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Biallelic Variants in Seven Different Genes Associated with Clinically Suspected Bardet-Biedl Syndrome

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Abstract and figures

Bardet–Biedl syndrome (BBS) is a rare clinically and genetically heterogeneous autosomal recessive multi-systemic disorder with 22 known genes. The primary clinical and diagnostic features include six different hallmarks, such as rod–cone dystrophy, learning difficulties, renal abnormalities, male hypogonadism, post-axial polydactyly, and obesity. Here, we report nine consanguineous families and a non-consanguineous family with several affected individuals presenting typical clinical features of BBS. In the present study, 10 BBS Pakistani families were subjected to whole exome sequencing (WES), which revealed novel/recurrent gene variants, including a homozygous nonsense mutation (c.94C>T; p.Gln32Ter) in the IFT27 (NM_006860.5) gene in family A, a homozygous nonsense mutation (c.160A>T; p.Lys54Ter) in the BBIP1 (NM_001195306.1) gene in family B, a homozygous nonsense variant (c.720C>A; p.Cys240Ter) in the WDPCP (NM_015910.7) in family C, a homozygous nonsense variant (c.505A>T; p.Lys169Ter) in the LZTFL1 (NM_020347.4) in family D, pathogenic homozygous 1 bp deletion (c.775delA; p.Thr259Leufs*21) in the MKKS/BBS5 (NM_170784.3) gene in family E, a pathogenic homozygous missense variant (c.1339G>A; p.Ala447Thr) in BBS1 (NM_024649.4) in families F and G, a pathogenic homozygous donor splice site variant (c.951+1G>A; p?) in BBS1 (NM_024649.4) in family H, a pathogenic bi-allelic nonsense variant in MKKS (NM_170784.3) (c.119C>G; p.Ser40*) in family I, and homozygous pathogenic frameshift variants (c.196delA; p.Arg66Glufs*12) in BBS5 (NM_152384.3) in family J. Our

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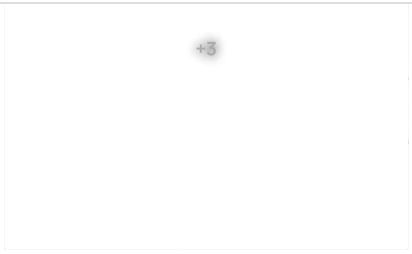

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Article

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Abstract: Bardet–Biedl syndrome (BBS) is a rare clinically and genetically heterogeneous autosomal recessive multi-systemic disorder with 22 known genes. The primary clinical and diagnostic features include six different hallmarks, such as rod–cone dystrophy, learning difficulties, renal abnormalities, male hypogonadism, post-axial polydactyly, and obesity. Here, we report nine consanguineous families and a non-consanguineous family with several affected individuals presenting typical clinical features of BBS. In the present study, 10 BBS Pakistani families were subjected to whole exome sequencing (WES), which revealed novel/recurrent gene variants, including a homozygous nonsense mutation (c.94C>T; p.Gln32Ter) in the *IFT27* (NM_006860.5) gene in family A, a homozygous nonsense mutation (c.160A>T; p.Lys54Ter) in the *BBIP1* (NM_001195306.1) gene in family B, a homozygous nonsense variant (c.720C>A; p.Cys240Ter) in the *WDPCP* (NM_015910.7) in family C, a homozygous nonsense variant (c.505A>T; p.Lys169Ter) in the *LZTFL1* (NM_020347.4)

in family D, pathogenic homozygous 1 bp deletion (c.775delA; p.Thr259Leufs*21) in the *MKKS/BBS5* (NM_170784.3) gene in family E, a pathogenic homozygous missense variant (c.1339G>A; p.Ala447Thr) in *BBS1* (NM_024649.4) in families F and G, a pathogenic homozygous donor splice site variant (c.951+1G>A; p?) in *BBS1* (NM_024649.4) in family H, a pathogenic bi-allelic nonsense variant in *MKKS* (NM_170784.3) (c.119C>G; p.Ser40*) in family I, and homozygous pathogenic frameshift variants (c.196delA; p.Arg66Glufs*12) in *BBS5* (NM_152384.3) in family J. Our findings extend the mutation and phenotypic spectrum of four different types of ciliopathies causing BBS and also support the importance of these genes in the development of multi-systemic human genetic disorders.

Keywords: ciliopathy; Bardet–Biedl syndrome; *IFT27*; *BBIP1*; *WDPCP*; *LZTFL1*; *MKKS*; *BBS1*; *BBS5*; polydactyly; obesity; intellectual disability

1. Introduction

Ciliopathy is a heterogeneous, multisystem genetic disorder caused by dysfunction of cilia. The cilia are hair-like outgrowths originating from the centriole and are of two types, motile and non-motile/primary. A typical cilium consists of mainly two parts, the basal body and the axoneme. Between them, a transitional zone/cilial domain is present, which acts like an enter and exit point for cilium and contains transitional fibers/alar sheets [1,2]. The basal body arises from the centriole providing an anchoring site for cilium, while the axoneme is composed of microtubules (the backbone of cilium) surrounded by a special membrane. In the axoneme of motile cilia, the two central doublets are surrounded by 09 pairs of outer doublets, making a (9 + 2) arrangement. In the case of non-motile/primary cilia, the central doublets are absent, making a (9 + 0) arrangement [2,3]. The intra-flagellar transport (IFT) mechanism is essential for trafficking the ciliary protein cargo for building, reabsorbing, and maintaining the newly synthesized cilium in the cell. In the cilium, the IFT cargo vesicle has anterograde and retrograde movement composed of different molecules such as IFT-B and kinesin particles, which are required for anterograde, while IFT-A and dynein are required for retrograde movement [3].

Regardless of the similarities, their function and effect are dramatically different from each other. Primary cilia function like antennae, sensing both mechanical and chemical changes in the microenvironment of the cell, through which they regulate fundamental biological processes, such as sensing extracellular cues and cell signaling (Wnt, Hedgehog, PDGF, etc.), leading to the transcription of target genes and playing a role in tissues homeostasis [2,4]. As already discussed, cilial function is notably associated with the BBS protein's stability and functionality. Any anomalies in primary cilial/BBS proteins will lead to diverse clinical manifestations, ranging from a single organ to multi-systemic disorders, termed as ciliopathy (Bardet–Biedl syndrome, Joubert Syndrome, polycystic kidney disease, etc.), while dysfunction of motile cilia usually causes disorder of situs-inversus, hydrocephalus, respiratory, infertility, etc. [5,6].

Bardet–Biedl syndrome (BBS; MIM, PS209900) is a rare genetic, pleiotropic, multisymptomatic autosomal recessive ciliopathy disorder. It is characterized by a wide spectrum of clinical features that affect different body systems, including polydactyly (hexadactyly), obesity (truncal), retinal degeneration (eye), renal dysfunction (kidney), genital anomalies (hypogonadism), and congenital impairment (learning disabilities). Minor or secondary features are also observed, though not always present in all BBS patients, including diabetes mellitus, liver disease, congenital heart defects, hearing loss, dental anomalies, facial dysmorphism, developmental delay, and many others [7]. Owing to the heterogeneous nature of BBS, clinical diagnosis is performed based on at least four primary features or three primary and two secondary features [8].

Two types of populations, geographically isolated and consanguineous families, have a high rate of BBS incidence. BBS is an autosomal recessive disorder; however, the occurrence of multi-gene involvement is common such as di-genic and tri-genic inheritance. BBS incidence is generally 1:25,000, while it is variable across different ethnicities, approximately 1:17,000 (Kowait), 1:156,000 (Tunisia), 1:65,000 (Arab population), and 1:37,000 (Foreislands) [3,7,9].

Defects in ciliogenesis (cilium formation) result in genetically heterogeneous multi-systematic disorders known as ciliopathies. As almost every cell contains cilia, any defects at the molecular level in the ciliary proteins archetypally affect different organ systems. Cilia play a vital role in signal transduction and enable cell-to-cell/ cell-to-surrounding communications [10].

BBS is a multi-systematic ciliopathy disorder characterized by mixed rod–cone dystrophy, with 40% of patients having renal and hypogonadism, 50% of patients suffering from intellectual disability, 2/3 patients having PAP, syndactyly, clinodactyly, or/and brachydactylic, and 70% of the patients experiencing truncal obesity associated with type 2 diabetes, hypertension, and dyslipidemia [3].

To date, genetic analysis has identified 22 genes implicated in causing BBS, among them *BBS1*, *BBS2*, *BBS4*, *BBS5*, *BBS7*, *BBS8* (*TTC8*), *BBS9* (*PTHB1*), *BBS17* (*LZTFL1*), and *BBS18* (*BBIP1*) are responsible for encoding the BBSome protein, while *BSS13* (*MKS1*), *BBS14* (*CEP290*; *TMEM67*), *BBS15* (*WDPCP*), and *BBS16* (*SDCCAG8*) produce the basal body interacting protein, *BBS6* (*MKKS*), *BBS10*, and *BBS12* form the chaperonin complex protein, *BBS11* (*TRIM32*) encodes E3 ubiquitin ligase, *BBS3* (*ARL6*), *BBS19* (*IFT27*), and *BBS20* (*IFT172*) give rise to a GTPase protein complex, *BBS21* (*C8ORF37*) plays an important role in primary cilia function and is involved in the development of BBS, and *BBS22* (*CEP19*) is responsible for encoding centrosomal and ciliary proteins [6,7,11].

Herein, we characterize 10 families with hallmark features of BBS. Genetic and molecular analysis revealed four novel and six already reported mutations in seven different genes previously associated with BBS.

2. Materials and Methods

2.1. Ethical Approval

The families presented here were ascertained from remote, poorly developed areas of Pakistan. Written informed consent for the publication of data and photographs were obtained from the parents (father) in compliance with the Helsinki Declaration. The IRB of UMT, Lahore [Dated: 03-01-2022; Ref# DLSBBC-2022-04], Pakistan and ethical research committee of the Kohat University of Science and Technology, Kohat, [dated: 16 July 2021; Certificate number: 1988] Pakistan, approved the research study.

2.2. DNA Isolation

Ten BBS families were examined for the primary and secondary features, pedigrees were constructed, and blood was collected in EDTA tubes from all the members labeled with asterisks in the pedigrees (Figure 1A–H). Patients' histories highlighted different prenatal, postnatal, and neonatal problems. Subsequently, DNA extraction and quantification were performed using standard methods [12].