Research Square

Preprints are preliminary reports that have not undergone peer review. They should not be considered conclusive, used to inform clinical practice, or referenced by the media as validated information.

Investigation of genetic variants causing Bardet–Biedl syndrome in Iranian families: Identification of a founder mutation in BBS2, p.T157T

Masoumeh Heidari Feizabadi
University of Social Welfare and Rehabilitation Sciences

Masoome Alerasool
Genetic Foundation of Khorasan Razavi

Atieh Eslahi
Mashhad University of Medical Sciences

Emran Esmaeilzadeh
Hope Generation Foundation

Mohammad Yahya Vahidi Mehrjardi
Shahid Sadoughi University of Medical Sciences

Mitra Saket
RP Eye patients Supporting Institute

Shima Farokhi
Mashhad University of Medical Sciences

Zohreh Fatahi
University of Social Welfare and Rehabilitation Sciences

Hamid Reza Khorram Khorshid
University of Social Welfare and Rehabilitation Sciences

Majid Mojarrad
mojaradm@mums.ac.ir

Mashhad University of Medical Sciences

Research Article

Keywords: Bardet–Biedl syndrome, Whole exome sequencing, ROH calling, Founder mutation, Copy Number Variation

Posted Date: October 18th, 2023

DOI: https://doi.org/10.21203/rs.3.rs-3445871/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
Read Full License

Additional Declarations: No competing interests reported.

Version of Record: A version of this preprint was published at Biochemical Genetics on February 26th, 2024. See the published version at https://doi.org/10.1007/s10528-023-10637-w.

Abstract

Background

Bardet-Biedl Syndrome (BBS) is a rare inherited ciliopathy disorder characterized by a wide range of clinical symptoms affecting multiple body systems. All BBS genes are involved in cilia function as a part of the BBSome complex. Mutations of BBS genes are not completely understood, suggesting that more research is needed to develop a molecular diagnostic strategy for this syndrome.

Methods and Results

Whole exome sequencing (WES) was performed on eighteen patients. A comparative study based on Runs of homozygosity (ROH (calling was performed using the BCFtools/RoH software on WES data. The potential pathogenicity of the detected mutations and the effect on splicing was predicted by *in-silico* analysis. Copy Number Variation (CNV) analysis was performed from the read depth of WES data using the ExomeDepth pipeline in unsolved BBS patients. Eight variants including four novel mutations, and a synonymous splicing variant (c.G471A) in *BBS2* were identified. By examining homozygous regions among these patients, the existence of common homozygous regions containing the identified mutation was proved in patients with Baloch ethnicity. *In-silico* analysis predicted the effect of the c.G471A mutations on BBS2 mRNA splicing, this mutation leads to broken wild-type donor site and intron retention in the mature mRNA. CNV analysis revealed a deletion of exons in the *BBS1* gene.

Conclusion

Our results declared the founder mutation c.G471A in the *BBS2* gene in the Baloch ethnicity of the Iranian population, which can determine the diagnostic approach of this syndrome in future studies.

Introduction

Bardet-Biedl Syndrome is a rare inherited ciliopathy disorder characterized by a wide range of clinical symptoms affecting multiple body systems [1]. Its primary features encompass polydactyly, obesity, hypogonadism, renal abnormalities, mental retardation, and rod-cone dystrophy. Also, secondary characteristics such as brachydactyly or syndactyly, developmental delay, congenital heart disease, diabetes mellitus, speech deficit, and ataxia have been observed in patients [2]. BBS prevalence shows a wide variety of 1 in 100,000 live birth in North America and Europe and 1 in 18,000 live birth on the island of Newfoundland [3]. Recent advances in NGS and genetic testing methods have significantly improved the identification of mutations and the diagnostic performance of such heterogeneous genetic conditions. So far, at least 26 BBS genes have been discovered that explain about 70–80% of clinically-diagnosed patients. However, the genetic basis of at least 20% of BBS patients remains undiagnosed [4]. All BBS genes are involved in cilia function as a part of the BBSome complex. As a cargo adapter, this

complex recognizes signaling proteins such as GPCRs[1] connecting them to the intraflagellar transport machinery [5].

Oligogenic inheritance such as triallelic is present in less than 10% of BBS [6]. Also, because of the complex genetic and clinical nature of ciliopathies, there is a wide variety of diseases that overlap with Bardet-Biedl syndrome, such as Sjogren-Larsson syndrome, Meckel–Gruber Syndrome, Ellis-van Creveld syndrome, Joubert syndrome. Therefore, the use of clinical and genetic analysis in the differential diagnosis of BBS from other ciliopathies is critical [7]. On the other hand, the genotype-phenotype association studies revealed that the identity of the causative gene and the character of the mutation partially predict the clinical consequence of the disease [8].

CNV is one of the major sources of genetic diversity in humans. CNVs involve the loss or gain of relatively large genomic DNA segments. Since the frequency of CNVs in pathogenicity is 12–16%, these changes play an important role in creating diversity in the population and disease phenotype [9]. The alteration in the copy number of one or more dosage-sensitive genes is the most common mechanism underlying CNV-mediated disease pathogenesis. Based on past studies, the role of CNV in causing BBS has been reported in 18.5% of patients, with this CNV including 13 different deletions in eight BBS genes and a deletion and a duplication in [2]*ALMS1* and *NPHP4*[3] genes, respectively [10].

This multisystem disorder primarily affects children of consanguineous marriages. According to the high consanguineous marriage frequency in Iranian society, investigating homozygous regions in the patient's families is fundamental.

Mutations of BBS genes in Middle Eastern countries- especially Iran, are not completely understood, suggesting that more research is needed to develop a molecular diagnostic strategy for this syndrome. This study was designed to characterize the responsible genes and mutation spectrums in a cohort of eighteen Iranian families with BBS, using whole exome sequencing.

Materials and Methods

Selection of the subjects and preparation of samples

This study was approved by the Institutional Review Boards of the University of Social Welfare and Rehabilitation Sciences (USWR) and Mashhad University of Medical Sciences (MUMS). According to BBS diagnostic criteria, a clinical examination is performed considering the presence of at least four major features or three primary features with at least two minor symptoms [11]. Clinical information was extracted from patients' medical records at Genetic Foundation, Khorasan Razavi, Hope Generation Foundation, Tehran, and Diabetes research center, Yazd. A family must have consanguineous marriages and at least one affected person, or at least two affected persons in non-familial situations, to be eligible for this research. Eighteen Iranian nonrelative families with suspicious symptoms of BBS were recruited for this investigation. Based on the tenets of the Declaration of Helsinki, informed consent was obtained from all the patient's parents. Eight patients belonged to Baloch ethnicity and the rest belonged to Persian

ethnicity. In ten families, only one affected child was present, whereas the other families had two or more children with the BBS phenotype. There was no consanguinity in four families (Table 1). Blood samples from all patients and their family members were collected in EDTA tubes, and genomic DNA was extracted from peripheral blood samples with a DNA extraction kit (Simbiolab, Iran).

Table 1
Clinical evaluation of all the patients in this study

Sample number	Gender	Age	Ethnicity	Consanguinity	Family history	Rod–cone dystrophya	RP	Obesity	Polydactyly	Genital abnormalities	ID	Renal and urinary abnormalities	Secondary features
04-1088	Female	6	Fars	✓	–	✓	✓	✓	–	✓	✓	✓	
10-1498	Male	22	Fars	✓	–	✓	✓	–	–	–	✓	✓	Hearing problems
11-1014	Female	22	Fars	✓	–	✓	✓	✓	✓	–	✓	✓	
10-2015	Female	9	Fars	✓	–	✓	✓	✓	✓	–	–	✓	
02-622	Male	32	Fars	✓	_	✓	✓	✓	✓	_	_	✓	
02-701	Male	8	Fars	✓	✓	✓	✓	✓	✓	_	_	✓	
15-723	Female	12	Fars	_	✓	✓	✓	✓	✓	–	_	✓	Diabetes mellitus
15-934	Male	3	Fars	_	✓	_	_	✓	✓	–	✓	✓	Diabetes mellitus
11-500	Male	7	Fars	_	–	–	–	✓	✓	–	✓	✓	Developmental delay,Epilepsy
01-556	Male	28	Fars	✓	✓	✓	✓	_	✓	–	✓	–	Nystagmus diabete type 1
4-770	Female	15	Baluch	✓	✓	✓	✓	✓	✓	–	✓	–	
11-792	Female	17	Baluch	✓	✓	✓	✓	✓		–	✓	✓	Brachydactyly
12-502	Male	12	Baluch	✓	_	✓	✓	✓	✓	–	–	✓	Strabismus
4-407	Female	24	Baluch	–	✓	✓	✓	✓	✓	–	✓	–	
4-408	Male	4	Baluch	✓	_	✓	✓	✓	✓	–	–	✓	
10-784	Male	8	Baluch	✓	✓	✓	✓	–	✓	✓	–	✓	
03-412	Male	17	Baluch	✓	_	_	_	✓	_	-	✓	✓	Brachydactyly
12-504	Male	11	Baluch	✓	–	✓	✓	✓	✓	–	✓	–	

Exome sequencing

Whole exome sequencing was performed on all eighteen patients from different families to look for causative variants of BBS. Target enrichment was carried out using the SureSelect Human All Exon V6 kit (Agilent, Santa Clara, CA, USA) and the HiSeq Rapid PE Cluster Kit v2 (Illumina, San Diego, CA, USA), and libraries were sequenced on a HiSeq 2500 instruments (Illumina). Adapter and low-quality reads were removed from the raw data by the Trimmomatic tools. Reads were aligned to the reference genome (build hg19) with the Burrows-Wheeler Aligner, and duplicate reads were then removed using Picard (v. 2.14.0-SNAPSHOT). The final results were converted to SAM and BAM files. Variant calling was performed to identify single-nucleotide polymorphisms (SNPs) and small insertions and deletions (indels) using The Genome Analysis Tool Kit (GATK v4.0) software package and the Best Practice Guidelines described by the developers. The readings in the modified area were recompiled and the resultant variant file was present as a VCF file. The VCF file was then annotated using the wANNOVAR tool. Synonymous

mutations, modifications in the intergenic, intronic, 3'UTR, and 5'UTR regions, as well as variants with a frequency higher than 0.01 based on the available information from databases such as ExAc, GnomAD, dbSNP, 1000 Genomes, and local database, Iranome were filtered. Furthermore, heterozygous mutations were filtered to analyze homozygous and compound heterozygous mutations in situations of autosomal recessive inheritance and parental consanguinity. Each variant is evaluated based on the available information from the databases (including HGMD, ClinVar, LSDBs, NHLBI Exome Sequencing Project, 1000 Genomes, and dbSNP), published literature, clinical correlation and its predicted functional or splicing impact using evolutionary conservation analysis and computational tools (including AlignGVGD, MAPP, MutationTaster, PolyPhen-2, SIFT, and SNAP).

ROH calling

A comparative study based on ROH frequency and identified potential genes that overlapped with ROH regions was performed using the BCFtools/RoH software on WES data. The BCFtools/RoH command detects regions of autozygosity using a Hidden Markov Model. ROH islands were defined based on consensus that overlapping homozygous areas with a frequency of more than 0.4.

Sanger sequencing

Primer-3 and Oligoanalyzer 3.1 were used for primers designing for the detected variants spanning regions. PCR reactions were performed under standard conditions. Purified PCR products were sequenced by Sanger sequencing using the Big Dye Terminator v3.1 Cycling Sequencing Kit (Applied Biosystem, Foster City, CA, USA) on an ABI 3730XL platform (Applied Biosystems). Data was reviewed using the Sequencher v4.8, SnapGene, and Chromas Lite v2.01 software, and compared with wild-type samples and reference sequences from the NCBI and Ensembl databases, and the frequency of candidate variants was evaluated in the Iranome database.

In-silico prediction

The potential pathogenicity of the detected mutations was predicted by *in-silico* analysis using two different tools: PolyPhen and SIFT. The potential effect on splicing was predicted by the Human Splicing Finder (HSF). The HSF[4] database was created using an Ensembl dataset that included all human genes containing introns and exons.

To analyze the predicted mutation on splice sites, we set mutation by HGVS nomenclature in the Mutation Analysis Tool of the HSF database. Finally, we got the reference sequence and mutant sequence. From the “Interpreted data”, we found the results of the predicted signal, prediction algorithm, cDNA position, and interpretation.

Copy Number Variation Analysis

CNV analysis was performed from the read depth of WES data using the ExomeDepth pipeline in unsolved BBS patients with a negative result through WES analysis according to the developers’ guidelines. ExomeDepth is a credible method for exome read depth analysis that uses an optimized set of

reference samples to compare CNV presence at the exon level and generate normalized read counts of the test sample. Using ClassifyCNV software, the pathogenicity of CNV was evaluated. This software facilitates CNV analysis by following the ACMG 2019 classification guidelines. The detected deletion was confirmed by PCR in one patient in comparison with healthy control. Real-time PCR analysis was performed to study the gene dosage changes in the deleted regions in heterozygous individuals compared to healthy controls. DNA was amplified with SYBR Green PCR Master Mix (Ampliqon, Denmark). The LightCycler® 96 Real-Time PCR System was used for relative quantification (Roche, Germany). All reactions were performed in triplicates. Melting curves were generated after amplification and were analyzed by LightCycler 96 software.

Results

Exome sequencing analysis

By analyzing WES in eighteen studied patients, we identified eight homozygous pathogenic variants in nine patients, including four novel mutations (Table 2). Pedigrees were drawn using Invitae software (Fig. 1).

Table 2 Mutations identified in the first step

Sample no	Gene	Mutation number	Accession no	Gene locus	Variation	Type of variation	Genotype	Pathogenicity	Reference
04-1088 10-1498	*BBS2*	M1	NM_031885	16q12.2	c.C1780T (P.R594X)	Nonsense	Homozygote	Pathogenic	[12]
11-1014	*BBS8 (TTC8)*	M2	NM_144596.4	14q31.3	c.901_905del (p.Ile301Ter)	Nonsense	Homozygote	Pathogenic	Novel
10-2015	*BBS7*	M3	NM_176824.3	4q27	c.602-12_622del (p.Gly201_Leu208delinsVal)	Deletion	Homozygote	Likely Pathogenic	Novel
02-622	*BBS5*	M4	NM_152384.3	2q31.1	c.790G>A (p.Gly264Arg)	Missense	Homozygote	Likely Pathogenic	[13]
02-701	*BBS6 (MKKS)*	M5	NM_170784.3	20p12.2	c.1334T>G (p.Leu445Ter)	Nonsense	Homozygote	Pathogenic	Novel
15-723	*BBS10*	M6	NM_024685.4	12q21.2	c.271dup (p.Cys91LeufsTer5)	Frame-shift	Homozygote	Pathogenic	[14]
15-934	*BBS12*	M7	NM_152618.3	4q27	c.1063C>T (p.Arg355Ter)	Nonsense	Homozygote	Pathogenic	[15]
4-770	*BBS17 (LZTFL1)*	M8	NM_020347	3p21.31	c.131-143del (p.L44Pfs)	Frame-shift	Homozygote	Likely Pathogenic	Novel

Identification of a founder mutation in BBS2

Reanalyzing of WES in unsolved nine patients showed a synonymous mutation in the *BBS2* gene in seven patients. Studying the pathogenicity of the c.G471A mutation in the databases provided sufficient confidence to confirm the pathogenicity of the mutation (Table 3). Variant segregation within healthy and affected members in patients' families confirmed co-segregation of the identified mutation with the

disease (Fig. 2). Analysis of homozygosity regions by ROH Calling was performed on VCF files in seven patients’ with Baluch ethnicity (one patient without mutation). A common locus of homozygosity in four patients was observed (Fig. 3). The results are collected in Table 4.

Table 3 The mutation identified in the next step

Sample no	Gene	Mutation Number	Accession no	Gene locus	Variation	Type of variation	Genotype	Pathogenicity	Reference
11-792	*BBS2*	M9	NM_031885.5	16q12.2	c.G471A (p.T157T)	Synonymous	Homozygote	Likely Pathogenic	[16], [17], [18]
12-502									
4-407									
4-408									
10-784									
03-412									
01-556									

Table 4 The information of patients with c.G471A mutations. The results of ROH calling

Sample no	Ethnicity	ROH result
11-792	Baluch	Positive
12-502	Baluch	Positive
4-407	Baluch	Positive
03-412	Baluch	Positive
4-408	Baluch	Negative
10-784	Baluch	Negative

In-silico analysis of c.G471A mutation

In-silico analysis with an online bioinformatics tool, the human splicing finder (HSF) predicted the effect of the c.471G > A mutations on *BBS2* mRNA splicing. The HSF analysis predicts disruption of the wild-type donor site, generating consensus values of 83.82 and 73.74 for the wild-type and mutant c.471G > A allele, respectively. The predicted consensus value deviation of -12.03% for the disrupted wild type donor site indicates the intron retention which would result in a frame-shift, and finally lead to a premature stop codon residue downstream of mutation.

Identification of a deletion in BBS1

CNV analysis led to the identification of the deletion of exons 14 to 17 in the *BBS1* gene of one patient. Quantitative Real-Time PCR analysis in other family members of this patient proved the decrease in the number of normal copies in heterozygous individuals compared to healthy individuals. The melting curve is shown in Fig. 4.

Discussion

In this study, we investigated the genetic factors involved in Bardet-Biedl syndrome in eighteen Iranian patients. In the first step, according to the results of WES, we were able to identify eight variants, Overall, four novel mutations including nonsense mutation in *BBS6* (c.1334T > G), partial deletion (c.602 – 12_622del) in the *BBS7*, frameshift deletion (c.131-143del) in *BBS17*, and nonsense mutation (c.901-905del) in *BBS8*, and four known mutations such as, premature nonsense mutation (c.C1780T) in *BBS2* in two patients, missense mutation (c.790G > A) in *BBS5*, frameshift mutation (c.271dup) in *BBS10*, and nonsense mutation (1063C > T) in *BBS12* were identified.

In the next step, by reanalyzing the unsolved cases, we identified a synonymous mutation (c.471G > A) in seven patients. Six patients were of Baloch ethnicity and one patient was of Persian ethnicity. The segregation study confirmed this mutation. Evaluating the homozygous regions in six patients showed the presence of a common homozygosity box in four patients belonging to the Baloch ethnicity, which suggests the possibility of the founder nature of this mutation. This approach is justified by the possibility that unaffected parents who are distantly related, come from an ethnic group with a high endogamy rate, or reside in a geographical isolate could be heterozygotes for the same recessive mutation from a common ancestor.

This mutation is a synonymous mutation, which at first glance looks like a benign mutation, but past population studies have shown that this mutation is associated with BBS disease [16]. Given the high conservation of the last guanine nucleotide and its possible role in splicing, this homozygous substitution in *BBS2*, c.G471A (p.T157T) is considered to have a strong effect on the pre-mRNA splicing process. The HSF analysis detected the disrupted wild-type donor site due to this mutation. Disruption in the splicing process probably leads to intron retention in the mature mRNA, and by creating frame-shift mutation and premature termination codon, it will probably lead to NMD. Thus, the interaction of the BBS2 protein with other proteins in the BBSome complex, a complex of proteins involved in early cilia development, is disrupted.

There is no precise information about the frequency of this mutation in Iranian databases, and the allelic frequency of this mutation is reported as 0.000014 in the GnomAD database [19]. According to the geographical location of patients with this mutation in this study and previous studies, this mutation seemingly is very old in this region. This raises the possibility of a high frequency of this mutation in Iranian society. Thus, population screening of this mutation in the southeast community of Iran can have a good effect on preventing the recurrence of this disease.

In the last decade, copy number variations (CNVs) have been recognized as a major contributor to genetic burden in rare and common disorders. CNV is a phenomenon caused by genomic rearrangement and its length usually exceeds 1 kb [20]. These changes play a significant role in creating the necessary diversity in the population and disease phenotype. In this study, the investigation of CNV in one patient led to the identification of a large deletion in the BBS1 gene that includes exons 14 to 17. This deletion was confirmed using Quantitative real-time PCR in the family members of the patients. Deletion of this region leads to the removal of terminal domains and the creation of a shortened and ineffective protein.

Additionally, due to the heterogeneous nature of the BBS, the distribution of causative genes varies across populations. Even though the highest proportion of genes involved in BBS in different populations belong to *BBS1* and *BBS10* [21], in this study, 50% of patients of Iranian origin carried the *BBS2* mutation, because of the *BBS2* founder mutation phenomenon. This information is completely different from Caucasian populations. These results propose a new approach for diagnosing BBS using molecular genetics in Iran and perhaps other Middle Eastern countries, particularly Pakistan and Afghanistan. Finding the population-specific frequent, or founder mutations could be a vital tool to develop an efficient algorithm for clinical genetic practice that would refine screening protocols before using more advanced technologies like next-generation sequencing.

Conclusions

BBS diseases is the most important member of ciliopathies, which lead to syndromic mental retardation with retinopathy, polydactyly, obesity, reproductive and kidney disorders. So far, a large number of genes involved in ciliogenesis have been identified in connection with BBS; however, a significant part of this disease remains idiopathic. More studies are needed to identify unusual variants in known genes in addition to identifying new mutations associated with this disease. Our data in this study suggest the c.G471A variant is a founder mutation that causes Bardet-Biedl syndrome in In the Baloch ethnicity of the Iranian population. So, the Baloch ethnicity BBS suspected patients may be evaluated for this mutation as the first diagnostic step. Also, according to the identification of CNV in a patient with a negative-WES result, CNV analysis must be performed in these patients.

Abbreviations

BBS: Bardet–Biedl syndrome; WES: Whole exome sequencing; ROH: Runs of homozygosity; CNV: Copy Number Variation; NGS: next-generation sequencing

Declarations

Acknowledgements

We would like to thank our patients and their families for their collaboration in this research and the colleagues who helped us in this study.

Funding

This research was supported by Mashhad University of Medical Sciences and University of Social Welfare and Rehabilitation Sciences.

Competing interests

The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by Masoumeh Heidari Feizabadi, Masoome Alerasool, Atieh Eslahi, Emran Esmaeilzadeh, Mohammad Yahya Vahidi Mehrjardi, Mitra Saket, Zohreh Fatahi, Hamid Reza Khorram Khorshid, Majid Mojarrad. The first draft of the manuscript was written by Shima Farokhi and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Research Ethics Committee of University of Social Welfare and Rehabilitation Science, Tehran, Iran (Date: 2021-12-27/No: IR.USWR.REC.1400.235).

Consent to participate

Informed consent to participate in this research was obtained from all the patient's parents.

Consent to publish

Informed consent for publication of the results was obtained from all the patient's parents.

References

1. Waters AM, Beales PL. Ciliopathies: an expanding disease spectrum. Pediatr Nephrol. 2011;26(7):1039-56.
2. Forsythe E, Beales PL. Bardet-Biedl syndrome. Eur J Hum Genet. 2013;21(1):8-13.
3. 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(4):352-60.
4. M'Hamdi O, Ouertani I, Chaabouni-Bouhamed H. Update on the genetics of bardet-biedl syndrome. Mol Syndromol. 2014;5(2):51-6.
5. Tobin JL, Beales PL. Bardet-Biedl syndrome: beyond the cilium. Pediatr Nephrol. 2007;22(7):926-36.
6. 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(1):41.
7. Grochowsky A, Gunay-Aygun M. Clinical characteristics of individual organ system disease in non-motile ciliopathies. Transl Sci Rare Dis. 2019;4(1-2):1-23.
8. Esposito G, Testa F, Zacchia M, Crispo AA, Di Iorio V, Capolongo G, et al. Genetic characterization of Italian patients with Bardet-Biedl syndrome and correlation to ocular, renal and audio-vestibular

phenotype: identification of eleven novel pathogenic sequence variants. BMC Med Genet. 2017;18(1):10.

9. Komlósi K, Gyenesei A, Bene J. Editorial: Copy Number Variation in Rare Disorders. Front Genet. 2022;13:898059.
10. Lindstrand A, Frangakis S, Carvalho CM, Richardson EB, McFadden KA, Willer JR, et al. Copy-Number Variation Contributes to the Mutational Load of Bardet-Biedl Syndrome. Am J Hum Genet. 2016;99(2):318-36.
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(6):437-46.
12. Bravo-Gil N, Méndez-Vidal C, Romero-Pérez L, González-del Pozo M, Rodríguez-de la Rúa E, Dopazo J, et al. Improving the management of Inherited Retinal Dystrophies by targeted sequencing of a population-specific gene panel. Sci Rep. 2016;6:23910.
13. Weisschuh N, Mayer AK, Strom TM, Kohl S, Glöckle N, Schubach M, et al. Mutation Detection in Patients with Retinal Dystrophies Using Targeted Next Generation Sequencing. PLoS One. 2016;11(1):e0145951.
14. Janssen S, Ramaswami G, Davis EE, Hurd T, Airik R, Kasanuki JM, et al. Mutation analysis in Bardet-Biedl syndrome by DNA pooling and massively parallel resequencing in 105 individuals. Hum Genet. 2011;129(1):79-90.
15. Mary L, Chennen K, Stoetzel C, Antin M, Leuvrey A, Nourisson E, et al. Bardet-Biedl syndrome: Antenatal presentation of forty-five fetuses with biallelic pathogenic variants in known Bardet-Biedl syndrome genes. Clin Genet. 2019;95(3):384-97.
16. Fattahi Z, Rostami P, Najmabadi A, Mohseni M, Kahrizi K, Akbari MR, et al. Mutation profile of BBS genes in Iranian patients with Bardet-Biedl syndrome: genetic characterization and report of nine novel mutations in five BBS genes. J Hum Genet. 2014;59(7):368-75.
17. Sanchez-Navarro I, L RJdS, Blanco-Kelly F, Zurita O, Sanchez-Bolivar N, Villaverde C, et al. Combining targeted panel-based resequencing and copy-number variation analysis for the diagnosis of inherited syndromic retinopathies and associated ciliopathies. Sci Rep. 2018;8(1):5285.
18. Wang F, Wang H, Tuan HF, Nguyen DH, Sun V, Keser V, et al. Next generation sequencing-based molecular diagnosis of retinitis pigmentosa: identification of a novel genotype-phenotype correlation and clinical refinements. Hum Genet. 2014;133(3):331-45.
19. https://grch37.ensembl.org/Homo_sapiens/Variation/Population
20. Bhanuprakash V, Chhotaray S, Pruthviraj DR, Rawat C, Karthikeyan A, Panigrahi M. Copy number variation in livestock: A mini review. Vet World. 2018;11(4):535-41.
21. Suspitsin EN, Imyanitov EN. Bardet-Biedl Syndrome. Mol Syndromol. 2016;7(2):62-71.

Footnotes

1. G-protein-coupled receptors

2. Alstrom Syndrome Protein 1
3. Nephrocystin 4
4. Human Splicing Finder

Figures

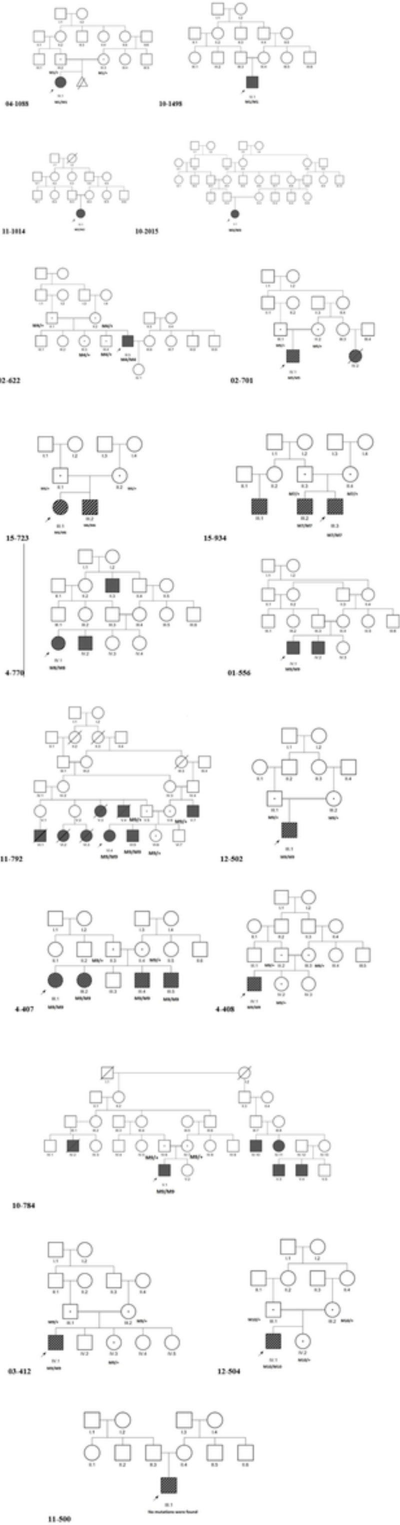


Figure 1

Overview of the pedigrees of the 18 families presented in this study. Probands who undergo WES analysis are indicated with an arrow

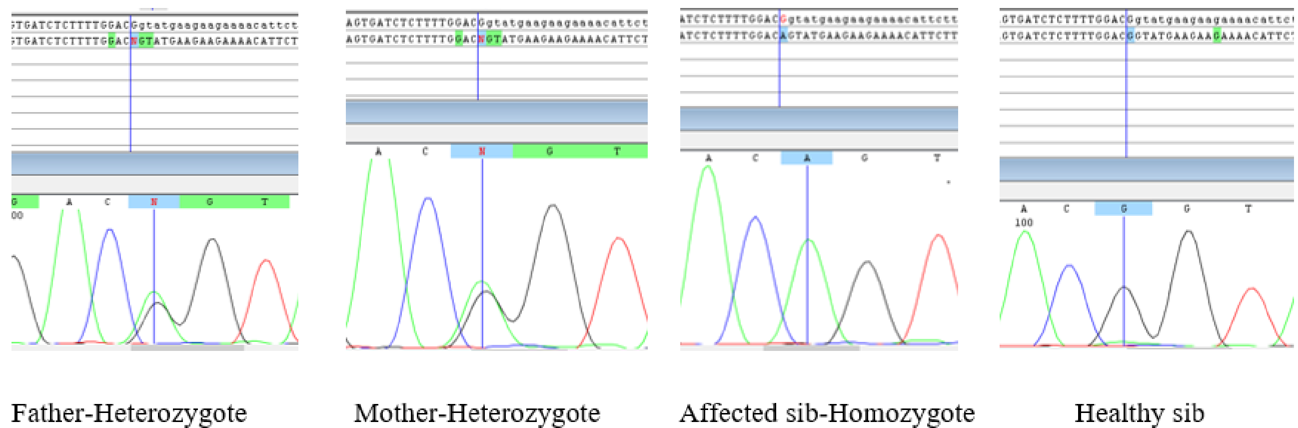


Figure 2

Sanger sequencing result on the genomic level confirmed co-segregation of the splicing variant (c.G471A) in the families of patients

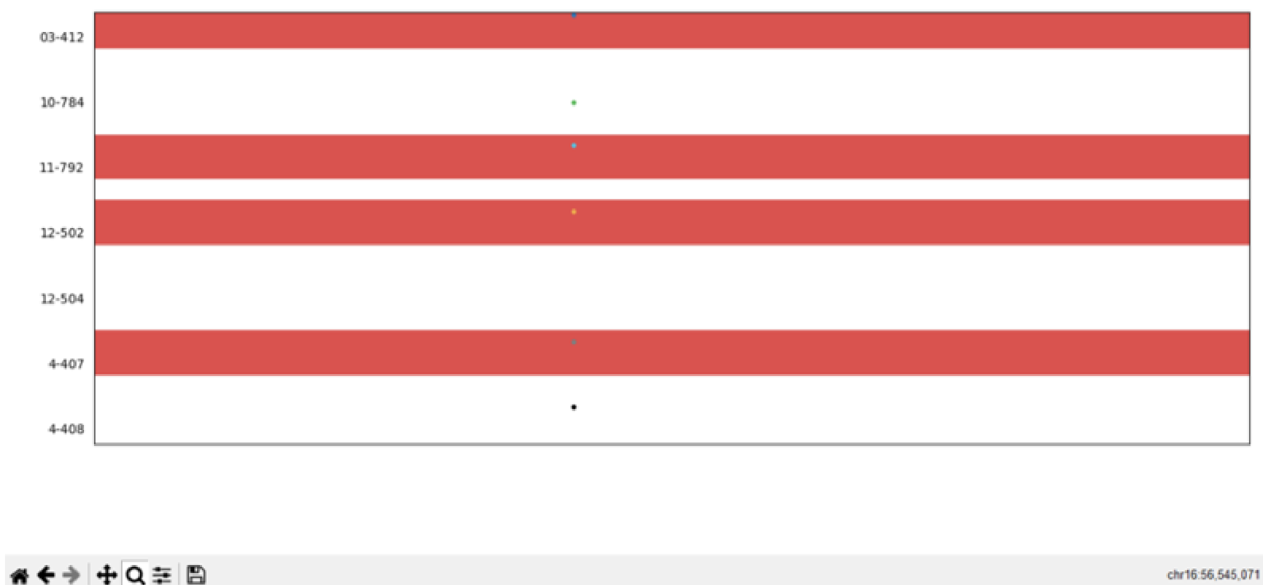
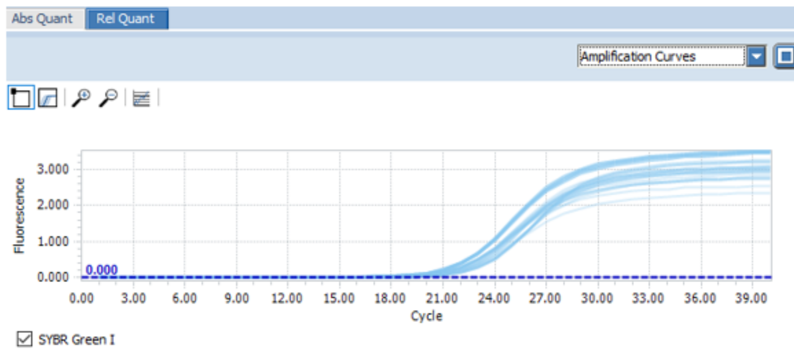


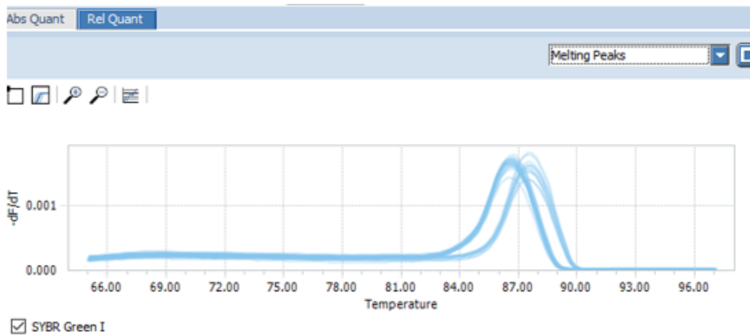
Figure 3

ROH plot to compare homozygous boxes between six patients with c.G471A mutation at position 56545071 and one patient without mutation as negative control (12-504), all patients are of Baloch ethnicity. The homozygous box is observed in four patients

(a)



(b)



(c)

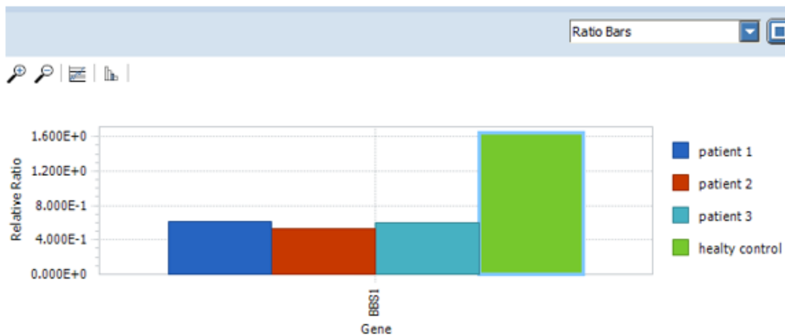


Figure 4

SYBR Green Real-Time PCR results to compare gene dosage changes in heterozygous individuals with exon deletion and healthy control sample. A, amplification curve, Dilution of the DNA sample of the heterozygous family members and the control sample was performed and the Real-Time PCR reactions were performed in triplicates. B, melting curve. C, the ratio bar shows that the gene dosage in a healthy control without deletion is almost twice as much as in heterozygous people, including the father, mother and sister of the patient with deletion of exons 14 to 17