



Screening for mutation hotspots in Bardet–Biedl syndrome patients from India

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Received November 21, 2015

Background & objectives: Bardet–Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder characterized by multiple organ defects involving retina, kidney, liver and brain. Disease-causing mutations in *BBS* genes narrowed down by homozygosity mapping in small consanguineous and non-consanguineous pedigrees were reported in 80 per cent of the study population. This study was aimed to screen these genes (*BBS3*, *BBS10*) and specific exons of *BBS* genes (*BBS1*, *BBS5*, *MKKS*, *BBS9*, *BBS11* and *BBS12*) for recurrent mutations in a selected sample of BBS patients.

Methods: The recurrent mutations in *BBS* genes were screened in the BBS affected individuals by PCR based direct sequencing. The pathogenicity of the observed mutations were confirmed by co-segregation analysis, screening of healthy unrelated controls and *in silico* analysis.

Results: In the 64 BBS patients (44 males, 20 females) were studied, mutations were predominant in *BBS10* and *ARL6* genes; the c.272T>C; p.(I91T) mutation in *ARL6* gene was a recurrent mutation. One novel non-sense mutation c.425T>G; p(L142*) was obtained in *BBS5* gene (family BSI-31).

Interpretation & conclusions: *BBS10* gene mutations clustered in exon 2 of the gene suggesting the exon as a probable hotspot for mutations in Indian population. A cost- and time-effective strategy for the molecular diagnosis of BBS was designed based on these results.

Key words *ARL6* - Bardet–Biedl syndrome - *BBS10* - India

Bardet–Biedl syndrome (BBS) is a genetically heterogeneous autosomal recessive disorder manifesting with major clinical features of retinal degeneration, obesity, hypogonadism, polydactyly, renal dysfunction and learning disability, and minor features such as cardiac abnormalities, diabetes,

hypertension, hearing defects and anosmia. The diagnosis for BBS is well established by the clinical manifestations; the presence of three primary with two secondary or four primary features confirms the diagnosis of BBS¹. The prevalence of BBS is estimated as 1 in 150000 individuals worldwide, but its

incidence increases in isolated/inbred/consanguineous populations². This syndrome is mapped to 20 genes (*BBS1-21*)³⁻⁵; these genes code for proteins involved in ciliary biogenesis and functions. Mutation in these genes affect the structure and function of cilia, the characteristics of any ciliopathy disorders and BBS is grouped one among them.

Mutations in *BBS* genes (*BBS1-18*) account for about 70-80 per cent of the disease with frequent recurrent *BBS1* and *BBS10* gene mutations as reported in certain populations of European and Caucasian descents⁶. The role of founder effect has also been indicated for mutations such as the M390R in *BBS1* and C91fs*95 in *BBS10* genes in specific populations⁷. Mutations in *BBS4*, *BBS5* and *TTC8/BBS8* are most commonly seen in the Middle East and North African populations⁸. *BBS4* gene is reported as the most prevalent gene among Turks and Pakistanis³. Among Saudi BBS patients, there is an unusual predominance of *ARL6/ARL6* gene mutations, all of which are novel, in striking contrast to the BBS reports from Caucasians in which *ARL6* gene mutations are extremely rare⁹.

The divergent clinical/syndromic features of BBS and variable age of onset often complicates the diagnosis of BBS. Retinal degeneration involving the macula by the second to third decade, with decreased visual acuity 20/200 or worse is the major primary feature of the disease¹⁰. In patients with progressive renal impairment, end-stage renal disease renal transplantation is required in 10 per cent of affected individuals¹¹. A higher frequency of sterility was observed in male BBS patients when compared to the female BBS patients². Cardiovascular and renal diseases are the major causes of death and morbidity¹². Hence, diagnosing the disease as early as possible will have an impact on disease management for the patients as there are no treatment options available for BBS.

Screening of Indian BBS families, we have earlier observed disease-causing mutations in 80 per cent of the study population¹³. In Indian population, *ARL6* gene accounted for 18 per cent of the mutations, as against worldwide reports (0.4%), followed by *BBS10* (11%), *BBS9/PTHB1* and *BBS6/MKKS* gene (8% each)¹³. The frequently reported mutations C91fsX95 (*BBS10*) and M390R (*BBS1*) genes observed in Caucasians were not more common in Indians¹³. To validate our earlier observation that >10 per cent of mutations were in *ARL6* (inclusive of a recurrent mutation c.272T>C; p.(I91T)) and *BBS10* genes, the current study was undertaken in a larger sample.

Material & Methods

Clinical examination was performed on all the patients and family members included in the study. All the patients manifesting at least four primary features or three primary features with at least two minor symptoms¹⁴ were included. The study protocol was approved by the Institutional Research and Ethics Committee of Vision Research Foundation, Chennai, India. The patients were selected by consecutive sampling method during December 2012 to May 2015 after a detailed clinical evaluation at the vitreoretina clinic of Sankara Nethralaya, Chennai, or department of Paediatrics, Amrita Institute of Medical Sciences, Kochi. The ophthalmological and other morphological features were documented in a questionnaire based on the details provided by the probands/parents medical history. Power of 80 and 96.1 per cent was achieved for the sample size of 50 cases and 100 controls for screening the *BBS3* and *BBS10* genes when calculated (<http://osse.bii.a-star.edu.sg>) based on the allele frequency observed in our initial study¹³.

Blood collection and DNA extraction: The pedigree was constructed followed by blood collection (8 ml of whole blood collected in Acid Citrate Dextrose Vacutainer) from the proband and the family members after their written consent. The genomic DNA of the patients was extracted using NucleoSpin XL DNA extraction kit as per the manufacturer's instruction (Machery-Nagel, Duren, Germany).

Mutational analysis: Polymerase chain reaction (PCR)-based direct sequencing was performed in ABI Prism 3100 AVANT Genetic analyzer (Applied Biosystems, USA) for the entire coding regions of *ARL6*, *BBS10* and the specific exons harbouring the reported mutations from Indian patients (*BBS1*, *BBS5*, *MKKS*, *BBS9*, *BBS11* and *BBS12*). The sequences were compared with the reference sequences NM_001278293.1 and ENST00000463745 (*ARL6*), NM_024685.3 and ENST00000393262 (*BBS10*), NM_018848.3 and ENST00000347364 (*MKKS*), NM_152384.2 and ENST00000295240 (*BBS5*) and ENST00000450136 and NM_001099679 (*BBS11*). The primer sequences and PCR conditions were as mentioned earlier¹³.

Bioinformatic analysis: The mutation nomenclature was in accordance with the guidelines of Human Genome Variation Society (HGVS v.2.0, <http://www.hgvs.org/>) and checked with the Mutalyzer. The functional effect

Table I. Frequency of primary and secondary clinical features in the study population	
Clinical features	Frequency % (n=64)
Primary features	
Retinal dystrophy	97
Polydactyly	86
Obesity	89
Learning disabilities	66
Hypogonadism	66
Renal anomalies	16
Secondary features	
Speech disorder/delay	53
Developmental delay	48
Ataxia	22
Diabetes mellitus	28
Mild spasticity	23
Congenital heart disease	23
Behavioral problems	45
Hearing loss	22
Hypertension	13
Other remarks	33
Consanguinity	47

of the novel missense mutations was evaluated using PolyPhen (<http://genetics.bwh.harvard.edu/pph2/>), SIFT (http://sift.bii.a-star.edu.sg/www/SIFT_seq_submit2.html) and ConSurf server (<http://consurf.tau.ac.il/>).

Results

In total, 64 BBS patients (males 44 and females 20) with a mean age of 13.4±6.42 yr (range 6-30) were studied. Table I describes the clinical features of these patients. Thirty one of the 64 families showed a history of consanguinity. Retinal dystrophy was seen in 97 per cent. The frequencies of obesity, polydactyly and brachydactyly were 89, 86 and 29 per cent, respectively. Twenty two per cent of the cases had hearing loss, and 48 per cent showed delayed milestones.

Mutational analysis of *ARL6* and *BBS10* genes: Three mutations (3/64) were observed in the study population [c.272T>C; p.(I91T) *ARL6* gene (family BSI-42); c.1249G>A; p.(A417T) (family BSI- 25), and c.1334_1338del;p. (L445_L722delinsH) (family BSI-46) in *BBS10* gene]. The novel mutations co-segregated with the disease phenotype (Fig. 1A-C) and predicted to be pathogenic and deleterious by the bioinformatic tools. (Table II). The c.1334_1338del; p. (L445_L722delinsH) resulted in 446 amino acid (aa) product as against the 721

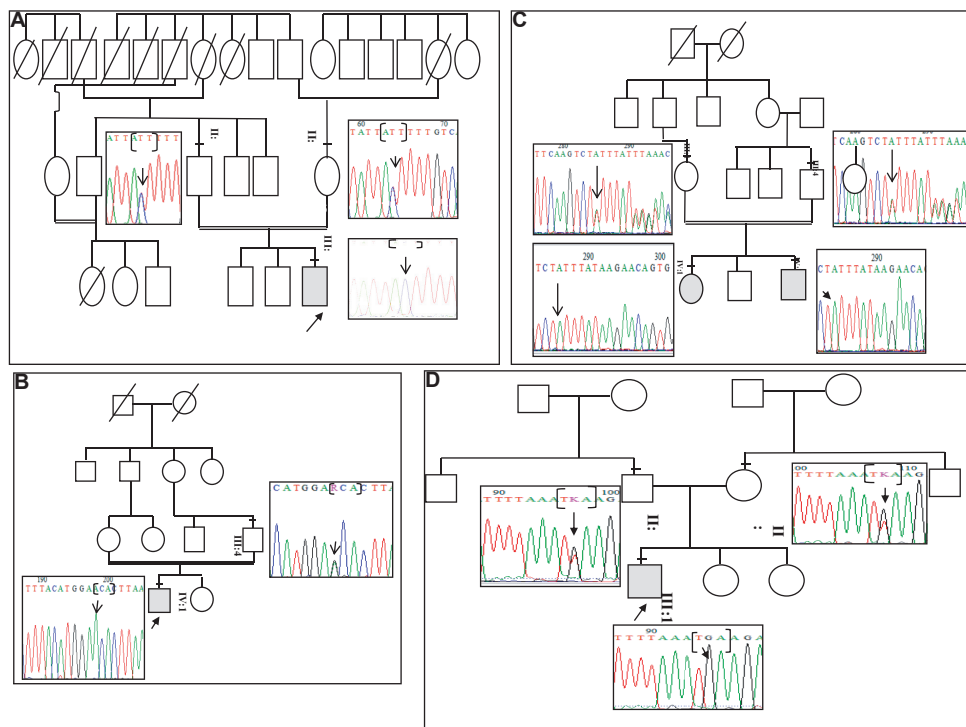


Fig. 1. Co-segregation analysis for Bardet–Biedl syndrome gene mutations: (A) c.272T>C in *ARL6* gene (BSI-42 family) (B) c.1249G>A in *BBS10* gene (family BSI-25) (C) c.1334_1338del in *BBS10* gene (family BSI-46) (D) c.425T>G in *BBS5* gene (family BSI-31).

Table II. *In silico* analysis of *BBS* gene mutations identified in this study

Proband ID	Predicted protein change	Nucleotide alteration	Gene	SIFT score	PolyPhen	Consurf	Co-segregation
BSI-42	p.I91T	c. 272T>C	<i>BBS3</i>	0	Probably damaging	Yes	Yes
BSI-25	p.A417T	c. 1249G>A	<i>BBS10</i>	0	Probably damaging	Yes	Yes
BSI-46	p. L445_L722delinsH	c. 1334_1338del	<i>BBS10</i>	-	-	Yes	Yes
BSI-31	p.*142Lext*?	c. 425T>G	<i>BBS5</i>	-	-	Yes	Yes

SIFT, sorting intolerant from tolerant

aa in wild-type protein. Mutation screening of specific exons in *BBS1*, *BBS5*, *MKKS*, *BBS9*, *TRIM32/BBS11* and *BBS12* genes revealed one novel non-sense mutation c.425T>G; p.(L142*) in *BBS5* gene (family BSI-31) (Fig. 1D). These mutations segregated with the disease and the parents were heterozygous for the same; none of the novel mutations were seen in 100 healthy individuals.

Discussion

The clinical diagnosis of BBS is often made when the patient reports for their visual distress. Some of the features prompting a clinical diagnosis of BBS include (i) childhood-onset retinal dystrophy that occurs in more than 90 per cent of BBS patients, (ii) prenatal appearance of enlarged hyperechoic kidneys without corticomedullary differentiation (CMD) along with polydactyly¹⁵, and (iii) positive family history of BBS. Even in families without a history of BBS, *in utero* findings of CMD and polydactyly has always prompted a differential diagnosis for BBS. However, these primary or secondary features, such as polydactyly (congenital) and obesity (that develops by 2-3 yr), are often ignored. Due to the high incidence of renal developmental anomalies and renal cell carcinoma in relatives of BBS patients, a combination of renal ultrasound study and intravenous pyelography is advised for early identification of renal anomalies in these families¹⁶. A detailed clinical history of other systemic features has also been recommended¹⁷. There are reports on diagnosis of BBS through prenatal ultrasonography which was subsequently confirmed postnatally through clinical evaluation and molecular confirmation¹⁸.

Genetic diagnosis often helps in disease confirmation, effective clinical management, severity prediction, carrier risk identification with potential counselling benefits. A practical approach by prioritizing the frequently mutated genes has been earlier reported for an efficient genetic screening⁸. High throughput sequencing by panel testing has proven to be very

effective in identifying the disease-causing mutations in a single platform¹⁹; however, a strategic and cost-effective approach is necessary for countries like India.

On screening 30 Indian families using homozygosity-based gene mapping, we reported a different mutation profile earlier¹³. *ARL6* and *BBS10* gene mutations were predominant with a common variant, p.I91T, in *ARL6* gene. The present study was performed to validate these observations in an increased sample number that might result in strategical mutation screening system at an affordable cost. To achieve this, a direct sequencing was adopted for entire coding regions of *ARL6* and *BBS10* genes contributing to >10 per cent of mutations and specific exons in genes (*BBS1*, *BBS5*, *MKKS*, *BBS9*, *BBS11* and *BBS12*) that were mutated in <10 per cent of the study population.

The study revealed that *BBS10* gene mutations were more common when compared to the other genes as has been reported earlier¹⁸. However, we were unable to replicate the results of other genes, probably due to genetic heterogeneity of the disease. The c.272T>C; p.(I91T) mutation in *ARL6* gene recurred in the present study similar to our earlier results¹³ thus suggesting the probability of I91T being a recurrent mutation in Indian population; p.(M390R) in *BBS1* gene was not seen in any of the BBS patients.

It was observed that patients with *BBS10* gene mutations manifested retinitis punctata albescens, polydactyly, obesity and learning difficulties, and those with *ARL6/BBS5* gene mutations correlated with retinal dystrophy, polydactyly, obesity and hypogonadism. With these data, we propose here a mutation screening strategy for the genetic diagnosis of BBS in Indian population that has potential counselling benefits (Fig. 2) in Indian population. The feasibility of the strategy has, however, to be tested after screening all the *BBS* genes in larger sample size. On successful validation of this strategy, an affordable molecular test for patients and their

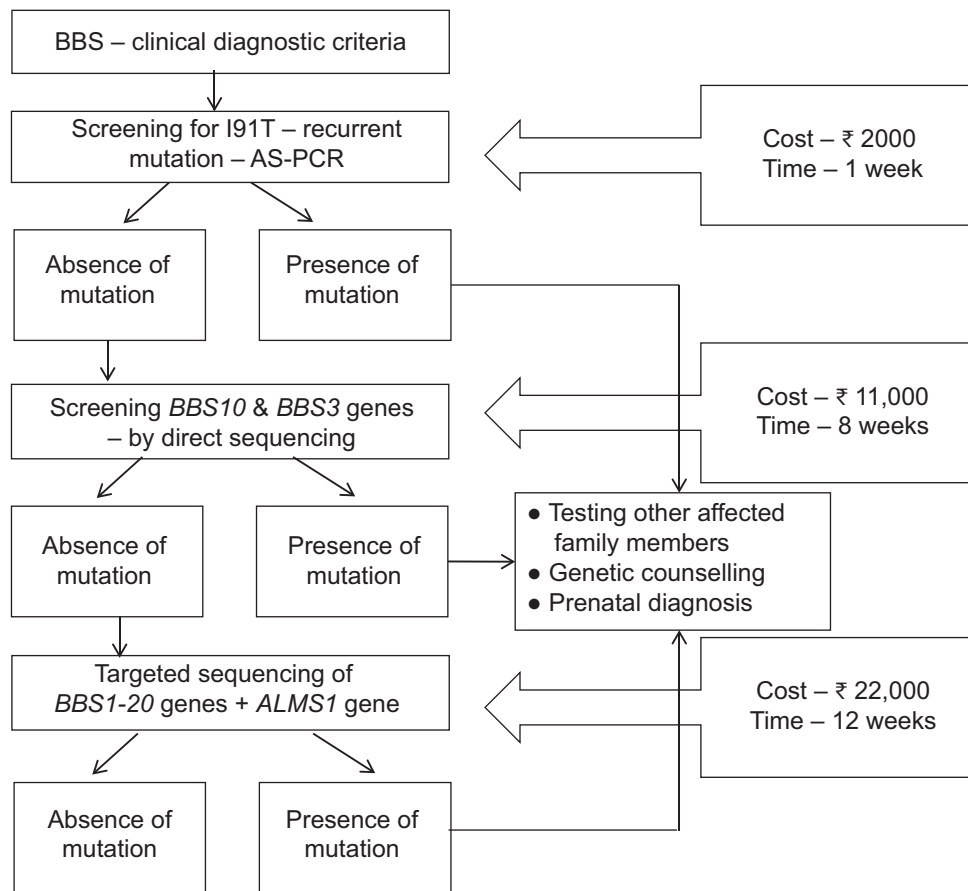


Fig. 2. Strategy designed for Bardet-Biedl syndrome diagnosis in Indian Bardet-Biedl syndrome patients.

afflicted family would be available having potential for a timely assistance in prenatal diagnosis and genetic counselling benefits.

The limitation of the study was sample size. By increasing the sample size the mutation data observed in our previous study¹³ could have been validated. On screening the exons of other *BBS* genes, the complete mutation spectrum for 100 families could have been achieved.

To summarize, the c.272T>C; p.(I91T) mutation in *ARL6* gene was a recurrent mutation, and the intermediate domain of exon-2 of *BBS10* gene as a probable hotspot region for Indian population. Varying frequency of clinical features and the novel mutations might be attributed to the ethnicity/a different mutation spectrum. Similar studies in larger samples representing various parts of the country are required to validate the observed results and also to increase the knowledge on varying expression of clinical features in Indian BBS patients.

Financial support & sponsorship: The first author (SPC) acknowledges the Indian Council of Medical Research (ICMR),

New Delhi, for providing financial support as Senior Research Fellowship (Project number: 45/08/2012-HUM-BMS).

Conflicts of Interest: None.

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