Exclusion of autosomal dominant polycystic kidney disease type II (ADPKD2) from 160 cM of chromosome 1

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Abstract

Autosomal dominant polycystic kidney disease is a heritable disorder and recent studies have shown genetic heterogeneity, with some, but not all, families showing linkage with markers on chromosome 16p. Members of a large ADPKD family, unlinked to chromosome 16, have been typed for 12 marker loci located on both arms of chromosome 1. Multipoint analysis excluded ADPKD2 from the region between *D1S81* (pTHH33) and *D1S67* (pHHH106) on the long arm and between *Rh* and *PGM1* on the short arm. This excludes the disease locus from about 61% of chromosome 1.

Autosomal dominant polycystic kidney disease (ADPKD) is a systemic disorder characterised by cysts in the kidneys, liver, pancreas, and ovaries and by structural abnormalities in the gastrointestinal tract, vascular tree, and cardiac valves. ADPKD is one of the most common genetic disorders, occurring with a frequency of about 1:1000, and is responsible for 8 to 9% of end stage renal disease in Europe and North America.¹⁻³

One gene responsible for ADPKD has been localised on the short arm of chromosome 16 by virtue of showing tight linkage between the cluster of genes coding for the α chain of haemoglobin⁴ and the gene for the enzyme phosphoglycolate phosphatase (PGP).^{5,6} However, subsequent studies showed genetic heterogeneity in ADPKD with the observation of a lack of

linkage with chromosome 16 markers in some ADPKD families.⁷⁻¹⁰ The type of ADPKD localised to the short arm of chromosome 16 is known as ADPKD1, while the type unlinked to chromosome 16 is known as ADPKD2. In the latter case, there is a possibility that families with 'ADPKD2' may also be genetically heterogeneous in which case further subdivision may be warranted. The proportion of families unlinked to chromosome 16 (ADPKD2) has been estimated to be 7%.¹¹

The discovery of the linked markers has provided a method for diagnosis of ADPKD before the development of detectable renal cysts. This method also permits prenatal detection of gene carriers. However, the accuracy of prediction by linkage is dependent upon the relative frequency of the unlinked type of ADPKD. The greater the proportion of ADPKD2, the greater the likelihood of diagnostic error. This is especially true in small families since large families can provide enough information to verify the presence of linkage with known chromosome 16 markers. This difficulty in small families could be largely avoided if the linkage relationship of the ADPKD2 gene were known. Hence, we have continued our studies on localising the ADPKD2 gene. The present paper concerns the results of analysis of several markers, spanning chromosome 1, used for linkage studies with the ADPKD2 gene.

Materials and methods

The family studied in the present investigation is a large kindred with over 250 members. The original ancestors emigrated to America from Sicily and the family has been followed by the Renal Division at the University of Colorado Health Sciences Center for the past 20 years. The pedigree has already been published elsewhere.⁷ The diagnosis of ADPKD was confirmed by ultrasonography. Any persons having at least one cyst in each kidney and at least two cysts in one kidney was considered affected.¹²

Genomic DNA was isolated from either lymphocytes or transformed lymphoblasts. The digestion of 5 to 10 μ g of DNA from each family member was performed under standard conditions.¹³ The appro-

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Table 1 Characterisation of genomic probes used in ADPKD gene localisation.

Probe (locus)	Map location	Restriction enzyme	Allele size (kb)	Allele frequencies
pJA110 (PND)	1p36	BglII	A1 10.0 A2 6.0	0.87 0.13
PGD (PGD)	1p34	PGD	A C	0.98 0.02
Rhesus (RH)	1p36.2-p34	Rhesus	DCe dce DcE Dce DCE dCe dCE	0.39 0.46 0.12 0.01 0.01 0.00 0.01
pTHI54 (DIS62)	1p	PvuII	A1 6.0 A2 5.0	0.51 0.49
PGM1 (PGM1)	1p34-p33 or p31.1 or p31.1-p22.1	PGM1	1+ 1- 2+ 2-	0.63 0.12 0.21 0.04
N8C6 (NGFB)	1p22.1 or p13	BglII	A1 6.0 A2 4.1, 1.9	0.22 0.78
pHHH106 (DIS67)	1	MspI	A1 2.3 A2 2.0	0.46 0.54
Duffy (FY)	1p21-q25	Duffy	Fya Fyb	0.45 0.55
pAT3c (AT3)	1q23-q25.1	PstI	A1 10.5 A2 5.5, 5.0	0.50 0.50
pHRnES1.9 (REN)	1q32 or 1q42	HindIII	A1 8.7 A2 6.2	0.70 0.30
pEKH7.4 (DIS65)	1	TaqI	A1 5.0 A2 3.8	0.47 0.53
pTHH33 (DIS81)	1q	RsaI	VNTR with 6 alleles 4.0-7.0	

Table 2 Results of two point analysis between ADPKD and different marker loci.

Probe	Lod scores at recombination fraction					
	0.0	0.05	0.1	0.2	0.3	0.4
JA110	-∞	-1.694	-0.845	-0.163	0.039	0.052
PGD	-0.218	-0.174	-0.138	-0.082	-0.041	-0.142
RH	-∞	-5.559	-2.732	-0.491	0.269	0.349
THI54	-∞	-1.834	-1.202	-0.617	-0.323	-0.138
PGM1	-∞	-2.907	-1.403	-0.225	0.166	0.214
NGFB	0.137	0.116	0.096	0.058	0.028	0.008
HHH106	-∞	-4.383	-2.317	-0.592	-0.003	0.093
FY	-∞	-2.062	-1.184	-0.389	-0.075	0.123
AT3C	-∞	-1.101	-0.767	-0.418	-0.215	-0.083
HRNES1.9	-∞	-2.550	-1.737	-0.867	-0.397	-0.130
EKH7.4	-∞	-5.004	-3.297	-1.490	-0.643	-0.249
THH33	-∞	-7.986	-5.045	-2.233	-0.906	-0.275

Lod scores were calculated assuming that θ male = θ female.

priate restriction endonucleases were used for digestion depending on the polymorphic sites of the various probes. DNA fragments were separated by electrophoresis using 0.7 to 1% agarose gel and thereafter transferred to GeneScreen Plus.¹⁴ The filters were

prehybridised according to manufacturer's (Du Pont, USA) instructions and then hybridised with an appropriate probe, labelled with ³²P by nick translation for 10 to 24 hours. Probes which contained multicopy human repeat sequences were hybridised with human

(A) Physical assignments of chromosome 1.²⁰ (B) Genetic map based on sex average summarization of HGM10 data on chromosome 1.²⁰ The distance is given in centimorgans. (C) The shaded region represents the exclusion of the ADPKD2 gene by a lod score of less than -2 using multipoint analysis.

DNA to reduce the background or probe hybridisation to repeat sequences within the sample.¹⁵ Hybridised membranes were washed in $2\times\text{SSC}$ and 0.1% SDS for one hour with three changes, and eventually with $0.2\times\text{SSC}$ and 0.1% SDS for one hour with two changes, before autoradiography. The genomic probes used in this study are shown in table 1.

Linkage analysis was performed using version 5.03 of the LINKAGE program.¹⁶ Lod scores were calculated using MLINK and LINKMAP options for pairwise and multipoint linkage analysis, respectively. Unaffected family members under the age of 15 years

were eliminated from the linkage analysis. All unaffected subjects who had not recently had ultrasonography were considered to have an unknown diagnosis. Persons over 15 years were assigned to one of the three liabilities classes: 15 to 20 years, 20 to 30 years, or over 30 years with penetrances of 0.32, 0.72, and 0.90 respectively.¹²

Results

Pairwise lod scores are presented in table 2. Close linkage to ADPKD could be excluded from 12 marker loci: JA110 (PND), PGD (PGD), Rhesus (RH), TH154 (DIS62), PGM1 (PGM1), NGFB (N8C6), HHH106 (DIS67), Duffy (FY), AT3C (AT3), HRNES1.9 (REN), EKH7.4 (DIS65), and THH33 (DIS81).

With multipoint analysis, using the genetic map of chromosome 1,¹⁷⁻¹⁹ the ADPKD2 locus was excluded from the region between DIS81 and DIS67 which covers a distance of about 96 cM on the long arm of chromosome 1 (figure). The exclusion by multipoint analysis is based on constant sex specific difference in recombination frequency of $\theta_f:\theta_m$ ratio of 1.9 and the disease locus was considered to be excluded wherever the scores were below the $\log_{10}$ likelihood of -2. On the short arm, the ADPKD locus was excluded around the PND region and also from a distance of about 58 cM between the Rh and PGM1 loci (figure). Although the region around PND has been excluded, it is not known exactly how far the genetic map extends beyond the distal part of PND. However, it has been suggested that the male genetic map extends at least 23 cM beyond PND.¹⁸

Discussion

The localisation of the ADPKD1 gene to chromosome 16 made possible the indirect diagnosis of gene carrier status by linkage analysis. However, genetic heterogeneity complicated the issue. Linkage diagnosis may be confounded by the uncertainty of not knowing the ADPKD type in a specific family. A direct diagnosis of the ADPKD1 gene by DNA analysis may not be possible until the ADPKD gene is cloned and characterised. The genetic heterogeneity, carrying the ADPKD2 gene at unknown locations in the human genome, stresses the need for careful studies in families in order to establish which disease gene is present by genetic linkage using diagnostic genetic markers. In our first attempt to localise the ADPKD2 gene, the results based on multipoint analysis have excluded the disease locus from 96 cM on the long arm and about 64 cM on the short arm of chromosome 1.

Present knowledge of the clinical features and pathogenesis of ADPKD is equivocal. The localisation of the ADPKD2 gene would help to distinguish the

effects of the ADPKD genes in terms of their clinical interpretation as well as for diagnostic purposes. The importance of multipoint analysis in excluding the disease locus using linkmaps from whole chromosomes has been reported earlier.^{21 22} The results of the present study exclude at least 61% of the region from chromosome 1. Further studies are required to map the other regions in order to localise the ADPKD2 gene.

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