Assocation Between Missense Mutations in the BBS2 Gene and Nonsyndromic Retinitis Pigmentosa

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**Bardet-Biedl syndrome (BBS; OMIM 209900)** is an autosomal recessive disease that is heterogeneous both clinically and genetically and is characterized by a wide and variable spectrum of clinical features that can be categorized as primary features (including retinal degeneration, polydactyly, renal and gonadal malformations, obesity, and learning disabilities) or secondary features (including speech disorders, developmental delay, ataxia, diabetes mellitus, dysmorphic features, and variable cognitive impairment). The clinical diagnosis of BBS is based on the presence of at least 4 primary features or a combination of 3 primary and 2 (or more) secondary features. Bardet-Biedl syndrome is considered to be one of the ciliopathies, a group of inherited diseases caused by mutations in genes encoding ciliary proteins. Owing to an overlap of clinical features with other ciliopathies, an accurate diagnosis of BBS is sometimes difficult.

The prevalence of BBS was estimated to be 1 in 77,000 persons in Denmark, 1 in 50,000-65,000 persons in Kuwait, and a relatively high prevalence of 1 in 3700 persons in the Faroe Islands due to a common founder mutation. Bardet-Biedl syndrome is a genetically heterogeneous disorder that can be caused by mutations in at least 15 genes. The inheritance pattern is autosomal recessive, but triallelic inheritance was reported as a possible mechanism in some families. The genes that are known to cause BBS when mutated encode proteins that localize to the basal body of the cilium and are associated with ciliogenesis and intraflagellar transport. Most BBS proteins form the BBSome complex, which is localized to nonmembranous centriolar satellites in the cytoplasm, but also to the membrane of the cilium, and is required for ciliogenesis and functions in ciliary membrane biogenesis.
Although the vast majority of patients with biallelic mutations in one of the BBS genes show most of the clinical features of this syndrome, some patients were recently reported to have nonsyndromic retinitis pigmentosa (RP).9-14 Retinitis pigmentosa (OMIM #268000) is an inherited retinal degeneration that affects photoreceptors and pigment epithelial function and is considered one of the most heterogeneous genetic diseases in humans. Retinitis pigmentosa displays different inheritance patterns: autosomal recessive (50%-60% of cases), autosomal dominant (30%-40% of cases), or X-linked (5%-15% of cases).15 A total of 36 genes have been identified as the cause of nonsyndromic autosomal recessive RP. In the present study, we show that specific missense mutations in the BBS2 gene, known to cause BBS, can cause nonsyndromic RP.

Methods

Participants
Ethical approval for our study was obtained from the relevant local research ethics committees (the Hadassah-Hebrew University Medical Center in Jerusalem, Israel, and St James’s University Hospital in Leeds, England). All participants in our study signed an informed consent that adhered to the tenets of the Declaration of Helsinki before we obtained blood samples for molecular analysis.

Clinical Evaluation
The ocular diagnosis was determined using a full ophthalmologic examination; full-field electroretinography; electro-oculography; color vision testing using the Panel D-15 test; optical coherence tomography; color, infrared, and fundus autofluorescence imaging; and fluorescein angiography, as detailed previously.16

Homozygosity Mapping
Genomic DNA was extracted from peripheral blood samples obtained from the participants using FlexiGene DNA kit (Qiagen). DNA samples of affected patients were genotyped using the Affymetrix 250K single-nucleotide polymorphism (SNP) microarray platform. The array data were analyzed using the HomozygosityMapper online program (http://www.homzygositymapper.org/). A homozygous region was defined as harboring at least 1000 consecutive homozygous SNPs (corresponding, on average, to a 12-megabase [Mb] homozygous region).

Exome Analysis
Whole-exome sequencing evaluations for patients from 2 families (MOL0369 and MOL0970) was performed at the Otogenetics Corporation in Norcross, Georgia, using the Roche NimbleGen version 2 (44.1-megabase pair) paired-end sample preparation kit and Illumina HiSeq2000 at a mean coverage ×31. Sequence reads were aligned to the human genome reference sequence (build hg19), and variants were identified and annotated using the DNAnexus software package. Data set files including the annotated information were analyzed using ANNOVAR (http://wannovar.usc.edu/), which included the database of SNPs (dbSNP; build 135). The following filtering steps were applied: autosomal recessive inheritance; variant type (including missense, nonsense, and splice-site); not within segmental duplications; minor allele frequency less than 0.01; SIFT (Sorting Intolerant From Tolerant) score less than 0.05 when available; and PolyPhen-2 (Polymorphism Phenotyping v2) score greater than 0.85 when available. Patient II:1 from family RD158 was analyzed by targeted exome analysis at GeneDx in Gaithersburg, Maryland, of 113 retinal disease genes (XomeDx slice).

Independently, patient II:2 from the Great Britain (GB) family was evaluated by use of a custom-designed targeted capture reagent consisting of 162 genes in the Retinal Information Network (as of July 2010) (Agilent Technologies).17 Sequence variants were filtered to exclude those that were outside the coding regions, as well as those that were 5 base pairs adjacent to the splice site junction. Variants were also excluded if they were synonymous or if the allele frequency was greater than 0.01 in dbSNP or the 1000 Genomes Project catalog. For missense variants, the following additional criteria were used when available: a SIFT score of less than 0.05 and a PolyPhen-2 score of greater than 0.85.

Mutation Analysis
Exons and exon-intron boundaries of BBS2 (accession number NM_031885.3) were amplified by polymerase chain reaction. Primers were designed using the Primer3 program (http://bioinfo.ut.ee/primer3-0.4.0/). The possible pathogenicity of missense changes was evaluated using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), and SIFT (http://sift.jcvi.org/).

Evolutionary Analysis
The following accession numbers were used: human (NP_114091.3), chimpanzee (XP_5109080.2), horse (XP_001493033.1), elephant (XP_006897174.1), mouse (NP_080392.1), rabbit (XP_002711574.1), dog (XP_535296.3), bat (XP_006908350.1), cow (NP_001033249.1), and zebrafish (NP_690843.1).

Results
Aiming to identify novel causes of nonsyndromic RP, we performed exome analysis on index cases from autosomal recessive RP families. In 4 families, missense mutations were identified in BBS2 (Table 1). Mutations in this gene were previously reported to cause BBS.18 We therefore performed a detailed analysis aiming to examine the involvement of BBS2 in nonsyndromic RP.

Analysis of a Moroccan Jewish Family With Autosomal Recessive RP
For our study, we recruited a nonconsanguineous Moroccan Jewish family (family MOL0369) with 3 siblings affected by nonsyndromic RP (Figure 1). The index case (patient II:1) received a diagnosis of RP at 20 years of age following complaints of impaired night vision and constricted visual fields.
At 32 years of age, her electroretinographic responses were nondetectable (Table 1). At 49 years of age, her visual acuity (VA) was at the level of hand motions, and posterior polar cataract and posterior subcapsular opacity were evident. Fundus findings were compatible with advanced RP, including moderate pallor of the optic disc, widespread atrophy, and pigmentary changes. Optical coherence tomography revealed severe thinning of the outer nuclear layer and the epiretinal membranes. By 53 years of age, her VA decreased to the level of light perception with partial projection in both eyes. Her medical health record includes carcinoma of the cervix, which was diagnosed when she was 47 years of age and treated surgically with no sequela. She has mild hypertension, which is controlled by medication, and mild reflux. The results of a hearing test performed when she was 48 years of age were normal.

Her brother (patient II:2) also had a rather severe course of retinal degeneration (Table 1), in which RP was diagnosed when he was 18 years of age. Cataract surgery was performed when he was 36 years of age. An experimental telescopic lens was implanted that was associated with a reduction in vision and thus was replaced with a regular intraocular lens 13 years later. At 53 years of age, his VA was at the level of hand motions. Fundus findings included pallor of the optic disc, narrowing of retinal blood vessels, and widespread retinal atrophy. His medical history included polycythemia vera and ischemic heart disease, which was the status of his health after coronary artery bypass surgery at the age of 46 years (it should be noted that ischemic heart disease is highly prevalent in this family, even among family members who do not have a retinal disease). At 51 years of age, he developed autoimmune hepatitis, which was treated with immune suppression. A second affected brother (patient II:3) was not clinically examined at our center but, per anamnesis, also manifested a severe course of nonsyndromic retinal degeneration. Notably, all 3 affected siblings did not manifest the characteristic systemic features of BBS (Table 1).

Aiming to identify the genetic cause of disease in family MOL0369, we performed a series of mutation analyses, including APEX (arrayed primer extension)–based screening and Sanger sequencing. Missense mutations in BBS2 were identified in all 3 affected siblings. These included heterozygous mutations (c.98C>A, p.A33D; c.401C>G, p.P134R) and homozygous mutations (c.311A>C, p.D104A; c.1895G>C, p.R632P) in the BBS2 gene. These findings support the hypothesis that BBS2 mutations are a common cause of nonsyndromic RP.
of all known RP mutations (http://www.asperbio.com/asper-ophthalmics) and screening for a set of mutations that we previously identified in patients with RP of the same origin (including mutations in the following genes: \textit{ABCA4}, \textit{AIPL1}, \textit{DHDDS}, \textit{EYS}, \textit{GUCY2D}, \textit{NR2E3}, \textit{RAB28}, \textit{RPE65}, and \textit{USH2A}). Both analyses did not yield a possible RP-associated mutation. Although no consanguinity has been reported in the family, the parents shared the same origin. We therefore assumed an identical-by-decent disease-causing mutation and performed homozygosity mapping on the 3 affected individuals using the Affymetrix 250K platform. Although a few large homozygous regions were detected, none harbored an RP-associated gene, and none of the regions were shared by the 3 affected individuals. A search for shared heterozygous regions revealed 7 large genomic regions, one of which contains the \textit{BBS2} gene. We subsequently performed a whole-exome sequencing analysis of the index case and did not identify any potential disease-causing mutations in genes that were previously associated with nonsyndromic retinal degeneration.

A subsequent analysis of other genes associated mainly with syndromic RP revealed the identification of 2 novel heterozygous missense variants in \textit{BBS2} (c.98C>A leading to p.A33D and c.401C>G leading to p.P134R) within the largest shared heterozygous region (Table 1). A segregation analysis showed that the mutations are in \textit{trans} (ie, compound heterozygous mutations) and perfectly cosegregated with the disease (Figure 1). The results of a search for potential additional sequence variants in other known BBS genes were negative. The results of a screening test for both mutations in a set of 83 North African Jewish families with retinal degeneration were negative. The c.98C>A mutation was absent in 160 control chromosomes, and the c.401C>G mutation was identified in 1 of 160 control chromosomes. Both mutations were predicted by use of online prediction software to be pathogenic (Table 2) and were absent in the Exome Variant Server database, which includes data on approximately 13 000 control chromosomes, and the affected amino acids are highly conserved through evolution (Figure 2).

### BBS2 Mutation in the Ashkenazi Jewish Population

In parallel, for Ashkenazi Jewish families with autosomal recessive RP, we performed whole-exome sequencing analysis and mutation screening, and in 3 families, we identified \textit{BBS2} mutations. Family MOL0970 (Figure 1 and Table 1) includes 2 brothers affected by nonsyndromic RP with early macular involvement. The older sibling (patient II:1) received a diagnosis of retinal degeneration at the age of 15 years. His electroretinographic responses at 29 years of age were nondetectable, A subsequent analysis of other genes associated mainly with syndromic RP revealed the identification of 2 novel heterozygous missense variants in \textit{BBS2} (c.98C>A leading to p.A33D and c.401C>G leading to p.P134R) within the largest shared heterozygous region (Table 1). A segregation analysis showed that the mutations are in \textit{trans} (ie, compound heterozygous mutations) and perfectly cosegregated with the disease (Figure 1). The results of a search for potential additional sequence variants in other known BBS genes were negative. The results of a screening test for both mutations in a set of 83 North African Jewish families with retinal degeneration were negative. The c.98C>A mutation was absent in 160 control chromosomes, and the c.401C>G mutation was identified in 1 of 160 control chromosomes. Both mutations were predicted by use of online prediction software to be pathogenic (Table 2) and were absent in the Exome Variant Server database, which includes data on approximately 13 000 control chromosomes, and the affected amino acids are highly conserved through evolution (Figure 2).

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his VA was 0.1, mild posterior subcapsular cataract was present, and his visual fields were very severely constricted. Fundus findings included pallor of the optic disc, narrowing of the blood vessels, and marked macular involvement manifested by atrophy and pigmentation (including the foveal area). Midperipheral salt-and-pepper changes were accompanied by mild bone spicule-like pigmentation. He was generally healthy and so was not prescribed any medication. His younger brother (patient II:2) received a diagnosis of retinal degeneration at the age of 4 years, following a similar diagnosis for his older brother who was 15 years of age at the time. The younger brother’s electroretinographic responses at 21 years of age were nondetectable, and his visual fields were severely constricted (5°-15°). Cataract surgery was performed on both eyes when he was 18 years of age. His VA at the age of 21 years was 0.6 OD and 0.03 OS. Fundus findings included waxy pallor of the optic disc, narrowing of blood vessels, and atrophy surrounding the fovea. There were salt-and-pepper changes in the midperiphery accompanied by bone spicule-like pigmentation. Optical coherence tomography revealed an epiretinal membrane with thinning of the photoreceptor layer. At 23 years of age, his VA decreased to 0.02 OD owing to the extension of atrophy into the fovea (Table 1).

To identify the cause of disease in family MOL0970, the index case was screened for a set of mutations previously identified in patients with RP of the same origin (DHDDS, EYS, FAM161A, MAK, NR2E3, and RPGR) and was found to be negative. We subsequently performed whole-exome sequencing on the DNA sample of his affected brother (patient II:2) and identified a homozygous BBS2 mutation c.311A>C (p.D104A>G). The patient did not carry any other possible mutation in any known retinal degeneration gene, including all known genes associated with BBS. Both affected siblings did not have any nonocular diseases, nor did they exhibit any of the signs of BBS (Table 1).

The second family (family GB) (Figure 1) was a consanguineous Ashkenazi Jewish family residing in Great Britain, including 2 siblings affected with RP (Figure 1). The index case (patient II:2 [patient 1267 in our records]) first presented in the ophthalmic clinic with poor VA at 5 years of age (Table 1). She had high myopia (−4.5 diopters [D]), and her corrected VA was 0.5. Abnormal retinal pigmentation was noted at 37 years of age, when her VA decreased to 0.25. Her vision deteriorated further, and her VA was 0.1 by 45 years of age with −15 D. She was also reported to have learning difficulties and polydactyly that was removed from 1 hand as a child. Her brother (patient II:1 [patient 2093 in our records]) had relatively mild RP and received a diagnosis at the age of 39 years with a corrected VA of 1.0. Bull’s-eye maculopathy and bone spicule-like pigmentation were noted with increasing light sensitivity. At 50 years of age, his VA decreased to 0.3 and kept deteriorating to counting fingers and 0.01 by the age of 56 years. He did not have any other manifestations of BBS. Aiming to identify the cause of disease in family GB, we performed homozygosity mapping on both affected individuals, followed by targeted exome analysis of 162 genes. The homozygosity analysis revealed 3 large shared homozygous regions on chromosomes 11 (45.6-55.4 Mb, for a total of 9.8 Mb) and 16 (19.0-
null phenotype of patient MOL0565-1 (who is homozygous for the mutations). We also reported to have a carrier frequency of 0.473% and 0.261%, respectively, in a very large sample size of Ashkenazi Jewish controls. The 2 mutations were extremely rare in the Exome sequencing data within the homozygous regions and identified a homozygous BBS2 missense mutation (c.1895G>C; p.R632P-Rs128043021). This mutation was previously reported with an incorrect nomenclature. The third family (family RD158) was a nonconsanguineous Ashkenazi Jewish family, including an isolated case of retinitis pigmentosa (RP). The index case (patient II:1) first presented to the ophthalmic clinic with poor VA at childhood. She had high myopia (−10.0 D), and her corrected VA was 0.1 at age of 34 years) since childhood. Targeted exome sequencing of 113 genes revealed 2 heterozygous BBS2 mutations (p.D104A and p.R632P). The 2 mutations (p.D104A and p.R632P) that we identified in patients of the same origin (Ashkenazi Jewish) are predicted to be pathogenic (Table 2), and the affected amino acids are highly evolutionarily conserved (Figure 2). The 2 mutations were extremely rare in the Exome Variant Server database (1 of 12 995 alleles) and were recently reported to have a carrier frequency of 0.473% and 0.261%, respectively, in a very large sample size of Ashkenazi Jewish controls.

A screening test for both mutations in 37 Ashkenazi Jewish patients with syndromic or nonsyndromic RP revealed 2 cases. The first was an isolated case, patient II:1 from family MOL0714 who was compound heterozygous for both mutations (Table 1 and Figure 1). The second case was patient MOL0714 who is compound heterozygous for both mutations (p.D104A and p.R632P) but the mutation did not cosegregate with the disease in the family because his affected brother did not carry the mutation. A subsequent analysis revealed that both siblings are heterozygous for the p.K42E mutation in DHDDS, which was previously reported to cause autosomal recessive RP. The retinal phenotype in patient MOL0565-1 was therefore due to the DHDDS mutation, with p.D104A-BBS2 as a possible disease modifier in this family. It should be noted that the retinal phenotype of patient MOL0565-1 (who is homozygous for DHDDS-p.K42E and heterozygous for BBS2-p.D104A) was more severe than that of his affected brother who does not carry the BBS2 mutation (with a VA of light perception at 38 years of age compared with 0.63 at age 37 year of age).

Discussion

To date, mutations in 5 BBS genes are known to cause nonsyndromic retinitis pigmentosa: CEP290 mutations can cause nonsyndromic Leber congenital amaurosis, a BBS2 missense mutation was reported to cause nonsyndromic RP in a single family, a nonsense BBS2 mutation was reported to cause late-onset retinal dysfunction with postaxial polydactyly in a single family, a splice-site BBS8 mutation in a retina-specific alternatively spliced exon was reported to cause nonsyndromic RP in a single family, and BBS1 mutations represent a wide spectrum of phenotypes, including nonsyndromic RP. We provide evidence, for the first time to our knowledge, for BBS2 as a sixth BBS gene that can cause nonsyndromic retinal degeneration when mutated.

Mutations in BBS2 were identified in 2001 as a cause of BBS. Since then, specific BBS2 mutations were reported to be involved in triallelic inheritance in 4 BBS families, and the observation that some individuals harboring biallelic BBS2 mutations were asymptomatic suggested that BBS2 may play a modifier role in contributing to complex disease. It is interesting to note that one of the patients reported in this previous study (patient AR171) was a compound heterozygote for p.D104A and p.R632P (misreported as p.R634P) in BBS2 and had exhibited some of the clinical features of BBS, including obesity and polydactyly. We describe 2 patients who share the same BBS2 genotype; however, one of the 2 patients (patient II:1 from family MOL0714) does not exhibit the additional clinical features (besides RP), and the other (patient II:1 from family RD158) is obese. This result further demonstrates the complexity of the BBS2-related phenotypes and the probable involvement of modifier genes. Although the patients that we identified in our study do not show the major clinical features of BBS, the retinal involvement in our 2 patients seems to be as severe as that in patients with BBS due to BBS2 mutations.
Conclusions

The \textit{BBS2} missense mutations that we report here as the cause of nonsyndromic RP do not cluster in a specific region, and a summary of all reported \textit{BBS2} mutations does not provide a clear genotype-phenotype correlation (Figure 3). All nonsyndromic RP cases, however, have missense mutations on both copies of the gene, and it seems that a null mutation on one gene copy with either a missense or null mutation on the counter allele leads to the classic BBS phenotype. Due to the complexity of the BBS phenotype and the variable expression of the disease, we predict that other genetic and nongenetic factors modulate the involvement of other clinical features of this syndrome. A study of a large number of patients with nonsyndromic RP would be required to identify the polymorphic variants in candidate modifier genes for BBS.

\textbf{REFERENCES}