Adult polycystic kidney disease and linked RFLPs at the α globin locus: a genetic study in the South Wales population

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SUMMARY Thirteen South Wales kindreds with adult polycystic kidney disease have been studied for genetic linkage using the α globin and 3'HVR probes. A maximum lod score of 24.187 was found at a recombination fraction of 0.03. This study supports the existence of a single locus on chromosome 16 responsible for the disorder.

Adult polycystic kidney disease (APKD) is an autosomal dominant disorder, displaying variable age at diagnosis and severity.^{1,2} The incidence is around 1 in 1000 births³ with a mutation rate of 6.5 to 12×10^{-5} .⁴ It is a common and often potentially fatal multisystem disorder, the major abnormality being the progressive enlargement of cysts in several organs, primarily the kidney, resulting in chronic renal failure. It accounts for approximately 9.5% of the total dialysis and transplant workload of the renal units in South Wales, thus proving a substantial economic burden.^{5,6} The basic biochemical defect is as yet unknown,⁷ and current therapy is ineffective in the retardation of cyst development. In the past, gene carriers were discovered when they were symptomatic or had affected offspring. Recently, ultrasonographic scanning (USS) has been used to identify patients presymptomatically, but a normal result cannot be relied upon under the age of 30 years. The earlier detection of gene carriers is important in allowing accurate genetic counselling and giving reproductive choice, including prenatal diagnosis, to those found to carry the gene. Those found not to carry the gene can be reassured and can be considered, if they wish, as potential transplant donors for affected relatives. Earlier detection of the gene may also encourage regular monitoring for the recognition and treatment of such problems as hypertension and urinary infection, thus helping to preserve renal function.

Until recently the chromosomal localisation of

APKD was unknown, but through DNA technology it has been localised by Reeders *et al*⁸ to chromosome 16, using a hypervariable sequence (3'HVR) which lies ~8 kb from the α globin gene cluster. The study was based on nine families giving a maximum lod score (Z) of 25.85 at a recombination fraction (θ) of 0.05. Since this linkage was published, a protein polymorphism of phosphoglycolate phosphatase (PGP)⁹ has been found to be closely linked to APKD (PGP vs α globin cluster, $Z=8.21$ at $\theta=0$; PGP vs APKD $Z=11.61$ at $\theta=0$). The results obtained by Reeders *et al*⁹ suggest a single locus for the APKD gene; however, the families were limited in number. Further family studies in different populations are required to confirm their original findings.

This report analyses South Wales APKD families with the 3'HVR marker and polymorphisms within the α globin locus, the study forming part of a broader genetic and population study of the disorder in the region.

Materials and methods

A total of 139 families was identified from the dialysis and transplant registers of the Cardiff Royal Infirmary Renal unit (serving a population of 2.1×10^6). From these, 13 were found to have the necessary structure for linkage analysis. These families showed typical clinical features of the disease with the development of end stage renal failure at a mean age of 49.5 years. A total of 243 family members and spouses was included in the

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genetic linkage analysis, of whom 81 were affected and 67 were at risk of carrying the disease, the total including those whose DNA typing was inferred. Diagnosis of symptomatic cases was confirmed by USS. Asymptomatic at risk subjects were only included when aged over 20, after informed consent had been obtained. Diagnostic criteria were the finding of a minimum of two cysts in one kidney and one cyst in the other.¹ Only three subjects were found to be equivocal using USS, of whom one was rescreened independently from the study using computerised tomography (CT) and found to be affected; the other two were not included in the analysis.

DNA ANALYSIS

Blood samples were taken in EDTA tubes and frozen at -20°C . Total genomic DNA was prepared from the leucocytes of 20 ml of blood by a triton X-100 method.¹⁰ Restriction enzymes *PvuII* and *RsaI* (BRL Ltd, Cambridge) were used to digest DNA, under conditions recommended by the manufacturers, and fragments separated by size using a gel of 0.8% agarose-ultrapure (BRL Ltd, Cambridge). Southern blots were performed to transfer fragments onto a membrane (N-Hybond, Amersham). DNA was fixed to the membrane by UV irradiation for five minutes and baked for one hour at 80°C . ^{32}P labelled probe 3'HVR was used for hybridisation. The probe was prepared from a 4 kb *HinfI* restriction enzyme fragment, containing the entire 3'HVR, subcloned into a vector pSP64^{11 12} and from the α globin gene as a 1.3 kb fragment in PBR322 (TET Resistant, MRC Haematology Unit, Oxford).¹³

Hybridisation was carried out for approximately 18 hours at 65°C , with 5×10^7 CPM/ml in N-Hybond recommended conditions. Filters were washed for one hour in $2 \times \text{SSC} + 0.1\%$ SDS at room temperature, in $0.1 \times \text{SSC} + 0.1\%$ SDS at 65°C for one hour, and were exposed at -70°C to Fuji x ray film for one to five days, using an X-ograph intensifying screen. Allele inheritance was assessed directly.

Results

Figs 1 and 2 show the band patterns obtained using the α globin and the 3'HVR probes respectively. No cross overs were seen between the inheritance of alleles detected by these two markers⁶; the regions they recognise have therefore been considered one locus for analysis, allowing haplotypes to be used. Fig 3 shows the pedigrees analysed with the marker genotypes.

FIG 1 Autoradiography of α globin gene probe hybridised to 5 μg genomic DNA digested with *RsaI*. There is a two allele system (1,2), the frequency of allele 2 being 0.40 in the South Wales population.

The results show linkage between the α globin gene cluster and *APKD* locus, with three recombinants out of 83 phase known meioses and a maximum lod score of $\hat{Z}=24.187$ at $\theta=0.03$ (based on a haplotype between α globin and the 3'HVR allele inheritance in 12 families).

Non-symptomatic at risk subjects with a prior risk of 50% that they carried the *APKD* gene had their risks altered as a function of their age at negative screening with USS¹ as follows: age 15 to 20 years 0.34; 20 to 30 years 0.14; 30+ years 0.05. These risks were used in the age of onset correction option of MLINK and LIPED.^{14 15} The LIPED programme was used for the calculation of linkage when segregating for sex, and MLINK for calculation of haplotypes. Subjects not at risk were governed by the disease frequency in the general population of 1 in 1000.

The families studied were analysed with and without using the age of onset probability (tables 1 to 4). No significant difference in recombination fraction was observed, even when analysed separately for each sex (tables 5 to 8). Our results show only three recombinations, all of which were in male meioses.

Subject III.22 in *APKD* family 1 (1–III.22) and subject II.1 in *APKD* family 12 (12–II.1) were found

to carry the affected haplotype, but on USS were observed to be normal, therefore appearing to be cross overs. It must be taken into account though that subject 1-III.22 was scanned using USS at the

age of 20 and therefore still has a 14% risk of expressing the disease in later life. Subject 12-II was aged 33 when scanned using USS and therefore only has a <5% risk.

FIG 2 Autoradiograph of 3'HVR probe hybridised to 5 µg DNA digested with PvuII. Members of APKD family 6 (fig 3) are identified above each track. Alleles are named A-I and are specific to the family. The large number of alleles can be attributed to the fact that the 3'HVR region consists of a tandem repeat of a 17 nucleotide base sequence, variability of copy number of the element resulting in polymorphism. The probe hybridises to two allelic fragments in each subject, fragments varying in size from 1.8 kb to 8 kb and in all meioses tested, following Mendelian segregation. As the identity of electrophoretically indistinguishable alleles in a highly polymorphic system cannot be assumed, allele frequencies have to be estimated. Because of the great number of alleles being seen in a family using the 3'HVR (in APKD family 1, 22 were seen), analysis of the inheritance of the disease with a particular allele is complex. Alleles entering a pedigree from outside, that is, those alleles brought in by the unrelated spouses, were given a common designation to cut down on the number of alleles in a pedigree as far as possible, to aid the computer analysis of the results.

TABLE 1 Individual family lod score, APKD vs α globin, when at risk subjects were simply coded as normal.

Family	Recombination fraction (θ)										$\hat{\theta}$	$\hat{Z}$
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40		
1	—	5.169	5.356	5.417	5.425	5.405	5.179	4.100	2.824	1.363	0.04	5.425
2	3.311	3.263	3.215	3.166	3.116	3.066	2.861	2.245	1.607	0.871	0.00	3.311
3	2.160	2.121	2.083	2.044	2.004	1.965	1.803	1.327	0.848	0.331	0.00	2.160
4	1.204	1.182	1.160	1.138	1.115	1.093	0.999	0.721	0.444	0.173	0.00	1.204
5	0.903	0.881	0.859	0.837	0.815	0.793	0.703	0.449	0.228	0.061	0.00	0.903
6	1.517	1.488	1.458	1.428	1.398	1.368	1.243	0.879	0.531	0.201	0.00	1.517
7	0.903	0.886	0.868	0.850	0.832	0.814	0.739	0.517	0.298	0.094	0.00	0.903
8	0.039	0.037	0.036	0.034	0.033	0.032	0.026	0.014	0.006	0.001	0.00	0.039
9	0.301	0.292	0.284	0.275	0.266	0.258	0.223	0.134	0.064	0.017	0.00	0.301
10	-2.876	-1.349	-1.063	-0.898	-0.783	-0.695	-0.439	-0.220	-0.116	-0.051	0.45	-0.025
11	-0.021	-0.019	-0.018	-0.016	-0.015	-0.013	-0.007	-0.001	-0.004	0.003	0.30	-0.004
12	—	—	—	—	—	—	—	—	—	—	—	—
13	0.903	0.890	0.877	0.863	0.850	0.836	0.766	0.612	0.438	0.238	0.00	0.903

FIG 3 Pedigrees used in the study with the genotypes at the 3'HVR (H) locus and the α globin (A) locus given for each subject tested and for those that could be inferred (*). An example of the inheritance of these loci with the disease gene is given by family APKD 6, where the APKD phenotype is seen to segregate with the B12 haplotype.

TABLE 2 Individual family lod score, APKD vs 3'HVR, when at risk subjects were simply coded as normal.

Family	Recombination fraction (θ)										$\hat{\theta}$	$\hat{Z}$
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40		
1	$-\infty$	8.464	8.886	9.055	9.121	9.128	8.767	7.272	5.247	2.768	0.05	9.128
2	4.214	4.419	4.083	4.016	3.949	3.880	3.529	2.276	1.924	0.999	0.00	4.214
3	2.896	2.846	2.795	2.744	2.693	2.641	2.376	1.808	1.184	0.496	0.00	2.896
4	1.204	1.182	1.160	1.138	1.115	1.093	0.975	0.721	0.444	0.173	0.00	1.204
5	0.903	0.881	0.859	0.837	0.815	0.793	0.680	0.449	0.229	0.061	0.00	0.903
6	1.540	1.495	1.449	1.403	1.358	1.312	1.082	0.626	0.206	-0.045	0.00	1.540
7	1.204	1.182	1.160	1.138	1.115	1.093	0.975	0.721	0.444	0.173	0.00	1.204
8	0.000	3.167	3.383	3.473	3.511	3.520	3.371	2.712	1.856	0.924	0.05	3.520
9	0.903	0.886	0.868	0.851	0.833	0.815	0.725	0.542	0.357	0.175	0.00	0.903
10	-1.922	-0.695	-0.426	-0.275	-0.173	-0.098	0.095	0.171	0.119	0.039	0.19	0.172
11	0.323	0.314	0.306	0.297	0.288	0.280	0.237	0.157	0.087	0.033	0.00	0.323
12	$-\infty$	-1.402	-1.106	-0.934	-0.814	-0.721	-0.444	-0.194	-0.076	-0.018	0.45	-0.004
13	1.204	1.182	1.160	1.138	1.116	1.094	0.981	0.746	0.503	0.255	0.00	1.204

TABLE 3 Individual family lod score, APKD vs α globin, when at risk subjects were given a prior probability from their age.

Family	Recombination fraction (θ)										$\hat{\theta}$	$\hat{Z}$
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40		
1	$-\infty$	4.869	5.060	5.125	5.138	5.121	4.841	3.887	2.668	1.279	0.04	5.138
2	3.170	3.123	3.076	3.029	2.981	2.932	2.683	2.140	1.528	0.825	0.00	3.170
3	1.982	1.946	1.909	1.872	1.835	1.797	1.605	1.197	0.752	0.283	0.00	1.982
4	1.090	1.070	1.049	1.028	1.007	0.985	0.875	0.639	0.389	0.153	0.00	1.090
5	0.861	0.840	0.818	0.797	0.776	0.754	0.645	0.423	0.214	0.057	0.00	0.861
6	1.436	1.434	1.405	1.376	1.346	1.317	1.164	0.841	0.504	0.190	0.00	1.463
7	0.840	0.823	0.806	0.789	0.771	0.754	0.664	0.471	0.266	0.082	0.00	0.840
8	0.039	0.037	0.036	0.034	0.033	0.032	0.025	0.014	0.006	0.001	0.00	0.039
9	0.244	0.237	0.229	0.222	0.214	0.207	0.171	0.104	0.049	0.013	0.00	0.244
10	-2.876	-1.349	-1.063	-0.898	-0.783	-0.695	-0.439	-0.220	-0.11	-0.051	>0.50	0.000
11	-0.021	-0.019	-0.018	-0.016	-0.015	-0.013	-0.007	0.001	0.001	0.003	0.35	0.004
12	—	—	—	—	—	—	—	—	—	—	—	—
13	0.825	0.813	0.800	0.788	0.775	0.763	0.697	0.555	0.395	0.212	0.00	0.825

TABLE 4 Individual family lod score, APKD vs 3'HVR, when at risk subjects were given a prior probability from their age.

Family	Recombination fraction (θ)										$\hat{\theta}$	$\hat{Z}$
	0.00	0.01	0.02	0.03	0.04	0.05	0.10	0.20	0.30	0.40		
1	$-\infty$	9.004	9.155	9.178	9.147	9.085	8.550	6.997	4.987	2.607	0.03	9.178
2	4.073	4.009	3.944	3.879	3.813	3.746	3.403	2.662	1.844	0.953	0.00	4.073
3	2.662	2.614	2.566	2.518	2.469	2.420	2.169	1.635	1.053	0.426	0.00	1.982
4	1.090	1.070	1.049	1.028	1.007	0.985	0.875	0.639	0.389	0.153	0.00	1.090
5	0.861	0.840	0.818	0.797	0.776	0.754	0.645	0.423	0.214	0.057	0.00	0.861
6	1.462	1.419	1.376	1.333	1.290	1.247	1.030	0.596	0.192	-0.051	0.00	1.463
7	1.084	1.063	1.042	1.021	1.000	0.979	0.869	0.633	0.381	0.143	0.00	1.084
8	$-\infty$	2.900	3.118	3.212	3.253	3.265	3.133	2.513	1.707	0.840	0.05	3.265
9	0.846	0.829	0.813	0.796	0.779	0.761	0.675	0.500	0.325	0.157	0.00	0.846
10	-1.964	-0.737	-0.468	-0.316	-0.213	-0.138	-0.058	0.142	0.101	0.033	0.19	0.142
11	0.323	0.314	0.306	0.297	0.288	0.280	0.237	0.157	0.087	0.033	0.00	0.323
12	$-\infty$	-1.402	-1.106	-0.934	-0.814	-0.721	-0.444	-0.194	-0.076	-0.018	>0.50	0.000
13	1.126	1.105	1.084	1.063	1.042	1.020	0.912	0.688	0.459	0.299	0.00	0.825

TABLE 5 *Linked analysis between APKD and α globin, when at risk subjects were simply coded as normal.*

		Recombination fraction (θ)									95% confidence limit	
		0-00	0-01	0-02	0-03	0-04	0-05	0-06	0-07	0-08		0-09
α globin	M	—	5-608	5-791	5-846	5-848	5-819	5-771	5-709	5-635	5-553	(0-00-0-16)
	F	10-212	10-037	9-861	9-683	9-503	9-322	9-139	8-954	8-768	8-580	(0-00-0-06)
	M+F	—	14-843	15-115	15-139	15-059	14-920	14-743	14-539	14-314	14-073	(0-00-0-09)
3'HVR	M	—	8-549	9-236	9-546	9-698	9-765	9-776	9-748	9-690	9-608	(0-01-0-15)
	F	—	15-304	15-295	15-159	14-966	14-747	14-503	14-244	13-973	13-690	(0-00-0-07)
	M+F	—	23-652	24-578	24-882	24-928	24-830	24-639	24-381	24-074	23-728	(0-01-0-08)
Haplotype M+F		—	24-012	24-935	25-237	25-280	25-178	24-984	24-723	24-412	24-062	(0-00-0-08)

TABLE 6 *Linked analysis between the α globin locus and APKD, when at risk subjects were given a prior probability from their age.*

		Recombination fraction (θ)									95% confidence limit	
		0-00	0-01	0-02	0-03	0-04	0-05	0-06	0-07	0-08		0-09
α globin	M	—	5-190	5-379	5-440	5-447	5-425	5-383	5-327	5-260	5-184	(0-00-0-16)
	F	9-623	9-455	9-285	9-114	8-941	8-767	8-591	8-413	8-235	8-054	(0-00-0-06)
	M+F	—	13-823	14-108	14-145	14-078	13-953	13-790	13-601	13-390	13-163	(0-00-0-09)
3'HVR	M	—	8-844	9-264	9-429	9-489	9-490	9-452	9-386	9-299	9-196	(0-00-0-15)
	F	—	14-386	14-391	14-267	14-089	13-879	13-649	13-402	13-144	12-876	(0-00-0-07)
	M+F	—	23-029	23-699	23-872	23-837	23-685	23-457	23-175	22-852	22-496	(0-00-0-08)
Haplotype M+F		—	23-338	24-056	24-225	24-187	24-031	23-779	23-513	23-186	22-825	(0-00-0-08)

TABLE 7 *Linked analysis between α globin locus and APKD, when at risk subjects were simply coded as normal.*

		Recombination fraction (θ)								$\hat{\theta}$	$\hat{Z}$
		0-10	0-15	0-20	0-25	0-30	0-35	0-40	0-45		
α globin	M	5-464	4-937	4-316	3-626	2-880	2-096	1-306	0-583	0-04	5-848
	F	8-390	7-415	6-395	5-331	4-228	3-097	1-964	0-891	0-00	10-212
	M+F	13-817	12-389	10-779	9-033	7-177	5-245	3-302	1-485	0-03	15-139
3'HVR	M	9-510	8-815	7-898	6-827	5-622	4-305	2-888	1-417	0-06	9-776
	F	13-400	11-851	10-187	8-439	6-648	4-848	3-081	1-413	0-01	15-304
	M+F	23-350	21-118	18-499	15-611	12-523	9-303	6-034	2-844	0-04	24-928
Haplotype M+F		23-679	21-422	18-772	15-846	12-715	9-445	6-123	2-882	0-04	25-280

TABLE 8 *Linked analysis between α globin locus and APKD, when at risk subjects were given a prior probability from their age.*

		Recombination fraction (θ)								$\hat{\theta}$	$\hat{Z}$
		0-10	0-15	0-20	0-25	0-30	0-35	0-40	0-45		
α globin	M	5-101	4-609	4-027	3-378	2-679	1-946	1-212	0-541	0-04	5-447
	F	7-872	6-939	5-967	4-958	3-917	2-857	1-806	0-820	0-00	9-623
	M+F	12-923	11-574	10-052	8-404	6-659	4-851	3-047	1-371	0-03	14-145
3'HVR	M	9-078	8-341	7-429	6-388	5-239	3-995	2-669	1-303	0-05	9-490
	F	12-600	11-123	9-535	7-874	6-176	4-481	2-833	1-294	0-02	14-391
	M+F	22-113	19-910	17-372	14-602	11-664	8-623	5-563	2-610	0-03	23-872
Haplotype M+F		22-438	20-208	17-640	14-833	11-852	8-762	5-650	2-646	0-03	24-225

Discussion

These results, like those of Reeders *et al.*,⁸ are conclusive for linkage between *APKD* and the α globin gene cluster, suggesting no genetic difference between the disease seen in the South Wales families and those used by Reeders *et al.*⁹ This strengthens the feasibility of using these markers for the presymptomatic prediction of *APKD* and for prenatal diagnosis.¹⁶ The high degree of polymorphism at the α globin/3'HVR locus (in combination 96% of persons are heterozygous) makes this locus highly informative. There is a risk of error of recombination of around 3% (confidence limits 0.00 to 0.08) but this risk estimate is not age dependent as is USS, which has been the usual screening test until now. At risk subjects under 30 years of age, who are negative on USS, still have a 15% risk of carrying the gene.¹ This can now be greatly reduced by the use of DNA markers in combination with USS in suitable families. Closer genetic markers, in particular those flanking the *APKD* locus, are currently being sought and should reduce still further the error rate in prediction. Isolation of the gene itself should not only eliminate error due to recombination, but will give rise to a greater understanding of the pathogenesis of the disease.

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