Supplement

PCR and Sanger Sequencing

Forward Sequence	Location	Reverse Sequence	Location	Expected Band Size (bp)
Genomic DNA- Variant Validation				
GACTTTGTGTAAACTTCGCGG	Intron 1	AAGAAACAAGTCACTCGTCTGA	Intron 2	407
cDNA – Canonical Transcript/ Exon 2 Skipping				
CAGTTTAATTCTCCGGCGGC	Exon 1	ACATGTCTGTCTGTGGTGGT	Exon 5	601
cDNA – Intron Retention				
AGTCTCTTTGCAGTCGGGAA	Intron 1	TGCCATCAATTTCAGCCTGC	Exon 3	345

Next Generation Sequencing*

Forward Sequence	Location	Reverse Sequence	Location	Expected Band Size (bp)
cDNA – Canonical Transcript/ Exon 2 Skipping				
CTGCAGATTGACGGGACGAGAT	Exon 1	TGCCATCAATTTCAGCCTGC	Exon 3	346
cDNA – Intron Retention				
TAGTCTCTTTGCAGTCGGGAA	Intron 1	TGCCATCAATTTCAGCCTGC	Exon 3	346

*Standard Illumina adaptors plus unique barcodes were added to each NextGen primer

Supplemental Figure S1. Genomic DNA Chromatogram. Sanger sequencing of DNA from the proband validated 2 variants in *RTTN:* c.32-3C>T and c.190G>T; p.V64F indicated by black arrows. The c.190G>T; p.V64F variant is inherited from the mother and the c.32-3C>T variant is inherited from the father.

Supplemental Figure S2. We designed primers to amplify cDNA region from exon 1 to exon 5 (wild type transcript). **S2a.** All 3 samples (proband, mother (M), and father (F) revealed an expected band size of 601 bp (wild type transcript) as well as a lighter, smaller band at 407 bp (transcript lacks exon 2)

S2b. PCR amplification of cDNA using a primer pair designed to identify a cryptic splice site in intron 1 revealed bands only in the samples from the proband and father. Gel purification and sequencing of these bands demonstrated aberrant splicing and retention of intron 1.

Supplemental Figure S3. Sequence chromatograms of gel purified PCR bands from the proband in Figure 2 (main text).

S3a. Upper band (601 bp): Sequence demonstrates canonical exon 1-exon 2 splicing.

S3b. Lower band (407 bp) Sequence demonstrates exon 1- exon 3 sequence indicating aberrant splicing and exon 2 skipping.

S3c. Sequence demonstrates intron 1 retention indicating aberrant splicing from upstream cryptic splice site

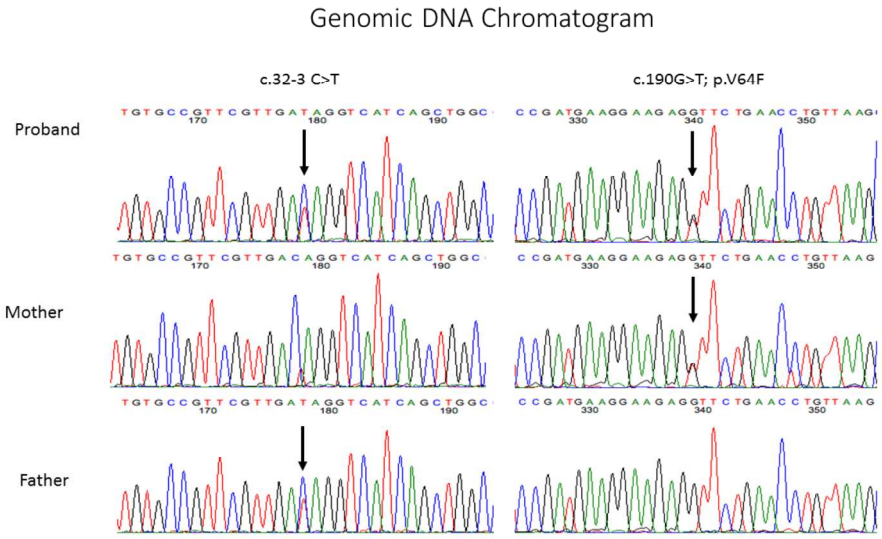
S3d. Sequence demonstrates canonical exon 2-3 splicing indicating this is cDNA and not genomic DNA contamination.

Supplemental Figure S4. Canonical and aberrant splicing of *RTTN*

Schematic representation of canonical splicing and aberrant splicing resulting in exon 2 skipping or intron 1 retention.

Supplemental Table. Next Generation Sequencing of PCR Products from Amplification of cDNA Region Exons 1-5 of *RTTN*

Sample	Coverage (x)	WT Transcript	Aberrantly Spliced Transcript
Proband	8977	0.29	0.71
Mother	682	0.71	0.29
Father	2374	0.39	0.61
Control 1	2003	0.62	0.38
Control 2	349	1.0	0
Control 3	1515	0.80	0.20
Control 4	1769	0.77	0.23



Supplemental Figure S1. Genomic DNA Chromatogram. Sanger sequencing of DNA from the proband validated 2 variants in RTTN: c.32-3C>T and c.190G>T; p.V64F indicated by black arrows. The c.190G>T; p.V64F variant is inherited from the mother and the c.32-3C>T variant is inherited from the father.

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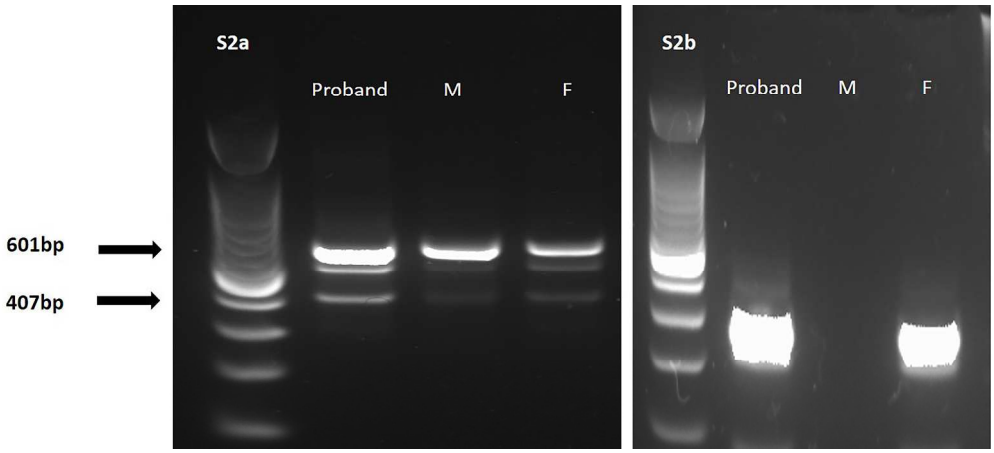

Supplemental Figure S2. We designed primers to amplify cDNA region from exon 1 to exon 5 (wild type transcript). S2a. All 3 samples (proband, mother (M), and father (F) revealed an expected band size of 601 bp (wild type transcript) as well as a lighter, smaller band at 407 bp (transcript lacks exon 2)
S2b. PCR amplification of cDNA using a primer pair designed to identify a cryptic splice site in intron 1 revealed bands only in the samples from the proband and father. Gel purification and sequencing of these bands demonstrated aberrant splicing and retention of intron 1.

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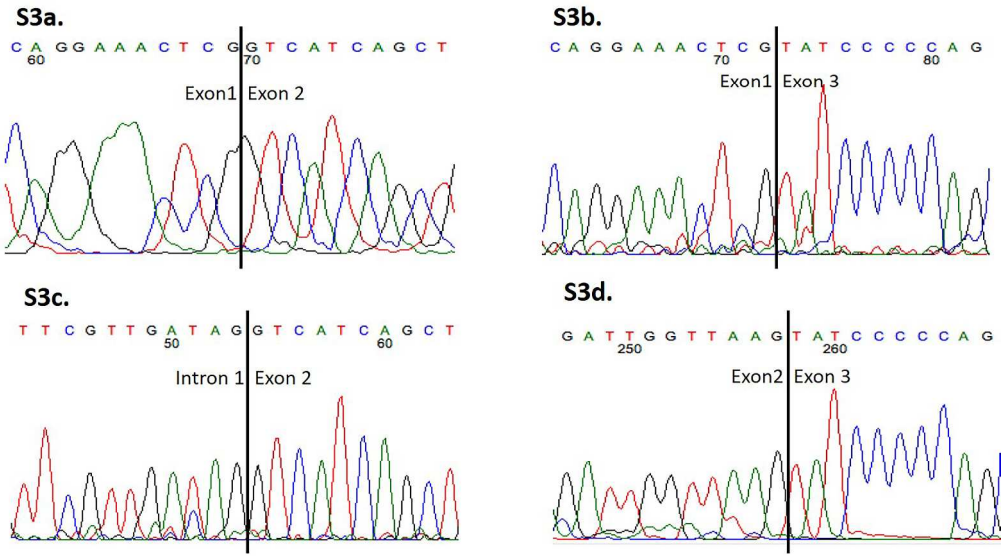

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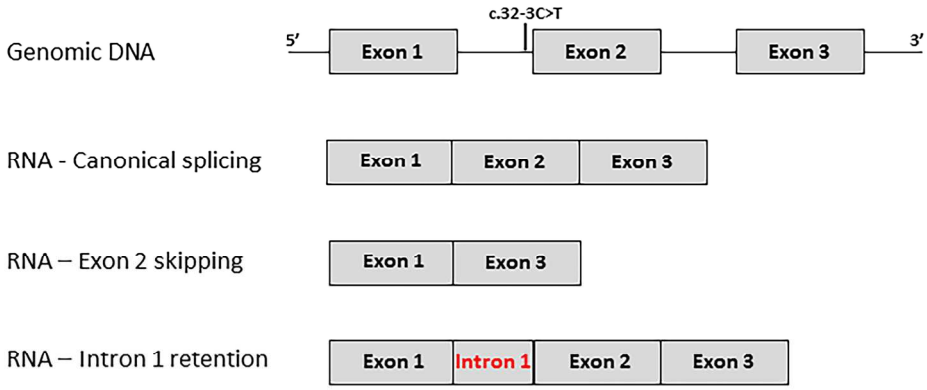

Supplemental Figure S4. Canonical and aberrant splicing of RTTN
Schematic representation of canonical splicing and aberrant splicing resulting in exon 2 skipping or intron 1 retention.

188x91mm (600 x 600 DPI)