CLINICAL RESEARCH

Two genetic markers closely linked to adult polycystic kidney disease on chromosome 16

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Abstract

The genetic locus for autosomal dominant adult polycystic kidney disease was recently assigned to chromosome 16 by the finding of genetic linkage to the α globin gene cluster. Further study showed that the phosphoglycolate phosphatase locus is also closely linked to both the locus for adult polycystic kidney disease and the α globin gene cluster.

These findings have important implications for the prenatal and presymptomatic diagnosis of adult polycystic kidney disease and for a better understanding of its pathogenesis.

Introduction

Adult polycystic kidney disease is one of the commonest dominant diseases of man.¹ Although improvements in dialysis and transplantation have led to increased survival, morbidity from the disease continues to represent a considerable burden on families and the community. No specific treatment is known to retard the development of cysts or the consequent loss of renal function. In addition to renal failure, sufferers with the disease have an increased risk of

subarachnoid haemorrhage,² which often affects family members who are otherwise asymptomatic. Strategies for preventing subarachnoid haemorrhage have been analysed and found to be ineffective.²

For these reasons there is considerable demand for a reliable method of presymptomatic diagnosis of adult polycystic kidney disease.³ Currently the best method is ultrasonography,^{4,5} but this suffers from a major disadvantage in that its sensitivity depends on age; Bear *et al* estimated that 34% of at risk family members in the second decade carry the gene for adult polycystic kidney disease despite normal ultrasonographic results.⁶ Their criteria for diagnosis require the finding of at least one cyst in each kidney and at least two cysts in one kidney; relaxing the stringency of these criteria will increase the sensitivity of the test but will, concomitantly, reduce the specificity as patients with one or more "simple" cysts will be misdiagnosed. Thus ultrasonography is least reliable when diagnostic information for genetic counselling is most valuable—that is, in the second and third decades. A closely linked genetic marker would overcome the problem of the sensitivity of diagnosis being dependent on age and would also provide, for the first time, a method for prenatal detection of adult polycystic kidney disease. As the result of an attempt to identify a marker for this disease we recently demonstrated close linkage between it and the α globin cluster,¹ at a recombination frequency of approximately 5%. As α globin had previously been assigned to the short arm of chromosome 16 (*16p12-16pter*)⁸ this finding localised the gene for adult polycystic kidney disease to this chromosome.

As part of the initial search for the locus for adult polycystic kidney disease we investigated several enzyme and other protein polymorphisms. One of these, phosphoglycolate phosphatase, had previously been mapped to *16p12-pter*.⁹ Here we show that it is closely linked to both adult polycystic kidney disease and α globin.

Families and methods

We studied nine northern European families, four from the Oxford Renal Unit (NDM A, B, C, D) and five from various units in The Netherlands (NL 1, 2, 3, 4, 5). Affected members of the families show the commonest clinical type of adult polycystic kidney disease, developing end stage renal failure at a mean age of 48. Symptomatic patients were diagnosed by intravenous pyelography or ultrasonography. Asymptomatic relatives at risk over 15

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years old were assessed by ultrasonography after informed consent had been obtained. We used the diagnostic criteria of Bear *et al*⁶ as there are corresponding data concerning sensitivity.

Blood was taken from 183 members of the families and typed for 17 red cell enzyme and serum protein polymorphisms: phosphoglucomutase 1, acid phosphatase 1, transferrin, group specific component, glyoxalase, properdin factor B, glutamic pyruvate transaminase, adenylate kinase 1, esterase D, α_1 -antitrypsin, Gm, phosphoglycolate phosphatase, haptoglobin, peptidase D, C3, glucose phosphate isomerase, and adenosine deaminase. In addition families were typed for 10 blood group markers:

FIG 1—Autoradiograph of 3' hypervariable region hybridised to 5 μ g genomic DNA digested with *Pvu*II. Part of the pedigree in family NL 2 is drawn so that each subject appears above the corresponding gel track. The affected mother has two 3' hypervariable region alleles of 5.7 and 3.4 kb. Her unaffected spouse is homozygous for a 2.4 kb allele. The disease (indicated by solid symbols in the pedigree chart) segregates with the 3.4 kb allele.

ABO, MNSs, P₁, rhesus, Lutheran, Kell, Lewis, Duffy, Kidd, Colton, Auberger, and LKE; Dutch families were also typed for 6-phosphogluconate dehydrogenase, δ -aminolaevulinic acid dehydrogenase, and NADPH-diaphorase; and English families were typed for HLA. Phosphoglycolate phosphatase phenotypes were distinguished by starch gel (or Cellophane) electrophoresis.¹⁰ Methods for detecting a DNA polymorphism at a highly polymorphic site 3' to the α globin cluster were reported previously.⁷

Results

The 3' hypervariable region is a highly polymorphic DNA sequence situated 8 kilobases (kb) downstream (3') from the α globin gene cluster.¹¹ This region, which has been cloned and sequenced (R D Nicholls, A P Jarman, and D R Higgs, paper in preparation), consists of a core sequence that is tandemly repeated. The repeat number varies considerably between chromosomes, which accounts for the highly polymorphic nature of this

locus. To identify each allele total genomic DNA from each subject was digested with the restriction enzyme *Pvu*II, which has sites close to both ends of the 3' hypervariable region, so that the size of the *Pvu*II fragment detected by the 3' hypervariable region probe closely reflects the number of repeats in a particular allele. Figure 1 shows that the probe hybridised to two fragments in each subject.

Polymorphism at the phosphoglycolate phosphatase locus was detected by starch gel electrophoresis. There are three electrophoretically distinguishable phosphoglycolate phosphatase alleles (PGP*1,2,3) with frequencies of 0.83, 0.13, and 0.04 respectively,¹⁰ so that 29% of a European population is heterozygous at this locus. An affected parent who is heterozygous for phosphoglycolate phosphatase is "informative" in that the inheritance of each phosphoglycolate phosphatase allele can be followed in the progeny. Members of four families (NDM A, NL 1, NL 2, NL 4) were informative for phosphoglycolate phosphatase. Figure 2 shows the phosphoglycolate phosphatase genotypes of members of family NDM A. In this pedigree the PGP*2 allele cosegregates with the disease, and, as we showed previously, a 4.8 kb 3' hypervariable region fragment also segregates with the disease (except in subject IV:26, who is a recombinant between the 3' hypervariable region and adult polycystic kidney disease⁷).

FIG 2—Phosphoglycolate phosphatase genotypes in pedigree NDM A. Affected subjects are indicated by solid symbols; subjects at risk who do not fulfil the ultrasonographic criteria for diagnosis of adult polycystic kidney disease are indicated by open symbols. One of these, V:22, was aged under 20 at the time of study. Subjects not at risk (also indicated by open symbols) were assumed to have an adult polycystic kidney disease gene frequency of 0.001.

Where phase is known, linkage is detected by counting the number of recombinants in all the informative meioses: no recombinants were observed between phosphoglycolate phosphatase and adult polycystic kidney disease in 22 meioses, or between phosphoglycolate phosphatase and the α globin (3' hypervariable region) locus in 27 meioses. As a negative ultrasonographic diagnosis is unreliable in subjects under the age of 20 meioses were scored again after exclusion of "normal" family members under this age: no recombinants were seen between phosphoglycolate phosphatase and adult polycystic kidney disease in 21 meioses. As reported previously,⁷ there were seven probable recombinants between adult polycystic kidney disease and the 3' hypervariable region in 127 meioses. None of these recombinants, however, was informative for phosphoglycolate phosphatase, so it was not possible to determine whether these subjects showed recombination between phosphoglycolate phosphatase and adult polycystic kidney disease, or to deduce a gene order for the three loci.

To permit analysis of extended but fragmented pedigrees of the type shown in figure 2 we used the computer program Liped (version 3)¹² to calculate lod scores. The lod score, $z(\theta)$, is computed as

$$\log_{10} \frac{\text{likelihood of linkage at a specified recombination frequency } (\theta)}{\text{likelihood that the loci are unlinked}}$$

It is thus a measure of the probability that linkage is present and reaches a maximum at the most likely recombination frequency (θ). The table gives lod scores summated for all families. The maximum lod score was 8.21 at $\theta=0$ for linkage between adult polycystic kidney disease and phosphoglycolate phosphatase; 11.61 at $\theta=0$ for phosphoglycolate phosphatase *v* the 3' hypervariable region; and 25.85 at $\theta=0.05$ for adult polycystic kidney disease *v* the 3' hypervariable region.⁷ This indicates that the odds in favour of linkage between phosphoglycolate phosphatase and adult polycystic kidney disease are approximately $10^{8.21}:1$, and that the best estimate of the recombination fraction between these loci is zero (99% confidence limits place the phosphoglycolate phosphatase locus within 12 centiMorgans (cM) of the locus for adult polycystic kidney disease and within 7 cM of the α globin cluster).

Lod scores for linkage between adult polycystic kidney disease (APCKD), the 3' hypervariable region (3' HVR), and phosphoglycolate phosphatase (PGP)

	Recombination fraction (θ)*						
	0.00	0.05	0.10	0.15	0.20	0.30	0.40
APCKD <i>v</i> 3' HVR	— ∞	25.85	24.59	22.25	19.48	12.81	5.48
APCKD <i>v</i> PGP	8.21	7.82	7.11	6.29	5.38	3.44	1.50
PGP <i>v</i> 3' HVR	11.61	10.58	9.44	8.23	6.97	4.36	1.84

* $\theta=0_{M-F}$. Lod scores for each sex supplied on request.
A correction for age at onset was used to allow for the age dependent sensitivity of ultrasonography.⁷

Discussion

About one in 1000 people carries the mutant gene responsible for dominantly inherited polycystic kidney disease,¹³ which accounts for about 10% of all requirements for dialysis and transplantation.¹⁴ Despite the importance of this disease nothing is known about its pathogenesis and, hitherto, nothing was known about the chromosomal localisation of its genetic determinants. Recombinant DNA technology offers a new approach to the pathogenesis of inherited diseases, such as adult polycystic kidney disease, in which the underlying biochemical defect is unknown. In this approach, termed "reverse genetics,"²¹ the mutation producing the disease is defined and then the effect of this mutation on the expression of the gene is elucidated. This process begins with the chromosomal localisation of the disease gene by linkage analysis, followed by the assignment of other polymorphic loci to the chromosomal region of interest and the ordering of these loci to create a genetic map of the region. We have taken the first step in this process by establishing a linkage group of three loci for adult polycystic kidney disease on the short arm of chromosome 16.

Further studies are under way to determine whether adult polycystic kidney disease is genetically homogeneous. If this can be established then the linkage markers described here will have an immediate application in the presymptomatic and prenatal diagnosis of the disease. The usefulness of a marker in this context depends on the heterozygote frequency, or "informativeness," of the marker locus. The 3' hypervariable region polymorphism is of particular value in this respect because of the high heterozygosity of this locus: only five homozygotes have been found in 61 unrelated northern Europeans. Moreover, homozygotes at the 3' hypervariable region locus may sometimes be informative if haplotypes are obtained by combining genotypes at several of the polymorphic restriction sites within the α globin cluster. The phosphoglycolate phosphatase polymorphism, on the other hand, has a limited diagnostic usefulness because of low heterozygosity and because its gene has not been cloned. This locus, however, will facilitate both the search for "flanking" markers for the locus for adult polycystic kidney disease and the ordering of further markers as they become available. Occasionally phosphoglycolate phosphatase may also be helpful diagnostically when the 3' hypervariable region and α globin loci are both uninformative: in pedigree NDM A, for example, subject IV:1 was homozygous for the 3' hypervariable region and four restriction sites within the α globin cluster but informative for phosphoglycolate phosphatase. In all nine families only one affected parent was not informative for either locus.

The reliability of diagnosis by linkage analysis depends on the phase information available and the recombination frequency between marker and disease. Highly polymorphic markers, such as the 3' hypervariable region, provide considerable phase information as each allele is relatively rare so that its inheritance may be followed through the pedigree. When phase is known (or can be inferred with a high degree of confidence) the reliability of the 3' hypervariable region marker is roughly 95%, and diagnosis will be possible in about 92% of cases. Phosphoglycolate phosphatase typing may be contributory in a few additional cases. Whether it is appropriate to try to detect adult polycystic kidney disease using a test with this degree of reliability must be decided for each particular case. We are currently searching for further markers as the availability of a flanking marker will greatly increase the reliability of this method of detection.^{15 16}

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100 YEARS AGO

Dr. Bedoin, in a paper communicated to the Belgian Royal Academy of Medicine on antiseptic dressings suitable for military purposes, said that the requisites to be kept in mind were that any dressing to be used on the field of battle must be simple, occupying but small space, inexpensive, and capable of being used for all surgical necessities. Dr. Bedoin believes that he has found a substance which combines in itself all these requisite qualities—a substance, too, which is well known, easily procurable, and in constant use—being, in fact, no other than blotting- or filtering-paper. Before being used for surgical purposes, it should be disinfected by a lengthened exposure to a heat of 120° Cent., and by immersion in an antiseptic solution, and afterwards dried. Wounds are dressed by the application of seven or eight layers of this paper, the whole being covered with gutta-percha tissue, and a bandage applied. The dressing weighs only about forty grammes, so that each soldier can carry one. In the ambulance, this dressing can be applied by the surgeon with any others that it is thought well to employ. (*British Medical Journal* 1886;ii:33.)