JCI The Journal of Clinical Investigation

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J Clin Invest. 2024. https://doi.org/10.1172/JCI178919.

Research Letter In-Press Preview Genetics Neuroscience

Bi-allelic variants in *RINT1* present as early-onset pure hereditary spastic paraplegia

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Conflict of interest statement: The authors have declared that no conflict of interest exists.

Research Letter

The hereditary spastic paraplegias (HSPs) are a group of over 80 neurogenetic disorders that share the feature of progressive lower limb spasticity. Bi-allelic loss-of-function variants in the *RINT1* gene have been implicated in acute liver failure in the pediatric population (1) and were recently described to lead to a complex form of HSP in three children with early-onset spastic paraplegia, ataxia, optic nerve hypoplasia with significant vision impairment, dysmorphic features, and a thin corpus callosum (2). We would like to add a fourth case due to novel *RINT1* variants presenting with a largely 'pure' form of HSP (Supplementary Table_1). Methods are detailed in Supplementary File_1.

The patient is a 4-year-old female, who was 2.5-years old at the time of initial referral. She was born after an uncomplicated pregnancy and delivery to non-consanguineous healthy parents of Swedish heritage. Family history was unremarkable. No dysmorphic features were identified. While she met all early developmental milestones appropriately, first concerns arose when she was found to have progressive in-toeing and toe walking, starting at around 15-months of age (Video_1 & Supplementary File_1). Symptoms rapidly progressed with a decline in gait and frequent falls, leading her to stop walking at 18-months. She regained the ability to walk with assistance at 20-months of age (Video_1). At 3.5-years, her exam was notable for distal lower extremity spasticity (Modified Ashworth Scale 3 in gastrocnemius and soleus bilaterally) and weakness, prominent pyramidal signs, and a spastic-ataxic gait (Video_1), necessitating ankle-foot-orthoses for walking and making running or climbing stairs impossible.

Now at 4-years of age, clinical features have remained stable with no new manifestations or progression. Notably absent are features of complex HSP such as delays in speech and cognitive domains. Brain and spine MR imaging, including MR spectroscopy, liver function tests and routine laboratory studies were normal. Clinical exome sequencing was non-diagnostic. Research trio genome sequencing revealed rare compound heterozygous truncating variants in *RINT1* (NM_021930.6: c.1501C>T, p.(Arg501Ter); c.1671_1671+2del, p.(Val557del)). Direct gene sequencing of the proband and parents confirmed these

variants in trans. Both variants are observed at low frequencies in gnomAD 4.0 (MAF 1.098E-5, 1.602E-5). The nonsense variant (c.1501C>T, p.(Arg501Ter)) was predicted to result in premature termination and nonsense-mediated decay. The 3-bp deletion (c.1671_1671+2del, p.(Val557del)) had been previously identified in two individuals by two diagnostic laboratories and classified as a variant of uncertain significance (ClinVar_ID: 224920). No phenotypic information was available. This variant was predicted to maintain the reading frame and preserve the canonical splice junction, moving the splice donor site 2-bp upstream (SpliceAI Donor Loss 0.98 at 1bp, Donor Gain 0.85 at -2bp) and to result in a mutant protein missing a single amino acid at p.(Val557del) (Figure_1A). RNA sequencing in the patient's fibroblasts confirmed these predictions: At the splice junction associated with the 3-bp deletion, loss of the codon for Val557 was demonstrated (Supplementary Figure_1A). The absence of transcripts containing the codon for Val557 also supported the prediction that the transcripts from c.1501C>T, p.(Arg501Ter) indeed undergo nonsense-mediated decay. Overall, these data indicate that the *RINT1* variants in our patient allow for residual expression of a mutant protein p.(Val557del) (Supplementary Figure_1B), which contrasts variants report by Launay et al. (2).

To determine the functional impact of this, further experiments in patient fibroblasts were conducted. Protein levels of RINT1 were decreased compared to control samples, similar to results in patients P1 and P3 reported by Launay et al. (2) (Figure_1B&C). Plasma lipidomic profiles showed reduced levels of diacylglycerol and cholesterol esters, as well as elevated levels of phosphatidylcholine, phosphatidylethanolamine, and phosphatidylserine (Figure_1D). The free cholesterol to cholesterol ester ratio was significantly reduced in patient cells, suggesting a higher degree of cholesterol esterification (Figure_1D). Consistent with an overall increase in phospholipid levels, the concentration of lysophospholipid derivatives was decreased (Figure_1E), supporting the hypothesis that RINT1 deficiency leads to a shift toward the use of diacylglycerols to synthesize phospholipids and an inhibition of Lands cycle. Taken together, these findings support a common molecular mechanism in bi-allelic variants in *RINT1* alter NRZ complex function leading to reduced triglyceride and diglyceride synthesis, impaired

cholesterol turnover, reduced phosphatidylcholine/phosphatidylserine ratios and an inhibition of Lands cycle.

Following the molecular diagnosis of *RINT1* deficiency in our patient, additional investigations including repeat liver function tests and a hepatic ultrasound revealed no abnormalities. Clinically, a concern for cortical visual impairment was raised but a detailed ophthalmological assessment (including optical coherence tomography and electroretinogram) was normal. The proband will undergo annual visits with an ophthalmologist and hepatologist, and parents have been instructed about symptoms of liver dysfunction and precautions during fever/illness.

Our case illustrates that bi-allelic variants in *RINT1* should be considered in children presenting with 'pure' and 'complex' hereditary spastic paraplegia (Supplementary Table_1), many of whom might be initially misdiagnosed as having cerebral palsy. A pattern of relatively normal early motor development followed by rapid onset and progression of lower limb spasticity in early childhood is uncommon among the recessive forms of HSP. The exon-intron boundaries around intron 11 may represent a mutational hotspot, given variation at c.1671+2 and 1672-1 in all reported cases thus far. A prompt diagnosis is important as this allows for monitoring for liver dysfunction during periods of metabolic stress (fever or infection) and avoidance of hepatotoxic agents, as well as genetic counseling and monitoring of siblings. Future research in prospective longitudinal natural history studies (NCT04712812) is needed to delineate the full phenotypic spectrum of *RINT1*-associated disease and possible genotype-phenotype correlations.

Figure Legend.

Figure 1. (A) Representation of the new splice site 2bp upstream because of c.1671_1671+2del. **(B&C)** Western blot analysis of RINT1, ZW10, and NBAS and their quantification in fibroblasts from controls (CTL, n=4), the patient (P4), and the previously published patients (P1 and P3). Lipidomic analysis of **(D)** glycerides and **(E)** phospholipids in plasma from controls (CTL, n=6), the patient (P4), and the previously published patients (P1 and P3). All experiments were done in biological duplicates. Data are presented as box-and-whisker plots (median, interquartile interval, minimum, maximum). **P*<0.05, ***P*<0.01, ****P*<0.001, 2-tailed Student's *t* test. CE, cholesteryl esters; DAG, diacylglycerides; FC, free cholesterol; Lyso-PC, lysophosphatidylcholine; Lyso-PE, lysophosphatidylethanolamine; Lyso-PS, lysophosphatidylserine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS, phosphatidylserine; TAG, triacylglycerides.

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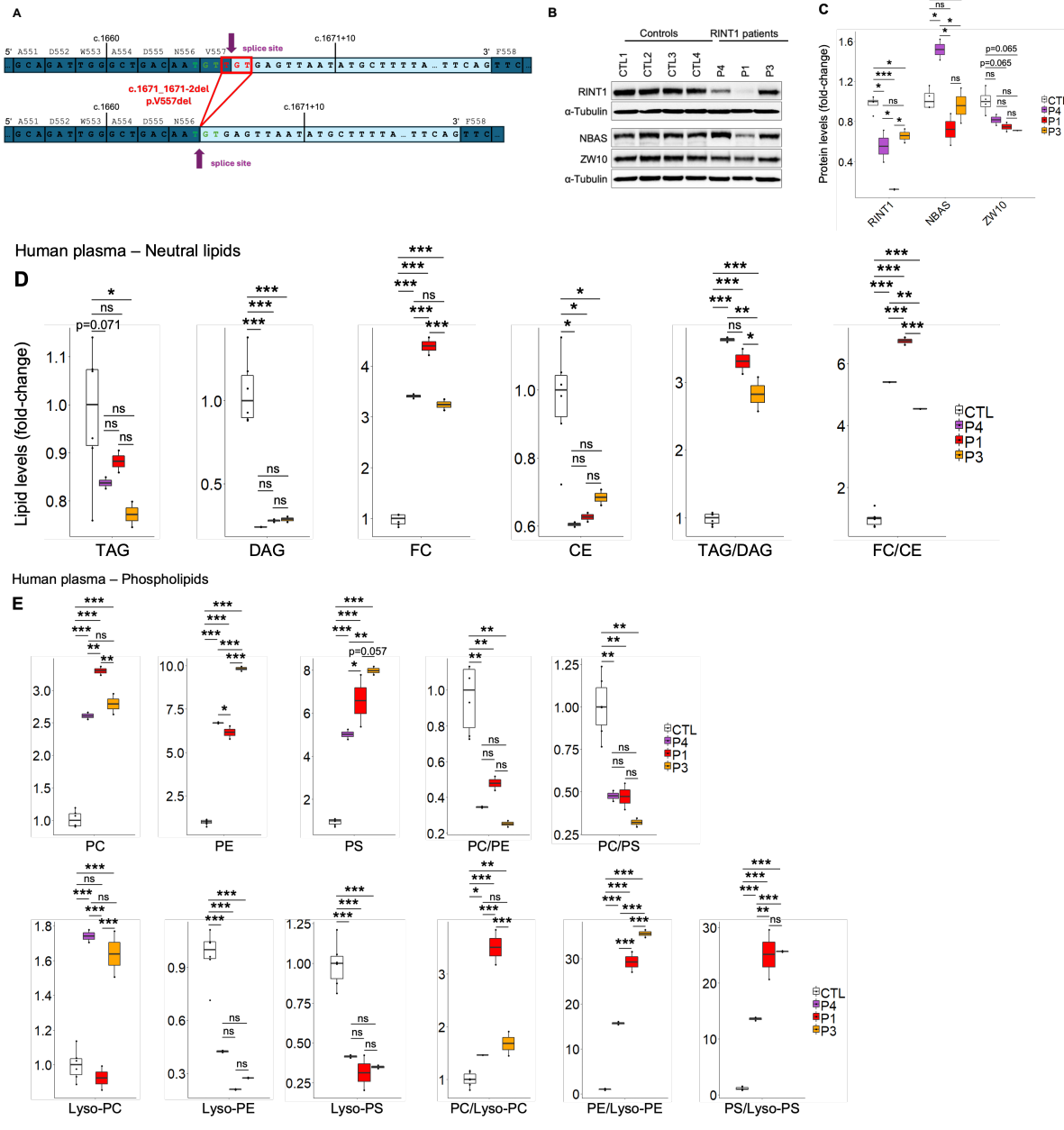
A
c.1660
c.1671+10
splice site
c.1671_1671-2del
p.V557del
B
Human fibroblasts
Controls
RINT1 patients
CTL1
CTL2
CTL3
CTL4
P4
P1
P3
RINT1
α-Tubulin
NBAS
ZW10
C
Protein levels (fold-change)
p=0.065
CTL
P4
P1
P3
Human plasma – Neutral lipids
D
Lipid levels (fold-change)
p=0.071
TAG
DAG
FC
CE
TAG/DAG
FC/CE
Human plasma – Phospholipids
E
p=0.057
PC
PE
PS
PC/PE
PC/PS
Lyso-PC
Lyso-PE
Lyso-PS
PC/Lyso-PC
PE/Lyso-PE
PS/Lyso-PS