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Genetic testing for Bardet-Biedl syndrome

Andi Abeshi[1,2], Francesca Fanelli[3], Tommaso Beccari[4], Munis Dundar[5], Fabiana D'Esposito[3,6,7] and Matteo Bertelli[2,3]

Abstract

We studied the scientific literature and disease guidelines in order to summarize the clinical utility of genetic testing for Bardet-Biedl syndrome (BBS). The disease has autosomal recessive inheritance, a prevalence varying from one in 13 500 to one in 160 000, and is caused by mutations in the *ARL6, BBIP1, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, CEP290, IFT172, IFT27, LZTFL1, MKKS, MKS1, NPHP1, SDCCAG8, TRIM32, TTC8* and *WDPCP* genes. The clinical diagnosis of BBS is based on four primary features or three primary features plus two secondary features. The genetic test is useful for confirming diagnosis, and for differential diagnosis, couple risk assessment and access to clinical trials.

[1]MAGI Balkans, Tirana, Albania
[2]MAGI'S Lab, Rovereto, Italy
[3]MAGI Euregio, Bolzano, Italy
[4]Department of Pharmaceutical Sciences, University of Perugia, Perugia, Italy
[5]Department of Medical Genetics, Erciyes University Medical School, Kayseri, Turkey
[6]Head and Neck Department, School of Medicine and Surgery, University of Naples "Federico II", Italy
[7]ICORG (Imperial College Ophthalmology Research Group), Western Eye Hospital, London, United Kingdom

Corresponding author: M. Bertelli
E-mail: info@assomagi.org

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Bardet-Biedl syndrome

(other synonyms: BBS; Laurence–Moon–Bardet–Biedl syndrome; Laurence–Moon–Biedl syndrome) (1)

General information about the disease

Bardet-Biedl syndrome (BBS) is a rare, genetically and phenotypically heterogeneous syndromic disorder characterized by six primary and several secondary features (1-7). Primary features are the following: rod-cone dystrophy presenting as reduced visual acuity, defective dark adaptation and constriction of the visual field (over 90% of cases), truncal obesity (72%-92% of cases), hypogonadism (59%), polydactyly (63-81%), cognitive impairment (61%) and renal anomalies (53%). Other less frequent secondary findings include developmental delay, diabetes mellitus, eye abnormalities (strabismus, astigmatism and cataract), anosmia, cardiovascular anomalies (50% of cases), ataxia, Hirschsprung disease, hepatic fibrosis, craniofacial dysmorphism and orodental abnormalities (5).

The clinical diagnosis of BBS is based on the presence of four primary features or three primary features plus two secondary features. (5-8) Clinical diagnosis can be confirmed by molecular genetic analysis of the related genes.

The prevalence of BBS ranges from one in 13,500 (Kuwaiti Bedouins) to one in 160,000 (Swiss population).

Differential diagnosis should consider Alström syndrome, McKusick Kaufman syndrome, Leber congenital amaurosis, Joubert syndrome, Biemond syndrome type II and Senior Løken syndrome because there is significant phenotypic and molecular overlap between Bardet-Biedl syndrome and other cilopathies. Pathogenic variants in several genes that cause BBS can also lead to other distinct ciliopathy traits.

BBS is a genetically heterogeneous disorder inherited in an autosomal recessive manner and is very common in populations with a high level of consanguinity (1). The evidence for additional locus heterogeneity is given by the fact that approximately 20% of persons with BBS do not have identifiable pathogenic variants in any of the known BBS-related

genes. These genes are: *ARL6* (OMIM gene: 608845; OMIM disease: 600151), *BBIP1* (OMIM gene: 613605; OMIM disease: 615995), *BBS1* (OMIM gene: 209901; OMIM disease: 209900), *BBS2* (OMIM gene: 606151; OMIM disease: 615981), *BBS4* (OMIM gene: 600374; OMIM disease: 615982), *BBS5* (OMIM gene: 603650; OMIM disease: 615983), *BBS7* (OMIM gene: 607590; OMIM disease: 615984), *BBS9* (OMIM gene: 607968; OMIM disease: 615986), *BBS10* (OMIM gene: 610148; OMIM disease: 615987), *BBS12* (OMIM gene: 610683; OMIM disease: 615989), *CEP290* (OMIM gene: 610142; OMIM disease: 615991), *IFT172* (OMIM gene: 607386; OMIM disease: 615630), *IFT27* (OMIM gene: 615870; OMIM disease: 615996), *LZTFL1* (OMIM gene: 606568; OMIM disease: 615994), *MKKS* (OMIM gene: 604896; OMIM disease: 605231), *MKS1* (OMIM gene: 609883; OMIM disease: 615990), *NPHP1* (OMIM gene: 607100) (9), *SDCCAG8* (OMIM gene:613524; OMIM disease: 615993), *TRIM32* (OMIM gene: 602290; OMIM disease: 615988), *TTC8* (OMIM gene: 608132; OMIM disease: 615985) and *WDPCP* (OMIM gene: 613580; OMIM disease: 615992). Penetrance was originally thought to be complete; however, several examples of unaffected individuals with two pathogenic variants in the same gene have been reported.

Moreover it is well accepted that, in addition to variants in a known gene, variants in an heterozygous state at a different locus may affect the phenotype (10).

Pathogenic variants may consist in small intragenic deletions/insertions, as well as splice-site, missense and nonsense variants and also deep intronic variants (such as c.2991+1655A>G in *CEP290*); Exon or whole-gene duplications/deletions have also been reported(1).

Aims of the test

- To determine the gene defect responsible for the pathology
- To confirm clinical diagnosis of the disease
- To determine carrier status for the disease.

Test characteristics

Expert centers/Published guidelines

The test is listed in the Orphanet database and is offered by 24 accredited medical genetic laboratories in the EU, and in the GTR database, offered by 22 accredited medical genetic laboratories in the US.

The guidelines for clinical use of the test are described in "Genetics home reference" (ghr.nlm.nih.gov) and "Gene reviews".

Test strategy

A multi-gene NGS panel is used for the detection of nucleotide variations in coding exons and flanking introns known genes. Potentially causative variants and region with low coverage are Sanger-sequenced. MLPA is used to detect duplications and deletions in *CEP290*. Sanger sequencing is also used for family segregation studies.

The test identifies variations in known causative genes in patients suspected to have BBS. To perform molecular diagnosis, a single sample of biological material is normally sufficient. This may be 1 ml blood in a sterile tube with 0.5 ml K3EDTA or 1 ml saliva in a sterile tube with 0.5 ml ethanol 95%. Sampling rarely has to be repeated. Gene-disease associations and interpretation of genetic variants are rapidly developing fields. It is therefore possible that the genes mentioned in this note may change as new scientific data is acquired. It is also possible that genetic variants today defined as of "unknown or uncertain significance" may acquire clinical importance.

Genetic test results

Positive

Identification of biallelic pathogenic variants in *ARL6, BBIP1, BBS1, BBS2, BBS4, BBS5, BBS7, BBS9, BBS10, BBS12, CEP290, IFT172, IFT27, LZTFL1, MKKS, MKS1, NPHP1, SDCCAG8, TRIM32, TTC8* or *WDPCP* confirms the clinical diagnosis and is an indication for family studies.

A pathogenic variant is known to be causative for a given genetic disorder based on previous reports or predicted to be causative based on the loss of protein function or expected significant damage to protein or protein/protein interactions. In this way it is possible to obtain a molecular diagnosis in new/other subjects, establish the risk of recurrence in family members and plan preventive and/or therapeutic measures.

Inconclusive

Detection of a variant of unknown or uncertain significance: a new variation and/or without any evident pathogenic significance or with insufficient or significant conflicting evidence to indicate it is likely benign or likely pathogenic for a given genetic disorder. In these cases, it is advisable to extend testing to the patient's relatives in order to assess variant segregation and clarify its contribution. In some cases it could be necessary to perform further examinations/tests or to do a clinical reassessment of pathological signs.

Negative

The absence of variations in the genomic regions investigated does not exclude a clinical diagnosis but suggests the possibility of:

- sequence variations in gene regions not investigated by this test, such as regulatory regions (5' and 3' UTR) and deep intronic regions;
- variations in other genes not investigated by the present test;
- alterations that cannot be identified by sequencing, such as large rearrangements that cause loss (deletion) or gain (duplication) of extended gene fragments.

Unexpected

Unexpected results may come out from the test, for example information regarding consanguinity; absence of family correlation or the possibility of developing genetically based diseases.

Risk for progeny

Autosomal recessive transmission needs that both healthy carrier parents transmit their disease variant to his/her children. In this case, the probability of having an affected boy or girl is therefore 25%. Although a second locus effect is described, even in the presence of the so-called "triallelic" genetic asset, the risk should be evaluated as for a pure recessive disease (11).

Limits of the test

The test is limited by current scientific knowledge regarding the genes and disease.

Analytical sensitivity (proportion of positive tests when the genotype is truly present) and analytical specificity (proportion of negative tests when the genotype is not present)

NGS: Analytical sensitivity: >99% (with a minimum coverage of 10X); Analytical specificity: 99.99%.

MLPA: Analytical sensitivity: >99.99%; Analytical specificity: 99.99%.

Clinical sensitivity (proportion of positive tests if the disease is present) and clinical specificity (proportion of negative tests if the disease is not present)

Clinical sensitivity: variations in known causative genes are identified in about 80% of cases (1).

Clinical specificity: is estimated at about 99.99% [Author's laboratory data] (12).

Prescription appropriateness

The genetic test is appropriate when:

a) the patient meets the diagnostic criteria for the disease;

b) the genetic test has diagnostic sensitivity greater than or equal to other published tests.

Clinical utility

Clinical management	Utility
Confirmation of clinical diagnosis	yes
Differential diagnosis	yes
Access to clinical trial (13)	yes
Couple risk assessment	yes

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