Triallelic Inheritance in Bardet-Biedl Syndrome, a Mendelian Recessive Disorder


Bardet-Biedl syndrome (BBS) is a genetically heterogeneous disorder characterized by multiple clinical features that include pigmentary retinal dystrophy, polydactyly, obesity, developmental delay, and renal defects. BBS is considered an autosomal recessive disorder, and recent positional cloning efforts have identified two BBS genes (BBS2 and BBS6). We screened our cohort of 163 BBS families for mutations in both BBS2 and BBS6 and report the presence of three mutant alleles in affected individuals in four pedigrees. In addition, we detected unaffected individuals in two pedigrees who carry two BBS2 mutations but not a BBS6 mutation. We therefore propose that BBS may not be a single-gene recessive disorder but a complex trait requiring three mutant alleles to manifest the phenotype. This triallelic model of disease transmission may be important in the study of both Mendelian and multifactorial disorders.

Locus heterogeneity in Mendelian disorders is the phenomenon whereby mutations in different genes result in a similar or identical clinical phenotype. In most reported instances, mutations at a single locus suffice to cause disease, although rare cases have been reported where mutations at two loci are necessary for pathogenesis (1) or exacerbate the severity of the phenotype (2, 3). BBS is a typical example of a rare, genetically heterogeneous disorder. BBS patients manifest a complex and variable phenotype that includes pigmentary retinal dystrophy, polydactyly, central obesity, hypogonadism, learning difficulties, and renal dysplasia; additional features such as asthma and diabetes mellitus may also be present (4, 5). The segregation of the disorder in families and population isolates led to the hypothesis that the syndrome is inherited in an autosomal recessive manner. On the basis of this model, six BBS loci have been identified: BBS1 on 11q13 (6), BBS2 on 16q21 (7), BBS3 on 3p12 (8), BBS4 on 15q22-2q23 (9), BBS5 on 2q31 (10, and BBS6 on 20p12 (11), with evidence for at least one more locus (12). Two BBS genes have been cloned recently: BBS6 (11, 13), which encodes a putative chaperonin (14), and BBS2, which encodes a protein of unknown function (15).

During our analysis of BBS6 in 163 patients, we identified eight pedigrees with mutations. A high frequency of these (seven of eight) harbored only a single mutant BBS6 allele (12). Furthermore, we described one consanguineous pedigree in which both the affected and unaffected sibs carried a heterozygous A242S mutation in BBS6, but the affected sib also exhibited homozygosity by descent (HBD) across the BBS2 locus. This observation suggests either that BBS arises through triallelic inheritance or that the A242S allele is a rare polymorphism (12).

The recent identification of BBS2 enabled us to test the former hypothesis by screening the same cohort of BBS patients (16) for coding sequence alterations in BBS2, irrespective of haplotype-inferred chromosomal assignment for any given pedigree or previously obtained mutational data for BBS6. Upon deducing the intron-exon structure of BBS2 (17), we designed suitable primers and amplified and sequenced each of the 17 exons of this gene in our entire patient cohort (18).

We identified numerous sequence alterations. In 19 unrelated patients (Table 1), the alterations fulfilled our minimal criteria for mutations, in that they caused a nonconservative amino acid change and that they were not found in a minimum of 192 control chromosomes from ethnically matched samples (19). Initial segregation analyses confirmed that BBS2 mutations were likely to be pathogenic. We identified six pedigrees (AR171, PB005, PB020, PB026, PB058, and K059) in which two independent mutations segregated with the disease (Table 1) (20). However, despite complete DNA sequence coverage of the BBS2 open reading frame (ORF) and exon-intron boundaries, we identified only a single mutant allele in eight pedigrees. We considered four possible explanations for this result: (i) the single alleles found may not be pathogenic but rare polymorphisms; (ii) by chance alone, we were detecting rare BBS2 carriers, (iii) the second mutation might reside in the regulatory regions or introns of BBS2 or might be undetectable by sequenc-
mutations, and BBS2 fore, the association of five mutations with
124, AR237, AR238, and AR596; Table 1). Likewise, the mutation found in pedi-
gree AR050 was an arginine to STOP (R275X), also causing premature ter-
mination because of deletions of the whole or part of the
ance pattern of the parental chromosomes in
any given pedigree with a single mutation was consistent with linkage to BBS2 (22). Six (of
eight) pedigrees provided sufficient information for this investigation. Only one pedigree,
AR029, was consistent with linkage to BBS2.

The first hypothesis is unlikely because
changing causes by several of the “singleton”
migrant alleles are predicted to be severe (Table
1). For instance, conceptual translation of the
single mutation in pedigree AR124 causes a
frameshift affecting a valine at position 158 and
then results in premature termination of trans-
lation at position 200 (V158fsX200; Fig. 1 and
Table 1). Likewise, the mutation found in pedi-
gree AR124 causes a
frameshift affecting a valine at position 158 and
then results in premature termination of trans-
lation at position 200 (V158fsX200; Fig. 1 and
Table 1). Furthermore, this R275X mutation
was also found in the homozygous state and
segregated with the disorder in pedigree PB005
(20). None of these alterations were found in
192 ethnically matched control chromosomes.

The possibility of incidental detection of
BBS carriers is even less likely. The frequen-
cy of BBS is 1:125,000 to 1:150,000 in the
European and North American populations (4).
Furthermore, the contribution of BBS2 to
all BBS has been estimated at about 10 to
20% (12, 15, 21), which at Hardy-Weinberg equi-
librium leads to an expected carrier fre-
quency of 1: 400 to 1: 700 for
BBS. Therefore, the association of five mutations with
BBS2 (in North American pedigrees AR050,
AR124, AR237, AR238, and AR596; Table 1)
(20) in a sample of 163 patient DNAs is signif-
ificant (χ² = 52.9; P < 0.0001).

We explored the possibility that the second
mutation was not located in the ORF or was a
large deletion by genotyping microsatellites
around BBS2 and examining whether the seg-
mation pattern for some mutations indicates a frameshift; IVS, intervening sequence.

Table 1. Mutational, genetic, and phenotypic analysis of pedigrees with
mutations in BBS2. The different aspects of the BBS phenotype in each
pedigree are indicated as present (+), normal (Nml), or not recorded (NR).
The primary features of BBS were examined: retinitis pigmentosa (RP), obesity
(Ob), polydactyly (PD), developmental delay (Dev), and renal (Ren) and
gonadal malformations (Gon). HBD, homozygous by descent; the “fs” anno-
tation for some mutations indicates a frameshift; IVS, intervening sequence.
*, **, ***, and **** indicate recurrent mutations.

<table>
<thead>
<tr>
<th>Pedigree</th>
<th>Allele 1</th>
<th>Allele 2</th>
<th>Allele 3</th>
<th>RP</th>
<th>Ob</th>
<th>PD</th>
<th>Dev</th>
<th>Ren</th>
<th>Gon</th>
</tr>
</thead>
<tbody>
<tr>
<td>AR171†</td>
<td>D104A (BBS2)*</td>
<td>R634P (BBS2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
<td>Nml</td>
<td>NR</td>
</tr>
<tr>
<td>PB005†</td>
<td>R275X (BBS2)**</td>
<td>R275X (BBS2)**</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>PB020†</td>
<td>Y24X (BBS2)**</td>
<td>Y24X (BBS2)**</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>PB026†</td>
<td>D170fsX171 (BBS2)</td>
<td>D170fsX171 (BBS2)</td>
<td>(HBD for BBS1)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>PB059§</td>
<td>C210fsX246 (BBS2)</td>
<td>C210fsX246 (BBS2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>KS3†‡</td>
<td>R315W (BBS2)</td>
<td>R315W (BBS2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>AR029†</td>
<td>Y24X (BBS2)*</td>
<td>BBS2-linkage</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AR724*</td>
<td>Q59X (BBS2)****</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>PB045§</td>
<td>IVS1-1G—C</td>
<td>Unknown</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AR050†</td>
<td>R275X (BBS2)**</td>
<td>BBS1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
</tr>
<tr>
<td>AR124†</td>
<td>V158fsX200 (BBS2)</td>
<td>BBS1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
<td>+</td>
<td>Nml</td>
</tr>
<tr>
<td>AR238†</td>
<td>D104A (BBS2)*</td>
<td>BBS1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
<td>+</td>
<td>Nml</td>
</tr>
<tr>
<td>AR596†</td>
<td>IVS4+1G—C</td>
<td>BBS3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AR237†</td>
<td>N705 (BBS2)</td>
<td>Y37C (BBS6)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>AR579†</td>
<td>L168fsX170 (BBS2)</td>
<td>R216X (BBS2)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>NFB14†</td>
<td>Y24X (BBS2)*</td>
<td>Y24X (BBS2)*</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>+</td>
<td>NR</td>
</tr>
<tr>
<td>AR241†</td>
<td>IVS1-1G—C/R315Q</td>
<td>Unmapped</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
<td>NR</td>
<td>+</td>
</tr>
<tr>
<td>AR259†</td>
<td>Y24X (BBS2)*</td>
<td>Q59X (BBS2)****</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
</tr>
<tr>
<td>PB043§</td>
<td>T560I (BBS2)</td>
<td>(HBD for BBS4)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Nml</td>
</tr>
</tbody>
</table>

Pedigrees have been divided into the following: †two BBS2 mutations segregating with the disease, ‡one BBS2 mutation but excluded genetically from the BBS2 locus, §three BBS mutations, and ¶two BBS2 mutations found in unaffected individuals.
The remaining five pedigrees could be excluded genetically from BBS2 either because the affected individuals shared only one parental chromosome at the locus (e.g., AR124, Fig. 1) or because the affected and unaffected sibs had inherited the same parental chromosomes and both were carriers for the single mutation found. Despite the dense microsatellite spacing, there remained a remote chance that a recombination between any marker and the locus might have led to a deceptive exclusion; we eliminated this possibility by genotyping all individuals with a highly informative single nucleotide polymorphism in exon 3 of BBS2 (Fig. 1). When we used polymorphic microsatellites from all BBS loci to monitor the segregation of the parental chromosomes, we observed that three out of five pedigrees could be excluded from all BBS loci except for BBS1, the major BBS locus, suggesting that these three pedigrees may harbor BBS1 mutations. One pedigree was consistent with mapping only to BBS2, and one pedigree, AR237, could be assigned to BBS6 (Fig. 2A).

These data suggest that mutations at more than one locus may be present in BBS patients. We therefore integrated and expanded all sequence data from the BBS2 and BBS6 mutation screens that yielded a complete scan of the ORFs of the two genes, and we constructed detailed haplotypes across both genomic regions. Of the 19 pedigrees with one or two BBS2 mutations, we identified four pedigrees with a third potential mutation (AR237, AR579, NFB14, and PB043). AR237 was reported in our initial discovery of BBS6, because it harbored a homozygous Tyr24→Cys (Y37C) mutation in BBS6 (11). We then found a heterozygous Asn70→Ser (N70S) alteration in BBS2 in this family (Fig. 2A and Table 1). In AR259, we found two heterozygous BBS2 nonsense mutations [Tyr24→X (Y24X) and Gln59→X (Q59X)] and one BBS6 nonsense mutation [Gln147→X (Q147X)]. Similar findings were seen in pedigrees AR579 and NFB14 (Table 1 and Fig. 2, B and C).

The probability that we had detected three mutant alleles by chance is minuscule, given the rarity of this disorder and the observation that BBS6 contributes only ~4% to BBS (11, 12). That the third allele is not a mutation is possible but is not likely. First, nonsense mutations are unlikely polymorphisms. Second, the Ala232→Ser (A242S) allele identified in NFB14 has been observed on two other occasions, once in the Old Order Amish, associated with McKusick-Kaufman syndrome (14), and subsequently in a BBS patient, but not in 384 control chromosomes (Table 2). The N70S (BBS2) and Cys999→Ser (C499S) (BBS6) alleles are more challenging to interpret in the absence of a functional assay for either gene, but neither has been found in 384 control chromosomes. More importantly, however, the cumulative data indicate that, among 19 pedigrees with BBS2 mutations, sequence or genetic evidence for involvement of another locus occurs in at least nine families (47.3%). The same is also true for BBS6: Of the eight outbred pedigrees where mutations have been identified (12), three harbor BBS2 mutations (37.5%). The combination of the genetic data described earlier and the mutational data for the only two genes known to be mutated in BBS suggest that multiple alleles may act in concert to cause pathogenesis. We therefore propose a model of triallelic inheritance, in which three mutant alleles segregate with the disorder.

In the absence of either complete gene identification for all BBS loci or an animal model, it is not clear whether two mutations at a single locus may sometimes suffice to cause BBS. The recessive segregation pattern in several BBS2 pedigrees initially suggests that this may be the case and that “biallelic” inheritance may occur for some instances. However, the BBS6 homozygous Y37C mutation in pedigree AR237 segregates perfectly with the disease, yet this pedigree harbors an additional BBS2 mutation (Fig. 2A). In fact, in three additional pedigrees, three disease alleles are identified in affected individuals: two BBS2 alleles with one BBS6 allele (Figs. 2, B and C, and 3). Consistent with our triallelic hypothesis, individuals with two mutant alleles at BBS2 who are either wild type for BBS6 (AR259-05; Fig. 3) or map genetically to another locus (BBS4 in PB043; Table 1) are unaffected. The absence of these alterations in 384 control chromosomes and the nature of the BBS2 mutations in AR259-05, in particular (Y24X and Q59X), suggest that these are not polymorphisms (Table 2). Inheritance of multiple alleles has been documented previously. Paired single mutations in ROM1 and RDS, for example, are the cause of some cases of retinitis pigmentosa (1). Subse-
quent functional studies confirmed these genetic
observations by modeling the mutations in vitro and showing definitive assembly of the
ROM/RDS complex (23). Dominant Hirschsprung disease also provides a similar, poten-
tially digenic example, where mutations in both SPRW and RET are necessary for patho-
genesis (15, 16). Our observations on BBS mutations but 

regressive, genetically heterogeneous disorders is unclear. A model of a dominant loss-of-function
mutation requisite to unmask regressive mutations may explain, however, the genetic hetero-
geneity seen in many instances in inbred popula-
tions, where both one dominant and two re-
cessive mutations are required for pathogenesis.

This model is similar to the inheritance patterns seen in some complex traits, such as type I diabetes (IDDM1), where a dominant ILA geno-
type is necessary but not sufficient to cause disease; a second, recessively inherited alter-
tation must also be present (31). The presence of a common dominant “master locus” but variable “partners” may also explain the inconsistency in replying genetic linkage data from discrete populations in various complex traits such as asthma, diabetes, and schizophrenia, because each population isolate will be enriched for a limited number of the recessive mutations that, by themselves, will not satisfy all genetic criteria for pathogenicity. A modification of the theoretical model of disease transmission in these disorders may provide important insights.

To our knowledge, there is only one other clearly documented mechanistic example with a three-allele requirement to manifest a Mendelian trait, exemplified by the segmental dupli-
cations (32). The sequence spanning all coding exons
for pathogenicity. A modification of the theoret-
ical model of disease transmission in these dis-
orders may provide important insights.

References and Notes
16. The diagnosis of BBS was based on established crite-
ria in which three of six cardinal features must be present (4). In several cases, the diagnosis was ascer-
tained by local physicians and verified through exten-
sive examination of medical records by one or more of the coauthors.
17. The published cDNA sequence for BBS2 (GenBank accession number AF342736) was screened through the assembled human genomic sequence with the BLAST algorithm (http://www.ncbi.nlm.nih.gov/genome/seq/HsBlast.html). Assembly scaffold NT_024816.3 exhibited >99.5% identity to the cDNA sequence. The sequence was downloaded and aligned with the BBS2 cDNA with programs from the GCG software package (17). The sequence flanking all coding exons was identified, and primers were designed to amplify both exons and intronic splice junctions with the Primer v3 program (www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi).
18. Amplified polymerase chain reaction products from patients, relatives, and control samples were se-
19. Sequences were aligned with the wild-type cDNA and genomic sequences, and alterations were confirmed on both strands and evaluated by the Sequencer sequence alignment program (GeneCodes, Ann Arbor, MI).
20. Examples of Mendelian regressive segregation of mu-
tations are shown for pedigrees AR171 and PB005 and are available as supplemental Web material on Science Online at www.sciencemag.org/cgi/content/ full/293/5538/2256/DC1.
22. Microsatellites D16S417, D16S415, D16S419, D16S390, D16S408, D16S526, D16S3034, D16S408, D16S3057, D16S514, D16S503, D16S400, D16S421, and D16S515 were genotyped in all available pedigrees, and haplotypes were con-
tstructed (33).
33. We thank the families and the referring physicians for their cooperation and A. Beaudet, H. Bellen, and J. Belmont for constructive comments. Supported by grants EY1266 from the National Eye Institute (NIH) (N.K.), the March of Dimes (N.K. and J.R.L.), the Foundation Fighting Blindness, USA (R.A.L. and J.R.L.), the Canadian Institutes of Health Research (W.D.), the National Kidney Research Fund (B.E.H.), the Birth Defects Foundation, and the Wellcome Trust (P.L.B.).
18 June 2001; accepted 6 August 2001