

# Identification of A Novel Compound Heterozygous Mutation in *BBS12* in An Iranian Family with Bardet-Biedl Syndrome Using Targeted Next Generation Sequencing

Emad Nikkhah, Ph.D.<sup>1</sup>, Reza Safaralizadeh, Ph.D.<sup>1\*</sup>, Javad Mohammadiasl, Ph.D.<sup>2</sup>, Maryam Tahmasebi Birgani, Ph.D.<sup>2\*</sup>, Mohammad Ali Hosseinpour Feizi, Ph.D.<sup>1</sup>, Neda Golchin, M.Sc.<sup>3</sup>

1. Department of Animal Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

2. Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

3. Noor Genetics Lab, Ahvaz, Iran

\*Corresponding Addresses: Department of Animal Biology, Faculty of Natural Science, University of Tabriz, Tabriz, Iran

Department of Medical Genetics, School of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Emails: safaralizadeh@tabrizu.ac.ir, tahmasebi-ma@ajums.ac.ir

Received: 8/Jan/2017, Accepted: 21/Mar/2017

## Abstract

Bardet-Biedl syndrome (BBS) is a pleiotropic and multisystemic disorder characterized by rod-cone dystrophy, polydactyly, learning difficulties, renal abnormalities, obesity and hypogonadism. This disorder is genetically heterogeneous. Until now, a total of nineteen genes have been identified for BBS whose mutations explain more than 80% of diagnosed cases. Recently, the development of next generation sequencing (NGS) technology has accelerated mutation screening of target genes, resulting in lower cost and less time consumption. Here, we screened the most common *BBS* genes (*BBS1-BBS13*) using NGS in an Iranian family of a proposita displaying symptoms of BBS. Among the 18 mutations identified in the proposita, one (*BBS12 c.56T>G* and *BBS12 c.1156C>T*) was novel. This compound heterozygosity was confirmed by Sanger sequencing in the proposita and her parents. Although our data were presented as a case report, however, we suggest a new probable genetic mechanism other than the conventional autosomal recessive inheritance of BBS. Additionally, given that in some Iranian provinces, like Khuzestan, consanguineous marriages are common, designing mutational panels for genetic diseases is strongly recommended, especially for those with an autosomal recessive inheritance pattern.

**Keywords:** BBS12, Bardet-Biedl Syndrome, Mutation, Sequence Analysis

Cell Journal (Yakhteh), Vol 20, No 2, Jul-Sep (Summer) 2018, Pages: 284-289

**Citation:** 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(2): 284-289. doi: 10.22074/cellj.2018.5012.

## Introduction

Bardet-Biedl syndrome (BBS, MIM#209900) is a rare genetic condition diagnosed with a wide range of major and minor symptoms including learning difficulties, obesity, rod-cone dystrophy, polydactyly, genital anomalies and renal abnormalities. In addition, other symptoms including speech and developmental delay, diabetes, dental anomalies, congenital heart disease, brachydactyly/syndactyly, ataxia, deafness and anosmia have also been reported (1).

Usually, BBS can be diagnosed by the presence of at least four major features or the combination of three major and at least two minor features (2). The incidence of BBS varies among different populations and is increased in regions with a high level of consanguinity. For instance, in North America and Europe, the prevalence of BBS is estimated around 1/160,000 (3) while this frequency rises to 1/13,500 in Kuwait, most likely due to the high level of consanguinity and founder effects (4-6).

The syndrome shows an autosomal recessive inheritance pattern, however, oligogenic patterns have also been

observed (7, 8). Until now, a total of nineteen genes have been identified for BBS which play specific roles in cilium biogenesis and function (8-12). These genes are *BBS1*, *BBS2*, *BBS3* (*ARL6*), *BBS4*, *BBS5*, *BBS6* (*MKKS*), *BBS7*, *BBS8* (*TTC8*), *BBS9* (*PTHB1*), *BBS10*, *BBS11* (*TRIM32*), *BBS12*, *BBS13* (*MKS1*), *BBS14* (*CEP290*), *BBS15* (*C2orf86*), *BBS16* (*SDCCAG8*), *BBS17* (*LZTFL1*), *BBS18* (*BBIP1*) and *BBS19* (*IFT27*) (8, 12). Mutations in this gene panel explain more than 80% of identified cases (7, 13-15). Furthermore, the distribution of BBS-causative mutations varies among different geographical regions; *BBS1* and *BBS10* are the most frequently mutated genes in European and North American populations, whereas *BBS2*, *BBS4*, *BBS5* and *BBS12* are common in Middle East and North Africa (7, 16-18).

Recently, robust genomic analysis including homozygosity mapping and high-throughput sequencing holds the promise of identifying novel causative mutations in such a heterogeneous condition (1). Targeted next generation sequencing (NGS) is one of the favorite strategies for medical geneticists to screen known

genes across the whole genome affordably (19). The present study was aimed to screen *BBS* genes in an Iranian female with symptoms of BBS. Targeted NGS identified a novel compound heterozygous mutation in *BBS12*.

## Case report

A 13-year-old Iranian female was admitted to the Noor Medical Genetic Clinic for truncal obesity and blindness. She was the first offspring of a consanguineous marriage. Her parents were healthy as was her younger brother. Initial evaluation confirmed polydactyly (specifically hexadactyly) of all four limbs, congenital heart disease, blindness and obesity. We also found hypothyroidism and dental anomalies such as crowding of the teeth, however urinalysis, complete blood count and renal function tests were found to be normal. She had a rather normal facies and hearing impairment was not identified. She had experienced normal maturation at puberty and showed secondary sexual characteristics such as pubic hair and regular menses. At one year of age, she had undergone surgery for correcting the postaxial polydactyly of the four limbs (Fig.1).

She had learned to walk and speak at the age of two but had difficulty in finding words. Learning disabilities was noted at the age of eight, when she had also started to complain of night blindness. Two years later, at the age of ten, she had become blind. There was a family history of death due to renal dysfunction in her maternal uncle, who had displayed similar phenotypic characteristics. According to the clinical background and consanguineous nature of

the relationship of her parents, BBS was diagnosed by the physician and therefore genetic screening was undertaken.

## Patient recruitment

This study was Ethically approved by Tabriz University, Tabriz, Iran. All the participants signed an informed consent prior to joining the project. We studied all the available members who were informative for tracking the origin of mutation(s) in the pedigree, namely the probanda, father, mother, brother and the uncle's nuclear family (i.e. uncle's wife and daughter).

## DNA extraction

Blood sample (5 ml) was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes from each participant and genomic DNA was extracted from peripheral blood samples using the salting out method (20). The quality of extracted DNA was checked by 1% agarose gel (KBC, Iran) electrophoresis followed by ethidium bromide staining (Merck, Germany). The optical density of extracted DNA was also examined at 260 nm and 280 nm using the Nanodrop Analyzer (ND-1000) spectrophotometer (Thermo Fisher Scientific, USA) to evaluate the purity of each sample and detect possible contamination.

## Targeted next generation sequencing

DNA extracted from the probanda was submitted to BGI (BGI-clinical laboratories, China) for whole genome amplification using a custom designed chip to capture the genes *BBS1-BBS13* to identify potentially pathogenic variants in these genes.



**Fig.1:** The patient had undergone surgery for correcting the postaxial polydactyly at the age of one. The above photograph was taken with the consent of the parents of patient at the Noor Genetics Laboratory.

## In silico mutation analysis

Criteria used to assign a mutation as novel and pathogenic were previously described by Chen et al. (21). Accordingly, the genomic variants were considered as novel if not previously reported in dbSNP or the literature. Polyphen (<http://genetics.bwh.harvard.edu/pph2/>), PROVEAN (<http://provean.jcvi.org/index.php>) and SIFT (<http://sift.bii.a-star.edu.sg/>) were used to predict if any variant is pathogenic by potentially affecting the protein structure.

Additionally, to evaluate if the novel mutation had occurred in a conserved domain of a target gene, the protein sequence of that gene were obtained for different species from the NCBI protein database (<http://www.ncbi.nlm.nih.gov/protein/>) and aligned using ClustalW2 (<http://www.ebi.ac.uk/Tools/clustalw2>). The novel variants were eventually traced in the family of the proposita to uncover their parental origin.

## Polymerase chain reaction and Sanger sequencing

To confirm the mutations detected based on targeted NGS, Sanger sequencing of the regions containing the mutations was undertaken. First, genomic DNA was amplified with polymerase chain reaction (PCR) using specific primers flanking the mutation regions. The primer sequences and their related amplicon are illustrated (Table 1). PCR reactions were carried out in a total volume of 25 µl containing 1X reaction buffer (Merck, Germany), 0.5 µg of genomic DNA template, 1.5 U of Taq DNA polymerase (KBC, Iran), 2 pmol/L of each primer (Macrogen, Korea) and 0.25 mM of each dNTP (KBC, Iran). PCR cycling conditions were 5 minutes denaturation at 95°C for initial denaturation, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 60°C for 30 seconds and extension

at 72°C for 30 seconds, followed by a final extension at 72°C for 2 minutes. Additionally, a negative control (no template DNA sample) was included in all PCR reactions. PCR products were then analyzed on a 1.5% agarose gel dyed with ethidium bromide (2%) and product bands were visualized under ultraviolet light (UV Tec, USA). Finally, using the same primers, Sanger sequencing was undertaken by the means of Big Dye Terminators (Applied Bio systems 3130 Genetic Analyzer, Applied Bio systems, Foster City, CA, USA).

## A novel pathogenic variant in *BBS12* were detected in targeted NGS of the proposita

Targeted NGS was conducted on 13 common *BBS* genes of the proposita. A total of twenty two genetic variants were detected, of which one was novel (Table 2). The novel variant *BBS12* c.56T>G (p.Leu19Arg) and *BBS12* c.1156C>T (p.Arg386Trp) occurred in exon 2 of *BBS12* and the proposita was heterozygote for both variants. The frequency of these two variants in single nucleotide polymorphism database (dbSNP), HapMap, 1000 Genomes and BGI's database is very low (<1%) (Table 2).

In silico mutation analysis using SIFT, PolyPhen and PROVEAN predicted that the mutation *BBS12* c.56T>G (p.Leu19Arg) is damaging and localized in a conserved domain of *BBS12*. However the mutation *BBS12* c.1156C>T (p.Arg386Trp) is predicted to be either damaging or benign and also not confined in a conserved domain of *BBS12* (Fig.2). No damaging mutations were found in other *BBS* Genes. In specific, defects in *BBS12* cause BBS type 12. There is ample evidence showing the causal relationship of *BBS12* variants with BBS, however, in the Iranian population, only two studies have reported this relationship (Table 3).

**Table 1:** List of the primer sets and related amplicons

| Mutation                 | Primer         | Sequence (5'-3')         | PCR product (bp) |
|--------------------------|----------------|--------------------------|------------------|
| <i>BBS12</i> c.56 T>G    | bbs12ex1-1F584 | CCTCTGTTGGGTGGAGTGTT     | 584              |
|                          | bbs12ex1-1R584 | ACAAAAGTTTAAAGCCTTCTGACA |                  |
| <i>BBS12</i> c. 1156 C>T | bbs12ex1-3F500 | TGAGTCATGGAGATCACAGCA    | 500              |
|                          | bbs12ex1-3R500 | CACACTGCCATTCACTGAGC     |                  |

PCR; Polymerase chain reaction.



**Fig.2:** Sequence alignment of *BBS12* of several species showing the conserved position of Leu19 and the non-conserved Arg386.

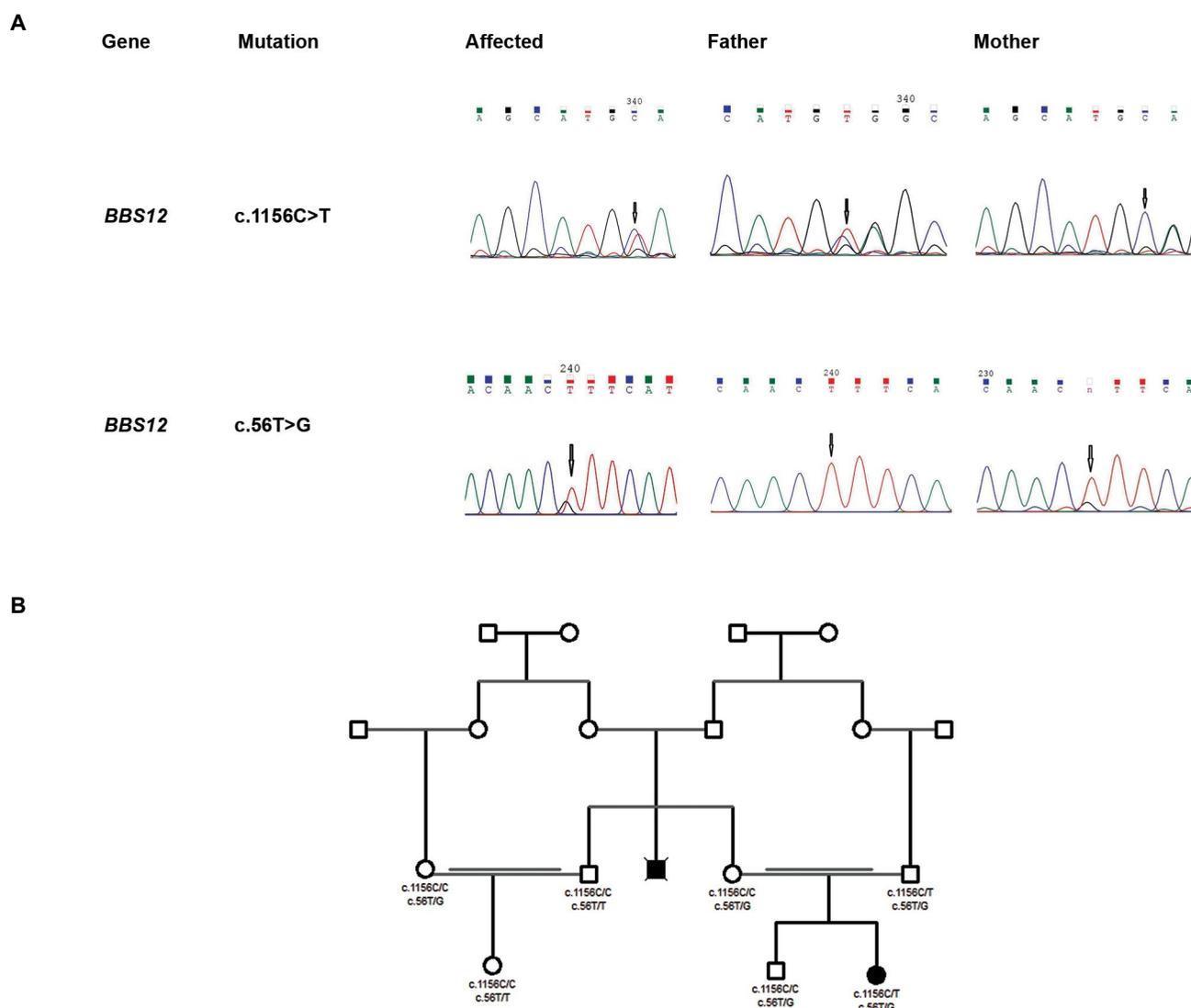
**Table 2:** Variants identified in all targeted *BBS* genes in the probanda

| Gene         | Mutation name         | SubRegion   | Nucleotide change | RS ID       | Het /Hom   | Mutation type | Freq_HapMap | Freq_dbSNP | Clinical significance  |       |
|--------------|-----------------------|-------------|-------------------|-------------|------------|---------------|-------------|------------|------------------------|-------|
| <i>BBS4</i>  | c.77-6G>A             | IN2         | c.77-6G>A         | rs8033604   | Hom        | Splice        | 1           | 0.908      | Benign                 |       |
|              | p.Phe302Phe           | EX12/CDS12  | c.906T>C          | rs12914333  | Hom        | Synonymous    | 1           | 0.94       | Benign                 |       |
|              | p.Ile354Thr           | EX13/CDS13  | c.1061T>C         | rs2277598   | Hom        | Missense      | 0.051       | 0.203      | Likely benign          |       |
| <i>BBS6</i>  | p.Pro39Pro            | EX3/CDS1    | c.117C>T          | rs16991547  | Het        | Synonymous    | 0.299       | 0.323      | Likely benign          |       |
|              | p.Ile178Ile           | EX3/CDS1    | c.534C>T          | rs17852625  | Het        | Synonymous    | 0           | 0.284      | Other                  |       |
|              | p.Arg517Cys           | EX6/CDS4    | c.1549C>T         | rs1547      | Het        | Missense      | 0.307       | 0.287      | Likely benign          |       |
|              | p.Gly532Val           | EX6/CDS4    | c.1595G>T         | rs1545      | Het        | Missense      | 0.307       | 0.286      | Likely benign          |       |
| <i>BBS10</i> | p.Pro539Leu           | EX2/CDS2    | c.1616C>T         | rs35676114  | Het        | Missense      | 0           | 0.068      | Likely benign          |       |
| <i>BBS11</i> | p.Val418Val           | EX2/CDS1    | c.1254G>A         | rs1661300   | Het        | Synonymous    | 0.228       | 0.19       | Other                  |       |
| <i>BBS12</i> | p.Leu19Arg            | EX2/CDS1    | c.56T>G           | Novel       | Het        | Missense      | 0           | 0          | -                      |       |
|              | p.Arg386Trp           | EX2/CDS1    | c.1156C>T         | rs202225266 | Het        | Missense      | 0           | 0          | uncertain significance |       |
|              | p.Arg386Gln           | EX2/CDS1    | c.1157G>A         | rs309370    | Hom        | Missense      | 0.382       | 0.229      | Benign                 |       |
|              | p.Val460Val           | EX2/CDS1    | c.1380G>C         | rs13135766  | Het        | Synonymous    | 0           | 0.198      | Likely benign          |       |
|              | p.Gly466Gly           | EX2/CDS1    | c.1398C>T         | rs2292493   | Het        | Synonymous    | 0.46        | 0.399      | Benign                 |       |
|              | p.Asp467Asn           | EX2/CDS1    | c.1399G>A         | rs13135778  | Het        | Missense      | 0.007       | 0.194      | Likely benign          |       |
|              | p.Cys470Cys           | EX2/CDS1    | c.1410C>T         | rs13135445  | Het        | Synonymous    | 0           | 0.244      | Likely benign          |       |
|              | p.Gln624Gln           | EX2/CDS1    | c.1872A>G         | rs13102440  | Het        | Synonymous    | 0           | 0.193      | Likely benign          |       |
|              | <i>INPP5E (JBTS1)</i> | p.Pro324Pro | EX3/CDS3          | c.972A>G    | rs10870199 | Het           | Synonymous  | 0.277      | 0.21                   | Other |
|              |                       | p.Thr416Thr | EX5/CDS5          | c.1248T>C   | rs10781542 | Het           | Synonymous  | 0.321      | 0.471                  | Other |
| p.Gly428Gly  |                       | EX6/CDS6    | c.1284T>C         | rs10870194  | Het        | Synonymous    | 0.313       | 0.47       | Other                  |       |
| p.His507His  |                       | EX7/CDS7    | c.1521C>T         | rs10870188  | Het        | Synonymous    | 0           | 0.215      | Other                  |       |
| p.Gly598Gly  |                       | EX9/CDS9    | c.1794G>T         | rs33982662  | Het        | Synonymous    | 0           | 0.3        | Other                  |       |

dbSNP; Single nucleotide polymorphism database.

**Table 3:** *BBS12* variation identified in different populations

| Nucleotide change | Amino acid change | Type of variation | Ethnic origin | References |
|-------------------|-------------------|-------------------|---------------|------------|
| c.56T>G           | p.L19R            | Missense          | Iranian       | This study |
| c.1156C>T         | p.R386W           | Missense          | Iranian       | This study |
| c.1156_1157 CG>TA | p.R386X           | Nonsense          | Iranian       | (22)       |
| c.1507G>A         | p.V503M           | Missense          | Egyptian      | (23)       |
| c.1560G>A         | p.W520X           | Nonsense          | Tunisian      | (21)       |
| c.1589T>C         | p.L530P           | Missense          | Pakistani     | (24)       |
| c.1619G>T         | p.G540D           | Missense          | Gypsy         | (25)       |
| c.1620 G>A        | p.G540D           | Missense          | Caucasian     | (26)       |
| c.1993_1996del    | p.V665Lfs*14      | Deletion          | Arabs         | (27)       |
| c.2019del         | p.W673Cfs*7       | Deletion          | Iranian       | (22)       |
| c.2023C>T         | p.R675X           | Nonsense          | Caucasian     | (21)       |
| c.2103C> A        | p.S701X           | Nonsense          | Pakistani     | (18)       |
| c.3232C>T         | p.P108L           | Missense          | Caucasian     | (26)       |



**Fig.3:** Sequence analysis and pedigree of the Bardet-Biedl syndrome case. **A.** Sequence analysis of c.1156C>T and c.56T>G in *BBS12* of the proposita and her parents. The proposita carries both mutations as a compound heterozygote and **B.** Pedigree of the Bardet-Biedl syndrome case: proposita has received c.1156C>T from her father and c.56T>G from her mother.

### Sanger sequencing revealed that the proposita carries the novel variants as a compound heterozygote

Sanger sequencing was carried out on the proposita and her family to validate the NGS-based variants and their parental origin. We found that the affected girl was compound heterozygote for the two variants; the mother and the father harbored *BBS12* c.56T>G and *BBS12* c.1156C>T respectively. The variant status in the maternal uncle's nuclear family members is shown (Fig.3A). The *BBS12* c.56T/G variant originates from a maternal ancestor (Fig.3B).

### Discussion

This case report provided data of a genetic screening of BBS in an Iranian proposita suffering from this syndrome. Due to the heterogeneous nature of BBS, targeted NGS was applied to screen any causal mutations in thirteen *BBS* (1-13) genes. We identified a novel *BBS12* mutations as compound heterozygote c.56T>G (p.Leu19Arg)

and c.1156C>T (p.Arg386Trp), this mutation was not previously reported in SNP database.

The *BBS12* gene, located on 4q27, is one of the key genes involved in pathogenicity of BBS. The gene structurally only contains two exons (25). The protein encoded by *BBS12* is not only part of a complex involved in cilia movement, but it is also involved in adipocyte differentiation. Three proteins BBS6, BBS10 and BBS12 are key members of the chaperonin complex. This complex contributes to cilia movement and therefore its defect reduces the mobility of the cilia and result in BBS symptoms including retinopathy, polydactyly, mental retardation and obesity (12).

Using whole exome sequencing, the mutation profile of *BBS* genes in 14 Iranian families with Bardet-Biedl syndrome was reported by Fattahi et al. (22). They found five novel mutations of which most (28.6% of patients) occurred in *BBS2* with others occurring in *BBS4*, *BBS7* and *BBS12*. This finding was in contrast to that reported

in European and North American populations where *BBS1* and *BBS10* were the most frequently mutated genes accounting for 23% and 20% of BBS patients respectively. It is important to mention that *BBS12* c.1156C>T sequence variant was also observed in the study by Fattahi et al. (22) but in a more complex form of *BBS12* c.1156\_1157CG>TA, resulting in a nonsense mutation. In another study on 23 Iranian family members with BBS children, BBS was linked to markers at 3p13-p12 where the *BBS3* gene is located (28).

## Conclusion

We should stress that previous studies on Iranian BBS patients including ours have limited sample sizes which may be due to the rare prevalence of the disease in population, however, all have been informative on the Iranian population. Additionally, given that some Iranian provinces like Khuzestan have a higher rate of consanguineous marriages, designing population-specific mutational panels for genetic diseases especially those with an autosomal recessive inheritance pattern are strongly recommended. Finally, allelic and locus heterogeneity of diseases such as BBS further emphasizes the benefits of NGS technology to genetically confirm the clinical diagnosis.

## Acknowledgements

This project was financially supported by grants from Tabriz University, Tabriz, Iran and Ahvaz Jundishapur University of Medical Sciences, Ahwaz, Iran as well. We acknowledge our colleagues at the Noor Medical Genetic Clinic for their generous assistance in undertaking genetic counseling, PCR and Sanger sequencing. The authors declare that they have no conflict of interest.

## Author's Contributions

E.N., R.S., J.M., M.A.H.F.; Contributed to all experimental work, data analysis and interpretation of data. M.T.B.; Contributed extensively in interpretation of the data and the conclusion. N.G.; Contributed to experimental work (blood sampling and primer design). All authors read and approved the final manuscript.

## References

- M'hamdi O, Ouertani I, Chaabouni-Bouhamed H. Update on the genetics of Bardet-Biedl syndrome. *Mol Syndromol*. 2014; 5(2): 51-56.
- Beales P, 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): 437-446.
- Klein D, Ammann F. The syndrome of Laurence-Moon-Bardet-Biedl and allied diseases in Switzerland: clinical, genetic and epidemiological studies. *J Neurol Sci*. 1969; 9(3): 479-513.
- Farag TI, Teebi AS. High incidence of Bardet Biedl syndrome among the Bedouin. *Clin Genet*. 1989; 36(6): 463-464.
- Hjortshøj TD, Grønsvov K, Brøndum-Nielsen K, Rosenberg T. A novel founder *BBS1* mutation explains a unique high prevalence of Bardet-Biedl syndrome in the Faroe Islands. *Br J Ophthalmol*. 2009; 93(3): 409-413.
- 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): 352-360.
- Forsythe E, Beales PL. Bardet-Biedl syndrome. *Eur J Hum Genet*. 2012; 21(1): 8-13.
- 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): 368-375.
- Ansley SJ, Badano JL, Blacque OE, Hill J, Hoskins BE, Leitch CC, et al. Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature*. 2003; 425(6958): 628-633.
- Blacque OE, Reardon MJ, Li C, McCarthy J, Mahjoub MR, Ansley SJ, et al. Loss of *C. elegans* BBS-7 and BBS-8 protein function results in cilia defects and compromised intraflagellar transport. *Genes Dev*. 2004; 18(13): 1630-1642.
- Jin H, White SR, Shida T, Schulz S, Aguiar M, Gygi SP, et al. The conserved Bardet-Biedl syndrome proteins assemble a coat that traffics membrane proteins to cilia. *Cell*. 2010; 141(7): 1208-1219.
- Novas R, Cardenas-Rodriguez M, Irigoien F, Badano JL. Bardet-Biedl syndrome: is it only cilia dysfunction? *FEBS Lett*. 2015; 589(22): 3479-3491.
- Marion V, Stutzmann F, Gérard M, De Melo C, Schaefer E, Claussmann A, et al. Exome sequencing identifies mutations in *LZTFL1*, a BBSome and smoothed trafficking regulator, in a family with Bardet-Biedl syndrome with situs inversus and insertional polydactyly. *J Med Genet*. 2012; 49(5): 317-321.
- Scheidecker S, Etard C, Pierce NW, Geoffroy V, Schaefer E, Muller J, et al. Exome sequencing of Bardet-Biedl syndrome patient identifies a null mutation in the BBSome subunit BBIP1 (*BBS18*). *J Med Genet*. 2014; 51(2): 132-136.
- Sapp JC, Nishimura D, Johnston JJ, Stone EM, Héon E, Sheffield VC, et al. Recurrence risks for Bardet-Biedl syndrome: Implications of locus heterogeneity. *Genet Med*. 2010; 12(10): 623-627.
- 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: 644-653.
- Khan S, Ullah I, Touseef M, Basit S, Khan MN, Ahmad W. Novel homozygous mutations in the genes *ARL6* and *BBS10* underlying Bardet-Biedl syndrome. *Gene*. 2013; 515(1): 84-88.
- Pawlik B, Mir A, Iqbal H, Li Y, Nürnberg G, Becker C, et al. A novel familial *BBS12* mutation associated with a mild phenotype: implications for clinical and molecular diagnostic strategies. *Mol Syndromol*. 2010; 1(1): 27-34.
- Metzker ML. Sequencing technologies-the next generation. *Nature Rev Genet*. 2010; 11(1): 31-46.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988; 16(3): 1215.
- 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(8): 5317-5324.
- 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): 368-375.
- 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.
- Harville HM, Held S, Diaz-Font A, Davis EE, Diplas BH, Lewis RA, et al. Identification of 11 novel mutations in eight BBS genes by high-resolution homozygosity mapping. *J Med Genet*. 2010; 47(4): 262-267.
- Stoetzel C, Muller J, Laurier V, Davis EE, Zaghoul NA, Vicaire S, et al. Identification of a novel BBS gene (*BBS12*) highlights the major role of a vertebrate-specific branch of chaperonin-related proteins in Bardet-Biedl syndrome. *Am J Hum Genet*. 2007; 80(1): 1-11.
- Pereiro I, Valverde D, Piñeiro-Gallego T, Baiget M, Borrego S, Ayuso C, et al. New mutations in BBS genes in small consanguineous families with Bardet-Biedl syndrome: detection of candidate regions by homozygosity mapping. *Mol Vis*. 2010; 16: 137-143.
- Ghadami M, Tomita HA, Najafi MT, Damavandi E, Farahvash MS, Yamada K, et al. Bardet-Biedl syndrome type 3 in an Iranian family: Clinical study and confirmation of disease localization. *Am J Med Genet*. 2000; 94(5): 433-437.
- Abu-Safieh L, Al-Anazi S, Al-Abdi L, Hashem M, Alkuraya H, Alamm M, et al. In search of triallelism in Bardet-Biedl syndrome. *Eur J Hum Genet*. 2012; 20(4): 420-427.