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ORIGINAL INVESTIGATION

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A novel locus for Meckel-Gruber syndrome, *MKS3*, maps to chromosome 8q24

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Abstract Meckel-Gruber syndrome (MKS), the most common monogenic cause of neural tube defects, is an autosomal recessive disorder characterised by a combination of renal cysts and variably associated features, including developmental anomalies of the central nervous system (typically encephalcoele), hepatic ductal dysplasia and cysts, and polydactyly. Locus heterogeneity has been demonstrated by the mapping of the *MKS1* locus to 17q21-24 in Finnish kindreds, and of *MKS2* to 11q13 in North African-Middle Eastern cohorts. In the present study, we have investigated the genetic basis of MKS in eight consanguineous kindreds, originating from the Indian sub-continent, that do not show linkage to either *MKS1* or *MKS2*. We report the localisation of a third MKS locus (*MKS3*) to chromosome 8q24 in this cohort by a genome-wide linkage search using autozygosity mapping. We identified a 26-cM region of autozygosity between D8S586 and D8S1108 with a maximum cumulative two-point LOD score at D8S1179 (Z_{max}=3.04 at θ=0.06). A heterogeneity test provided evidence of one unlinked family. Exclusion of this family from multipoint analysis maximised the cumulative multipoint LOD score at locus D8S1128 (Z_{max}=5.65). Furthermore, a heterozygous SNP in *DDEF1*, a putative candidate gene, suggested that *MKS3* mapped within a 15-cM interval. Comparison of the clinical features of *MKS3*-linked cases with reports of *MKS1*- and *MKS2*-linked kindreds suggests that polydactyly (and possibly encephalocele) appear less common in *MKS3*-linked families. **Electronic Supplementary Material** is available if you access this article at http://dx.doi.org/10.1007/s00439-002-0817-0. On that page (frame on the left side), a link takes you directly to the supplementary material.

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Introduction

Autosomal recessive disorders represent an important cause of morbidity and mortality, particularly within the paediatric age range. A number of autosomal recessive conditions reach significant frequency within specific populations and ethnic groups. In Britain this is particularly true for some communities of Indian sub-continent origin, in which consanguinity is frequent (Bundey and Alam 1993). Meckel-Gruber syndrome [MKS (MIM 249000)] is an autosomal recessive lethal malformation syndrome characterised by large multicystic kidneys, central nervous system malformations (classically prosencephalic dysgenesis, occipital encephalocele and rhombic roof dysgenesis), bilateral upper and lower limb postaxial polydactyly and fibrocystic changes of the liver (Ahdab-Barmada and Claassen 1990; Salonen and Paavola 1998). Phenotypic expression is variable, but renal involvement appears to be a consistent finding (Fraser and Lytwyn 1981; Salonen 1984). In addition, hepatic developmental defects (e.g. arrested development of the intrahepatic biliary system and ductal plate malformations) are usually

present if sought (Blankenberg et al. 1987). Survival beyond the perinatal period is unusual. Estimates of the population frequency of MKS vary between 1:13,250 in the USA (Holmes et al. 1976) and 1:140,000 in Great Britain (Seller 1978), whilst higher frequencies have been reported in Finland and North Africa, 1:9,000 and 1:3,500, respectively (Salonen and Paavola 1998). MKS is considered the most frequent syndromic cause of neural tube defects (Simpson et al. 1991).

Locus heterogeneity has been documented previously for MKS. Linkage analysis of a cohort of Finnish MKS kindreds identified a locus (*MKS1*) at 17q21-24 (Paavola et al. 1995). Autozygosity mapping studies using inbred families from the Middle East and North Africa revealed a second locus, *MKS2*, at chromosome 11q13 with additional families evidently not linked to either locus (Roume et al. 1998). *MKS1* or *MKS2* genes at these loci have not been identified. We now report the mapping of a further locus (*MKS3*) to chromosome 8q24 in consanguineous families that originate from Pakistan and Northern India, and present evidence of yet further genetic heterogeneity in this disorder.

Patients and methods

Patients

Eight consanguineous MKS families from Pakistan and Northern India were ascertained through the West Yorkshire, West Midlands and Leicestershire Regional Clinical Genetics Services. Seven families originated from Pakistan and the remaining family (H), with two affected siblings, was from the Gujarat region of Northern India. Anonymised pedigrees of the families are shown in Fig. 1. DNA was available from parents, affected probands and unaffected siblings as shown in Fig. 1. In eight of nine probands, a therapeutic termination of pregnancy was performed following an ultrasound diagnosis of MKS during the second trimester of pregnancy (Silva and Jeantry 2000). One affected foetus was stillborn at 30 weeks. Prior to the start of the study, a diagnosis of MKS was confirmed by an experienced clinical geneticist (R.F.M., C.P.B.) following autopsy. All affected cases had cystic dysplasia of the kidneys and a normal cultured skin fibroblast karyotype. Informed consent was obtained from families and the study was approved by the relevant local research ethics committees (LRECs).

Molecular genetic studies

DNA was isolated from blood and frozen tissue by standard techniques (Müllenbach et al. 1989). Linkage to *MKS1* and *MKS2* was

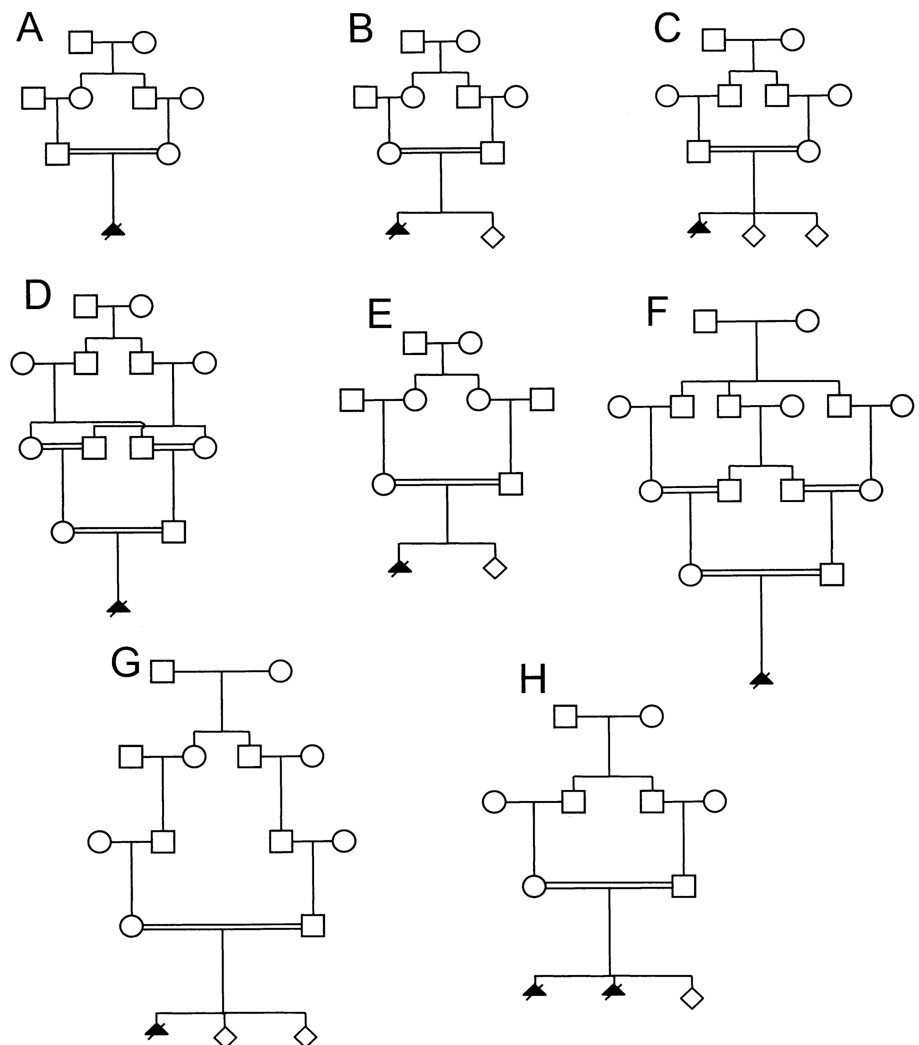

Fig. 1 Pedigrees of eight consanguineous MKS families from Pakistan and Northern India (denoted *A–H*). Affected probands are *shaded*

excluded by the finding of heterozygosity with microsatellite markers spanning *MKS1* and *MKS2* (see later) before a genome-wide linkage screen was undertaken, with 200 fluorescently labelled microsatellite markers from the Research Genetics version 10 mapping panel. PCR amplifications were performed using standard methods. PCR products were analysed on an ABI 377 DNA Analyzer and Genescan v3.1.2 and Genotyper v2.5.2 software (Applied Biosystems). The initial genome-wide screen used only affected probands.

Mutation analysis of candidate genes

Two genes mapping within the *MKS3* target region were selected for mutation analysis. Primers were designed from the *NOVH* (also known as *NOV*) genomic clone X78351-54 (GenBank) and the *DDEF1* BACs AC019167 and AC009682, from intronic sequences surrounding the exons. Primer sequences for the PCR products that span the five coding exons of *NOVH* and the 21 coding exons of *DDEF1* are available as electronic supplementary information from the website for this journal (http://link.springer.de). PCR products from proband samples were sequenced with a BigDye Terminator Cycle Sequencing Kit on an ABI 377 DNA Analyzer (Applied Biosystems).

Statistics

Two-point LOD scores were calculated with the LINKAGE (version 5.1) software package (Cottingham et al. 1993), assuming a fully penetrant autosomal recessive gene with a disease allele frequency of 0.001. Alleles for marker loci were assumed to be codominant and to occur at equal frequencies, because population allele frequencies were not available. Multipoint LOD scores were calculated using LINKMAP (version 5.1) software, again with assumption of equal allele frequencies.

Results

Exclusion of linkage to *MKS1* and *MKS2*

Eight nuclear families were typed with microsatellite markers spanning *MKS1* (D172180, D171606, D17S1290, D17S807, D17S2193, D17S1301 and D17S2195) over 40 cM and *MKS2* (D11S2006, D11S2371, D11S4079, D11S937, D11S2002 and D11S2000) over 42 cM. In each kindred there was no evidence of linkage to either *MKS1* or *MKS2*, with cumulative two-point LOD scores $Z<-2$ for all spanning markers (data not shown).

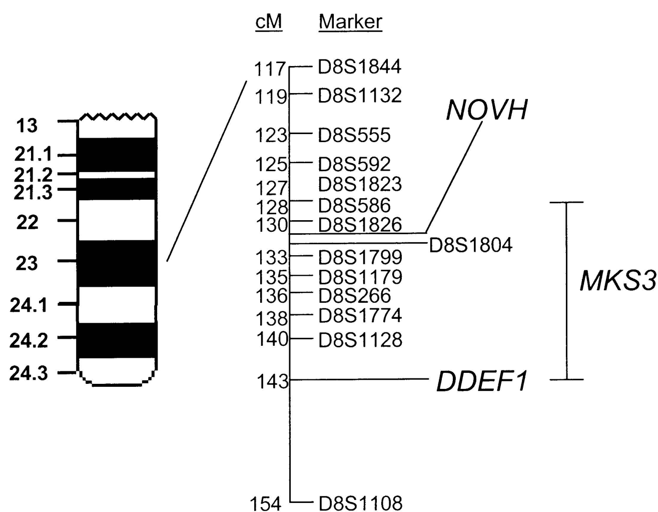

Fig. 2 Linkage map of microsatellite markers at chromosome 8q23-24. The locations of two candidate genes, *NOVH* and *DDEF1* are indicated. Approximate genetic distances in centiMorgans (cM) of the markers are taken from the Marshfield genetic map

Genome-wide linkage screen

After linkage to *MKS1* and *MKS2* was excluded, we performed a genome-wide search in the eight affected probands with 200 microsatellite markers, at an average spacing of 20 cM. At this stage, a region of homozygosity on chromosome 8q24 was apparent. Fourteen microsatellite

Table 1 Cumulative two-point LOD scores for the *MKS3* locus in a cohort of eight Pakistani and Northern Indian families with markers from chromosome 8q24

Locus	LOD score at θ=					
	Family	0.00	0.050	0.100	0.200	0.300
D8S1804	A	0.598	0.506	0.417	0.258	0.135
	B	0.848	0.712	0.581	0.348	0.176
	C	–∞	–0.165	0.004	0.071	0.057
	D	0.641	0.535	0.435	0.260	0.132
	E	0.422	0.352	0.285	0.170	0.086
	F	–1.251	–0.495	–0.286	–0.114	–0.045
	G	–1.344	–0.360	–0.170	–0.044	–0.009
	H	–∞	–0.620	–0.365	–0.156	–0.068
	Total	–∞	0.464	0.901	0.793	0.463
D8S1799	A	0.630	0.535	0.443	0.277	0.146
	B	0.454	0.381	0.311	0.188	0.096
	C	0.454	0.381	0.311	0.188	0.096
	D	0.762	0.631	0.508	0.294	0.142
	E	0.444	0.370	0.300	0.172	0.075
	F	0.762	0.631	0.508	0.294	0.142
	G	–1.302	–0.536	–0.318	–0.131	–0.053
	H	–∞	–1.299	–0.763	–0.310	–0.117
	Total	–∞	1.096	1.300	0.971	0.526
D8S1179	A	0.695	0.596	0.498	0.318	0.171
	B	0.820	0.679	0.543	0.302	0.127
	C	0.644	0.555	0.466	0.300	0.163
	D	0.882	0.745	0.611	0.371	0.188
	E	0.983	0.840	0.700	0.442	0.235
	F	–1.304	–0.277	–0.092	0.013	0.023
	G	1.189	1.008	0.829	0.498	0.240
	H	–∞	–1.139	–0.655	–0.267	–0.103
	Total	–∞	3.005	2.901	1.977	1.043
D8S266	A	0.620	0.526	0.435	0.271	0.142
	B	0.897	0.758	0.624	0.381	0.197
	C	0.583	0.498	0.415	0.262	0.140
	D	0.744	0.615	0.493	0.283	0.136
	E	0.745	0.640	0.536	0.345	0.187
	F	–1.291	–0.318	–0.130	–0.013	0.008
	G	0.091	0.010	0.093	0.063	0.033
	H	–∞	–1.216	–0.708	–0.281	–0.101
	Total	–∞	1.603	1.758	1.310	0.743

Table 2 Genotypes in affected individuals of markers spanning the *MKS3* locus on chromosome 8q23-24 (*n.t.* not tested)

cM	Family proband																		
	Markers	A		B		C		D		E		F		G		H1		H2	
117	D8S1844	4	4	4	4	2	2	6	1	1	1	4	1	6	6	1	2	n.t.	n.t.
119	D8S1132	2	2	7	7	5	5	6	4	3	3	3	3	n.t.	n.t.	8	6	n.t.	n.t.
123	D8S555	7	7	7	7	5	5	5	4	4	4	5	5	5	5	5	2	n.t.	n.t.
125	D8S592	3	3	3	3	2	2	2	3	3	3	1	2	2	2	2	2	n.t.	n.t.
127	D8S1823	7	7	4	4	1	4	4	4	7	7	7	4	n.t.	n.t.	4	4	n.t.	n.t.
128	D8S586	2	2	2	2	2	1	2	2	7	7	2	1	n.t.	n.t.	7	2	n.t.	n.t.
130	D8S1826	11	11	12	12	11	11	11	11	10	10	10	11	12	12	11	10	12	12
132	D8S1804	1	1	3	3	2	2	1	1	5	5	4	1	n.t.	n.t.	1	1	4	1
133	D8S1799	20	20	9	9	20	20	15	15	19	19	9	9	n.t.	n.t.	9	9	15	15
135	D8S1179	7	7	3	3	8	8	8	8	4	4	3	6	5	5	8	5	5	6
136	D8S266	6	6	3	3	4	4	6	6	6	6	4	7	n.t.	n.t.	7	5	n.t.	n.t.
138	D8S1774	16	16	15	15	15	15	16	16	15	15	3	16	n.t.	n.t.	13	16	n.t.	n.t.
140	D8S1128	4	4	6	6	1	1	6	6	1	1	6	3	n.t.	n.t.	1	6	n.t.	n.t.
154	D8S1108	4	4	4	6	4	5	4	4	3	4	4	4	n.t.	n.t.	3	4	n.t.	n.t.

markers (see Fig. 2) mapping to 8q23-24 were genotyped to further investigate the candidate region. Markers were analysed in all available family members (probands, in addition to unaffected parents and siblings) in order to phase haplotypes. Significant linkage was detected at D8S1179 (Z_{max}=3.04 at θ=0.06; refer to Table 1). Genotype results for each proband are shown in Table 2. Genotypes for kindred C placed the *MKS3* locus in a 28-cM interval between D8S586 and D8S1108. The Smith admixture test (Smith 1963) was performed to test for heterogeneity. The estimated proportion of families consistent with linkage to 8q23-24 was 0.70. Rejection of homogeneity was mainly due to kindred H, since $Z<-2$ for all markers in the critical interval (see Tables 1 and 2), and each affected sibling had different haplotypes. This excludes linkage of kindred H to the *MKS3* locus. To provide details on the likely location of *MKS3*, we excluded family H and performed a multipoint analysis. A maximum cumulative multipoint LOD score was obtained at locus D8S1128 (Z_{max}=5.65; Fig. 3). Family F showed a small region of autozygosity at D8S1799 that needs further investigation, as it would significantly reduce the MKS critical interval. Inspection of haplotypes in families A to G did not show evidence of a founder mutation (Table 2). For marker D8S586, homozygous alleles were found to be common to three families (A, B and D). Allele 11 for marker D8S1826 was homozygous and common to families A, C and D; allele 12 was homozygous in two other families, and family H which is unlinked to *MKS3* (Table 2).

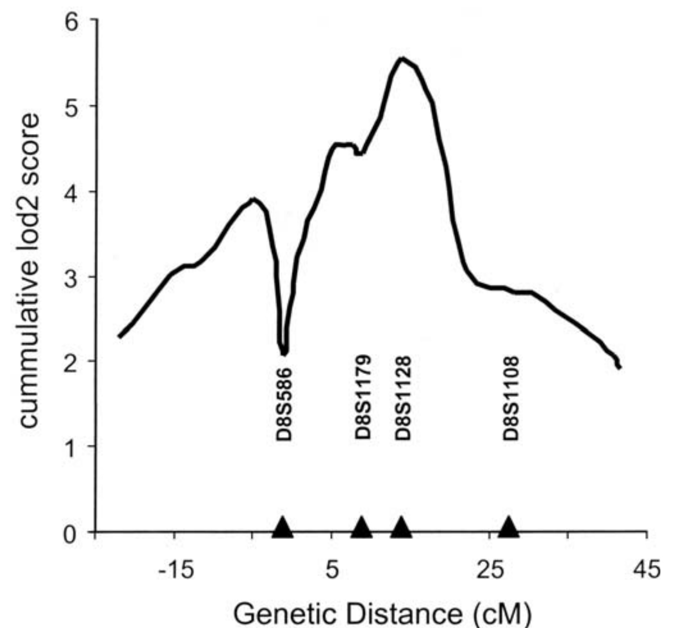

Fig. 3 Cumulative multipoint analysis of four chromosome 8 markers in MKS families. Family H was excluded from the analysis

Candidate gene analysis

Two potential candidate genes, *NOVH* (nephroblastoma overexpressed gene) and *DDEF1* (development and differentiation enhancing factor 1 gene), were analysed for germline mutations. Sequencing of the five coding exons and adjacent splice sites of *NOVH* did not reveal any sequence variants. Sequence analysis of *DDEF1* revealed a single heterozygous SNP in intron 15 of the gene (IVS15–102 G>A), in the proband of family E. This suggested that the *MKS3* locus mapped between D8S586 and *DDEF1*, an interval of approximately 15 cM (see Fig. 2).

Discussion

Autozygosity mapping in inbred families is a powerful strategy for localising recessive genes, even in the presence of locus heterogeneity (Lander and Botstein 1987; Mueller and Bishop 1993; Gschwend et al. 1996). In ad-

dition to the mapping of *MKS2* in North African and Middle-Eastern kindreds, Roume et al. (1998) reported one Tunisian kindred unlinked to either *MKS1* or *MKS2*. To reduce the likelihood of locus heterogeneity in our cohort, we studied eight MKS families from the Indian sub-continent, in which linkage to *MKS1* and *MKS2* was excluded. Our results suggest that mutations in *MKS3* account for most cases of Meckel-Gruber syndrome within the British ethnic population that has originated from the Indian sub-continent, in which MKS is comparatively common (Young et al. 1986). We note that Roume et al. (1998) reported one MKS kindred linked to *MKS2* that was of Pakistani origin, and we also report one kindred from Northern India (family H) that did not show evidence of linkage to *MKS1*, *MKS2* or *MKS3*. This suggests that there may be further locus heterogeneity in both the Pakistani and Northern Indian populations.

MKS demonstrates marked intra- and inter-familial phenotypic variability. Although strict diagnostic criteria requiring the presence of a triad of major features (cystic kidneys, CNS malformation, usually occipital encphalocele, and polydactyly) have been proposed, in practice this definition is likely to be conservative. Comparison of probands and sibs in MKS kindreds revealed that, in general, sibs showed lesser degrees of malformation and that the only common feature in 65 patients was renal cystic dysplasia (Fraser and Lytwyn 1981). A similar conclusion was reached by Salonen (1984), but in addition, delayed development of the intrahepatic biliary tree (ductal plate malformation) also appeared to be a constant feature of MKS when the liver has been examined (Salonen 1984; Blankenberg et al 1987). In the study by Fraser and Lytwyn (1981), the criteria for selection of probands was renal cystic dysplasia, CNS malformation and at least one other anomaly known to occur in the syndrome. This study noted that the *siblings* of the probands had a broader phenotypic spectrum, with only 63% of siblings presenting with encephalocele (vs 89% in the probands) and 55% polydactyly (vs 85% in probands) (Fraser and Lytwyn 1981). Among the nine cases of our cohort, all had cystic dysplastic kidneys, seven had a CNS malformation (five of the seven with encephalocele) and one proband had polydactyly. In the *MKS1*-linked families described by Paavola et al. (1995), all had cystic dysplastic kidneys, a CNS malformation and polydactyly. The *MKS2*-linked families that were reported by Roume et al. (1998) were selected for linkage analysis by the presence of a triad of classical CNS abnormalities (prosencephalic dysgenesis, occipital exencephalocele and rhombic roof dysgenesis); renal cystic dysplasia and polydactyly were also reported to be present in all cases. Although the incidence of CNS malformations and polydactyly is less in *MKS3*-linked cases than in *MKS1*- and *MKS2*-linked cases, it is not clear if this reflects differing criteria for selection for linkage studies, or a real genotype-phenotype correlation. Phenotypic overlap between MKS and other cerebro-acro-visceral syndromes has been observed (Brueton et al. 1990; Lurie et al. 1991; Walpole et al. 1991; Verloes et al. 1991), but no other similar disorder has been mapped at chromosome 8q.

We have mapped a novel disease locus, which we have termed *MKS3*, to a 15-cM interval between D8S586 and *DDEF1* at chromosome 8q24. There are >50 genes in the *MKS3* critical interval, and none of these appear obvious candidates for MKS. Among the genes that we have excluded as *MKS3* are *NOVH* (nephroblastoma overexpressed) and *DDEF1* (development and differentiation enhancing factor 1), which both map within the target interval for *MKS3*. *NOVH* is thought to have a role in the control of cell proliferation and has also been shown to bind extracellular matrix proteins such as fibulin 1C (Perbal et al. 1999). To assess *NOVH* as a candidate gene, we undertook mutation analysis by direct sequencing in the eight probands (see Materials an methods), but did not detect any sequence variants. However, sequencing of *DDEF1* revealed a heterozygous sequence change in the intronic sequence in one proband. Since all SNPs within the critical interval should be identical-by-descent, and hence homozygous, the *MKS3* candidate region was reduced to 15 cM.

A functional role for mediating extracellular interactions by the MKS protein might be suggested by comparison with the putative functional roles of fibrocystin and nephrocystin. Fibrocystin, a putative receptor protein, is encoded by the gene mutated in autosomal recessive polycystic kidney disease (Ward et al. 2002), a monogenic fibrocystic syndrome similar to MKS. Nephrocystin, the product of the nephronophthisis type I gene (*NPHP1*), appears have a role in mediating cell adhesion (Hildebrandt and Omram 2001). Nephronophthisis, at least for the juvenile or type I form, manifests with cyst formation in the kidneys and occasional hepatic fibrosis, and therefore also resembles MKS.

The mapping of *MKS3* will enable molecular genetic testing to be offered to large families that can be confidently linked to *MKS3*. This is particularly important for British families, as the phenotypic spectrum for MKS appears to be broad and could create problems in genetic counselling and prenatal diagnosis in the absence of a reliable diagnostic test. To narrow the critical interval we plan to ascertain and investigate additional families. The genotyping of further microsatellite markers and SNPs within the critical interval may identify evidence for a common ancestral haplotype in additional families. To date, no MKS genes have been identified and the identification of *MKS3* will provide key insights into important developmental pathways, and may facilitate the identification of *MKS1* and *MKS2*.

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