RIPPLY2: Believe it or not

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CLARITAS GENOMICS

Here we discuss the case of a newborn male who presented with multiple vertebral anomalies. Whole exome sequencing was performed when the patient was 9 months old and uncovered compound heterozygous *RIPPLY2* variants. This case report expands the understanding of *RIPPLY2* to be a gene that impacts vertebral development.

Clinical Description

The proband was born to non-consanguineous parents via vaginal delivery. Cervical spine instability due to an abnormal C2 vertebra, hypoplasia of the other cervical vertebrae, craniocervical junction stenosis, and spinal cord compression led to a spinal cord injury during labor and delivery, resulting in respiratory failure, hypotonia and decreased mobility. Upon close inspection of CT images, it appears the proband had an abnormal C2 vertebra due to absence of the posterior elements. He subsequently became hypertonic with spasticity and muscle spasms and continues to be trach-dependent. The vertebral anomalies present were most similar to, yet distinctly different than those involved in Klippel-Feil syndrome. Prior to exome sequencing, a Klippel-Feil genetic testing panel was performed, which included sequencing and deletion/ duplication testing for *GDF6*, *MEOX1*, & *GDF3*, but was negative. This vertebral anomaly is extremely rare and no genetic causes have been identified to date.

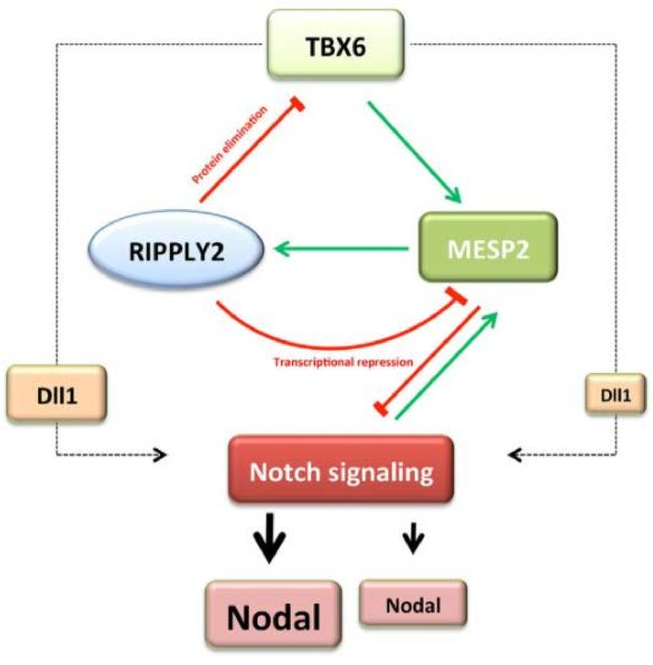

Figure A. RIPPLY2-TBX6-MESP2 signaling pathway. Am J Med Genet A. 2015; 167A(11): 2795–2799.

Molecular Genetics of *RIPPLY2*

Clinical exome testing, performed at Claritas Genomics using their orthogonal sequencing approach[1], identified two missense variants in *RIPPLY2* in a *trans* configuration; a c.240-4T>G and a c.286G>A (p.Ala96Thr) variant. *RIPPLY2* encodes a transcriptional repressor which functions in the NOTCH signaling pathway via a negative feedback loop and is essential for vertebrate somitogenesis and rostrocaudal patterning of somites; several other genes involved in this pathway have been associated with segmentation defects of the vertebrae (SVD) in humans.

In mouse somitogenesis, the somite boundary is formed by a genetic cascade activated by the Notch signaling pathway. This pathway begins with *Mesp2* periodically being activated by Notch in a Tbx6 dependent cascade in the anterior domain of the somite. The expression of Mesp2 creates the new posterior segment boundary of the somite. Mesp2 is subsequently downregulated in the caudal half of the somite while maintained in the rostral half. Once Mesp2 protein is produced, Tbx6 signal is repressed, creating the anterior border of the somite[2]. *Ripply2* is a direct transcriptional target of Mesp2 and Tbx6. As such, the Ripply2 protein forms a negative feedback loop, by repressing *Tbx6*-mediated transcription from the *Mesp2* promoter. In the absence of Ripply2, Mesp2 is expressed over a longer period of time[3,4].

In *Ripply2*-null mice, embryos show somite segmentation defects, resulting in a rostralized phenotype, as well as a reduction in the number of ribs and fused or missing pedicles of the neural[4,5]. However, heterozygous mice were viable and lacked skeletal abnormalities[4].

The c.240- 4T>G variant identified in this individual was previously reported in the compound heterozygous state with a nonsense variant in two siblings with multiple regional segmentation defects of the vertebrae. One sibling had failure of formation of the posterior elements of C1 to C4 resulting in canal stenosis and cord compression, while the other sibling had deficiency of the posterior elements of C1–C3. Both siblings had hemivertebrae resulting in kyphoscoliosis. Although the effects of the splicing variant could not be tested, as *RIPPLY2* expression could not be detected in peripheral blood, this variant was predicted to affect the splice acceptor consensus sequence[6].

The c.286G>A variant in *RIPPLY2* is unreported in the literature and control datasets. It occurs at a highly conserved nucleotide and amino acid in the conserved Ripply homology domain, which interacts with the T-box domain of *TBX6* and is required for transcriptional repression. In addition, this variant is predicted to be deleterious by in silico prediction models. The two identified variants were observed on opposite strands in the NGS data, suggesting a *trans* configuration. However, due the limited information available regarding these variants, they were classified as uncertain.

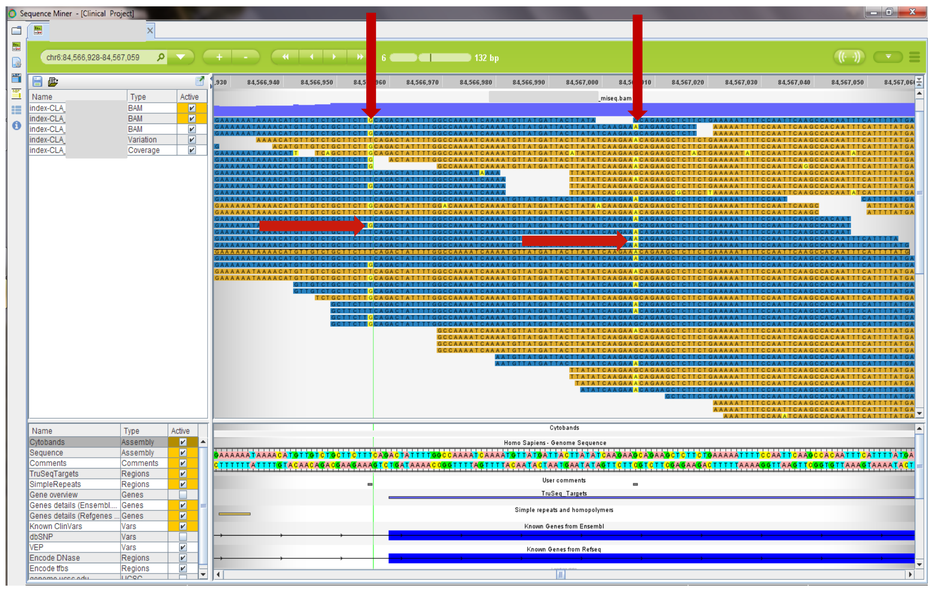

Figure B. NGS reads showing the RIPPLY2 variants on opposite alleles visualized in the WuXi NextCODE SequenceMiner software.

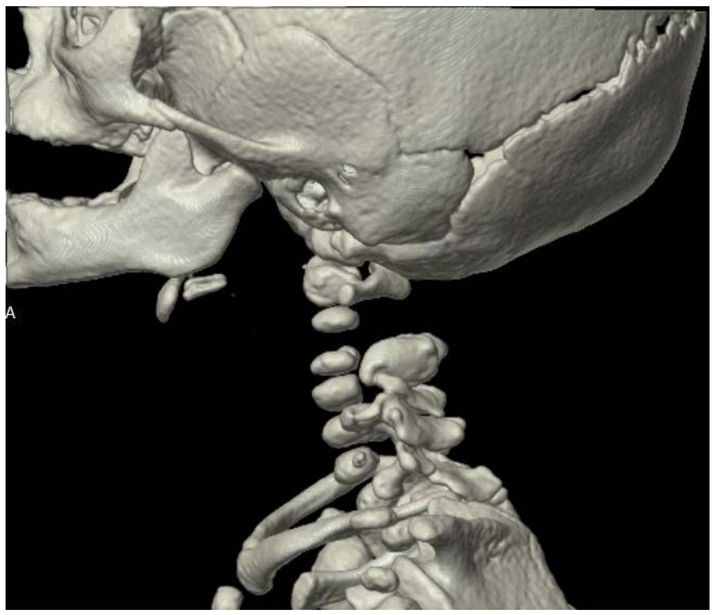

Figure C. CT scans showing a partial but abnormal C2 vertebral body of with the posterior elements missing.

Discussion

In this current patient, we describe a rare disorder with clinical implications and suggest a biological mechanism for disease. This case highlights the role of *RIPPLY2* in vertebral development and provides further evidence that *RIPPLY2* is a gene responsible for autosomal recessive malformation of the cervical vertebrae. Although biallelic variants in *RIPPLY2* have been reported in an individual with classic Klippel-Feil syndrome[7], it has also been observed in individuals with other segmentation defects of the vertebrae. While Klippel-Feil is typically characterized by fusion of the cervical vertebrae, here we present a case characterized by absence of elements of the cervical vertebrae. The biological similarities between the previously reported affected individuals and our patient suggest *RIPPLY2* may have biological implications in a new syndrome. However, functional studies are necessary to determine the pathogenicity of the identified variants and to fully evaluate the phenotypic spectrum of disease associated with this gene.

Conclusion

Here we described a rare, but clinically manageable phenotype, and suggest a possible molecular cause rooted in developmental biology, which highlights the clinical utility of whole exome sequencing and genomic testing.

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