Title: RNA splicing analysis reclassified two variants of uncertain significance in an autosomal recessive Noonan syndrome case

Authors & Affiliations: Nora Urraca (Division of Genetics, University of Tennessee Health Science Center), Holly Lydigsen (Division of Genetics, University of Tennessee Health Science Center), Shoji Ichikawa (Ambry Genetics), Victoria Suslovitch (Ambry Genetics), Roya Mostafavi (Le Bonheur Children's Hospital)

Abstract: Noonan Syndrome (NS) is a genetic disorder with an estimated prevalence of 1 in 1,000-2,500 live births. It is characterized by distinctive facial features, short stature, congenital heart defects, and varying degrees of developmental delay. To date, variants in 15 genes involved in the RAS/MAPK signaling pathway are associated with NS. NS is usually inherited in an autosomal dominant manner; however, LZTR1 is the only gene known to cause autosomal recessive NS.

We present a 3-year-old female referred to Genetics at one month of age due to a prenatal finding of cystic hygroma. She was born full term to non-consanguineous parents, and her birth weight (50ile) and length (25ile) were within normal range. She required NICU admission for 3 days due to respiratory distress and intubation. Initial exam showed bilateral epicanthal folds, posteriorly rotated low-set ears, a short, webbed neck, and widely spaced nipples. An echocardiogram identified an atrial septal defect. Karyotype was 46,XX. A RASopathy panel identified two variants of uncertain significance (VUS) in the LZTR1 gene: paternally inherited c.898G>A (p.G300R) and maternally inherited c.1149+20T>G. In silico models predicted that these variants create a novel acceptor and donor splice site, respectively. Due to strong clinical suspicion for NS, RNA analysis was performed and showed both variants disrupted normal splicing. The paternal variant caused an in-frame deletion of 36 amino acids; the maternal variant caused partial intron retention, resulting in an out-of-frame transcript. This led to their reclassification as likely pathogenic.

Currently, the patient has characteristic NS facial features, short stature, left ventricular hypertrophy, and speech delay. RNA analysis was key in establishing a definitive diagnosis by demonstrating aberrant splicing, which led to upgrading both VUSs to likely pathogenic. VUSs are not considered medically actionable, which limits their utility in clinical decision-making and genetic counseling. Therefore, without the functional evidence from RNA in this case, diagnosis would not have been confirmed. Functional assays can clarify the impact of variants on the protein product. When RNA analysis demonstrates aberrant splicing, variants may be reclassified as pathogenic. Without RNA analysis in this patient, accurate recurrence risk for autosomal recessive inheritance would not have been

possible. RNA analysis is thus essential in interpreting suspected splice-affecting VUSs when gene expression is adequate in blood.