

Molecular genetic diagnosis of autosomal dominant polycystic kidney disease in a newborn with bilateral cystic kidneys detected prenatally and multiple skeletal malformations

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Abstract

We report a case of an unusual prenatal presentation of polycystic kidneys associated with multiple skeletal limb defects, including polydactyly, syndactyly, bilateral agenesis of the tibia, and club foot. The ultrasonographic picture was consistent with a diagnosis of polycystic kidney disease, either the adult onset autosomal dominant type (ADPKD) or the early onset autosomal recessive form (ARPKD). However, there was a positive family history for ADPKD. Linkage analysis was performed in 10 family members, of whom four were affected, using six flanking DNA markers tightly linked to the PKD1 locus on chromosome 16p, and one marker linked to the putative PKD2 locus on chromosome 2p. Lod score determinations indicated that the affected gene in the family is most likely PKD1. The patient inherited the disease linked haplotype from his affected mother.

(*J Med Genet* 1993;30:419-22)

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common hereditary single gene disorders in humans, occurring in approximately 1 in 500 to 1 in 1000 live births. The biochemical defect of the disease is as yet unknown. The gene whose mutations are responsible for the most frequent form of the disease has been named PKD1 and is located on the short arm of chromosome 16.¹ It has been estimated that around 1 to 4% of families with ADPKD have mutations unlinked to chromosome 16 markers, thus confirming previous reports of genetic heterogeneity.^{2,3} Recently, a putative PKD2 locus has been tentatively mapped to chromosome 2,⁴ even though there has been no other confirmation of linkage to this locus in an ADPKD family. ADPKD is typically a late onset disorder, the first clinical symptoms usually occurring between 40 and 50 years of age.⁵ Nevertheless, there have been several reports of ADPKD diagnosed by ultrasonography in a newborn or infant and fewer cases detected prenatally.^{6,7}

Autosomal recessive polycystic kidney disease (ARPKD), the infantile type of the disease, commonly develops in infancy or childhood,⁸ and is caused by mutation(s) in a gene still unknown, but different from PKD1.⁹

We report here an unusual case of bilateral polycystic kidneys, detected prenatally by ultrasonography, in a newborn with multiple skeletal malformations and a family history of ADPKD.

Case report

An obstetric ultrasound examination was performed on a 28 year old woman with ADPKD (subject III-15 in fig 1) at 20 weeks' gestation. The fetal ultrasonography showed bilateral microcystic kidneys. A male infant was delivered by caesarean section because of breech presentation at 40 weeks' gestation. At birth, the baby had enlarged and low set ears, micrognathia (fig 2A), normal weight and head circumference, and slightly reduced length. The abdomen appeared significantly and irregularly enlarged with easily palpable kidneys. No respiratory distress was present. The urine was normal. At birth, a sonogram showed enlarged, diffusely hyperechogenic kidneys (about 7 cm in length), with tiny, multiple, bilateral cortical cysts and disappearance of the structural differentiation pattern of the renal cortex and medulla. Several larger cortical cysts later appeared, of which the largest was localised at the inferior pole of the left kidney and had a diameter of more than 15 mm at 3 months of age and of 25 mm at 5 months. No liver cysts or bile duct abnormalities were detected. The baby's blood pressure, renal and liver functions were normal at 5 months of age. He has been successfully treated for a urinary tract infection.

In addition to the renal involvement, the newborn had complex skeletal malformations including bilateral complete syndactyly of the hands and feet, bilateral polydactyly of the feet (seven metatarsal phalangeal structures on the right and eight on the left), with fusion of the soft tissues, shortening and bowing of the lower limbs, bilateral agenesis of the tibia, and bilateral club foot (fig 2B and C, fig 3B and C). No vertebral, hip, rib, or skull defects were detected by x ray examinations (fig 3A). A cerebral CT scan showed no brain defects. Karyotypic analysis showed no chromosome abnormalities. The extended prometaphase chromosome banding pattern was normal. There was no history of skeletal malformations in either the maternal or paternal families. The

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Received 4 July 1992.
Revised version accepted 2 September 1992.

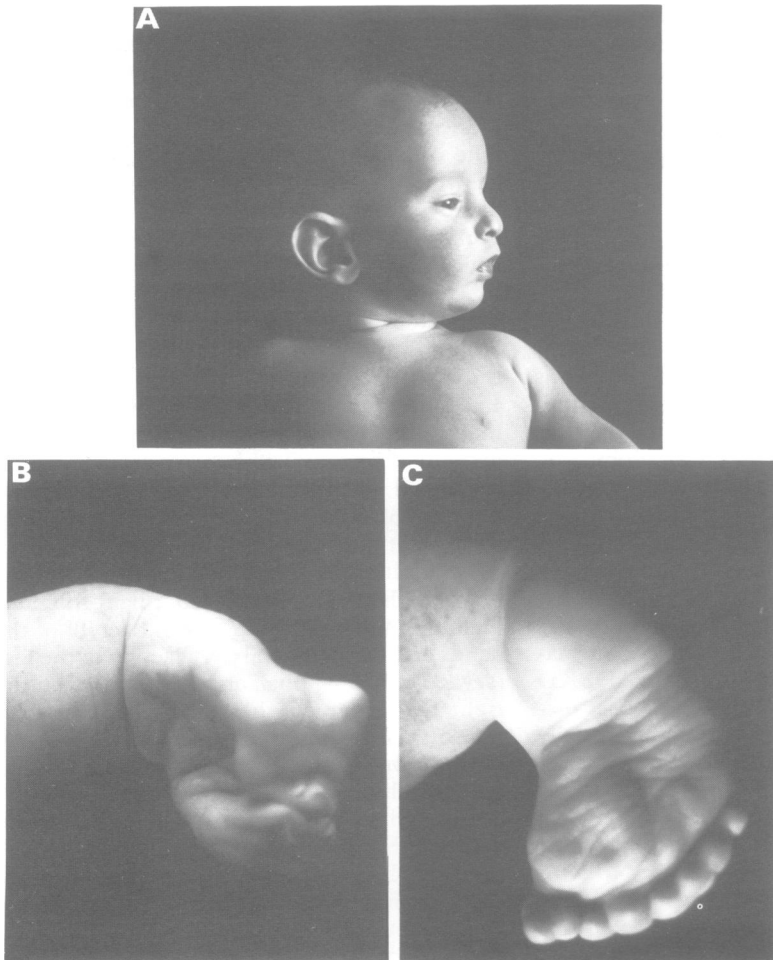
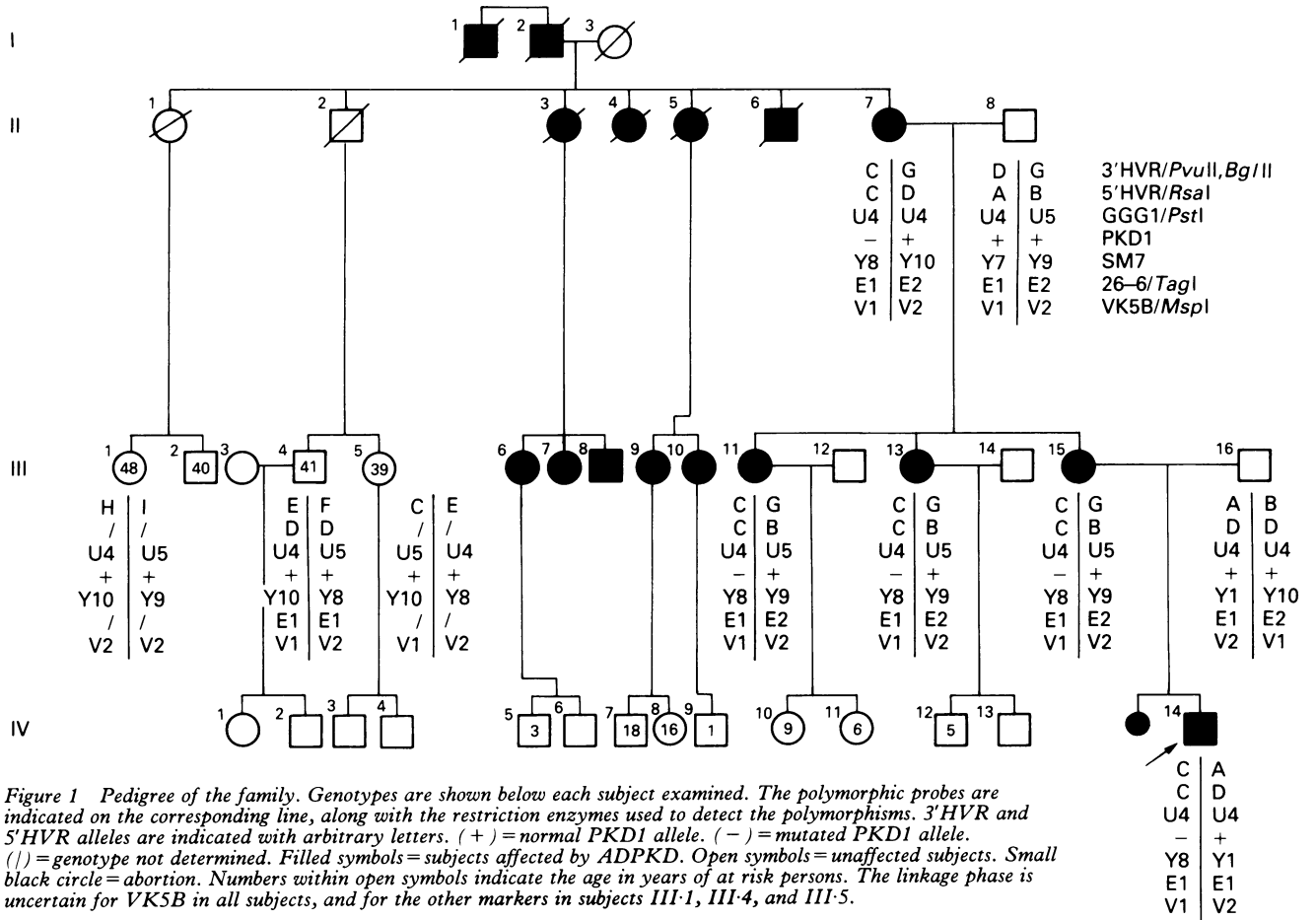


Figure 2 The patient at 3 months of age showing (A) low set ears and micrognathia, (B) polydactyly-syndactyly of the left hand, (C) polydactyly of the left foot.

family comes from north-eastern Italy and there is no indication of consanguinity. Further information on the family is available on request from the authors.

The genotypes obtained at the various 16p polymorphic loci^{3,10} are shown in fig 1 under each subject tested. The other affected members in generation III were unwilling to cooperate. The disease appears to segregate with the haplotype (tel-3'HVR/5'HVR-GGG1-PKD1(-)-SM7-26-6-VK5-cen): C/C/U4/Y8/E1/V1(or V2). No recombination events were identified between PKD1 and any of the informative markers used.

An example of the results obtained with the newly described SM7 microsatellite marker¹¹ is shown in fig 4. Five different alleles were detected in this family. Allele Y8 segregates with the disease.

The pairwise lod scores for linkage to PKD1 are shown in the table. The maximum lod scores (Zmax) for informative markers 3'HVR, 5'HVR, SM7, and GGG1 were 0.983, 1.074, 0.888, 0.197, respectively, obtained at $\theta=0.0$ for 3'HVR, 5'HVR, and SM7, and at $\theta=0.02$ for GGG1. The lod scores for 26-6 were slightly negative, owing to the low informativity of the marker in the family. The lod scores for VK5B were equal to zero for all values of θ owing to the complete uninformativity of this probe. The highest relative likelihood (odds ratio) for linkage to PKD1 (5'HVR) was 11.85 to 1. The multipoint lod score for linkage of the disease to the PKD1 gene in the family, considering simultaneously

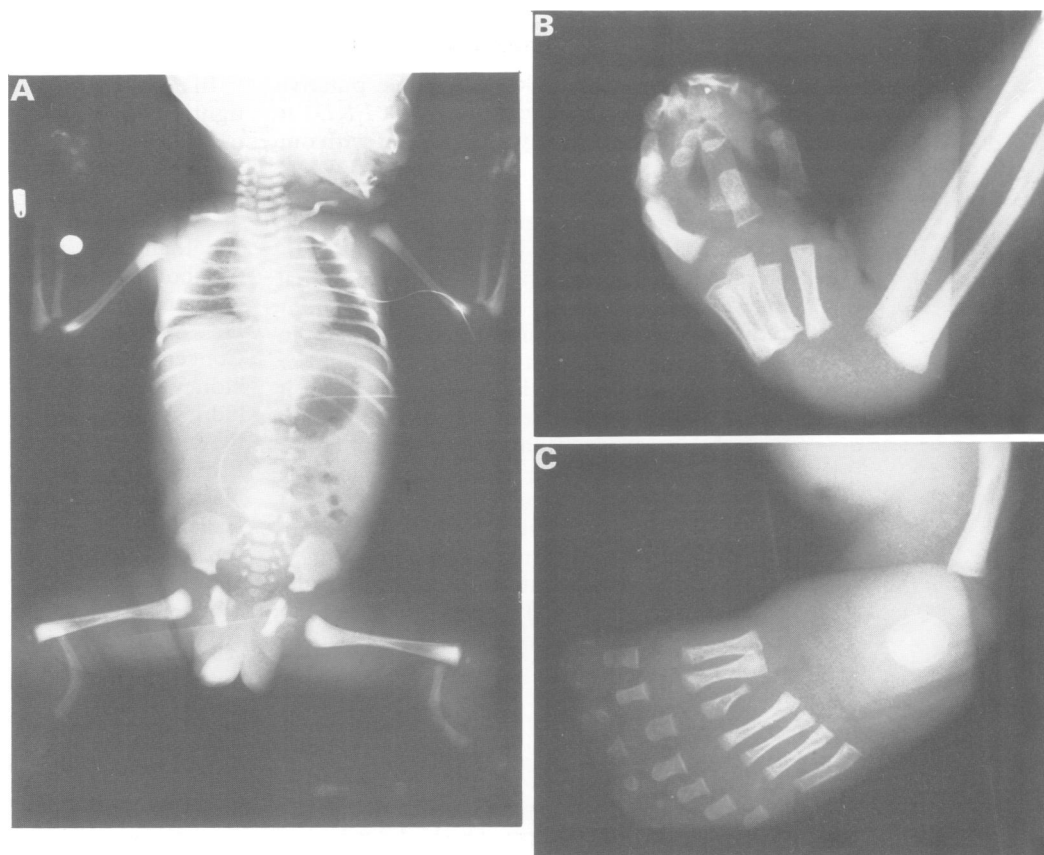


Figure 3 Radiographs of the patient showing (A) whole body (anteroposterior view), (B) left hand, (C) left foot. Note the absence of the tibiae.

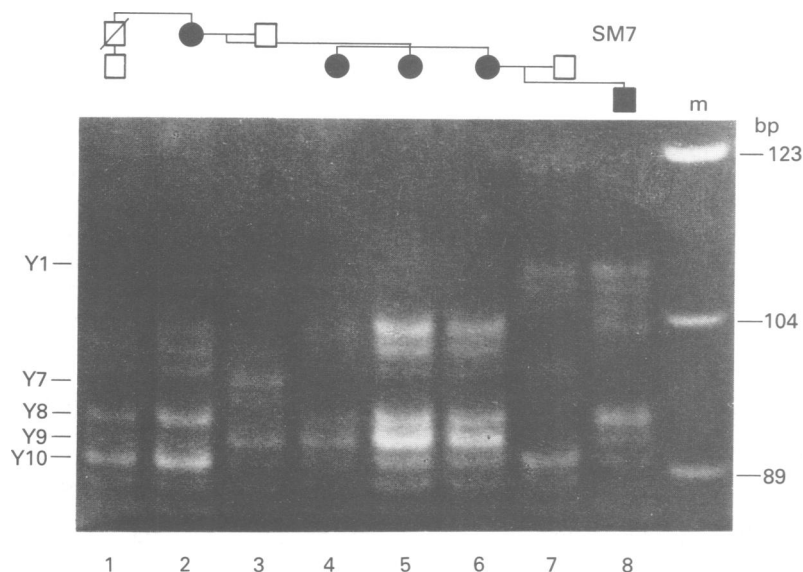


Figure 4 Direct analysis of SM7 genotypes in the family after PCR and ethidium bromide staining. Lane 1 III-4, lane 2 II-7, lane 3 II-8, lane 4 III-11, lane 5 III-13, lane 6 III-15, lane 7 III-16, lane 8 IV-14. M = molecular weight markers in base pairs (bp). Alleles are indicated on the left hand side.

Two point lod scores for linkage to PKD1 or PKD2.

ADPKD	Recombination fraction (θ)						Zmax	θ_{max}
	0.0	0.001	0.02	0.05	0.10	0.20		
v								
3'HVR	0.983	0.960	0.942	0.880	0.774	0.552	0.983	0.00
5'HVR	1.074	1.052	1.028	0.957	0.837	0.591	1.074	0.00
GGG1	0.196	0.196	0.197	0.195	0.191	0.169	0.197	0.02
SM7	0.888	0.841	0.854	0.802	0.711	0.517	0.888	0.00
26-6	-0.152	-0.150	-0.126	-0.096	-0.059	-0.020	0.000	0.50
VK5B*	0.000	0.000	0.000	0.000	0.000	0.000	0.000	
YNH24	$-\infty$	-4.999	-2.415	-1.647	-1.089	-0.561		

* The VK5B linkage data were completely uninformative; therefore the lod scores equal zero for all values of θ .

the hypervariable flanking markers 5'HVR and SM7, was 0.927, indicating that the odds for linkage were 8.46 to 1. The lod scores obtained with the VNTR probe YNH24 ($Z = -3.007$ at $\theta = 0.01$) indicated absence of linkage to the putative PKD2 locus on chromosome 2. These results strongly suggest that the disease is most likely of the PKD1 type.

Discussion

The finding of a fetus with polycystic kidneys suggests the diagnosis of the perinatal form of autosomal recessive polycystic kidney disease (ARPKD). Given that ADPKD and ARPKD may be clinically and ultrasonographically indistinguishable, the positive family history of ADPKD, and the unusual skeletal features, the issue in this case was whether the patient had the rarer and more severe recessive form, or the adult type disease, or still another unrelated disorder. A few cases of adult type ADPKD have been reported so far with early onset in childhood or even in the neonate.⁶ Fewer cases have been diagnosed in the fetus. For example, in a large series of paediatric nephrology patients surveyed over a 15 year period, 22 ADPKD cases were observed with onset before the age of 15, of which two cases were diagnosed prenatally.¹²

By means of molecular haplotype identification, we have determined the presence of the ADPKD carrying chromosome in the family, showing that a PKD1 gene mutation was segregating in the affected family members, and that the same gene was inherited by the

proband. Negative lod scores were obtained with probe YNH24 for linkage to *PKD2*, indicating absence of linkage to this putative second PKD locus. The finding that *PKD1* is segregating in the family is consistent with our previous report on six large ADPKD kindreds.³

SM7, a recently developed microsatellite polymorphism tightly linked to *PKD1*, increases the informativeness of proximal markers, and allows a more rapid and non-radioactive linkage analysis by PCR in ADPKD families. Furthermore, being closer to *PKD1* than any of the existing markers, it carries the least possible risk of recombination with the disease locus, and represents a better starting point for the identification of the *PKD1* gene by positional cloning.

ARPKD is rare,¹³ and there are usually no associated malformations. Liver involvement is considered an obligatory finding, with bile duct proliferation and periportal fibrosis.¹⁴ The lack of hepatic involvement in the patient would exclude the diagnosis of ARPKD; moreover, the presence of the ADPKD gene makes it highly unlikely that the disease might be the recessive variety.

The occurrence of multiple congenital skeletal malformations in association with ADPKD has not been previously described to the best of our knowledge. In this family, it seems to represent a sporadic event, associated by chance with ADPKD. We could reasonably exclude the use of known teratogens, drugs, alcohol, ionising radiation, and infection during the course of the pregnancy. A case of fetal onset ADPKD diagnosed by ultrasonography has been reported in which the baby was born with congenital absence of the right hand.⁶ Loh *et al*¹⁵ reported an association between infant onset ADPKD and hypertrophic pyloric stenosis.

Factors leading to early manifestations of ADPKD are unknown. The involvement of modifying alleles has been postulated¹⁶ to explain 'familial' clustering of early onset ADPKD. However, in this case there is no evidence of early onset of the disease in the living affected members. For the dead members who were affected, no information is available as to the age of onset of the disease. The search for the *PKD1* gene is in progress.¹⁷ Once the *PKD1* gene is known, it will be of great interest to determine whether particular mutations may also be responsible for an earlier onset subgroup of the disease.¹⁸

The prognosis for ADPKD in the fetus and in the infant is not easily predictable, but it is better than for ARPKD.⁶ Early diagnosis of ADPKD allows for proper clinical care and treatment of complications, with the potential for long term benefits for the patient.¹⁹ A high proportion of ADPKD patients suffering from intracranial haemorrhage have untreated or poorly treated hypertension.²⁰ Considering that in this family two out of nine patients with a known history suffered from subarachnoid haemorrhages, and taking into account that seven out of eight patients have hypertension, early detection and treatment of high blood

pressure may well decrease the risk of intracranial bleeding.¹⁹

In conclusion, segregation analysis with tightly linked flanking DNA markers enabled us to detect the ADPKD chromosome in the patient, confirming the clinical diagnosis of the adult type disease at the genetic level.

We thank the members of the family who collaborated in this study. The probes on chromosome 16p were made available by Dr M Breuning through the European Concerted Action 'Towards prevention of kidney failure caused by inherited polycystic kidney disease'. Primer sequences for SM7 PCR were kindly provided by Dr P Harris. Probe YNH24 was kindly provided by Dr Y Nakamura. This work was supported by the Italian National Research Council (CNR) Target Projects 'Biotechnology and Bioinstrumentation' and 'Genetic Engineering', and by a Veneto Region Sanitary Research Grant.

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