Haplotype analysis in autosomal dominant polycystic kidney disease

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Abstract

Haplotype analysis was performed in 35 autosomal dominant polycystic kidney disease (ADPKD) families typed with 13 markers close to the PKD1 locus. The identification of recombinants close to the PKD1 gene on chromosome 16p indicates that PKD1 lies between CMM65 distally and 26-6 proximally. In addition, three unlinked (PKD2) families and two families with potential new mutation were identified.

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Following the initial demonstration of linkage between autosomal dominant polycystic kidney disease (ADPKD) and 3'HVR on the short arm of chromosome 16,¹ many linkage studies have been performed on ADPKD kindreds. The gene present in chromosomal region 16p13.3 has been designated PKD1. Genetic heterogeneity has now been confirmed with the demonstration that ADPKD families unlinked to 16p13.3 (PKD2) show linkage to markers on chromosome 4q.² Two recent studies estimate the proportion of PKD1 out of all ADPKD linked families to be 0.81³ and 0.86,⁴ although the confidence limits were wide in both studies.

In order to facilitate accurate genetic mapping of PKD1, markers have been isolated from the short arm of chromosome 16. These have been ordered with respect to each other by a combination of linkage analysis, physical mapping to human-rodent hybrids, and long range restriction mapping,⁵⁻⁹ and are summarised in fig 1. All the probes used by Reeders *et al*⁵ lie in the region 16p13.11-16pter. Genetic linkage analysis of PKD1^{5,7,8,10,11} localises PKD1 to between GGG1 and 26-6, although one person

in the study of Somlo *et al*¹¹ gave a conflicting localisation for the gene. Conflicting localisations by mapping of recombinants have also been seen, for example, in Huntington's disease.¹² Several pitfalls exist which can result in a misleading genetic assignment: false positive and false negative diagnoses can lead to misinterpretation of results; double recombinants, although very rare in such small genetic distances, can occur and may be mimicked by gene conversion events; non-paternity can arise and lead to erroneous interpretation of results; and mistyping can occur, so that it is important that each apparent recombinant is checked, preferably by an independent laboratory or by typing with other markers. It is therefore important to accumulate as much genetic evidence on PKD1 localisation as possible.

Materials and methods

Thirty-five families with ADPKD were ascertained through the Medical Renal Unit, Royal Infirmary of Edinburgh and the Renal Unit, Western Infirmary, Glasgow, and characterised clinically as described by Wright *et al*.³ DNA was extracted and analysed for markers as described by Pignatelli *et al*,¹⁰ except that in addition to 3'HVR (D16S85), CMM65 (D16S84), and VK5 (D16S94), the 26-6 (D16S125)¹³ and SM7 (D16S283)¹⁴ loci were analysed. Haplotypes were drawn up for each pedigree, so as to allow the identification of recombinants. Eight additional markers were then used to identify the point at which recombination had taken place. Markers used were MS205,¹⁵ 16AC2.5 (D16S291),¹⁶ SM6 (D16S665), Blu-24 (D16S662), CW3 (D16S664), CW2 (D16S663) as described by Peral *et al*,¹⁷ KG8, and W5.2 as described by Snarey *et al*.¹⁸ These markers are summarised in fig 2.

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Figure 1 A map of the distal part of chromosome 16. The long vertical bars indicate breakpoints in chromosome 16 present in cell lines, the short vertical lines indicate polymorphic loci. The map is not to scale and is taken from Breuning *et al*.⁷

Figure 2 Approximate location of the markers used for typing. The positions of the markers are shown in relation to the CMM65 (D16S84), 26-6 (D16S125), and VK5 (D16S94) loci which are thought to flank the PKD1 gene (taken from Germino et al¹⁹ and Snarey et al¹⁶).

Results

ANALYSIS OF PKD1 RECOMBINANTS

One hundred and ninety two informative meioses were typed in the PKD1 families. Twelve people from 10 different kindreds, were identified as having a single recombination event

between PKD1 and flanking markers.

Three subjects from three different families were recombinant for 3'HVR and non-recombinant for CMM65 and VK5. One person was unaffected with a negative ultrasound scan at the age of 37 years, and is therefore unlikely to have a false negative diagnosis; the other two are affected. In these three families, the crossovers have occurred between 3'HVR and CMM65, placing the disease gene proximal to 3'HVR.

Five people from five different families were recombinant for 3'HVR, uninformative for CMM65, and non-recombinant for SM7. All these people are affected with a clearly positive ultrasound scan. Since CMM65 was uninformative in all these recombinants, probe MS205 (fig 2) was used to type the families. MS205 detects an RFLP and maps close to EKMDA2²⁰ which is distal to CMM65. It was informative in all of the above recombinants. All were non-recombinant for MS205, which localised the crossovers to between 3'HVR and MS205, and the PKD1 gene to a position proximal to 3'HVR.

In family PK27 (fig 3A), three recombinants have occurred, all in affected subjects. II.4 is recombinant with 3'HVR and non-recombinant with KG8, 16AC2.5, CW2, and 26-6. This places the crossover between KG8 and 3'HVR and the PKD1 locus proximal to 3'HVR. II.1 and II.3 are both non-recombinant with 3'HVR, KG8, 16AC2.5, and CW2, and recombinant with 26-6. This places both crossovers between CW2 and 26-6, and the PKD1 locus distal to 26-6. The 26-6 typings were repeated and found to be consistent. Confirmation of these recombinants with other proximal markers was not possible, since I.1 was uninformative for VK5. The probability that this family is linked to PKD1 is only 0.57, based on a previous heterogeneity analysis.³ However, exchange of flanking markers has been shown to occur in all three recombinant meioses, suggesting that it may be linked to PKD1.

Subject III.5 in family PK19 (fig 3B) is recombinant for 3'HVR and CMM65, and non-recombinant for CW3, CW2, and VK5. The other markers are uninformative. This places the crossover between CMM65 and CW3, and PKD1 proximal to CMM65. III.5 is, however, unaffected with a negative ultrasound scan at the age of 23 years, and therefore the possibility remains that he may develop the disease.

Taken together, the above results localise PKD1 distal to 26-6, and proximal to CMM65.

Figure 3 (A) Haplotype analysis of family PK27 showing three recombinants, all in affected subjects II.1, II.3, and II.4. II.1 and II.3 are both non-recombinant with 3'HVR, KG8, 16AC2.5 and CW2, and recombinant with 26-6. II.4 is recombinant with 3'HVR and non-recombinant with KG8, 16AC2.5, CW2, and 26-6. (B) Haplotype analysis of family PK19 in which III.5 is recombinant for 3'HVR and CMM65, and non-recombinant with CW3, CW2, and VK5. III.5 is unaffected with a negative ultrasound scan at the age of 23 years. III.6 refused permission for DNA studies. (C) Extended haplotype analysis of family PK52 showing no exchange of flanking markers between 3'HVR and CRI-090.

Figure 4 (A) Haplotype analysis of family PK29, where initial typing with three markers suggested a recombinant with 3'HVR and SM7 in III.3. Further typing, however, suggests that this family is unlinked to PKD1. In III.3, recombination between SM7 and 26-6 has occurred. (B) Haplotype analysis of family PK8. The haplotypes of I.1 and I.51 are inferred. II.1 appears to be a new mutation, with the affected chromosome being inherited by both III.1 and III.2. II.4 has the same "affected" chromosome, but without the mutation, since he is unaffected with a normal ultrasound scan at the age of 53 years. (C) Haplotype analysis of family PK54 showing a new PKD1 mutation in II.1. I.1 is assumed to be unaffected having died at the age of 74 years of cerebrovascular accident and prostatic carcinoma. His blood urea, electrolytes, and creatinine were all normal. I.51 is unaffected with a normal ultrasound scan at 84 years. The mutation has occurred on the maternal chromosome. II.3 and II.4 are recombinant between 3'HVR and SM6, and II.6 is recombinant between CW2 and SM7.

FAMILIES THAT APPEAR UNLINKED TO PKD1

As described elsewhere, only two small families out of the 35 analysed showed statistically significant evidence of non-linkage to PKD1.³ One family (PK53) would require either two or three double recombinants out of five meioses to be consistent with a PKD1 locus. Since no exchange of flanking markers was seen, the family appeared unlinked from haplotype analysis. The other family (PK52) was initially thought to be unlinked, but only a single recombinant out of three meioses needed to be invoked to be consistent with a PKD1 locus.³ Further typing was carried out with CA repeat polymorphisms isolated from the SM6, CW2, VK5, and CRI-090 (D16S45) loci which were all informative (fig 3C). No exchange of flanking markers was seen between 3' HVR and CRI-090. This is therefore consistent with non-linkage in this family also, since the alternative explanations of either a double crossover between CW2 and VK5, which are located approximately 400 kb apart, or a location for

PKD1 proximal to CRI-090 are both very unlikely.

In a third family (PK29), typing with four loci indicated that subject III.3 was recombinant with 3' HVR and SM7, and non-recombinant with 26-6 and VK5. Further typing with other markers, however, suggests that this family is unlinked to PKD1, since no common haplotype can be identified (fig 4A). Subjects III.1, III.2, and III.3 have negative ultrasound scans at ages 30, 28, and 40 respectively. Subject III.4 refused an ultrasound scan, but is clinically unaffected at the age of 38 years.

IDENTIFICATION OF NEW MUTATIONS

Following haplotype analysis, two subjects who had apparently inherited a new mutation were identified. These were in families PK8 and PK54. In family PK8 (fig 4B), both parents were unaffected as far as can be determined from death certificates. Subject II.1 appears to

be a new mutation, with the affected chromosome being inherited by both III.1 and III.2. II.4 has the same "affected" chromosome, but is unaffected with a normal ultrasound scan at the age of 53 years. The possibility exists, however, that the family is unlinked to chromosome 16 (PKD2). Family PK54 (fig 4C) was excluded from linkage analysis in view of the fact that there was only one affected person and no clear linkage to chromosome 16. I.1 is thought to have been unaffected, and I.51 is clearly so with a negative ultrasound scan at the age of 84 years. Two of the three children of II.1 are affected and have inherited the same paternal chromosome from II.1. This chromosome originated from I.51 and has also been inherited by II.2 and II.6, although II.6 is recombinant for SM7 and VK5. Both of these sibs are unaffected. II.2 has a negative ultrasound scan at the age of 52 years.

Discussion

RECOMBINANTS WITH PKD1

A meiosis showing a single recombination event between the closest informative flanking markers contains information that is useful in localising PKD1. In this set of 32 PKD1 families (excluding unlinked families PK29, PK52, and PK53) 13 single recombinants were identified between PKD1 and the flanking markers, out of a total of 192 informative meioses. Nine of these recombinants place PKD1 proximal to 3'HVR, which is in keeping with the results of previous multipoint analyses.^{5,7,8,10,21} Two recombinants place PKD1 distal to 26-6: these are subjects II.1 and II.3 from family PK27, both of whom are clearly affected in a kindred with typical clinical features of ADPKD. The presence of recombinants with 26-6 in a family that is not unequivocally PKD1 raises some doubts as to their validity, although exchange of flanking markers has occurred. Confirmation of these recombinants with another proximal marker has not been possible. The final recombination event in subject III.4 of family PK19 places PKD1 proximal to CMM65. This kindred also shows typical features of ADPKD, but III.4 is unaffected with a clearly negative ultrasound scan at the age of 23 years. At this age, the probability of detecting cysts in a gene carrier by ultrasound scanning is 0-92.²² The possibility therefore exists that he may yet develop ADPKD, but it is more likely that he will remain unaffected. These four recombinants therefore localise the PKD1 gene to the interval between CMM65 distally and 26-6 proximally.

Somlo *et al.*¹¹ identified 11 single recombinant meiotic events in eight linked families, out of over 200 families originally studied. Of these 11 recombinants, three placed PKD1 proximal to GGG1 and two placed PKD1 distal to 26-6. Since GGG1 is located approximately 10 kb distal to CMM65⁸ the recombinants identified in this study place PKD1 in exactly the same interval as that obtained by Somlo *et al.*¹¹ This interval is known to span approximately 750 kb of DNA,¹⁹ and within this interval there is some doubt regarding the localisation of PKD1 as defined by recombinants. Somlo¹¹ found that

the two recombinants locating PKD1 distal to 26-6 also placed the disease distal to the marker 92.6SH1.0, but they also identified a recombinant which located PKD1 proximal to 92.6SH1.0. This man is unaffected at the age of 33 years, which leaves open the possibility that he may yet develop renal cysts. This is unlikely, however, given that two studies have shown that all renal cysts in gene carriers are detectable by ultrasound by the age of 30 years.^{23,24} However, the brother of this man, who should be affected according to flanking markers, remains unaffected at the age of 40 years, and it may be that this family has a late onset form of the disease owing to allelic variation. Other possibilities exist to explain the results in this family, including non-linkage to PKD1, a gene conversion event, or a large PKD1 gene which straddles 92.6SH1.0. If the gene does straddle the 92.6SH1.0 locus, the three recombinants would be intragenic, suggesting a recombinational hot spot. There are no recombinants in the families analysed in this study which throw any further light on the problem.

UNLINKED FAMILIES

Phenotypic differences between PKD1 and PKD2 families have been described^{23,25,26} in which PKD2 families have a milder phenotype, with delayed appearance of cysts, and a lower risk of developing hypertension or progressing to renal failure. The phenotype in family PK53 is consistent with a milder form of the disease, with the mother having normal renal function and only borderline hypertension at the age of 59 years. However, this is not uniformly so within the family, since the two affected sons required renal dialysis at the ages of 33 years and 27 years respectively. Family PK52 (fig 3C) shows a more severe phenotype: the father (I.1) received a renal transplant at the age of 64 years, and the affected daughters (II.1 and II.2) are hypertensive with normal renal function at the ages of 43 and 39 years respectively. In family PK29 (fig 4A), II.1 required dialysis at the age of 51 years and was transplanted the following year. II.2 has been lost to follow up. All three families are of Scottish ancestry. The three unlinked families seen in this study are similar to those first described by Romeo *et al.*²⁷ and Kimberling *et al.*²⁸ in that they show a more severe ADPKD phenotype. The identification of family PK29 as unlinked only occurred after analysis of the family with further markers because of a potentially interesting recombinant. More families may therefore be found to be unlinked if they are typed with a large number of loci.

NEW MUTATIONS

Dalgaard²⁹ calculated the rate of new mutations in ADPKD to be between 6.5×10^{-5} and 12×10^{-5} per gene per generation, one of the highest mutation rates in genetic disease. This is likely to be an overestimate since many patients with ADPKD are asymptomatic with normal renal function, but can be diagnosed

on ultrasound scan. Since this technology was not available in 1957, it is possible that many parents were given a false negative diagnosis, thereby increasing the apparent mutation rate. A high mutation rate is inconsistent with the presence of linkage disequilibrium, which has been detected in this population.³⁰ Only two possible new mutations have been seen in the 36 families studied, consistent with a relatively low mutation rate and high reproductive fitness in ADPKD.³¹ Direct determination of the mutation rate from these data is difficult, however, because (1) the size of the population from which the 36 families were ascertained is uncertain because of incomplete ascertainment; and (2) other families with only a single affected member were excluded from the study.

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