Original articles

Map of 16 polymorphic loci on the short arm of chromosome 16 close to the polycystic kidney disease gene (PKD1)


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Abstract

To define the PKD1 locus further, the gene involved in the most frequent form of adult polycystic kidney disease, probes from 16 polymorphic loci were mapped on 16p13.1–pter with the combined use of cell lines containing rearranged chromosomes and family studies. Five breakpoints in the distal part of 16p arbitrarily subdivided the loci into five groups. By analysing 58 recombination events among 259 informative meioses in 12 large families with PKD, we were able to construct a linkage map for the distal part of 16p. The order of the markers obtained with chromosomal rearrangements was confirmed by the family studies. The D16S85 locus near α globin, D16S21, and D16S3 map distal, or telomeric, to PKD1. The polymorphic red cell enzyme phosphoglycolate phosphatase (PGP), D16S84, D16S259, and D16S246 showed no recombination with PKD1. The remaining nine RFLPs all map proximal to the PKD1 gene. By cosmid walking, additional RFLPs were detected at the D16S21 locus. A single intrahaploype recombinaton observed defines the orientation of D16S21 relative to PKD1.

The new polymorphisms are valuable for presymptomatic and prenatal diagnosis of PKD1. Furthermore, our map is both a good starting point for the physical map of 16p and a useful tool for the isolation of the PKD1 gene.

The adult form of polycystic kidney disease (PKD1), one of the most frequent inherited diseases of man,1 is caused by a gene defect located on the short arm of chromosome 16. Close linkage has been found between PKD1 and 3'HVR, a highly polymorphic DNA marker located 8 kilobases downstream from the 3' end of the haemoglobin α gene cluster.2 3 Both 3'HVR and PKD1 are also closely linked to the polymorphic red cell enzyme phosphoglycolate phosphatase (PGP), which also maps to 16p.4 Before any linkage between PKD and genetic markers on chromosome 16 could be applied to early diagnosis of
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<th>Insert ends</th>
<th>Insert size (kb)</th>
<th>RFLP enzyme</th>
<th>Fragment (kb)</th>
<th>Frequency</th>
<th>PIC a</th>
<th>PIC b</th>
<th>PIC c</th>
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<td>pBR322</td>
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<td>EcoRI</td>
<td>1.7</td>
<td>MspI</td>
<td>V1:1.6</td>
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<td>pKUN1</td>
<td>PstI</td>
<td>0.5</td>
<td>PvuII</td>
<td>Hae III:2.8</td>
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<td>C2:5.8</td>
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<td>+</td>
<td>ND</td>
<td>+</td>
</tr>
</tbody>
</table>

HGM=human gene mapping library, PIC=polyorphism information content, ND=not determined.

*(1) Somatic cell hybrids containing parts of chromosome 16.
*(2) More restriction enzymes are known to show polymorphism (see also ref 33).
*(3) Also polymorphic with EcoRI (Reeders and Gillespie, unpublished data).
*(4) From CRI-327 a 0.7 kb fragment obtained by EcoRI + HindIII digestion was used.
*(5) Detected in a family with cystic kidneys.
*(6) CY13 positive for 16p13.1–qter.
*(7) CY19 positive for 16p13.1–qter.
*(8) GM2324 c16:22Xp13.3.q12.3.
*(9) N-OH1 positive for 16p13.3–qter.

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this disease, the question had to be resolved whether mutations at one or more loci were responsible for the disease. Initially, no evidence for genetic heterogeneity in PKD was found among 27 families from four countries. The peak lod score obtained in these families was 59.72 at a recombination frequency of 5%. Recently, however, several groups have reported families of Italian origin in which PKD did not segregate with markers from chromosome 16, proving that genetic heterogeneity in PKD does indeed exist.

The close genetic linkage between 3' HVR and PKD1 has been used for presymptomatic and prenatal diagnosis of the disease. However, recombination between 3' HVR and the gene for PKD may lead to erroneous diagnosis and false predictions. Such recombination events can be detected with additional restriction fragment length polymorphisms (RFLPs) located on the opposite side of the PKD gene. Several probes which detect RFLPs on the short arm of chromosome 16 have been isolated. One of these, D16S80, was clearly found to be flanking the gene, with a recombination frequency of approximately 5%. As this polymorphism is informative in only 31% of random subjects, more polymorphic markers are needed to make diagnosis routine and informative. The purpose of this study was to combine the probes isolated by a number of different groups and to study them in large families with polycystic kidney disease. The resulting linkage map of the short arm of chromosome 16 will later be used to design a standard protocol for diagnosis of PKD with linked DNA markers.

**Materials and methods**

**PROBES FROM LEIDEN, THE NETHERLANDS**

Probes for chromosome 16 were isolated using a protocol described by Hofker et al. Briefly, DNA from a hybrid cell line (CY18) containing a single human chromosome 16 was partially digested with MboI and cloned in pc0s2EMBL. Colonies hybridising to DNA from human patients were selected. After rescreening with mouse and human DNA, 219 cosmids were pooled, digested with PstI, and subcloned in pKUN. Probes negative on the cell line CY13, which contains a chromosome 16 deleted for the 16p13.1-pter region, were selected for further study (table 1). The four subclones which detected RFLPs were used in family studies. Subsequently, the 219 cosmids from the pool were mapped individually relative to the chromosome 16 breakpoint in the hybrid cell line CY19, and by in situ hybridisation, relative to the breakpoint of the GM2324 cell line. A total of 166 cosmids contained human DNA. The cosmids distal to the breakpoint of GM2324 were subcloned in a plasmid vector. Two subclones, 26-6 and 218EP6, detected polymorphisms (fig 1, table 1).

**PROBES FROM SALT LAKE CITY/YALE, USA**

Two probes detecting RFLPs on 16p were studied, pEKMDA-2 and CMM65. By cosmid walking, the probe pGGGI was isolated 10 kb distal to CMM65 (fig 1, table 1).

**PROBE FROM ADELAIDE, AUSTRALIA**

A large number of clones for chromosome 16 has been isolated. One of these, VK5, detected an RFLP with MspI (fig 1h).

**PROBES FROM BEDFORD, USA/OXFORD, UK**

The isolation of probes on chromosome 16 has been described previously. We used CRI-090, CRI-133, and CRI-327 (fig 1, table 1).

**PROBE FROM FREIBURG, WEST GERMANY**

Fr3-42 (D16S21) is a 1.8 kb EcoRI/HindIII insert cloned in pSP65 showing several RFLPs (table 1, fig 1). By screening a cosmid library we obtained five cosmids overlapping the region detected by Fr3-42. One subclone from this cosmid walk, 2BP5, detects two RFLPs, one with PstI S6, S7 (fig 1b), and one with XmnI S8, S9 (not shown). Cosmid 2B itself was also used as a probe, competing out the signal from repetitive sequences with an excess of unlabelled sonicated human DNA. In this way a highly polymorphic region could be detected in DNA digested with enzymes RsaI, PvuII, TaqI, and SacI (fig 1c).

We subsequently isolated a single copy subclone HMJ1 specific for this polymorphic region (table 1).

**FAMILIES**

DNA was isolated from peripheral blood. Lysis of erythrocytes was accomplished by resuspension of the cells in three volumes of an ice cold isotonic NH4Cl solution. The cells were left for 20 minutes on ice with gentle shaking every few minutes, after which the intact white cells were spun down. Excess red cell ghosts were washed away with the same buffer. If necessary the incubation with NH4Cl was repeated. The white cell pellet was resuspended in sodium EDTA buffer with pronase and SDS. DNA was isolated using standard methods.

Ultrasoundography of kidney and liver was performed on asymptomatic at risk family members as described previously. To correct for false negative diagnosis we used the criteria formulated by Bear et al. The 'unaffected' family members were divided into three age groups: below 20 years, diagnosis unknown; 20 to 30 years, 85% chance of detecting the disease; older than 30 years, 95% chance of detecting PKD.
Figure 1  RFLPs in the distal part of the short arm of chromosome 16, 16p13.1–pter. RFLPs detected by 3’ HVR and 5’ HVR are not shown. The order is from telomere to centromere (see fig 2). For designation of the alleles see also table 1.
LINKAGE ANALYSIS

The ILINK program from the LINKAGE program package version 4.7 was used to calculate the likelihoods of alternative orders for the markers and PKD. This program uses an iterative procedure to optimise the recombination fractions for a given order. Where necessary, different polymorphisms detected by a single probe, or sets of probes, from one locus were used to construct haplotypes.

RESULTS

BREAKPOINTS ON THE SHORT ARM OF CHROMOSOME 16

During the study, five cell lines containing rearrangements in the short arm of chromosome 16 (table 1, fig 2) were available. CY13, CY14, CY19, and NOH-1 were somatic cell hybrids with a human chromosome 16 deleted for part of the short arm as a result of a reciprocal translocation. GM2324 is an EBV-transformed cell line from a reciprocal translocation carrier t(16;22) (p13.3;q12.2). Probes were mapped by in situ hybridisation. In this way five clusters of probes could be discerned (table 1, fig 2).

FAMILY STUDIES

The inheritance of RFLPs was studied in large families with PKD (figs 3, 4, and 5). The family studies were performed in two phases. First, a set of seven probes was tested on 12 informative families. By analysing the chromosomes derived from the unaffected parents as well as those with PKD, we could score 259 meioses which were informative for at least one marker on either side of the gene involved in PKD (table 2). At least 58 recombinations were present. In table 2, the probes are listed in order from telomere to centromere as defined by multipoint analysis (see below). The results give a rough approximation of the recombination frequencies between the various markers on 16p. During these studies new polymorphic probes were isolated. These were mapped by collective use of the cell lines, the recombinations characterised earlier, and several new families (fig 2). The order shown in fig 2 leads to the absence of double recombinants between the polymorphic markers themselves. In contrast, apparent double recombinants between flanking markers and PKD

...
were observed in five families, indicating the possibility of a second unlinked locus for PKD. Indeed, in two families, the mutation causing PKD and chromosome 16p markers segregated independently: one family showed three non-recombinants and three double recombinants, while the other showed four non-recombinants and four double recombinants, plus a single recombinant between PGP and VK5 (pedigrees not shown). One family still being studied showed nine non-recombinants and one apparent double recombinant with a negative ultrasound result at the age of 55 (pedigree not shown). In two families, the mutation causing PKD was clearly linked to markers on chromosome 16, but no cysts were seen on ultrasonography, although the patient inherited the markers from the affected chromosome (figs 3 and 4). In fig 3, II.2 and II.3 are both unaffected, but both have a recombination between 3'HVR and pEKMDA-2, which leads to different sets of markers proximal to PKD1. Two additional recombinants map pEKMDA-2 distal to PKD1 (fig 4, II.8 and pedigree not shown) giving odds against inversion calculated on the total set of families of 1:5 (table 3). False negative ultrasound in II.3 in fig 4 may be the explanation. A similar reason may apply to III.2 in fig 4.

MULTIPOINT ANALYSIS

Breakpoints in 16p

Since it has been notoriously difficult to define breakpoints in the distal part of 16p by cytogenetic methods, leading to controversy over the localisation of the α globin gene cluster, we first investigated whether the genetic order obtained by multipoint analysis was in agreement with the order of the breakpoints in the cell lines reported...
Five clusters of polymorphic loci could be distinguished in the 16p31.1—pter region (table 3, fig 2). The most proximal marker is 36–1 (D16S79), a highly polymorphic locus, just distal to the fragile site FRA16A. The most distal marker is 3'HVR (D16S85). Although not always the same probes were used, no results at variance with other reports were obtained.

Localisation of PKD1

In analysing the map from centromere to telomere, PKD1 clearly maps distal to CRI–090 (D16S45), CRI–133 (D16S63), and 24–1 (D16S80) (table 3). It also maps distal to 26–6 (D16S125), but the odds against inversion are not very high. In the families studied, PKD1 was not separated from PGP, 218EP6 (D16S246), pGGG1 (D16S259), or CMM65 (D16S84). Reenders et al (unpublished data) have observed one crossover mapping the PKD1 gene proximal to pGGG1. pEKMDA–2 (D16S83) maps distal to PKD1, but the odds against inversion are low (see above). In this set of families, the D16S21 locus is the first marker clearly distal to PKD1.

Figure 4 Family PK12. Subject III.2 may have had a false negative ultrasound.
Figure 5  Family PK3. Intrahaplotype recombination within the D16S21 locus most probably in the meiosis of the affected parent of III.5.
Table 2 Informative meioses scored using 3'HVR at the tip of chromosome 16p as a reference.

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<th>Female NR</th>
<th>Female Rec</th>
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<th>Unknown Rec</th>
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<th>Total Rec</th>
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<td>4</td>
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<td>58</td>
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NR = non-recombinant, Rec = recombinant.

Table 3 Relative likelihood of selected possible orders of polymorphic markers on 16p.

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<td>Order relative to breakpoint</td>
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<td>S85-S84</td>
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<tr>
<td>GM2324</td>
<td>S85-S83</td>
<td>-PKD1</td>
</tr>
<tr>
<td>NOH-1</td>
<td>S85-S84</td>
<td>S125</td>
</tr>
<tr>
<td>Order within</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CY19</td>
<td>S85-S80</td>
<td>-S45</td>
</tr>
<tr>
<td>GM2324</td>
<td>S85-S125</td>
<td>S94</td>
</tr>
<tr>
<td>CY14</td>
<td>S85-S21</td>
<td>-S45</td>
</tr>
<tr>
<td></td>
<td>S85-cos2B</td>
<td>-2BP5</td>
</tr>
</tbody>
</table>

*This order is excluded by physical mapping. No informative recombinants between S21 and S83, S84 and PKD1, S63 and S80. S81 and S82 map on independently isolated, overlapping cosmids. The S246 locus was not included in this analysis.

Computer program used: ILINK, LINKAGE package 4.7.40

Order of markers within clusters
Distal to the breakpoint of CY13, we find from centromere to telomere CRI-133 (D16S58), 3-15 (D16S81) and 41-1 (D16S82), CRI-90 (D16S45), 24-1 (D16S80) and CRI-327 (D16S63). The latter two markers were not separated by recombination in this set of families.

Distal to the GM2324 breakpoint, VK5 (D16S94) maps proximal to 26-6 (D16S125); these loci were not separated from 218E6P (D16S246) by recombination.

Distal to the CY14 breakpoint, we have mapped pEKMDA-2 (D16S83) and the D16S21 locus represented by several probes, Fr3-42, cosmid 2B, HM1J, and 2BP5. pEKMDA-2 was not separated by recombination from D16S21 in these families. However, Bachner et al (unpublished data) observed an apparent recombination placing pEKMDA-2 proximal to D16S21. In one family we detected an apparent intrahaplotype recombination event within the D16S21 locus (fig 5). From this recombination, it could be deduced that 2BP5 is closer to PKD1 than the polymorphism detected by cos2B (odds against inversion 1:42, table 3). Unfortunately, this branch of the family was not informative for pEKMDA-2. The map ends on the telomeric side with the alpha globin region, represented in our studies by 3'HVR (D16S85).

In two branches of families uninformative for 3'HVR, we used 5'HVR. Recombinations between 3'HVR and 5'HVR were not sought.

In agreement with earlier observations,10 12 a striking excess of recombination in meioses of males over females was observed in the distal part of the map (table 2, fig 2).

Discussion
We have presented here a detailed map of 16 polymorphic loci on the distal part of the short arm of chromosome 16. Genetic studies were combined with physical mapping of probes relative to translocation breakpoints. The PKD1 gene is now surrounded by an extensive set of polymorphic markers that can be used for presymptomatic and prenatal diagnosis.45

The computer programs used for the construction of linkage maps have been the subject of controversy.46 It has been argued that linkage maps can be constructed by multiple pairwise scoring of polymorphic markers. Although this may be true in principle, in practice the material for such an analysis would have to be extremely large to be sufficiently reliable. In smaller samples, especially with closely linked markers, the stochastic nature of recombination will lead to inaccurate orders of polymorphic loci. This problem is best illustrated by the following example. The D16S81 and D16S82 polymorphisms have been detected by independent subclones from a pool of cosmids.18 Both were localised proximal to PKD1. Relative to 3'HVR, D16S81 has a recombination frequency of 18% (table 2) and D16S82 of 10%.
Unexpectedly, however, both probes were found to originate from two independent, but overlapping, cosmids, as shown by digestion of the cosmids, thus revealing shared fragments hybridising with both subclones (data not shown). To circumvent these problems, we have used mostly three point analysis to establish gene order. The ILINK program was used, assuming no interference and no difference between male and female recombination rates. Our main reason for doing this was the impossibility of determining the degree of interference in 16p with reasonable accuracy. Similarly, the number of informative meioses available for each pair of informative markers precluded separate estimates for male and female recombination rates. However, if we consider the order of polymorphic loci depicted in fig 2 as the correct one, no double crossovers were found in 259 meioses tested for the first set of markers.

The odds against inversion (table 3) were not very high in all cases. Therefore our linkage map needs to be confirmed by other methods used in parallel on the same chromosomal region. The number of naturally occurring breakpoints used for physical mapping (table 1) is being extended further. In our hands, the mapping data obtained with these breakpoints and the order indicated by family studies have always been the same. An extensive set of radiation hybrids is now available for chromosome 16.47 Using an algorithm described by Goss and Harris,48 DNA fragments can be ordered along the chromosome at high resolution. A complete physical map of the tip of the short arm of chromosome 16 is now being constructed using pulsed field gel electrophoresis. Ultimately, overlapping cosmids clones and artificial yeast chromosomes will resolve the remaining uncertainties on the map of the chromosome.49

The research on the short arm of chromosome 16 will now focus on the genes present in the region in order to obtain candidates for the PKD1 gene. Once this gene has been isolated, its mutations can be studied.

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19 Konings RH, Luiten RGM, Peeters BPH. Mike, a chimeric filamentous phage designed for the separate production of either DNA strand of PKUN vector plasmids by F+ cells. Gene 1986;46:269–76.
27 Snijwedit FGM, Saris JJ, Dauwerse GR, Breuning MH, van Ommen GJB. Probe 218EP6 (D16S246) detects RFLPs close to...


