Analysis of a large family with the second type of autosomal dominant polycystic kidney disease

Barbera Veldhuisen1, Martijn H. Breuning1, Eveline Wesby-van Swaay2, Jaap Boersma1 and Dorien J. M. Peters1

1Department of Human Genetics, Leiden University, Leiden and 2Department of Clinical Genetics, Dijkzigt Hospital, Erasmus University, Rotterdam, The Netherlands

Abstract. Autosomal dominant polycystic kidney disease (ADPKD) is a genetically heterogeneous disorder. A mutation in at least three different genes can cause the disease. A mutation in the first gene, the PKD1 gene, which has been identified on chromosome 16p13.3, accounts for ADPKD in ~86% of the families with this disorder. In the majority of the other ADPKD families the disease is caused by a mutation in a second gene, the PKD2 gene. This gene has been mapped to chromosome 4q21-22, but has not yet been identified. In a few families ADPKD is not caused by a mutation in either the PKD1 or the PKD2 gene. The locus for a possible third gene has not yet been determined. Now that haplotype analysis with polymorphic markers at the ADPKD 1 and ADPKD2 loci is possible, we can easily distinguish between both forms of ADPKD. We describe a large Dutch family in which ADPKD is linked to chromosome 4. Compared with ADPKD1 families, the disease in this family tends to run a milder course, as has been described previously for other ADPKD2 families.

Key words: ADPKD; linkage analysis; PKD2; Polycystic kidney disease

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is one of the most frequently occurring genetic diseases. The incidence in the Caucasian population has been estimated to be 1 per 1000 individuals [1]. ADPKD is a multi-system disorder, mainly characterized by the formation and enlargement of fluid-filled cysts in the kidneys, which can finally result in complete renal failure. Cysts can also be found in the liver, the pancreas and other organs [2,3]. Additional manifestations of the disease are the occurrence of cerebral aneurysms and hypertension [4,5].

The disease is genetically heterogeneous and can be caused by a mutation in at least three different genes. In the majority of ADPKD families (~86%), the gene responsible is located on chromosome 16p13.3 [6]. This gene, the PKD1 gene, has been identified but the function of the gene product is still unknown [7-10]. The gene encodes a 14 kb transcript, of which ~75% shares a very strong homology to a locus more proximal on the short arm of chromosome 16. To date only a few mutations have been found [7,11-13].

A second gene has been assigned to chromosome 4q21-22, using linkage analysis of families in whom ADPKD was not linked to chromosome 16 [14,15]. Initially, the PKD2 gene was localized to an interval of ~7 cM between the markers D4S231 and D4S423. This region has been cloned in overlapping yeast artificial chromosomes (YACs). Polymorphic markers published in the literature and in databases or newly isolated markers were mapped in the region and ordered with respect to each other by polymerase chain reaction (PCR) analysis on these YACs. The polymorphic markers were used to construct chromosome 4 haplotypes in families with ADPKD in whom linkage of the disease to chromosome 16 was excluded. Recombination events in these families were studied to reduce the ADPKD2 interval. In the case of a recombination event, the affected individual inherits part of the normal and part of the mutated chromosome from its affected parent. The inherited normal region can then be excluded as the possible locus for the PKD2 gene. Recently, several families have been described with recombination events, resulting in a confinement of the ADPKD2-region to an interval of 3 cM between the markers D4S1534 and D4S423. This region has been cloned in overlapping yeast artificial chromosomes (YACs). Polymorphic markers published in the literature and in databases or newly isolated markers were mapped in the region and ordered with respect to each other by polymerase chain reaction (PCR) analysis on these YACs. The polymorphic markers were used to construct chromosome 4 haplotypes in families with ADPKD in whom linkage of the disease to chromosome 16 was excluded. Recombination events in these families were studied to reduce the ADPKD2 interval. In the case of a recombination event, the affected individual inherits part of the normal and part of the mutated chromosome from its affected parent. The inherited normal region can then be excluded as the possible locus for the PKD2 gene. Recently, several families have been described with recombination events, resulting in a confinement of the ADPKD2-region to an interval of 3 cM between the markers D4S1534 and D4S423. To identify the PKD2 gene, further confinement of the region is necessary in order to reduce the number of candidate genes that needs to be
screened for disease-causing mutations in ADPKD2 patients.

Since only a few families have been identified whom are not linked to either chromosome 4 or 16 [16,19,20], the majority of non-PKD1 families seem to be families with ADPKD2 [unpublished observations]. A putative third gene has not yet been localized.

In order to find more recombinants near the PKD2 gene, we tested a large number of new families with polymorphic markers from the PKD1 and PKD2 regions. In addition, families with chromosome 4-linked ADPKD were expanded as much as possible.

A large family in which linkage to the PKD1 gene was excluded has been analysed with polymorphic markers at the ADPKD2 locus. In this family the disease tends to run a milder course than in families with PKD1. This is in agreement with previous reports describing a diagnosis at an older age, fewer cysts at time of diagnosis, less hypertension and a slower progression towards end-stage renal failure in non-PKD1 patients [21–24]. In this family three cases of ADPKD2 patients with intracranial aneurysms have been described [25], which may be an indication that clustering of intracranial aneurysms also occurs in families with ADPKD2.

Subjects and methods

Family material

Blood samples were collected of 89 members of a large Dutch family with ADPKD. Twenty-six individuals have been diagnosed with ADPKD. 19 members of the family have a 50% chance and 34 members have a 25% chance of carrying the ADPKD-causing mutation. The remaining 10 persons are spouses. Most of the asymptomatic at-risk individuals were examined by ultrasound, using the diagnostic criteria described by Bear et al. [26]. Forty-one at-risk individuals showed no cysts on ultrasound made over the age of 25 years. DNA was isolated from 10 ml of peripheral blood using standard procedures.

Polymorphic markers

Markers flanking the PKD1 and PKD2 loci were selected to establish linkage of the family to either chromosome 16 or 4.

The polymorphic markers used to construct chromosome 16 haplotypes were D16S85 (3' HVR) [27], D16S83 (pEKMDA) [28], D16S84 (pGGG1) [29], D16S125 (26-6prox) [30,31], D16S246 (218EP6) [32], D16S63 (CRI-327) [33] and D16S45 (CRI-090) [33] (Figure 1). The microsatellite markers used to show linkage to chromosome 4 were D4S231 [34], D4S1534 [35], D4S1542 and D4S423, 53°C (JV106), 55°C (JSTG3, AICA1) or 60°C (D4S1563), and extension for 1 min at 72°C. The PCR was completed with a final extension of 9 min at 72°C. The samples were loaded on a 6% denaturing polyacrylamide gel. After electrophoresis, the different alleles were visualized on a Kodak XAR5 film.

Results

Construction of chromosome 16 haplotypes

Haplotypes of a part of the family were constructed using polymorphic chromosome 16 markers (Figure 1). The order of the markers is shown in Figure 2.

For part of the family analysed with flanking markers of both the PKD1 as PKD2 loci, multipoint lod scores were calculated [15].
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lod score of -5.11 (D16S85 and D16S125), suggested that in this family ADPKD was not caused by a mutation in the PKD1 gene [15]. Therefore, this family was expanded and analysed with chromosome 4 markers [15].

Construction of chromosome 4 haplotypes

Chromosome 4 haplotypes of the complete family were constructed using the polymorphic markers shown in Figure 2. The results for a large part of the family are shown in Figure 4. All affected family members share the same haplotypes for the markers D4S1534, D4S1542, JV106, JSTG3, AICA1 and D4S1563 (the alleles: 1–3–1–5–4–5). In three affected individuals a recombination event was observed. Two recombinants place the disease at the centromeric side of marker D4S1544. The affected parent (III-3) of the persons IV-6, IV-8 and IV-10 must have had a recombination between the markers D4S423 and JSTG3 (AICA1 and D4S1563 are not informative). Further analysis with more markers in the region maps the recombination between D4S1544 and TMCA1. Both markers are located between D4S423 and D4S1563 (data not shown), thus placing the PKD2 gene proximally to D4S1544. Similarly, the affected parent (III-27) of individuals IV-42, IV-43, IV-44, IV-45 and IV-46 must have carried a recombination between D4S1544 and AICA1, placing the disease proximally from D4S1544. A different recombination event was observed in the affected individual V-4, where a crossing-over between D4S231 and D4S1534 places the PKD2 gene telomeric from D4S231.

Phenotypical analysis

In this family 25 individuals are alive with the diagnosis ADPKD. At present only one person receives renal replacement therapy, which was initiated at the age of 58 years. One affected person of 82, one of 75, three of 60–70 and eight of 50–60 years have functioning kidneys without symptoms related to renal failure.

Eleven family members have died, leaving offspring with ADPKD and the chromosome 4 haplotype known to harbour the mutation. Three persons died at 80–90, one at 77, two at 60–70 and two at 40–50 years, and three persons died at an unknown age. Unfortunately, medical records were not available for most of these.
Fig. 4. Segregation of chromosome 4 markers in part of a large ADPKD2 family. For confidentiality male and female symbols are not distinguished. • Affected family member with ADPKD; ○ family member without ADPKD symptoms. Deceased persons are indicated by a slash through the symbol. The alleles of the different haplotypes are:

family members. Several female members of this family have had symptomatic polycystic liver disease. However, this was not studied in detail.

Two family members (34 and 50 years of age) have had a subarachnoid bleeding due to intracranial aneurysms and there was strong evidence of subarachnoid bleeding [25] in a third family member who died at 49 years of age.

Discussion

Using a dense set of polymorphic markers in the PKD2 region, we have been able to analyse comprehensively the inheritance of the mutation-carrying haplotype in a very large Dutch family with ADPKD.

This type of analysis can be performed for several purposes. Firstly, the use of highly polymorphic markers flanking the mutation makes prenatal and presymptomatic diagnostics possible for almost all meioses in the family. Secondly, haplotype analysis may reveal recombination events crucial for the refinement of the disease interval. In this family three recombinants were identified which do not narrow down the PKD2 interval as reported previously [16-18]. More recently the region was further refined [B. Veldhuisen et al., unpublished data]. Thirdly, a detailed haplotype construction in several families may reveal a common haplotype, suggesting a common founder for these families. In total we analysed five Dutch ADPKD2 families in whom at least three different haplotypes were recognized, indicating the possibility that several mutations will be found in the Dutch ADPKD2 population.

Many members of the family described in this paper have lived to old age without renal failure. This is in agreement with previous reports on a milder progression of the disease in affected members of non-PKD1 families [21–24]. However, slow progression has also been reported for some PKD1 families [36]. We chose another approach to assess the impact of ADPKD on health. Florijn et al. [37] were able to show excess mortality of PKD1 patients compared with the general population. They analysed five large PKD1 families, with 348 individuals contributing to the study. Since excess mortality in ADPKD2 families might be substantially lower, a very large set of families and individuals needs to be examined to answer the question of whether the mutation causing ADPKD2 affects lifespan.

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