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Refining the Map and Defining Flanking Markers of the Gene for Autosomal Recessive Polycystic Kidney Disease on Chromosome 6p21.1-p12

To the Editor:

Autosomal recessive polycystic kidney disease (ARPKD) is one of the most important hereditary nephropathies in childhood. The reported incidence is 1:6,000–1:40,000 live births. The clinical spectrum of ARPKD is variable, with severe forms presenting in early childhood and with the milder forms presenting at later ages and with survival into adulthood (Cole 1990; Zerres 1992). Specific pathoanatomic changes for ARPKD are dilated collecting ducts in the kidneys and biliary dysgenesis and portal fibrosis, which often cause clinical symptoms.

The ARPKD families of our linkage study were collected by members of the German study group for pediatric nephrology and by several European investigators. The diagnoses were reliably verified by pediatric nephrologists, on the basis of familial and medical history. None of the parents showed cysts on renal ultrasound, and in most cases the diagnosis was confirmed, in addition, by at least one of the following criteria: clinical signs of hepatic fibrosis, parental consanguinity, and pathoanatomic proof of ARPKD in at least one affected sibling. Our study included 36 families—12 multiplex and 24 simplex families—with a total of 49 affected children. The spectrum of clinical manifestations among these families ranges from severe prenatally diagnosed cases to those with onset in later childhood. The clinical spectrum of liver involvement is variable, with severe hepatic fibrosis in young children and with missing clinical signs of liver involvement in young adults.

The procedures for DNA isolation and PCR amplification have been described elsewhere (Miller et al. 1988; Zerres et al. 1994). The two-point and multipoint linkage analyses were performed assuming no sex difference and using the LINKAGE package, version 5.1 (Lathrop et al. 1984, 1985; Ott 1985). The gene frequency was set at .001, and penetrance was set at 100% for affected individuals and at 0% for unaffected individuals. The alleles in each family were scored as 1–4, and allele frequencies were listed as 25% for each allele.

We recently mapped the gene for ARPKD to chromosome 6p21-cen (Zerres et al. 1994) by linkage analysis. In a more extensive study, we analyzed two additional microsatellite markers of the region 6p21 (AFM184xa11 at D6S282 and AFM123xe1 at D6S269; table 1) in 12 multiplex and 4 simplex ARPKD families (30 affected and 16 unaffected siblings), which have previously been published by Zerres et al. (1994). Because of additional typing, more families have become informative for single markers. The results of the two-point linkage analysis with ARPKD are given in table 2. The highest lod score Z_{max}, 8.76 at θ_{max} = .00, was obtained with AFM142xe7 at D6S272. Three loci—D6S269, D6S272, and D6S295—were examined for linkage with additional 20 simplex families (one affected and one unaffected child). AFM123xe1 at locus D6S269 close to D6S272 showed recombinations in 2 multiplex families and in 1 more of the 20 simplex families (Z_{max} = 5.51 at θ_{max} = .05). Only one recombination was observed with AFM206xc11 at locus D6S295. Two multiplex families were not informative for this probe (Z_{max} = 4.59 at θ_{max} = .03).

In addition, we performed a two-point linkage analysis of all eight loci. Significant results were obtained between

Table 1

Polymorphic Microsatellites Localized to Chromosome 6

Locus	Marker Name	Primer Sequence 5′–3′	Heterozygosity Frequency	Allele Size (bp)	Physical Location on Chromosome[b] 6
D6S282	AFM184xa11	ATGGCCCAGACAGTGGGTAT ATGGTTTGTGCAGGTTCAGA	.87	108–126	p21.2-p21.1
D6S269	AFM123xe1	CCTTGCTCATGGTTTTACAA CAGAAAGACATGGTAGAAGAGG	.72	178–192	p21.2-p21.1

[a] Additional primer information has been published by Zerres et al. (1994).
[b] Based on the map published by the second international workshop on human chromosome 6 (Volz et al. 1994).

the following loci: D6S269, D6S272, D6S295, and D6S294; Z_{max} = 12.88 at θ_{max} = .01 occurred between D6S269 and D6S272; Z_{max} = 9.85 at θ_{max} = .05 occurred between D6S272 and D6S295; and Z_{max} = 8.37 at θ_{max} = .02 occurred between D6S272 and D6S294. With the program ILINK we performed a multipoint linkage analysis between the closest marker loci with a fixed location corresponding to the map from Weissenbach et al. (1992) and with the ARPKD gene locus placed in different positions between these loci. The likelihood of the best order versus the next most likely order is not significantly different, because of the lack of recombinants in this region. The most likely position for ARPKD is between D6S272 and D6S295, and it is only 1.55 times more likely than the next most likely position, between D6S269 and D6S272. The other two orders are slightly more unlikely. Therefore the markers AFM123xe1 at D6S269, AFM142xe7 at D6S272, and AFM206xc11 at D6S295 can be defined as flanking markers of ARPKD.

We constructed possible haplotypes of five key-recombinant families. The two important families are shown in figure 1. All recombinations were confirmed by repeating the PCR twice. Figure 1 shows the two most informative families. The order of the loci is as follows: D6S282-TCTE-D6S269-D6S272-D6S295-D6S294-D6S257-D6S313. Under the assumption of no double recombination in one chromosome, the haplotypes of each family show no region of unknown breakpoint, even though there are some markers not informative. The marker locus D6S272 is the only one without a recombination in all families.

The positional cloning approach is complicated by the lack of multiplex kindreds, which most often is due to the high rate of neonatal mortality. Our linkage study was based on 36 families, which were from different European countries and represented a broad clinical spectrum of ARPKD. Most of the analyzed families had the milder form of ARPKD, with survival beyond the first years of life, although in at least two families the affected children belong to the perinatal phenotype. Among the 20 additionally included simplex families, 4 belong to the perinatal phenotype. The linkage of ARPKD to 6p21.1-p12 markers, without evidence of genetic heterogeneity, underlines the hypothesis of ARPKD being a single-gene defect. Before use of DNA markers for prenatal diagnosis, linkage results have to be confirmed in a greater number of pedigrees with early-onset ARPKD. The set of polymorphic markers with close linkage to ARPKD is a good tool for prenatal diagnoses in families with proved ARPKD.

Table 2

Two-Point Linkage Analysis of the ARPKD Gene with Chromosome 6 Microsatellites

	Z at Assumed Value of θ =							
Marker	.0	.01	.05	.1	.2	.3	Z_{max}	θ_{max}
D6S282	$-\infty$	4.47	5.52	5.12	3.55	1.86	5.52	.05
TCTE1	$-\infty$	4.24	4.87	4.50	3.16	1.69	4.87	.05
D6S269	$-\infty$	4.39	5.51	5.19	3.68	1.98	5.51	.05
D6S272	8.76	8.52	7.54	6.31	3.97	1.97	8.76	.00
D6S295	$-\infty$	4.37	4.55	4.12	2.84	1.49	4.59	.03
D6S294	$-\infty$	4.60	5.20	4.78	3.36	1.82	5.20	.04
D6S257	$-\infty$	3.19	4.44	4.28	3.07	1.64	4.47	.06
D6S313	$-\infty$	1.77	2.61	2.52	1.80	.98	2.63	.06

All markers used in our linkage study are now genetically and physically mapped (Volz et al. 1994), with the following distances between the loci: D6S282–7.1 cM–TCTE–5.6 cM–D6S269–0.6 cM–D6S272–4.8 cM–D6S295–1.9 cM–D6S294–1.8 cM–D6S257–6 cM–D6S313. Our results from the two-point linkage analysis of the markers support these orders (despite the small size of the families that we studied and the few recombinants), and so does the result of the analysis of recombinants (fig. 1). However, a smaller distance between TCTE and D6S296 was obtained in the families that we studied. In the recently published map by Matise et al. (1994), D6S272 is not exactly genetically mapped but is assigned to the most likely position, in the interval between D6S269 and D6S294. A similar assignment is given to the Kirsten rat sarcoma 1 viral oncogene homologue, i.e., the processed pseudogene (KRAS1P) (Popescu et al. 1985), and to methylmalonyl–coenzyme A mutase (MUT) (Nham et al. 1990). The physical assignment of the flanking markers is 6p21.2-p21.1 for D6S269 and D6S272 and 6p12 for D6S295. Figure 2 summarizes these results. The region flanked by D6S269 and D6S295 spans ~5.4 cM (Volz et al. 1994) (fig. 2).

None of the following genes, which have been localized to the chromosomal region 6p21-p11, can be considered as candidate genes: KRAS1P, MUT, and PGK1P2 (phosphoglycerate kinase 1 pseudogene 2) (Matise et al. 1994); and RDS (retinal degeneration), MAPTL (microtubule-associated protein tau-like), FTHP1 (ferritin heavy-polypeptide pseudogene 1, and GSTA1 (glutathione S-transferase alpha) (Volz et al. 1994). KRAS1P, PGK1P2, and FTHP1 are pseudogenes; RDS and MAPTL are not expressed in kidneys; GSTA1 is involved in the metabolism of xenobiotics; and MUT is involved in the degradation of branched-chain amino acids.

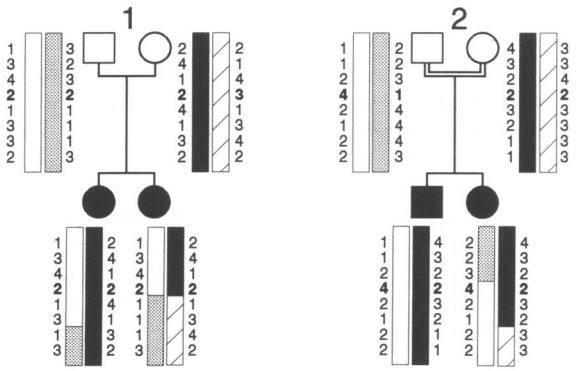

Figure 1 Haplotypres of two key-recombinant multiplex families. The order of the genotypes corresponds to the following locus order: D6S282 - top - TCTE - D6S269 - D6S272 - D6S295 - D6S294 - D6S257 - D6S313-bottom.

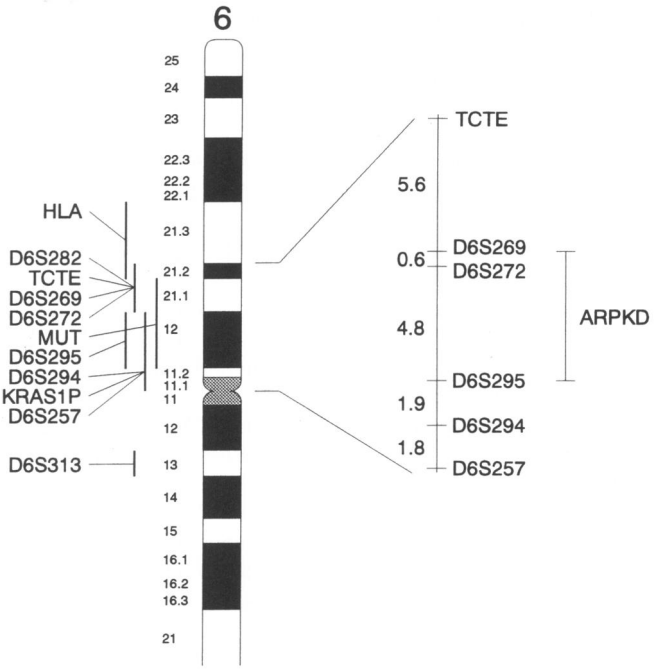

Figure 2 Ideogram of human chromosome 6p, with an expanded genetic map of the analyzed region including the position of locus ARPKD. Distances are those published by Volz et al. (1994).

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On the Diversity of β-Globin Mutations, a Reflection of Recent Historic Events in Israel

To the Editor:

In a recent issue of the *Journal*, Filon et al. (1994) report on the characterization of 500 β-thalassemia genes in the Israeli population and explain the broad heterogeneous spectrum of mutations they found by the various ethnic groups present in the country and by the history of their migration. This approach is particularly interesting from an anthropological point of view and also for the design of an efficient diagnostic strategy in all countries with highly mixed populations.

On the basis of their outstanding technical results, the authors provide a convincing explanation for the mutations found among Israeli Jews, which indeed reflects recent historic events of the state of Israel. However, they surely oversimplify their interpretation concerning the Arabs. One can presume that the group they call Bedouins are truly Arabs from the Arabian Peninsula and that the Druze are probably an autochthonous people. But they refer to other groups as being Moslem Arabs or Christian Arabs, which by far are not ethnic categories. The diffusion of Islam is a sociocultural fact, starting from the seventh century, and people from extremely varied origins have become Moslem since then.

Our own investigations have not so much concerned the Middle East Arabs as northern Africa Arab countries, the populations of which are Moslem and Arab speaking. There, too, we found a high heterogeneity of mutations (Bennani et al. 1993, 1994). When comparing those mutations, as well as their genetic background, to the data of the literature, we indeed observe a coincidence with major historical influences, but first they relate to more ancient events, and, second, they are not at all associated with the Arabs.

Roman veterans settled for several centuries wherever the Roman Empire extended, including the land of the present state of Israel. More recently, from the 16th to the 19th century, almost all the Arab countries were under the domination of the Ottoman Empire, and we find among Arabs many thalassemic mutations originally described among Turks. A third clear influence is that of the Greek community, which was a world of travelers.

Considering the historical data, these considerations should be as true for Israeli Arabs as they are for the Algerians or Tunisians, not to mention Moroccans, who are more complex still, in that they present also with remnants of Spanish and African migrations. Thus, a more in-depth interpretation of the many thalassemic mutations found among Israeli Arabs, which will provide information of great interest, should be sought.

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