A *BBS1* branchpoint variant is associated with non-syndromic retinitis pigmentosa

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Background & Aim

- **Retinitis pigmentosa (RP)** shows a high degree of genetic heterogeneity with more than 90 genes involved[1,2].
- The **Bardet-Biedl syndrome 1 (*BBS1*)** gene is associated with syndromic and non-syndromic autosomal recessive RP[3].
- Using **Whole Genome Sequencing (WGS),** a *BBS1* **branchpoint variant**, c.592-21A>T, has been found in four unrelated individuals with non-syndromic RP. All cases carry the common *BBS1* c.1169T>G variant in *trans*.

Assess pathogenicity of the *BBS1* branchpoint variant by midigene *in vitro* splice assay in HEK293T cells

Four families with non-syndromic RP carry *BBS1*, c.1169T>G and c.592-21A>T variants in *trans*

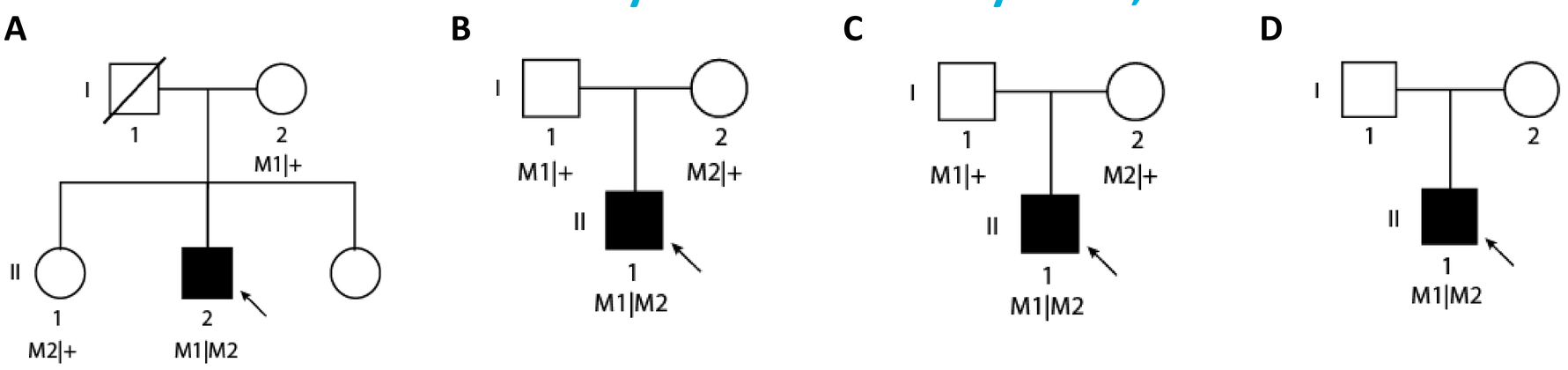

Figure 1: Pedigrees of four unrelated individuals. All affected individuals carry the *BBS1* variants c.1169T>G and c.592-21A>T which segregate with the disease in three studied families. The c.1169T>G is the most frequent pathogenic variant in *BBS1* which is associated with syndromic and non-syndromic RP. The arrow indicates the proband in each family.
M1: c.1169T>G; p.(Met390Arg)
M2: c.592-21A>T; p.[Thr198_Lys207del,=]

BBS1 c.592-21A>T variant causes partial and complete exon 8 skipping

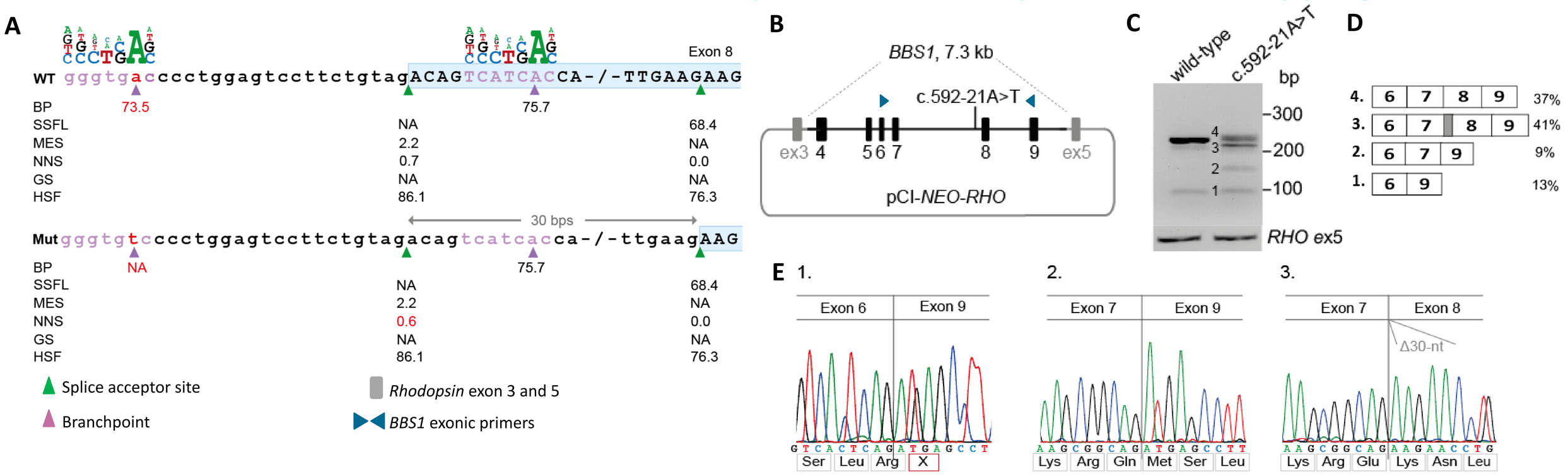

Figure 1: Molecular genetic characterization of c.592-21A>T variant in *BBS1*. A) Schematic representation of the intron 7 – exon 8 boundary in the wild-type and mutant sequences. The purple sequences highlight the 7-mer branchpoint motif and the sequence above shows the general branchpoint motif. The sequences in blue boxes represent the exonic region and smaller letters are intronic sequences. **B)** Schematic representation of the mutant pCI-NEO-RHO vector, containing exon 4-9 of *BBS1* flanked by *RHO* exons 3 and 5, which was used to transfect HEK293T cells, parallel to the wild-type construct. **C)** The RT-PCR product of the wild-type midigene reveals the wild-type fragment along with a smaller fragment (#1) corresponding to natural exon 7/8 skipping. In the mutant midigene, two additional fragments were observed in which the larger one (#3) corresponds to a 30-bp deletion of exon 8 and the smaller fragment (#2) to a complete deletion of exon 8. **D)** Quantification shows partial exon 8 deletion (41% of total RNA) to be the most abundant event for the mutant construct. **E)** Skipping of exon 7/8 results in a premature stop codon (p.(Phe174*)), while a 30-nt deletion of exon 8 and complete deletion of this exon cause in-frame deletions ((p.(Thr198_Lys207del), and p.(Thr198_Lys241del), respectively). SSFL, SpliceSiteFinder-like (0–100); MES, MaxEntSCan (0–12); GS, GeneSplicer (0–24); HSF, Human Splicing Finder (0–100); BP, Branch Points (0–100); WT, wild-type; Mut, mutant; nt, nucleotide; bp; base-pairs.

Conclusions

- **We identified a complex splice defect caused by a pathogenic branchpoint variant c.592-21A>T in *BBS1* in *trans* with the most frequent pathogenic missense variant p.(Met390Arg) in four unrelated individuals.**
- **It is the first report of a pathogenic branchpoint variant in IRD-associated genes as well as the first report of natural exon 7/8 skipping in *BBS1* mRNA.**

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References:
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