Letters to the Editor

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Autosomal dominant polycystic kidney disease unlinked to the *PKD1* and *PKD2* loci presenting as familial cerebral aneurysm

EDITOR—Autosomal dominant polycystic kidney disease (ADPKD) is a multisystem disease with intrafamilial phenotypic heterogeneity. Cerebral aneurysms develop in 10-20% of patients with ADPKD.[1 2] Aneurysm rupture may precede the development of hypertension or renal manifestations of the disease.[2] Two ADPKD loci (*PKD1* and *PKD2*) map to chromosomes 16p and 4q, respectively. Reports of unlinked pedigrees have suggested the existence of a third unmapped locus.[3–7] We report a three generation white family with an ADPKD like disease, unlinked to the *PKD1* or *PKD2* loci (fig 1, table 1). Other genetic causes of renal cysts, such as tuberous sclerosis and von Hippel-Lindau disease, have been excluded clinically and linkage to the known loci for these diseases is unlikely. This family supports the case for a third locus for ADPKD, but differs from the previously described unlinked pedigrees by its presentation with cerebral aneurysm rupture.

Ethical approval for this study was granted by the local research ethics committee. The family was identified because three members had sustained a subarachnoid haemorrhage. All family members who were older than 18 were invited for examination and screening for intracranial aneurysms by magnetic resonance angiography (MRA). There was no clinical evidence of tuberous sclerosis, von Hippel-Lindau disease, or autosomal recessive polycystic kidney disease (diseases associated with cerebral aneurysm formation). MRA scans that were suspicious for cerebral aneurysm were followed up by conventional cerebral angiography. In view of advanced age, MRA screening was not offered to I.2, but it was noted that she had previously suffered a CVA of undetermined aetiology. II.2 and II.4 had both sustained a subarachnoid haemorrhage as a result of rupture of an anterior communicating artery aneurysm (ACoA), and II.6 had a ruptured posterior communicating artery aneurysm (PCoA). All three were normotensive with normal serum urea and creatinine concentrations when they presented with subarachnoid haemorrhage. There was no past history of ADPKD within the family. Subjects III.8, III.9, III.10, and III.12 declined examination.

Subjects meeting recognised criteria for diagnosis of polycystic kidney disease were classed as affected.[8 9] II.1, although designated normal, declined MRA and ultrasound investigation but was happy to provide blood. II.2 and II.6 did not meet these criteria. II.2 at the age of 57 had no renal cysts but was hypertensive, had a ruptured cerebral aneurysm, and had a son with renal cysts. II.6 had marked hepatic cystic disease, was also hypertensive, and had a ruptured cerebral aneurysm. In addition, her son, III.7, had renal cysts and was mildly hypertensive at the age

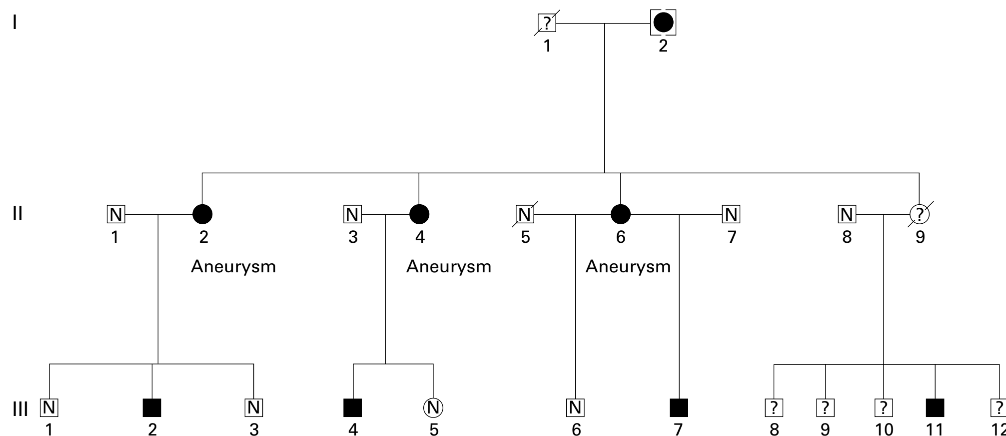

Figure 1 Pedigree with autosomal dominant polycystic kidney disease.

Table 1 Clinical details of family members

	Age	*Aneurysm*	*BP (mm Hg)*	*Creatinine (μmol/l)*	*Ultrasound*
I.2	88	Unknown	130/80	117	Multiple cysts in R kidney, single cyst in L kidney
II.2	57	ACoA	HT	99	No cysts
II.4	65	ACoA	HT	87	Multiple cysts
II.6	54	PCoA	HT	64	Several liver cysts, no renal cysts
III.1	36	Normal	130/80	89	No cysts
III.2	25	Normal	120/80	98	2 cysts L kidney, 1 cyst R kidney
III.3	32	Normal	120/84	85	No cysts
III.4	43	Normal	170/120	92	Multiple cysts in both kidneys
III.5	37	Normal	120/80	85	1 cyst in right kidney
III.6	27	Normal	120/84	69	Normal
III.7	19	Normal	140/100	90	1 cyst in each kidney
III.11	35	Normal	130/90	90	2 cysts in each kidney, renal calculi

ACoA = anterior communicating artery aneurysm, PCoA = posterior communicating artery aneurysm, HT = hypertension diagnosed and treated.

of 19 years. Both II.2 and II.6 were designated affected because they both had many phenotypic characteristics of the disease, had an affected parent, and also had affected offspring. Absence of renal cysts in a subject with ADPKD proven by linkage has been described previously.[8]

Linkage analysis was carried out. Microcrosatellite markers flanking the known loci of *PKD1*/*TSC2*, *PKD2*, and *TSC1* were chosen.[10–13] CI-46 was used as a marker for the *VHL* locus.[14] Results were analysed in two separate fashions using the LINKAGE (v 5.04) program.[15] First, we tested the hypothesis that this family had classical ADPKD linked to either the *PKD1* or *PKD2* loci using recognised liability classes for ADPKD.[9] At risk but unaffected family members were assigned to three liability classes, less than 10 years, 20-25 years, and greater than 25 years, with penetrances of 0.22, 0.66, and 0.85, respectively.[9] Linkage to the *PKD1* and *PKD2* loci was also assessed using an affecteds only analysis. Multipoint lod scores were consistently less than −2.0 across the *PKD1*/*TSC2* and *PKD2* loci using both methods. The disease is therefore not linked to these loci. Further linkage analysis was restricted to affected subjects only, as there are no data on the penetrance of ADPKD unlinked to *PKD1* and *PKD2*.

We considered linkage to other loci associated with renal cystic disease or intracranial aneurysm formation. Multipoint lod scores plotted at *TSC1*/*HHT1* (<-1.5) and *ELA1*/*HHT2* (<-2.5) suggested non-linkage. Two point lod scores for the *VHL* locus (<-2.0 within 0.08 cM from CI-46) also showed non-linkage.

Since subjects I.1 and II.9 were non-smokers who died at an early age from emphysema, we considered the diagnosis of α1-antitrypsin deficiency. Serum α1-antitrypsin levels and phenotypes were recorded in I.2, II.2, II.4, II.6, and III.11. Results lay within the normal range and all subjects typed had the MM phenotype.

There are previous reports of unlinked pedigrees.[3–7] Daoust *et al*[3] reported a two generation family with six affected subjects. Although only one member in this pedigree showed evidence of renal insufficiency, affected subjects had renal, hepatic, splenic, and ovarian cysts and were hypertensive. Cerebral aneurysms were not reported in this pedigree.[3] These authors reported negative lod scores at *TSC1* and *VHL*. In the pedigree reported by de Almeida *et al*,[4] there were 25 subjects available for linkage analysis with 12 affected subjects in four generations. The phenotypic characteristics of all pedigree members were not presented. Affected subjects were noted to meet recognised ultrasonographic diagnostic criteria, to be variously hypertensive, and to show slow progression to renal replacement therapy. The authors specifically noted the absence of cerebral aneurysms or cerebral haemorrhage in the pedigree.[4] *TSC1* and *VHL* were not excluded by linkage analysis.

In conclusion, this family has an inherited condition similar to classical autosomal dominant polycystic kidney disease, with vertical transmission of disease. This is characterised by renal cysts, hepatic cysts, hypertension, and cerebral aneurysms. Within the pedigree there are no affected males of sufficient age to have allowed male to male transmission to be shown, so the possibility of X linked inheritance cannot be excluded. The family is not linked to the *PKD1*/*TSC2* or *PKD2* loci and supports the case for a third locus for ADPK-like conditions. This depends on us assuming that affected members of this pedigree are suffering from the consequences of the same monogenic disease mutation; it is possible (but unlikely) that the pedigree is a rare coincidental clustering of ADPKD-like phenotypes and that the condition is behaving as an autosomal dominant trait. The maximum and mean simulated lod scores for this pedigree at $\theta=0$ were 2.9 and 1.6, respectively, Thus, the available samples will not be able to show linkage at a lod score >3.

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Detection of heterozygous *SMN1* deletions in SMA families using a simple fluorescent multiplex PCR method

EDITOR—With a prevalence of 1/6000 live births, spinal muscular atrophy (SMA) represents the second most common fatal autosomal recessive disorder after cystic fibrosis.[1 2] SMA is characterised by the degeneration of anterior horn cells of the spinal cord, resulting in progressive, symmetrical limb and trunk paralysis associated with muscular atrophy. This condition is clinically heterogeneous and has been subdivided into three types according to age of onset and clinical course[3]: type I (Werdnig-Hoffmann disease, MIM 253300), type II (intermediate form, MIM 253550), and type III SMA (Kugelberg-Welander disease, MIM 253400). The *SMA* locus has been mapped to chromosome 5q11.2-q13.3 within a region characterised by the large inverted duplication of a 500 kb element.[4–6] The survival motor neurone (*SMN*) gene, which lies within this element, is duplicated and both copies are expressed. The telomeric gene (*SMN1*) has been shown to be deleted or mutated in all three types of SMA.[4] *SMN1* encodes almost the full length transcript whereas the centromeric copy

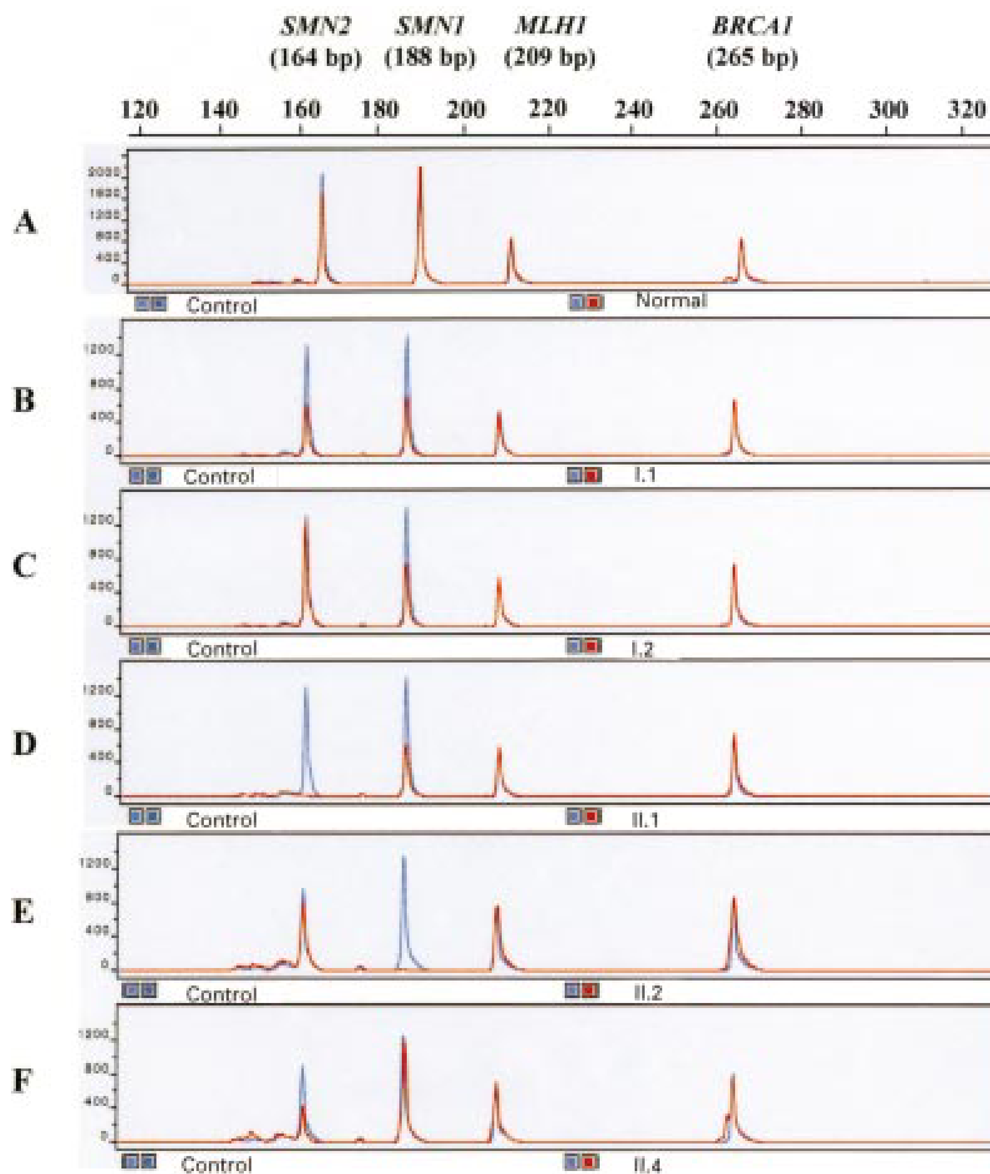

Figure 1 Quantification of SMN1 and SMN2 copies using a non-competitive fluorescent multiplex PCR assay. Genomic fragments were PCR amplified using dye labelled primers (table 1). After DraI digestion, fragments were separated by electrophoresis on an ABI 377 DNA automated sequencer and electropherograms from two subjects were superimposed. In each electropherogram, the y axis displays fluorescence intensity in arbitrary units and the x axis indicates the size (in bp) of the fragments. The expected sizes are indicated. The superimposition of two distinct samples, a control sample with two copies of SMN1 and two of SMN2 (blue) and the analysed sample (red), indicates the number of SMN1 and SMN2 copies. The result of this quantification for one normal subject and five relatives of a SMA family (fig 2) are presented. (A) Normal subject with two copies of SMN1 and two copies of SMN2. (B) Index case's father (I.1) with one copy of SMN1 and one copy of SMN2. (C) Mother (I.2) with one copy of SMN1 and two copies of SMN2. (D) Unaffected sib (II.1) with one copy of SMN1 and no copy of SMN2. (E) Affected sib (II.2) with no copy of SMN1 and two copies of SMN2. (F), Unaffected sib (II.4) with two copies of SMN1 and one copy of SMN2.