

Studies of genetic linkage between adult polycystic kidney disease and three markers on chromosome 16

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SUMMARY Adult polycystic kidney disease (APKD) is a common genetic disorder that is inherited as an autosomal dominant trait. Recent reports show that, in some families, the APKD gene shows close genetic linkage to two chromosome 16 specific genetic markers. We have been conducting a genetic linkage study using 29 polymorphic isoenzyme and antigenic markers in 184 members of 12 APKD families. We present here the results of linkage analysis using three of these markers which have also been reported to be located on chromosome 16: phosphoglycolate phosphatase (PGP), glutamate pyruvate transaminase (GPT), and haptoglobin (HP). The results show that *APKD* is closely linked to the *PGP* locus on the short arm of chromosome 16 (16p13→p12), which is consistent with the previously reported linkage both to *PGP* and to the α globin locus. The genetic distance between *PGP* and *APKD* shows a maximum likelihood value of the recombination fraction at zero with a lod score of 5.5. There is no evidence of linkage between *APKD* and either *GPT* or *HP*. The *PGP* polymorphism potentially provides a useful predictive test to complement the use of α globin probes in genetic counselling. These tests should provide an efficient means of primary screening of family members at risk, as well as introducing the possibility of prenatal diagnosis.

Polycystic kidney disease in adults (APKD) is an autosomal dominant trait which shows a high penetrance. It is a common clinical abnormality affecting 80/100 000 of the general population¹ and in many renal replacement therapy programmes represents the third commonest cause of chronic renal failure.²

Many patients do not present until the onset of symptoms, commonly after the age of 30, by which time target organ damage from hypertension may be severe.³ Diagnosis before clinical presentation can be made by ultrasound, computed tomography, or intravenous urography with nephrotomography. As ultrasound does not require the use of radiation or the injection of contrast medium, it is to be preferred as a screening method. The sensitivity of detection is limited in the younger age groups, being only 22% in the 0 to 9 year age group, but rising to

86% in the 20 to 29 year age group.⁴ Satisfactory genetic counselling for members of affected families can therefore be difficult,⁵ which has prompted the search for a closely linked genetic marker to assist in the prediction of polycystic kidney disease.

Previous genetic linkage studies have looked at the relationship of histocompatibility complex (HLA) and certain blood group antigens and have excluded close linkage to these loci.¹⁻⁶ We initiated a broad based genetic linkage study using a variety of polymorphic markers, which at the outset included blood groups, enzyme and other protein markers, and DNA probes mapped to the full range of human chromosomes. However, during the early phase of our study, Reeders *et al*⁷⁻⁸ reported genetic linkage between *APKD* and a highly polymorphic DNA locus situated 8 kb 3' to the α globin cluster and the phosphoglycolate phosphatase (*PGP*) locus on chromosome 16. Here we present therefore the results of our analysis of three polymorphic markers

whose loci are all reported to be on chromosome 16 (*PGP*, *GPT*, and *HP*).⁹

Methods

One hundred and eighty-four relatives of 12 APKD probands were admitted to the study after giving informed consent, as approved by the local Ethical Committee. Each member was visited at home and 50 ml EDTA anticoagulated blood was taken by venepuncture for measurement of nine blood group antigens, 20 plasma, red and white blood cell isoenzymes, and for extraction of DNA. Blood for separation of red and white cells was centrifuged at 3000 rpm for 10 minutes and the plasma and buffy coat were removed. The red cells were washed three times in 0.9% sodium chloride and stored at -70C before isoenzyme electrophoresis.

ISOENZYME ELECTROPHORESIS

Plasma and washed red blood cells, lysed by freezing and thawing, were used for the analyses using previously described methods for *PGP* and *GPT*¹⁰ and for *HP*.¹¹

PGP shows six electrophoretically distinguishable phenotypes attributable to three polymorphic alleles, *PGP1*, *PGP2*, and *PGP3*, which occur in the European population at frequencies of 0.826, 0.129, and 0.045, respectively.¹² *GPT* shows three electrophoretic phenotypes associated with alleles *GPT1* and *GPT2* with frequencies 0.52 and 0.47 respectively.¹³ Haptoglobin shows three electrophoretic phenotypes associated with alleles *HP1* and *HP2* with frequencies of about 0.4 and 0.6 respectively (Galton Laboratory data).

ULTRASOUND TECHNIQUE

Sonography was performed by an experienced sonologist (PLA) using realtime equipment. Most of the examinations were done using a 3.5 MHz transducer but a 5.0 MHz transducer was used if it provided adequate penetration of the subject. Both kidneys were examined in their long axes and also transversely, with additional planes being used as necessary to obtain the best possible visualisation. The liver, pancreas, and spleen were also examined for the presence of cysts. All at risk family members sampled were given ultrasonic scans to help confirm or exclude the presence of APKD. The presence of at least two cysts in one kidney and one cyst in the contralateral kidney was considered diagnostic of APKD.⁴

LINKAGE ANALYSIS

Pedigrees were drawn up on the basis of family histories and by consultation with the Centralised

Register for Births, Marriages and Deaths for Scotland. Linkage was analysed using the computer programme LIPED,¹⁴ using an age of onset correction.¹⁵ Confidence limits were obtained by reducing the maximum likelihood value of the recombination fraction by a factor of 100. On the basis of the renal ultrasound data of Bear *et al*,⁴ the likelihoods of a subject with the APKD gene showing a normal ultrasound scan were estimated to be 0.9, 0.4, 0.2, and 0.1 in the first, second, third and fourth decades. Those aged over 40 years and negative on the basis of ultrasound and clinical criteria were taken to be unaffected.

Results

Of the 12 families examined, four were informative for linkage to *PGP*. There is no evidence of recombination between *APKD* and *PGP* in these families, indicating that the two loci are closely linked on chromosome 16. The results from two of these families are shown in the figure. In family PK-3, *APKD* is segregating with the allele *PGP2* such that all affected subjects have *PGP2* and

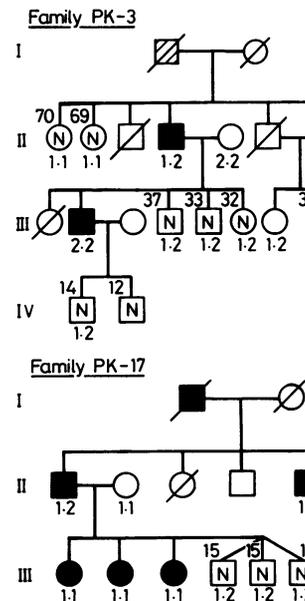


FIGURE Pedigree of two families with APKD. Affected subjects indicated by shaded boxes. N indicates unaffected on the basis of ultrasound scanning. Superscript numbers indicate ages. Allele type in relation to *PGP* isoenzyme is indicated numerically. *APKD* segregates with allele 2 in family PK-3 and allele 1 in family PK-17. The triplets in family PK-17 are trizygotic and for statistical purposes have been treated as three separate sons.

TABLE Results of linkage analysis between adult onset polycystic kidney disease and phosphoglycolate phosphatase (PGP), glutamate pyruvate transaminase (GPT), and haptoglobin (HP) loci, showing lod scores for different values of the recombination fraction (θ). The results for the sexes are shown separately, since recombination is generally greater in human female meioses, and combined (assuming equal rates). A lod score is the log 10 of the likelihood ratio of observing the family data at each value of θ compared with a value of 0.5 (random assortment).

	Recombination fraction (θ)					
	0.00	0.05	0.10	0.20	0.30	0.40
<i>PGP</i>						
Male	3.57	3.23	2.88	2.14	1.36	0.55
Female	1.18	1.13	1.04	0.77	0.44	0.13
Combined	5.50	4.99	4.44	3.26	2.01	0.75
<i>GPT</i>						
Male	−∞	−1.49	−1.09	−0.53	−0.19	−0.02
Female	−∞	−1.88	−1.51	−0.87	−0.38	−0.09
Combined	−∞	−4.56	−3.04	−1.42	−0.55	−0.11
<i>HP</i>						
Male	−∞	−0.69	−0.52	−0.27	−0.12	−0.03
Female	−∞	−2.04	−1.31	−0.55	−0.18	−0.03
Combined	−∞	−2.61	−1.72	−0.76	−0.27	−0.06

unaffected subjects receive PGP1 from their affected heterozygous parent. The marker alleles indicate whether a subject inherits the APKD bearing or the normal chromosomal homologue, without this association being disrupted by recombination during meiosis, implying that the two loci are physically close on the same chromosome. A similar result is obtained in family PK-17 (figure), in which the disease is segregating with allele PGP1. In generation II, however, the association with allele PGP1 is less obvious, perhaps because the affected parent in generation I was homozygous for allele PGP1, while the unaffected parent was heterozygous. The conventional statistic for measuring linkage between two loci is the maximum likelihood lod score and the value of the recombination fraction that produces it. Combining the results from each family in this way shows that the maximum lod score is 5.50 at a recombination fraction (θ max) of zero with 99% confidence limits extending to 0.18.

In contrast to the results with PGP, there is no evidence of genetic linkage between *APKD* and either *GPT* or *HP* (table). The maximum likelihood values of the recombination fraction are 0.5 at a lod score of zero in each case, with 99% confidence limits of 0.15 to 0.5 (*GPT*) and 0.07 to 0.5 (*HP*), indicating that these loci are likely to be at least 50 cM away from the *APKD* locus.

Discussion

Phosphoglycolate phosphatase (PGP) is expressed in a variety of tissues including red and white blood

cells.¹² On the basis of somatic cell hybrid panels which express PGP activity, its location has been assigned to the short arm of chromosome 16.¹⁶ The α globin cluster, containing the hypervariable site (3' HVR) linked to *APKD*,^{7,8} has also been assigned to this region (16pter→p13). Three polymorphic alleles (PGP1, PGP2, and PGP3) are thought to determine the six electrophoretic phenotypes observed in the general population, making it a useful marker for genetic studies. To be informative either for genetic counselling or for mapping studies, an affected parent must be heterozygous for PGP, so that on the basis of the allele frequencies in the general population, one third of *APKD* families are expected to be informative with this marker. Only when a person is heterozygous both for disease and marker loci is there an opportunity to follow the segregation of the normal and disease alleles in the offspring.

No definite recombinations were observed between *PGP* and *APKD* in these families so that the maximum likelihood genetic distance between these loci is estimated to be at a recombination fraction of zero, with a lod score of 5.5. The result is therefore statistically significant and shows that the PGP polymorphism does indeed provide a potentially useful predictive test for *APKD*. However, before PGP or 3'HVR is applied clinically, a number of issues need to be resolved.

Firstly, it will be necessary to obtain a larger sample of informative families on which to base an accurate estimate of the genetic distance between *PGP* and *APKD*. The latter will form the basis for risk estimation in genetic counselling, whether for

preclinical detection or for prenatal diagnosis. The confidence limits for the recombination fraction between *APKD* and *PGP* in this study extend from 0 to 0.18, implying that a predictive error as high as 18% is possible. When these results are combined with those of Reeders *et al.*,⁸ the maximum likelihood value of the recombination fraction remains at zero (99% confidence limits of 0.09) with a lod score of 13.71 (sexes combined).

Secondly, the possibility of genetic heterogeneity of the locus must be considered. This is true of virtually all genetic disease in which the biochemical defect is unknown, particularly when there is a high prevalence, as in adult polycystic kidney disease.¹ However, at present, combined results with the 3' HVR probe indicate that the disease is likely to be genetically homogeneous.¹⁷

Thirdly, it will be important to establish the order of the *PGP*, 3'HVR, and *APKD* loci on chromosome 16. Error in predictive counselling on the basis of a linked genetic marker arises from recombination between marker and disease locus. As discussed above, accurate estimation of the recombination fraction is essential, but predictive error can be reduced considerably by the simultaneous use of markers that are located on either side of the disease gene (bridging markers). It remains to be determined whether *PGP* and a globin bridge the *APKD* locus.

How useful is the *PGP* polymorphism likely to be in prenatal diagnosis? *PGP* has been detected in all tissues examined so far.¹² It is therefore possible that it is also present in amniocytes and chorionic villus samples, although it is not yet clear whether at a sufficiently high level for reliable detection.

There is some controversy surrounding the precise location of the *GPT* locus since linkage has been reported both to chromosome 16 and to other chromosomes.⁹ A substantial amount of new data has been reported recently which still does not resolve the issue but shows that there is 44% recombination with *PGP*, which is consistent with our failure to detect linkage between *APKD* and *GPT*. The *HP* locus has been assigned to the long arm of chromosome 16 (16q21→q22)⁹ and shows at least 35% recombination with *PGP*. The failure to find evidence for linkage between *HP* and *APKD* in this study is therefore consistent both with *APKD* being close to *PGP* on the short arm and with *HP* and *PGP* being well separated.¹⁸

A number of clinical issues arose in the course of the study that highlight the need for reliable predictive markers in *APKD*. One problem arose when subjects at risk for *APKD* were found to have only one or two renal cysts, raising doubts as to their genetic status. Misclassification of normals as

being affected will lead to inaccurate genetic mapping data and seriously compromise genetic counselling. The diagnosis was only made, therefore, if certain criteria were met on renal ultrasound examination. Because single and multiple cysts occur in normal subjects and their prevalence increases with age,¹⁹ scans were only considered to be positive if two or more transonic cysts with well defined back walls and some acoustic enhancement were seen in one kidney and a single cyst in the other. In early cases, when the cysts are small (<6 to 8 mm), they may not fulfil these criteria for technical reasons. In these cases, small, abnormal, cyst-like anechoic or very hyperechoic areas were considered to represent cysts provided that they did not correspond in size, shape, or position to the renal pyramids. However, subjects at risk showing only one or two clearly defined renal cysts can cause considerable diagnostic problems that would be greatly relieved by the availability of genetic markers.

Another issue that became increasingly apparent as the study progressed was the need for genetic counselling in *APKD* families, as reported by Sahney *et al.*⁵ Many of the family members were not aware of the condition in their relatives, and if they were poorly informed about its genetic implications. Far from preferring to remain in ignorance of the risk to themselves or their offspring, most family members did want to know their disease status and took advantage of the informal genetic counselling offered. A substantial number of previously undiagnosed affected subjects were also found to have untreated hypertension, so that all new cases were encouraged to attend a hypertension clinic for long term follow up.

The expensive and time consuming effort of exhaustively screening all relatives at risk by clinical and ultrasound examinations has limited this approach in most centres, despite the obvious benefits to families with *APKD*. The availability of preclinical predictive tests such as *PGP* or 3'HVR for families, which can be carried out on blood samples, even when despatched from remote areas by the public postal service, might provide the ideal primary screen for *APKD*. Secondary screening of those 'affected' on the basis of their marker status alone can then be carried out by clinical and ultrasound examination. Early identification of affected subjects will facilitate the early diagnosis and appropriate treatment of hypertension and permit effective genetic counselling for all those at risk.

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