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Identification of 11 Novel Mutations in 8 *BBS* Genes by High-Resolution Homozygosity Mapping

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Abstract

Bardet-Biedl syndrome (BBS) is primarily an autosomal recessive disorder characterized by the five cardinal features retinitis pigmentosa, postaxial polydactyly, mental retardation, obesity and hypogenitalism. In addition, renal cysts and other anomalies of the kidney and urinary tract can be present. To date, mutations in 12 *BBS* genes as well as in *MKS1* and *CEP290* have been identified as causing BBS. The vast genetic heterogeneity of BBS renders molecular genetic diagnosis difficult in terms of both the time and cost required to screen all 204 coding exons. Here, we report the use of genome-wide homozygosity mapping as a tool to identify homozygous segments at known *BBS* loci in BBS individuals from inbred and outbred background. In a worldwide cohort of 45 families, we identified, via direct exon sequencing, causative homozygous mutations in 20

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SUPPLEMENTAL DATA One supplementary figure and one supplementary table are available with this paper online at <http://jmg.bmj.com>.

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WEB RESOURCES The URLs for data presented herein are as follows: <http://genome.ucsc.edu>; <http://www.biobase-international.com>.

families. Eleven of these mutations were novel, thereby increasing the number of known *BBS* mutations by 5% (11/218). Thus, in the presence of extreme genetic locus heterogeneity, homozygosity mapping provides a valuable approach to the molecular genetic diagnosis of BBS and will facilitate the discovery of novel pathogenic mutations.

Keywords

Molecular Genetics

INTRODUCTION

Bardet-Biedl syndrome (BBS, [OMIM #209900]) is a clinically heterogeneous primarily autosomal recessive disorder characterized by structural and functional defects in numerous organs. The five cardinal features are retinitis pigmentosa, postaxial polydactyly, mental retardation, obesity and hypogenitalism. In addition, renal cysts and other anomalies of the kidney and urinary tract can be present^{1–4}. Secondary features include developmental delay, ataxia, diabetes mellitus, congenital heart defects, hearing loss, and anosmia^{4–7}. Diagnosis of BBS is often achieved only once vision begins to deteriorate. Night blindness from rod-cone dystrophy usually manifests around eight years of age and loss of peripheral vision develops by the age of 15 years⁴. Clinical manifestations such as cystic kidneys, type II diabetes, hypertension, and hypercholesterolemia are frequent causes of morbidity and mortality in individuals suffering from BBS⁴.

In addition to the broad clinical heterogeneity of BBS, early linkage studies have also revealed a high level of gene locus heterogeneity. Thus far, a total of 12 genes have been identified that, when mutated, cause BBS (*BBS1*, *BBS2*, *BBS3* [*ARL6*], *BBS4*, *BBS5*, *BBS6* [*MKKS*], *BBS7*, *BBS8* [*TTC8*], *BBS9*, *BBS10*, *BBS11* [*TRIM32*], and *BBS12*)^{7–17}. In addition, mutations in *MKS1* and *CEP290* have been implicated in BBS¹⁸. The proteins BBS1, 2, 4, 5, 7, 8, 9 and 10 are part of a protein complex, termed the “BBSome” and localize to primary cilia, and the basal body of primary cilia or the peri-centriolar region. This indicates a role of ciliogenesis and intraflagellar transport (IFT) in BBS pathogenesis^{7–13,19}. BBS6, 10, and 12 are likely type II chaperonins, which may facilitate in the folding of proteins essential for ciliogenesis or IFT^{12,15,17}. BBS9 encodes a parathyroid hormone-responsive protein¹⁴. Furthermore, *BBS11* encodes an E3 ubiquitin ligase, suggesting a role for basal body protein dysregulation in the pathogenesis of BBS¹⁶.

Given the observed pleiotropy associated with BBS, resolving a correlation between phenotype and genotype poses a significant challenge. Moreover, the vast clinical and genetic heterogeneity of this syndrome makes molecular genetic diagnosis difficult with regards to the time, labor, and cost required to screen all 204 coding exons. Finally, only ~40% of known pathogenic alleles among BBS patient cohorts of Northern European descent are contributed by two common mutations (M390R in *BBS1* and C91LfsX5 in *BBS10*), with the remainder composed of rare variants. In non-European families, in which the prevalence of BBS is typically higher, the contribution of M390R is modest, exacerbating the problem of molecular diagnosis (reviewed in²⁰). Here, we report the use of prescreening by genome-wide homozygosity mapping, in a multiethnic cohort of 45 families from both inbred and outbred backgrounds, as a tool to identify homozygous segments at known *BBS*-loci. Using this strategy, we were able to identify, by subsequent exon sequencing, causative mutations of eight different *BBS* genes in 20 families. As eleven of these mutations are novel, we increase the number of known *BBS* mutations by 5% (11/218).

PATIENTS AND METHODS

Patients

We performed a total genome search in a worldwide cohort of 45 families with 68 individuals clinically diagnosed with Bardet-Biedl syndrome. The diagnoses were ascertained on the basis of previously established criteria⁴. For all kindreds, blood samples, and pedigrees were ascertained following informed consent. The study was done in accordance with ethics approval from the Internal Review Boards of the University of Michigan, the Ethics Committee of the UCL Institute of Child Health and the Internal Review Board of the Johns Hopkins University.

Homozygosity Mapping

For all affected individuals, genome-wide scans for homozygosity were performed and analyzed as described previously²¹. We used single nucleotide polymorphism (SNP) arrays (GeneChip) from Affymetrix™, Inc. All individuals were genotyped at 250 K resolution (Human Mapping 250 K *StyI* Array). Samples were processed, hybridized, and scanned using the manufacturer's standard methods at the University of Michigan Core Facility (www.michiganmicroarray.com). Using the program ALLEGRO²², non-parametric likelihood ratio Z-scores (ZLR scores) were calculated with the following parameters: a disease allele frequency of 0.001, a hypothetical standard pedigree structure assuming first cousin marriage for parents of affected individuals using marker allele frequencies for Northern Europeans from the Affymetrix data set. For single affected individuals a non-existent sibling was included to enable non-parametric ALLEGRO runs. ZLR scores were plotted over genetic distance across the entire human genome using GNUMPLOT (<http://www.gnumplot.info/>). In this way, maxima of ZLR scores, "ZLR peaks", are expected to reflect segments of homozygosity by descent, which may harbor the homozygous mutation of the recessive disease gene²¹. Using ALOHOMORA²³, ZLR scores were calculated using one marker every 100,000 nucleotides of human genomic sequence, i.e. for minor allele frequencies of >0.2, >0.3, and >0.4. We have reported previously²¹ that ZLR peaks, which exceed the value of 2.0 under two out of the three conditions (minor allele frequencies of >0.2, >0.3, and >0.4), did in fact contain the known homozygous disease-causing mutation in one of these ZLR peaks in 93% of the cases and exhibited continuous segments of homozygosity upon haplotype inspection. We refer to peaks that fulfill these criteria as "consistent ZLR peaks" ("cZLR" peaks).

Mutation analysis

Individuals exhibiting a cZLR peak at a known *BBS* locus or showing homozygosity >500 kb at a known *BBS* locus upon inspection of their haplotype data underwent mutation analysis of the coding region of the respective *BBS* gene. However, no mutations were found in homozygous segments shorter than 2.1 Mb, the previously described detection limit for cZLR peaks (Hildebrandt et al. *PLoS Genet* 5:e1000353, 2009). Following the previously established protocol exon PCR and direct sequencing was performed using exon flanking primers (Supplementary Table 2)²⁴. For sequence analysis we used SEQUENCHER 3.8 (Gene Codes, Ann Arbor, Michigan); the reference sequences used are shown in Supplementary Table 1. All novel mutations were shown to be absent from at >90 ethnically matched healthy control individuals by direct sequencing.

RESULTS

We performed genome wide homozygosity mapping in an ethnically diverse cohort of 45 families with BBS (Table 1 and Supplementary Figure 1). 27 families were known to be consanguineous (group c; Table 1) and 18 were from outbred populations with no evidence

for consanguinity (group o; Table 1). Total genome homozygosity mapping yielded segments of likely homozygosity by descent “cZLR peaks”²¹ in 32 of the 45 families. Of these 32 families total genome homozygosity mapping data are depicted in Supplementary Figure 1 for the 20 families, in whom we detected the causative homozygous *BBS* mutations. The number of cZLR peaks of homozygosity is compared for consanguineous (group c) and outbred (group o) families in Table 1 and was as follows: In the 27 families with known consanguinity (group c) all exhibited cZLR peaks of homozygosity and the median number of cZLR peaks of homozygosity was 13 (range 1 – 36), whereas in the 18 families from outbred populations and no known consanguinity (group o) only 5 (27%) exhibited cZLR peaks with a median number of cZLR peaks of homozygosity was 2 (range 1 – 4) (Table 1). This is consistent with the previous finding that individuals with homozygous mutations in recessive disease genes exhibit four or more cZLR peaks of homozygosity, if they are from inbred populations, whereas they exhibit four or less cZLR peaks of homozygosity if they are from outbred populations²¹. The 12 families that exhibited cZLR peaks but in whom we did not detect the causative mutations were not homozygous at any known *BBS* locus. These families are candidates for the identification of novel *BBS*-causing genes within those homozygous candidate regions.

For each family, homozygous segments that harbored a known *BBS* gene (*BBS1–BBS12*) (arrow heads in Supplementary Figure 1) were examined further using direct exon sequencing of all coding exons and splice sites of that gene. Using this strategy we detected both disease-causing alleles in 20 families. Eighteen of these families were knowingly consanguineous, two were not consanguineous. There were 17 homozygous mutations in *BBS 1, 2, 4, 5, 7, 8, 10* and *12* in these 20 families (Table 2 and Figure 1). Three mutations occurred twice in two different families each; eleven of the 17 homozygous mutations were novel (Table 2 and Figure 1). These eleven novel mutations resulted in various predicted changes on the protein level (Table 2 and Figure 1): i) three presumed null mutations (p.M1K in *BBS5*, p.R238fsX296 in *BBS7*, and p.W41X in *BBS8*); ii) five missense mutations (p.I66M in *BBS7*, p.W320R in *BBS7*, p.E596K in *BBS7*, p.Y177C in *BBS10*, and p.L530P in *BBS12*) and; iii) three splice site mutations (IVS1–1 g>a and IVS2+1g>a in *BBS1* and IVS3–3 c>g in *BBS4*). All 17 mutations were absent from >90 ethnically matched healthy control individuals. We thereby increased the number of known *BBS* mutations by 5% (11/218) using only homozygosity mapping information as a hierarchical tool for screening (<http://www.biobase-international.com>).

The novel homozygous missense *BBS12* mutation (c.1589T>C, p.L530P) was found in two consanguineous Pakistani individuals (A2003-II1 and A2015-II1) (Table 2 and Figure 1). Haplotype analysis confirmed that this mutation represents a likely Pakistani founder effect, as it occurs on a shared 9-Mb haplotype that extends across *rs7690104* to *rs2049092* (data not shown). Two additional individuals from Pakistan, A2002-II1 and A2027-II1, harbored a novel homozygous missense mutation of the *BBS5* start codon (p.M1K) (Table 2 and Figure 1). This mutation is likely to represent a null mutation, resulting either in failure of protein translation or in the translation of an illegitimate transcript. The parents of both individuals were known to be related. Since the alteration is embedded in a 0.89 Mb homozygous haplotype between markers *rs10199676* and *rs2355351* (data not shown), this mutation also likely represents a Pakistani founder effect (Supplementary Figure 1).

Discussion

Disorders of striking genetic heterogeneity, such as *BBS*, represent a major diagnostic challenge as mutations at more than 12 loci have been shown to be necessary and sufficient for pathogenesis, rendering medical resequencing of >204 known exons laborious and expensive. Other viable alternatives, such as specific interrogation of known mutations by

primer extension is practical, but can only capture less than 50% of the mutational load^{25–28} (our unpublished data). In the present study, we have demonstrated that whole genome homozygosity mapping followed by direct sequencing is an effective alternative means of identifying causative mutations in BBS. We have shown recently that homozygosity mapping can also be applied to individuals who reside within “outbred” populations but are “homozygous by descent” from a remote ancestor at the locus of the disease-causing gene²¹. We here successfully apply this approach to patients from both, inbred and outbred populations, confirming that there can be broad utility of homozygosity mapping in the absence of ancestry information²¹. Specifically, in families A2875 and A2876 who derive from outbred populations (Table 2), we were able to detect the disease causing mutations (Supplementary Figure 1).

Alternatively, the “homozygosity peaks” could be generated by hemizyosity, i.e. a large heterozygous deletion. In a recessive disease, a second mutant allele would be expected to be present in the gene affected by the disease-relevant heterozygous deletion giving rise to a compound heterozygous state. However, especially in individuals with known consanguinity of the parents, compound heterozygosity is unlikely, because consanguinity poses an *a priori* risk for the affected individual for homozygous mutations but not for compound heterozygous mutations. In molecular diagnostics the presence of a large heterozygous deletion would have the same practical consequence, i.e. to perform exon sequencing to detect the second recessive mutation.

Notably, none of the families studied had a sufficient number of affected individuals to allow mapping of a disease locus by classic linkage analysis, further emphasizing the fact that homozygosity mapping can solve an otherwise experimentally intractable problem, especially as the cost for total genome SNP analysis has considerably dropped recently. In the families who do not yield cZLR peaks, which were 13 families in this study, mutation analysis for compound heterozygous mutations will still have to be performed for all exons of all known BBS genes.

In conclusion, we increased the number of BBS-associated mutations by 5% (11/218) employing homozygosity mapping. Our study will likely catalyze the identification of additional *BBS* loci, since 12 families exhibited homozygous regions outside a known *BBS* locus or were negative upon mutation analysis (data not shown). Alternatively, these families may have compound heterozygous mutations in known *BBS* genes, because there is a possibility of false positive “homozygosity peaks” at a rate of less than 35% in individuals from “outbred” background (Hildebrandt et al. *PloS Genet* 5:e1000353, 2009). This rate was been determined by examining parents of children with recessive mutations for “homozygosity peaks”. However, as parents of children with mutations homozygous by descent have themselves a somewhat increased likelihood that their own parents are related, this rate of 35% may be somewhat of an overestimate.”

Ultimately, comprehensive analysis of the entire BBS-associated gene spectrum will be necessary, because of the presence of modifier alleles (even in consanguineous families) that can modify the penetrance and expressivity of BBS endophenotypes^{18,29,30}. Nonetheless, the rapid identification of the primary genetic lesion will have a clear and immediate benefit with regard to patient management and reproductive choices and is likely to prove useful in the study of other disorders of marked genetic heterogeneity such as retinitis pigmentosa, sensorineural deafness and others.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

1. Laurence J, Moon R. Four cases of retinitis pigmentosa occurring in the same family accompanied by general imperfection of development. *Ophthalmol Rev* 1866;2:32–41.
2. Bardet G. On congenital obesity syndrome with polydactyly and retinitis pigmentosa (a contribution to the study of clinical forms of hypophyseal obesity). 1920. *Obes Res* 1995;3(4):387–99. [PubMed: 8521156]
3. Biedl A. A pair of siblings with adiposo-genital dystrophy. 1922. *Obes Res* 1995;3(4):404. [PubMed: 8521158]
4. Beales PL, Elcioglu N, Woolf AS, et al. New criteria for improved diagnosis of Bardet-Biedl syndrome: results of a population survey. *J Med Genet* 1999;36(6):437–46. [PubMed: 10874630]
5. Moore SJ, Green JS, Fan Y, 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;132(4):352–60. [PubMed: 15637713]
6. Lorda-Sanchez I, Ayuso C, Ibanez A. Situs inversus and hirschsprung disease: two uncommon manifestations in Bardet-Biedl syndrome. *Am J Med Genet* 2000;90(1):80–1. [PubMed: 10602122]
7. Kulaga HM, Leitch CC, Eichers ER, et al. Loss of BBS proteins causes anosmia in humans and defects in olfactory cilia structure and function in the mouse. *Nat Genet* 2004;36(9):994–8. [PubMed: 15322545]
8. Nishimura DY, Fath M, Mullins RF, et al. Bbs2-null mice have neurosensory deficits, a defect in social dominance, and retinopathy associated with mislocalization of rhodopsin. *Proc Natl Acad Sci U S A* 2004;101(47):16588–93. [PubMed: 15539463]
9. Fan Y, Esmail MA, Ansley SJ, et al. Mutations in a member of the Ras superfamily of small GTP-binding proteins causes Bardet-Biedl syndrome. *Nat Genet* 2004;36(9):989–93. [PubMed: 15314642]
10. Kim JC, Badano JL, Sibold S, et al. The Bardet-Biedl protein BBS4 targets cargo to the pericentriolar region and is required for microtubule anchoring and cell cycle progression. *Nat Genet* 2004;36(5):462–70. [PubMed: 15107855]
11. Li JB, Gerdes JM, Haycraft CJ, et al. Comparative genomics identifies a flagellar and basal body proteome that includes the BBS5 human disease gene. *Cell* 2004;117(4):541–52. [PubMed: 15137946]
12. Kim JC, Ou YY, Badano JL, et al. MKKS/BBS6, a divergent chaperonin-like protein linked to the obesity disorder Bardet-Biedl syndrome, is a novel centrosomal component required for cytokinesis. *J Cell Sci* 2005;118(Pt 5):1007–20. [PubMed: 15731008]
13. Blacque OE, Reardon MJ, Li C, 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):1630–42. [PubMed: 15231740]
14. Nishimura DY, Swiderski RE, Searby CC, et al. Comparative genomics and gene expression analysis identifies BBS9, a new Bardet-Biedl syndrome gene. *Am J Hum Genet* 2005;77(6):1021–33. [PubMed: 16380913]
15. Stoetzel C, Laurier V, Davis EE, et al. BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. *Nat Genet* 2006;38(5):521–4. [PubMed: 16582908]

16. Chiang AP, Beck JS, Yen HJ, et al. Homozygosity mapping with SNP arrays identifies TRIM32, an E3 ubiquitin ligase, as a Bardet-Biedl syndrome gene (BBS11). *Proc Natl Acad Sci U S A* 2006;103(16):6287–92. [PubMed: 16606853]
17. Stoetzel C, Muller J, Laurier V, 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. [PubMed: 17160889]
18. Leitch CC, Zaghloul NA, Davis EE, et al. Hypomorphic mutations in syndromic encephalocele genes are associated with Bardet-Biedl syndrome. *Nat Genet* 2008;40(4):443–8. [PubMed: 18327255]
19. Ansley SJ, Badano JL, Blacque OE, et al. Basal body dysfunction is a likely cause of pleiotropic Bardet-Biedl syndrome. *Nature* 2003;425(6958):628–33. [PubMed: 14520415]
20. Zaghloul NA, Katsanis N. Mechanistic insights into Bardet-Biedl syndrome, a model ciliopathy. *J Clin Invest* 2009;119(3):428–37. [PubMed: 19252258]
21. Hildebrandt F, Heeringa SF, Ruschendorf F, et al. A systematic approach to mapping recessive disease genes in individuals from outbred populations. *PLoS Genet* 2009;5(1):e1000353. [PubMed: 19165332]
22. Gudbjartsson DF, Jonasson K, Frigge ML, et al. Allegro, a new computer program for multipoint linkage analysis. *Nat Genet* 2000;25(1):12–3. [PubMed: 10802644]
23. Ruschendorf F, Nurnberg P. ALOHOMORA: a tool for linkage analysis using 10K SNP array data. *Bioinformatics* 2005;21(9):2123–5. [PubMed: 15647291]
24. Otto EA, Helou J, Allen SJ, et al. Mutation analysis in nephronophthisis using a combined approach of homozygosity mapping, CEL I endonuclease cleavage, and direct sequencing. *Hum Mutat* 2008;29(3):418–26. [PubMed: 18076122]
25. Katsanis N, Eichers ER, Ansley SJ, et al. BBS4 is a minor contributor to Bardet-Biedl syndrome and may also participate in triallelic inheritance. *Am J Hum Genet* 2002;71(1):22–9. [PubMed: 12016587]
26. Myktyyn K, Nishimura DY, Searby CC, et al. Evaluation of complex inheritance involving the most common Bardet-Biedl syndrome locus (BBS1). *Am J Hum Genet* 2003;72(2):429–37. [PubMed: 12524598]
27. Fauser S, Munz M, Besch D. Further support for digenic inheritance in Bardet-Biedl syndrome. *J Med Genet* 2003;40(8):e104. [PubMed: 12920096]
28. Karmous-Benailly H, Martinovic J, Gubler MC, et al. Antenatal presentation of Bardet-Biedl syndrome may mimic Meckel syndrome. *Am J Hum Genet* 2005;76(3):493–504. [PubMed: 15666242]
29. Karmous-Benailly H, Martinovic J, Gubler MC, et al. Antenatal presentation of Bardet-Biedl syndrome may mimic Meckel syndrome. *Am J Hum Genet* 2005;76(3):493–504. [PubMed: 15666242]
30. Khanna H, Davis EE, Murga-Zamalloa CA, et al. A common allele in RPGRIP1L is a modifier of retinal degeneration in ciliopathies. *Nat Genet*. 2009

Figure 1. Six known and 11 novel mutations found in *BBS1*, 2, 4, 5, 7, 8, 10 and 12
Families are listed in the same order as in Table 2. Seventeen different homozygous missense, nonsense, or splice site mutations in *BBS1*, 2, 4, 5, 7, 8, 10 or 12 were found in 20 families with BBS. Three mutations (M390R, M1K, and L530P) occur in 2 different families each. Family numbers, (mutated gene), altered nucleotides, and amino acid changes are given above sequence traces. Wild type control sequences are shown below mutated sequences. The codon triplets in each chromatogram are underlined to indicate reading frame. All mutations were absent from >90 ethnically matched healthy controls.

Table 1

Number of cZLR peaks of homozygosity in 45 families with BBS

Group	# of families in group	# of families with cZLR peaks out of 45 families total (% of group)	# of cZLR peaks per family (median; range)
Known consanguinity (c)	27	27 (100%)	(13; 1 – 36)
No consanguinity (o)	18	5 (27%)	(2; 1 – 4)
Total	45	32/45 (71%)	

Table 2

correlation in 20 BBS families with 17 different homozygous disease-causing mutations of **BBS1, 2, 4, 5, 7, 8, 10 and 12**. Eleven (shown in bold) and 6 have been published previously.

Ethnic Origin (Consanguineous) ^a	Gene	Nucleotide Alteration(s) ^b	Alteration(s) in Coding Sequence ^c	Mutation Reference	Phenotype							
					Primary Signs ^d							Secondary Signs ^e
					Retinitis pigmentosa	Polydactyly	Obesity	Hypogonadism	Renal Anomalies			
Turkey (Y)	<i>BBS1</i>	IVS1-1g>a	Splice site	present study	N	Y	Y	Y	Y	Y	Y	
Saudi Arabia (Y)	<i>BBS1</i>	IVS2+1g>a	Splice site	present study	Y	Y	Y	ND	ND	MR,SP,DD,SS	MR,SP,DD,SS	
					Y	Y	Y	ND	Y	MR,DP,DD,SS	MR,DP,DD,SS	
N. Europe (N)	<i>BBS1</i>	1169T>G	M390R	Myktyyn, et al. 2003	Y	N	N	Y	N	DD,SZ	DD,SZ	
					Y	Y	Y	Y	Y	MR,SP,DD,S	MR,SP,DD,S	
N. Europe (Y)	<i>BBS1</i>	1169T>G	M390R	Myktyyn, et al. 2003	Y	Y	Y	N	Y	MR,SP,DD,BC,HT,OM	ND	
					ND	Y	Y	N	ND	ND	ND	
Turkey (Y)	<i>BBS2</i>	68G>C	R23P	Karmous-Banaïly, et al.	Y	Y	Y	N	Y	Y	Y	
Pakistan (Y)	<i>BBS2</i>	1237C>T	R413X	Fausser, et al 2003	Y	Y	Y	N	N	Y	Y	
UAE (Y)	<i>BBS4</i>	IVS3-2a>g	Splice site	Katsanis et al. 2002	Y	Y	Y	Y	N	Y	Y	
Turkey (Y)	<i>BBS4</i>	IVS3-3c>g	Splice site	present study	Y	Y	Y	Y	Y	Y	Y	
Pakistan (Y)	<i>BBS5</i>	2T>A	M1K	present study	Y	Y	Y	Y	N	Y	Y	
Pakistan (Y)	<i>BBS5</i>	2T>A	M1K	present study	Y	Y	N	Y	N	Y	Y	
N. Europe (N)	<i>BBS7</i>	188T>G	I66M	present study	Y	Y	Y	Y	Y	MR,DD,HT,SF	MR,DD,HT,SF	
					Y	Y	Y	ND	ND	MR,HT,SF	MR,HT,SF	
Turkey (Y)	<i>BBS7</i>	688T>C	W320R	present study	N	Y	Y	Y	Y	Y	Y	
Turkey (Y)	<i>BBS7</i>	711_714delGAGA	R238fsX296	present study	Y	Y	Y	Y	N	Y	Y	
Israel Arab (Y)	<i>BBS7</i>	1786G>A	E596K	present study	Y	Y	Y	ND	N	ND	ND	

Ethnic Origin (Consanguineous) ^a	Gene	Nucleotide Alteration(s) ^b	Alteration(s) in Coding Sequence ^c	Mutation Reference	Phenotype						
					Primary Signs ^d						
					Retinitis pigmentosa	Polydactyly	Obesity	Hypogonadism	Renal Anomalies	Secondary Signs ^e	
					Y	Y	Y	ND	ND	MR, SP, DD, SF	
Turkey (Y)	<i>BBS8</i>	122G>A	W41X	present study	Y	Y	Y	Y	N	Y	
Pakistan (Y)	<i>BBS8</i>	IVS10+2_4delTGC	Splice site	Ansley, et al. 2003	Y	Y	Y	Y	ND	MR, SP, DD, BC, HT	
Turkey (Y)	<i>BBS10</i>	530A>G	Y177C	present study	Y	Y	Y	Y	N	Y	
Turkey (Y)	<i>BBS12</i>	1483_1484delGA	E495fsX498	Stoetzel, et al 2007	Y	Y	Y	N	N	Y	
Pakistan (Y)	<i>BBS12</i>	1589T>C	L530P	present study	Y	Y	N	Y	Y	Y	
Pakistan (Y)	<i>BBS12</i>	1589T>C	L530P	present study	Y	Y	N	Y	Y	Y	

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n at least 90 ethnically matched healthy controls.

ated / data not available

cephaly, DD = Developmental delay, DP = Delay in pubescence, HT = hypotonia, MR = mental retardation, OM = chronic otitis media, S = Seizures, SF = short fingers, SI =

e, SZ = schizoid/bipolar disorder.,