Whole Genome Sequencing Identified Deep-Intronic *COL4A5* Splice Variants in Two Pediatric Cases of Alport Syndrome Undetected by Targeted Exome Analysis

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COI disclosure

presenter : Asahi Yamamoto

I have no relevant financial relationship
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Introduction

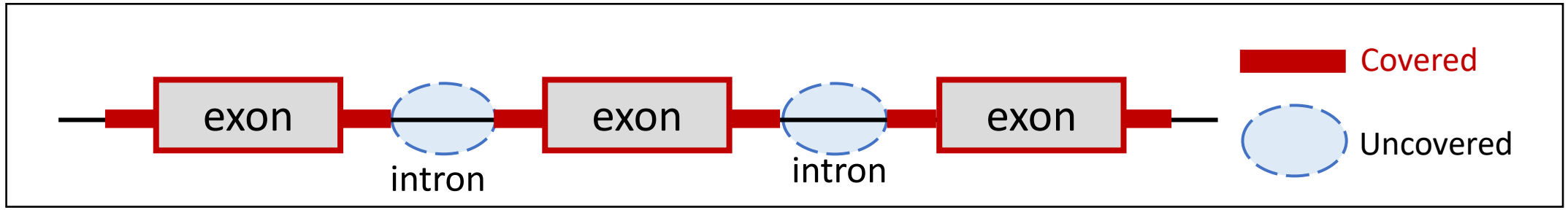
- **Alport syndrome** is a hereditary glomerular disorder caused by pathogenic variants in *COL4A3*, *COL4A4*, or *COL4A5*, which encode type IV collagen.

- Clinically, It is characterized by **hematuria**, **progressive kidney dysfunction**, **sensorineural hearing loss**, and **ocular abnormalities**.

- **Targeted exome sequencing** is widely used for genetic diagnosis.

Introduction

<targeted exome sequencing >



Its coverage is limited to exons and flanking intronic regions.

Deep-intronic variants cannot be detected by target exome sequencing.

→To detect them, **whole genome sequencing (WGS)** is required.

We report two pediatric cases of X-linked Alport syndrome
in which **WGS** identified **deep-intronic COL4A5 splice-altering variants**.

Case 1 : 16-year-old girl

【History of Present Illness】

・At 9 months, she had an episode of **gross hematuria**. Since then, she had persistent **microscopic hematuria and proteinuria.**

・At 2 years, a kidney biopsy showed a **basket-weave appearance of the glomerular basement membrane (GBM)** and a **mosaic α5 staining pattern.**

【Laboratory Findings】

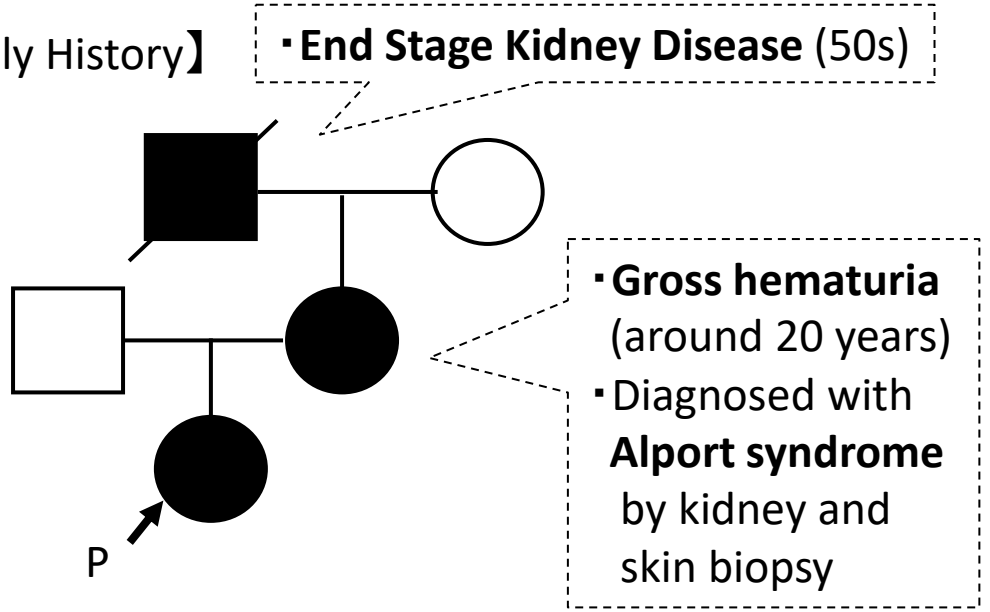
・Urinalysis : RBC 50-99 /HPF, TP/Cre 0.20 g/gCr

・Blood tests : Cr 0.63 mg/dL (eGFR 97.9 ml/min/1.73m^2)

【Target exome sequence (at 8 years)】

・**No pathogenic variant was detected.**

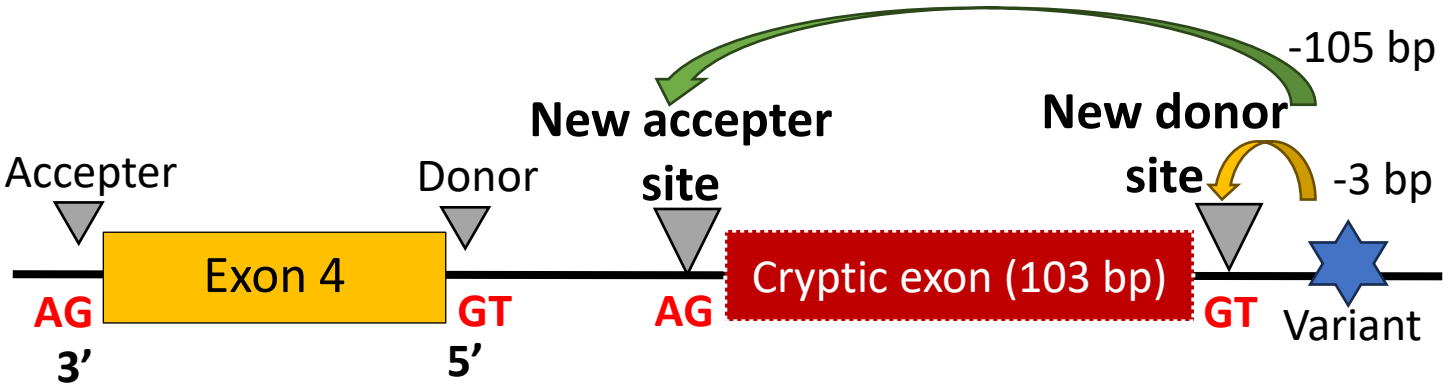
【Family History】



Case 1 : 16-year-old girl

【WGS】 *COL4A5* (NM_000495.5): c.276+1306G>A , located in intron 4

< Schematic overview of the splicing mechanism >

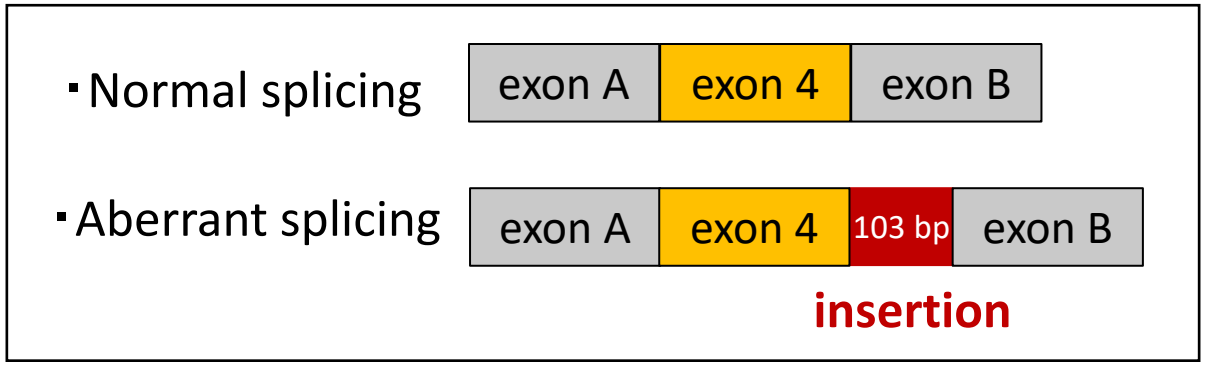


SpliceAI			
A-Loss	D-Loss	A-Gain	D-Gain
0.00 (779bp)	0.00 (-1306bp)	0.29 (-105bp)	0.61 (-3bp)

(A: Acceptor, D: Donor)

※SpliceAI: In-silico tool to predict aberrant splicing

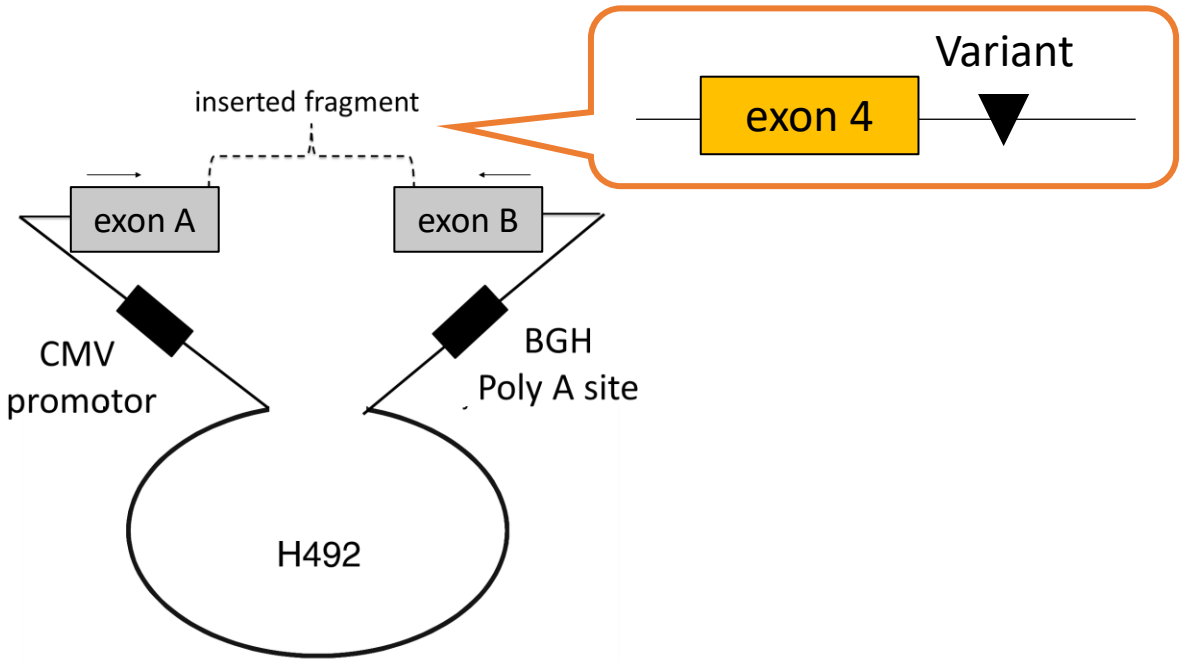
Kishore Jaganathan, et al. Cell. 2019; 176(3):535-548.



The variant was predicted causing aberrant splicing (insertion of a **103-bp cryptic exon**).

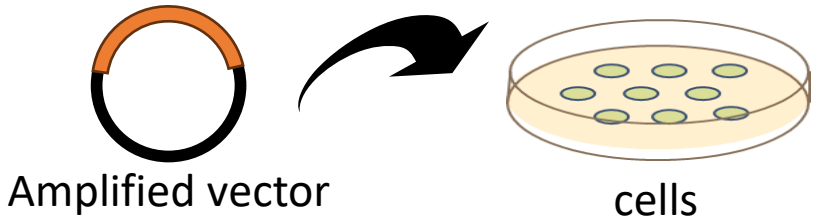
＜Minigene assay＞

Step 1 : Creating **DNA fragment** by PCR amplification



Step 2 : Inserting the fragment into **H492V vector** (Wild type or Mutant type)

Step 3 : Transfecting vectors into HEK293T or Hela cells



Step 4 : **RNA extraction** and **reverse-transcribed PCR**

- Normal splicing

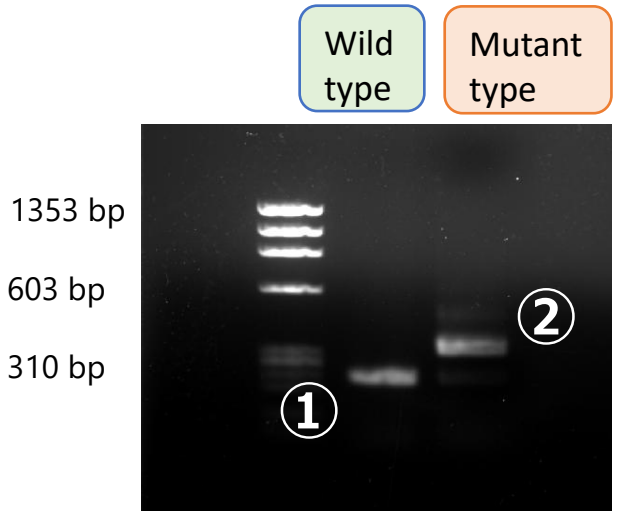
- Aberrant splicing

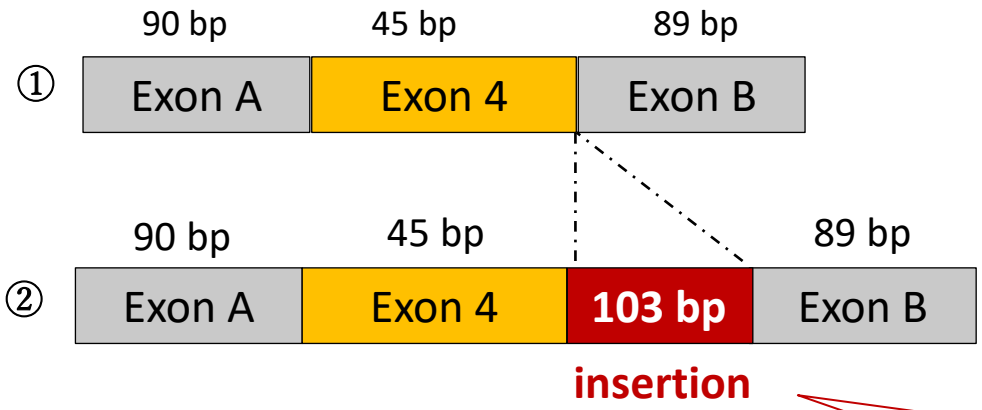
insertion

Predicted pattern of splicing in Case 1

Case 1 : 16 years-old girl

【Minigene assay】 *COL4A5* (NM_000495.5): c.276+1306G>A , located in intron 4





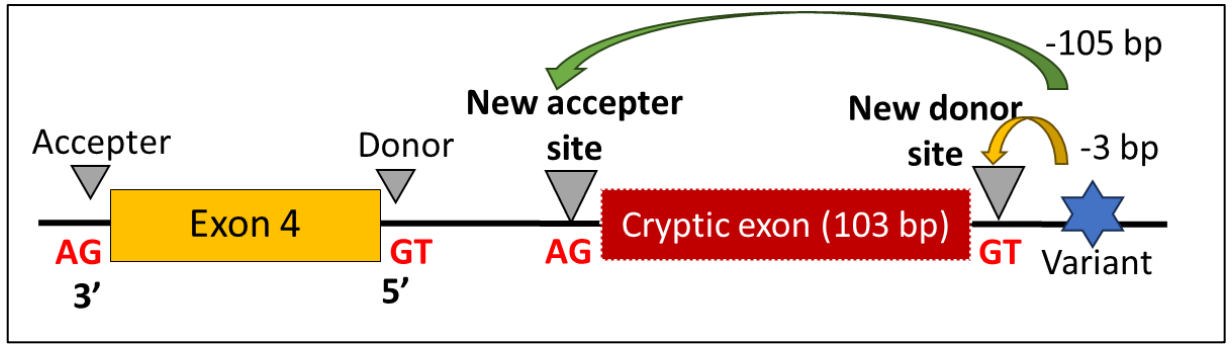
Wild type

224 bp

Mutant type

327 bp

Sanger sequencing confirmed the inserted sequence originated from **intron 4**.



Result

- Wild type (①) : Normal splicing
- **Mutant type (②) : Aberrant splicing with insertion of a 103-bp insertion pattern**

Case 1：16-year-old girl

【Summary of Case 1】

・Whole-genome sequencing identified **a deep-intronic *COL4A5* variant.**

・A minigene assay confirmed that the variant caused aberrant splicing with insertion of **a 103-bp cryptic exon**.

Final diagnosis

X-linked Alport syndtome

COL4A5(NM_000495.5): c.276+1306G>A　p.Gly93Phefs*99

Case 2：3 years-old girl

【History of Present Illness】

・Since infancy, **green-colored urine during upper respiratory infections**.

・At 2 years, **gross hematuria** was noted at nursery school.
Urinary test at local clinic showed hematuria (RBC 50-99～≧100 /HPF) and proteinuria (TP/Cre 0.56～1.1 g/gCr).

・She had no family history.

【Laboratory Findings 】

・Urinalysis: TP/Cre 0.78～3.1 g/gCr ・Blood Tests: Cre 0.22 mg/dL
（eGFR 148.4 ml/min/1.73m^2）

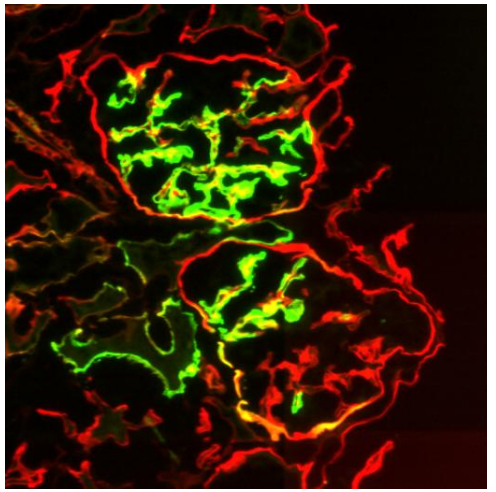
【Kidney Biopsy】

・**α5 mosaic pattern** ・Lamellation and basket-weave changes

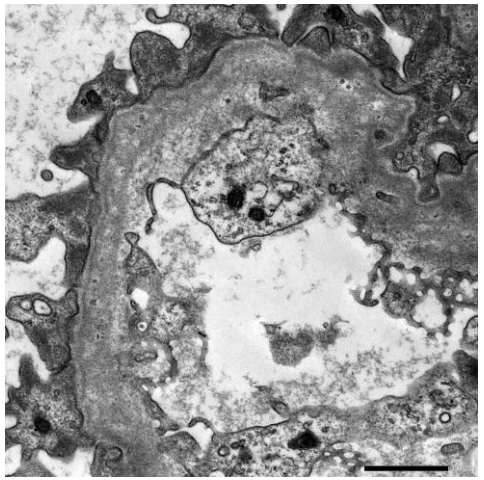
【Target exome sequence】**No pathogenic variant was detected.**

※These findings are from age 2.

▼ IF (α5 staining)



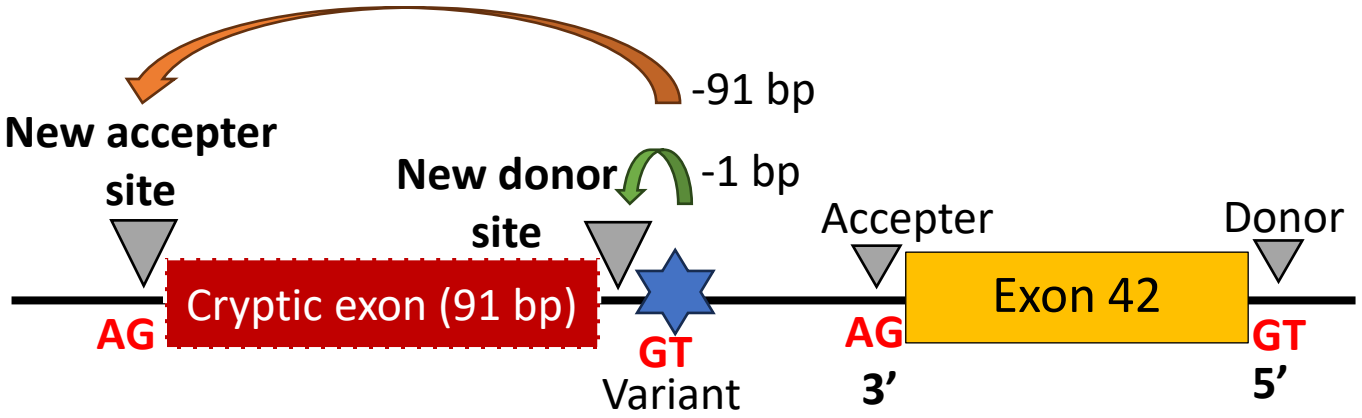
▼ EM



Case 2 : 3 years-old girl

【WGS】 *COL4A5* (NM_000495.5): c. 3791-1066A>G, located in intron 41

< Schematic overview of the splicing mechanism >



Splice AI			
A-Loss	D-Loss	A-Gain	D-Gain
0.00	0.00 (-368bp)	0.14 (-91bp)	0.31 (-1bp)

(A: Acceptor, D: Donor)

※SpliceAI: In-silico tool to predict aberrant splicing

Kishore Jaganathan, et al. Cell. 2019; 176(3):535-548.

- normal splicing: exon A | exon 41 | exon B
- Aberrant splicing: exon A | 91 bp | exon 41 | exon B

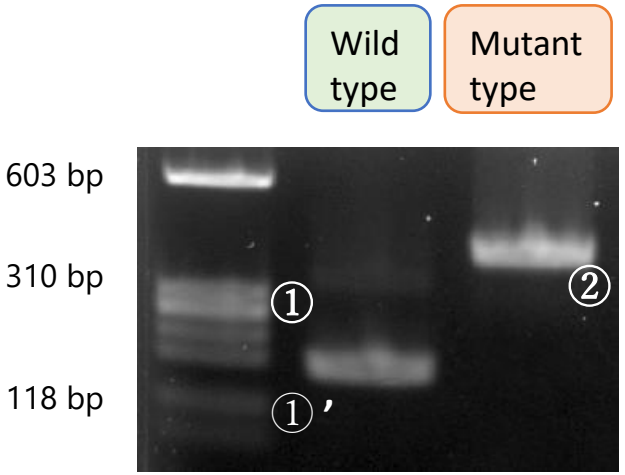
insertion

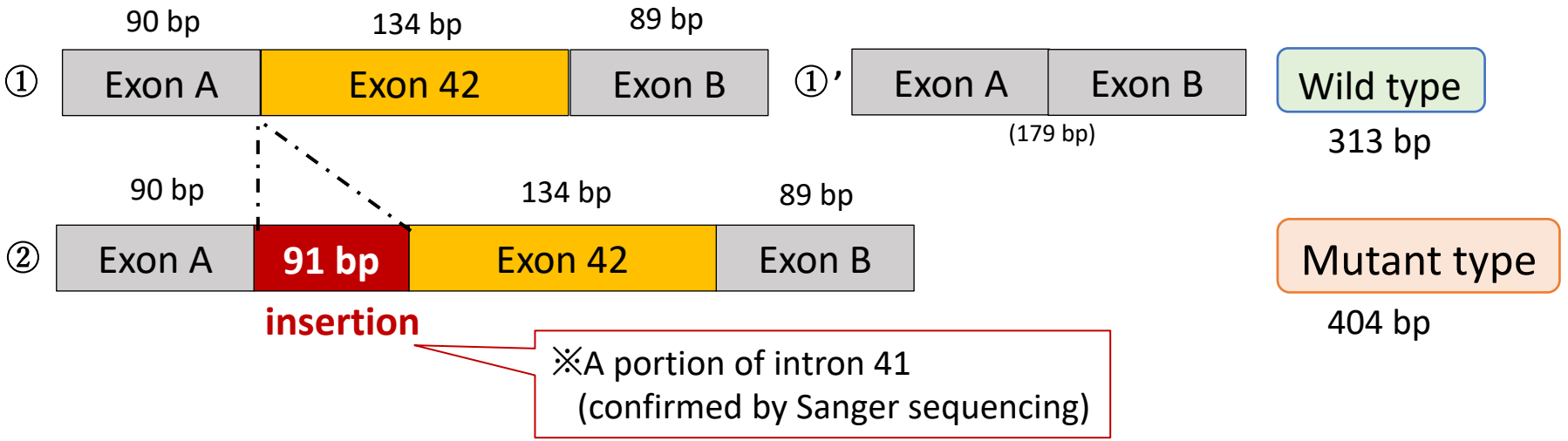
The variant was predicted causing aberrant splicing (insertion of a **91-bp cryptic exon**).

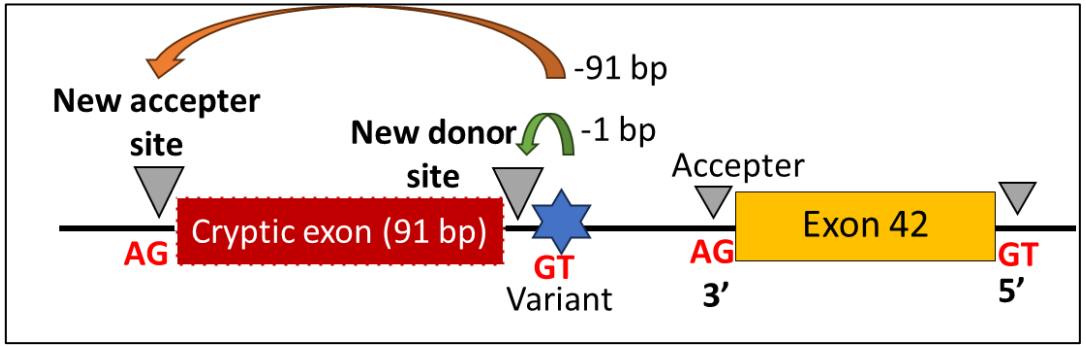
Case 2：3 years-old girl

【Minigene assay】

COL4A5 (NM_000495.5): c. 3791-1066A>G, located in intron 41







Result

- Wild type (①) : Normal splicing pattern
- **Mutant type (②) : Aberrant splicing with insertion of a 91-bp insertion**

Case 2：3-year-old girl

【Summary of Case 2】

・Whole-genome sequencing identified **a deep-intronic *COL4A5* variant.**

・A minigene assay confirmed that the variant caused aberrant splicing with insertion of **a 91-bp cryptic exon**.

Final diagnosis

X-linked Alport syndtome

COL4A5(NM_000495.5): c.3791-1066A>G p.Gly1264_Leu1265insArgIleThr*

Discussion : Deep-intronic variants of Alport syndrome

・**Deep intronic *COL4A5* variants can cause cryptic exon inclusion**, but are <u>not detectable by exome-based methods</u>.

・Previous RNA-based studies have shown that pathogenic variants may reside far from canonical splice sites *(e.g., Yamamura et al., 2019; Nozu et al., 2014)*.

・Recent studies, including WGS, have confirmed such deep intronic splice-altering variants *(e.g., Boisson et al., 2023; Qian et al., 2023)*.

Our two cases further expand this spectrum and demonstrate that
WGS is essential when exome sequencing is negative despite strong clinical suspicion.

Conclusion

- WGS identified deep-intronic *COL4A5* variants in both clinically suspected cases.

- These cases highlight **the diagnostic utility of WGS** for detecting deep-intronic pathogenic variants in patients with clinically suspected Alport syndrome.

- When targeted exome sequencing is negative but histological or clinical findings strongly support the diagnosis of Alport syndrome, WGS followed by functional validation should be considered to achieve a definitive genetic diagnosis.