Further support for digenic inheritance in Bardet-Biedl syndrome

S Fauser, M Munz, D Besch

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 0.32). Among North American patients an allele frequency of 0.14. Among North American patients an allele frequency of 0.32 was found.

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 hypothesis 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

ONLINE MUTATION REPORT

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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 hypogonadism. 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: 11q13 (BBS1), 16q21 (BBS2), 3p13 p12 (BBS3), 15q22.3q23 (MKKS), 7p12 (BBS5), 20p12 (BBS6), and 4q27 (BBS7). Five genes have been cloned so far: BBS1, BBS2, BBS4, MKKS (BBS6), and BBS7. 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 archaeobacterial 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 not have little effect on the outcome.

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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. Another recent report came to a similar conclusion. 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.

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. 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 additional 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.

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

<table>
<thead>
<tr>
<th>Patient</th>
<th>Gene</th>
<th>Exon</th>
<th>Nucleotide change</th>
<th>Predicted effect</th>
<th>Reference</th>
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<tbody>
<tr>
<td>1</td>
<td>BBS1</td>
<td>12</td>
<td>1169T&gt;G</td>
<td>M390R</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>BBS1</td>
<td>15</td>
<td>1552T&gt;C</td>
<td>L518P</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>BBS1</td>
<td>10</td>
<td>IVS10+1G&gt;A</td>
<td>Splice site</td>
<td></td>
</tr>
<tr>
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<td>BBS1</td>
<td>9</td>
<td>IVS9+1G&gt;A</td>
<td>Splice site</td>
<td></td>
</tr>
<tr>
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<td>BBS1</td>
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<td>436C&gt;T</td>
<td>R146X</td>
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<tr>
<td>6</td>
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<td>12</td>
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<td>M390R</td>
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</tr>
<tr>
<td>7</td>
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<tr>
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<td>MKKS</td>
<td>3</td>
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<td>A181P</td>
<td></td>
</tr>
<tr>
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<td>MKKS</td>
<td>6</td>
<td>1474G&gt;A</td>
<td>D492N</td>
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</tr>
</tbody>
</table>

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)

<table>
<thead>
<tr>
<th>Gene</th>
<th>Amino acid change</th>
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<tr>
<td>BBS2</td>
<td>643</td>
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<td>HS</td>
<td>K/TKMK S YMELY</td>
<td></td>
</tr>
<tr>
<td>MM</td>
<td>K/TKMK S YMELY</td>
<td></td>
</tr>
<tr>
<td>DR</td>
<td>R/CAKK R YIELY</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>46</td>
<td>503</td>
</tr>
<tr>
<td>HS</td>
<td>I/ILYR K DYEAC</td>
<td>EPE P AVES</td>
</tr>
<tr>
<td>MM</td>
<td>I/ILYR K DYEAC</td>
<td>EPE PE P YEA</td>
</tr>
<tr>
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<td>G/YTR K HFEQC</td>
<td></td>
</tr>
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<td>DM</td>
<td>I/YTR R REFR</td>
<td></td>
</tr>
<tr>
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<td>181</td>
<td>492</td>
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<td>A/ALIK A FLTI</td>
<td>VANWP D LLSQC</td>
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<td>DR</td>
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<td>ISSQT E VKHTC</td>
</tr>
</tbody>
</table>

**REFERENCES**


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