ONLINE MUTATION REPORT

Further support for digenic inheritance in Bardet-Biedl syndrome

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Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterised by the primary features of obesity, retinal dystrophy, polydactyly, renal malformations, mental retardation, and hypogenitalism. Patients with BBS also have an increased risk for developing diabetes mellitus, hypertension, and congenital heart disease. Seven loci have been mapped with evidence of at least one additional locus[1]: 11q13 (*BBS1*),[2] 16q21 (*BBS2*),[3] 3p13 p12 (*BBS3*),[4] 15q22.3q23 (*BBS4*),[5] 2q31 (*BBS5*),[6] 20p12 (*BBS6*),[7] and 4q27 (*BBS7*).[8] Five genes have been cloned so far: *BBS1*,[9] *BBS2*,[10] *BBS4*,[11] *MKKS* (*BBS6*),[7 12 13] and *BBS7*.[8] The function of these genes and the disease mechanism remain unclear. Whereas the BBS6 protein has similarity to a bacterial chaperonin, the other BBS proteins have no significant similarity to archebacterial chaperonins or other known proteins.

Before the *BBS1* gene had been cloned, a report had suggested that three mutated alleles (two at one locus, and a third at a second locus) may be required for manifestation of BBS, a so called triallelic inheritance.[14] Also, many cases with only one mutant allele suggested an unusual mechanism of inheritance.[15]

In this study, we have analysed whether there is evidence for multiallelic inheritance in patients with BBS by sequencing the complete coding region and exon-intron boundaries of four cloned *BBS* genes (*BBS1, BBS2, BBS4,* and *MKKS*) which represent most of the mapped loci. The study was completed before the gene *BBS7* was published and thus it is not included in the analysis. As *BBS7* seems to be a minor locus, this will have little effect on the outcome.

PATIENTS AND METHODS

Twenty-one unrelated European patients with the clinical diagnosis of BBS were used in the study. The diagnosis was based on the presence of at least three of the major features of BBS (obesity, retinal dystrophy, polydactyly, renal malformations, mental retardation, and hypogonadism). Genomic DNA was prepared from peripheral blood by a standard salting out procedure.

Mutation screening was performed for *BBS1, BBS2*, *BBS4*, and *MKKS*. The analysis was done by polymerase chain reaction (PCR) amplification and sequencing of genomic DNA. A complete list of primers used for PCR and sequencing is available from the authors.

A PCR protocol was carried out using 100 ng DNA and 10 pmol of each primer in a standard 50 µl reaction. The profile used for amplification in a GeneAmp 2400 PCR cycler was two minutes at 94°C, 37 cycles at 94°C for 15 seconds, 48°C for 30 seconds, 72°C for 30 seconds, and a final extension step at 72°C for two minutes. Direct sequencing of PCR products was carried out using the ABI Prism BigDye Terminator Cycle Sequencing ready reaction kit (Applied Biosystems) and an ABI automated DNA sequencer 310 (Applied Biosystems).

RESULTS AND DISCUSSION

Direct sequencing of the *BBS1* gene in 21 patients led to the identification of mutations in five (24%) (table 1). This confirms that mutations in *BBS1* account for most cases of BBS among the six mapped loci. Our finding is comparable with a report that linked mutations in *BBS1* to 32% of patients with BBS.[16] The previously described M390R mutation was found in all patients on at least one allele (allele frequency 0.14). Among North American patients an allele frequency of 0.32 was found.[9] One patient was heterozygous for the L518P mutation.

Two new splice site mutations in *BBS1* were identified at the splice donor site at the end of exon 9 and 10, respectively. Both mutations were found in affected patients together with the M390R mutation.

All available family members were included in the analysis. Consistent with an autosomal recessive model of inheritance,

Key points

- We have analysed whether there is evidence for multiallelic inheritance in patients with Bardet-Biedl syndrome (BBS) by sequencing the complete coding region and exon-intron boundaries of four cloned BBS genes (*BBS1, BBS2, BBS4, MKKS*) which represent most of the mapped loci. Twenty one unrelated European patients were studied.
- Mutations were identified in the *BBS1* gene in five (24%) patients, two novel mutations were found in *BBS2* and *BBS6* (*MKKS*), and one mutation in *BBS4*. Overall, mutations were found in nine (43%) patients suggesting that either a large number of cryptic mutations are present in these genes or a sizeable proportion of genes have yet to be identified.
- Five patients had mutations in *BBS1* and each had at least one M390R mutant allele in combination with a second mutation. This confirms that mutations in *BBS1* account for most cases of BBS among the mapped loci. No support for the involvement of *BBS1* in triallelic inheritance was found.
- The remaining four patients had mutations in *BBS2*, *BBS4*, or *MKKS*. However, in three of these four patients, two mutant alleles were not present in the same gene. Two patients had one mutation in two different *BBS* genes (*BBS2* and *BBS4*), one patient had only one mutated allele, in *MKKS*. This strongly supports a digenic diallelic pattern of inheritance in two if not three patients. This phenomenon would also be expected if a three allele hypthesis were correct. These data add to the increasing evidence that BBS has a complex mode of inheritance.

Abbreviations: BBS, Bardet-Biedl syndrome; PCR, polymerase chain reaction

Table 1 Mutations found in *BBS1*, *BBS2*, *BBS4*, and *MKKS*

Patient	Gene	Exon	Nucleotide change	Predicted effect	Reference
1	BBS1	12	1169T>G	M390R	9
	BBS1	15	1552T>C	L518P	16
2	BBS1	10	IVS10+1G>A	Splice site	
	BBS1	12	1169T>G	M390R	9
3	BBS1	12	1169T>G	M390R	9
	BBS1	12	1169T>G	M390R	9
4	BBS1	9	IVS9+1G>A	Splice site	
	BBS1	12	1169T>G	M390R	9
5	BBS1	5	436C>T	R146X	17
	BBS1	12	1169T>G	M390R	9
6	BBS2	11	1237C>T	R413X	
	BBS4	16	1508C>T	P503L	
7	BBS2	16	1928G>A	R643H	
	BBS4	3	147A>G	K46R	16
8	MKKS	3	541G>C	A181P	
	MKKS	3	541G>C	A181P	
9	MKKS	6	1474G>A	D492N	

Table 2 Evolutionary conservation of BBS genes surrounding mutation sites showing local alignment of amino acid sequence A, *BBS2*. B, *BBS4*. C, *MKKS* (the number indicates the position of the missense mutation)

A		
BBS2	643	
HS	KTMKS R YMELY	
MM	KTMKS R YMELY	
DR	RNMKK R YIELY	
B		
BBS4	46	503
HS	LHYIR K DYEAC	EPE P AVESS
MM	LHYIR K DYEAC	EPEPE P TVEAS
AG	GLYTR K HFEQC	
DM	IYFTR R REFTR	
C		
MKKS	181	492
HS	ALILR A FLLTI	VANWP D LLSQC
MM	ALILK A FLLTI	VGNWS D TLSRC
DR	SLITQ A FLYSI	ISSQT E VKHTC

HS, *Homo sapiens*; MM, *Mus musculus*; DR, *Danio rerio*; DM, *Drosophila melanogaster*; AM, *Anopheles gambiae*.

unaffected family members of three patients were either heterozygous for a mutant allele or had wild type alleles. An affected sister of one patient showed the same mutations as her sib.

In *BBS2*, two novel mutations were found and in both cases only one mutant allele was identified. The two mutations were R643H and R413X. One of the mutations introduces a stop codon, the other one changes a highly conserved amino acid (table 2) and was not found among 60 controls. The R643H mutation occurred in combination with the sequence change K46R in *BBS4*. K46R was suggested to be a polymorphism.[16] The other patient was a compound heterozygote for R143X (*BBS2*) and P503L (*BBS4*). P503L is a highly conserved residue and was not found in 60 controls. Eight out of 21 patients had the I123V polymorphism. In *BBS4*, two polymorphisms were found: V284A and T354I.[16]

In *MKKS* two novel mutations were found, a homozygous A181P mutation and a heterozygous D492N mutation. Both mutations occur at highly conserved residues and were not present among 60 healthy controls. No second mutation was identified in the patient with the D492N mutation. In two patients, two polymorphisms (R517C and G532V) were found in *MKKS*.[16]

Among our patients we cannot find support for the hypothesis that *BBS1* is involved in triallelic inheritance. The disease segregated as an autosomal recessive disorder including the duplex family. Although we cannot rule out the possibility that the patients have a mutation in one of the unidentified genes (*BBS3* or *BBS5*), this would be unlikely as the remaining genes account only for a very small proportion.[1] Another recent report came to a similar conclusion.[16] Because of the relatively few patients, this study may have missed (rare) cases where *BBS1* is involved in triallelism. Another recent study indicates that *BBS1* can participate in triallelic inheritance but that other BBS loci, especially *BBS2* and *BBS6*, participate more often in this mode of inheritance.[17]

Apart from the five patients with mutations in *BBS1*, four other patients harboured mutations in *BBS2*, *BBS4*, or *MKKS*. However, in three of these patients, two mutant alleles were not present in the same gene. Two patients had one mutant allele in two genes (*BBS2* and *BBS4*), suggesting a digenic diallelic mode of inheritance. If in one of these cases the K46R (*BBS4*) mutation is considered a non-disease causing sequence change, then this patient would harbour only one mutant allele in four *BBS* genes. Another patient was identified with only one mutant allele in *MKKS*. The detection of only one mutant allele was reported in 10 cases in *MKKS*.[15] One possibility is that additional mutations were not detected with the methodology used. These mutations would include changes in the promoter region, in introns, or in additional exons. But additonal unidentified mutations would not account for the fact that patients were found with two mutations in two different genes associated with BBS. Although these data do not support triallelic inheritance per se, they strongly support multiallelic inheritance. It provides good evidence for digenic diallelic inheritance in BBS. This phenomenon would also be expected if the three allele hypothesis were correct. Particularly, the patient with the two significant mutations R143X (*BBS2*) and P503L (*BBS4*) would be a strong indication for diallelic digenic inheritance.

Another interesting finding is the relatively high percentage of patients with no mutations. At least one mutation was found in nine out of 21 patients (43%). The two remaining mapped loci (*BBS3* and *BBS5*) should only account for a very small percentage of cases. Either a large number of cryptic mutations are present in the five known genes or additional unidentified loci are mutated in most cases.

There is increasing evidence that BBS is involved in a complex mode of inheritance. This is confirmed by the results of our study, which support a digenic mode of inheritance. Experiments of gene interactions will be required to understand the exact mechanism of the disease.

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