

## Genetic Interaction of *BBS1* Mutations with Alleles at Other *BBS* Loci Can Result in Non-Mendelian Bardet-Biedl Syndrome

Philip L. Beales,<sup>1,\*</sup> Jose L. Badano,<sup>3,\*</sup> Alison J. Ross,<sup>1</sup> Stephen J. Ansley,<sup>3</sup> Bethan E. Hoskins,<sup>1</sup> Brigitta Kirsten,<sup>2</sup> Charles A. Mein,<sup>2</sup> Philippe Froguel,<sup>2,5</sup> Peter J. Scambler,<sup>1</sup> Richard Alan Lewis,<sup>6,7,8,9</sup> James R. Lupski,<sup>6,8</sup> and Nicholas Katsanis<sup>3,4</sup>

<sup>1</sup>Molecular Medicine Unit, Institute of Child Health, University College London, <sup>2</sup>Genome Centre, Barts and the London, Queen Mary's School of Medicine and Dentistry, London; <sup>3</sup>Institute of Genetic Medicine and <sup>4</sup>Wilmer Eye Institute, Johns Hopkins University, Baltimore; <sup>5</sup>CNR-Institute of Biology, Pasteur Institute, Lille, France; and Departments of <sup>6</sup>Molecular and Human Genetics, <sup>7</sup>Ophthalmology, <sup>8</sup>Pediatrics, and <sup>9</sup>Medicine, Baylor College of Medicine, Houston

Bardet-Biedl syndrome is a genetically and clinically heterogeneous disorder caused by mutations in at least seven loci (*BBS1–7*), five of which are cloned (*BBS1*, *BBS2*, *BBS4*, *BBS6*, and *BBS7*). Genetic and mutational analyses have indicated that, in some families, a combination of three mutant alleles at two loci (trialelic inheritance) is necessary for pathogenesis. To date, four of the five known *BBS* loci have been implicated in this mode of oligogenic disease transmission. We present a comprehensive analysis of the spectrum, distribution, and involvement in non-Mendelian trait transmission of mutant alleles in *BBS1*, the most common *BBS* locus. Analyses of 259 independent families segregating a *BBS* phenotype indicate that *BBS1* participates in complex inheritance and that, in different families, mutations in *BBS1* can interact genetically with mutations at each of the other known *BBS* genes, as well as at unknown loci, to cause the phenotype. Consistent with this model, we identified homozygous M390R alleles, the most frequent *BBS1* mutation, in asymptomatic individuals in two families. Moreover, our statistical analyses indicate that the prevalence of the M390R allele in the general population is consistent with an oligogenic rather than a recessive model of disease transmission. The distribution of *BBS* oligogenic alleles also indicates that all *BBS* loci might interact genetically with each other, but some genes, especially *BBS2* and *BBS6*, are more likely to participate in triallelic inheritance, suggesting a variable ability of the *BBS* proteins to interact genetically with each other.

### Introduction

Oligogenic inheritance occurs when specific alleles at more than one locus affect a genetic trait by causing and/or modifying the severity and range of a phenotype. The rapidly expanding number of known disease-causing genes and an improved understanding of the cellular bases of mutational mechanisms have suggested that many disorders previously thought to be monogenic are, rather, the products of the genetic interaction of a small number of loci (Badano and Katsanis 2002; Ming and Muenke 2002).

Bardet-Biedl syndrome (BBS [MIM 209900]) is a clinically heterogeneous pleiotropic disorder characterized by progressive retinal dystrophy, central obesity, poly-

dactyly, renal dysplasia, reproductive tract anomalies, and cognitive impairment (Schachat and Maumenee 1982; Green et al. 1989). Additional features include short stature, endocrinopathies (including diabetes mellitus and thyroid deficiency), and congenital heart malformations, as well as speech and behavioral disturbances (Green et al. 1989; Beales et al. 1999). BBS is also genetically heterogeneous; on the basis of an autosomal recessive transmission model, seven loci have been mapped in the genome: *BBS1* on 11q13 (Leppert et al. 1994), *BBS2* on 16q21 (Kwitek-Black et al. 1993), *BBS3* on 3p12 (Sheffield et al. 1994), *BBS4* on 15q22.2–q23 (Carmi et al. 1995), *BBS5* on 2q31 (Young et al. 1999a), *BBS6* on 20p12 (Katsanis et al. 2000), and *BBS7* on 4q27 (Badano et al. 2003). However, the cloning of the first *BBS* locus, *BBS6* (Katsanis et al. 2000; Slavotinek et al. 2000), followed by mutational analyses on a large multiethnic cohort, implied that some mutations do not conform to a traditional model of autosomal recessive disease transmission (Beales et al. 2001). After the identification of two additional *BBS* loci, *BBS2* (Nishimura et al. 2001) and *BBS4* (Mykytyn et al. 2001), sequence analyses showed that, in some families, a total of three mutations in two genes

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Address for correspondence and reprints: Dr. Nicholas Katsanis, McKusick-Nathans Institute of Genetic Medicine, Johns Hopkins University, 600 North Wolfe Street, 2-127 Jefferson Street Building, Baltimore, MD 21287. E-mail: katsanis@jhmi.edu

\* These two authors contributed equally to this work.

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are necessary for pathogenesis (Katsanis et al. 2001a, 2002), thus establishing a triallelic model of disease transmission and suggesting that BBS might be a useful model to study oligogenic traits (Badano and Katsanis 2002).

More recently, two new *BBS* genes have been cloned: *BBS1* (Mykytyn et al. 2002) and *BBS7* (Badano et al. 2003), each encoding a protein of unknown function but exhibiting modest similarity to the other. Mutational analysis of *BBS7* suggested that this locus might also participate in non-Mendelian inheritance. However, the reported initial analyses of *BBS1* in 60 families with BBS yielded no evidence for triallelic inheritance, because of both the absence of patients with three mutations at two loci and the lack of asymptomatic individuals with two *BBS1* mutations (Mykytyn et al. 2002).

We performed comprehensive genetic and mutational analyses of 259 families, of various ethnicities, with BBS and describe the characteristics, frequency, and position of *BBS1* mutations. Our data provide compelling evidence for the participation of *BBS1* in triallelic inheritance and indicate that BBS can arise from the pairing of different combinations of mutations in which each locus can contribute either one or two mutant alleles at varying frequencies.

## Patients and Methods

### Patients

Two hundred and fifty-nine families with BBS, of various ethnic origins, were screened for mutations in *BBS1*. The diagnosis of BBS was based on established criteria requiring that four of six cardinal features be present (Anderson and Lewis 1997; Beales et al. 1999). In several cases, the diagnosis was ascertained by local physicians and was verified through examination of medical records by one or more of us (R.A.L., P.L.B.). Blood was obtained with consent, in accordance with protocols approved by the appropriate human subjects ethics committees at each participating institution, and DNA was extracted as described elsewhere (Katsanis et al. 2000).

### Genetic and Mutational Analysis

The reported cDNA sequence of *BBS1* (GenBank accession number AF503941) (Mykytyn et al. 2002) was aligned to genomic sequence from the June 2002 human genome assembly with the nucleotide-nucleotide BLAT algorithm at the University of California, Santa Cruz. We determined intron-exon boundaries for 17 exons, which is consistent with the published data. We extracted 100–300 bp of sequence flanking each coding exon and designed amplicons that span each exon and both splice junctions, as described elsewhere (Katsanis et al. 2000).

We purified the amplified PCR products from patients, relatives, and unrelated but ethnically matched controls, with either the Exo-SAP cleanup kit (USB) or the QIAquick PCR purification kit (Qiagen), and performed direct sequencing of the PCR products with dye-primer (Applied Biosystems) or dye-terminator (Amersham Biosciences or Applied Biosystems) chemistry. Sequencing was performed on an ABI 377 and ABI 3700 sequence analyzer (Applied Biosystems) or a MegaBACE1000 sequence analyzer (Amersham Biosciences).

We performed genetic analyses by generating genotypes for each relevant patient or relative with fluorescent STRs and constructing haplotypes across each *BBS* locus, as described elsewhere (Katsanis et al. 1999; Beales et al. 2001). Microsatellite sequences were obtained from either the Genome Database or the Whitehead Institute Center for Genome Research.

### Evolutionary Conservation Analyses

We identified the *Drosophila melanogaster*, *Caenorhabditis elegans*, *Mus musculus*, *Rattus norvegicus*, and *Danio rerio* orthologs of *BBS1*, *BBS2*, *BBS4*, and *BBS6* through the search utility of Swissprot and TrEMBL with the human peptide sequence. The sequences were aligned with the ClustalW software to return a graphical representation of the conserved and nonconserved residues. We also identified partial sequences of the *Sus scrofa*, *Bos taurus*, *Xenopus laevis*, and *Gallus gallus* orthologs of *BBS6* by conducting TBLASTN searches of the *BBS6* peptide sequence against the nonhuman/nonmouse EST database. We aligned relevant sequences with programs from the GCG v.9.0 software analysis package, as described elsewhere (Katsanis and Fisher 1998).

## Results

### Spectrum of *BBS1* Mutations

To determine the range and type of mutations in *BBS1*, we sequenced a total of 259 families of various ethnicities, which were divided into two cohorts depending on the laboratory responsible for collection and maintenance of DNA stocks: a U.S. cohort (147 families) and a U.K. cohort (112 families). Furthermore, because of the complex inheritance of BBS (Katsanis et al. 2001a, 2002), we investigated all available families irrespective of previous genetic or mutational data.

We identified numerous alterations, which we investigated further for pathogenicity by (a) segregating them with the phenotype in families; (b) ascertaining whether they might disrupt splicing; (c) evaluating whether they represented a nonconservative amino acid substitution or predicted premature termination codon; (d) investigating whether they were present in 200–400 unrelated, ethnically matched control chromosomes; and (e) deter-

**Table 1****Summary of *BBS1* Mutational Data For Each Cohort**

Mutations	DATA FROM COHORT								
	U.K.			U.S.			Combined		
	No. of Families ( <i>n</i> = 112)	% of All Families with BBS	% of Families with <i>BBS1</i> Mutations	No. of Families ( <i>n</i> = 147)	% of All Families with BBS	% of Families with <i>BBS1</i> Mutations	No. of Families ( <i>n</i> = 259)	% of All Families with BBS	% of Families with <i>BBS1</i> Mutations
Two <i>BBS1</i> mutations (recessive)	16	14.3	69.6	29	19.7	78.4	45	17.4	75.0
Two <i>BBS1</i> mutations and one mutation elsewhere	4	3.6	17.4	2	1.4	5.4	6	2.3	10.0
One <i>BBS1</i> mutation and two mutations elsewhere	0	.0	.0	2	1.4	5.4	2	.8	3.3
Total mutations at two loci	4	3.6	17.4	4	2.7	10.8	8	3.1	13.3
One <i>BBS1</i> mutation and linked to 11q13	1 <sup>a</sup>	.9	4.3	1	.7	2.7	2 <sup>a</sup>	.8	3.3
One <i>BBS1</i> mutation and not linked to 11q13	0	.0	.0	2	1.4	5.4	2	.8	3.3
One <i>BBS1</i> mutation and no linkage data	3	2.7	13.0	1	.7	2.7	4	1.5	6.7
All families with <i>BBS1</i> mutations	23	20.5		37	25.2		60	23.2	

<sup>a</sup> One arm of family PB086 has two *BBS1* mutations, whereas, in a second arm, we have detected a single *BBS1* mutation, despite a haplotype-based expectation for two 11q13 mutations. Therefore, this family was counted both as recessive and as "One *BBS1* mutation and linked to 11q13."

mining the level of evolutionary conservation for each residue.

Overall, we detected at least one *BBS1* disease-associated mutation in 60 of 259 pedigrees (23.2% of families, 111 mutant alleles) (tables 1 and 2), despite an expectation of ~40%–56% of disease-associated alleles, based on previous analyses of the contribution of this locus to BBS by us and others (Bruford et al. 1997; Katsanis et al. 1999). Of the 24 different *BBS1* mutant alleles detected in our two cohorts, 2 were splice junction mutations and 5 were insertions or deletions (table 2). One of these is a 3-bp deletion eliminating the isoleucine at position 389, whereas the other four result conceptually in a frameshift and the premature termination of translation of the encoded peptide. Most changes were either 1-bp transitions or transversions resulting in missense substitutions (*n* = 9) or nonsense mutations introducing a premature stop codon (*n* = 8). One of the missense mutations was a contiguous 2-bp substitution, TT→AC (L503H); subsequent subcloning and sequencing of each allele indicated that the two alterations lie in the same strand, occupying the second and third positions in the same codon.

With the exception of the M390R mutant allele, most mutations were found at a low frequency in each cohort (table 2). Only one allele, D148N, was found in both the U.K. and U.S. patient collection, even though the prevalence of some mutations was elevated in one or the other cohort (e.g., four R146X U.S. alleles and three Y284fsX288 U.K. alleles), suggesting that each patient collection might have a distinct genetic composition. Notably, *BBS1* mutations were primarily detected in white families; only 2 of the 24 families of Saudi Arabian descent had *BBS1* mutations (8%), in contrast to ~20% of whites, indicating that *BBS1* may not be the major *BBS* locus in this population.

Mapping the position of the mutations in *BBS1* re-

**Table 2****Prevalence of *BBS1* Mutations in the U.S. and U.K. Cohorts**

MUTATION	NO. OF MUTANT ALLELES IN COHORT	
	U.K. ( <i>n</i> = 112)	U.S. ( <i>n</i> = 147)
H35R	1	0
K53E	1	0
L75fsX98	0	1
Y113X	0	1
Q128X	0	1
R146X	0	4
D148N	2	2
E234K	0	1
IVS9-3C→G	0	2
Y284fsX288 <sup>a</sup>	3	0
Q291X	0	1
G305S	0	4
389ΔI	0	1
M390R <sup>a</sup>	33	41
R429X	0	1
Y434S	0	1
R440X*	2	0
IVS13-2A→G	0	2
R483X	0	1
L503H	1	0
L505fsX556	0	1
L518Q	0	1
L548fsX579	0	1
E549X <sup>a</sup>	0	1

NOTE.—Numbers of mutant alleles are shown; therefore homozygous mutations were scored as two alleles. Mutations are listed in a N- to C-terminal order, with position "1" being the first methionine in sequence AF503941 (Mykytyn et al. 2002).

<sup>a</sup> These mutations were also found by Mykytyn et al. (2002).

vealed a nonuniform distribution (fig. 1), in which none of the 26 mutations lies at the extreme N or C termini of the predicted protein. In addition, the region extending from exon 6 to exon 9 is devoid of mutations, except for a single allele, E234K.

### M390R is the Predominant BBS1 Mutation

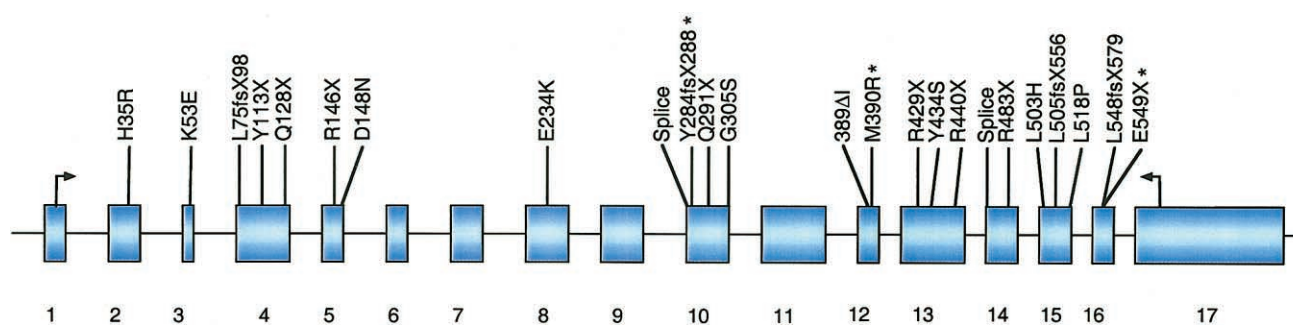
Previous mutational analyses of a smaller patient cohort indicated that a single missense alteration in exon 12 of *BBS1*, M390R, is the most common *BBS1* mutant allele, accounting for 32% (38/120 mutant alleles, under the assumption of an exclusively recessive model) of all BBS mutations (Mykytyn et al. 2002). Although the contribution of M390R to BBS in our cohort is lower (18%), we found the M390R allele to be the most frequent *BBS1* mutation in each of our cohorts. In the U.S. cohort, M390R is present in 75.7% of all families with *BBS1* mutations, and, in the U.K. cohort, we observed a slightly higher proportion of 82.6% (table 4). Overall, 45% of pedigrees with *BBS1* mutations were homozygous for the G nucleotide (encoding arginine), whereas 33.3% were heterozygous. Independent analyses of each patient cohort also revealed a slight enrichment for M390R heterozygotes (40.5%) compared with homozygotes (35.1%) in the U.S. cohort, in contrast to the U.K. cohort, in which homozygotes (60.9%) are more than twice as common as heterozygotes (21.7%). This difference may be due, in part, to the background ethnicity of the two family collections; in each cohort, the M390R allele was found almost exclusively in pedigrees of European origin. This observation, coupled with the high frequency of this allele in BBS, suggests that M390R might be an ancient founder, propagated through the population either by genetic drift or by some as-yet-unidentified selective advantage in the heterozygous state.

### Evaluation of Complex Inheritance in BBS1

The availability of 259 families with BBS, with associated data for each of the other known loci, offered the opportunity to evaluate comprehensively the genetic contribution of *BBS1* alleles to the phenotype. We postulated that supportive evidence for the involvement of *BBS1* in complex inheritance would include the following observations. First, patients with *BBS1* mutations should have mutations at other *BBS* loci. Second, examples of asymptomatic individuals who are carriers of two bona fide *BBS1* mutations should be present in our cohorts. Third, the prevalence of *BBS1* mutations involved in triallelism might be elevated in the general population beyond a value expected under a Mendelian recessive model. To minimize potential scoring and population bias for testing these predictions, each laboratory analyzed its respective cohort independently in a masked fashion and the cumulated data were shared only after the completion of the experimental phase.

*Patients with BBS1 mutations have mutations at other BBS loci.*—By performing sequence analyses for all known *BBS* genes, we found direct mutational evidence for non-Mendelian trait inheritance in eight families with *BBS1* mutations (13.3% of all such families) (tables 1 and 3). Of these families, six (10% of all families with *BBS1* mutations) have two mutations in *BBS1* and one mutation at another locus, and two families (3.3% of all families with *BBS1* mutations) present the reciprocal combination. Furthermore, half of these families originated from the U.K. cohort and half from the U.S. cohort, suggesting similar prevalences of non-Mendelian BBS in the two collections.

In one English pedigree, PB056, both mother and daughter are affected, which resembles a pseudodominant pattern of inheritance. Subsequent sequencing revealed the presence of two M390R alleles in both patients (fig. 2A). Furthermore, each affected individual



**Figure 1** Distribution of mutations along *BBS1*. Exons are depicted as blue bars and the position of each mutation is shown above. Arrows indicate the positions of the start and termination codons, whereas asterisks indicate mutations reported previously by Mykytyn et al. (2002); scale is approximate. The *BBS1* protein has no discernible domains, with the exception of a region of similarity between residues 187–377 with *BBS2* and *BBS7* that potentially encodes a six-bladed  $\beta$ -propeller (Badano et al. 2003).

has a third heterozygous M472V alteration in *BBS4*, inherited from the maternal grandfather, who also carries a heterozygous M390R allele but is unaffected. Moreover, the methionine residue involved in M472V has been found, on subsequent evolutionary analysis, to be highly conserved (fig. 5). Because the father in this family was unavailable, we excluded the possibility of M390R hemizygoty in the proband or uniparental disomy (UPD) as alternative explanations, by genotyping regional STRPs and intragenic SNPs (fig. 2A).

Examples of triallelic inheritance and the involvement of mutations at two distinct loci were also found in the U.S. cohort. The proband in family AR396 was a compound heterozygote for the M390R allele and a nonsense mutation Q291X and also carries a heterozygous S236P allele in *BBS6* (fig. 2B). Conversely, the Puerto Rican consanguineous family AR69 carries a heterozygous E234K allele in *BBS1* but is excluded genetically from this locus. During the course of our studies, we determined that this family has inherited a homozygous T211I mutation at a highly conserved residue in *BBS7* (Badano et al. 2003).

In the families described above, the third mutant allele is a missense alteration. As such, despite its absence from some 400 ethnically matched control chromosomes, the possibility remains that some of these alleles represent benign polymorphisms and, given the small size of pedigrees, cosegregation of these alleles with the disorder might be argued to be coincidental. However, AR241, a pedigree of European descent from the U.S. cohort, provides compelling evidence for the requirement of mutant alleles at two loci. Both affected brothers, -05 and -06, are heterozygous for the M390R *BBS1* mutation. In addition, each sib carries two *BBS2* mutant alleles: a missense R315Q mutation, which is a disease-associated residue in a Saudi Arabian family with BBS (Katsanis et al. 2001a), and a splice junction mutation in the acceptor site of exon 2 (IVS1+1G→C; fig. 2C). In the unlikely event that the R315Q allele proved benign, the patients

**Table 3**

**Families with Mutations in *BBS1* and Other Loci**

FAMILY	ALLELE			HAPLOTYPE MAPPING <sup>a</sup>
	1	2	3	
AR69	E234K	T211I ( <i>BBS7</i> )	T211I ( <i>BBS7</i> )	4q27
AR241	M390R	IVSx2 ( <i>BBS2</i> )	R315Q ( <i>BBS2</i> )	Unmapped
AR396	Q291X	M390R	S236P ( <i>BBS6</i> )	Unclear
AR710 <sup>b</sup>	M390R	...	...	16q21
AR729 <sup>b</sup>	M390R	...	...	Not 11q13
AR768	M390R	L548fsX579	T325P ( <i>BBS6</i> )	11q13
PB006 <sup>c</sup>	M390R	M390R	<i>BBS8?</i>	11q13
PB009	M390R	M390R	L349W ( <i>BBS2</i> )	11q13
PB029 <sup>c</sup>	M390R	M390R	<i>BBS8?</i>	11q13
PB056	M390R	M390R	M472V ( <i>BBS4</i> )	Unclear

<sup>a</sup> Whenever possible, families were excluded from recessive mutations at a particular locus by constructing extended haplotypes. These were centered either at the gene (when known) or the polymorphic marker with the highest LOD score and extended beyond the first reported recombinant marker for each *BBS* locus.

<sup>b</sup> Families with one *BBS1* mutation excluded from 11q13 by haplotype analysis. In family AR710, haplotype analysis across all known *BBS* loci is consistent with linkage to *BBS2*, but no mutations have been identified. Family AR729 has been excluded from all known *BBS* loci.

<sup>c</sup> Families in which an unaffected individual is homozygous for M390R mutations; in these instances, a mutation at a novel *BBS* locus is postulated (i.e., *BBS8?*) that may exert either a causal or a protective effect.

in this family still have inherited bona fide mutations in both *BBS1* and *BBS2*.

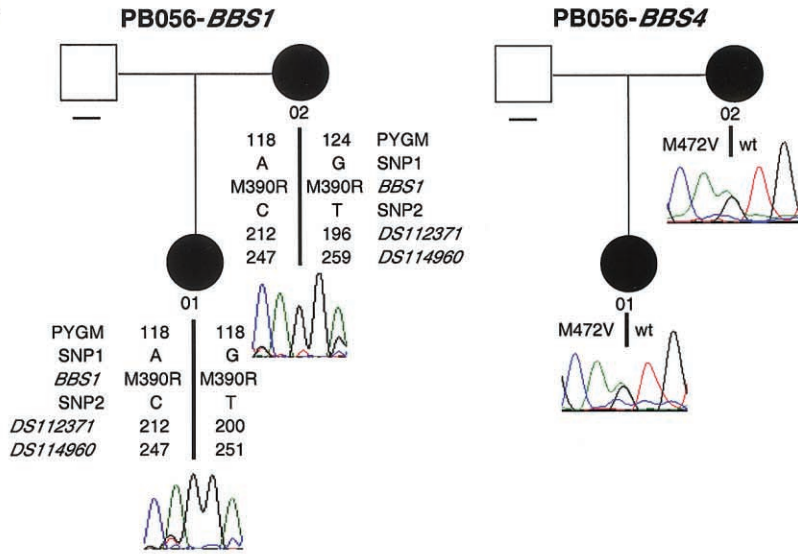
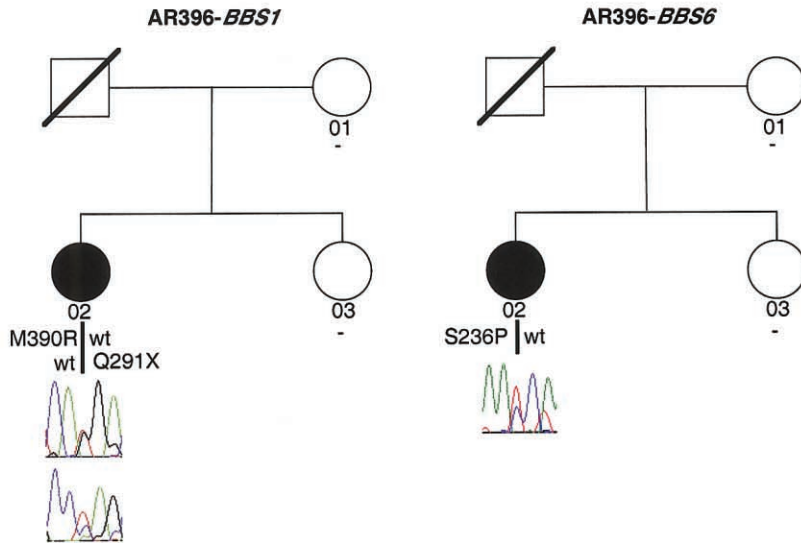
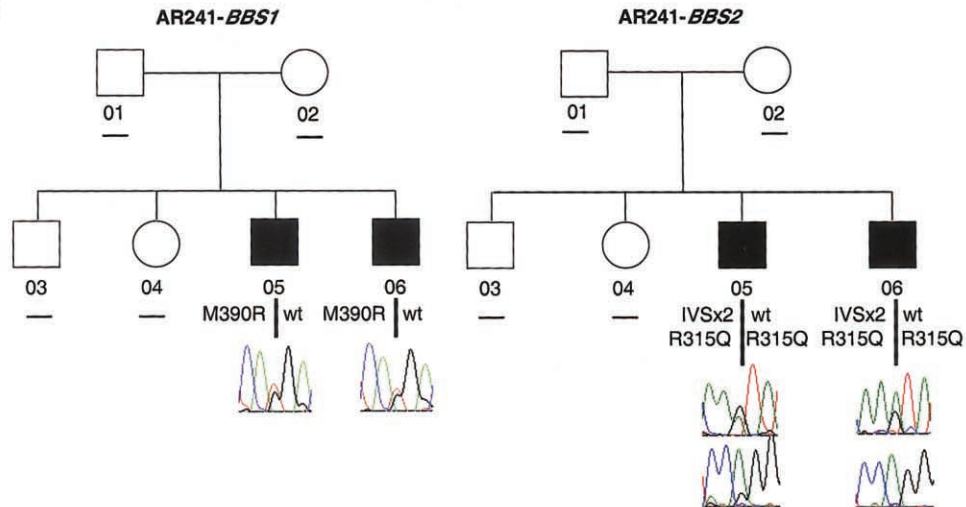
*Unaffected individuals with two BBS1 mutations.*—A second prediction of the triallelic hypothesis is that, if mutations at two loci are necessary for pathogenesis, unaffected individuals must exist who have two mutations at one locus. This has been found previously to be true for other *BBS* loci (Katsanis et al. 2001a; Katsanis et al. 2002), but not *BBS1* (Mykytyn et al. 2002). To examine this aspect of our model, we determined the segregation of each mutant *BBS1* allele. Although in several instances parents and/or unaffected sibs were un-

**Table 4**

**Relative Prevalence and Distribution of the M390R Allele**

M390R STATUS	DATA IN COHORT						M390R PREVALENCE	
	U.K.		U.S.		Combined		No. of Families ( <i>n</i> = 259)	% of All Families with BBS
	No. of Families with <i>BBS1</i> Mutations ( <i>n</i> = 23)	% of All Families with <i>BBS1</i> Mutations	No. of Families with <i>BBS1</i> Mutations ( <i>n</i> = 37)	% of All Families with <i>BBS1</i> Mutations	No. of Families with <i>BBS1</i> Mutations ( <i>n</i> = 60)	% of All Families with <i>BBS1</i> Mutations		
Homozygotes	14	60.9	13	35.1	27	45.0	27	10.4
Heterozygotes	5	21.7	15	40.5	20	33.3	20	7.7
Total	19	82.6	28	75.7	47	78.3	47	18.1
M390R and mutations elsewhere <sup>a</sup>	4	17.4	5	13.5	9	15.0	9	3.5

<sup>a</sup> These families have also been counted as M390R homozygotes and heterozygotes, depending on the genotype of each family.

**A****B****C**

available, we could ascertain genotypes for most of each cohort. Consistent with a non-Mendelian mode of disease transmission, we identified two British pedigrees (PB006 and PB029) segregating two copies of M390R in the probands, in which the fathers in each case were homozygous for the common *BBS1* mutation, M390R (figs. 3A and 3B). Despite careful clinical re-evaluation of these individuals, neither parent displays any features of the BBS phenotype, and marker segregation excluded the possibility of missampling. This raises two possibilities: either (1) a third allele at an unidentified locus is necessary for pathogenesis in these two families or (2) a third allele carried by the father but not by the children confers protection against the M390R mutation.

*Elevated M390R allele frequency in the population.*—A third prediction of a multilocus causality model for BBS is that the carrier frequency of the mutations involved in complex inheritance must be greater than predicted for a recessive disorder. Evaluation of this possibility has been difficult to date, since the low frequency of the known *BBS2*, *BBS4*, *BBS6*, and *BBS7* mutations in patients with BBS could require querying a large number of healthy, unrelated individuals. The presence of a common M390R allele in some 18% of BBS patients, however, and the involvement of this allele in several cases of complex inheritance (tables 3 and 4) provided the opportunity to conduct this study.

We assayed by direct sequencing 658 unrelated control chromosomes for the M390R alteration, 75% of which were of European origin. We detected two heterozygous carriers, indicating that an approximate carrier frequency in European and North American whites is ~1:325. From our mutational analyses, we concluded that 10.4% of BBS patients are homozygous for this allele (table 4). The prevalence of BBS in North America and Europe ranges from 1:100,000 to 1:160,000 (Klein and Ammann 1969; Croft et al. 1995; Beales et al. 1997). Therefore, under the idealized conditions of Hardy-Weinberg equilibrium, the carrier frequency of M390R under a recessive model should range from 1:1,000 to 1:1,200 chromosomes in the general population. This difference is statistically significant, since the probability of finding two M390R mutations in 658 chromosomes by chance alone is only 2% (binomial exact test;  $P = .022$ ). From the observed carrier frequency, one would predict that, if the M390R allele always segregated with a monogenic phenotype, BBS would be ~10 times more prevalent than observed (1:11,000).

## Discussion

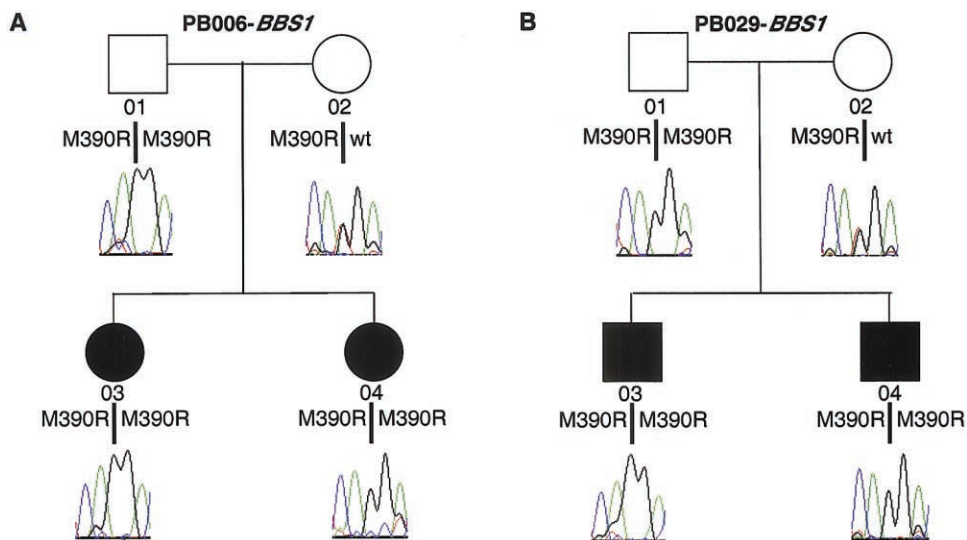
We report the results of a large study between two laboratories to screen for mutations in *BBS1*. We investigated 259 pedigrees of multiethnic origin, irrespective of the prior detection of mutations in other genes. In these families, we identified 26 different *BBS1* mutant alleles, accounting for 23.2% of families in our combined cohort. The predominance of M390R as the main *BBS1* mutation is striking (present in 78.3% of families with *BBS1* mutations), suggesting that the M390R allele might be an ancient mutation, especially since the mutation is not due to a CpG dinucleotide transition. Furthermore, genetic analysis across the *BBS1* locus indicates a common haplotype for all arginine 390-carrying chromosomes (Mykytyn et al. 2003; authors' unpublished data). This might reflect positive selective advantage for M390R heterozygotes or genetic drift. Notably, we found the arginine 390 allele almost exclusively in families of European descent, suggesting this allele might have been fixed in this population.

### *Evidence that BBS1 Participates in Triallelic Inheritance*

Our analyses provide three independent lines of evidence supportive of the hypothesis that *BBS1* participates in triallelic inheritance. First, we identified several *BBS1* families that segregate a total of three mutant alleles. In some instances, two *BBS1* alleles cosegregate with a single *BBS2*, *BBS4*, or *BBS6* allele, whereas, in other cases, a single *BBS1* mutation is present in the patients, in conjunction with two mutations in *BBS2* or *BBS7* (Badano et al. 2003). Given the rarity of the syndrome and the low contribution of pathogenic *BBS2*, *BBS4*, *BBS6*, and *BBS7* alleles to BBS, the likelihood that these patients are coincidental carriers of mutations in more than one *BBS* genes is negligible (Katsanis et al. 2001a). Second, we determined that, in two unrelated families, the father is in each case unaffected and homozygous for the common M390R allele, indicating that two *BBS1* mutations are not always sufficient for pathogenesis. Finally, we determined that the carrier frequency of the M390R allele in the general population is inconsistent with an exclusively autosomal recessive mode of inheritance. The 10-fold difference between the predicted and experimentally determined frequency of BBS in European populations is difficult to explain as merely the result of a diagnostic or selection bias. A more

**Figure 2** Triallelic inheritance involving *BBS1*. A, Pedigree PB56, in which both the affected mother and affected daughter are M390R homozygous and also carry an M472V mutation in *BBS4*. B, Pedigree AR396, in which the affected sib carries two *BBS1* mutations (M390R and Q291X) and a *BBS6* mutation, S326P. C, Family AR241, in which the affected sibs have two *BBS2* mutations (R315Q/R315Q; IVS1+1G) and a single *BBS1* mutation (M390R). Dashes indicate that these individuals were either unavailable or unwilling to participate in the study.





**Figure 3** Two M390R mutations are not sufficient for pathogenesis. In pedigrees PB006 (A) and PB029 (B), the unaffected father is homozygous for the common M390R allele, as are all affected individuals. A third mutation has not yet been found in these two families.

likely explanation is that the M390R allele is not always fully penetrant. We propose that an oligogenic mode of inheritance in BBS may account for some of the non-penetrance of M390R. Consistent with this hypothesis, we have determined that 15% of families with *BBS1* mutations that carry at least one M390R allele exhibit non-Mendelian trait inheritance (tables 3 and 4). We further suggest that nonpenetrance of M390R might stem from a requirement of either a third pathogenic allele in the patients or a third protective allele in the unaffected individuals. Since additional *BBS* loci are likely to be present in the genome (>50% of our pedigrees bear no mutations in the five known and tested genes, and most can be excluded by linkage from *BBS3* and *BBS5*, the two remaining mapped loci), these alternative hypotheses cannot yet be evaluated fully.

A recent study concluded that *BBS1* does not exhibit complex inheritance based on the analysis of 43 unrelated probands with two *BBS1* mutations from a cohort of 129 families with BBS (Mykytyn et al. 2003). Our data are consistent with these observations in that *BBS1* does not appear to be commonly involved in complex inheritance with the other known loci. In contrast to the conclusions of Mykytyn and colleagues, however, we report several examples in which mutations in *BBS1* alone are insufficient for pathogenesis. This discrepancy might be due to the difference in the relative size of the cohort in each study. Furthermore, our cohort is likely more genetically diverse, since the M390R allele accounts for 18% of mutations in our collection (versus >30% in the study by Mykytyn et al.), thereby increasing the probability of detecting more rare allele combina-

tions. We also note, however, that some analytical aspects presented by Mykytyn et al. (2003) are, a priori, incompatible with testing a hypothesis of complex inheritance. First, evaluation of complex inheritance in families used to establish the presence of a *BBS* locus by conventional linkage is unlikely to provide useful information, since the presence of complex inheritance may have precluded linkage in the first place. Second, traditional examination of control chromosomes alone is insufficient evidence to determine the pathogenic effect of any given allele, since mutations participating in complex inheritance are expected to have elevated carrier frequency in the general population. Finally, we note with interest that Mykytyn et al. reported two families with a single *BBS1* mutation with no evidence for a second *BBS1* allele. More significantly, they report missense alleles in other *BBS* loci, which neither represent conservative substitutions nor have been found in control chromosomes but might not segregate with the disorder. We have found two instances in which a third mutant allele exerts a modifying effect on the phenotype and have shown a cellular phenotype for such “nonsegregating” third alleles (authors’ unpublished data). We therefore suggest that some of the mutations reported in table 2 of the recent report by Mykytyn et al. might be not benign polymorphisms but pathogenic mutations.

#### *Prevalence and Distribution of Triallelic Inheritance in BBS*

From our mutational, genetic, and population data, we conclude that *BBS1*, like the other known *BBS* loci,



participates in both autosomal recessive and oligogenic inheritance. However, in contrast to some other *BBS* loci, the majority of *BBS1* mutations appear to segregate in an autosomal recessive fashion. Overall, in some 75% of families with *BBS1* mutations, two alleles appear sufficient for pathogenesis (table 1). Comparing these data with the other *BBS* loci suggests that each gene has a distinct level of involvement in triallelism (fig. 4A). Although the numbers of families with complex mutations in *BBS4* and *BBS7* are too small for accurate comparison, it appears, on the basis of the limited data presently available, that mutations in *BBS2* and *BBS6* are most frequently insufficient to cause disease by themselves. In the triallelic combinations identified to date, *BBS6* contributes a single mutant allele three times as often as *BBS2* and twice as often as *BBS4* (fig. 4A). Not surprisingly, the most frequent *BBS* gene pairing is between *BBS2* and *BBS6* (fig. 4B), although this observation will be corroborated only as additional *BBS* genes are cloned and more triallelic mutation combinations are ascertained.

We also note that not all instances of triallelic inheritance in *BBS1* are associated with causality, but some alleles might modify the phenotype. We have observed two instances in which the third mutant allele potentially exerts an epistatic effect, since some but not all of the affected sibs harbor three mutant *BBS* alleles. In family PB009, all three affected sibs are homozygous for the common M390R *BBS1* mutation, but two of those also have an additional L349W allele in *BBS2* (table 3). Intriguingly, the two sibs with the three mutations exhibit substantially more-severe retinal dystrophy, suggesting that the L349W *BBS2* allele might act as a severity modifier. Likewise, in a second family, AR768, the presence of a third T325P mutant allele in *BBS6* in addition to two *BBS1* mutations (table 3) correlates with a substantially more severe phenotype (authors' unpublished data). Given the infrequent occurrence of such genotypes, these correlations will require further substantiation. It is notable, however, that immunohistochemical analyses have indicated that the T325P substitution has a significantly detrimental effect on the cellular localization of the *BBS6* protein and is thus more likely to be a loss-of-function allele, rather than a benign variant (authors' unpublished data).

#### *The Nature of Mutations in Triallelic Inheritance*

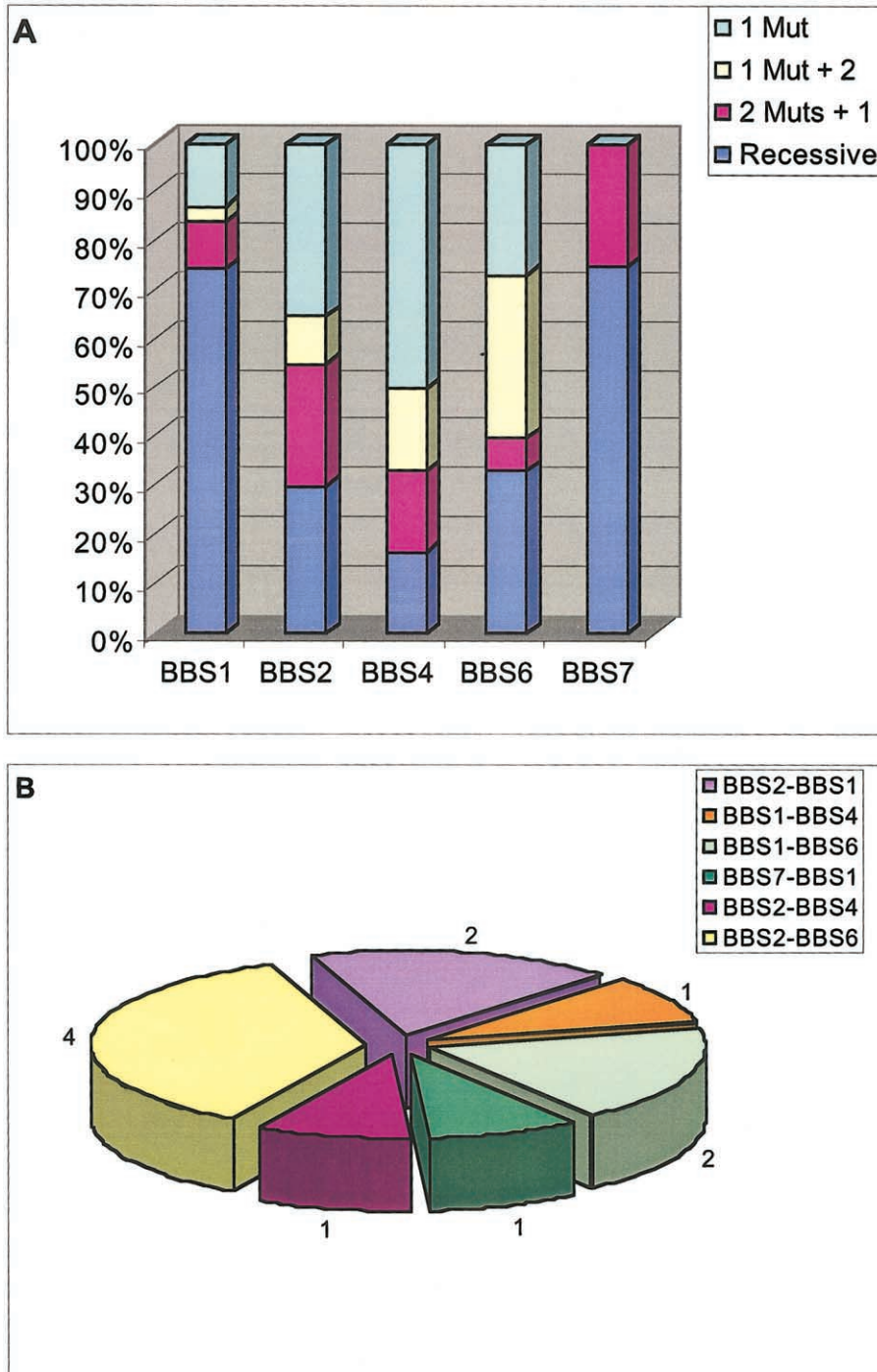
Among the oligogenic families with *BBS* identified to date, there is one example of alleles in which a null effect is the most probable functional outcome. In family AR259, a Q147X *BBS6* allele is coupled to two nonsense *BBS2* alleles (Y24X and a Q59X) in exons 1 and 2, respectively (Katsanis et al. 2001a). In the present study, we have found that both the single alleles contributed

by *BBS1* and the single alleles from other *BBS* loci coupled to two *BBS1* mutations are missense alterations. In some cases, the pathogenicity of such alleles can be substantiated by their presence in other families, such as the triallelic M390R allele in family AR241 (fig. 2C). Otherwise, evolutionary and domain analyses are currently the only means of assessing the potential functional importance of the affected residues. For instance, the methionine 472 of *BBS4*, disrupted by the missense M472V mutation in family PB056, lies in the predicted 13th tetratricopeptide (TPR) motif of *BBS4* (our unpublished observations) and is highly conserved during evolution (fig. 5).

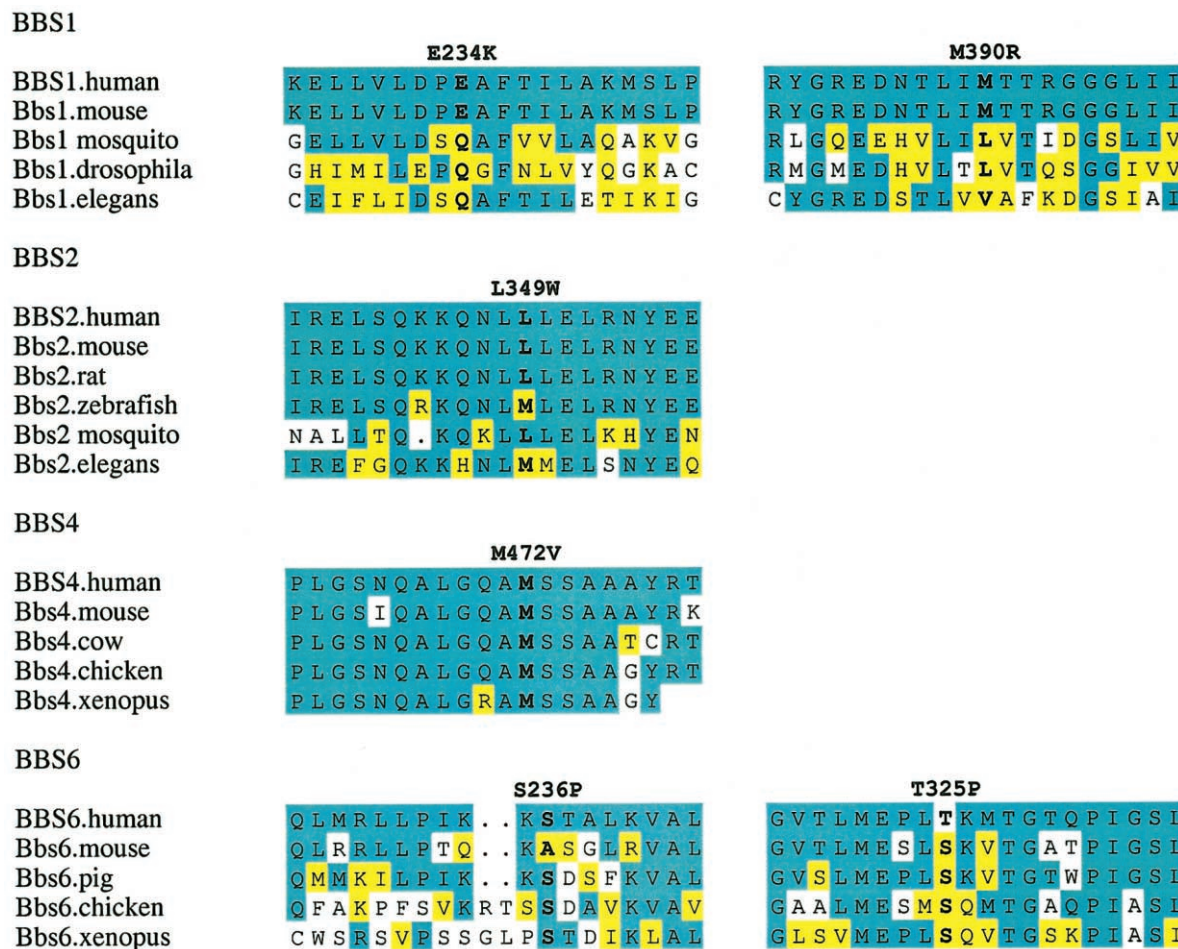
#### *Is BBS1 the Only BBS Locus on 11q13?*

A surprising finding in our studies was the relative paucity of mutations in the *BBS1* ORF. Genetic heterogeneity (HOMOG) testing and haplotype analyses have suggested that the contribution of *BBS1* to *BBS* would range from 40% to 56% (Beales et al. 1997; Bruford et al. 1997; Katsanis et al. 1999). In the present study, 20.5% of U.K. families and 25.2% of U.S. families have mutations in *BBS1*, which is significantly different from the expected values (total 23.2% vs. 40%;  $P < .0001$ ) (table 1). Several possibilities might account for this apparent discrepancy. First, larger deletions or insertions not detectable by sequencing could account for some of the missing mutations. Second, mutations at regulatory elements or cryptic splice sites would also be missed by our methodology. Third, the expected 40%–50% contribution for *BBS1* might be an overestimation, especially since it is based largely on data from small nuclear families. Fourth, there may exist additional *BBS1* exons that either were missed by the initial computational assembly of this locus or are expressed in a narrow temporal window and have thus not been detected by RT-PCR. Finally, there might be a second *BBS* locus on 11q13.

Genetic and mutational data suggest that the first possibility is unlikely to be a major factor, because (a) analysis of *BBS1* SNPs did not yield examples of hemizygosity and (b) in only two instances did we find a single mutation in pedigrees whose haplotypes are consistent with linkage to 11q13. An alternative possibility is that of regulatory element mutations. Several pedigrees harboring a single mutation at another *BBS* locus (families AR50, AR124, and AR238 for *BBS2*; family AR153 for *BBS4*) are predicted, by haplotype analysis, to carry two mutations on 11q13 (Katsanis et al. 2001a, 2002) yet do not contain mutations in the *BBS1* ORF. This is reminiscent of another oligogenic trait, Hirschsprung disease, in which noncoding mutations in *RET* on chromosome 10q11.2 are associated genetically with mutations at a second locus on chromosome 9q31 (Bolk et



**Figure 4** Analysis of triallelism. *A*, Bar graph demonstrating the distribution of recessive and complex alleles in each of the five cloned *BBS* genes. The relative contribution of one or two alleles is also indicated. *B*, Pie chart depicting the prevalence of locus combinations in families with complex BBS. Combinations were scored irrespective of the number of alleles provided by each locus. Numbers outside each slice indicate how many families exhibit each locus combination.



**Figure 5** Evolutionary analysis of mutations involved in complex inheritance. The conservation of each of the mutant *BBS1*, *BBS2*, *BBS4*, and *BBS6* alleles involved in complex inheritance was examined by comparing the predicted protein sequences of all known *BBS* genes with orthologs and homologs from all available species. The region around each relevant residue is also shown. Blue represents identities, and yellow represents conservative substitutions.

al. 2000). Finally, the possibility of a second *BBS* locus on 11q13 must be considered, similar to other instances in which two genetically unresolvable loci cause the same phenotype independently (Wang et al. 2001; Ramoz et al. 2002). Overall, 15% of pedigrees with haplotypes consistent with mapping to 11q13 bear no mutations in the *BBS1* ORF. Although the mapping of any of these families to 11q13 might be fortuitous in light of the typically small pedigree size, it is unlikely that 15% of our cohort maps to 11q13 by chance (and simultaneously has been excluded from each of the other six *BBS* loci).

More importantly, *BBS1* lies outside the previously described critical interval (Katsanis et al. 1999; Young et al. 1999b). Re-evaluation of the ancestral recombination events indicates that of the four key families with historical recombinants at D11S913, a marker 400 kb proximal to *BBS1*, three bear no *BBS1* coding muta-

tions (AR37, AR603, and PB10 (Katsanis et al. 1999), whereas the fourth family, PB13, is homozygous for M390R but remains genetically inconsistent (by haplotype analysis) with the locus, raising the possibility of complex inheritance. Of particular note is family AR37, since it generates a multipoint LOD score of 1.8 (the theoretical maximum) for 11q13 (peaking at *PYGM*) and is recombinant for *BBS1*, on the basis of both microsatellite and exonic SNP data. Whether this is due to a second *BBS* gene or a long-range control element for *BBS1* will require additional experiments.

*BBS as a Model of Oligogenic Inheritance*

Oligogenic inheritance appears to be a widespread phenomenon, and examples of genetic interaction of mutant alleles at different loci have been reported in numerous species, including humans (Badano and Katsanis

2002; Ming and Muenke 2002). Previously, we documented triallelic inheritance involving *BBS2*, *BBS4*, *BBS6* (Katsanis et al. 2000, 2001*b*), and, more recently, *BBS7* (Badano et al. 2003). These observations may reflect functional aspects of each protein, such as the ability of cellular redundancy mechanisms to rescue mutations at each locus. *BBS6*, a putative type II chaperonin (Stone et al. 2000), is frequently involved in triallelism, and most families with BBS analyzed to date with mutations at this locus carry a single mutation (Beales et al. 2001; Katsanis et al. 2001*a*; Slavotinek et al. 2002). This raises the possibility that this molecule might exert its primary effect as a modifier of two mutations at another *BBS* locus.

Thus far, each identified BBS gene has been implicated in a complex mode of inheritance at a differing frequency, including *BBS1*. In vitro and in vivo dissection of the cellular and biochemical consequences of each *BBS* allele will be required to understand the precise contribution of each gene to the phenotype. Such modeling will improve our understanding of oligogenicity, will enhance our ability to predict the phenotype from any given genotype, and will provide genetic, molecular, and statistical tools to model traits of increasing complexity of transmission.

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## Electronic-Database Information

Accession numbers and URLs for data presented herein are as follows:

BLAT search engine, <http://genome.ucsc.edu/cgi-bin/hgBlat?command=start>  
 Genome Database, <http://www.gdb.org>  
 Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim/> (for BBS)

Whitehead Institute Center for Genome Research, <http://www-genome.wi.mit.edu/>

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