RNA Splicing Analysis Reclassified Two Variants of Uncertain Significance in an Autosomal Recessive Noonan Syndrome Case

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INTRODUCTION

Noonan Syndrome (NS) is a genetic disorder with a 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.

CASE DESCRIPTION

A one-month-old female was referred to Genetics due to a prenatal finding of cystic hygroma. She was born at full term to non-consanguineous parents, with a birth weight of 3.2 kg (50th %ile) and a length of 48.2 cm (25th %ile). She required a 3-day NICU admission due to respiratory distress and intubation. Initial examination revealed NS facial features (**Figure 1**) and widely spaced nipples. An echocardiogram identified an atrial septal defect.

Figure 1. Front and profile pictures at 1 month of age demonstrating bilateral epicanthal folds, a short neck, and posteriorly rotated, low-set ears

RESULTS

- Karyotype revealed a 46,XX chromosomal complement.
- RASopathy panel identified two variants of uncertain significance (VUS) in *LZTR1*: paternally inherited c.898G>A (p.G300R) and maternally inherited c.1149+20T>G.

In silico models predicted that these variants create novel acceptor and donor splice sites, respectively. To exclude other genetic syndromes and further explore the impact of the *LZTR1* variants, WES + RNA analysis was performed. WES was negative for other reportable variants. RNA demonstrated abnormal splicing caused by both variants (**Figure 2**), which led to reclassification of both VUSs to likely pathogenic.

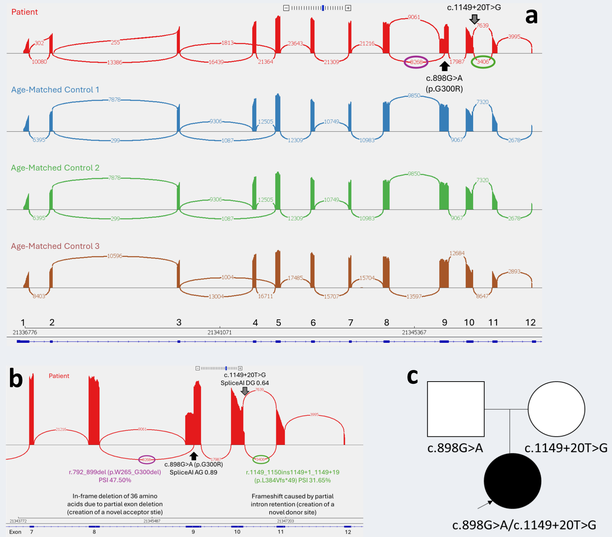

Figure 2. Sashimi plot of RNA-seq analysis. a) Comparison of the patient sample with three wild-type blood controls for the *LZTR1* gene. **b)** Magnified view demonstrates disruption of normal splicing in both variants. **c)** Pedigree shows biparental inheritance of the *LZTR1* variants in this autosomal recessive NS case.

Currently, at 4 years of age, the patient presents with characteristic NS facial features (**Figure 3**), short stature (106.5 cm; 0.1st percentile), left ventricular hypertrophy, and speech delay.

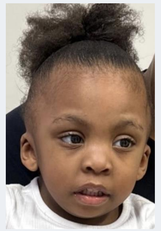

Figure 3. Front picture at 3-years. Tall forehead, bilateral epicanthal folds, short neck and low-set ears

DISCUSSION

- Genetic testing approach was guided by strong clinical suspicion of NS.
- RNA analysis was key in establishing a definitive diagnosis by demonstrating aberrant splicing, which resulted in the reclassification of both VUSs to likely pathogenic.
- VUSs are not considered medically actionable, which limits their utility in clinical decision-making and genetic counseling.
- Functional assays can clarify the impact of genetic variants on the protein product.

CONCLUSION

- An accurate recurrence risk for autosomal recessive inheritance would not have been possible without RNA analysis in this patient.
- RNA analysis is thus essential to interpreting VUSs suspected to impact splicing when gene expression is adequate in blood.

We would like to thank this family for allowing us to present this case.