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Identification of 28 novel mutations in the Bardet–Biedl syndrome genes: the burden of private mutations in an extensively heterogeneous disease

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Abstract

Bardet–Biedl syndrome (BBS), an emblematic disease in the rapidly evolving field of ciliopathies, is characterized by pleiotropic clinical features and extensive genetic heterogeneity. To date, 14 BBS genes have been identified, 3 of which have been found mutated only in a single BBS family each (BBS11/TRIM32, BBS13/MKS1 and BBS14/MKS4/NPHP6). Previous reports of systematic mutation detection in large cohorts of BBS families ($n > 90$) have dealt only with a single gene, or at most small subsets of the known BBS genes. Here we report extensive analysis of a cohort of 174 BBS families for 12/14 genes, leading to the identification of 28 novel mutations. Two pathogenic mutations in a single gene have been found in 117 families, and a single heterozygous mutation in 17 families (of which 8 involve the BBS1 recurrent mutation, M390R). We confirm that *BBS1* and *BBS10* are the most frequently mutated genes, followed by *BBS12*. No mutations have been found in BBS11/TRIM32, the identification of which as a BBS gene only relies on a single missense mutation in a single consanguineous family. While a third variant allele has been observed in a few families, they are in most cases missenses of uncertain pathogenicity, contrasting with the type of mutations observed as two alleles in a single gene. We discuss the various strategies for diagnostic mutation detection, including homozygosity mapping and targeted arrays for the detection of previously reported mutations.

Introduction

Bardet–Biedl syndrome (BBS; OMIM 209900) is a clinically pleiotropic, primarily autosomal recessive disorder whose hallmarks include obesity, progressive early-onset retinal degeneration, polydactyly, hypogenitalism, cognitive impairment and kidney dysplasia. Recent functional investigations of BBS genes and their protein products allowed the characterization of BBS as a ciliopathy, an expanding group of clinically distinct but overlapping disorders caused by defects in proteins involved in the centrosomal/primary cilia organelles (Badano et al. 2006). Homozygosity mapping in consanguineous BBS families demonstrated a surprisingly high level of non-allelic genetic heterogeneity. Since the identification of the first gene in 2000 (*BBS6*) (Katsanis et al. 2000; Slavotinek et al. 2000), mutations have been found, to date, in a total of 12 genes (*BBS1-12*) (Ansley et al. 2003; Badano et al. 2003; Chiang et al. 2004; Fan et al. 2004; Li et al. 2004; Mykytyn et al. 2001, 2002; Nishimura et al. 2001, 2005; Stoetzel et al. 2006a, 2007). In addition, single BBS cases have been described carrying homozygous or compound heterozygous mutations in two genes (*MKS1* and *CEP290/NPHP6/MKS4*) associated in general to other ciliopathies, and it was proposed that these define *BBS13* and *BBS14* (Leitch et al. 2008). *BBS1* and *BBS10* each account for about 20–25% of the mutational load in families of European descent, *BBS12* for about 8% of the families whereas each of the other nine genes accounts for ≤5% of the cases and some of them were found mutated in only few families (Katsanis 2004; Stoetzel et al. 2006a, 2007) or even, for *BBS11*, in a single family (Chiang et al. 2006). Two wide-spread recurrent mutations resulting from founder effects have been described: M390R in BBS1 (Badano et al. 2003; Mykytyn et al. 2002, 2003) and C91fsX95 in BBS10 (Stoetzel et al. 2006a). Taken together, the known BBS genes account for about

75% of families, suggesting that mutations in known genes have not yet been detected by current investigations and/or that additional BBS genes remain to be identified. A further complication is the finding that in rare cases, inheritance departs from classic autosomal recessive inheritance and involves three mutated alleles in two genes defining oligogenic inheritance (Katsanis 2004; Katsanis et al. 2001). Such third alleles may also modulate expressivity of the clinical phenotype (Katsanis 2004). We have previously calculated that given the overall frequency of BBS and the contribution of known genes to the mutation load, one can expect that 1 in 50 patients will carry a third bona fide mutation in a BBS gene by chance alone, as this is the cumulative carrier frequency of such mutations (Laurier et al. 2006). The genetic heterogeneity is a burden for identifying mutations as the full sequencing of the 12 BBS coding sequences implies more than 150 amplicons and is time-consuming with routine techniques of diagnostic laboratories.

Herein, we analyzed a cohort of 174 families, found BBS mutations in 134 of them and describe 28 novel mutations. We confirm the high level of private mutations in this heterogeneous condition and highlight the difficult task of routine mutation identification in the context of genetic counseling for the families. We discuss the available diagnostic strategies, impact on genetic counseling and some features of our observations, such as the unexpected number of heterozygotes for the recurrent BBS1 M390R mutations for which a second mutation was not found, and the lack of confirmatory mutation for the implication of the TRIM32/BBS11/LGMD in BBS.

Materials and methods

Patients

Since 2002, DNA samples from 350 BBS families, selected on the classical clinical criteria for the Bardet–Biedl syndrome (Beales et al. 1999), have been steadily referred to our laboratories for mutation screening. To date, half of the cohort (174 families) has been thoroughly investigated: mutations have been identified in 134 families (77%), whereas in 40 families (22%) no mutation was detected and are currently being explored for undetected mutations (deletions, promoter sequencing) and new gene identification. The purpose of this paper is to describe the mutational load of the explored cohort of 134 families. Validation of mutations was performed by sequencing of a control population of 96 DNA. We previously reported mutation analysis for part of this cohort (see Supplementary data 1 for more details) (Hichri et al. 2005; Stoetzel et al. 2006a, b,2007).

Initial mutation screening of the BBS1, BBS2, BBS4, BBS6, BBS7 and BBS8 genes

Mutation screening of the six BBS genes first identified was performed by DHPLC analysis using at least two melting temperatures for each amplicon, followed by direct sequencing of the variant PCR fragments as described by Hichri et al. (2005), or for some initial work on BBS1, 2, 4 or 6, by SSCP. To detect homozygous mutations, subsequent analysis was performed by SSCP and/or by mixing PCR products for DHPLC. When a BBS gene with a mutation was identified, its entire coding sequence, including splice sites, was analyzed by direct sequencing.

DNA sequencing and mutation screening

PCR amplification was performed on 50 ng of genomic DNA. Bidirectional sequencing of the purified PCR products was performed using the ABI Big Dye Terminator Sequencing kit on an ABI3100 automated capillary sequencer (Applied Biosystems). Detailed protocols are available on request. The long range PCR was carried out according to the manufacturer's protocol (Elongase Amplification System by Invitrogen). Primers are available upon request.

Screening for the M390R BBS1 recurrent mutation

Screening for the BBS1 recurrent mutation was performed by a direct digestion PCR-RFLP test according to Hichri et al. (2005).

Analysis of microsatellite markers

Genotyping of Xuorescent microsatellite markers around BBS loci was performed on a CEQ8800 genetic analysis system (Beckman Coulter). Primers for the 12 BBS genes and experimental conditions are available on request. Microsatellite sequences were obtained from the UCSC Genome Bioinformatics website (http://genome.ucsc.edu/cgi-bin/hgGateway).

SNP homozygosity mapping

Families were studied with the Affymetrix GeneChip® Mapping 10 or the 50K Array Xba 240 (Affymetrix, Santa Clara, CA). Sample processing and labeling were performed according to the manufacturer's instructions (Affymetrix Mapping 10K 2.0 Assay Manual, Version 1.0, 2004 or Affymetrix Mapping 100K Assay Manual). Arrays were hybridized on a GeneChip Hybridization Oven 640, washed with the GeneChip Fluidics Station 450 and scanned with a GeneChip Scanner 3000 Data were processed by the GeneChip DNA Analysis Software version 3.0.2 (GDAS) to generate SNP allele calls. An average call rate >99% was obtained. Homozygosity regions were identified as regions of homozygosity longer than 25 adjacent SNPs (Stoetzel et al. 2007) for the 10K arrays, 30 for the 50K arrays and 35 for the 250K arrays.

Detection of deletions or duplications with the Affymetrix 6.0 array

Samples were processed by Affymetrix using the Automated Target Preparation protocol P/N 702561 and the Affymetrix 6.0 microarray. Analysis has been carried with the Affymetrix Genotyping Console 3.0.2.

Asper Ophthalmics BBS array

Forty-seven families have been tested onto a dedicated chip from Asper Ophthalmics (http://www.asperophthalmics.com/BBSgenetest.htm). The Bardet–Biedl syndrome test array has been established for screening 237 mutations (including some polymorphisms/rare variants of uncertain pathogenicity) from 12 genes: BBS1, BBS2, BBS3, BBS4, BBS5, BBS6, BBS7, BBS8, BBS10, PHF6 (Borjeson–Forssman–Lehmann syndrome), *ALMS1* (Alstrom syndrome, which shows some clinical overlap with BBS) and *GNAS1* (Albright hereditary osteodystrophy). DNA samples were processed directly by Asper Biotech. Some of the mutations assayed in the Asper array were integrated by Asper based on our initial data from this cohort.

Bioinformatics

For each of the mutated BBS genes in our study, the protein sequences were retrieved from the HomoloGene database (Wheeler et al. 2008) and UniProt (2008) database. When available, we extracted representative sequences for metazoan organisms including: Caenorhabditis elegans, Caenorhabditis briggsae, Drosophila melanogaster, Anopheles gambiae, Ciona intestinalis, Tetraodon nigroviridis, Brachydanio rerio, Takifugu rubripes, Xenopus laevis, Bos taurus, Gallus gallus, Rattus norvegicus, Mus musculus, Canis familiaris, Homo sapiens. Missing proteins in some organism were predicted when possible on the basis of the available sequences and using TBLASTN and the corresponding genome. The genome sequences were retrieved from generic databases such as NCBI (Wheeler et al. 2008), UCSC (Karolchik et al. 2008) or ENSEMBL (Flicek et al.2008).

Multiple alignments were computed using ClustalW (Thompson et al. 1994) and were further manually inspected. The BBS6, BBS10 and BBS12 multiple alignments are a subset of the multiple alignment described initially in (Stoetzel et al. 2007). The newly described mutations in this study are positioned according to the protein sequences onto their respective multiple alignment. Sequence conservation of the mutated amino acids residues has been analyzed within metazoans. We first looked if the residue was strictly conserved at the position concerned, or the amino acid properties (i.e., hydrophobic or charged) and then if the mutation observed has been already found during the evolution at this position. The results are available at http://bips.u-strasbg.fr/BBS/BBS_NovelMutations_2009.html.

All missense mutations have been tested for pathogenicity using both SIFT (Ng and Henikoff 2003) and the alignments built for this study as queries, and the PolyPhen web server via batch submission mode (Ramensky et al. 2002).

Splice sites scoring programs such as SpliceView (Rogozin and Milanesi 1997) or NNSPLICE (Reese et al.1997) were used to evaluate the effect of various mutations (e.g., silent, or missense changes and intronic variations) affecting splice sites. Rescue ESE web server (Yeo et al.2004) was used to predict potential exonic splicing enhancers in polymorphic exonic variants (silent or missense variants).

Results

We describe here the results of systematic mutation screening in *BBS1-12* in 174 families. As this work extended over several years, different screening strategies were used, including heteroduplex screening by DHPLC, homozygosity mapping in consanguineous families, and use of a dedicated microarray for assaying previously reported BBS mutations (Asper Ophthalmics). In addition, Affymetrix 6.0 arrays were used to detect genome rearrangement with a potential pathogenic effect. Together, our work led to the identification of mutations in 134 families (77%) of this cohort. Initial results for six BBS genes on a small subset of families were reported earlier (Hichri et al. 2005), while an analysis of a larger subset were reported for *BBS8, BBS10* and *BBS12* (Stoetzel et al. 2006a, b, 2007). We have referenced 89 different mutations in 10 of the 12 known BBS genes (for a complete description of all mutations see Supplementary Data 1), including 28 alleles that have not been reported previously. These mutations are the basis of all subsequent analyses.

We excluded from our novel pathogenic mutations list two missense observed in two families: two rare missense most likely to be non-pathogenic found as a third allele, R122Q in BBS3 and S574C in BBS7, respectively, in a single family homozygote for a BBS12 frameshift mutation (T257fsX266) and in a single family compound heterozygote for BBS2 (L168fsX200/C307Y) (see Supplementary Data 1).

Our calculation of the mutation load includes for each BBS gene, the count of families (not taking into account occasional third allele to avoid overestimation of families identified) and the number of mutated alleles observed in the families. The two most frequently mutated genes are BBS1 in 44 families (32.6%)/79 alleles (30.6%) including nine families with only one mutation identified and *BBS10* in 44 families (32.6%)/87 alleles (33.7%) with only one family with one mutation identified (Fig. 1). The third most frequently mutated gene is *BBS12* with 14 families (10.4% of the families)/28 alleles (10.9%). Overall, *BBS1, BBS10* and *BBS12* account for about 75% of the identified mutational load in our series (or 58% of the total load including the families with no identified mutations), consistent with previous studies (Badano et al. 2003; Mykytyn et al. 2002; Stoetzel et al. 2006a, 2007). The other BBS genes contribution to the identified mutation load is the following: *BBS2* in eight families (5.9%)/14 alleles (5.4%); *BBS4* in eight families (5.9%)/14 alleles (5.8%) and once

as a third allele; *BBS5* in three families (2.2%)/6 alleles (2.3%); *BBS6* in five families (3.7%)/11 alleles (4.3%) and three missense variants as third alleles; *BBS7* for two families (1.5%)/5 alleles (1.9%) and one splice mutation as a third allele; *BBS8* in three families (2.2%)/5 alleles (1.9%); *BBS9* for four families (3.0%)/8 alleles (3.1%). Except for a single missense variant as a third allele of uncertain pathogenicity (see above), no mutations were found for *BBS3* encoding the smallest coding sequence (only 187 aa) of the know BBS genes. No mutation was identified for BBS11.

Overall, the 28 novel mutations (Table 1) cover 8 BBS genes and are distributed as following: 13 nonsense mutations, 5 deletions, 10 missense mutations and 1 splice mutation. Of them, 16 are found at the homozygous state and 11 at the heterozygous state including 7 as a second allele of a recurrent mutation (BBS1:M390R or BBS10:C91fsX95).

For *BBS1*, four novel mutations were observed: three as a second allele for the recurrent M390R mutation and one (A107fsX) at the homozygous state. For *BBS10*, all four mutations were the second allele of the recurrent C91fsX95 mutation. For the other BBS genes, the novel mutations had the following distribution at the homozygote state: three for *BBS2*, four for *BBS4*, three for *BBS5*, one for *BBS6* and three for *BBS9*.

Two recurrent mutations have been reported previously, respectively, M390R for BBS1 and C91fsX95 for BBS10 (Mykytyn et al. 2002; Stoetzel et al. 2006a). The BBS1 M390R mutation was found at the homozygous state in 18 families and as a compound heterozygote allele in 21 families; overall this mutation represents 73.4% (58/79 alleles) of the BBS1 mutational load, in agreement with previous observations (Badano et al. 2003; Mykytyn et al. 2002) (Fig. 1). One intriguing observation is the presence of a heterozygous M390R mutation in eight probands in whom no second mutation was detected by sequencing of all BBS1 coding exons. The C91fsX95 mutation represents 48.3% (42/87 alleles) of the BBS10 mutational load encompassing 29 families: 13 families at the homozygous state and 16 at the heterozygous state. This mutation was observed only once as a single heterozygous allele with no second mutation detected in the gene, in contrast to the observation on the recurrent status of heterozygote BBS1 M390R mutation.

Although the newly identified mutations were not observed in a panel of 96 control DNA and are not recorded in the single nucleotide polymorphism database (dbSNP) (Wheeler et al. 2008), we cannot exclude that some of them are not fully pathogenic or may represent normal rare variants. Splice mutation effect has been checked and except a BBS7 missense allele (discussed later) none of the other novel missense mutations or novel third allele variants were predicted as affecting splicing. Therefore, we tested these missense mutations using bioinformatic prediction software such as SIFT (Ng and Henikoff 2003) and PolyPhen (Ramensky et al. 2002), and further assessing sequence conservation around the variant position among metazoan species of each BBS genes. The results are shown in Table 1 and detailed in Supplementary Data 2. Among the nine prospective mutations tested, seven were clearly predicted as deleterious and 1 mutation (BBS9:I154M) is predicted as benign by PolyPhen (however with a score close to the “possibly damaging” threshold) and deleterious by SIFT (see Supplementary Data 2). Although SIFT and PolyPhen already take the conservation of sequences into account, we further investigated evolutionary conservation of the residues affected by these mutations in metazoan species, based on their positioning onto their respective multiple sequence alignments (Table 1). Detailed views of the alignments have been prepared using Jalview (Waterhouse et al. 2009) (Supplementary Data 3).

With the exception of BBS6, BBS10 and BBS12, which are only present in vertebrates (Kim et al. 2005; Stoetzel et al. 2007), all BBS genes do exist in all metazoan species used in our analysis. Among the nine missense mutations, three affect positions conserved in vertebrates

only (BBS2:C307Y, BBS6:I297T, BBS10:G43D), one in chordates and insects (BBS1:L288R) and five across all metazoans (BBS2:L221P as hydrophobic residue, BBS4:N309K, BBS5:R56G, BBS9:I154M as hydrophobic residue and BBS9:V81E as hydrophobic residue). The BBS9 I154M where PolyPhen and SIFT give apparently contradictory prediction is located in a stretch of eight amino acids strictly conserved in vertebrates.

Oligogenic interaction

One BBS7 (S574C) missense variants was present as a third allele and in a family with 2 BBS2 mutations (L168fsX200/C307Y). Apart from that, five other families carried three already known mutations or rare missense variant: one is a compound heterozygote family for BBS1 (M390R; R146X) and carries a third BBS6 allele (T57A) (the position is strictly conserved across metazoan and PolyPhen is describing the variation as “possibly damaging” with a score of 1.825) (Katsanis et al. 2000); one family is homozygous for a BBS8 mutation (splice mutation T153T) and carries a third BBS7 mutation (M114V mutation also affecting splicing) (Stoetzel et al. 2006b); three families carry two BBS10 mutations and a third missense allele in either BBS4 [BBS10:C91fsX95/C91fsX95 and BBS4:L351R] (strictly conserved except in *C. elegans*, and PolyPhen describing it as “probably damaging” with a score of 2.018) or BBS6 (BBS10:R34P/C91fsX95 with BBS6:I339V) [not likely pathogenic based on previous segregation data (Slavotinek et al. 2002), the poor conservation across metazoan and the PolyPhen prediction as “benign” with a score of 0.959], and BBS10:C91fsX95/C195W with BBS6:T237A (Hichri et al. 2005; Stoetzel et al. 2006a) (not likely pathogenic given the high variability of that position among metazoan and the PolyPhen prediction as “benign” with a score of 0.051).

Discussion

A growing number of inherited disorders show extensive genetic heterogeneity implying difficulties in genotyping patients for diagnosis confirmation and/or genetic counseling. Leber congenital amaurosis (at least 14 genes involved) (den Hollander et al. 2008), Usher syndrome (at least 11 genes involved) (Saihan et al. 2009) or BBS are examples of numerous gene identifications in the last years and continuous struggle for efficient genotyping. The extensive genetic heterogeneity of BBS has profound implications for diagnostic and genetic counseling applications.

Herein, we present a cohort of 134 fully explored BBS patients carrying mutations in the known genes for BBS and report 28 novel mutations. Genotyping investigations have improved in the last years. Ongoing since 2002, our mutation detection strategy has evolved according to novel gene identifications and the availability of new methods of investigation. Overall, this evolution combined chronologically the use of single strand conformation polymorphism (SSCP) or denaturing high-performance liquid chromatography (DHPLC) (Hichri et al. 2005), sequencing of known BBS genes (primers available on request), screening for recurrent mutations for BBS1 (M390R screened by restrictive test) and for BBS10 and BBS12 because of a single exon (both screened by direct sequencing). In parallel, analysis of consanguineous families was converted from the initial microsatellite analysis into SNP analysis in the last 3 years. More recently, some families were screened by the arrayed primer extension technology for 161 previously described mutations (Asper Ophthalmics) and screened for some of them for genomic rearrangements with the Affymetrix 6.0. Overall, our study confirms the major contribution of BBS1, BBS10 and (to a lower degree) BBS12 to the mutation load, as these genes account for three quarters of the detected mutant alleles. The importance of the recurrent mutations accounting herein for at least half of the respective mutations of BBS1 and BBS10 highlights the potential value of a

preliminary screening for these mutations. Thus, for efficient and rapid genotyping, it is rewarding to test in priority BBS1, BBS10 and BBS12.

Furthermore, the 28 novel mutations described herein can be added to the global mutational load of the BBS genes and complement previously designed genotyping arrays. In this series, 256 mutated alleles are described of which 49 are newly described alleles (19.1%) and 136 are found only in a single family (53.1% of the mutational load). A similar figure was reported by Harville et al. recently, with 11 novel homozygous mutations out of 20 identified in consanguineous families (and none of these mutations have been observed in the present study) (Harville et al. 2009). This underlines the “private” (one mutation found only in one family) characteristics of BBS mutations in more then half of the cases disclosing a limit for rapid mutation detection in half of the cases. The nine novel mutations found in compound heterozygotes were detected following preliminary screening for a recurrent mutation in BBS1 or BBS10.

Intriguingly, we have an excess of heterozygote mutations especially for BBS1. This can be explained by either an undetected *BBS1* mutation (large deletion, mutation in promoter) or if the M390R is the third allele accompanying mutations in a yet not identified gene in these families. Seventeen families are in this situation and eight are heterozygotes for the M390R mutation with no other mutation detected to date.

We carried an Affymetrix SNP 6.0 array study on four of the latter and failed to identify any rearrangement in the vicinity of BBS1 or anywhere else in the genome. These results prompted us to check the sensitivity of this approach in detecting known deletions in BBS patients. In this respect, we performed the analysis on two BBS4 deleted samples: VII.28 (del exon 4-5-6) and II.24 (del exon 7-8). Interestingly, the first could be validated using the Affymetrix array (and further validated by RT-PCR), whereas the second one was not detected due to the lack of oligonucleotide probes on the array within the deleted region (see Fig. 2).

Validation of missense mutation can be a delicate task and uncertainty about pathogenicity is a major problem for genetic counseling and in particular for prenatal diagnosis. Sequence conservation studies can be rewarding and may be usefully complemented by functional testing. For one missense mutation at the homozygous state in BBS5, we were able to validate its pathogenicity by way of in vivo complementation analysis (Leitch et al. 2008) (see Supplementary Data 4) opening the way for future prenatal diagnosis required for the family. Another revealing case is one missense identified in a patient who was homozygous for the BBS4 region using microsatellite analysis. We observed for the first time the previously described rare variant K46R in BBS4 (Mykytyn et al. 2003) at the homozygous state (see Supplementary Data 1), which is predicted as very unlikely to be the pathogenic mutation in this family (see Supplementary Data 1). Nevertheless, functional studies (N.K., manuscript submitted) by way of in vivo complementation analysis reveal that this variation is indeed a real mutation, pointing out some limitation of bioinformatics analysis and conservation studies. Preliminary SNP study for consanguineous families was extremely useful to detect homozygosity in regions with known BBS genes prompting sequencing of the coding sequence of the gene of interest. Seventeen novel mutations were identified using this strategy and would have been missed by the available mutation array. While this manuscript was completed, Harville et al. also reported the value of preliminary homozygosity mapping in identifying target BBS genes for sequencing and diagnosis. This strategy is useful for recessive disease with extensive genetic heterogeneity (Cossee et al. 2009).

Efficient BBS genotyping to date is based either on classical mutation detection (RFLP for the recurrent M390R mutation and direct sequencing) or on sequencing chip array designed (Asper Ophthalmics) to detect previously reported mutations. However, this latter method of investigations does not cover novel mutations.

Given the high number of private mutations identified in BBS patients, and the small numbers of reported mutations for some BBS genes these arrays are certainly useful but have presently a limited power. Herein, we suggest a strategy that could be applied to efficient mutations detection in BBS patients (see Fig. 3). An initial screening encompassing at least the recurrent mutations of BBS1 and BBS10 and/or the full sequence of BBS10 can detect potentially 30–50% of the BBS patients. This could be complemented by the sequencing of the unique coding exon of BBS12, which would increase the detection power by 8%. The recurrent mutation tests have been described previously (Hichri et al. 2005; Stoetzel et al. 2006a). If the family is consanguineous (or if the parents are from the same region or population group), we advocate a SNP homozygosity study and sequence the BBS gene (s) found in regions of homozygosity. This strategy has permitted us to detect 80% of mutations in our cohort including novel mutations especially associated to recurrent mutations or at the homozygote state in consanguineous families.

For genetic counseling purposes, the spouse of a confirmed or putative heterozygote patient for a recurrent BBS1 or BBS10 mutations can benefit from recurrent mutation screen in order to exclude heterozygosity and reduce risk of recurrence. To date, genetic counseling is performed on the basis of a classical autosomal recessive condition with a 25% risk of recurrence. Prenatal diagnosis by molecular analysis of chorionic villi can be offered if two pathogenic mutations in one BBS gene are clearly and unambiguously identified. If no mutation is identified the only way to seek for a recurrence in case of a couple with an affected child is ultrasound detection of polydactyly and/or enlarged kidneys.

A number of questions remain to be answered. First, how many BBS patients disclose oligogenic inheritance in BBS genes or related ciliopathy genes? In this respect we are currently extensively screening 288 BBS samples in a high-throughput sequencing project on ciliopathies. Undetectable mutations with classical direct sequencing screen such as deletions or duplication or promoter mutation are currently under investigation using the latter strategy and high-density arrays or quantitative PCR analysis. On the basis of homozygosity mapping in families where no gene has been identified yet, it is very likely that the unidentified BBS genes each account for a small percentage of families. Exon capture combined to high-throughput sequencing will provide identification of the unknown BBS genes and may even be used in the future for diagnostic purpose (Ng et al. 2009).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, Kim JC, Ross AJ, Eichers ER, Teslovich TM, Mah AK, Johnsen RC, Cavender JC, Lewis RA, Leroux MR, Beales PL, Katsanis N.

Basal body dysfunction is a likely cause of pleiotropic Bardet–Biedl syndrome. Nature. 2003; 425:628–633. [PubMed: 14520415]

Badano JL, Ansley SJ, Leitch CC, Lewis RA, Lupski JR, Katsanis N. Identification of a novel Bardet–Biedl syndrome protein, BBS7, that shares structural features with BBS1 and BBS2. Am J Hum Genet. 2003; 72:650–658. [PubMed: 12567324]

Badano JL, Mitsuma N, Beales PL, Katsanis N. The ciliopathies: an emerging class of human genetic disorders. Annu Rev Genomics Hum Genet. 2006; 7:125–148. [PubMed: 16722803]

Beales PL, Elcioglu N, Woolf AS, Parker D, Flinter FA. New criteria for improved diagnosis of Bardet–Biedl syndrome: results of a population survey. J Med Genet. 1999; 36:437–446. [PubMed: 10874630]

Chiang AP, Nishimura D, Searby C, Elbedour K, Carmi R, Ferguson AL, Secrist J, Braun T, Casavant T, Stone EM, Sheffield VC. Comparative genomic analysis identifies an ADP-ribosylation factor-like gene as the cause of Bardet–Biedl syndrome (BBS3). Am J Hum Genet. 2004; 75:475–484. [PubMed: 15258860]

Chiang AP, Beck JS, Yen HJ, Tayeh MK, Scheetz TE, Swiderski RE, Nishimura DY, Braun TA, Kim KY, Huang J, Elbedour K, Carmi R, Slusarski DC, Casavant TL, Stone EM, Sheffield VC. Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet–Biedl syndrome gene (BBS11). Proc Natl Acad Sci USA. 2006; 103:6287–6292. [PubMed: 16606853]

Cossee M, Lagier-Tourenne C, Seguela C, Mohr M, Leturcq F, Gun-desli H, Chelly J, Tranchant C, Koenig M, Mandel JL. Use of SNP array analysis to identify a novel TRIM32 mutation in limb-girdle muscular dystrophy type 2H. Neuromuscul Disord. 2009; 19:255–260. [PubMed: 19303295]

den Hollander AI, Roepman R, Koenekoop RK, Cremers FP. Leber congenital amaurosis: genes, proteins and disease mechanisms. Prog Retin Eye Res. 2008; 27:391–419. [PubMed: 18632300]

Fan Y, Esmail MA, Ansley SJ, Blacque OE, Boroevich K, Ross AJ, Moore SJ, Badano JL, May-Simera H, Compton DS, Green JS, Lewis RA, van Haelst MM, Parfrey PS, Baillie DL, Beales PL, Katsanis N, Davidson WS, Leroux MR. Mutations in a member of the Ras superfamily of small GTP-binding proteins causes Bardet–Biedl syndrome. Nat Genet. 2004; 36:989–993. [PubMed: 15314642]

Flicek P, Aken BL, Beal K, Ballester B, Caccamo M, Chen Y, Clarke L, Coates G, Cunningham F, Cutts T, Down T, Dyer SC, Eyre T, Fitzgerald S, Fernandez-Banet J, Graf S, Haider S, Hammond M, Holland R, Howe KL, Howe K, Johnson N, Jenkinson A, Kahari A, Keefe D, Kokocinski F, Kulesha E, Lawson D, Longden I, Megy K, Meidl P, Overduin B, Parker A, Pritchard B, Prlic A, Rice S, Rios D, Schuster M, Sealy I, Slater G, Smedley D, Spudich G, Trevanion S, Vilella AJ, Vogel J, White S, Wood M, Birney E, Cox T, Curwen V, Durbin R, Fernandez-Suarez XM, Herrero J, Hubbard TJ, Kasprzyk A, Proctor G, Smith J, Ureta-Vidal A, Searle S. Ensembl 2008. Nucleic Acids Res. 2008; 36:D707–D714. [PubMed: 18000006]

Harville HM, Held S, Diaz-Font A, Davis EE, Diplas BH, Lewis RA, Borochowitz ZU, Zhou W, Chaki M, Macdonald J, Kayserili H, Beales PL, Katsanis N, Otto E, Hildebrandt F. Identification of 11 novel mutations in 8 BBS genes by high-resolution homozygosity mapping. J Med Genet. 2009 doi:10.1136/jmg.2009.071365.

Hichri H, Stoetzel C, Laurier V, Caron S, Sigaudy S, Sarda P, Hamel C, Martin-Coignard D, Gilles M, Leheup B, Holder M, Kaplan J, Bitoun P, Lacombe D, Verloes A, Bonneau D, Perrin-Schmitt F, Brandt C, Besancon AF, Mandel JL, Cossee M, Dollfus H. Testing for triallelism: analysis of six BBS genes in a Bardet–Biedl syndrome family cohort. Eur J Hum Genet. 2005; 13:607–616. [PubMed: 15770229]

Karolchik D, Kuhn RM, Baertsch R, Barber GP, Clawson H, Diekhans M, Giardine B, Harte RA, Hinrichs AS, Hsu F, Kober KM, Miller W, Pedersen JS, Pohl A, Raney BJ, Rhead B, Rosenbloom KR, Smith KE, Stanke M, Thakkapallayil A, Trumbower H, Wang T, Zweig AS, Haussler D, Kent WJ. The UCSC genome browser database: 2008 update. Nucleic Acids Res. 2008; 36:D773–D779. [PubMed: 18086701]

Katsanis N. The oligogenic properties of Bardet–Biedl syndrome. Hum Mol Genet. 2004; 13:R65–R71. Spec No 1. [PubMed: 14976158]

Katsanis N, Beales PL, Woods MO, Lewis RA, Green JS, Parfrey PS, Ansley SJ, Davidson WS, Lupski JR. Mutations in MKKS cause obesity, retinal dystrophy and renal malformations associated with Bardet–Biedl syndrome. Nat Genet. 2000; 26:67–70. [PubMed: 10973251]

Katsanis N, Ansley SJ, Badano JL, Eichers ER, Lewis RA, Hoskins BE, Scambler PJ, Davidson WS, Beales PL, Lupski JR. Triallelic inheritance in Bardet–Biedl syndrome, a Mendelian recessive disorder. Science. 2001; 293:2256–2259. [PubMed: 11567139]

Kim JC, Ou YY, Badano JL, Esmail MA, Leitch CC, Fiedrich E, Beales PL, Archibald JM, Katsanis N, Rattner JB, Leroux MR. MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet–Biedl syndrome, is a novel centrosomal component required for cytokinesis. J Cell Sci. 2005; 118:1007–1020. [PubMed: 15731008]

Laurier V, Stoetzel C, Muller J, Thibault C, Corbani S, Jalkh N, Salem N, Chouery E, Poch O, Licaire S, Danse JM, Amati-Bonneau P, Bonneau D, Megarbane A, Mandel JL, Dollfus H. Pitfalls of homozygosity mapping: an extended consanguineous Bardet–Biedl syndrome family with two mutant genes (BBS2, BBS10), three mutations, but no triallelism. Eur J Hum Genet. 2006; 14:1195–1203. [PubMed: 16823392]

Leitch CC, Zaghloul NA, Davis EE, Stoetzel C, Diaz-Font A, Rix S, Alfadhel M, Lewis RA, Eyaid W, Banin E, Dollfus H, Beales PL, Badano JL, Katsanis N. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet–Biedl syndrome. Nat Genet. 2008; 40:443–448. [PubMed: 18327255]

Li JB, Gerdes JM, Haycraft CJ, Fan Y, Teslovich TM, May-Simera H, Li H, Blacque OE, Li L, Leitch CC, Lewis RA, Green JS, Parfrey PS, Leroux MR, Davidson WS, Beales PL, Guay-Woodford LM, Yoder BK, Stormo GD, Katsanis N, Dutcher SK. Comparative genomics identifies a Xagellar and basal body proteome that includes the BBS5 human disease gene. Cell. 2004; 117:541–552. [PubMed: 15137946]

Mykytyn K, Braun T, Carmi R, Haider NB, Searby CC, Shastri M, Beck G, Wright AF, Iannaccone A, Elbedour K, Riise R, Baldi A, Raas-Rothschild A, Gorman SW, Duhl DM, Jacobson SG, Casavant T, Stone EM, Sheffield VC. Identification of the gene that, when mutated, causes the human obesity syndrome BBS4. Nat Genet. 2001; 28:188–191. [PubMed: 11381270]

Mykytyn K, Nishimura DY, Searby CC, Shastri M, Yen HJ, Beck JS, Braun T, Streb LM, Cornier AS, Cox GF, Fulton AB, Carmi R, Luleci G, Chandrasekharappa SC, Collins FS, Jacobson SG, Heckenlively JR, Weleber RG, Stone EM, Sheffield VC. Identification of the gene (BBS1) most commonly involved in Bardet–Biedl syndrome, a complex human obesity syndrome. Nat Genet. 2002; 31:435–438. [PubMed: 12118255]

Mykytyn K, Nishimura DY, Searby CC, Beck G, Bugge K, Haines HL, Cornier AS, Cox GF, Fulton AB, Carmi R, Iannaccone A, Jacobson SG, Weleber RG, Wright AF, Riise R, Hennekam RC, Luleci G, Berker-Karauzum S, Biesecker LG, Stone EM, Sheffield VC. Evaluation of complex inheritance involving the most common Bardet–Biedl syndrome locus (BBS1). Am J Hum Genet. 2003; 72:429–437. [PubMed: 12524598]

Ng PC, Henikoff S. SIFT: predicting amino acid changes that affect protein function. Nucleic Acids Res. 2003; 31:3812–3814. [PubMed: 12824425]

Ng SB, Turner EH, Robertson PD, Flygare SD, Bigham AW, Lee C, Shaffer T, Wong M, Bhattacharjee A, Eichler EE, Bamshad M, Nickerson DA, Shendure J. Targeted capture and massively parallel sequencing of 12 human exomes. Nature. 2009; 461:272–276. [PubMed: 19684571]

Nishimura DY, Searby CC, Carmi R, Elbedour K, Van Maldergem L, Fulton AB, Lam BL, Powell BR, Swiderski RE, Bugge KE, Haider NB, Kwitek-Black AE, Ying L, Duhl DM, Gorman SW, Heon E, Iannaccone A, Bonneau D, Biesecker LG, Jacobson SG, Stone EM, Sheffield VC. Positional cloning of a novel gene on chromosome 16q causing Bardet–Biedl syndrome (BBS2). Hum Mol Genet. 2001; 10:865–874. [PubMed: 11285252]

Nishimura DY, Swiderski RE, Searby CC, Berg EM, Ferguson AL, Hennekam R, Merin S, Weleber RG, Biesecker LG, Stone EM, Sheffield VC. Comparative genomics and gene expression analysis identifies BBS9, a new Bardet–Biedl syndrome gene. Am J Hum Genet. 2005; 77:1021–1033. [PubMed: 16380913]

Ramensky V, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. Nucleic Acids Res. 2002; 30:3894–3900. [PubMed: 12202775]

Reese MG, Eeckman FH, Kulp D, Haussler D. Improved splice site detection in Genie. J Comput Biol. 1997; 4:311–323. [PubMed: 9278062]

Rogozin IB, Milanesi L. Analysis of donor splice sites in different eukaryotic organisms. J Mol Evol. 1997; 45:50–59. [PubMed: 9211734]

Saihan Z, Webster AR, Luxon L, Bitner-Glindzicz M. Update on Usher syndrome. Curr Opin Neurol. 2009; 22:19–27. [PubMed: 19165952]

Slavotinek AM, Stone EM, Mykytyn K, Heckenlively JR, Green JS, Heon E, Musarella MA, Parfrey PS, Sheffield VC, Biesecker LG. Mutations in MKKS cause Bardet–Biedl syndrome. Nat Genet. 2000; 26:15–16. [PubMed: 10973238]

Slavotinek AM, Searby C, Al-Gazali L, Hennekam RC, Schrander-Stumpel C, Orcana-Losa M, Pardo-Reoyo S, Cantani A, Kumar D, Capellini Q, Neri G, Zackai E, Biesecker LG. Mutation analysis of the MKKS gene in McKusick–Kaufman syndrome and selected Bardet–Biedl syndrome patients. Hum Genet. 2002; 110:561–567. [PubMed: 12107442]

Stoetzel C, Laurier V, Davis EE, Muller J, Rix S, Badano JL, Leitch CC, Salem N, Chouery E, Corbani S, et al. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. Nat Genet. 2006a; 38(5):521–524. [PubMed: 16582908]

Stoetzel C, Laurier V, Faivre L, Megarbane A, Perrin-Schmitt F, Verloes A, Bonneau D, Mandel JL, Cossee M, Dollfus H. BBS8 is rarely mutated in a cohort of 128 Bardet–Biedl syndrome families. J Hum Genet. 2006b; 51:81–84. [PubMed: 16308660]

Stoetzel C, Muller J, Laurier V, Davis EE, Zaghloul NA, Vicaire S, Jacquelin C, Plewniak F, Leitch CC, Sarda P, et al. Identification of a novel BBS gene (BBS12) highlights the major role of a vertebrate-specific branch of chaperonin-related proteins in Bardet-Biedl syndrome. Am J Hum Genet. 2007; 80(1):1–11. [PubMed: 17160889]

Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 1994; 22:4673–4680. [PubMed: 7984417]

UniProt. The universal protein resource (UniProt). Nucleic Acids Res. 2008; 36:D190–D195. [PubMed: 18045787]

Waterhouse AM, Procter JB, Martin DM, Clamp M, Barton GJ. Jalview Version 2—a multiple sequence alignment editor and analysis workbench. Bioinformatics. 2009; 25:1189–1191. [PubMed: 19151095]

Wheeler DL, Barrett T, Benson DA, Bryant SH, Canese K, Chetvernin V, Church DM, Dicuccio M, Edgar R, Federhen S, Feolo M, Geer LY, Helmberg W, Kapustin Y, Khovayko O, Landsman D, Lipman DJ, Madden TL, Maglott DR, Miller V, Ostell J, Pruitt KD, Schuler GD, Shumway M, Sequeira E, Sherry ST, Sirotkin K, Souvorov A, Starchenko G, Tatusov RL, Tatusova TA, Wagner L, Yaschenko E. Database resources of the National Center for Biotechnology Information. Nucleic Acids Res. 2008; 36:D13–D21. [PubMed: 18045790]

Yeo G, Hoon S, Venkatesh B, Burge CB. Variation in sequence and organization of splicing regulatory elements in vertebrate genes. Proc Natl Acad Sci USA. 2004; 101:15700–15705. [PubMed: 15505203]

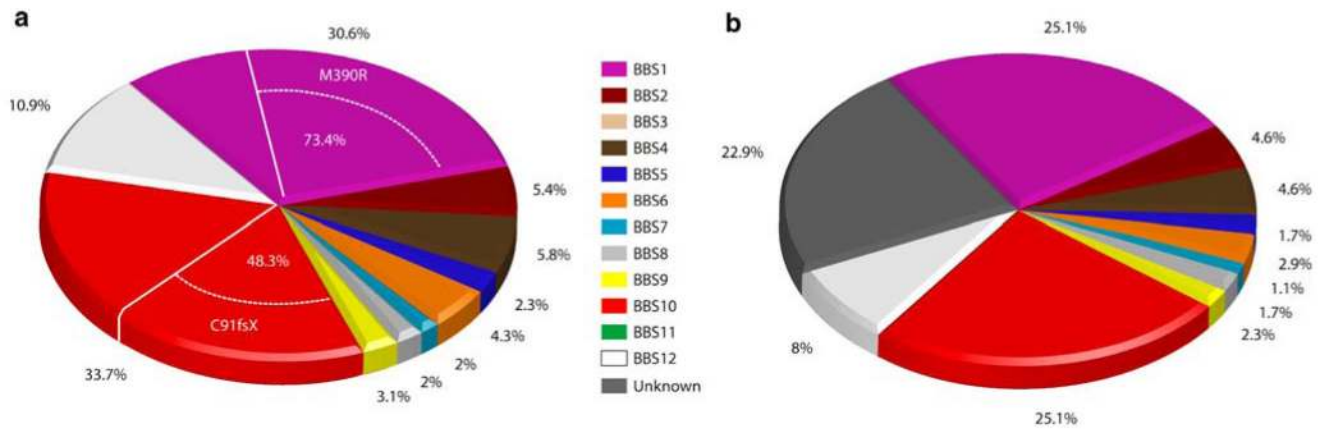

Fig. 1.

a Distribution of mutated alleles for each BBS genes in the 134 families analyzed. Details are given for the two recurrent mutations M390R and C91fsX, respectively, in BBS1 and BBS10. **b** Distribution of the fraction of BBS genes mutated in our cohort of 174 families

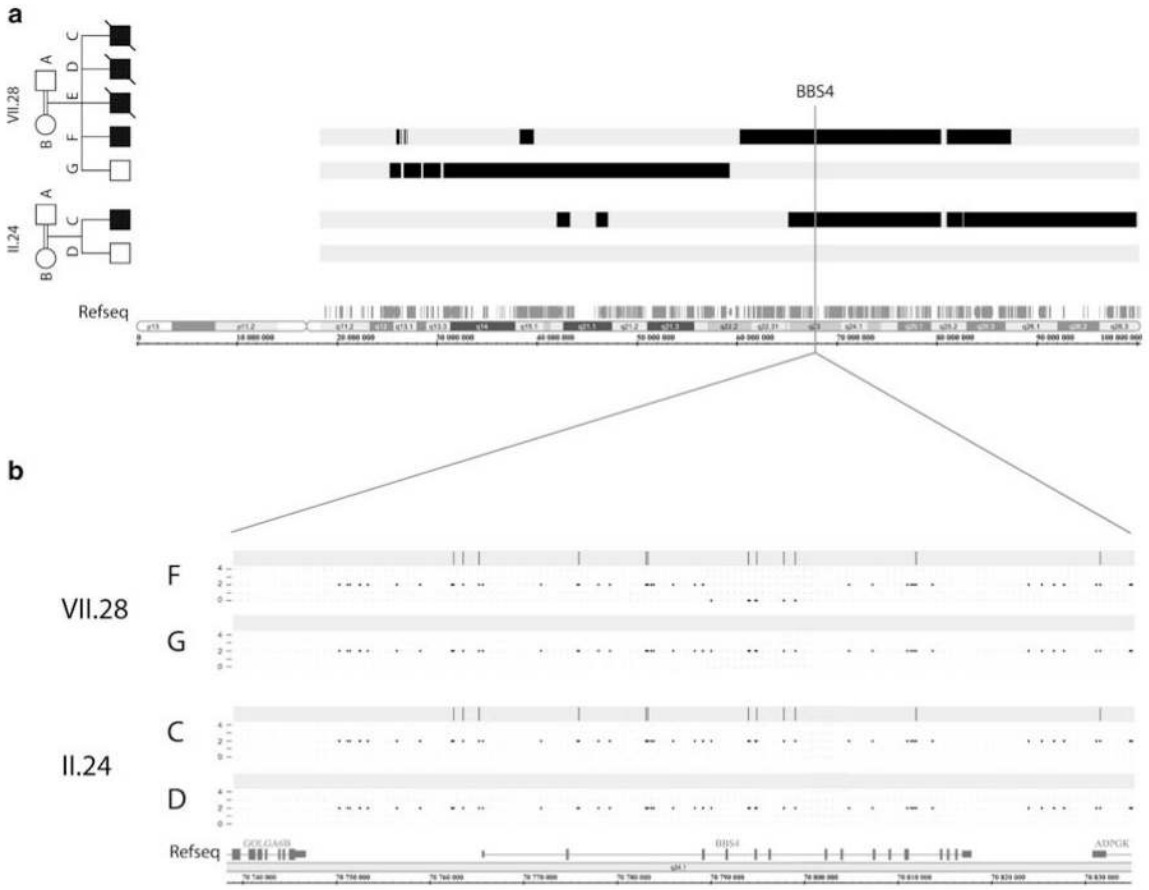

Fig. 2.

a Mapping of the BBS4 locus on Chromosome 15. The areas of homozygosity are colored in *black*, whereas heterozygosity regions are in *gray*. **b** Detailed BBS4 region. For each patient, the *first line* represents the homozygous and heterozygous state, respectively, in *black* and in *gray*. The *following lines* indicate the copy number variation status with hypomorph SNPs (i.e., a *black dot* if one SNP and the level indicates the CNV status). The *gray boxes* highlight, respectively, the deletion of the two alleles of the exons 4, 5 and 6 (i.e., *lower black dots*) from the BBS patient F in respect to the patient G in the family VII.28 and the absence of SNPs information (i.e., absence of black dots) for the family II.24

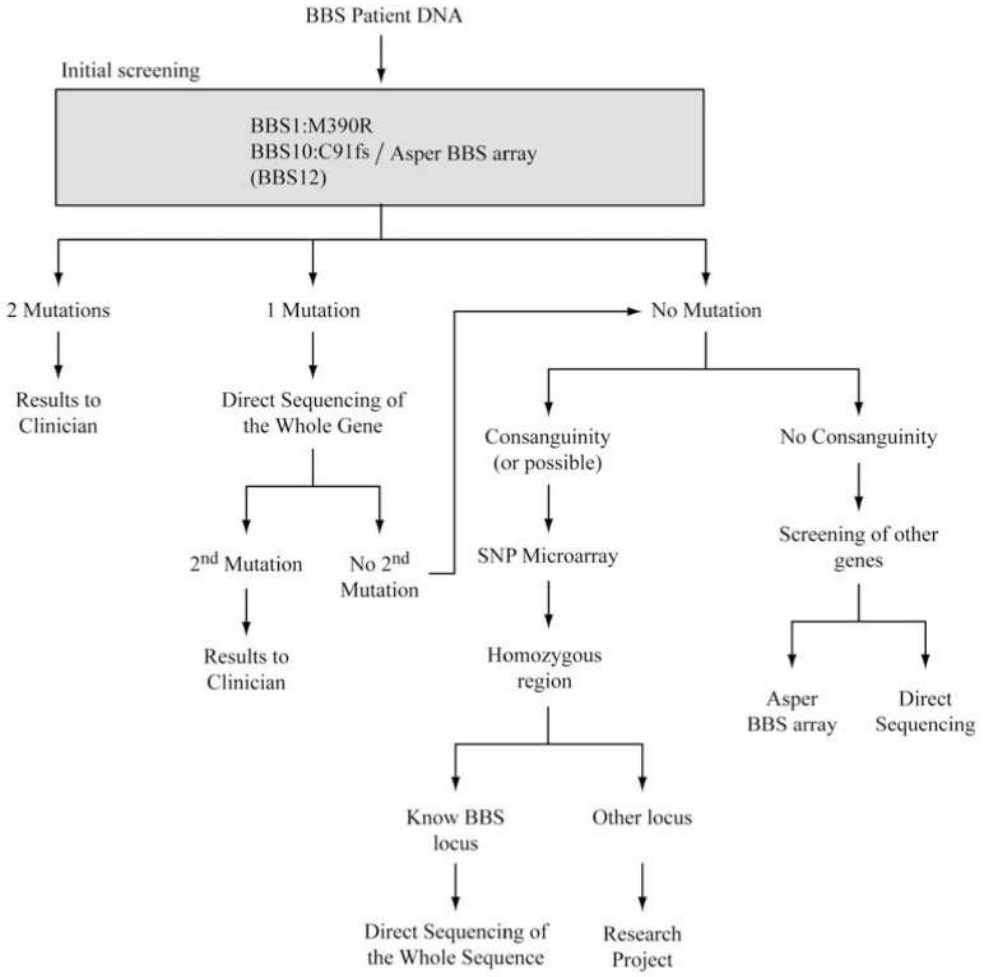

Fig. 3.

Decision tree for identification of mutation in BBS patients. The initial screening can be achieved by several methods, either by the direct sequencing of recurrent mutations or using the Asper array. The complete BBS10 sequence could be sequenced to ensure a full coverage of this gene. Because accounting for 8% of the BBS patients and the presence of a single coding exon, BBS12 can be added to the pool of initial screened genes. Then if no mutation or no second mutation is found, the established consanguineous or possibly consanguineous families (i.e., patients with parents from the same region or population group) should be analyzed by SNP array to perform homozygosity mapping and identify a possible BBS locus

Table 1

The 28 new mutations described for the 134 families with BBS

Gene	Family	Affected	Segregation		Amino acid change		Nucleotide change		Method	Pathogenicity		
			Mother	Father	Allele 1	Allele 2	Allele 1	Allele 2		PolyPhen	SIFT	Sequence conservation
BBS1	III.28	1	Htz (M390R)	NA	**G73X**	*M390R*	c.217G>T	c.1169T>G	Di + S			
	V.21	2	Htz	Htz	**A107fsX**	**A107fsX**	320–332Dup 13pb	320–332Dup 13pb	M + S			
	II.9	1	Sporadic	Sporadic	**Q170X**	*M390R*	c.508C>T	c.1169T>G	Di + S			
	II.12; II.13	2; 1	Htz (M390R)	Sporadic	**L288R**	*M390R*	c.863T>G	c.1169T>G	Di + S	Possibly damaging	Deleterious	All but worms
BBS2	II.24	1	Hz L168fst	NA	*L168fsX200*	**C307Y**	c.504delG	c.920G>A	D + S	Probably damaging	Deleterious	Vertebrates
	II.16	2	Htz	Htz	**L221P**	**L221P**	c.662T>C	c.662T>C	M + S	Possibly damaging	Deleterious	All (hydrophobic)
	III.1	4 (1 nephew)	Htz	Htz	**I314fsX324**	**I314fsX324**	c.940del A	c.940del A	M + S			
	I.12	1	NA	NA	**Del exon 8+9+10**	**Del exon 8+9+10**			A			
BBS4	II.8	2	N/N	Htz	**L114fsX118**	–	c.341insA		D + S			
	V.13	1	Htz	NA	**Q247X**	**Q247X**	c.739C>T	c.739C>T	D + S			
	II.2; III.2bis	4; 1	Htz	Htz	**N309K**	**N309K**	c.927T>G	c.927T>G	M + S	Probably damaging	Deleterious	All
	VI. 28	4 (3 fetuses)	Htz	Htz	**Del exon 4+5+6**	**Del exon 4+5+6**			M + S			
	I.24	1	Htz	Htz	**Del exon 7+8**	**Del exon 7+8**			M + S			
BBS5	II.22	2	Htz	Htz	**MIL**	**MIL**	c.lA>T	c.lA>T	M + S			
	I.5	5	Htz	Htz	**K41fsX52**	**K41fsX52**	c.l23delA	c.l23delA	D + S			
	I.29	2 (1 fetus)	Htz	Htz	**R56G**	**R56G**	c.l66A>G	c.l66A>G	A	Probably damaging	Deleterious	All
BBS6	V.31	1	Sporadic	Sporadic	*Y37C*	**I297T**	c.110A>G	c.890T>C	D + S	Possibly damaging	Deleterious	All (hydrophobic)
	II.3	2	NA	NA	**Q550X**	**Q550X**	c.1648C>T	c.1648C>T	D + S			
BBS7	III.18	1	NA	NA	**IVS5+1G>A**	**1656fsX673**	c.528+lG>A	c. 1967–1968 del TA ins C	D + S			
BBS9	VI.23	1	Htz	Htz	**V81E**	**V81E**	c.242T>A	c.242T>A	A	Possibly damaging	Deleterious	All (hydrophobic)
	IV.2	2	NA	Htz (I154M)	**I154M**	**I154M**	c.462C>G	c.462C>G	D + S	Benign	Deleterious	All (hydrophobic)
	III.27	2	Htz (R278X)		**R278X**	**Del exon 9**	c.832C>T		M + S			
	VI. 1	1	NA	NA	**Del exon 8+9**	**Del exon 8+9**			M + S			
BBSIO	VII.23	1	Htz	Htz	**G43D**	*C91fsX95*	c.l28A>G	c.271ins T	S + Mi	Possibly damaging	Deleterious	Vertebrates[a]
	I.10	1	Htz (N364fs)	NA	*C91fsX95*	**N364fsX368**	c.271ins T	c. 1091 del A	S			
	VIII. 1 1B; VIII. 1 1D	2; 2	NA	NA	*C91fsX95*	**198delF**,199delF	c.271ins T	c.591 del CTTTTT	S			

Gene	Family	Affected	Segregation		Amino acid change		Nucleotide change		Method	Pathogenicity		
			Mother	Father	Allele 1	Allele 2	Allele 1	Allele 2		PolyPhen	SIFT	Sequence conservation
	VII. 25	1	Sporadic	Sporadic	*C91fsX95*	**L533fsX554**	c.271ins T	c.1599 del AACT	S			

The family numbering is done according to the laboratories' coding. New mutations are in regular bold

Method for identification: *S* sequencing, *D* DHPLC, *Di* direct digestion, *A* Affymetrix, *M* microsatellite, *Mi* ASPER microarray. Segregation: *Htz* heterozygous, *Hmz* homozygous, *Sporadic and NA* DNA not available

[a] BBS6, BBSIO and BBS 12 are present in only vertebrates genomes

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