Mutations in *MKKS* cause Bardet-Biedl syndrome

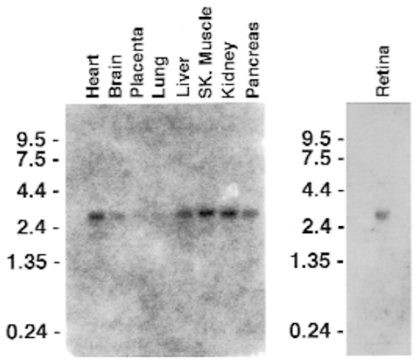

Fig. 1 Human multiple tissue northern blots analysed with a probe from exons 3-6 of the full-length *MKKS* cDNA showing a 2.4-kb transcript in tissues affected by BBS. The numbering of mutations is based on the 5′ end of the *MKKS* cDNA with exon 1B (Genbank AF221993).

Bardet-Biedl syndrome (BBS) is an autosomal recessive disorder with locus heterogeneity[5–9]. None of the 'responsible' genes have previously been identified. Some BBS cases (approximately 10%) remain unassigned to the five previously mapped loci[10]. McKusick-Kaufma syndrome (MKS) includes hydrometrocolpos, postaxial polydactyly and congenital heart disease, and is also inherited in an autosomal recessive manner[11,12]. We ascertained 34 unrelated probands with classic features of BBS including retinitis pigmentosa (RP), obesity and polydactyly. The probands were from families unsuitable for linkage because of family size. We found *MKKS* mutations in four typical BBS probands (Table 1). The first is a 13-year-old Hispanic girl with severe RP, PAP, mental retardation and obesity (BMI >40). She was a compound heterozygote for a missense (1042G→A, G52D) and a nonsense (1679T→A, Y264stop) mutation in exon 3. Cloning and sequencing of the separate alleles confirmed that the mutations were present in *trans*. A second BBS proband (from Newfoundland), born to consanguineous parents, was homozygous for two deletions (1316delC and 1324-1326delGTA) in exon 3, predicting a frameshift. An affected brother was also homozygous for the deletions, whereas an unaffected sibling had two normal copies of *MKKS*. Both the proband and her affected brother had RP, PAP, mild mental retardation, morbid obesity (BMI >50 and 37, respectively), lobulated kidneys with prominent calyces and diabetes mellitus (diagnosed at ages 33 and 30, respectively). A deceased sister (DNA unavailable) had similar phenotypic features (RP with blindness by age 13, BMI >45, abnormal glucose tolerance test and IQ=64, vaginal atresia and syndactyly of both feet). Both parents and the maternal grandfather were heterozygous for the deletions. Genotyping with markers from the *MKKS* region[12] confirmed homozygosity at 20p12 in both affected individuals.

A third family included a 4-year-old male proband (the offspring of consanguineous parents) with reduced visual acuity, PAP, obesity and cystic kidneys, and a sibling with hypospadias, PAP, obesity and lobular cystic kidneys who died at age 18 months (eye examination and DNA were unavailable). A fourth BBS family consisted of a female proband diagnosed at age five years because of severe RP, PAP, morbid obesity and diabetes mellitus, and a male sibling with RP, PAP, obesity, lobulated cystic kidneys and diabetes mellitus. Although not known to be related, both families are from the same region of Newfoundland. Sequencing revealed that the three affected individuals in these two families from whom DNA is available are homozygous for a frameshift mutation (1167 delT). Both parents of family 3 and the mother of family 4 were heterozygous for this mutation. No DNA was available from the father of family 4. Families 3 and 4 share a haplotype of 5 SNPs within *MKKS* and 5 STRPs flanking the gene, indicating that they probably inherited the 1167delT mutation from a common ancestor. The mutations reported here were not found in 102 Northern European control chromosomes[13] or, in the case of the mutations in family 1, in 102 chromosomes from Hispanic controls. *MKKS* is expressed in tissues affected by BBS, including retina, pancreas, brain and kidney (Fig. 1).

MKKS represents a sixth locus for BBS and is the first gene shown to be mutated in patients with BBS. Mutations in *MKKS* cause MKS in the Amish[4], in whom retinal degeneration, obesity and learning disability have not been reported[12,14]. We hypothesize that MKS is a distinct phenotype caused by hypomorphic alleles of *MKKS*, whereas BBS is caused by null alleles. Although the numbers are small, it is of interest that all known MKS patients have at least one missense mutation, whereas homozygous frameshift mutations are present in three BBS families. Alternatively, amino-acid polymorphisms in *MKKS* (ref. 4) or variations in the promoter region may influence the phenotype. The frequency of *MKKS* mutations in BBS patients (4/34 =11.8%; 95% CI 1–24%) is consistent with the percentage of cases unassigned to the five previously known loci.

Identification of *MKKS* as a BBS gene may assist in the identification of other BBS genes. We hypothesize that the clinical features of BBS may be caused by the inability of the MKKS putative chaperonin to maintain protein integrity in the retina, brain, pancreas and other organs. Our results suggest that genes encoding chaperonins and their substrates are candidates for other BBS loci, RP, diabetes, obesity and mental retardation.

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Table 1 • Diagnosis of BBS and MKS

Clinical feature	Primary (P) or secondary (S) diagnostic criteria for BBS	Major diagnostic criteria for MKS	Family 1	Family 2	Family 3	Family 4
pigmentary retinopathy	P		+	+	+	+
polydactyly	P	+	+	+	+	+
obesity	P		+	+	+	+
learning disability	P		+	+	+	–
hypogonadism	P		–	–	–	–
renal anomalies	P		–	+	+	+
diabetes mellitus	S		–	+	–	+
hydrometrocolpos		+	–	–	–	–
congenital heart disease	S	+	–	–	–	–

A diagnosis of BBS requires four primary features or three primary features plus two secondary features[2]. A diagnosis of MKS requires all three major criteria[12].

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Methylation of the *CDH1* promoter as the second genetic hit in hereditary diffuse gastric cancer

Aberrant promoter methylation and the associated loss of gene expression is a common accompaniment of human cancers[1]. Nonetheless, it has been challenging to demonstrate in any given tumour that methylation of a specific gene was causal and not consequent to malignant transformation. In this regard, our attention was drawn to the genesis of gastric cancers in individuals with hereditary diffuse gastric cancer (HDGC). These individuals harbour germline mutations in the gene encoding E-cadherin, *CDH1* (refs 2–4), but their cancers have consistently demonstrated absence of loss of heterozygosity at the *CDH1* locus[2–4]. These findings suggested the hypothesis that *CDH1* promoter methylation might function as the 'second genetic hit' in the genesis of these cancers.

Our study was carried out in two previously identified HDGC kindreds bearing known germline *CDH1* mutations[4] (Fig. 1*a*). Immunohistochemistry showed absence of E-cadherin protein expression from five of six gastric cancers from these families (Fig. 2*d–f*), suggesting these

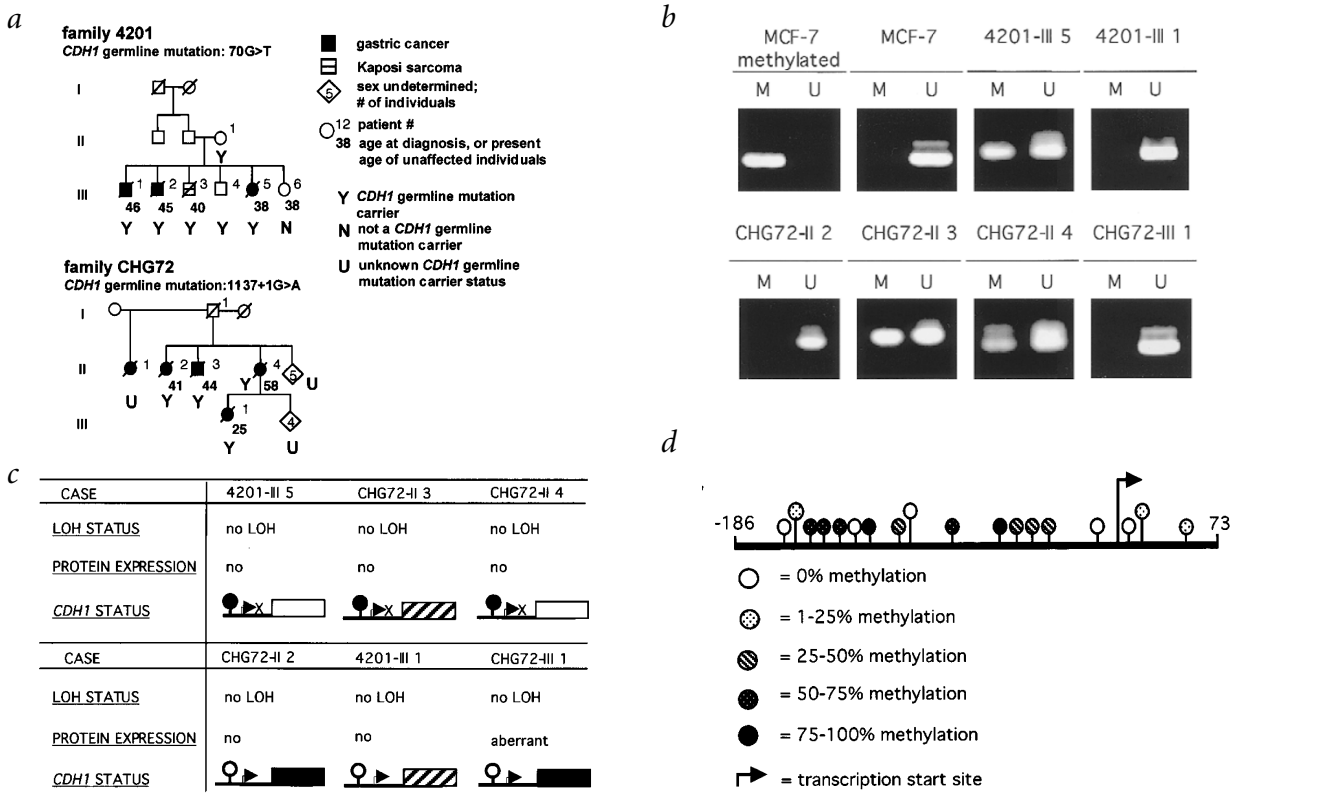

Fig. 1 *CDH1* inactivation in HDGC tumours. ***a***, Pedigrees of families 4201 and CHG72. 'Y', 'N' and 'U' indicate *CDH1* germline mutations as present, absent or undetermined. ***b***, Methylation-specific PCR (ref. 8) of the *CDH1* promoter[5,10]. Following sodium bisulphite treatment of genomic DNA, the *CDH1* promoter was amplified using PCR primers specific for methylated (M) or unmethylated (U) promoters. *CDH1* methylation is demonstrated in HDGC tumours 4201-5, CHG72-II-3 and CHG72-II-4. 'MCF7 methylated' and 'MCF7' are positive and negative controls. ***c***, HDGC tumour summary showing *CDH1*-LOH, E-cadherin expression, *CDH1* promoter methylation (filled circle, methylated; open circle, unmethylated) and *CDH1* sequence (open box, wild type; filled box, somatic mutation; diagonal hashed box, unknown). 'X' designates presumptive silencing of methylated promoters. ***d***, Map of the methylation status of the CpG dinucleotides (circles) located in the *CDH1* promoter.