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SHORT COMMUNICATION

Identification of a novel mutation confirms the implication of *IFT172* (*BBS20*) in Bardet–Biedl syndrome

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Bardet–Biedl syndrome (BBS; MIM 209900) is a recessive heterogeneous ciliopathy characterized by retinitis pigmentosa (RP), postaxial polydactyly, obesity, hypogonadism, cognitive impairment and kidney dysfunction. So far, 20 BBS genes have been identified, with the last reported ones being found in one or very few families. Whole-exome sequencing was performed in a consanguineous family in which two affected children presented typical BBS features (retinitis pigmentosa, postaxial polydactyly, obesity, hypogonadism and cognitive impairment) without any mutation identified in known BBS genes at the time of the study. We identified a homozygous splice-site mutation (NM_015662.2: c.4428+3A>G) in both affected siblings in the last reported BBS gene, namely, *Intraflagellar Transport 172 Homolog (IFT172)*. Familial mutation segregation was consistent with autosomal recessive inheritance. *IFT172* mutations were initially reported in Jeune and Mainzer–Saldino syndromes. Recently, mutations have also been found in isolated RP and Bardet–Biedl-like ciliopathy. This is the second report of *IFT172* mutations in BBS patients validating *IFT172* as the twentieth BBS gene (*BBS20*). Moreover, another *IFT* gene, *IFT27*, was already associated with BBS, confirming the implication of *IFT* genes in the pathogenesis of BBS.
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INTRODUCTION

Bardet–Biedl syndrome (BBS; MIM 209900) is an emblematic ciliopathy characterized by the association of retinitis pigmentosa (RP), postaxial polydactyly, obesity, hypogonadism, kidney dysfunction and cognitive impairment.[1]

Most of the BBS genes code for proteins of the BBSome, a complex involved in trafficking to and from cilia, or implicated in its assembly.[2] However, intraflagellar transport (IFT) has been recently implicated in the pathogenesis of BBS as mutations in two *IFT* genes, *IFT27* and *IFT172*, have been identified in two BBS families.[3,4]

IFT is a bidirectional (anterograde and retrograde) transport process essential for the assembly and the maintenance of the cilia. Most of mutations in *IFT* genes, such as *IFT172*, have been initially reported in skeletal ciliopathies, such as the Mainzer–Saldino syndrome (MIM 266920), the Sensenbrenner syndrome (MIM 218330) and the Jeune asphyxiating thoracic dystrophy (MIM 208500).[5]

Here, whole-exome sequencing led us to identify a novel homozygous mutation in *IFT172* in a consanguineous family with a BBS phenotype.

MATERIALS AND METHODS

Subjects

The patients are the third and the fifth children of a related couple of Melanesian origin (Figure 1).

Patient IV.3

The patient presented with early developmental delay as he walked after the age of 2. He had no polydactyly but a syndactyly between the second and the third toes. The diagnosis of RP was suspected at 43 months because of hemeralopia and confirmed at 8 years on ophthalmologic examination. He presented early as overweight and, at last medical examination, at 18 years, he weighed 116.5 kg (>+3SD) for 163 cm (−2SD). Moreover, he had learning difficulties that implied the need for special education. Asthma was also diagnosed.

X-rays of the skeleton showed no anomaly of the thorax, no polydactyly or brachydactyly. Auditory evoked potential and audiogram found no deafness. Cerebral MRI and electroencephalogram were normal. Renal ultrasound showed no renal or hepatic anomalies. Biological follow-up found normal renal and hepatic functions and no diabetes.

Patient IV.5

At birth, postaxial polydactyly of the right hand and bilateral preaxial polydactyly of the feet were noticed. As for his brother, he presented with

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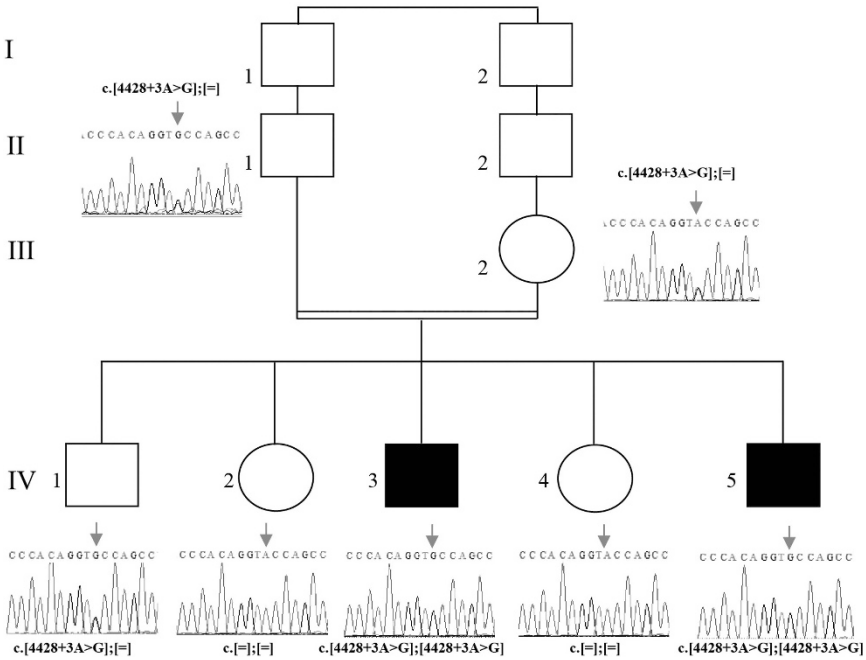

Figure 1 Mutation segregation analysis. Electropherogram of a part of exon 40 of *IFT172* encompassing the identified mutation (c.[4428+3A>G];[4428+3A>G], p.[(Tyr1439_Asn1477del)];[(Tyr1439_Asn1477del)]) in the affected patients, their unaffected parents, brother and sisters. The familial segregation showed that the mutated was found in the homozygous state in the affected patient, in the heterozygous state in the parents and was either in the heterozygous state or absent in the unaffected sibs. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

delayed development and obesity. Hypogonadism with micropenis and bilateral cryptorchidism was diagnosed at 2 years. The diagnosis of RP was established at 3 years. At last examination, at 13 years, the patient weighed 111 kg (>+3SD) for 157 cm (+0.5SD), and presented with orthopedic and respiratory complications.

X-rays of the skeleton confirmed the postaxial polydactyly of the hand and the preaxial polydactyly of the feet with duplication of the metatarsus and the phalanges without other abnormality. Brain imaging, abdominal ultrasound, and biological renal, hepatic and pancreatic functions were normal.

Whole-exome sequencing

Genomic DNA (2 μg) was sheared to obtain a mean fragment size of 150 bp using the Covaris E210 (KBioscience, Herts, UK) followed by library preparation using the Agilent SureSelect All Exon XT2 kit (Agilent Technologies, Santa Clara, CA). Sequencing was performed on an Illumina HiSeq 2500 (Illumina, San Diego, CA, USA) to generate 100-bp paired-end reads following the manufacturer's protocols.

Bioinformatic analysis

Exome data processing, variant calling and variant annotation were performed using VaRank (Strasbourg, France)[6] as previously described.[7] We excluded variants (i) present in dbSNP138 and annotated as non-pathogenic (using the 'ClinicalSignificance' field) validated by at least two methods (using the 'Validation Status' field) and (ii) variants with an allele frequency of more than 1% in the dbSNP database, the Exome Variant Server (http://evs.gs.washington.edu/EVS/), the 1000Genomes (http://www.1000genomes.org/), the ExAC browser database or our internal exome database. Variant effect on the nearest splice site was predicted using MaxEntScan (Cambridge, MA, USA),[8] NNSplice (Berkeley, CA, USA)[9] and Splice Site Finder (Montpellier, France) (based on Shapiro and Senapathy[10]). We focused on compound heterozygous and homozygous variants consistent with a recessive transmission.

Sequencing

Bidirectional sequencing of the purified PCR products was performed by the GATC Sequencing Facilities (Konstanz, Germany). Primers are summarized in Supplementary Table 1.

RNA extraction, cDNA synthesis

Reverse transcription of 300 ng total RNA obtained from blood using the PAXgene Blood RNA Kit (PreAnalytiX GmbH, Hombrechtikon, Switzerland) was performed using the BioRad iScript cDNA Synthesis Kit (BioRad, Hercules, CA, USA). Primers used are summarized in Supplementary Table 1.

RESULTS

Whole-exome sequencing was performed for both affected brothers (IV.3 and IV.5) and a healthy sister (IV.4). From 58 307 to 58 733 genetic variants (SNV+Indel) were identified per proband. Bioinformatic analyses narrowed down the number of variants to 29 homozygous and 12 compound heterozygous in 38 genes (Supplementary Table 2). We focused on a homozygous variation (NM_015662.2: c.4428+3A>G) in intron 40 of *IFT172*. Sanger sequencing confirmed the mutation and familial segregation analysis was consistent with disease transmission (Figure 1). This mutation is absent from dbSNP, 1000Genomes, EVS, ExAC browser database and our internal database. According to the prediction program Alamut (Interactive Biosoftware, Rouen, France), the c.4428+3A>G variant affects splicing with an abolition of the donor splice site (Supplementary Figure 1). We analyzed *IFT172* mRNA from the affected patients and we showed that the mutation led to a variable splicing with an in-frame deletion of 39 amino acids (p.Tyr1439_Asn1477del), corresponding to exon 40, in the major alternative splicing isoform of IFT172 (Figure 2). We were not able to perform protein quantification, as we did not have sufficient quantities

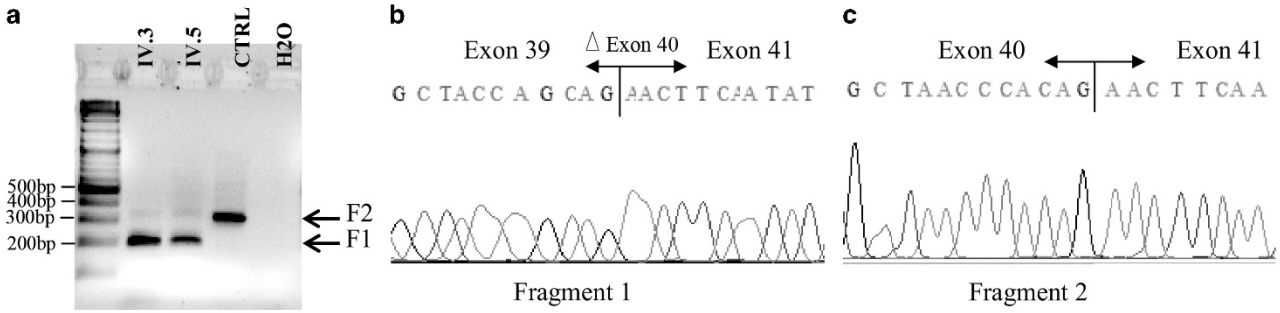

Figure 2 Effects of the *IFT172* c.[4428+3A>G];[4428+3A>G] mutation on splicing. (**a**) Amplification of the cDNA fragment between exons 39 and 42 of *IFT172* showing one band for the control (normal allele) and two bands for the individuals IV.3 and IV.5 (normal allele with a weak signal and a pathologic allele of 200 bp). (**b**) Sequencing of amplified cDNA fragment between exons 39 and 42 of *IFT172* in affected individuals showing skipping of exon 40 (117 bp). (**c**) Sequencing of amplified cDNA fragment between exons 40 and 42 of *IFT172* in affected individuals showing normal splicing with integration of exon 40. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

of blood for protein extraction and skin biopsies of the patients were not available.

DISCUSSION

We report the second *IFT172* mutation identified in a BBS family presenting with five major and two minor criteria (RP, obesity, cognitive impairment, polydactyly, hypogonadism, conductive deafness and asthma). Clinically, one patient has preaxial polydactyly of the feet. In both BBS and skeletal ciliopathies (including patients with *IFT172* mutations), polydactyly is primarily postaxial.[1,11] Interestingly, the murine model of *Ift172* has preaxial polydactyly.[12] Only one BBS patient has been reported with preaxial polydactyly, but no molecular analysis was possible at the time of that diagnosis.[13]

Our mutation, located within the +3 position of the consensus donor splice site, leads to variable skipping of exon 40. Similar mutations have been previously shown to cause aberrant splicing.[14] The 39-amino-acid deletion encompasses part of the eighteenth and nineteenth tetratricopeptide repeat domains of IFT172.[15] Tsao and Gorovsky[16] demonstrated that partial truncations of the C-terminal region containing one or more tetratricopeptide repeat domains affect the assembly efficiency and/or the mechanism that regulates ciliary formation or length.

Bujakowska *et al.*[4] also reported an *IFT172* mutation located outside the consensus splice site (c.3112-5T>A) in isolated RP. This mutation led to an alternative splicing with a predominant population of correctly spliced *IFT172* mRNA transcripts and a minority (20%) with a new splice acceptor site located three nucleotides upstream of the original one, leading to the insertion of a glutamine. The authors hypothesized that residual wild-type protein may explain the milder isolated RP phenotype. Similarly, we hypothesize that the presence of some remnant wild-type protein may explain the milder phenotype seen in our patients compared to the severe skeletal ciliopathies associated with *IFT172* mutations. To date, there is no correlation between the genotype and the different *IFT172*-associated phenotypes (RP, BBS, JATD and MZSD).[4] Various mutations are reported and widespread over the coding sequence. We can only notice that non-sense mutations are not found in milder phenotypes (RP and BBS) (Supplementary Table 3).

A direct relation between IFT172 and the BBSome has not been demonstrated to date. As mutations in *IFT172* were reported not only in BBS but also in other ciliopathies, the roles and the interactions of IFT172 could be diverse. More studies are necessary to understand the exact functions of IFT172 and determine the functional link between IFT172 and the BBSome as patients mutated in this gene presented with a typical BBS phenotype.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Supplementary Information accompanies the paper on Journal of Human Genetics website (http://www.nature.com/jhg)