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A *BBS1* SVA F retrotransposon insertion is a frequent cause of Bardet-Biedl syndrome

Running title: Mobile element insertion in *BBS1*

Delvallée Clarisse[1], Nicaise Samuel[1], Antin Manuela[2], Leuvrey Anne-Sophie[2], Nourisson Elsa[2], Leitch C. Carmen[3], Kellaris Georgios[3], Stoetzel Corinne[1], Geoffroy Véronique[1], Scheidecker Sophie[1,2], Keren Boris[4,5], Depienne Christel[4,6], Klar Joakim[7], Dahl Niklas[7], Deleuze Jean-François[8], Génin Emmanuelle[9], Redon Richard[10], Demurger Florence[11], Devriendt Koenraad[12], Mathieu-Dramard Michèle[13], Poitou-Bernert Christine[14], Odent Sylvie[15,16], Katsanis Nicholas[3,17], Mandel Jean-Louis[2,18], Davis E. Erica[3,17], Dollfus Hélène[1,19,20], Muller Jean[1,2].

[1]Laboratoire de Génétique Médicale, Institut de génétique médicale d'Alsace IGMA, INSERM U1112, Fédération de Médecine Translationnelle de Strasbourg (FMTS), Université de Strasbourg UMRS_1112, Strasbourg, France.
[2]Laboratoires de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.
[3]Advanced Center for Translational and Genetic Medicine (ACT-GeM), Stanley Manne Children's Research Institute, Ann & Robert H. Lurie Children's Hospital of Chicago, Chicago, IL, USA
[4]Institut du Cerveau et de la Moelle épinière (ICM), Sorbonne Université, UMR S 1127, Inserm U1127, CNRS UMR 7225, F-75013 Paris, France.
[5]AP-HP, Hôpital de la Pitié-Salpêtrière, Département de Génétique, F-75013, Paris, France.
[6]Institute of Human Genetics, University Hospital Essen, University of Duisburg-Essen, Hufelandstraße 55, 45147 Essen, Germany
[7]Department of Immunology, Genetics and Pathology, Science for Life Laboratory, Uppsala University, Uppsala, Sweden.
[8]Centre National de Recherche en Génomique Humaine (CNRGH), Institut de biologie François Jacob, CEA, 91000, Evry, France.
[9]Inserm UMR1078, CHRU Brest, Univ Brest, Brest, France
[10]Université de Nantes, CNRS, INSERM, l'institut du thorax, F-44000 Nantes, France.
[11]Service de Génétique Médicale, Centre Hospitalier Bretagne Atlantique, Vannes, France.
[12]Center for Human Genetics, University Hospital Leuven and KU Leuven, Leuven, Belgium.
[13]Centre d'activité de génétique clinique, CLAD nord de France, CHU Amiens, Amiens, France.
[14]Assistance Publique Hôpitaux de Paris, Nutrition Department Pitié-Salpêtrière Hospital; Sorbonne Université, INSERM, NutriOmics Research Unit, Paris, France;
[15]Service de Génétique Clinique, CHU Rennes, Rennes, France.
[16]CNRS, IGDR (Institut de Génétique et Développement de Rennes) UMR 6290, Université de Rennes, Rennes, France.
[17]Department of Pediatrics, Feinberg School of Medicine, Northwestern University, Chicago, IL USA
[18]Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS UMR 7104, INSERM U964, Université de Strasbourg, Dept Transl Med and Neurogenetics Illkirch, France
[19]Service de Génétique Médicale, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.
[20]Filière SENSGENE, Centre de Référence pour les affections rares en génétique ophtalmologique, CARGO, Hôpitaux Universitaires de Strasbourg, Strasbourg, France.

Corresponding author: Jean Muller, jeanmuller@unistra.fr, +33 3 69 55 07 77
ORCID: Jean Muller, 0000-0002-7682-559X

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CONFLICT OF INTEREST STATEMENT

NK is a significant shareholder in Rescindo Therapeutics. The other authors declare no conflict of interest.

AVAILABILITY OF DATA AND MATERIAL

Data generated or analyzed during this study are included in the published article and the corresponding supplementary data. The raw sequencing data generated in the course of this study are not publicly available due to the protocol and the corresponding consents used that did not include such information. The variants are available in ClinVar (SCV001427246, SCV001427247) and the SVA F sequence in GenBank (MT113356).

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ABSTRACT

Bardet-Biedl syndrome (BBS) is a ciliopathy characterized by retinitis pigmentosa, obesity, polydactyly, cognitive impairment and renal failure. Pathogenic variants in 24 genes account for the molecular basis of >80% of cases. Toward saturated discovery of the mutational basis of the disorder, we carefully explored our cohorts and identified a hominid-specific SINE-R/VNTR/*Alu* type F (SVA F) insertion in exon 13 of *BBS1* in 8 families. In six families, the repeat insertion was found *in trans* with c.1169T>G, p.Met390Arg and in two families the insertion was found in addition to other recessive BBS loci. Whole genome sequencing, *de novo* assembly and SNP array analysis were performed to characterize the genomic event. This insertion is extremely rare in the general population (found in 8 alleles of 8 BBS cases but not in >10,800 control individuals from gnomAD-SV) and due to a founder effect. Its 2,435 bp sequence contains hallmarks of LINE1 mediated retrotransposition. Functional studies with patient-derived cell lines confirmed that the *BBS1* SVA-F is deleterious as evidenced by a significant depletion of both mRNA and protein levels. Such findings highlight the importance of dedicated bioinformatics pipelines to identify all types of variation.

Keywords: Bardet-Biedl syndrome, BBS1, Mobile element insertion, SVA F, Founder effect.

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INTRODUCTION

Bardet-Biedl syndrome (BBS, MIM# 209900) is a rare autosomal ciliopathy associating retinitis pigmentosa, polydactyly, obesity, cognitive impairment and renal dysfunction. Its prevalence ranged from 1/160,000 live births [1] to <1/50,000 in consanguineous or isolated populations [2]. Recent studies have delineated the antenatal and postnatal presentation of BBS patients [3,4] with pathogenic variants in 24 genes accounting for the molecular basis of a majority of cases [5]. Among the known BBS genes, *BBS1* contributes the highest fraction of BBS patients (~20%) [6] thanks to a founder variant (c.1169T>G, p.Met390Arg) [7]. Among the unresolved cases in our cohort, several patients were heterozygous carriers of this variant suggesting two hypothesis: (1) a missed second primary driver allele in *BBS1*; or (2) participation of this variant as a second-site modifier in the presence of a different primary BBS driver locus [8,9]. Notably, due to the high frequency of this variant in the general population (0.27% in non-Finnish Europeans; gnomAD) this variant could be detected coincidently.

In this study, we report the identification of a mobile element insertion of SVA F type in 8 families, a portion of which are *in trans* to the recurrent c.1169T>G in *BBS1* (n=6), and a subset as secondary sites in addition to a known recessive BBS locus (n=2). Already described as a single case report [10], here we estimate the frequency of this variant in BBS (131 families) identifying this variant as the second most common pathogenic variant in *BBS1*. We also determined its full-length sequence using WGS and performed functional analysis to understand its consequences. In our cohorts, the insertion always occurs at the same genomic location and its recurrence in BBS populations is the result of a founder effect.

SUBJECTS AND METHODS

Subjects

Written informed consent was obtained from each participant and/or parent or legal guardian. The study protocols were approved by local Institutional Review Boards: "Comité de Protection des Personnes" (EST IV, N°DC-20142222), or the Lurie Children's Hospital IRB (IRB 2019-3057 or IRB 2019-2950). Our research complies with the Declaration of Helsinki. All patients were diagnosed with BBS according to Beales' clinical diagnostic criteria [11] (Supplementary Table 1). Patients (n=564) have been assembled into several cohorts (Supplementary Table 2).

Methods

All experimental procedures are reported as supporting information.

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RESULTS

Identification of an SVA F insertion in BBS1

According to our tiered diagnostic paradigm, we screened the recurrent BBS variants (*BBS1:*p.Met390Arg and *BBS10:*p.Cys91Leufs*5) in cohort 1 (n=217) and cohort 2 (n=192) followed by targeted exome sequencing (TES) in cohort 1. We identified seven individuals who carry heterozygous p.Met390Arg changes without a second pathogenic *BBS1* variant identified *in trans*. In one family (family I), we identified a novel heterozygous deletion encompassing exons 4 to 11 (Supplementary Figure 1). Among the six p.Met390Arg variation-bearing cases, we leveraged a high depth of coverage to identify another putative pathogenic variant in exon 13 of *BBS1* with the deletion/insertion of 2 bases, c.1215_1216delinsT (p.(Ala406Glnfs*4)) in family A.II-3 (Figure 1B and supplementary Figure 2) and 2 others (B.II-1, C.II-1). Careful analysis of the genomic region revealed a more complicated event leading to incorrect variant calling (Figure 1C). Indeed, soft clipped reads part (82 bp) revealed the insertion of an SVA F retrotransposon with a possible truncation of 311 bases 5' of the consensus sequence (Figure 1D).

To screen additional patients not yet available by TES (cohort 2; 213 cases from 192 families), or an independent group with no pre-selection bias for BBS variations other than exclusion of *BBS1* recessive cases (cohort 3; 134 cases from 126 families), we designed a duplex PCR including the SVA insertion point (Supplementary Figure 3). This led to the identification of 5 additional cases (family D to H) including 3 *in trans* to the p.Met390Arg and 2 as third or fourth allele (Figure 1A). Biallelic status of the variants and the exact same insertion point was confirmed for 7 individuals (Figure 1E).

To estimate the overall incidence of the SVA F as a contributor of the *BBS1* mutational burden, we explored all our *BBS1* positive cases (e.g. biallelic pathogenic variants). Data from 131 unrelated cases of European ancestry corresponding to 262 pathogenic alleles in total (cohort 4) could be retrieved and account for 44 different pathogenic variations. Among those, the c.1169T>G represents 72.1% (189 alleles) while the second most frequent variant is the SVA F with a frequency of 2.3% (6 alleles).

Determination of the full length SVA F sequence

To understand the consequences of this insertion, we determined its full-length sequence. Given the repeated sequence of the SVA F, alignment of short reads onto the human genome generate artifacts (multimapped reads, split reads, discordant read pairs) that can be rescued using a dedicated bioinformatics pipeline. The initial TES aligned reads allowed us to identify 99 bp from the SVA F (Figure 2B). Assembled reads from WGS data (A.II-3) output a 387 bp contig composed of *BBS1* (38 bp before the breakpoint in exon 13), a TSD (17 bp) and 330 bp that matched the SVA F consensus sequence. In conclusion, WGS data enabled accurate characterization of an additional 250 bp SVA F sequence (Figure 2). Based on this and the consensus SVA F sequence [12], we walked on the genome and determined an inserted sequence of 2,435 bp (Figure 2B and Supplementary Figure 4 and 5). With the exception of a missing 311 bases (Figure 2A) in the 5' part, hallmarks of L1 mediated retrotransposition could be identified (Figure 2). The variant can now be described as c.1214_1215ins[MT113356], p.(Ala406Glnfs*47).

Functional impact of the SVA F insertion on BBS1

Predicted to create a premature stop codon, a reduced or absent mRNA expression due to nonsense-mediated decay potentially leading to a shorter protein (452 vs 593 AA) is likely to happen. Using primary cells from individual A.II-3, we demonstrated a significantly reduced *BBS1* expression corresponding *a priori* to the loss of the SVA F allele (Figure 3A). However, a transcript carrying the SVA F is expressed (Supplementary Figure 6), albeit likely minor compared to the p.Met390Arg allele. Western blot analysis on protein extracted from A.II-3 cells revealed a single band corresponding to the normal BBS1 protein (Figure 3B) demonstrating the absence of a putative BBS1 polypeptide carrying the SVA F.

Founder effect or recurrent mechanism?

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Given the multiple carriers of this insertion in our cohorts (8 families), we wondered whether this MEI occurred as an independent (recurrent) mechanism or as the same mutational event derived from common ancestry (founder effect). First, no occurrence could be observed either from 1299 healthy individuals of European ancestry or from the 14,891 gnomAD SV controls [13]. Second, genotyping of 4 different families with SVA F alleles, along with 3 patients carrying the c.1169T>G change in the homozygous state (Supplementary Table 5) revealed the common c.1169T>G haplotype (~1.614 Mb) as well an SVA F allele of a much smaller size (~56.6kb) (Supplementary Table 5). PCA analysis confirmed the geographical distribution of the patients (Supplementary Figure 7). Haplotypes shared by the four SVA F carriers were compared to find the position where recombination events are likely to have happened. The estimation of the most recent common ancestor age was 74 generations 95% CI [33;190]. If we assume that one generation lasts 25 years, this will indicate an age of 1850 years 95% CI [825;4750]. Overall, this MEI is an ultra-rare event that arose from a common ancestor on one of the most common haplotypes at this locus and more recently than the Met390Arg haplotype.

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DISCUSSION

Although the diagnostic yield in BBS is relatively high, (60-100%), a substantial proportion of cases remain unresolved [3,5]. This could be related to the attributes of the cohort (sample size and geographic origin), the clinical inclusion criteria (strict or more inclusive) or the genetic test used (TES, WES, WGS). In addition to the first BBS case with MEI reported by our group [14] and the recent SVA F description [10], this is the largest description of such cases in BBS patients. Although rarely described, a number of MEI cases (>100) have been reported in rare diseases [15] or in cancer [16]. The majority are *Alu* insertions [17] and only a few SVA (n=16). Recently, large studies have evaluated the impact of MEI in patients between 0.04% to 0.15% of the cases [18,19].

BBS1 is often the target of structural variations [20]. Interestingly, the *BBS1* locus is GC rich and has a high density of L1 and *Alu* insertions that might explain the SVA F insertion (Supplementary methods). Given the nature of the SVA F sequence, which contains highly repeated elements, GC rich content and a poly(A) tail, complete amplification was not easily performed. We thus used the breakpoints identified by the TES and WGS to walk across the patient's genome and determine a 2,435bp insertion in exon 13 of *BBS1*. This inserted sequence is identical to the one already described [10]. Unlike most of the SVA elements in the human genome (63%) [21], this inserted SVA F is incomplete lacking 311 bases in 5' and possibly as almost all SVA it is inactive [17]. Notably, beside short read standard WGS we also performed linked-read sequencing (10x genomics) to see whether this technology could overcome any of the technical challenges encountered through WGS and analysis (Supplementary Figure 8). Consistent with other reports, we did not find any significant advantage and it is now discontinued [22]. Interestingly sequencing of the insertion point revealed the exact same sequence for all patients and the genotyping of some individuals revealed a common shared haplotype. All families carrying the SVA F are of European ancestry: 4 originated from the western part of France (family A, C, E and F), one from the northern part of France (family B) and one from Denmark and Sweden (family D). These observations were highly suggestive of a single founder genomic event that we confirmed and estimated its age close to 1850 years.

In conclusion, we identified the recurrence of a specific SVA F element in exon 13 of *BBS1* in multiple BBS families highlighting the importance of detecting such genetic events even by TES. We determined the full length sequence of the inserted SVA F, show its pathogenicity and demonstrated the founder effect. This rare allele is the second most frequent cause of *BBS1* after the c.1169T>G variant. We have also developed a duplex PCR, offering a simple and inexpensive way to detect this variant prior to any other large genetic screen. Accordingly we have modified our BBS diagnostic strategy (Supplementary Figure 10).

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FIGURES LEGENDS

Figure 1. Pedigree of BBS families and variant analysis
(A) 8 families carrying the SVA F insertion in exon 13 of *BBS1*.
(B) Next generation sequencing data displayed from the Integrative Genomics Viewer [23] surrounding the *BBS1* locus from A.II-3 and one control individual.
(C) Magnified view of the incorrectly called deletion insertion with the sorting and coloring of the reads according to the "insert size". The sequence reveals all reads ending at the exact same position with a "AG" deletion and a "T" insertion (c.1215_1216delinsT).
(D) Region of interest with the "Show soft-clipped bases" turned off revealing multiple reads with aberrant alignments on their right side and corresponding to an SVA F insertion. The spacing between the reads on the left (including the 3' end of the SVA F with the poly(A) tail) and the reads on the right (including the 5' end of the SVA F) corresponds to the TSD.
(E) Segregation analysis using Sanger sequencing for M1 (left) and M2 (right).

Figure 2. Schematic of the inserted SVA F element in exon 13 of *BBS1.*
(A) Canonical SVA F structure composed of 6 parts: (1) hexameric CCCTCT repeats (2) *Alu*-like domain, (3) a Variable Number Tandem Repeats (VNTR) domain, (4) a Short Interspersed Nucleotide Elements (SINE-R) domain, (5) a poly(A) tail and (6) the target site duplication (TSD) at both extremities. Numbers above the scheme indicate the size of each element.
(B) Schematic of both *BBS1* alleles in A.II-3 with primer positioning and amplification products highlighting each contribution to the resolution of the full-length sequence.

Figure 3. Quantification of BBS1 mRNA and protein levels in A.II-3
(A) Reduced *BBS1* mRNA expression by RT-qPCR (A.II-3) compared to control or homozygous p.Met390Arg patient. The major fraction of *BBS1* expression likely corresponds to the p.Met390Arg allele. Normalization was performed using both *GAPDH* and *HPRT* as reference genes. Mean of both results are shown. Controls are in gray (n=3), homozygous p.Met390Arg in black (n=2), and patient A.II-3 (p.Met390Arg and SVA F) in white (n=2). Bars show the mean of 2 independent experiments +/- SEM (n=2, *t*-test *: $p<0.05$, ns: not significant).
(B) Western blot analysis of BBS1 protein level. No truncated version of BBS1 could be detected. Half reduced BBS1 protein level in A.II-3 compared to control. Bars show mean of 3 independent experiments +/- SEM (n=3, *t*-test *: $p<0.05$, ns: not significant). M: molecular weight ladder (kDa).

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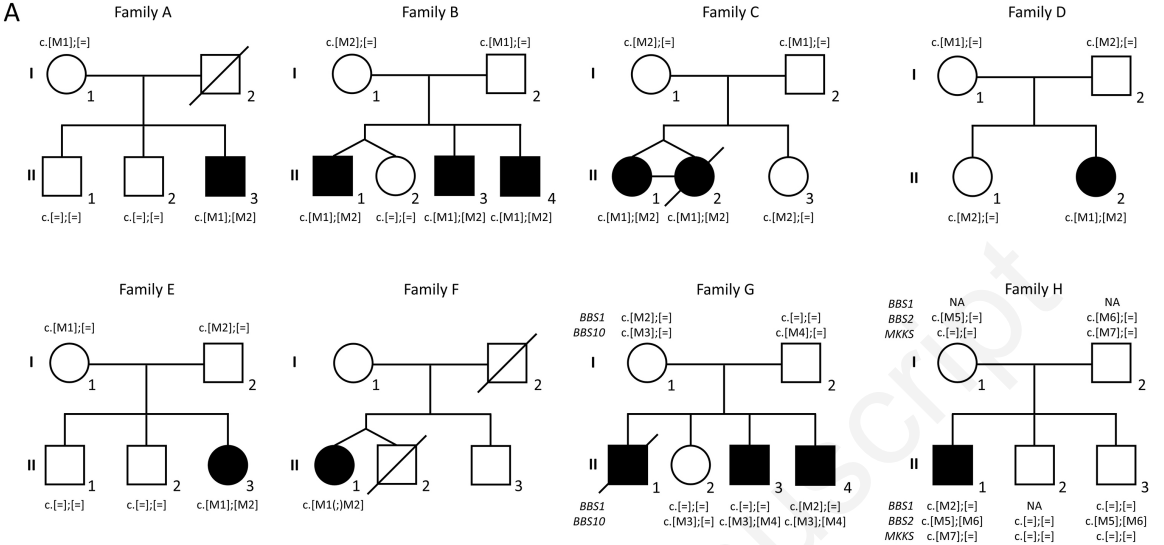
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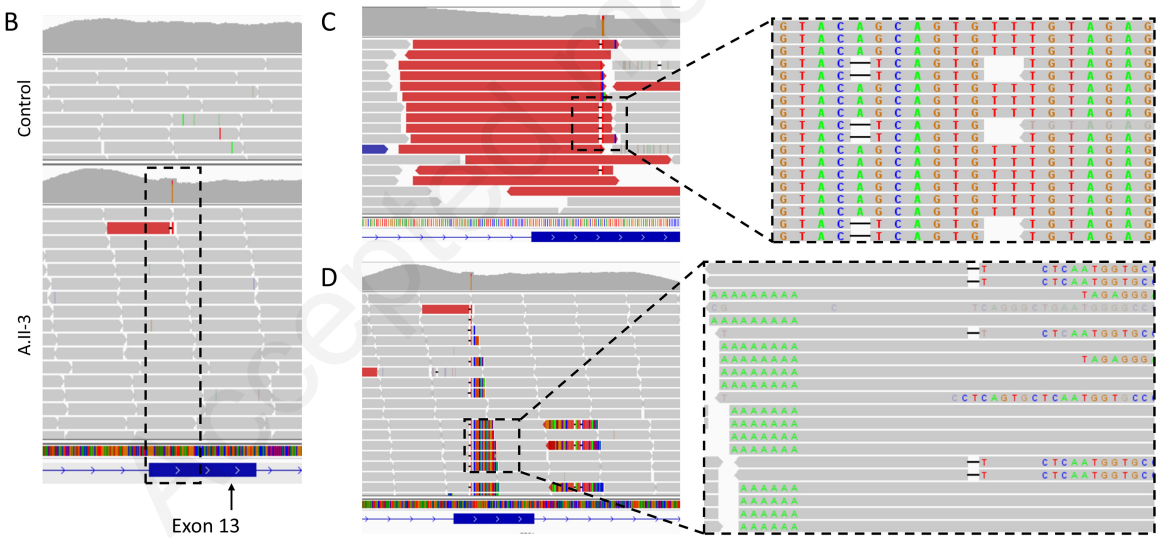
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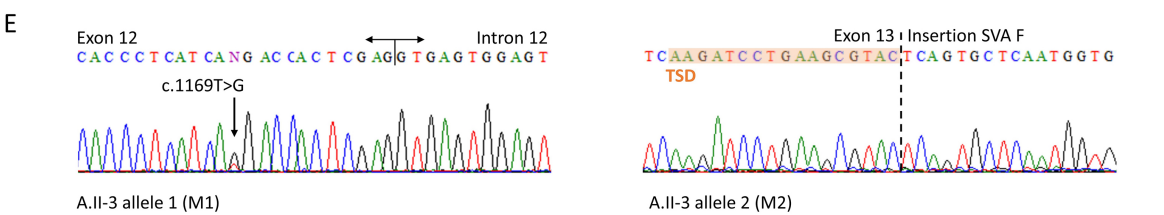
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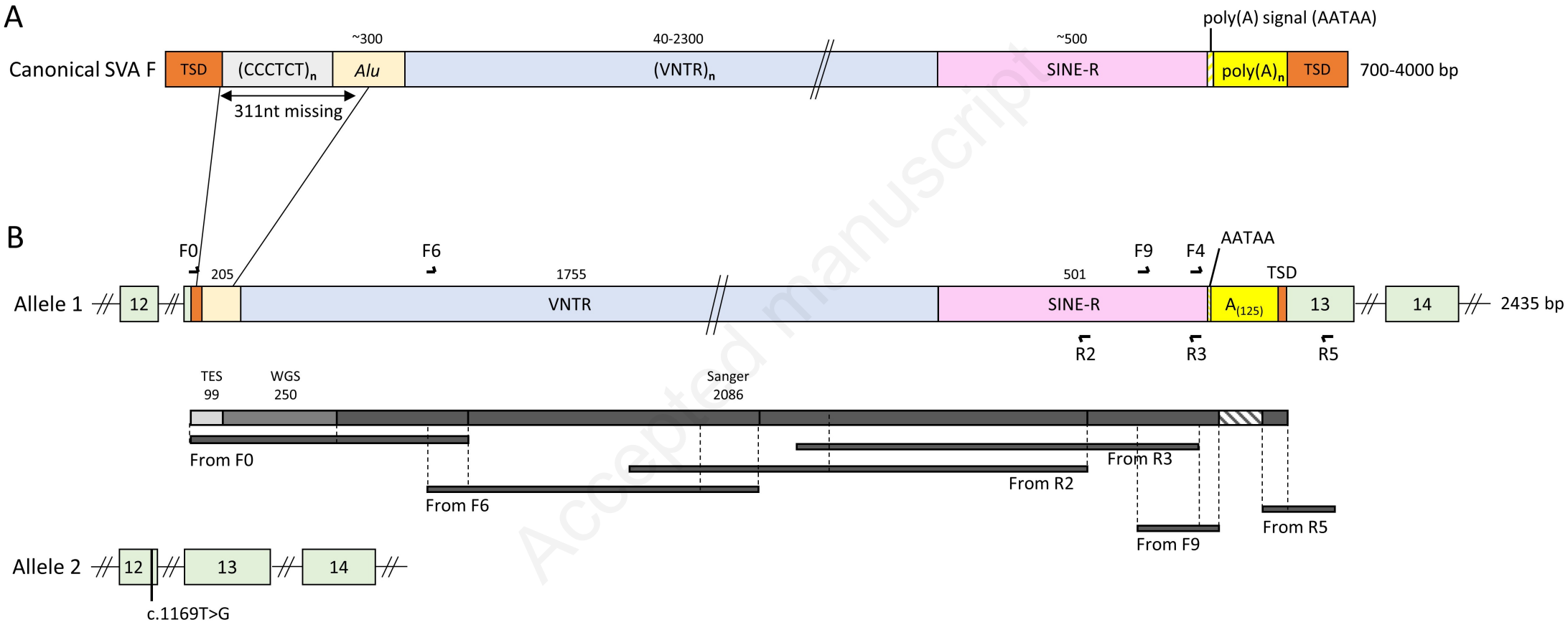


M1 : *BBS1* c.1169T>G, p.Met390Arg
M2 : *BBS1* c. 1214_1215ins[MT113356], p.Ala406Glnfs*47
M3 : *BBS10* c. 271dupT, p.Cys91Leufs*5
M4 : *BBS10* c.145C>T, p.Arg49Trp
M5 : *BBS2* c.72C>T, p.Tyr24*
M6 : *BBS2* c.175C>T, p.Gln59*
M7 : *MKKS/BBS6* c.442C>T, p.Gln148*

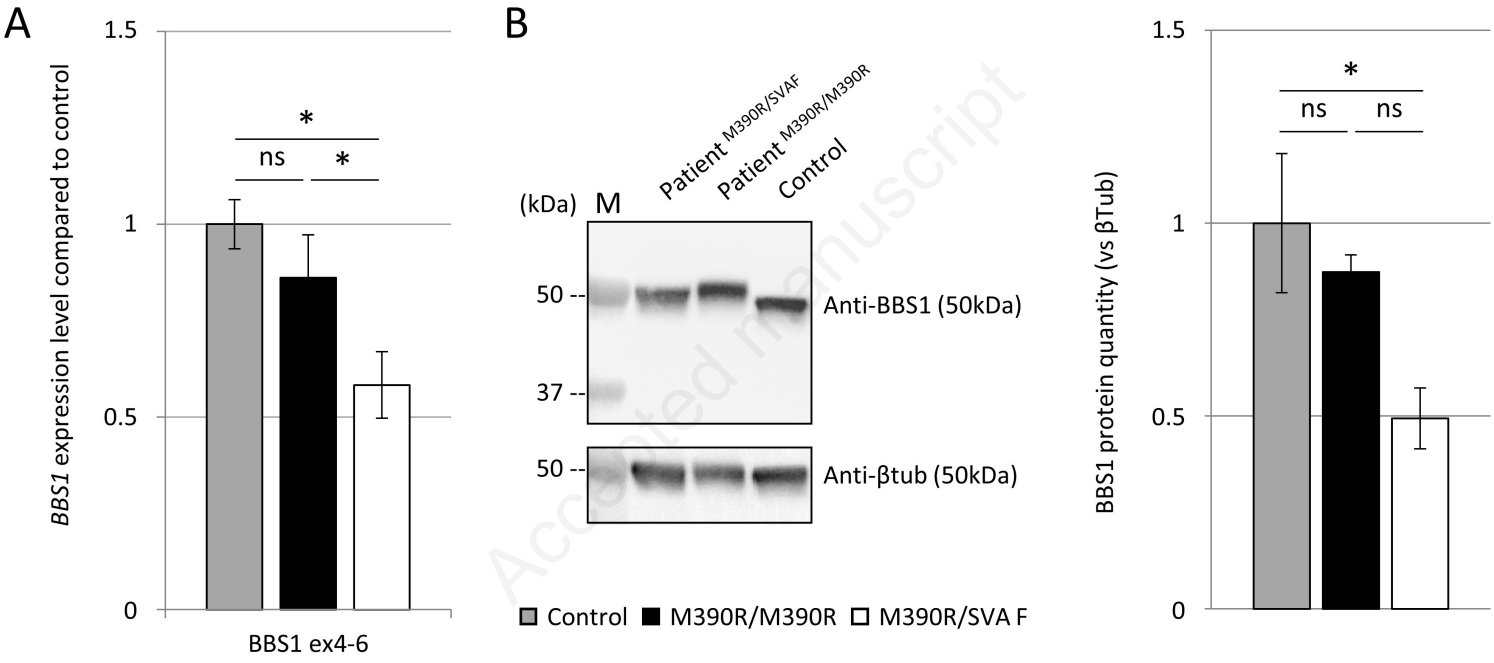




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