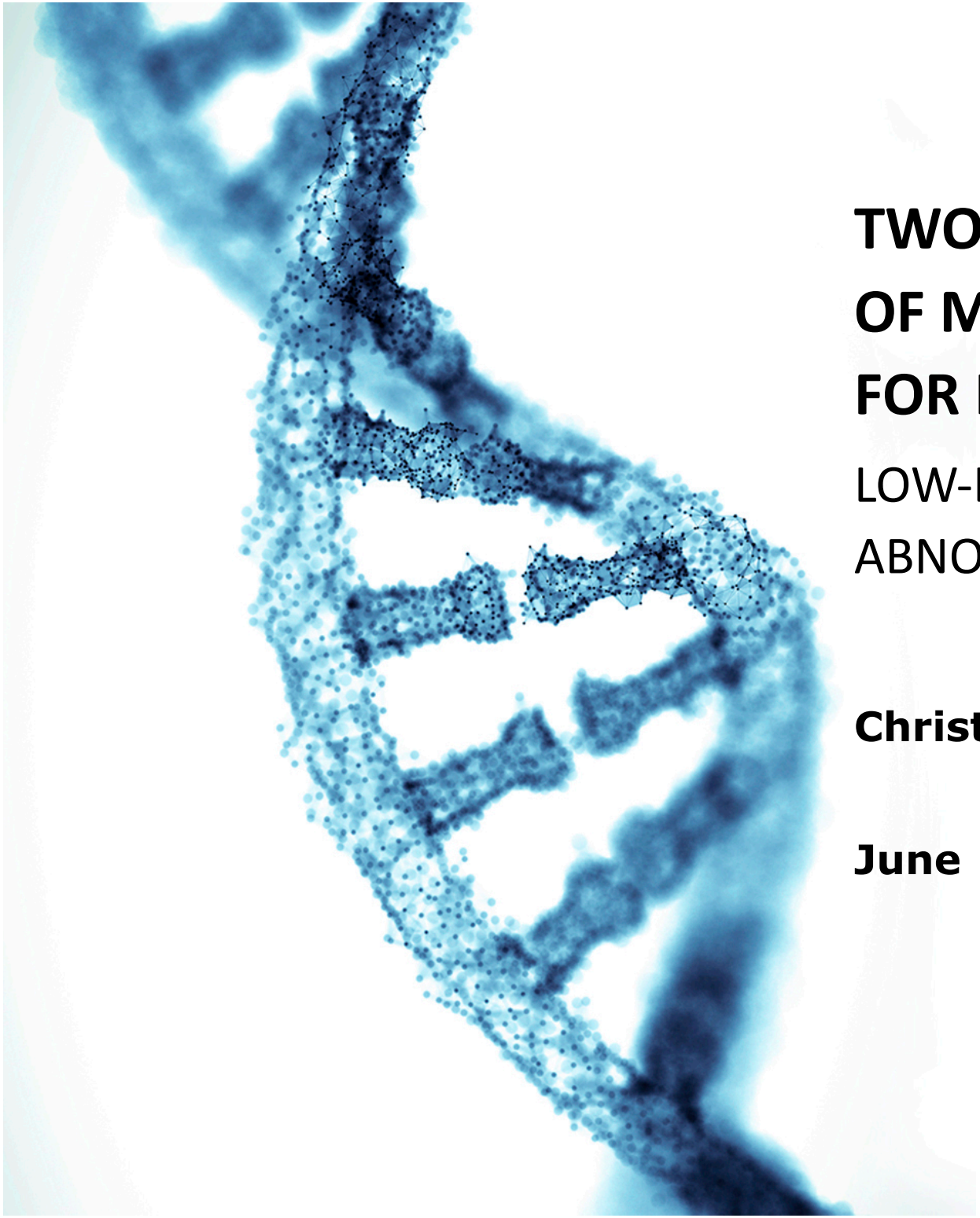

TWO INTERESTING CASES OF MOLECULAR DIAGNOSIS FOR HHT:

LOW-LEVEL MOSAICISM AND ABNORMAL SPLICING OF *ACVRL1*

Christopher Trevors, MSc, CGC

June 10, 2017

LabCorp
impact genetics

Disclosures:

Full time paid employee of Impact Genetics, Dynacare, LabCorp

Impact Genetics *Current* HHT Test Methods

ENG, ACVRL1, SMAD4	• Sequence analysis (by Sanger) • *ENG, ACVRL1:* Entire coding, flanking intronic, 5' UTR of *ENG* • *SMAD4*: Exons 8-11
ENG, ACVRL1	• Deletion/duplication analysis (by MLPA)
VUS Uncertain	• RNA analysis (by reverse-transcriptase PCR)

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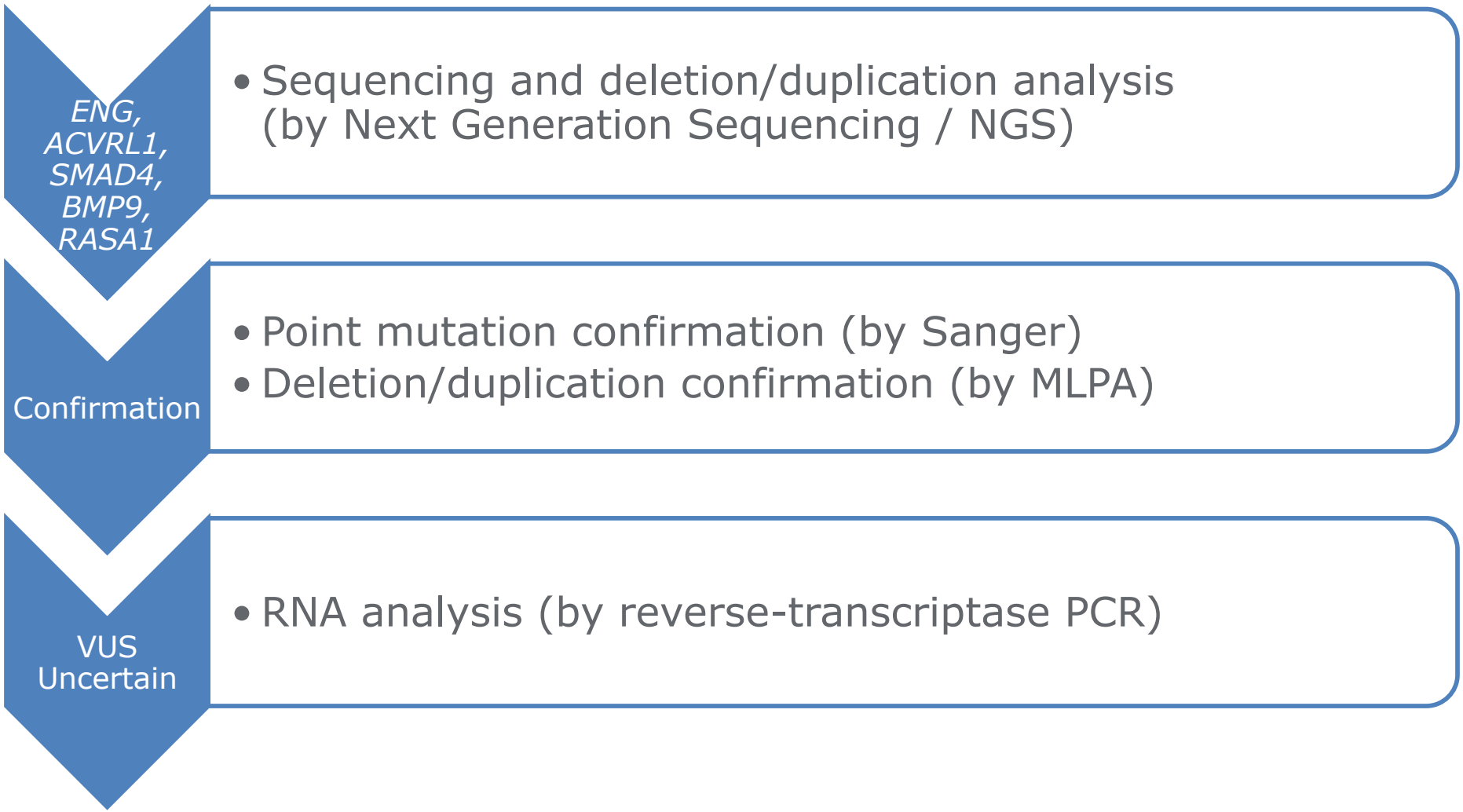
Impact Genetics *Future* HHT Testing



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Impact Genetics: Statistics (as of Jan 2017)

Including only patients meeting Curaçao criteria clinical sensitivity is 89.7%

Gene	Meet Curaçao criteria	Distribution
ENG	85%	46.2%
ACVRL1	79%	43.0%
SMAD4	1%	0.5%

Analytical sensitivity 99.9%

1.6% of pathogenic mutations were confirmed/detected via RNA analysis

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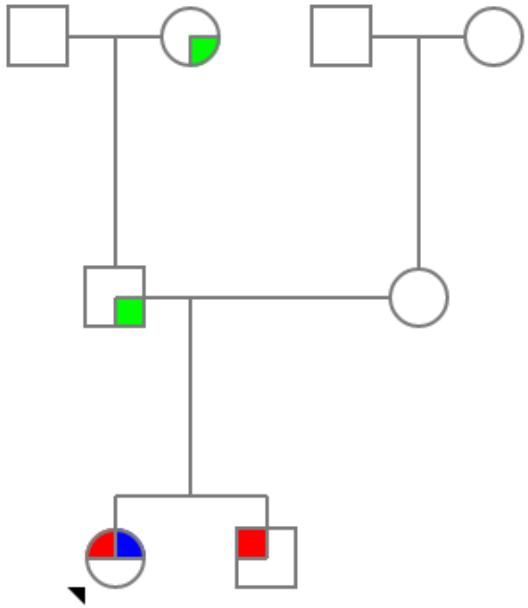
Case 1

Clinical Presentation

- 62 year old female
- Personal history (Suspected HHT – 2 Curaçao Criteria)
  - Epistaxis
  - Telangiectasias (nares, fingertips, superficial cutaneous)
- Family history (questionable)

Pedigree

Paternal Uncle – died aneurysm
Paternal Grandmother – died aneurysm
Full sister – epistaxis as child



Genetic test results

Initial analysis

- *ENG, ACVRL1* (sequencing, del/dup)
- *SMAD4* (sequencing exons 8-11)
- **No mutation found**

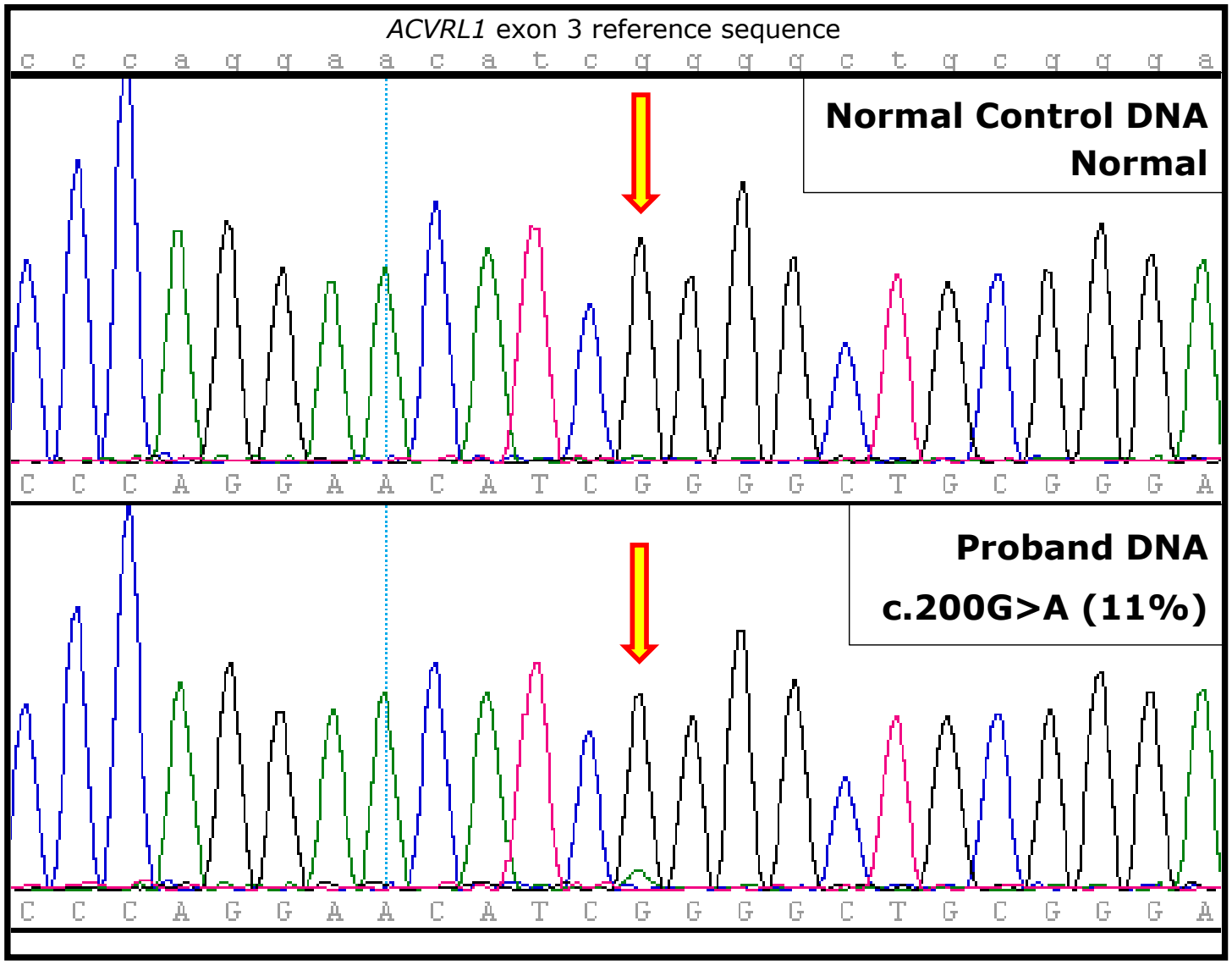
Additional Clinical Info Obtained

- Patient had telangiectasias, all on the RIGHT side of her body
- Suspicious of mosacism?

Follow-up Analysis

- All data reassessed for low level genetic changes
- Alternative primers and allele-specific PCR used to confirm **mosaic (~11%)** finding of a known, missense mutation **c.200G>A(p.Arg67Gln) in *ACVRL1***

Mosaic *ACVRL1* c.200G>A



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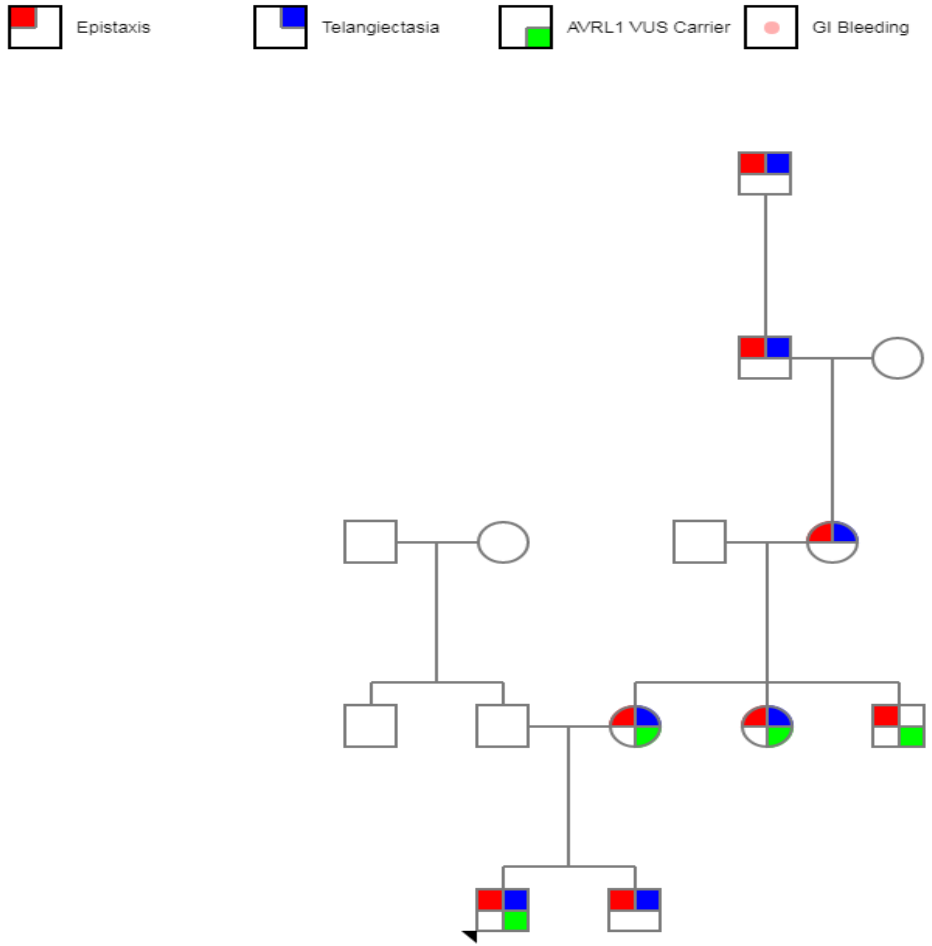
Case 2

Clinical Presentation

- 14 year old male
- Personal history (2 Curaçao Criteria)
  - Epistaxis
  - Telangiectasias
- Strong family history
- Previous genetic testing
  - *ACVRL1* – VUS found in other family member c.625+56G>A
  - *ENG* - Normal
  - *SMAD4* - Not performed

Pedigree



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Genetic test results

Initial Analysis

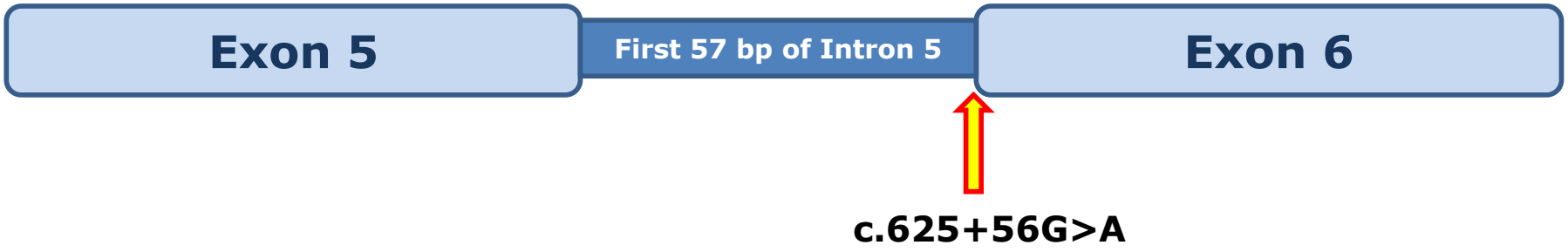
- *SMAD4* performed at Impact Genetics – normal
- *ACVRL1* – VUS present in our patient which segregated strongly through family

Functional (RNA) Analysis

- *ACVRL1* RNA analysis identified proportion of *ACVRL1* transcripts with abnormal splicing
- Causing retention of *ACVRL1* intron 5 nucleotides from c.625+1 to c.625+57
- Cryptic donor site created by the G>A substitution was used preferentially over the canonical donor site = shift in the reading frame

Supports that this substitution is likely to be pathogenic

Effect of *ACVRL1* c.625+56G>A VUS on mRNA splicing



ACVRL1 mRNA reference	Exon 5 | Exon 6
Proband cDNA r.625_626ins625+1_625+57	Minor sequence: Intron 5
Normal control cDNA No aberrations detected	

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Conclusion

- Clinical information is essential in maximizing the potential of genetic testing
- Approach to genetic testing must be flexible
- Understanding the pros and cons of different laboratory techniques is important when selecting diagnostic laboratories
- Genetic Counselors are pivotal in patient care
- New genetic technology, applied in the right way, is prerequisite for diagnosis and therapeutics
  - Pharmaceutical therapy
  - CRISPR

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