Recombination or heterogeneity: is there a second locus for adult polycystic kidney disease?

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Abstract
Twenty-four families with adult onset polycystic kidney disease were typed for markers flanking the PKD1 locus on chromosome 16. The aggregated results gave a significant lod score in favour of linkage to PKD1. Within this group of families two showed unusual features: recombinations, including double recombinations, and, in one family, an unexpectedly high proportion of affected people. We consider the evidence that in these families the disease might result from a mutation at a different locus, PKD2, not linked to PKD1. We suggest that a useful test is to compare the relative numbers of meioses apparently non-recombinant and doubly recombinant for markers flanking the normal disease locus, ignoring meioses recombinant for only a single marker. Using this test, neither our two families nor the data published so far on other families provide compelling evidence for the existence of a second locus for adult polycystic kidney disease. For genetic counselling in families too small to give internal evidence for or against linkage, the extra uncertainty can be handled by using a higher recombination rate.

It has been suggested\(^{1,2}\) that adult polycystic kidney disease (APKD) may be caused by mutation at more than one locus. One locus (PKD1) has been unambiguously mapped\(^{3}\) to chromosome 16p13. The 3'HVR and 24-1 (D16S80) probes have been shown to flank PKD1\(^{4}\) and a detailed map of this region has been constructed by multipoint linkage mapping combined with physical mapping.\(^{5}\) Other workers have confirmed the linkage of APKD to 16p markers in independently ascertained families.\(^{6}\)

These studies showed that APKD usually maps to the PKD1 locus on chromosome 16. However, two families have been reported\(^{1,2}\) in which there are many recombinants between APKD and 16p markers, and which are claimed to show the existence of a second locus (PKD2) unlinked to PKD1. Interestingly, both families are of Italian extraction. Since the disease in these two families is not clinically distinguishable from normal APKD, this presents an added uncertainty in any clinical use of the 16p markers, and renews interest in any unusual inheritance patterns seen in APKD families.

We have studied the segregation of 16p markers in 24 APKD families. Two of the families show unusual features. We report the overall evidence for linkage of APKD to chromosome 16p in our families, and consider possible interpretations of the two unusual families.

Methods
Twenty-one of the families presented to the North Western Regional Genetic Service or the Renal Unit at Manchester Royal Infirmary. All of these are in regular contact with us through our voluntary genetic register for APKD. Three families originated from the Institut de Pathologie Moleculaire, Paris. APKD was diagnosed by clinical assessment of symptoms and ultrasound imaging of renal cysts using the criteria of Bear et al.\(^{7}\)

Blood samples of 20 ml were collected using EDTA as anticoagulant. Lymphocyte DNA was extracted and restriction fragment length polymorphisms were scored as described.\(^{8}\) The polymorphisms studied are shown in table 1. Lod scores were calculated using the MLINK program\(^ {10} \) version 4.7. Calculations were

\[ \text{Table 1 Polymorphisms studied in the APKD families.} \]

<table>
<thead>
<tr>
<th>Probe</th>
<th>Restriction enzyme</th>
<th>Allele</th>
<th>Size (kb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>3'HVR (locus HBA)</td>
<td>PvuII</td>
<td>A-M</td>
<td>2.0-10.0</td>
<td>9</td>
</tr>
<tr>
<td>24-1 (locus D16S80)</td>
<td>TaqI</td>
<td>1</td>
<td>3.8</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>1.5+1.3</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>1.5</td>
<td>4</td>
</tr>
</tbody>
</table>

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performed using two alternative models for the penetrance of APKD (table 2). In the more stringent model, all at risk but unaffected people were excluded; in the less stringent model, at risk but unaffected people were excluded if aged under 15, and otherwise the age dependent penetrances used by Bear et al were applied.

For calculating the likelihood that any one pedigree shows independent segregation of APKD and chromosome 16, only meioses triply informative for APKD and for two markers flanking the PKD1 locus were considered. Meioses showing recombination between the flanking markers were excluded, and the remaining meioses were scored as apparently non-recombinant (NR) or apparently doubly recombinant (DR).

On the assumption that the disease maps to the PKD1 locus, and neglecting interference, the likelihood of each DR meiosis is

$$P_{DR} = \frac{\theta A \theta B}{[1 - \theta A - \theta B + 2\theta A \theta B]}$$

where $\theta A$ and $\theta B$ are the recombination fractions between PKD1 and the flanking markers. The likelihood of each NR meiosis is 1–PDR. On the alternative assumption that the disease is not linked to the PKD1 locus, the likelihood of each NR or DR meiosis is 1/2.

The overall probability that APKD can be caused by mutation at the PKD1 locus can then be calculated using Bayes's formula. An example calculation is shown in table 3.

### Results

Table 2 shows the two point lod scores calculated on all 24 families, assuming equal recombination rates in males and females. The peak scores (calculated using the MLINK program) are 7.71 at $\theta = 0.062$ (PKD1 v HVR) and 4.18 at $\theta = 0.057$ (PKD1 v D16S80).

Two families (R.158 and R.106) showed unexpected features (figs 1 and 2). All other families studied were either non-recombinant or showed only one crossover in informative meioses. There is no discernible difference in the clinical presentation or progress of renal disease in these two families compared to the others studied.

In family R.158 (fig 1) the dead affected man I.1 passed E1 and E2 to his two affected sons, and B1 and E1 to his two unaffected children. The phase known affected son II.4 passed one non-recombinant and one singly recombinant chromosome to his two affected children. The phase of I.1 can be assigned in two alternative ways. Most probably he is B+1/E A 2, and there are three single recombinants (II.1, II.2, and III.1). Alternatively, if the phase of I.1 is B+2/E A 1, then there is one double recombinant (II.3) and three single recombinants (II.2, II.4, and III.1). Subject II.3 is healthy and shows no ultrasound evidence of renal cysts at the age of 32 years.

Family R.106 (fig 2) includes a remarkable number of affected people: all eight sibs in generation II are clinically affected and all four daughters of affected

### Table 2

<table>
<thead>
<tr>
<th>Loci</th>
<th>0.001</th>
<th>0.01</th>
<th>0.05</th>
<th>0.10</th>
<th>0.20</th>
<th>0.30</th>
<th>0.40</th>
</tr>
</thead>
<tbody>
<tr>
<td>APKD v 3'HVR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>1.41</td>
<td>4.23</td>
<td>5.80</td>
<td>5.60</td>
<td>4.09</td>
<td>2.34</td>
<td>0.85</td>
</tr>
<tr>
<td>Model 2</td>
<td>2.10</td>
<td>4.98</td>
<td>7.02</td>
<td>7.10</td>
<td>6.51</td>
<td>3.42</td>
<td>1.26</td>
</tr>
<tr>
<td>APKD v D16S80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Model 1</td>
<td>-0.57</td>
<td>0.86</td>
<td>1.78</td>
<td>1.87</td>
<td>1.51</td>
<td>0.98</td>
<td>0.44</td>
</tr>
<tr>
<td>Model 2</td>
<td>1.99</td>
<td>3.28</td>
<td>4.09</td>
<td>3.99</td>
<td>3.13</td>
<td>1.98</td>
<td>0.90</td>
</tr>
<tr>
<td>3'HVR v D16S80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>-10.89</td>
<td>-4.03</td>
<td>0.24</td>
<td>1.52</td>
<td>1.89</td>
<td>1.34</td>
<td>0.54</td>
</tr>
</tbody>
</table>

### Table 3

Calculation of the probability that APKD is linked to the PKD1 locus in a family with six meioses non-recombinant and one doubly recombinant for PKD1 and flanking markers, each 5 cm from PKD1.

<table>
<thead>
<tr>
<th>Hypothesis: APKD and PKD1 are:</th>
<th>Linked</th>
<th>Not linked</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior probability</td>
<td>0.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Conditional probability</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 DR meiosis</td>
<td>$P_{DR} = 0.0028$</td>
<td>(1/2)</td>
</tr>
<tr>
<td>6 NR meioses</td>
<td>$(1 - P_{DR}) = 0.983$</td>
<td>(1/2)</td>
</tr>
<tr>
<td>Joint probability</td>
<td>$2 \times 478 \times 10^{-3}$</td>
<td>$7.812 \times 10^{-4}$</td>
</tr>
<tr>
<td>Final probability</td>
<td>0.760</td>
<td>0.240</td>
</tr>
</tbody>
</table>

$P_{DR}$ as defined in the text is $\theta A \theta B / [1 - \theta A - \theta B + 2\theta A \theta B] = 0.0025/0.9005 = 0.0028$ for $\theta A = \theta B = 0.05$. The prior probability of linkage is arbitrarily taken as 0.9.
subject II.8 show ultrasonic evidence of renal cysts. Assuming phases as shown for II.1, II.2, and II.8, then in generation II there are seven non-recombinant meioses and in generation III, 12 non-recombinants, two single recombinants (III.7 and either III.10 or III.11), and one double recombinant (III.18). All the presumed recombinations in both these families occurred in male meioses, which is consistent with the reported eightfold higher frequency of recombination in males in this chromosomal region.

**Discussion**

This linkage study confirms earlier and more extensive work mapping PKDI to the short arm of chromosome 16. A significant positive lod score is found for 3′HVR and APKD even when a stringent penetrance model is used, which eliminates all subjects not tested or negative on ultrasound examination. When an age dependent penetrance is used to bring these subjects into the calculations the flanking marker 24–1 also gives a significant lod score. The data presented here are consistent with earlier studies placing 24–1 flanking PKDI. In general, the families studied were small and each gave little linkage information. Most of these families were contacted to discover attitudes to prenatal diagnosis for APKD or to evaluate a presymptomatic carrier detection service rather than primarily for a linkage study. However, this does serve to prevent any bias resulting from the selection of large kindreds.

In clinical service, it often happens that those families showing unusual features are selected for further study, in order to try and clarify the risks for family members. This, for example, happened in our family R.106. We selected the family for study because of the remarkably high proportion of affected people; we started with the offspring of II.8 and immediately found a double recombinant. To assess the significance of one odd family requires an analysis that compares that family to the accepted linkage map, without the need to feed in and reanalyse all the raw data which established that map. We think our method achieves this in a simple way, and directs investigation to the crucial question, which is the proportion of apparent double recombinants.

It is essential to study these families with flanking markers because marker-marker recombinants do not constitute evidence for heterogeneity. An unusual cluster of marker-marker recombinants might be seen in a family for several reasons, including coincidence, ascertainment bias in favour of ‘interesting’ families, or a familial idiosyncrasy in the meiotic behaviour of chromosome 16, as suggested by Hultén. None of these possibilities has any relevance to the question of whether APKD in that family maps to chromosome 16p or not.

Our method of calculating the probability of heterogeneity (table 3) assumes that the currently accepted map is correct in most families, and particularly that the flanking markers really do flank the disease locus. Given the very high published lod scores and very few claimed non-linked families, we feel that this simple test can safely be used for APKD. It would apply
equally well to cystic fibrosis, but would not be appropriate for diseases where the normal map is uncertain, such as tuberous sclerosis.

The calculation given here ignores interference, which is the tendency of one crossover to inhibit other nearby crossovers. Interference suppresses close double recombinants, but gene conversion produces apparent double recombinants. In human meiosis the balance between these effects is unclear. If interference is significant then the calculation given here underestimates the probability of an unlinked locus.

**INTERPRETATION OF FAMILY R.158 (FIG 1)**

On the assumption of three single and no double recombinants, this family provides no evidence for segregation of a PKD2 locus. The alternative explanation, with two non-recombinants and one double recombinant, gives 83% probability that the disease is not linked to PKD1, but this is based on a small number of meioses and an implausible assignment of phase.

**INTERPRETATION OF FAMILY R.106 (FIG 2)**

Given 19 non-recombinants and one double recombinant, the probability we are seeing PKD1 linked disease is 0.9999, using the method of table 3 and a prior probability of 0.9. It is also evident from fig 2 that APKD is strongly associated with one particular haplotype (J1) in this family. Thus, this family does not provide evidence for a PKD2 locus.

A possible alternative explanation for this pedigree is that both parents in generation I carried APKD. We have been unable to obtain clinical information to confirm or refute this. If both the J1 and D2 chromosomes carry APKD, then four members of the family (II.3, II.4, II.7, and II.8) would be homozygous for APKD at the PKD1 locus. This would explain why all four children of II.8 are affected. III.18 would be non-recombinant rather than doubly recombinant: she has APKD because she has the D2 chromosome. However, on this hypothesis all the children of II.3 and II.4 should be affected. None of them has opted for ultrasound screening, but they are healthy at ages 30 (III.10), 33 (III.11), 25 (III.12), and 20 (III.14).

Since all but one (III.18) of the affected people tested has the J1 haplotype, the high proportion of affected people in this family is almost certainly a random quirk of segregation. Our ascertainment is no doubt biased in favour of families with more affected people.

A final possibility in this family is that a false positive ultrasound diagnosis of renal cysts was made in III.18. The frequency of such false positives has been claimed to be as high as 2%.5

It is interesting to compare these families with those previously reported by Romeo et al6 and Kimberling et al2. In the family of Kimberling et al2 163 subjects were typed for 3'HVR and two closely linked RFLPs. At least 24% of informative meioses showed recombination between APKD and one marker. No studies with flanking markers have yet been reported and so, though the pedigree is persuasive, the crucial test remains to be done. The family described by Romeo et al6 was studied with flanking markers, but because phase cannot be unambiguously assigned it is impossible to tell whether most of the reported recombinants are single (marker-marker) or double recombinants. Ten meioses include a minimum of one double and three single recombinants. On this minimum estimate our calculation gives the probability of non-linkage to PKD1 as 0.24 (table 3).

In conclusion, when the question arises of whether a single family with a well mapped disease has an unusual unlinked form of the disease, we suggest the crucial test should be the relative numbers of meioses apparently non-recombinant and doubly recombinant for markers flanking the normal map position of the disease locus. Using this test, neither our two families nor the two previously published provide compelling evidence for the existence of a second locus for adult polycystic kidney disease. Further study of the family of Kimberling et al2 might, however, provide such evidence.

Given the uncertainty about heterogeneity, how can one use linked markers for gene tracking in APKD families? If a pedigree is large enough it may be possible to confirm or refute 16p linkage within that family, but this will rarely happen. For an individual person, the only question that matters is whether he is recombinant or non-recombinant. Since we cannot know this, we quote an error rate equal to the probability that a given meiosis is recombinant. If a fraction x of families have disease owing to mutation at the PKD1 locus, with 5% average recombination risk, and the remainder have an unlinked disease with 50% chance of recombination with chromosome 16 markers, then the overall percentage risk of recombination is 5x+50(1-x). Thus, the practical error rate when a single marker is used is between 5% and 10%, and probably closer to 5%. Again, using flanking markers simplifies the problem. The likelihood, x, that the family has an unlinked disease can be calculated. Even if the disease is unlinked there is a 1 in 2 possibility of being right by chance, so the error rate is x/2+PDF. No prediction can be made if the consultant has a marker-marker recombination.

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