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Missed structural variation in a known BBS gene by targeted sequencing rescued by whole genome sequencing

Adella Karam[1*], Clarisse Delvallée[1*], Alejandro Estrada-Cuzcano[1], Véronique Geoffroy[1], Anne-Sophie Leuvrey[2], Elsa Nourisson[2], Julien Tarabeux[2], Corinne Stoetzel[1], Sophie Scheidecker[1,2], Emmanuelle Génin[3], Richard Redon[4], Steven McGinn[5], Anne Boland[5], Jean-François Deleuze[5,6], Hélène Dollfus[1,7,8], Jean Muller[1,2,9]

[1]Laboratoire de Génétique Médicale, Institut de Génétique Médicale d'Alsace (IGMA), INSERM U1112, Université de Strasbourg, Strasbourg, France, [2]Laboratoires de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, Strasbourg (HUS), [3]France, Inserm UMR1078, CHRU Brest, Univ Brest, Brest, France, [4]Université de Nantes, CNRS, INSERM, l'institut du thorax, Nantes, France, [5]CNRGH, Institut de Biologie François Jacob, DRF, CEA, Evry, France, [6]Centre d'études du polymorphisme humain-Fondation Jean Dausset, Paris, France, [7]Service de Génétique Médicale, IGMA, HUS, Strasbourg, France, [8]Centre de Référence pour les affections rares en génétique ophtalmologique, CARGO, Filière SENSGENE, HUS, Strasbourg, France, [9]Unité Fonctionnelle de Bioinformatique Médicale appliquée au diagnostic (UF7363), HUS, Strasbourg, France.

adella.karam@etu.unistra.fr

Introduction

Primary cilia has been considered for a long time as a cellular vestige[1]. However, it is now defined as a major sensory organelle involved in signalling pathways essentials for the cellular development such as Sonic Hedgehog signaling pathway (SHH). The term "ciliopathy" gathers together all diseases caused by ciliary proteins (~1500) impairment. In particular, the **Bardet-Biedl syndrome (BBS)** is an autosomal recessive disorder characterized by multiple affected organs (e.g. eye, kidney…)[2]. Variations in 24 genes have been identified to cause this rare syndrome (~1/150000)[3]. Among those, *BBS5* is a minor contributor of the disease (~2% of the patients), encoding a peripheral subunit of the BBSome a multiprotein complex located at the base of the cilium. Today 32 distinct pathogenic variations have been reported (8 nonsenses/frameshift, 2 startloss, 14 missenses, 5 splice, 3 large deletions). **In this study, we identified two novel variants in *BBS5* using whole genome sequencing. The two variants are representative of actual genetic challenges (1 missed by WES but rescued by WGS and 1 required functional assay).**

Patient and Methods

- The index patient is born from a Caucasian non-consanguineous union with typical **BBS phenotype** including:

Retinitis pigmentosa — Obesity — Intellectual disability
Renal abnormality — Polydactyly — Uterine atrophy

- **Molecular diagnosis**:
  Gene panel (58 genes), whole exome sequencing (WES) and whole genome sequencing (WGS) analysis.
- **Variant confirmation (biallelic status) and pathogenicity:**
  Sanger sequencing, duplex PCR, qRT-PCR and western blot (WB) using patient's RNA since parents' samples were not available.
- **Ciliary cellular phenotype (skin fibroblast)**
  Cilium staining by immunofluorescence, cell culture under ciliary condition (-FCS, 48H fetal calf serum deprivation). SHH pathway analysis using Smoothened AGonist (SAG) for induction +/- FCS

Results and discussion

- **Genetic analysis:**

Variant 1: large heterozygous deletion (5920 bp) encompassing exons 1 and 2 (c.1-2272_142+879del) (Fig.1B and 2) (**likely pathogenic variant, class 4). Missed by Panel and WES but not WGS.**

Variant 2: heterozygous duplication (c.550_552dupAAT, p.Asn184dup) in exon 7 (**variant of unknown significance, class 3)**

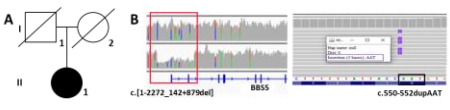

Figure 1: **A**. Pedigree. **B**. Visualization of the heterozygous deletion encompassing exons 1 and 2 using IGV (Integrative Genomics Viewer) and the AA duplication in *BBS5*

❖ **Variant 1 was only observed using WGS?**

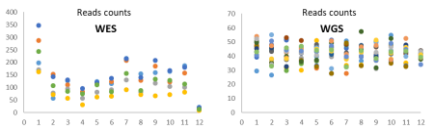

Figure 2: Distribution of the depth of coverage for each exon of *BBS5*

→WGS provides a **more uniform coverage distribution** despite less data

- **Variant 1 analysis**

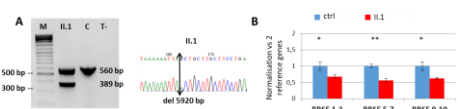

Figure 3: **A**. Duplex PCR and Sanger. **B**. qRT-PCR on RNA (ANOVA test). ****:p<0.0001

-Breakpoint sequencing and duplex PCR confirms the deletion (Fig.3A).
-Expression analysis revealed **only a single allele of *BBS5*** (Fig.3B).

Results and discussion

- **Variant 1 cohort screening**

-BBS cohort screening using duplex PCR is negative (n=430)
-Bioinformatics screening WGS cohort (FranceGenRef consortium) is negative (n=862)
-Despite being missed by panel & WES, the deletion of exon 1 and 2 is a rare variant.

- **Variant 2 analysis**

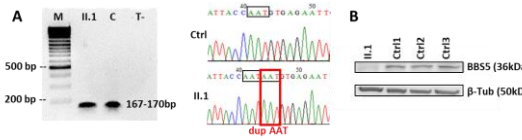

Figure 4: **A**. PCR and Sanger sequencing performed on RNA. **B**. WB of BBS5 protein expression

-Direct RNA sequencing revealed variant 2 at the homozygous state. Given the non expression of the allele carrier of variant 1, this demonstrate their **biallelic status (*in trans*)** (Fig.4A).
-No expression of BBS5 protein in patient's fibroblasts (Fig.4B). The duplication is likely destabilizing the protein. This redefines the variant 2 classification to **probably pathogenic (class 4)**

- **Ciliary cellular phenotype**

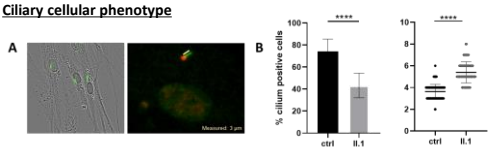

Figure 5: **A**. Cilia immunostaining using @ARL13B (green) and @γ-tub (red) of the patient's skin fibroblast. **B.** Quantification of ciliogenesis and measurement of cilia length. (Mann Whitney Test: ****: p<0.0001)

-The patient has 25% less ciliated cells compared to the control (n=900)
-The patients' cilia are longer than those of control (n=400)

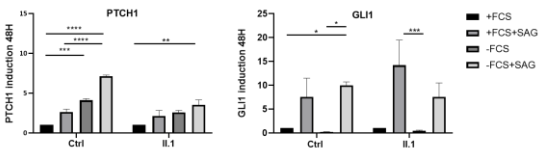

Figure 6: *PTCH1* and *GLI1* expression (qRT-PCR in 4 conditions, +/- FCS and SAG). (ANOVA test: ****:p<0.0001)

- SHH pathway activation is less important in the patient's fibroblasts

Conclusion

In this study we identified **two novel** variations in *BBS5*. We confirmed the biallelic status of the variant despite absence of parents' DNA as well as the **pathogenicity of both variations using the patient's cells (one variant reclassification)**. We also demonstrate alterations of the cilia morphology and function on the patient's cells. This study illustrates the usefulness of WGS sequencing to identify causative variations specially CNVs in highly heterogeneous genetic disorders.

[1] Goetz et al., 2009, [2] Gouronc et al., 2020, [3] Imani et al., 2019