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Multipoint mapping of adult onset polycystic kidney disease (PKD1) on chromosome 16

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Abstract

Analysis of genetic linkage data in 33 adult onset polycystic kidney (ADPKD) families was carried out using probes for the *D16S85, D16S84,* and *D16S94* loci. The data set of 33 families shows no evidence of genetic heterogeneity since one unlinked family was previously excluded. Two point linkage analysis showed maximum likelihood values of the recombination fraction of 0·07 for *ADPKD* and *D16S85* (lod score 18·78), 0·02 for *ADPKD* and *D16S84* (lod score 7·55), and 0·00 for *ADPKD* and *D16S94* (lod score 6·73). Multipoint analysis showed a maximum likelihood order of tel–*D16S85*–0·06–*D16S84*–0·02–(*PKD1, D16S94*)–cen with a multipoint lod score of 32·16. Analysis of rare recombinants lying close to *PKD1* gave results consistent with this order.

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Adult onset polycystic kidney disease (ADPKD) is an autosomal dominant condition characterised by progressive formation and enlargement of multiple cysts in the kidney and other organs leading to deterioration in renal function and the development of hypertension in middle life. End stage renal disease is a common outcome although renal replacement programmes have improved the prognosis considerably.[1] Premature deaths from the complications of renal failure and rupture of intracranial aneurysms are not uncommon.[2] ADPKD is one of the commonest genetic diseases in man affecting 1 per 1000 of the general population.[3] The pathophysiological basis is not well understood although the earliest changes appear to include hyperplasia of tubular epithelial cells and focal microcyst formation arising in any portion of the nephron from glomerulus to collecting tubules.[4] Abnormalities in tubular basement membrane, sodium pump orientation, and sensitivity to growth factors have recently been identified in ADPKD kidneys and cultured tubular epithelial cells.[5]

Linkage was first shown between *ADPKD* and *D16S85* close to α globin in chromosomal region 16p13 by Reeders *et al.*[6] This localisation has since been amply confirmed by several other groups.[7–9] The orientation of the *ADPKD* locus relative to *D16S85*, afterwards localised to band 16p13.3,[10 11] was shown in a multipoint linkage analysis[11] which indicated that the odds in favour of *PKD1* lying proximal to *D16S85* were >10 000:1 compared with a distal location. This group also showed that the *D16S63* and *D16S45* loci were located proximal to both *PKD1* and *D16S85* by a combination of linkage and somatic cell hybrid analyses.[11] Breuning *et al*[9] also showed that the disease locus is flanked by *D16S80* proximally and *D16S85* distally.

A previous analysis of 27 ADPKD families from four countries failed to show evidence of genetic heterogeneity.[12] However, two reports subsequently appeared describing families that failed to show linkage to the α globin region of chromosome 16.[13 14] Since accurate localisation of the gene first requires a genetically homogeneous sample, we extended our initial study of 10 ADPKD families, included in the study of Reeders *et al*,[12] to a total of 34 families. The results of this study are described elsewhere[15] and show that 81 to 83% of these families show linkage to this region of chromosome 16. However, only one family (PK53) showed clear evidence of non-linkage to *PKD1*[15] and was therefore removed from the data set for the purpose of this multipoint analysis. We now report the results of the analysis of the remaining 33 families using *D16S85, D16S84,* and *D16S94.*

Materials and methods

ASCERTAINMENT AND DIAGNOSIS OF FAMILY MEMBERS

Families were ascertained through the Medical Renal Unit, Royal Infirmary of Edinburgh and the Renal Unit, Western Infirmary, Glasgow. Diagnostic criteria included a family history of ADPKD (more than one affected member) and ultrasound findings of two or more cysts greater than 0·5 cm in diameter in one kidney and at least one such cyst in the contralateral kidney.[7] Pedigrees were drawn up after interviews with patients and were checked against the centralised Register for Births, Marriages, and Deaths for Scotland. Ethical approval was obtained and, after obtaining informed consent, 30 to 50 ml EDTA anticoagulated blood were drawn and frozen at −70°C before DNA extraction. Thirty-four probands and 311 family members were initially ascertained and their relatives sampled for biochemical and DNA analysis and examined clinically as described previously,[7] after which ultrasound examinations were arranged and carried out by experienced sonologists, one in each centre.

A previous study of linkage heterogeneity[15] identified one family (PK53) contributing disproportionately to the observed heterogeneity and so was excluded from this study. This family showed a conditional probability of 0·003 of being linked to the *D16S85,*

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D16S84, and *D16S94* loci. None of the other families showed good evidence of non-linkage to this region, so this set of 33 families was used in the study.

DNA EXTRACTION AND ANALYSIS

DNA was extracted by the method of Kunkel *et al.*[16] The following loci were used to type all family members.

(1) *D16S85* (3'HVR) is a hypervariable locus described by Jarman *et al*[17] and located in 16p13.3, which detects multiple alleles with several restriction enzymes. DNA samples were digested with the restriction enzyme *Pvu*II and allele frequencies were each set at 0·1.

(2) *D16S84* (pCMM65) was isolated by Nakamura *et al*[18] and detects two alleles of size 3·5 kb (A1) and 2·1 kb (A2) with frequencies of 0·40 and 0·60 with the enzyme *Pvu*II. It is localised to chromosomal region 16p.

(3) *D16S94* (pVK5B) was isolated by Hyland *et al*[19] and detects alleles of 1·6 kb (A1) and 1·3 kb (A2) with the enzyme *MspI* with frequencies of 0·55 and 0·45. It is localised to chromosomal region 16p13.3.

(4) *D16S45* (CRI-090) was isolated by Donis-Keller *et al*[20] and detects two alleles (20 kb and 13 kb) with frequencies of 0·47 and 0·53 with *Eco*RI. This probe was only used to probe families containing key recombinants. It is localised to chromosomal region 16pter-p13.

Probes were used without purification of inserts and labelled with ^{32}P-dCTP by random priming.[21] Unincorporated counts were separated by gel filtration. DNA from the patients was digested to completion with the above enzymes and separated by electrophoresis on 0·8% agarose gels in 1 × TBE buffer. Transfers were carried out by the method of Southern[22] using nylon (Nytran) filters. Filters were prehybridised for at least four hours in 5 × Denhardt's solution/4 × SSC/10% dextran sulphate/0·1% sodium pyrophosphate/1% sodium dodecylsulphate (SDS)/0·1 mg ml^{-1} denatured salmon sperm DNA. Filters were then hybridised in the same mixture containing 1 to 2 × 10^6 cpm ml^{-1} of labelled probe (2·5 ng ml^{-1}). After overnight hybridisation at 68°C, filters were washed in 2 × SSC/1% SDS down to a final stringency of 0·1 × SSC/1% SDS at 68°C. Filters were used to expose Kodak XAR-5 films in cassettes containing double intensifier screens for one to 14 days at −70°C.

LINKAGE ANALYSIS

Linkage was analysed using the LINKAGE program package version 5·03.[23] The ADPKD gene frequency was set at 0·0005 and male and female mutation rates assumed to be equal at 5 × 10^{-5} per locus per gamete. The female:male recombination ratio was assumed to be constant and was found to maximise the likelihood at a ratio of 0·2. This value was used for LINKMAP runs. MLINK was unable to support a fixed ratio of recombination in the two sexes, so sex averaged values were obtained. Two point linkage analyses were obtained using the MLINK subroutine. Multipoint analyses were run using LINKMAP with a fixed order of marker loci as follows: *D16S85*–0·06–*D16S84*–0·02–*D16S94*. The probabilities of ultrasonographic detection in gene carriers were assumed to be 0·22, 0·66, 0·86, and 0·95 during the first four decades of life and were taken to be 1·00 after the age of 40.[24]

Results

TWO POINT LINKAGE ANALYSES

The results of two point linkage analyses are shown in the table. The maximum likelihood value of the recombination fraction (sex averaged) was found to be 0·07 at a lod score of 18·78 for *PKD1-D16S85*. The corresponding values of the recombination fractions were 0.02 at a lod score of 7·55 for *PKD1-D16S84* and 0·00 at a lod score of 6·73 for *PKD1-D16S94*. All three loci show close linkage to *PKD1* and highly significant lod scores. No recombination was found with *D16S94* and only a single definite recombinant with *D16S84*.

MULTIPOINT LINKAGE ANALYSIS

The results of the multipoint analysis is shown in the figure. In a four point analysis with *PKD1, D16S85, D16S84,* and *D16S94*, the maximum likelihood is found to occur at the location *D16S85*–0·06–*D16S84*–0·02–(*PKD1, D16S94*) – cen (order 1) at a peak lod score of 32·16. Since no definite recombination was observed between *PKD1* and *D16S94*, the order of these two loci cannot be determined. This result is consistent with the presence of a single triply informative meiosis in an affected member of family PK52 which is recombinant with both *D16S85* and *D16S84*, although the family has not yet been found to be informative for *D16S94* or other proximal markers. Exchange of flanking markers has therefore yet to be shown. However, in the data set as a whole the likelihood of the order *D16S85*–0·04 – *PKD1* – 0·02 – *D16S84* – 0·02 – *D16S94* – cen (order 2) is only 2·6 times lower than order 1, with *PKD1* proximal to *D16S84*. However, order 1 is at least 2·7 × 10^5 times more likely than with *PKD1* distal to *D16S85*.

A total of 20 recombinants out of 226 informative meioses has been identified in the 33 APKD families using the *D16S85, D16S84, D16S94,* and *D16S45* loci. Only 12 of these are multiply informative and therefore provide information on the localisation of the *PKD1* locus. Five out of eighteen *D16S85* recombinants are also informative for *D16S84*, four of which are non-recombinant with *D16S84*, supporting the proposal that this locus is closer to *PKD1* than *D16S85*.[25] Five out of eighteen *D16S85* recombinants are informative for *D16S94*, each of which is non-recombinant with APKD, consistent with the hypothesis that *PKD1* lies distal or close to *D16S94*.

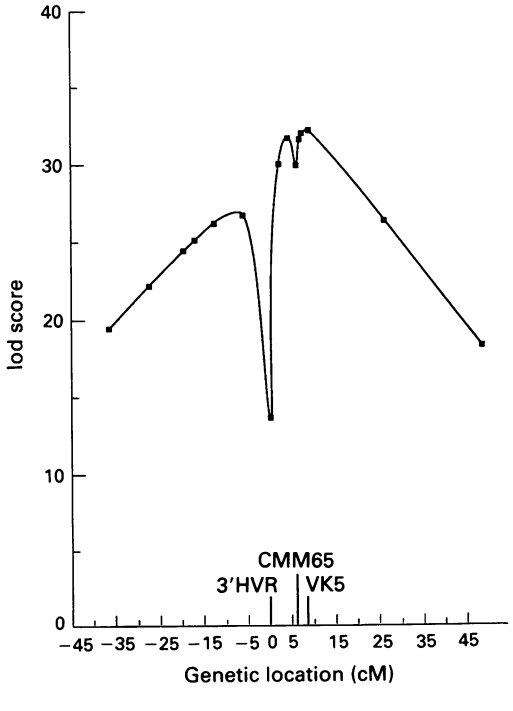
The results of two point linkage analyses of ADPKD and the D16S85, D16S84, and D16S94 loci. Recombination fractions and the corresponding lod scores are shown. The maximum likelihood values of the recombination fractions (θmax) and lod scores (Zmax) are indicated.

Locus	0·00	0·01	0·05	0·10	0·20	0·30	0·40	θmax	Zmax
D16S85 (3'HVR)	2·45	14·82	18·62	18·37	14·53	9·02	3·27	0·07	18·78
D16S84 (CMM65)	6·14	7·42	7·39	6·68	4·75	2·63	0·76	0·02	7·55
D16S94 (VK5B)	6·73	6·56	5·89	5·03	3·31	1·70	0·48	0·00	6·73

Discussion

Previous multipoint analyses of the *PKD1* locus have been described by Reeders *et al*,[11] Germino *et al*,[25] and Breuning *et al*.[9 26] A very substantial body of evidence supports the localisation of *PKD1* proximal to *D16S85*, the most distal marker known on chromosome 16, and distal to the *D16S45, D16S63*, and *D16S80* loci. Further refinement of the map of this region has come from the combined use of linkage and human-rodent hybrid cell lines which have distinguished four subregions by means of *23HA* (GM2324), *N-OH1*, and *CY14* breakpoints.[25 26] These regions separate the most important polymorphic loci into the following four groups, extending distally: (1) *D16S45, D16S63, D16S80* (proximal to *23HA*), (2) *D16S94, D16S125* (proximal to *N-OH1*, distal to *23HA*), (3) *D16S84, D16S259* (proximal to *CY14*, distal to *N-OH1*), (4) *D16S83, D16S21, D16S85* (distal to *CY14*).

The genetic relationship of *PKD1* with loci within groups 2 and 3 is less clear, however. Most of these loci show few if any recombinations with *PKD1*, which on the one hand supports the view that they lie closer to the gene than those in groups 1 and 4, but on the other hand makes ordering difficult. In the absence of ADPKD patients showing chromosomal rearrangements, the genetic definition of closely linked flanking markers is an essential prelude to successfully localising the gene by physical means. Initially, no recombinants were identified with *D16S84*,[25] although at least one has since been reported.[27] Similarly, at least one recombinant has been reported with *D16S125*,[26] which, together with the above, is consistent with a localisation for *PKD1* between *D16S84* and *D16S125*, an area of less than 750 kb as determined by pulsed field gel electrophoresis (PFGE) mapping.[28] However, several potential pitfalls exist which can lead to a misleading genetic assignment, so that it is important to accumulate as much genetic evidence as possible. Firstly, genetic heterogeneity is now known to exist in ADPKD so that genetic mapping should be carried out as far as possible on a genetically homogeneous population. We have attempted to do this by first carrying out a heterogeneity study and then by removal of unlinked families from further analysis.[15] Secondly, false positive[11] and false negative[24] diagnoses can lead to misinterpretation of results. Thirdly, double recombinants, although very rare in such small genetic distances, can occur and may be mimicked by gene conversion events. Fourthly, mistyping can occur, so that it is important that each apparent recombinant is checked, preferably by an independent laboratory. Finally, non-paternity can arise and lead to erroneous interpretation of results, so that this should ideally be checked by genetic fingerprinting.



Results of multipoint analysis of ADPKD and D16S85 (HVR), D16S84 (CMM), and D16S94 (VK5) loci. The multipoint lod scores are plotted against genetic location (based on recombination fractions in males) with reference to the D16S85 (3'HVR) locus at position O. The location of the D16S84 and D16S94 loci are also shown. Distances are given in centiMorgans (cM).

The results presented in this study confirm the tight genetic linkage of *D16S84* in region 3 which shows only a single definite recombinant with *PDK1* (θmax = 0·02, Zmax = 7·55), similarly with *D16S94* in region 2, which shows no definite recombination with *PDK1* (θmax = 0·00, Zmax = 6·73). Multipoint analysis showed the most likely order to be: tel–*D16S85–D16S84–(PKD1, D16S95)*–cen which gave a multipoint lod score of 32·16.

A single recombinant with *D16S84* was also recombinant for *D16S85*, arguing in favour of a location for *PKD1* proximal to both these loci. The recombinant subject is unequivocally affected, so that a false positive diagnosis seems highly unlikely, but the family has so far been uninformative for proximal markers such as *D16S94*. It will therefore be important to show that exchange of flanking markers has occurred in this instance, since the family is not large enough to be unequivocally linked to *PKD1*. Fine mapping these and other rare recombinants in regions 2 and 3, using new polymorphisms identified from these locations will help to confirm the location of *PKD1* and to narrow down the area of search by physical mapping methods.

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