Genetic linkage study of family members of a patient with adult polycystic kidney disease

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Objective. To study the feasibility of making an early diagnosis of adult polycystic kidney disease by using genetic linkage analysis in Hong Kong.
Design. Genetic linkage study.
Setting. University teaching hospital, Hong Kong.
Participants. Six members of a Chinese family with a history of adult polycystic kidney disease.
Main outcome measures. The inheritance pattern of adult polycystic kidney disease, as detected by polyacrylamide gel electrophoresis of polymerase chain reaction products using radioactively labelled primers specific to six microsatellite DNA markers that are closely linked to the *PKD1* gene on chromosome 16.
Results. Four of the six members of the family studied were shown to be positive for disease-linked markers, and the inheritance of adult polycystic kidney disease could be traced in this family with a higher degree of precision (93.7%) using genetic linkage analysis, than could be predicted otherwise.
Conclusion. The success of genetic linkage analysis in providing an early diagnosis of adult polycystic kidney disease is dependent on having a sufficient number of family members whose disease status has been established by imaging methods to allow the disease-linked marker haplotype to be determined. The establishment of a genetic data bank for families with adult polycystic kidney disease should be considered to maximise the effectiveness of this diagnostic approach.

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Key words: Chromosomes, human, pair 16; Genotype; Kidney, polycystic/genetics; Linkage (genetics); Prognosis

Introduction

Adult polycystic kidney disease (APKD) is characterised by bilateral multiple renal cysts and autosomal dominant inheritance.[1] The gene frequency in the general population has been estimated to be approximately 1 in 1000 in western countries[1] and is likely to be similar in Hong Kong.[2] The disease usually manifests in the fourth decade of life, when there is renal enlargement, loin pain, hypertension, haematuria, or chronic renal failure. Liver cysts are present in approximately 50% of patients.[1,2] Intracranial haemorrhage due to rupture of a berry aneurysm occurs in approximately 15% of patients.[2] Ultrasonography of the kidneys is the imaging technique of choice and allows an early diagnosis of APKD to be made for the first-degree relatives of an affected individual.[3] The early diagnosis of APKD and the adequate treatment of its treatable complications may help to improve the outcome of these patients.[4]

Recent studies have shown that APKD is genetically heterogeneous.[5-7] There are at least three genes involved in this disease. Mutations of the polycystic kidney disease (*PKD1*) gene on chromosome 16p13.3 account for approximately 85% to 95% of cases of APKD.[3,5] The *PKD1* gene has recently been sequenced, and the structure of its encoded protein—a complex molecule called polycystin—has been determined.[8,9] The second gene, *PKD2*, is located in the chromosome region 4q13-q23[10,11] and is defective in most of the non-*PKD1* cases of APKD.[5,6] A small number of families are affected by one or more genes of undetermined loci and which do not map to either *PKD1* or *PKD2*.[7]

With the elucidation of the genetic basis of APKD, it is possible to prevent the disease through

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early presymptomatic diagnosis, genetic counselling, antenatal diagnosis, and family planning. We are the first group in Hong Kong to develop a technique that allows a diagnosis of APKD to be made early and prenatally. The method and its application are reported in this paper. Problems that are associated specifically with this diagnostic approach are discussed.

Methods

Study subjects

The index patient with APKD in this study was a 52-year-old woman who was receiving treatment at the Queen Mary Hospital. Her spouse and four children were also recruited after informed consent had been obtained. The ages of the six family members ranged from 18 to 60 years. All six subjects underwent an ultrasonographic examination to detect renal cysts.

Blood collection and DNA extraction

From each subject, 15 to 20 mL of peripheral blood was collected and transferred to bottles that contained EDTA as an anticoagulant. The blood was mixed with Ficoll (Nycomed Pharma AS, Oslo, Norway) and mononuclear blood cells were isolated by centrifugation. The cells were digested with proteinase K at 37°C overnight. The DNA was then extracted using phenol, chloroform, and isoamyl alcohol. The concentration and purity of the extracted DNA were determined by measuring the absorbance (A) of ultraviolet light of wavelengths of 260 nm (A_{260}) and 280 nm (A_{280}). The DNA concentration (in ng/μL) was estimated by using the formula $A_{260}/20$, and the value of A_{260}:A_{280} ratio was kept between 1.8 and 2.0 to exclude phenol or protein contamination.

Microsatellite marker analysis

The haplotypes of six microsatellite markers within and around the *PKD1* gene were determined for each subject. The markers used were SM7,[12] CW2,[13] AC2.5,[14] SM6,[13] KG8,[15] and D16S521.[6] They have polymorphism information content (PIC) values that range from 0.24 to 0.76 in the local Chinese population (Wong HL, unpublished data, 1998). The locations of the markers relative to *PKD1* are shown in Fig 1.

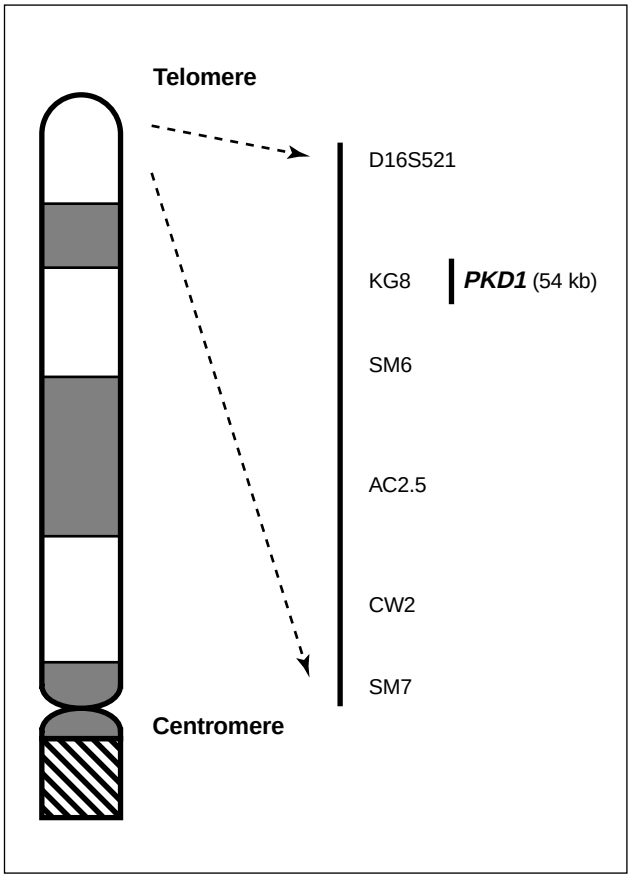

Fig 1. The locations of the markers relative to the *PKD1* gene on the short arm of chromosome 16

The microsatellite markers were amplified by the polymerase chain reaction (PCR) using specific primers. The DNA sequences of the primers are shown

Table 1. Primers used in the polymerase chain reaction amplification of six microsatellite markers (arranged according to their proximity to the centromere)

Study	Marker	Locus	Repeat	PCR primer
Harris et al[12]	SM7	D16S283	$(AC)_n$	5'-ACATATGTAGTCTTCTGCAGG 5'-ACAAAGAGTGAATCTCTGACAG
Peral et al[13]	CW2	D16S663	$(AC)_n$	5'-GTCTTTCTAGGAATGAAATCAT 5'-ATTGCAGCAAGACTCCATCT
Thompson et al[14]	AC2.5	D16S291	$(AC)_n$	5'-AGTGCTGGGATTACAGGCATGAACC 5'-GCAGCCTCAGTTGTGTTTCCTAATC
Peral et al[13]	SM6	D16S665	$(TG)_nC(CG)_nTGC(GT)_n$	5'-AGCTGGGGTCTCAGGGGAGCT 5'-GCGCACACAGCACTAACACG
Snarey et al[15]	KG8	-	$(AC)_n$	5'-CACAGCCAGCTCCGAGGGCCT 5'-GACTCCTCCTGGGGGCTGGCTC
Coto et al[6]	-	D16S521	$(AC)_n$	5'-CAGCAGCCTCAGGGTT 5'-GAGCGAGACTCCGTCTAAA

Table 2. Polymerase chain reaction conditions used to amplify microsatellite markers

Marker	Annealing temperature (°C)	[Mg^{2+}] (mmol/L)	Template (ng)	Taq Polymerase (U)	Number of cycles
SM7	56	1.5	80	0.5	25
CW2	61	2.5	20	1.0	25
AC2.5	68	1.5	20	1.0	35
SM6	65	1.5	20	0.5	35
KG8	70	1.5	40	0.5	25
D16S521	63	1.5	40	0.5	35

in Table 1. The sense strands of the primers were end-labelled with [γ-^{32}P]-dATP (Amersham LS Ltd., Buckinghamshire, United Kingdom) to a specific activity of 50 μCi/μg, so that the amplified markers could be detected by autoradiography. In each reaction, 0.4 nmol of labelled sense primer and 0.4 nmol of unlabelled antisense primer were mixed in a final reaction volume of 20 μL. The PCR commenced with 3 minutes of complete denaturation, followed by repeated cycles of 1 minute of denaturation, 30 seconds of annealing, and 1 minute of extension. The exact PCR conditions are shown in Table 2.

Polyacrylamide gel electrophoresis was used to resolve the markers according to their molecular size. The PCR products were mixed with 20 μL of formamide gel-loading buffer (10 mmol/L EDTA, 0.25% xylene cyanol, and 0.25% bromophenol blue).

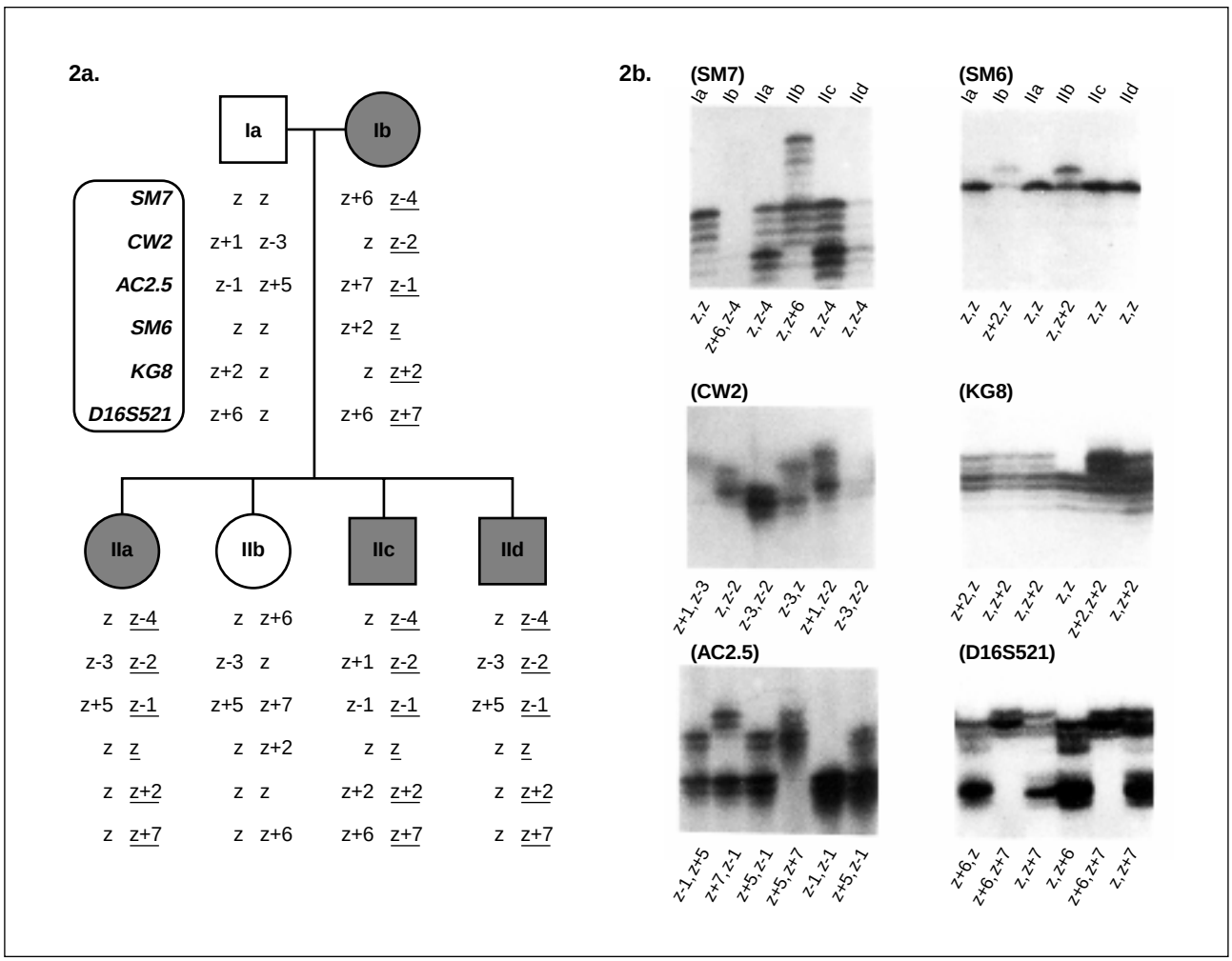

Fig 2. Pedigree and genetic linkage analysis of a family with a history of adult polycystic kidney disease
(2a) pedigree of the affected family and haplotypes of the markers SM7, CW2, AC2.5, SM6, KG8, and D16S521 that were identified (Z denotes the most common allele of the marker determined in a survey of the local population); (2b) auto-radiograms showing the results from the electrophoretic separation of PCR products amplified using primers specific to the six markers

The mixture was heated to 95°C for 5 minutes to denature the PCR product, 5 μL of which were then loaded onto a 6% denaturing polyacrylamide gel (19:1 ratio of acrylamide to bisacrylamide). The gel was electrophoresed in Tris-borate-EDTA buffer at 35 W until the xylene cyanol dye front reached approximately 5 cm from the bottom of the gel. The gel was vacuum-dried and exposed to X-ray film (Fuji Co., Tokyo, Japan) overnight. The film was then developed and kept as a permanent record.

Results

The pedigree of the family and the haplotypes of the panel of markers that were identified are shown in Figure 2A. There were four affected family members: subjects Ib, IIa, IIc, and IId. Large renal and liver cysts constituted a prominent feature in this family. Adult polycystic kidney disease had been diagnosed in subject Ib in her 40s. Three of her children aged in their 20s were shown to have APKD by ultrasonography.

Figure 2B shows the autoradiogram of the genotypes of this family. Subjects Ib, IIa, IIc, and IId possessed the haplotype of Z-4, Z-2, Z-1, Z, Z+2, and Z+7 for the markers SM7, CW2, AC2.5, SM6, KG8, and D16S521, respectively. Results of this genetic linkage analysis (Fig 2A) showed that it was unlikely (P=0.063) that APKD was not transmitted with the marker haplotype that was common to these four affected individuals (Ib, IIa, IIc, and IId) but was absent from the unaffected members of this family.

Discussion

Although there is no effective treatment that can decrease the rate of cyst progression and renal function deterioration in APKD, the major complications of APKD are treatable.[16] The presymptomatic diagnosis of APKD may help in the provision of early treatment or prevention of complications that may delay the onset of end-stage renal failure.[4] Appropriate genetic counselling can help families deal with psychological anxiety and proceed with family planning. The affected family should also be informed about the availability of antenatal diagnosis, even though some affected parents may not consider terminating a pregnancy.

The presymptomatic diagnosis of APKD is mostly achieved by performing ultrasonography.[3] A study showed that 22% of carriers of mutated *PKD1* who were younger than 30 years did not show any renal cysts.[17] The age-dependent manifestation of APKD thus renders this traditional diagnostic method less effective in young people. Genetic diagnostic methods that are independent of the development of phenotypes can overcome the limitations of ultrasonography.

In some genetic diseases, such as the thalassaemias and haemoglobinopathies, it is now easy to establish the diagnosis antenatally by directly identifying the genetic defects. The *PKD1* gene, on the other hand, is a large gene that has a 14-kb transcript and three regions at the 5' end that are highly homologous with the α-globin genes.[8,9] A large proportion of mutations that are associated with APDK fall within these homologous regions and thus cannot be readily identified. The realisation that polymorphic microsatellite markers are closely linked to some inherited diseases has meant that the PCR amplification of these markers can be used in the presymptomatic or even antenatal establishment of the diagnosis.[18] The method requires that several members of an affected family be studied. In the present study, we used six markers that were located within and on both sides of the *PKD1* locus on chromosome 16. The choice of these markers was based on their demonstrated polymorphism in the local population and the proximity of the markers to the *PKD1* locus. The marker KG8, in particular, had a low PIC value and was a marker of limited usefulness. The coverage of the markers made this method highly specific, because the chance of a double crossover occurring would be negligible owing to the proximity of the markers. Hence, once the marker haplotype that is associated with APKD has been determined in an affected family, it can be used to infer inheritance of the disease in individuals within the family.

When studying other families affected by APKD, we encountered some limitations of genetic linkage analysis. The greatest limitation was often the size of the family. Having more siblings within a family generally gives more information about the haplotype. Increased emigration rates and a tendency to have smaller families could make the establishment of the haplotype of the markers more difficult in some families. Adding to this problem is the genetic heterogeneity of APKD. As a result of insufficient data, we were unable to determine whether a defect of *PKD1* was responsible for APKD in some of the families studied. In the family presented in this study, the probability of a *PKD1* gene defect being responsible was determined to be 0.9375. A much higher probability value may be achieved if involvement of the *PKD2* gene can be excluded by performing further genetic linkage analysis using markers that flank *PKD2*.

It is possible that better techniques that could directly identify genetic defects may be developed in the future. In the mean time, genetic linkage analysis is a valuable method for the early diagnosis of APKD. The chance of its successful application can be increased by the creation of a genetic data bank for APKD families in Hong Kong. The genetic material collected from several generations of an affected family could then provide the necessary information to allow a diagnosis to be made by genetic linkage studies.

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