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SHORT COMMUNICATION

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BBS8 is rarely mutated in a cohort of 128 Bardet–Biedl syndrome families

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Abstract *BBS8* is one of the eight genes identified to date for Bardet–Biedl syndrome (BBS)—an autosomal recessive condition associated with retinitis pigmentosa, obesity, polydactyly, cognitive impairment and kidney failure. The identification of *BBS8* gave the key to the pathogenesis of the condition as a primary ciliary disorder. To date, only three families mutated in the *BBS8* gene have been reported. Here, we report on three additional families with *BBS8* mutations from a series of 128 BBS families. Two of the three families have homozygous mutations and one has a heterozygous mutation. Mutations in *BBS8* probably account for only a minority of BBS families (2%), underlining the difficulty of genotyping heterogeneous conditions.

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Introduction

Bardet–Biedl syndrome (BBS) is a genetically heterogeneous condition characterised by early-onset retinitis pigmentosa, obesity, polydactyly, hypogonadism, learning disabilities, and kidney malformation. A tremendous amount of knowledge about BBS has recently accumulated, demonstrating extensive genetic heterogeneity and giving clues as to the ciliary pathogenesis of this complex condition. It has also been suggested that its inheritance may depart from the classic autosomal recessive mode, and may involve, in some families, three mutated alleles in two genes (triallelic inheritance) (Katsanis et al. 2001).

To date, eight BBS genes have been identified: *BBS1* (11q13; Mykytyn et al. 2002), *BBS2* (16q21; Nishimura et al. 2001); *BBS3* (3p13-p12; Fan et al. 2004; Chiang et al. 2004), *BBS4* (15q22.3-q23; Mykytyn et al. 2001); *BBS5* (2q31; Li et al. 2004); *BBS6* (20 p12; Slaovotinek et al. 2000; Katsanis et al. 2000), *BBS7* (4q27; Badano et al. 2003) and *BBS8* (14q32.11; Ansley et al. 2003).

The identification of *BBS8*, encoding a centrosomal and basal body protein, designated BBS as a primary cilia disorder (Ansley et al. 2003). Since then, functional overlap between BBS genes at the level of basal body and cilia is now well recognized and various bbs transgenic mice have been reported.

BBS8 maps to the 14q32.11 region and spans 15 exons. The BBS8 protein (TTC8), like BBS4, contains several tetratricopeptide repeats (TPRs) involved in protein–protein interactions. This protein is associated with centriolar structures and interacts with PCM-1, which is involved with basal body function (Ansley et al. 2003).

To date, mutations in *BBS8* have been reported only for three families (Ansley et al. 2003): two families of Saudi Arabian lineage have a homozygous in-frame deletion of 6 bp in exon 6, and one family of Pakistani descent was reported to have a 3-bp deletion at the splice site junction of exon 10. Herein, we report *BBS8* mutations in three BBS families out of a cohort of 128 BBS families.

Materials and methods

Families

This study was performed on a series of 128 families containing one or more patients with a diagnosis of BBS (Beales et al. 1999).

Methods

Mutation screening in *BBS8* was performed by DHPLC analysis using at least three melting temperatures for each amplicon, followed by direct sequencing of the variant PCR fragments as described previously (Hichri et al. 2005). Splice site scoring programs (http://l25.itba.mi.cnr.it/~webgene/wwwspliceview.html, http://www.fruitfly.org/seq_tools/splice.html) were used to evaluate the effect of mutations affecting splice sites and to detect potential consequences on splicing of silent, or missense changes and intronic variations. ESE web servers (http://genes.mit.edu/burgelab/rescue-ese, http://ru-lia.cshl.edu/tools/ESE) were used to search for potential exonic splicing enhancers in exonic variants (silent or missense mutations).

Results

Three families with *BBS8* mutations were identified, of which two showed homozygous mutations and one showed a heterozygous mutation.

Family one is a consanguineous family of North-African descent with three BBS affected sibs and three non-affected sibs (Fig. 1). Affected sibs harboured a homozygous splice-site mutation (T153T, 459G > A) affecting the last G of exon 4 of *BBS8* and predicted to abolish the splice site of exon 4. Screening for other BBS genes for this family revealed an additional *BBS7* mutation (M114 V, 340A > G) for one sib only (Figs. 1, 2). This mutation is predicted to affect the donor splice site of exon 4 of *BBS7*. No clinical difference was recorded for the three BBS sibs and no cell-line is available.

Family two is a sporadic case of Lebanese lineage. The proband, born from a consanguineous marriage, showed a homozygous splice site mutation (IVS6 + 1-G > A) at the level of exon 6 of *BBS8* (the DNA of other members of the family is not available) (Fig. 2). No other mutation in any known BBS gene has been identified to date.

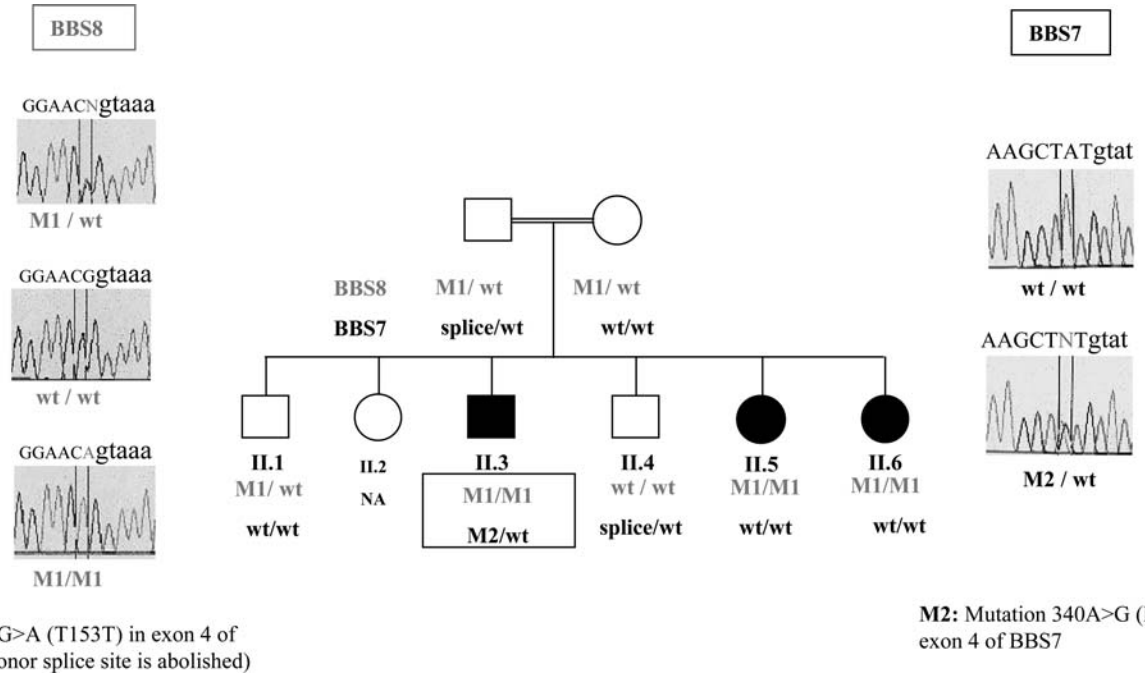

Fig. 1 Patient II.3 presented with retinitis pigmentosa, polydactyly at the level of the hands and feet, cognitive impairment and a micropenis. Patient II.5 had an initial presentation suggestive of McKusick–Kauffmann syndrome as she was operated on at birth for a hydrometrocolpos and had polydactyly at the level of the hands and feet. She developed retinitis pigmentosa and progressive renal failure. Patient II.6 was born with polydactyly at the level of the hands and feet, retinitis pigmentosa and kidney dysplasia

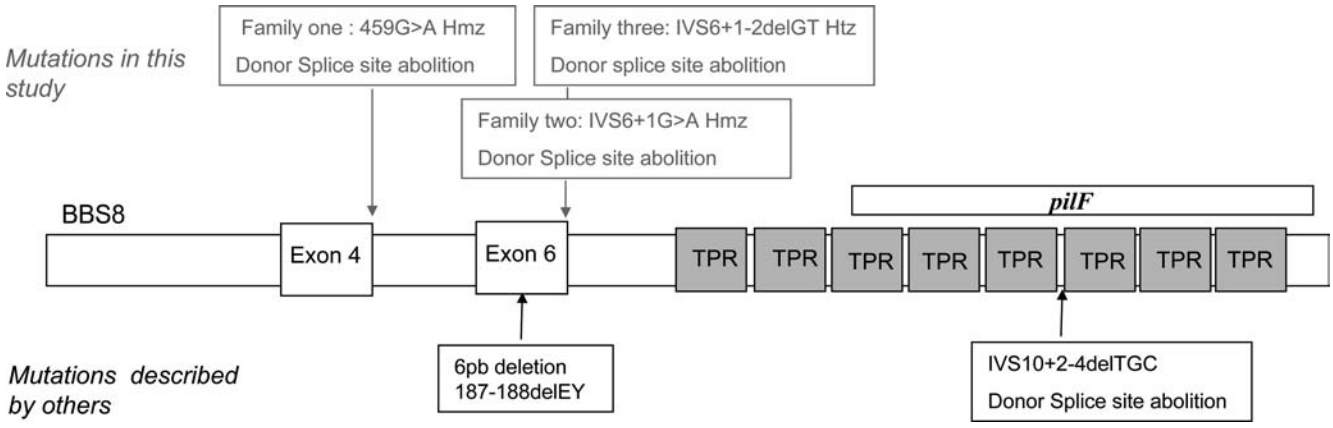

Fig. 2 Structure of the *BBS8* gene showing the tetratricopeptide repeats (TPR) and PilF domains. The positions of mutations previously reported in the literature and mutations described here are indicated

Table 1 Exonic variations in *BBS8* identified during this study, and silent exonic mutations and intronic variations in *BBS8* reported in the literature for Bardet–Biedl syndrome (BBS) families

Gene	Exon/intron	Nucleotide change	Predicted effect	Allele frequency in the families studied (%)	Reference
BBS8	Intron 2	235 + 49 del34bp	–	0.4	This paper
	Intron 2	235 + 71 delA	–	2.3	Hichri et al. 2005
	Exon 3	237C > A	R79R	0.4	This paper
	Exon 3	254A > G	K85R	1.2	This paper
	Intron 5	550–45 insA	–	0.8	Hichri et al. 2005
	Intron 7	673–81 A > G	–	2.7	Hichri et al. 2005
	Intron 7	673–5 C > T	–	1.9	Hichri et al. 2005
	Intron 8	758 + 75 G > C	–	2.3	Hichri et al. 2005
	Intron 8	758 + 78 G > A	–	0.4	This paper
	Intron 8	758 + 80 G > C		0.4	This paper
	Intron 10	958–51 T > G	–	0.4	Hichri et al. 2005
	Intron 14	1,479 + 81 A > G		0.8	This paper
	Intron 14	1,480–12 T > C	–	1.2	This paper

Family three, a sporadic case born from caucasian non-consanguineous parents carried a heterozygous mutation in the donor splice site of exon 6 of *BBS8* (IVS6 + 1−2delGT) (Fig. 2).

Two exonic variations were identified during this study. The R79R change, which creates a weak exonic splicing enhancer (ESE), found in one patient was not found in 137 matched controls. The K85R change found in three patients and in one control out of 137 matched controls is predicted to abolish two ESEs. Functional analysis was not feasible as no cell lines are available. Table 1 summarises the variations found in the *BBS8* gene during this study.

Conclusions

Mutations in *BBS8* remain rare events in BBS family cohorts. BBS8 mutations were found in only 1% (3/259) of the families studied in an earlier publication, and in 2% (3/128) of the families examined in this study. The Pakistani family in Ansley's paper presented with a BBS phenotype associated with *Situs Inversus*; however, to our knowledge, none of the patients reported herein exhibited this feature. *BBS7* and *BBS8* have been found to participate in the same process at the level of ciliary intraflagellar transport (Blacque et al. 2004). Interestingly, only one patient in family one harboured a "third mutated allele"; however, the *BBS7* heterozygous mutation predicted to affect splicing is not present in affected siblings, casting doubt on its pathogenicity as no clinical differences between the siblings were noted. To date, *BBS1* is the major BBS gene, accounting for at least 20% of BBS gene mutations found in affected families (Hichri et al. 2005). About 50% of the BBS families in various studies do not carry mutations in known BBS genes, suggesting that other genes remain to be identified. Numerous genes are probably involved in this unique syndrome and traditional genotyping remains a difficult task, stressing the need for automated genotyping for heterogeneous conditions.

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