney disease are not allelic. Hum Genet 77:221–222
17. Zerres K, Mücher G, Rudnik-Schöneborn S (1994) Autosomal recessive polycystic kidney disease does not map to the second gene locus for autosomal dominant polycystic kidney disease on chromosome 4. Hum Genet 93:697–698
18. Lathrop GM, Lalouel JM, Julier C, Ott J (1994). Strategies for multilocus linkage analysis in humans. Proc Natl Acad Sci USA 81:3443–3446
19. Hildebrandt F, Pohlmann A (1993) A computer program for the graphical evaluation of LODscore results in exclusion mapping of human disease genes. Comp Biomed Res 26:592–599
20. Zerres K, Mücher G, Bachner L et al (1994) Mapping of the gene for autosomal recessive polycystic kidney disease (ARPKD) to chromosome 6p21-cen. Nature Genetics 7:429–432
21. Guay-Woodford LM, Mücher G, Hopkins SD et al (1995). The severe form of autosomal recessive polycystic kidney disease (ARPKD) maps to chromosome 6p21.1-p12: implications for genetic counselling. Am J Hum Genet 56:1101–1107
22. Lens XM, Onuchic LF, Wu G et al (1997) An integrated genetic and physical map of the autosomal recessive polycystic kidney disease region. Genomics 41:463–466
23. Bond JS, Rojas K, Overhauser J, Zoghbi HY, Jiang W (1995) The structural genes, *MEP1A* and *MEP1B*, for the α and β subunits of the metalloendopeptidase meprin map to human chromosomes 6p and 18q, respectively. Genomics 25:300–303
24. Jiang W, Dewald G, Brundage E, Mücher G, Schildhaus H-U, Zerres K, Bond J (1995) Fine mapping of *MEP1A*, the gene encoding the α subunit of the metalloendopeptidase meprin, to human chromosome 6p21. Biochem Biophys Res Comm 216:630–635
25. Dewald G, Schildhaus H-U, Mücher G, Zerres K (1996) A *Hha*I polymorphism in the human *MEP1A* gene encoding the α subunit of the metalloendopeptidase meprin. Hum Hered (in press)
26. Board RG, Webb GC (1987) Isolation of a cDNA clone and localization of human glutathione S-transferase 2 genes to chromosome band 6p12. Proc Natl Acad Sci USA 84:2377–2381
27. Dib C, Fauré S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J (1996) A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380
28. Zerres K, Hansmann M, Mallmann R, Gembruch U (1988) Autosomal recessive polycystic kidney disease. Problems of prenatal diagnosis. Prenat Diagn 8:215–229
29. Morin PR, Potier M, Dallaire L, Melancon SB, Boisvert J (1981) Prenatal detection of the autosomal recessive type of polycystic kidney disease by trehalase assay in amniotic fluid. Prenat Diagn 1:75–79
30. Zerres K, Mücher G, Rudnik-Schöneborn S et al (1995) Early morphological evidence of autosomal recessive polycystic kidney disease. Lancet 345:987
31. Wisser J, Hebrisch G, Froster U. et al (1995) Prenatal sonographic diangosis of autosomal recessive polycystic kidney disease (ARPKD) during the early second trimester. Prenat Diagn 15:868–871