A novel compound heterozygous mutation in \textit{TTC8} identified in a Japanese patient

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\textbf{Abstract}

Bardet–Biedl syndrome (BBS), characterized by rod-cone dystrophy, postaxial polydactyly, central obesity, hypogonadism, renal abnormalities, and mental retardation, is a rare autosomal recessive disorder. To date, 21 causative genes have been reported. Here we describe a Japanese BBS patient with a novel compound heterozygous mutation in \textit{TTC8}. To the best of our knowledge, this is the first description of a BBS patient with a mutation in the \textit{TTC8} gene in Japan.

Bardet–Biedl syndrome (BBS) is a rare autosomal recessive disorder characterized by rod-cone dystrophy, postaxial polydactyly, central obesity, hypogonadism, renal abnormalities, and mental retardation. BBS is often complicated by strabismus/cataracts/astigmatism, diabetes mellitus, Hirschsprung disease, heart disease, and/or liver fibrosis. To date, 21 causative genes have been reported, comprising \textasciitilde80\% of BBS genetic abnormalities\textsuperscript{1,2}. The remaining 20\% of genetic abnormalities among BBS patients are not yet known. In the present study, we performed whole-exome sequencing (WES) of a classical BBS patient.

The patient was diagnosed with BBS at 8 years of age, in accordance with criteria reported previously\textsuperscript{3}. Primary and secondary signs of BBS in this patient are listed in Table 1. When the patient first visited Osaka University Hospital at 17 years of age, his best-corrected visual acuity (BCVA) was 0.07 in the right eye and 0.2 in the left eye. At 28 years of age, his BCVA was 0.01 in the right eye and 0.04 in the left eye; he exhibited bilateral diffuse retinal degeneration, including macular atrophy, attenuated retinal vessels, and optic nerve head pallor with little pigmentary dispersion. His parents were not consanguineous. His mother showed no sign of BBS or rod-cone dystrophy. His father did not have symptoms of BBS.

All experimental procedures were approved by the Ethics Committee at Osaka University (No. 719–2, Osaka, Japan) and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from the patient (at the time of the report, a 28-year-old male) and his 61-year-old mother. Both individuals underwent ophthalmologic examinations: BCVA in decimal units, slit-lamp biomicroscopy, fundoscopy, visual field testing with Goldmann perimetry, optical coherence tomography (SSOCT; DRI OCT1, Topcon Corp., Tokyo, Japan), and fundus autofluorescence (Optos, Optos KK, Tokyo, Japan). Genomic DNA was extracted from blood samples using NucleoSpin Blood XL (Macherey-nagel, Düren, Germany). DNA libraries were constructed using SureSelectXT Human All Exon Kit V6 and SureSelectXT Reagent Kit (Agilent, Santa Clara, CA, USA) and then subjected to 100 bp paired-end sequencing on an Illumina HiSeq2500 Platform (Illumina, San Diego, CA, USA). Sequence reads were aligned to the reference human genome (UCSC hg19) in BWA (http://www.bio-bwa.sourceforge.net/) to align short reads after adaptor sequences were removed by Cutadapt (https://cutadapt.readthedocs.io/en/stable/). SAM tools (Version 0.1.17; http://www.samtools.sourceforge.net/) were used for sequence data conversion, sorting, and indexing. To exclude duplicate reads, Picard (http://picard.sourceforge.net) was used. Variants were determined using GATK (http://www.broadinstitute.org/gatk/). ANNOVAR

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Table 1  Primary and secondary signs of BBS in this patient

| Primary signs                      | Age of onset | Clinical information                                                                 | Intervention                        |
|------------------------------------|--------------|---------------------------------------------------------------------------------------|--------------------------------------|
| Rod-cone dystrophy                 | 8 Years old  | Visual acuities: 0.01 (right), 0.04 (left), (with mild myopia and astigmatism)        | No medication                        |
| Fundus finding: binocular diffuse retinal degeneration |              | Visual field: centipede constriction (binocular)                                      |                                      |
| Optical coherence tomography: binocular diffuse thinning of outer retinal layer (+), macular atrophy (+), macular edema (–), cystic changes (–), ellipsoid zone (–) |              | Fundus autofluorescence: binocular mottled pattern (+), perifoveal ring (–)            |                                      |
| Polydactyly                        | At birth     | Both feet                                                                            | Plastic surgery (19 months old)      |
| Obesity                            | 9 Years old  | Height: 164 cm, Weight: 78.1 kg, Body mass index (BMI): 29 kg/m²                     | No medication                        |
| Hypogonadism                       | 8 Years old  | Testosterone: 300–600 ng/dl                                                        | No medication                        |
| Renal anomalies                    | 1 Week old   | Cystic kidney, Creatinine: 1.79 mg/dl, BUN: 21 mg/dl, eGFR cre: 37.2 mL/min/1.73 m² |                                       |
| Mental retardation                 | No           | -                                                                                    | -                                   |
| Secondary signs                    |              | Hirschsprung disease 3 Months old                                                    | Surgery (28 months old)             |
| Abnormal glucose tolerance         | 9 Years old  | HbA1c: 5.6%, 75 g oral glucose tolerance test: 82 mg/dl at 0 h, 185 mg/dl at 2 h     | No medication                        |
| Exotropia                          | NA           | -                                                                                    | Bilateral lateral rectus muscle recession (14 years old) |
| Hypertension                       | 27 Years old | Blood pressure = 145/83 mm Hg                                                       | Oral medicine (Amlodipine besilate 5 mg per day) |
| Cataract                           | NA           | -                                                                                    | -                                   |
| Heart diseases                     | No           | -                                                                                    | -                                   |
| Liver fibrosis                     | No           | -                                                                                    | -                                   |

(http://www.openbioinformatics.org/annovar/) was used to annotate the resulting genetic variants. Rare variants (minor allele frequency < 0.05) were selected using the Exome Sequencing Project, 1000 Genomes Project, and Human Genetic Variation databases; possible pathogenic variants, such as nonsynonymous, nonsense, and frameshift mutations, were extracted from among the retinal degenerative disease-related genes registered in the Ret.Net™ database.

Ten candidate pathogenic rare variants in genes related to retinal degenerative diseases were detected in this patient. All were heterozygous variants; however, two novel nonsense (NM_001288781.1 [TTCC8_v001]: c.226 C>T, p.Q76X) and frameshift (NM_001288781.1 [TTCC8_v001]: c.309_310insTA, p.T103fs) mutations were located in the TTCC8 gene (also known as BBS8). Both mutations were validated by direct sequencing of PCR products (Applied Biosystems 3730 DNA Analyzer; Thermo Fisher Scientific K.K., Tokyo, Japan). The primer sets used for PCR were as follows: c.226 C>T, 5’-TGG GTTITAGGCAGCTTGGAG-3’ and 5’-ACCATAAGGCA GAACAGAAACCA-3’; c.308_309insAT, 5’-TAGGCCTT GGAACGTCTTTG-3’ and 5’- ACCATAAGGCAAGAC AGAAACCA-3’. This mutation is likely to be pathogenic, because the TTCC8 gene has been reported as a causative gene for BBS8⁴. The nonsense mutation was located in exon 3 of the TTCC8 gene, thus producing a truncated protein without tetratricoptide repeats 11 and 15, which are involved in pilus formation and twitching mobility. The frameshift mutation in exon 5 (c.309_310insTA) generates a premature stop codon in exon 6, which also produces TTCC8 lacking normal tetratricoptide repeats 11 and 15. The premature stop codon is located before the last exon; notably, a mRNA transcribed from a gene with a truncating mutation often undergoes nonsense-mediated mRNA decay before translation⁵. Thus, transcripts with nonsense and frameshift mutations are likely to be rapidly degraded to reduce the translation of the truncated TTC8 protein. Therefore, this compound heterozygous patient would not have a functional TTC8 protein to support the formation of the BBSome, leading to the development of BBS. His mother exhibited the heterozygous nonsense mutation, but no frameshift mutation. Although the genetic and clinical data were not available from his father, this patient’s BBS was determined to result from a compound heterozygous TTCC8 gene mutation.

BBS patients with mutations in the TTCC8 gene comprise only 2.8% of all BSS patients⁶⁷. In Japan, the genetics of four BBS families have been reported: BBS2, BBS5, and BBS7 homozygotes, as well as a BBS10 compound heterozygote⁸⁰. To the best of our knowledge, this is the first BBS patient with a mutation in the TTCC8 gene in Japan. Thus far, 16 families with the TTCC8 genetic abnormality...
| Family  | Ethnic         | Consanguineous | Gene | Nucleotide alteration(s) | Zygosity state | Alteration(s) in coding sequence | Rod-cone dystrophy | Polydactyly | Obesity | Hypogonadism | Renal anomalies | Mental retardation | Secondary signs                                      | Reference |
|---------|----------------|----------------|------|--------------------------|----------------|----------------------------------|---------------------|-------------|---------|-------------|----------------|---------------------|-----------------------------------------------------|-----------|
| Family 1 | Japanese       | No             | TTC8 | 226 C > T & 308_309insAT | comp. het      | Q76X & T103fs                    | Yes                 | Yes         | No      | Yes         | No             | No                  | Hirschsprung disease, abnormal glucose tolerance, exotropia, hypertension | Present study |
| Family 2 | Pakistan       | Yes            | TTC8 | IVS10 + 2_4deTGC         | hom            | Splice site                      | Yes                 | Yes         | Yes     | Yes         | Yes            | NA                  | Speech impediment                                    | Ansley et al. |
| Family 2 | Pakistan       | Yes            | TTC8 | IVS10 + 2_4deTGC         | hom            | Splice site                      | Yes                 | Yes         | Yes     | Yes         | Yes            | NA                  | Speech impediment, developmental delay, brachycephaly | Ansley et al. |
| Family 2 | Pakistan       | Yes            | TTC8 | IVS10 + 2_4deTGC         | hom            | Splice site                      | Yes                 | Yes         | Yes     | Yes         | Yes            | NA                  | Speech impediment                                    | Ansley et al. |
| Family 3 | Saudi Arabian  | NA             | TTC8 | 187–188delEY             | hom            | 6 bp Inframe deletion            | Yes                 | Yes         | Yes     | NA          | NA             | NA                  | Speech impediment                                    | Ansley et al. |
| Family 3 | Saudi Arabian  | NA             | TTC8 | 187–188delEY             | hom            | 6 bp Inframe deletion            | Yes                 | Yes         | Yes     | NA          | NA             | NA                  | Speech impediment                                    | Ansley et al. |
| Family 3 | Saudi Arabian  | NA             | TTC8 | 187–188delEY             | hom            | 6 bp Inframe deletion            | Yes                 | Yes         | Yes     | NA          | NA             | NA                  | Speech impediment                                    | Ansley et al. |
| Family 4 | Saudi Arabian  | NA             | TTC8 | 187–188delEY             | hom            | 6 bp Inframe deletion            | Yes                 | Yes         | Yes     | NA          | NA             | NA                  | Speech impediment, developmental delay, brachycephaly, hemophilia | Ansley et al. |
| Family 4 | Saudi Arabian  | NA             | TTC8 | 187–188delEY             | hom            | 6 bp Inframe deletion            | Yes                 | Yes         | Yes     | NA          | NA             | NA                  | Speech impediment, developmental delay, brachycephaly, hemophilia | Ansley et al. |
| Family 5 | North African  | Yes            | TTC8 | 459 G > A                | hom            | Splice site                      | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 5 | North African  | Yes            | TTC8 | 459 G > A                | hom            | Splice site                      | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 6 | Lebanese       | Yes            | TTC8 | IVS6 + 1_G > A           | hom            | Splice site                      | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 7 | Caucasian      | No             | TTC8 | IVS6 + 1-2delGT          | het            | Splice site                      | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 8 | Tunisian       | NA             | TTC8 | 459 + 1 G > A            | hom            | Pro101LeufsX12                   | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 9 | Tunisian       | NA             | TTC8 | 459 + 1 G > A            | hom            | Pro101LeufsX12                   | Yes                 | Yes         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 10*| Tunisian      | NA             | TTC8 | 355_356insGGTGGA,AGGC_CAGGCA | hom             | Thr124ArgfsX43                   | NA                 | NA         | NA      | NA          | NA             | NA                  | Cognitive impairment                                 | Stoetzel al. |
| Family 11 | Turkish       | Yes            | TTC8 | 122 G > A                | hom            | W41X                             | Yes                 | Yes         | Yes     | Yes         | Yes            | No                  | Fatty liver, gall stones                              | Redin et al. |
| Family 12 | NA            | NA             | TTC8 | M52 + 1 G > A            | hom            | Splice site                      | Yes                 | Yes         | Yes     | Yes         | No             | No                  | Yes but details unknown                             | Janssen et al. |
| Family 13 | Hispanic      | NA             | TTC8 | 485delG & 1000delA       | comp. het      | G162fsX4 & I334fsX1             | Yes                 | Yes         | Yes     | Yes         | Yes            | Yes                  | Asthma, nasal polypeptide                            | Janssen et al. |
| Family 14 | Tunisian       | Yes            | TTC8 | 329 G > A                | hom            | Splice site                      | NA                 | NA         | NA      | NA          | NA             | NA                  | Dental anomalies, hypertension                      | Mhamdi Q, et al. |
| Family 15 | Tunisian       | Yes            | TTC8 | 459 + 1 G > A            | hom            | Splice site                      | Yes                 | Yes         | Yes     | Yes         | Yes            | NA                  | Fatty liver, gall stones                              | Janssen et al. |
have been reported (Table 2)\textsuperscript{4,7,10–15}. Most of these families have homozygous mutations; only our patient and a Hispanic family were compound heterozygotes. Although full clinical information was not available for some cases, most of the cases in these 16 families exhibit classical BBS without obvious differences in phenotypes.

In summary, we identified a novel compound heterozygous mutation in a Japanese BBS patient by WES. Our findings suggest that WES may be a useful tool for genetic diagnosis and characterization of BBS.

**HGV database**

The relevant data from this Data Report are hosted at the Human Genome Variation Database at https://doi.org/10.6084/m9.figshare.hgv.2528; https://doi.org/10.6084/m9.figshare.hgv.2531

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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