# TWO INTERESTING CASES OF MOLECULAR DIAGNOSIS FOR HHT: LOW-LEVEL MOSAICISM AND ABNORMAL SPLICING OF *ACVRL1*

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OBJECTIVES: Here we describe two cases demonstrating pathogenic changes in *ACVRL1* that can be difficult to identify using routine genetic testing procedures. For the first case, when subsequent clinical description of the proband indicated the potential for mosaicism, sequencing data was reassessed to investigate for low-level mutations. For the second case, a familial variant of uncertain significance (VUS) had been identified in intron 5 of the *ACVRL1* gene. Using RNA analysis, we investigated the functional impact of this previously unreported variant.

METHODS: To confirm low-level mosaicism, alternate primers were employed to bi-directionally re-sequence a suspicious alteration detected in the original *ACVRL1* sequencing trace. The sample was also tested by an allele-specific PCR assay. To determine splicing aberrations in the second case, the *ACVRL1* cDNA coding sequence was analyzed using Sanger sequencing to characterize splicing at the exon 5/6 junction.

RESULTS: We identified an *ACVRL1* mosaic c.200G>A mutation in the first patient with suspected HHT, estimated to be present on one allele in 22% of the patient's blood leukocytes. The previously observed familial VUS (c.625+56G>A) predicted to cause a cryptic preferred splice site in the second patient was shown by *ACVRL1* cDNA sequencing to cause a proportion of an affected patient's transcripts to have abnormal retention of nucleotides c.625+1 to c.625+57.

CONCLUSIONS: Communication of clinical symptoms to clinical laboratories greatly facilitates their investigations of pathogenetic changes. RNA analysis findings of aberrant splicing associated with a VUS can support molecular and clinical diagnosis of HHT.