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The Severe Perinatal Form of Autosomal Recessive Polycystic Kidney Disease Maps to Chromosome 6p21.1-p12: Implications for Genetic Counseling

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Summary

Autosomal recessive polycystic kidney disease (ARPKD) is a one of the most common hereditary renal cystic diseases in children. Its clinical spectrum is widely variable with most cases presenting in infancy. Most affected neonates die within the first few hours of life. At present, prenatal diagnosis relies on fetal sonography, which is often imprecise in detecting even the severe form of the disease. Recently, in a cohort of families with mostly milder ARPKD phenotypes, an ARPKD locus was mapped to a 13-cM region of chromosome 6p21-cen. To determine whether severe perinatal ARPKD also maps to chromosome 6p, we have analyzed the segregation of seven microsatellite markers from the ARPKD interval in 22 families with the severe phenotype. In the majority of the affected infants, ARPKD was documented by histopathology. Our data confirm linkage and refine the ARPKD region to a 3.8-cM interval, delimited by the markers D6S465/D6S427/D6S436/D6S272 and D6S466. Taken together, these results suggest that, despite the wide variability in clinical phenotypes, there is a single ARPKD gene. These linkage data and the absence of genetic heterogeneity in all families tested to date have important implications for DNA-based prenatal diagnoses as well as for the isolation of the ARPKD gene.

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Introduction

Autosomal recessive polycystic kidney disease (ARPKD) is a hereditary cystic disease that involves the kidneys and the biliary tract. Exact incidence figures for ARPKD are not available, and estimates vary widely, ranging from 1:6,000 live births in an American report to 1:40,000 in the European literature (Bosniak and Ambos 1975; Zerres et al. 1984). Taking all available data into account, the most representative incidence estimate is probably 1–2:10,000 live births (Bernstein and Slovis 1992).

The clinical spectrum of ARPKD is widely variable with most cases presenting in infancy (Zerres et al. 1984). Typically, ARPKD patients are identified either in utero or at birth. Prenatal diagnoses have been made either by screening fetal sonography or by serial sonograms in "high risk" pregnancies. Affected fetuses characteristically have enlarged echogenic kidneys and oligohydramnios due to poor renal output in utero (Reuss et al. 1991). As a result of the oligohydramnios, these infants develop the "Potter's phenotype," a syndrome consisting of pulmonary hypoplasia, a characteristic facies, and deformities of the spine and limbs (Osathanondh and Potter 1964; Zerres et al. 1988). In affected neonates, the clinical course is most often characterized by respiratory insufficiency and death within the first few hours of life.

Historically, there has been debate in the literature as to the genetic bases for ARPKD. In their classic report, Blyth and Ockenden (1971) postulated that ARPKD had four discrete phenotypes that were caused by four distinct gene mutations. Subsequent review of their patients refuted this rigid genetic classification (Kaplan et al. 1989). On the basis of family studies involving 73 affected children, Kaariainen et al. (1988) suggested that the perinatal form of ARPKD may represent a distinct genetic entity, whereas in neonatal survivors, a single

defective gene could cause the wide variability in phenotypic manifestations. Alternatively, Zerres et al. (1984, 1992) have proposed that the entire clinical spectrum of ARPKD can be explained by a single gene and multiple allelism. This genetic model suggests that a few mutant alleles of one mutant gene could account for the relatively high phenotypic concordance within families as well as the broad range of phenotypes that is evident among different families.

Recently, Zerres et al. (1994) mapped an ARPKD locus to chromosome 6p21-cen. These data positioned the ARPKD locus within a 13-cM region flanked by the markers TCTE1 and D6S294 and appear to confirm that the variable clinical manifestations of ARPKD result from a single-gene defect. Among the 32 families analyzed, the full spectrum of renal and hepatic manifestations were manifest, and no evidence of genetic heterogeneity was obtained. However, in the majority of these families, the affected children had milder forms of the disease with survival beyond the 1st year of life. Therefore, the authors cautioned that linkage must be confirmed for the severe perinatal form of the disease.

In this report, we confirm that the severe perinatal phenotype of ARPKD maps to the same chromosome 6p21-cen region. Our analyses demonstrate that ARPKD is a genetically homogeneous disorder, and, therefore, we have used recombination events to further refine the ARPKD interval. These data provide the genetic basis for both cloning the ARPKD gene and for DNA-based prenatal analyses in at-risk pregnancies.

Subjects and Methods

Pedigrees

Two cohorts of ARPKD families were analyzed. In both cohorts, the affected children presented in the perinatal period with massively enlarged kidneys, a history of oligohydramnios, and respiratory insufficiency. Most of the affected children died shortly after birth. The first cohort comprised 14 families of American and South African origin. In this cohort (families 1–14), the diagnosis was confirmed either by biopsy or by autopsy. Five of these families were multiplex, and the remaining nine were simplex families. Eight additional families (families 15–22) from a European cohort were added to the analyses. Of these families, four were multiplex and four were simplex. In the European cohort, the diagnosis was confirmed by the clinical history and negative renal ultrasound results in both parents. In addition, most index cases had one of the following criteria: (1) parental consanguinity, (2) sonographic signs of hepatic fibrosis, and (3) a pathoanatomic diagnosis of ARPKD in at least one affected sibling.

DNA Isolation

DNA was obtained from all families under informed consent. For living pedigree members, DNA was extracted from peripheral blood lymphocytes by using standard protocols. For deceased affected children, DNA was extracted from paraffin-embedded tissue by using a commercially available method (Oncor EX-WAX). In brief, the tissue was cut into 5-μm-thick sections, and three to five sections were used per extraction. The tissue was deparaffinized in 100% ethanol, the cells were lysed, and the DNA was solubilized. In a series of precipitation steps, the digested proteins were removed and the DNA extracted from residual debris and paraffin.

PCR Amplification and Linkage Analysis

Two of the microsatellite markers, D6S272 and D6S295, were used previously by Zerres et al. (1994). Five additional markers from the 6p21-centromere interval were also scored (Gyapay et al. 1994). The American families (families 1–14) were typed by the Guay-Woodford laboratory, whereas the European families (families 15–23) were typed by the Zerres laboratory. All microsatellite markers were examined by PCR amplification followed by separation of the amplified fragments on 6% polyacrylamide sequencing gel.

Primer pairs were either purchased from Research Genetics or synthesized using nucleotide sequences available in the Genome Data Base. For microsatellite amplification in the American families, one primer of each pair was end-labeled according to standard protocols with [γ^{32}P] using T4 polynucleotide kinase (Amersham). With lymphocyte-derived DNA, 50 ng DNA was amplified in a 25 μl PCR reaction with 200 nM of both labeled and unlabeled primers, dNTPs, reaction buffer, and 0.2 U AmpliTaq DNA polymerase, according to manufacturer's (Perkin Elmer Cetus) specifications. The reactions were performed with the following thermocycling protocol: initial denaturation at 94°C for 5 min, followed by 30 cycles of 94°C for 40 s and 55°C for 30 s, with a final cycle of 72°C for 2 min. In the European cohort, microsatellite markers were amplified as previously described (Zerres et al. 1994).

With DNA derived from paraffin-embedded tissues, 100 ng DNA was amplified in a 100 μl PCR reaction with 200 nM of both labeled and unlabeled primers, dNTPs, reaction buffer, and 0.8 U AmpliTaq DNA polymerase. The reactions were performed with the following thermocycling protocol: initial denaturation at 94°C for 10 min; followed by 40 cycles of 94°C for 1 min, 55°C for 2 min, and 72°C for 1 min; with a final cycle of 72°C for 7 min. The samples were concentrated to a 25-μl volume before electrophoresis.

Two-point and multipoint linkage analyses were performed using the LINKAGE package, version 5.1 (Lathrop et al. 1985). The gene frequency was set as .01, with a penetrance of 100% for affected individuals and 0% for unaffected family members. The alleles in

each family were scored 1–4, and the frequency of each allele was designated as .25. Haplotype studies were performed in all families, and the most likely haplotypes were inferred by minimizing the crossover events in each sibship.

Prenatal Analyses

In family 11 (American), two children were previously born with massively enlarged kidneys and died of respiratory insufficiency in the perinatal period. The diagnosis of ARPKD was confirmed by autopsy. Initial linkage analyses demonstrated that five of the seven microsatellite markers from the ARPKD interval were informative in this family and that specific haplotypes cosegregated with the disease in the two affected children. Subsequently, the mother became pregnant and requested genetic analysis of her fetus. Given that the maternal age was >35 years, amniocentesis was performed under informed consent at 14 wk for fetal karyotype analysis. Using standard techniques (Priest 1991), amniocytes were isolated, were cultured for 10 d, and were pelleted by centrifugation; and fetal DNA was extracted.

Similarly, in family 22 (British), two children died of respiratory insufficiency within the first few hours of life and ARPKD was subsequently confirmed by autopsy. Four of the seven microsatellite markers from the ARPKD interval were informative in this family. Specific haplotypes cosegregated with the disease in the two affected children, and these haplotypes were readily distinguished from those of the two unaffected children. In a recent pregnancy, chorionic villus sampling was performed at 10-wk gestation, and fetal DNA was extracted for linkage analyses. The chorionic cells were washed three times in Na-EDTA buffer, pH 8 (75 mM NaCl and 24 mM EDTA) and then subjected to overnight digestion with proteinase K (10 mg/ml in Na-EDTA buffer with 0.1% SDS) at 50°C. Following phenol extraction, the DNA was precipitated in ethanol and resuspended in TE (10 mM TRIS, pH 9 and 1 mM EDTA).

Results

Linkage Analyses

In a cohort of families with widely variable clinical phenotypes, Zerres et al. (1994) have mapped an ARPKD gene to chromosome 6p21-cen. In the present study, we have confirmed that in families with the severe perinatal phenotype the ARPKD gene maps to the same genetic interval. We analyzed seven microsatellite markers from the 6p21.1-p12 region. As shown in table 1, significant lod scores were obtained with six of these seven markers. In particular, all families were informative with D6S272, and a maximum lod score (Z_{max}) of 5.51 was obtained at a θ of .02. Given the uncertainty regarding the disease incidence, two-point linkage analyses were also performed with the gene frequency set as .001. There was no significant difference in the lod score results (data not shown). Haplotype analyses revealed no evidence of genetic heterogeneity, as all the family haplotypes were consistent with chromosome 6p linkage.

Preliminary data from a subset of American families had suggested that ARPKD may be linked to chromosome 2p25-24, the syntenic human linkage group for the mouse congenital polycystic kidneys (*cpk*) mutation (Guay-Woodford et al. 1993; Simon et al. 1994). Of interest for the current study, the *cpk* mouse model most closely resembles the severe perinatal phenotype of ARPKD (Preminger et al. 1982). Therefore, we typed our severely affected cohort of ARPKD families for the 2p25-24 markers, D2S319, D2S205, D2S281 and D2S162. Lod scores of <-2 for each marker excluded linkage between the human *cpk* synteny group and ARPKD (data not shown).

Haplotype Analyses and Refinement of the ARPKD Region

Haplotype analyses were consistent with the marker order proposed by Gyapay et al. (1994) and Muecher et al. (1994). Pedigrees and haplotypes of the key recombinant families are diagrammed in figure 1. In addition, as shown in figure 2, a key recombinant event occurred in the fetus of family 22. These specific recombination events are schematically represented in figure 3. The recombination between the ARPKD phenotype and D6S272 in family 3 is the first recombination event observed with this marker in the 60 families analyzed to date. In addition, recombinations occurred between ARPKD and D6S466 in family 9 and between ARPKD and D6S295 in family 1 and family 19. In family 13, recombination events were detected between D6S269 and ARPKD in a nonaffected individual (II:3) and between ARPKD and D6S466 in an affected individual (II:2). All recombinants were confirmed by performing the PCR amplification for the pertinent markers at least twice.

Both our two-point linkage data and our haplotype analyses support the locus order: 6pter–D6S269–D6S465/D6S427/D6S436/D6S272–ARPKD–D6S466–D6S295–6cen. In table 1, the two-point data suggest that D6S295 actually lies closer to ARPKD than D6S466. However, it is important to note that in family 9 two recombination events occurred between ARPKD and D6S466 and that these individuals were not informative for D6S295. Because the markers D6S427, D6S436, and D6S272 were not informative in family 6, our two-point data could not resolve the order of the markers, D6S465/D6S427/D6S436/D6S272 with respect to one another. Moreover, given the limited number of recombination events in the interval between

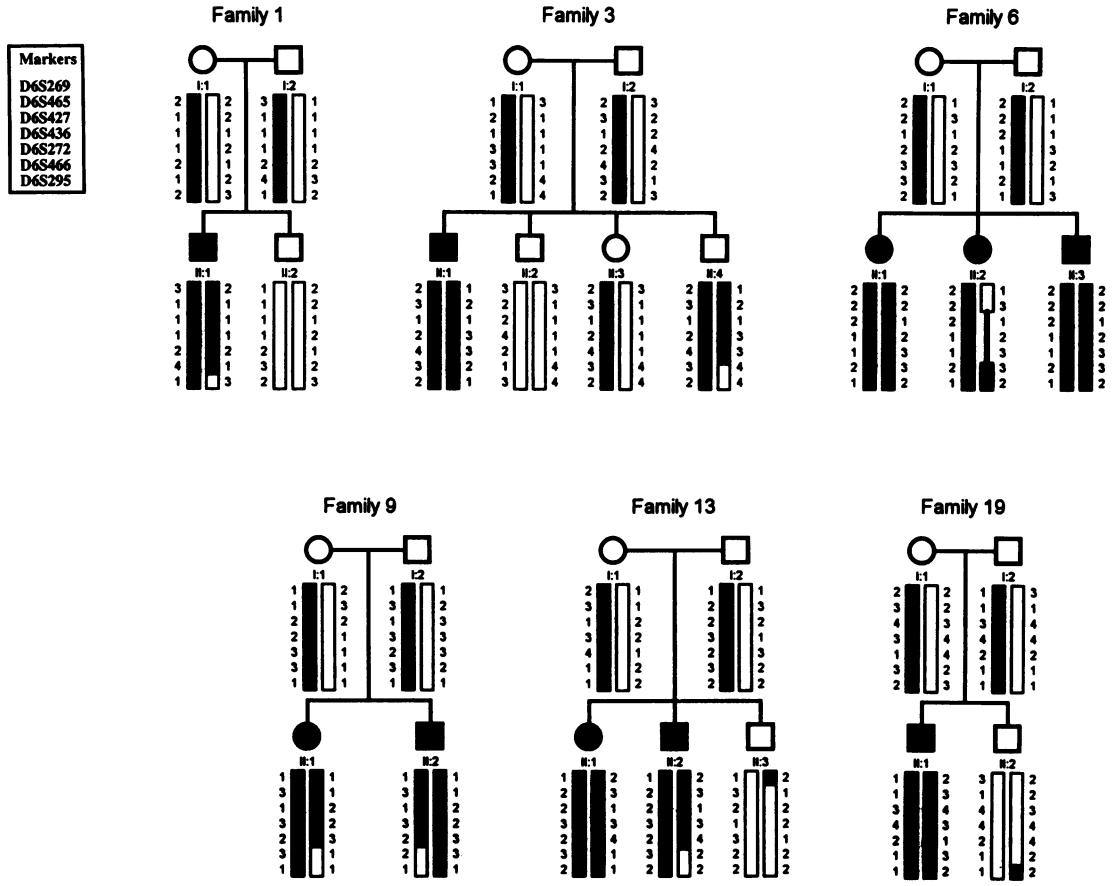

Figure 1 Pedigrees and haplotypes of ARPKD families with recombination events within the chromosome 6p21.1-p12 interval. For each sibling, the paternal chromosome is on the left and the maternal chromosome is on the right. The haplotype that segregates with ARPKD is depicted by the blackened bar. In family 6, the thin line represents an indeterminate region between the recombination breakpoints.

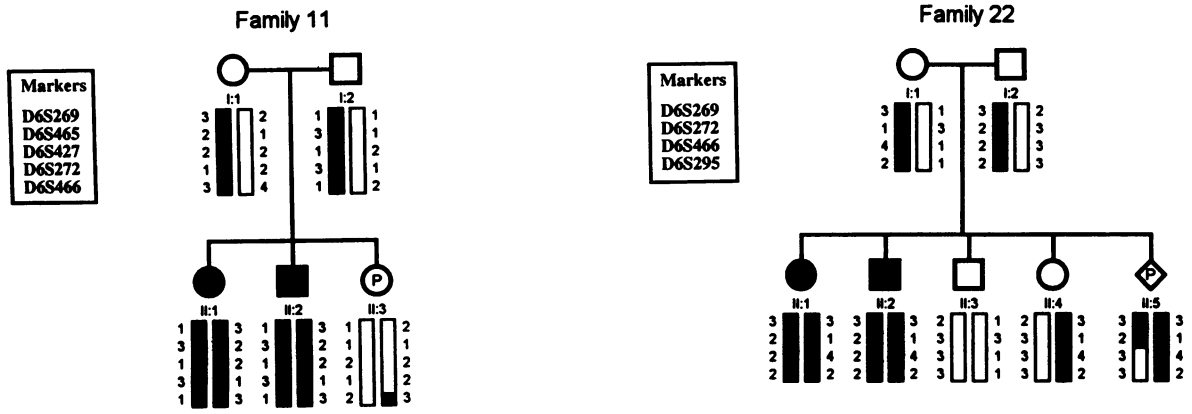

Figure 2 Pedigrees and haplotypes of two ARPKD families in whom prenatal analyses were performed. For each family, haplotypes included only those markers that were informative. The fetus is represented by a unblackened symbol with a central P.

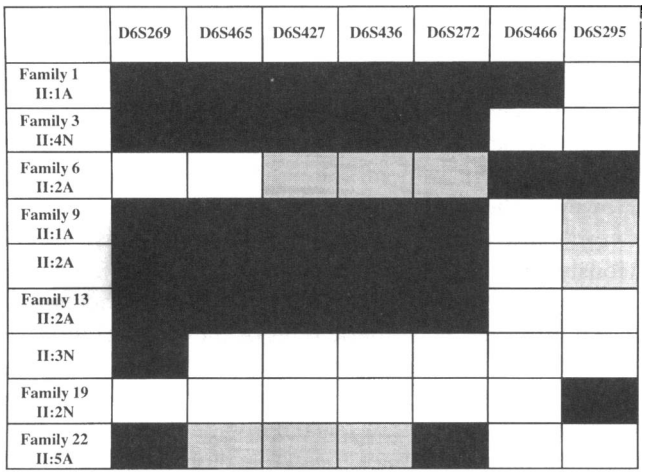

Figure 3 Schematic representation of the critical recombination events within the 6p21.1-p12 region. The specific individuals are listed on the left. Affected individuals are designated "A," and nonaffected individuals are designated "N." The unblackened boxes and the blackened boxes, respectively, represent marker alleles of the nonaffected and affected chromosomes. The stippled boxes represent noninformative markers. These recombination events delimit the ARPKD region to the interval between D6S465/D6S427/D6S436/D6S272 and D6S466.

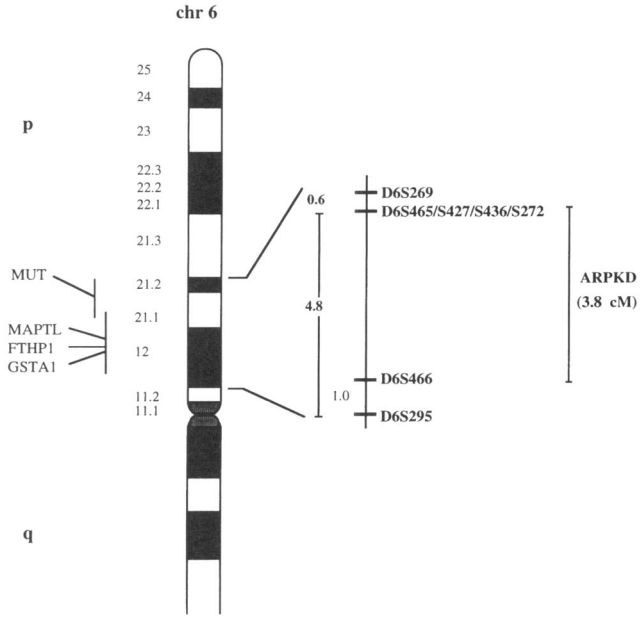

Figure 4 Refinement of the ARPKD region to a 3.8-cM interval on 6p21.1-p12. On the right, the boldface numbers represent genetic distances (in cM) reported between D6S269 and D6S272 and between D6S272 and D6S295 in the consensus map of chromosome 6p (Volz et al. 1994). In addition, the recent Généthon map established a 1-cM distance between D6S466 and D6S295 (Gyapay et al. 1994). Therefore, our data position the ARPKD locus within a 3.8-cM interval flanked by D6S465/D6S427/D6S436/D6S272 and D6S466, as indicated by the vertical line. Genes that have been localized to this region are indicated on the left (MUT = methylmalonyl-coenzyme A mutase; MAPTL = microtubule associated protein tau-like; FTHP1 = ferritin heavy polypeptide pseudogene 1; and GSTA1 = glutathione S-transferase alpha).

D6S269 and D6S295, multipoint analysis was not informative with our linkage data (data not shown).

In the recent consensus map of chromosome 6p, a genetic distance of 4.8 cM was reported between D6S272 and D6S295 (Volz et al. 1994). In addition, the recent Généthon map established a 1-cM distance between D6S466 and D6S295 (Gyapay et al. 1994). Therefore, we interpolated the ARPKD locus into the previously established marker order and positioned the gene within a 3.8-cM interval flanked by D6S465/D6S427/D6S436/D6S272 and D6S466 (fig. 4).

Prenatal Analyses

In Family 11, fetal cytogenetic analysis revealed a normal 46 XX female karyotype. As shown in figure 2, haplotype analyses predict that the fetus is unaffected. At ≥35 wk of gestation, the fetus remains normal.

In Family 22, the fetus inherited the affected maternal haplotype (fig. 2). However, given the recombination event between D6S272 and D6S466 on the paternal chromosome, the fetal haplotype analysis was not predictive. Given the significant probability that the fetus

Table 1

Two-Point Linkage Analysis of the ARPKD Gene with Chromosome 6 Microsatellites

	Lod Score at θ =							
Marker	.00	.01	.05	.10	.20	.30	Z_{max}	θ at Z_{max}
D6S269	−∞	2.72	3.42	3.16	2.13	1.08	3.42	.05
D6S465	−∞	3.08	3.73	3.41	2.28	1.15	3.73	.05
D6S427	2.40	2.35	2.13	1.82	1.19	.60	2.40	.00
D6S436	−∞	4.07	4.09	3.27	2.07	1.01	4.20	.03
D6S272	−∞	5.41	5.30	4.59	2.98	1.49	5.51	.02
D6S466	−∞	2.65	3.80	3.55	2.38	1.20	3.80	.06
D6S295	−∞	3.05	3.30	3.00	2.06	1.07	3.32	.04

could be affected, the parents elected to terminate the pregnancy at 14 wk gestation. Fetal pathologic examination revealed dilated collecting ducts and congenital hepatic fibrosis consistent with the diagnosis of ARPKD. Taken together, the pathologic diagnosis and the haplotype data confirm that the ARPKD gene lies in the interval between D6S465/D6S427/D6S436/D6S272 and D6S466.

Discussion

The data presented in this report confirm and substantially extend the findings of Zerres et al. (1994). The full spectrum of clinical phenotypes has now been analyzed. Genetic homogeneity in the linkage of 60 ARPKD families to 6p21.1-p12 supports the hypothesis that ARPKD results from a single gene defect. In addition, our data exclude linkage between ARPKD and the human homologues of the mouse *cpk* and Tg737 genes, two mouse mutations in which the phenotypes closely resemble severe ARPKD (Moyer et al. 1994; Simon et al. 1994). While genetic heterogeneity has been detected in most linkage studies with recessive diseases, cystic fibrosis and chronic childhood-onset spinal muscular atrophy represent similar situations in which a single defective gene gives rise to widely variable clinical phenotypes (Brzustowicz et al. 1990, Tsui 1992).

Our findings have important implications for both genetic counseling and the isolation of the ARPKD gene. To date, prenatal evaluation has had limited reliability due to the relative insensitivity of second trimester fetal sonography and its imprecision in distinguishing ARPKD in utero from other renal cystic diseases (Guay-Woodford, in press). In recessive diseases, single families are typically too small for definitive linkage analyses. However, given the evidence suggesting genetic homogeneity in ARPKD, we have performed haplotype analyses with closely linked markers in two families with previously affected children. In the first family, the haplotype data predict that the fetus is unaffected. The pregnancy has been monitored by serial sonograms, and the fetus remains normal at $\geq$35 wk gestation. In the second family, the haplotype analyses revealed that the fetus carried one affected chromosome and that a recombination event occurred within the ARPKD interval on the other chromosome. While not precisely predictive, these data suggested that the fetus was at significant risk for ARPKD, and the diagnosis was subsequently confirmed by histopathology. Given the generally poor prognosis for the majority of children with the severe perinatal ARPKD phenotype, we anticipate a strong demand for DNA-based prenatal diagnosis in families with proven ARPKD.

In the absence of genetic heterogeneity, recombination events can be used to further reduce the ARPKD genetic interval. The recombinants identified in this study have refined the ARPKD region to a 3.8-cM interval delimited by the markers D6S465/D6S427/D6S436/D6S272 and D6S466. Several markers were not optimally informative in our study cohort; therefore, multipoint analyses were not helpful in determining the relative locus order. However, the marker order and the genetic distance between markers in this interval have been established previously (Gyapay et al. 1994; Volz et al. 1994). The recombination events in family 9, family 13, and family 22 confirm the centromeric limit of the ARPKD interval while the recombinations in family 3 and family 6 establish the telomeric limit of this interval. Our genetic data combined with recent physical mapping data of chromosome 6p markers refines the cytogenetic assignment for the ARPKD locus to 6p21.1-12. Thus far, none of the genes mapped to this interval explain the pathogenesis of ARPKD (Muecher et al. 1994). However, these data provide the molecular framework for constructing a YAC-based physical map of the region. As a result, additional polymorphic markers will be identified for linkage analyses as well as prenatal testing, and, ultimately, the ARPKD gene will be identified.

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References

Bernstein J, Slovis TL (1992) Polycystic diseases of the kidney. In: CM Edelmann (ed) Pediatric kidney disease, 2d ed. Little, Brown, Boston. pp 1139–1153

Blyth H, Ockenden BG (1971) Polycystic disease of kidneys and liver presenting in childhood. J Med Genet 8:257–284

Brzustowicz LM, Lehner T, Castilla LH, Penchaszadeh GK, Wilhelmsen KC, Daniels R, Davies KE, et al (1990) Genetic mapping of chronic childhood-onset spinal muscular atrophy to chromosome 5q11.2-13.3. Nature 344:540–541

Guay-Woodford LM. Autosomal recessive disease: clinical and genetic profiles. In: Watson M, Torres VE (eds) Oxford University Press monograph on polycystic kidney disease Oxford University Press, Oxford (in press)

Guay-Woodford LM, D'Eustachio P, Bruns GAP (1993) Iden-

tification of the syntenic human linkage group for the mouse congenital polycystic kidney disease (*cpk*) locus. J Am Soc Nephrol 4:814A

Gyapay G, Morissette J, Vignal V, Dib C, Fizames C, Millaseau P, Marc S, et al (1994) Intermediary report of the Généthon human linkage map. Nat Genet 7:246–249

Kaariainen H, Koskimies O, Norio R (1988) Dominant and recessive polycystic kidney disease in children: evaluation of clinical features and laboratory data. Pediatr Nephrol 2:296–302

Kaplan BS, Fay J, Shah V (1989) Autosomal recessive polycystic kidney disease. Pediatr Nephrol 3:43–49

Lathrop GM, Lalouel J-M, Julier C, Ott J (1985) Multilocus linkage analysis in humans: detection of linkage and estimation in humans: detection of linkage and estimation of recombination. Am J Hum Genet 37:482–498

Moyer JH, Lee-Tischler MJ, Kwon H-Y, Schrick JJ, Avner ED, Sweeney WE, Godfrey VL, et al (1994) Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. Science 264:1329–1333

Muecher G, Wirth B, Zerres K (1994) Refining the map and defining flanking markers of the gene for autosomal recessive polycystic kidney disease on chromosome 6p21.1-p12. Am J Hum Genet 55:1281–1284

Osathanondh V, Potter EL (1964) Pathogenesis of polycystic kidneys: type 1 due to hyperplasia of interstitial portions of the collecting tubules. Arch Pathol 77:466–473

Preminger GM, Koch WE, Fried FA, McFarland E, Murphy ED, Mandell J (1982) Murine congenital polycystic kidney disease: a model for studying development of cystic disease. J Urol 127:556–560

Priest JH (1991) Prenatal chromosomal diagnosis and cell culture. In: Barch MJ (ed) The ACT cytogenetics laboratory manual, 2d ed. Raven Press, New York, pp 166–182

Reuss A, Wladimiroff JW, Niermeyer MF (1991) Sonographic, clinical and genetic aspects of prenatal diagnosis of cystic kidney disease. Ultrasound Med Biol 17:687–694

Simon EA, Cook S, Davisson MT, D'Eustachio P, Guay-Woodford LM (1994) The mouse congenital polycystic kidney (*cpk*) locus maps within 1.3 cM of the chromosome 12 marker *D12Nyu12*. Genomics 21:415–418

Tsui L-C (1992) The spectrum of cystic fibrosis mutations. Trends Genet 8:392–398

Volz A, Boyle JM, Cann HM, Cottingham RW, Orr HT, Ziegler A (1994) Report of the Second International Workshop on Human Chromosome 6. Genomics 21:464–472

Zerres K (1992) Autosomal recessive polycystic kidney disease. Clin Invest 70:794–801

Zerres K, Hansmann M, Mallmann R (1988) Autosomal recessive polycystic kidney disease: problems of prenatal diagnosis. Prenat Diagn 8:215–219

Zerres K, Muecher G, Bachner L, Deschennes G, Eggermann T, Kaariainen H, Knapp M, et al (1994). Mapping of the gene for autosomal recessive polycystic kidney disease (ARPKD) to chromosome 6p21-cen. Nat Genet 7:429–432

Zerres K, Volpel M-C, Weib H (1984) Cystic kidneys: genetics, pathologic anatomy, clinical picture and prenatal diagnosis. Hum Genet 68:124–167