RESEARCH ARTICLE

Assessment of genetic variation(s) in BBS10, BBS6, and BBS12 in a family from Sindh, Pakistan diagnosed with Bardet-Biedl Syndrome

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Abstract

Objective: To analyse the symptoms of Bardet-Biedl Syndrome, and to check the association of BBS10 (Bardet-Biedl syndrome 10 gene), BBS6 (Bardet-Biedl syndrome 6 gene) and BBS12 (Bardet-Biedl syndrome 12 gene) with the pathogenesis of Bardet-Biedl Syndrome.

Method: The case-control study was conducted in Karachi in 2019-20, and comprised Bardet-Biedl Syndrome patients and healthy controls from the same family. Blood was drawn from all the subjects for deoxyribonucleic acid extraction. Genotyping was performed by conventional and tetra primer amplification refractory mutation system polymerase chain reaction (T-ARMS-PCR) to identify the possible genetic variations. To validate the results, samples were randomly selected and sent for sequencing. Data was analysed using SPSS 20, while sequencing data was analysed using the Molecular Evolutionary Genetics Analysis X software.

Results: Of the 20 subjects with mean age 15.8+/-5.09 years (range: 9-25 years), 8(40%) were cases and 12(60%) were controls. The male-to-female ratio was 1:1. Pedigree analysis revealed that the pattern of inheritance was autosomal recessive. All the 8(100%) cases were obese compared to the controls. Truncal obesity, polydactyly, learning impairment and dysmorphic features, renal pain, olfactory dysfunction, respiratory tract infection and dysphasia were observed in all the 8(100%) cases. The globally reported genetic variants of BBS10 (rs1057516628 and rs1489342987) and BBS12 (rs121918327 and rs587777802) did not indicate any association with the clinical phenotype in the family concerned. The genetic variations of BBS6 (rs1547, rs1545 and rs1351192494), BBS12 (rs309370, rs2292493) and a BBS10 variant (rs35676114) had significant association with the disease.

Conclusion: The genetic variations showed confounding effects of BBS10, BBS6 and BBS12 genes which might indicate the epistatic effects of other variants present on different loci.

Key Words: Bardet-Biedl syndrome, BBS10, BBS6, BBS12, Genetic variations, Polymorphism.

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Introduction

Bardet-Biedl Syndrome (BBS) is an autosomal recessive pleiotropic disorder. Since it is a rare medical condition, its prevalence varies across the world. With a higher rate in populations where consanguineous marriages are more common, like in Newfoundland and Kuwait, it was reported to be 1:18,000 in Newfoundland and 1:13,500 is Kuwai.[1,2] However, limited data is available from Pakistan regarding BBS. So far, only a limited number of families have been reported from different areas of Pakistan, including Punjab, Dera Ismail Khan and other regions.[3-5] One study reported 4 families suffering from classical BBS and one from a BBS-like phenotype.[6] Another study reported two Pakistani families suffering from BBS.[7]

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Structural or functional defects in cilia may result in BBS. Cilia are responsible for cell motility and sensory reception, and they are involved in developmental signalling.[8] Malfunctioning cilia may result in the manifestation of several diseases and developmental disorders. Ciliary deficits affect multiple organs, including eye, limbs, kidney, liver and pancreas. Additionally, malfunctioning cilia can also affect odontoblasts, retinal photoreceptors and hypothalamic neurons.[9]

Symptoms of BBS are highly variable, including retinal degeneration, truncal obesity, polydactyly, reduced renal functions and genital abnormalities, in both genders.[10]

The criteria of diagnosis are based on the presence of miscellaneous primary and secondary features.[11] Primary features include polydactyly, rod con dystrophy, obesity, genital and renal abnormalities, and difficulty in learning. Developmental and speech delay, congenital heart diseases, diabetes, brachydactyly, dental abnormalities, anosmia and ataxia are considered secondary features.[12]

BBS symptoms are quite diverse as individuals who carry the same genotypes manifest symptoms differently.[13]

However, it has been reported that a correlation does exist between genotype and phenotype population-wise, but it is difficult to correctly predict the individual symptomatic manifestations.[14]

Mutations/variations in BBS genes have been reported to play an important role in the progression of this syndrome. It has been reported that BBS6, BBS10 and BBS12, which encode chaperonin-like proteins, are crucial to assembling the Bardet-Biedl Syndrome protein complex (BBSome complex) which plays a fundamental role in ciliary trafficking.[15] Multiple studies have reported that a significant number of clinically-diagnosed BBS families displayed variations in these genes compared to the other genes of the BBSome complex, which elucidates the importance of dysfunction in chaperonin proteins as pathogenic factors causing BBS.[16,17]

The current study was planned to analyse BBS symptoms, and to check the association of BBS10, BBS6 and BBS12 with BBS pathogenesis.

Patients and Methods

The case-control study was conducted in Karachi in 2019-20, and comprised BBS patients and healthy controls from the same family. Two individuals had been clinically diagnosed with BBS in a local tertiary care hospital, and they were later identified as probands of a family having eight individuals suffering from BBS. The family was the resident of Sakru village in the Sindh province. After approval from the institutional ethics review committees of the Dr A.Q. Khan Institute of Biotechnology and Genetic Engineering (KIBGE), University of Karachi, and the Jinnah Postgraduate Medical Centre (JPMC), Karachi, the family was approached for sample recruitment. All individuals diagnosed with BBS were recruited as the cases, while the healthy individuals in the same family were recruited as the controls. The purpose of this study was explained to each participant, and informed consent was obtained. BBS was diagnosed on the basis of either the presence of 4 primary, or 3 primary and 2 secondary features, with the criterion able to differentiate BBS from other overlapping phenotypic syndromes.[11]

A comprehensive history of the disease was recorded in the data forms along with age, gender, blood pressure. The body mass index (BMI) was calculated for each subject, and, as per the BMI range for Asians, >23kg/m2 was considered overweight and >25kg/m2 was considered obesity.[18] According to the World Health Organization (WHO), cut-offs for BMI aged 0-19 years are gender-specific.[19]

For the purpose of genotyping, whole blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers.

Genomic deoxyribonucleic acid (DNA) was isolated through the standard phenol-chloroform extraction method. Genotyping was performed by conventional and T-ARMS-PCR to identify the possible genetic variations. The PCR amplicons were separated on agarose gel stained with 7µl of visualaNA (Molequle-On, New Zealand) and were later analysed on a gel documentation system (BIO-Rad, United States). To confirm the PCR results, a few samples were randomly genotyped again. For further validation of the obtained results, the amplified products were purified by using a PCR purification kit (Molequle-On, New Zealand) and sent for DNA sequencing.

Data was analysed using SPSS 20 and Microsoft Excel. Sequences of all the samples were aligned and analysed using the Molecular Evolutionary Genetics Analysis (MEGA) X software.

Results

Of the 20 subjects with mean age 15.8+/-5.09 years (range: 9-25 years), 8(40%) were cases and 12(60%) were controls. The male-to-female ratio was 1:1. Among the cases, 3(37.5%) were aged 5-12 years, 2(25%) were aged 12-18 years, and 3(37.5%) were aged >18 years.

All the 4(100%) male cases had hypogonadism, while the female cases reported having irregular menstrual cycles. There were 5(62.5%) cases with dysphasia. Of the 4 female cases, 3(75%) had severe visual impairment. All the 8(100%) patients reported olfactory dysfunction. History of respiratory tract infection was also reported by all the 8(100%) cases. There were 4(50%) patients having asthma and chronic cough. The gait of the 8(100%) was suggestive of ataxia.

With respect to the genotyping of BBS10 gene polymorphism, rs1057516628 was amplified through the conventional PCR method. The required product of 151bp was observed in all the 20(100%) samples. No genetic change in rs1057516628 was observed in any of the samples. The electropherogram further validated the findings (Figure 1a).

As the amplification of rs1489342987 was performed through T-ARMS PCR, amplicons of different sizes were observed. None of the cases and controls showed the presence of homozygous TT genotype. The results were then confirmed through sequencing as well (Figure 1b). The amplicon generated by outer primers was sent for sequencing to check the presence of other single nucleotide polymorphisms (SNPs) located in that region.

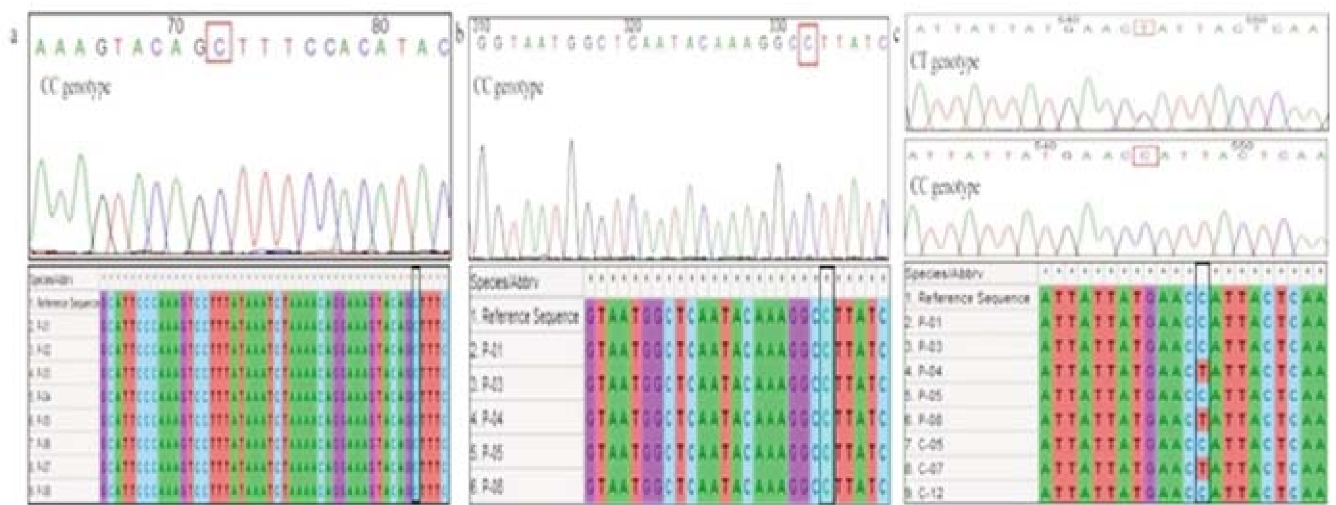

Figure-1: The electropherograms and multiple sequence alignment representation of the targeted single nucleotide polymorphisms (SNPs) in BBS10 gene. (a): rs1057516628; (b): rs1489342987; (c): rs35676114.

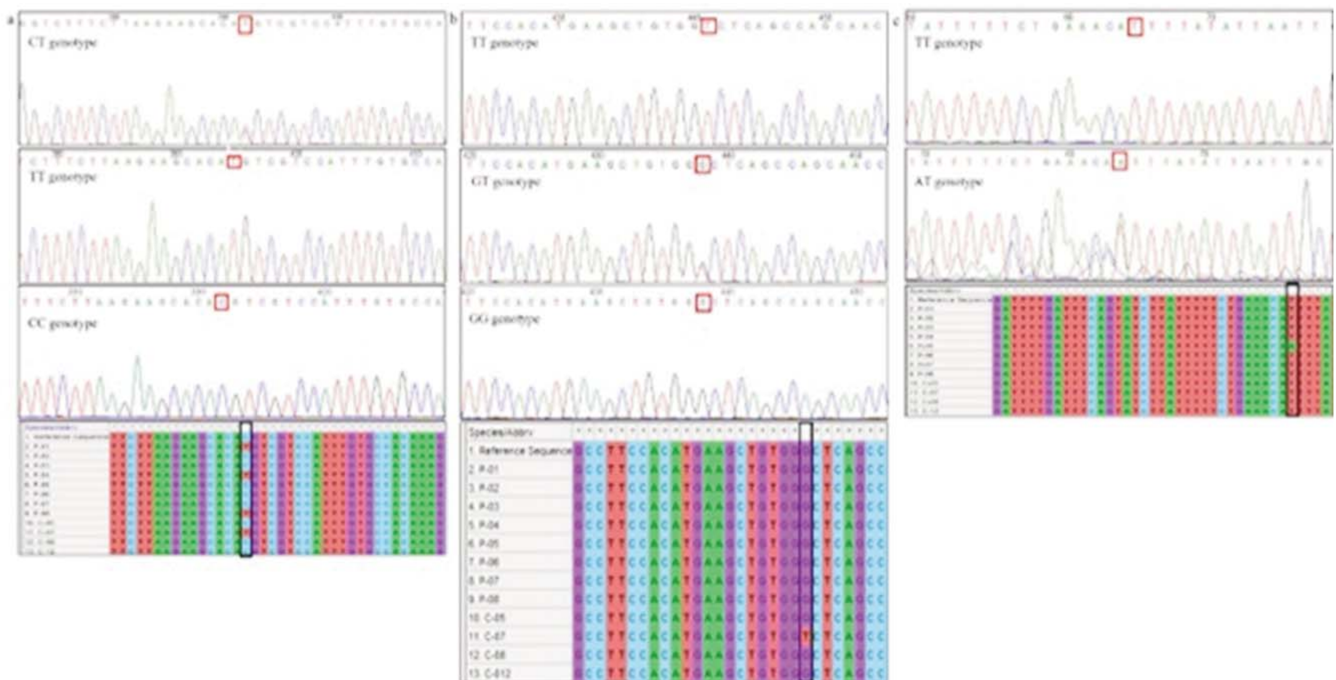

Figure-2: The electropherograms and multiple sequence alignment representation of the targeted single nucleotide polymorphisms (SNPs) in BBS6 gene. (a): rs1547; (b): rs1545; (c): rs1351192494.

Results revealed the presence of rs35676114, a missense variant, present at the second exon of the BBS10. This variation resulted in the alteration in the genetic code from CCA to CTA. There were 2(25%) cases and 1(8.3%) control who were carriers of the CT heterozygous genotype (Figure 1c).

With respect to the genotyping of BBS6 gene polymorphism, rs1547 was reported as a missense variant located on exon 6 of the BBS6 gene, resulting in the amino acid substitution of arginine with cysteine. Among the cases, 3(37.5%) showed C to T change (CT genotype) upon comparing the sequences with the reference sequence. There was 1(8.3%) individual in the control group who was a carrier of the TT homozygous genotype. These genetic changes were further validated for their heterozygosity or homozygosity by analysing the electropherograms (Figure 2a).

The rs1545 missense variant results in the change of

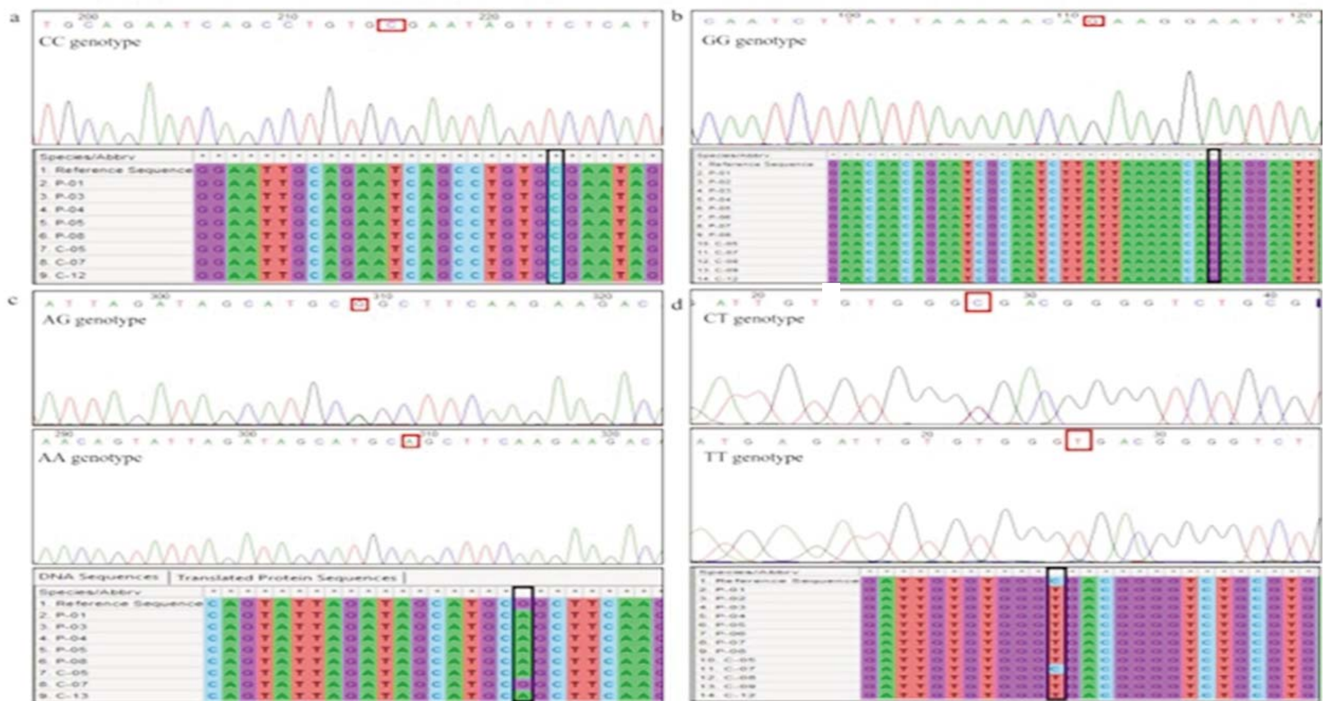

Figure-3: The electropherograms and multiple sequence alignment representation of the targeted single nucleotide polymorphisms (SNPs) in BBS12 gene. (a): rs121918327; (b): rs587777802; (c): rs309370; (d): rs2292493.

amino acid sequence by replacing Glycine with Valine at the 532nd position. Among the cases, 1(12.5%) was observed to be the carrier of heterozygous GT genotype upon examining the electropherogram. Also, 1(12.5%) control showed the variation of T in place of G in homozygous (TT) condition, with electropherogram analysis further validating the results (Figure 2b).

SNP rs1351192494 was found within the sequence of rs1547 upon analysing the electropherogram. In 1(12.5%) of the cases the T nucleotide was substituted with A, and the case was considered heterozygous for this SNP, while in the remaining subjects, no variation was observed, and they were the carriers of TT genotype (Figure 2c).

With respect to the genotyping of BBS12 gene polymorphism, rs121918327 was found on exon 2 of the BBS12 gene. During T-ARMS-PCR amplification of the variant, outer primers amplified that region were sequenced and the obtained results validated the results. No variation was observed, and all the 20(100%) subjects were carriers of homozygous CC genotype. The electropherograms further confirmed the results (Figure 3a).

The rs587777802, which is a frameshift mutation resulting from a two-base pair deletion, showed no variation, and it was further validated by electropherograms and multiple sequence alignment (Figure 3b).

The rs309370, which is a missense variant in BBS12, showed no variation, and the DNA sequence analysis showed that all the 8(100%) cases were carrying the homozygous AA genotype. Among the controls, 2(16.6%) were carriers of the AA genotype, and 1(8.3%) was carrier of the AG genotype. None of the samples showed homozygous GG genotype (Figure 3c).

Within the sequence of rs587777802, variation rs2292493 was observed that was synonymous in nature and C/T variation. Among the controls, 1(8.3%) was the carrier of heterozygous CT genotype, while the rest of the sample was carrier of homozygous TT genotype (Figure 3d).

Discussion

BBS patients carrying the pathogenic variants in BBS6, BBS10 and BBS12 genes have been observed to demonstrate more profound clinical features than in other genes encoding the BBSome complex.[17] Additionally, it has also been speculated that abnormalities in the chaperonin-like genes cause the early onset of BBS. The present study was designed to screen the selected family for variants present in BBS10, BBS6, and BBS12 genes.

BBS10 belongs to chaperones that play an important role

in protein folding as well as in regulating their activities. BBS10 protein is present at the basal body of the primary cilium in distinguished preadipocytes. In cultured cells, a deficiency of proteins (BBS6, BBS10 and BBS12) has been suggested as the primary cause of non-functional BBSome assembly.[20] A frameshift caused by rs1057516628 (BBS10 variation) may cause premature termination at the subsequent fourth amino acid. It was reported in a Pakistani family of Punjabi ethnicity.[3] However, no variation was observed in the focused family of the present study, suggesting the probability of ethnic variability.[4] Another studied SNP rs1489342987 (missense variant) changes the codon from CCT to CTT and results in the substitution of proline with leucine. Proline has been suggested to be a helix breaker, and another branched-chain amino acid, leucine, may induce conformational changes, thereby disrupting the BBS10 protein structure, and leading to its dysfunction. This variant was also not observed in the studied family. The rs35676114 is a missense variant which, due to codon alteration from CCA to CTA, results in the replacement of proline with leucine. In the current study, two cases and one control were observed to be the carriers of the heterozygous CT genotype, while the rest of the subjects did not show any variation.

BBS6 has been reported as a crucial determinant which aids in stabilising the two BBSome units (BBS2 and BBS7).[20] Two variants of BBS6 (rs1547 and rs1545) were targeted at first. A study reported the presence of these two SNPs in metabolic syndrome and BBS patients.[21] In the current study, three out of eight patients were found to be the carriers of the heterozygous genotype of rs1547, while others did not show any change in this position. This could be a result of an epistatic interaction, where the presence of a particular allele at a second gene has the potential to alter the impacts of the primary gene.

Earlier studies have also described the role of heterozygous mutations in BBS1, BBS2 and BBS6 to possess the potential epistatic effect.[22-23] On the other hand, rs1545 was found in heterozygous condition in one of the cases. Furthermore, one control was observed to be the carrier of TT genotype. Upon sequencing analysis, one more SNP was observed (rs1351192494) in one of the cases. Further studies are required to interpret the possible role of this SNP in BBS pathogenesis.

BBS12 encodes the third chaperonin-like BBS protein within the BBSome complex. It has been reported to be a large contributor to BBS. A large number of mutations, including indels, and missense/nonsense variations have been reported to date in the BBS12 gene playing a role in disease pathogenesis. Three reported mutations (rs587777802, rs121918327 and rs309370) were targeted in the present study. None of the patients were carrying the first two SNPs (rs587777802 and rs121918327), indicating that these variants were not linked with the BBS pathogenesis in the targeted family. However, rs309370 was observed in homozygous AA state in all subjects except for one control who was the carrier of AG genotype. This missense variant replaces adenine with guanine leading to a change of the amino acid arginine with glutamine. Furthermore, rs309370 has been classified as benign variant that is synonymous in nature.[4,16] However, it is imperative to note that the charges and sizes of these amino acids are different. Arginine has a positive charge and is bigger in comparison to glutamine which is smaller and neutral. This difference might impact the interactions with other residues and molecules that are important for ciliary functions.

Furthermore, a synonymous variant rs2292493 was observed in all except one control subject in homozygous TT condition, while the remaining controls were the carriers of CT genotype. Another study also reported the same result. This suggests the potential role of this SNP in manifestation of BBS in the studied family as all the studied members were the carriers of variant genotype in homozygous condition.

As BBS is characterised by inter- and intra-familial clinical variability, for the disease to manifest, some families describe the loss of function at one gene paired with haploinsufficiency at a second BBS locus.[22] In some families, the second-site locus serves as the modifier of expressivity and severity of the disease.[22,24-25] Therefore, it is necessary to investigate other BBS loci in the same family as well as in other families.

Conclusion

Genetic variants rs35676114, rs1547, rs1351192494, rs309370 and rs2292493 may contribute to the development of BBS. In a country with limited resources, the findings may not only help in the early identification of the disorder, but also lead to implementation of better healthcare management and treatment strategies of patients and individuals at risk with a focus on precision medications.

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References

1. Moore SJ, Green JS, Fan Y, Bhogal AK, Dicks E, Fernandez BA, et al.

Clinical and genetic epidemiology of Bardet-Biedl syndrome in Newfoundland: a 22-year prospective, population-based, cohort study. Am J Med Genet A 2005;132A:352-60. doi: 10.1002/ajmg.a.30406.

2. Farag TI, Teebi AS. High incidence of Bardet Biedl syndrome among the Bedouin. Clin Genet 1989;36:463-4. doi: 10.1111/j.1399-0004.1989.tb03378.x.
3. Agha Z, Iqbal Z, Azam M, Hoefsloot LH, van Bokhoven H, Qamar R. A novel homozygous 10 nucleotide deletion in BBS10 causes Bardet-Biedl syndrome in a Pakistani family. Gene 2013;519:177-81. doi: 10.1016/j.gene.2013.01.047.
4. Ajmal M, Khan MI, Neveling K, Tayyab A, Jaffar S, Sadeque A, et al. Exome sequencing identifies a novel and a recurrent BBS1 mutation in Pakistani families with Bardet-Biedl syndrome. Mol Vis 2013;19:644-53.
5. Muzammal M, Zubair M, Bierbaumer S, Blatterer J, Graf R, Gul A, et al. Exome sequence analysis in consanguineous Pakistani families inheriting Bardet-Biedle syndrome determined founder effect of mutation c.299delC (p.Ser100Leufs*24) in BBS9 gene. Mol Genet Genomic Med 2019;7:e834. doi: 10.1002/mgg3.834.
6. Maria M, Lamers IJ, Schmidts M, Ajmal M, Jaffar S, Ullah E, et al. Genetic and clinical characterization of Pakistani families with Bardet-Biedl syndrome extends the genetic and phenotypic spectrum. Sci Rep 2016;6:34764. doi: 10.1038/srep34764.
7. Chen J, Smaoui N, Hammer MB, Jiao X, Riazuddin SA, Harper S, et al. Molecular analysis of Bardet-Biedl syndrome families: report of 21 novel mutations in 10 genes. Invest Ophthalmol Vis Sci 2011;52:5317-24. doi: 10.1167/iovs.11-7554.
8. Ullah A, Khalid M, Umair M, Khan SA, Bilal M, Khan S, et al. Novel sequence variants in the MKKS gene cause Bardet-Biedl syndrome with intra- and inter-familial variable phenotypes. Congenit Anom (Kyoto) 2018;58:173-75. doi: 10.1111/cga.12264.
9. Forsythe E, Kenny J, Bacchelli C, Beales PL. Managing Bardet-Biedl Syndrome-Now and in the Future. Front Pediatr 2018;6:23. doi: 10.3389/fped.2018.00023.
10. Koscinski I, Mark M, Messaddeq N, Braun JJ, Celebi C, Muller J, et al. Reproduction Function in Male Patients With Bardet Biedl Syndrome. J Clin Endocrinol Metab. 2020 Dec 1;105(12):e4417–29. doi: 10.1210/clinem/dgaa551. Erratum in: J Clin Endocrinol Metab 2021;106:e1936. doi: 10.1210/clinem/dgaa897.
11. 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;36:437-46.
12. Melluso A, Secondulfo F, Capolongo G, Capasso G, Zacchia M. Bardet-Biedl Syndrome: Current Perspectives and Clinical Outlook. Ther Clin Risk Manag 2023;19:e115-32. doi: 10.2147/TCRM.S338653.
13. Jeziorny K, Antosik K, Jakiel P, Młynarski W, Borowiec M, Zmysłowska A. Next-Generation Sequencing in the Diagnosis of Patients with Bardet-Biedl Syndrome-New Variants and Relationship with Hyperglycemia and Insulin Resistance. Genes (Basel) 2020;11:1283. doi: 10.3390/genes11111283.
14. Forsythe E, Sparks K, Best S, Borrows S, Hoskins B, Sabir A, et al. Risk Factors for Severe Renal Disease in Bardet-Biedl Syndrome. J Am Soc Nephrol 2017;28:963-70. doi: 10.1681/ASN.2015091029.
15. Tomlinson JW. Bardet-Biedl syndrome: A focus on genetics, mechanisms and metabolic dysfunction. Diabetes Obes Metab 2024;26(Suppl 2):13-24. doi: 10.1111/dom.15480.
16. Nikkhah E, Safaralizadeh R, Mohammadiasl J, Tahmasebi Birgani M, Hosseinpour Feizi MA, Golchin N. Identification of A Novel Compound Heterozygous Mutation in BBS12 in An Iranian Family with Bardet-Biedl Syndrome Using Targeted Next Generation Sequencing. Cell J 2018;20:284-9. doi: 10.22074/cellj.2018.5012.
17. Álvarez-Satta M, Castro-Sánchez S, Valverde D. Bardet-Biedl Syndrome as a Chaperonopathy: Dissecting the Major Role of Chaperonin-Like BBS Proteins (BBS6-BBS10-BBS12). Front Mol Biosci 2017;4:e55. doi: 10.3389/fmolb.2017.00055.
18. Misra A, Jayawardena R, Anoop S. Obesity in South Asia: Phenotype, Morbidities, and Mitigation. Curr Obes Rep 2019;8:43-52. doi: 10.1007/s13679-019-0328-0.
19. World Health Organization (WHO). A healthy lifestyle - WHO recommendations. News release. The WHO Media Centre. [Online] 2010 [Cited 2024 August 12]. Available from URL: https://www.who.int/europe/news-room/fact-sheets/item/a-healthy-lifestyle---who-recommendations
20. Tian X, Zhao H, Zhou J. Organization, functions, and mechanisms of the BBSome in development, ciliopathies, and beyond. Elife 2023;12:e87623. doi: 10.7554/eLife.87623.
21. Sathya Priya C, Sen P, Umashankar V, Gupta N, Kabra M, Kumaramanickavel G, et al. Mutation spectrum in BBS genes guided by homozygosity mapping in an Indian cohort. Clin Genet 2015;87:161-6. doi: 10.1111/cge.12342.
22. Badano JL, Kim JC, Hoskins BE, Lewis RA, Ansley SJ, Cutler DJ, et al. Heterozygous mutations in BBS1, BBS2 and BBS6 have a potential epistatic effect on Bardet-Biedl patients with two mutations at a second BBS locus. Hum Mol Genet 2003;12:1651-9. doi: 10.1093/hmg/ddg188.
23. Badano JL, Leitch CC, Ansley SJ, May-Simera H, Lawson S, Lewis RA, et al. Dissection of epistasis in oligogenic Bardet-Biedl syndrome. Nature 2006;439:326-30. doi: 10.1038/nature04370.
24. Perea-Romero I, Solarat C, Blanco-Kelly F, Sanchez-Navarro I, Bea-Mascato B, Martin-Salazar E, et al. Allelic overload and its clinical modifier effect in Bardet-Biedl syndrome. NPJ Genom Med 2022;7:41. doi: 10.1038/s41525-022-00311-2.
25. Kousi M, Söylemez O, Ozanturk A, Mourtzi N, Akle S, Jungreis I, et al. Evidence for secondary-variant genetic burden and non-random distribution across biological modules in a recessive ciliopathy. Nat Genet 2020;52:1145-50. doi: 10.1038/s41588-020-0707-1.

AUTHOR'S CONTRIBUTION:

SF: Concept, methodology, formal analysis, investigation, pedigree analysis, writing, review and editing.

MA: Concept, formal analysis, investigation, writing and original draft preparation.

FZ: Concept, methodology, formal analysis and investigation.

KS & SK: Identification of proband, provision of samples and compilation of clinical profiles of patients.

SS & SZ: Pedigree analysis, writing, review and editing.