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Short Communication

A Homozygous c.1131G>A Missense Mutation in BBS9 Gene Manifesting Autosomal Recessive Bardet-Biedl Syndrome in Consanguineous Kashmiri Family

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Authors' Contribution
GA conceived the idea and supervised the research. KB and AK performed exome sequencing and data analysis. SAB and Sadia collected clinical samples, performed the experiments and drafted the manuscript.

Key words
Autosomal recessive, Bardet-Biedl syndrome9, Missense mutation, Kashmiri family, Whole exome sequencing.

ABSTRACT

Bardet-Biedl syndrome (BBS) is a rare autosomal recessive ciliopathic genetic disorder in humans. It is a multisystem disorder and is principally described by visual abnormalities, con-rod dystrophy, eyes exotropia, obesity, polydactyly, hypogonadism, and renal abnormalities. Few additional features of BBS also include delayed motor development, clumsiness, anosomia, ataxia, hypodontia, hearing impairment, hirschsprung disease, cardiovascular and liver disorders. So far 21 genes are reported that cause BBS (BBS1-BBS21). A consanguineous family having clinical symptoms of BBS9 is described in current study. Mutation was detected in BBS9 on chromosome 7p14.3 using whole exome sequencing (WES). A splice acceptor site mutation (c.1131G>A) in exon 3 was revealed by Sanger sequencing.

Bardet-Biedl Syndrome (BBS; MIM 209900) is an infrequent pleiotropic developmental disorder having visual impairments like con-rod dystrophy, retinitis pigmentosa, obesity, polydactyly, renal dysfunctions, hypogonadism and intellectual disability as primary features (Forsythe and Beales, 2013). Some other reported minor features of BBS also include anosmia (Kulaga *et al*., 2004), nystagmus, brachydactyly, syndactyly, truncal obesity, kidney dysfunctions, cryptorchidism (M'hamdi *et al*., 2013), puberty delay, poor articulation, anxiety, hypoplastic fallopian tubes, depression, obsessive-compulsive disorder, autism spectrum disorder, hypertension, anosmia, dental abnormalities, ataxia and hirschsprung disease (Pontual *et al*., 2009; Moore *et al*., 2005; Sahin *et al*., 2015). Inheritance is conventionally considered as an autosomal recessive, although remarkable exceptions exist, whereby BBS may be an oligogenic condition (M'hamdi *et al*., 2013; Katsanis *et al*., 2001). BBS is an assemblage of ciliopathies that share partial-overlapping phenotypes as well as common genes with significant intrafamilial and interfamilial phenotypic variation (Forsythe and Beales, 2013). It has very complex genetics and different variations are present within the phenotype and genotype of the disorder (White *et al*., 2007). Up till now, twenty one BBS genes (BBS1-21) have been recognized on different loci, which include 11q13 (BBS1) (Mykytyn *et al*., 2001), 16q21 (BBS2) (Nishimura *et al*., 2001), 3p13-p12 (BBS3) (Fan *et al*., 2004), 15q22.3q23 (BBS4) (Iannaccone *et al*., 2005), 2q31 (BBS5) (Young *et al*., 1999), 20p12 (BBS6) (Slavotinek *et al*., 2000), 4q27 (BBS7) (Badano *et al*., 2003), 14q32.11 (BBS8) (Ansley *et al*., 2003), 7p14 (BBS9) (Nishimura *et al*., 2013), 12q21.2 (BBS10) (Stoetzel *et al*., 2006), 9q33.1 (BBS11) (Chiang *et al*., 2006), 4q27 (BBS12) (Stoetzel *et al*., 2007), 17q23 (BBS13) (Leitch *et al*., 2008), 12q21.3 (BBS14) (Leitch *et al*., 2008), 2p15 (BBS15) (Kim *et al*., 2010), 1q43 (BBS16) (Otto *et al*., 2010), 3p21.31 (BBS17) (Leitch *et al*., 2008), 10q25.2 (BBS18) (Scheidecker *et al*., 2014), 22q12 (BBS19) (Aldahmesh *et al*., 2014), 2p23.3 (BBS20) (Schaefer *et al*., 2016), and 8q22.1 (BBS21) (Heon *et al*., 2016). In this study, we intended to find out the genetic

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cause of BBS9 in a patient from a consanguineous Kashmiri family using whole exome sequencing (WES).

Materials and methods

Permission to conduct the present research was obtained from the Director Advance Studies and Research (DASR) of University of Azad Jammu and Kashmir, Muzaffarabad and Charite University, Berlin. An informed written consent was obtained from the guardians of the affected individuals.

In the present study, a consanguineous family was investigated, from district Bagh, Azad Kashmir. Pedigree was constructed as per information provided by the family's elders. Generation pattern of pedigree indicates autosomal recessive inheritance (Fig. 1). Venous blood samples of four members of this family were collected in vacutainers (BD Biosciences, Franklin Lakes, NJ, USA) containing EDTA.

Genomic DNA was extracted from the collected blood samples using phenol-chloroform and GeneJET Genomic DNA Purification kit (Lithuania).

In order to identify the causative genes its direct exome sequencing was performed. DNA from a single proband (IV-1) in the family was subjected to WES. Exomes enrichment was accomplished using Agilent SureSelect Human All Exome 50 Mb kit and the sequencing was done on illuminaHiSeq 2000 systems. All the reads were aligned against the human assembly hg19 (GRCh38).

Results

Clinical phenotype of both effected individuals show mild type of intellectual disability along with vision

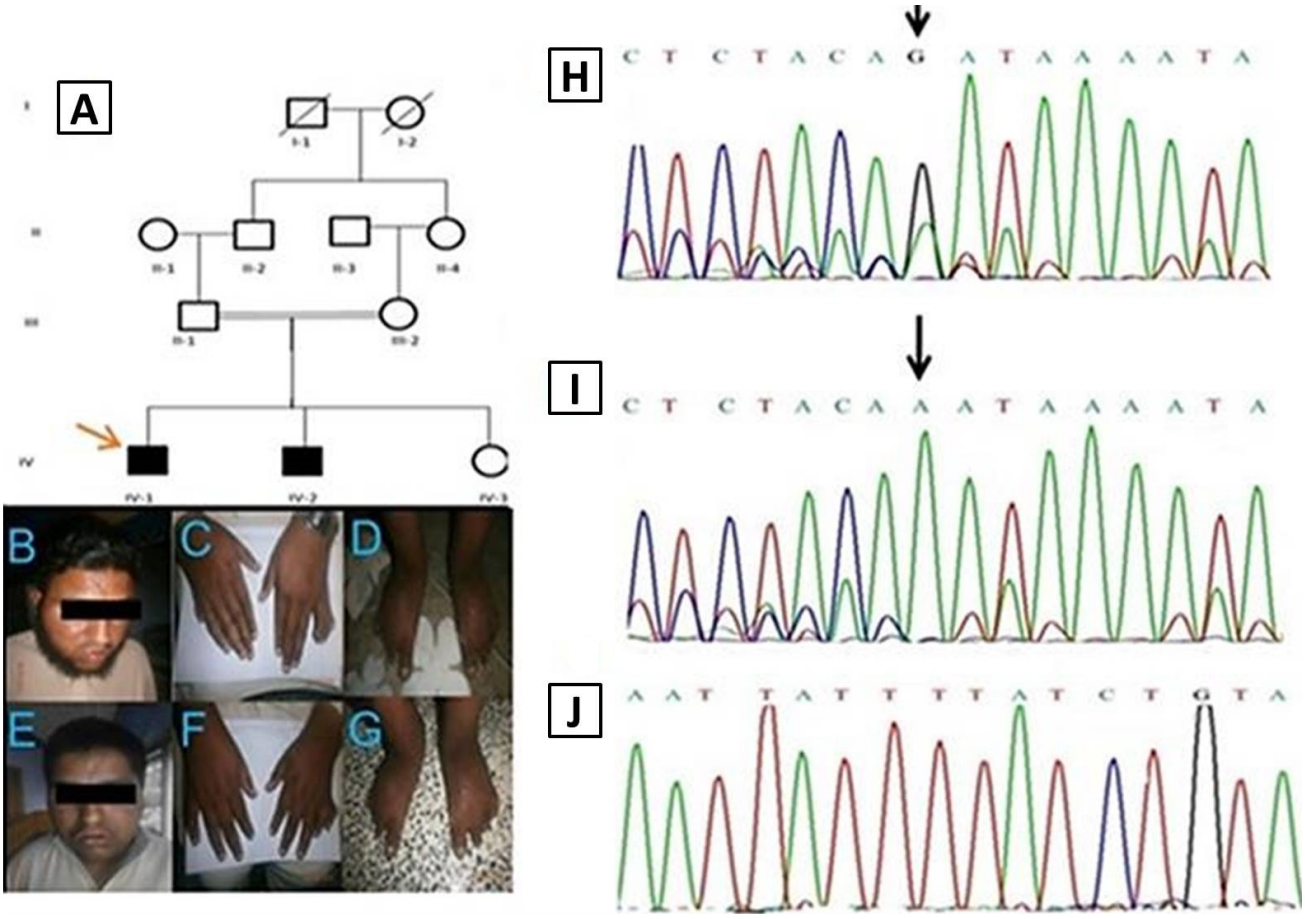

Fig. 1. **A,** pedigree of family (MR20) showing autosomal recessive inheritance, while red arrow indicates the affected individual for whom WES was done. **B,** affected individual IV-1, having typical features of BBS syndrome including hypertelorism, eyes exotropia and a flat nasal bridge. **C,** dorsal view of hands showing polydactyly in right hand while polydactyly and syndactyly is clearly visible in left hand. **D,** dorsal view of feet having polydactyly in both. **E,** typical BBS facial features shown by affected individual IV-2, having flat nasal bridge, poor eye sight, intellectual disability and small mouth. **F,** dorsal and palmar view of hands, and having post axial polydactyly in left hand. **G,** feet of affected individual IV-4, showing obesity and polydactyly in left foot. **H,** homozygous mutation in the patient IV-1 showing splice acceptor site change G>A on position 167. **I,** heterozygygous carrier and **J,** homozygous Normal.

Table I.- Clinical manifestations observed in affected members.

Patient	Sex	Age (years)	RP	Polydactyly (PAP)	Obesity	Hypogonadism	Eyes exotropia	CI	RI
IV-1	M	20	+	+	+	+	+	+	+
IV-2	M	24	+	+	+	+	+	+	+
III-2	F	40	-	-	-	-	-	-	-
IV-3	F	18	-	-	-	-	-	-	-

+, presence of feature; -, absence; RP, retinitis pigmentosa; PAP, postaxial polydactyly; ND, no data available; CI, cognitive impairment; RI, renal impairment.

loss, obesity, speech problems and learning disability. Hypogonadism was also found in both of the affected males. However, only one affected male (IV-2) have polydactyly. The age of the affected individuals was 20 (IV-1) and 24 (IV-2) years at the time of study (Table I).

Direct WES was performed in affected individual (IV-1) of the family under study, at the Department of Pediatric Neurology, Charite-Universit at smediz in Berlin, Germany.

Sanger sequencing of BBS9 gene exposed a homozygous G to A transition (c.1131G>A, p.38) resulting in splice acceptor site mutation at exon 3 in all affected individuals of this family. Unaffected members were either heterozygous for a mutant allele or had wild type alleles. Therefore, Sanger sequencing showed co-segregation of the variants with the disease phenotype.

Discussion

Bardet-Biedl syndrome is an autosomal recessive pleiotropic ailment (Muller *et al*., 2010). However, tri-allelic nature of BBS is reported to occur in less than 5% of cases having three mutated alleles in two genes (Katsanis *et al*., 2001; M'hamdi *et al*., 2013). In the recent study we have identified disease-causing allele in a consanguineous family of Azad Kashmir origin. The inheritance of disease has an autosomal recessive pattern hence, excluding tri-allelism in the present case. The observed clinical features in the studied family were alike to those reported before. WES analysis followed by Sanger sequencing revealed a variant in BBS9 gene. The BBS9 gene, mapped on chromosome 7p14.3, having 24 exons, which spans more than 700 kb genomic region (Vernon *et al*., 2003). BBSome is a group of seven BBS proteins (BBS1-BBS7) which promotes ciliogenesis. However, BBS7 and BBS8 are structurally different from others and work as intra flagellar transport (IFT) cohesion factors (Mykytyn *et al*., 2003; Nachury *et al*., 2007). Any change in the sequences of the BBSome results in defective ciliogenesis and its functions. The present study reported a splice acceptor site mutation (c.1131G>A, p.38) predicting a missense mutation gly-to-arg in exon 3 in the Kashmiri family from Pakistani origin Figure 1. Up till now eight mutations are reported in BBS9 gene including one of each splice site, stop, frame shift, missense, substitution and three deletion mutations.

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Statement of conflict of interest

The authors declare that they have no competing interests.

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