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Supplemental Data

RINT1 Bi-allelic Variations Cause Infantile-Onset Recurrent Acute Liver Failure and Skeletal Abnormalities

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Supplemental data:

Supplemental Note: Case Reports

Proband 1

Proband 1 was a male born to nonconsanguineous parents with mixed European ancestry at 33 6/7 weeks gestation by Cesarean delivery due to premature rupture of membranes and breech positioning. His birth weight was 2.53 kilograms (78th percentile), his length was 44.5 cm (49th percentile), and his head circumference was 33.5 cm (92nd percentile). He required a brief period of intubation and mechanical ventilation. He was born with a mild left clubfoot deformity that required casting, but was otherwise nondysmorphic. His weight tracked normally, but height was consistently below the 3rd percentile for age. Other than short stature, he developed normally for corrected gestational age and multiple prolonged hospitalizations, and had normal cognition.

At 8 months of age he presented to the pediatric intensive care unit with ALF, demonstrating evidence of hepatitis, cholestasis, hypoglycemia, and coagulopathy in the setting of a preceding gastrointestinal infection with fever. The laboratory findings upon admission are in **Table 1**. He was markedly hypoglycemic with significantly elevated liver enzymes, prothrombin time, and INR. Abdominal ultrasound revealed nonspecific findings of diffuse hepatic steatosis and gallbladder wall thickening. During this hospitalization, support was provided including dextrose containing fluids, fresh frozen plasma, and electrolyte replenishment. His coagulopathy normalized over 5 days. His transaminase levels improved but remained persistently elevated (300-400 U/L) on follow-up visits and never returned to normal. A subsequent liver biopsy showed a liver parenchyma containing 40% macrovesicular fat (**Figure 1)**. No ballooning or inflammation was noted with these fatty changes. There was evidence of periportal and pericellular fibrosis, defined as early bridging fibrosis. Electron microscopy was normal, without structural abnormalities of organelles or unusual storage material.

Retroperitoneal ultrasound revealed normal kidneys and urinary collecting system. Echocardiogram was normal. Multiple metabolic tests were completed and nondiagnostic including screening for congenital disorders of glycosylation, lactate, pyruvate, uric acid, ferritin, oxysterols, lipid panel including phytosterols, alpha-1-antitrypsin, plasma amino acid analysis, plasma acylcarnitine analysis, carnitine panel, urine organic acids, and urine quantification of polyols and carbohydrates. Given this, genetic testing to investigate over 50 disorders related to infantile-onset hypoglycemia and liver disease including hereditary fructose intolerance, various glycogen storage diseases, and leucyl-tRNA synthetase deficiency (*LARS)* as well as full sequencing of mtDNA was completed with no significant findings.

Proband 1 again presented at 12 months of age in ALF with an increase of liver transaminase levels, coagulopathy, and cholestasis in the context of another febrile illness. Less than two weeks after hospital discharge from his second episode he was readmitted for his third episode of liver failure, this time in the context of a rhinovirus infection. Recovery was similar to his previous episodes, including the continued elevation of transaminase levels (300-400 U/L) at subsequent clinic follow-ups. All three episodes were similar with regard to the liver abnormalities and elevated INR. In the second and third episodes, however, hypoglycemia was not a prominent feature, likely due to earlier intervention with glucose-containing fluids. His fourth episode of acute liver failure occurred approximately one year after the 3rd episode, in association with suppurative lymphadenitis. Although the levels of liver transaminases were as elevated as previous episodes, recovery was significant with the lowest recorded AST and ALT,

122 U/L and 282 U/L, respectively. His fifth episode occurred at 3 years of age when he presented at the emergency department in respiratory distress. He was found to have pneumococcal pneumonia and subsequent acute liver failure. He had hepatosplenomegaly and coagulopathy and was admitted for 40 days. On discharge, his liver transaminases had recovered to 159 and 93 U/L for ALT and AST, respectively, chest X-ray showed low lung volumes, but no pneumothorax or pleural effusion, and he was without acute infection. Five weeks later, however, he presented at the emergency department in severe respiratory distress reportedly having a cough for two days. He appeared pale and jaundiced, but no laboratory tests were pursued and he died of acute respiratory failure likely from severe sepsis with septic shock. No autopsy was performed.

Given the frequency and recurrence of liver failure, WES was performed revealing compound heterozygous variants in *RINT1*, a gene of uncertain clinical significance, reported due to the known interactions of RINT1 protein with NBAS, which causes a very similar condition. As *NBAS* alterations are associated with Short stature, Optic nerve atrophy, and Pelger-Huet anomaly (known as the SOPH) phenotype, an eye exam and skeletal survey were performed. The eye exam was normal. The skeletal survey showed findings of anterior vertebral body beaking, platyspondyly, wide ribs, and acetabular abnormalities, seen in **Figure 2A**.

Proband 2

Proband 2 is a female born at term after an uncomplicated pregnancy to nonconsanguineous parents with Manchu and Han Chinese ancestry. Her birth weight was 3.70 kg (83th percentile) and her length was 54.0 cm (99th percentile) with a normal head circumference. Her perinatal period was uncomplicated.

At 10 months of age she presented with a fever of 40°C after a measles vaccination and symptoms of a gastroenterological illness. An episode of acholic stool led to evaluation of liver function and testing showed significantly raised levels of transaminases, as well the elevation of total and direct bilirubin levels, however coagulation study results are not available. The symptoms resolved after one week of supportive therapy. The second episode of elevated liver transaminases and direct bilirubin levels, with normal coagulation, was secondary to bronchitis at the age of 16 months. About 2 months later, she developed recurrent vomiting, diarrhea, and fever that were believed secondary to an infection. While there was not a significant increase of transaminases (~100 U/L) and bilirubin levels compared to the in-flare interval, seizures developed with significant hypoglycemia and elevated blood ammonia level. It is unclear if the seizures were a result of liver failure or directly related to the diarrhea or infection. A CT scan at that time revealed severe liver steatosis, and a liver biopsy two months later revealed some minor abnormalities including macrovesicular steatosis in the lobule, but no active inflammation. The third significant elevation of transaminases (>1000 U/L) was documented at the age of about 2 years, without jaundice or coagulopathy. The fourth episode happened at the age of 2 years and 2 months. After one day of fever and cough, she developed vomiting and coma with hyperammonemia, elevated direct bilirubin level, and significant prolonged prothrombin time (99 s). With intensive support care, the symptoms disappeared, and bilirubin levels and prothrombin time normalized within 1 week. Four more episodes of liver crisis recurred at the ages of 3 years 5 months, 4 years 8 months, 5 years 6 months, and 6 years 8 months; each was precipitated by fever with significantly elevated transaminases (>1000 U/L), but normal prothrombin time. Except for mild conjugated hyperbilirubinemia in the episode at the age of 4 years and 8 months, the bilirubin level was normal during the other three episodes. Proband 2 is now 9 years old and has had no additional episodes of liver injury despite febrile illness since 6 years and 10 months of age.

During the workup for her liver disease, the following were completed and normal or uninformative: respiratory chain complex I – V activities, alpha-fetoprotein, urinary organic acid analysis, plasma amino acid analysis, carnitine panel, and sequencing for *SLC25A13*. WES revealed two VUS in *RINT1* and Sanger sequencing of the parental DNA confirmed biallelic inheritance with each parent carrying one variant.

In addition to her liver phenotype, this individual has short stature (<3rd percentile) and skeletal findings of platyspondyly and acetabular abnormalities (**Figure 2B**), but has experienced otherwise normal development and cognitive function.

Proband 3

Proband 3 is a female born at term to nonconsanguineous Caucasian parents of Bulgarian ancestry. Birth weight was 2.75kg (5th percentile). The neonatal period was unremarkable. There is no family history of liver disease. Psychomotor development was normal, she experienced no medical problems during the first two years of life, and was vaccinated. At 2 years of age she experienced the first episode of ALF and encephalopathy, in the setting of a viral infection. She recovered within 4 weeks, with a residual left sided motor deficit that eventually resolved. A diagnostic work-up revealed no infectious or metabolic cause. Newborn screening, acylcarnitine profile, organic acids, and alpha-1-antitrypsin testing were all unremarkable. She recovered well with normal laboratory values in the interim. Liver function tests were abnormal twice in her fifth year of life, without coagulopathy, both times coincident with a viral illness.

At 7 years of age she suffered her most severe episode of ALF preceded by a febrile (39°C) influenza A infection. After 3 days of fever and vomiting, she developed altered mental status, and was promptly intubated for stage 2-3 hepatic encephalopathy. Labs were remarkable for hyperammonemia, hyperbilirubinemia, metabolic acidosis, coagulopathy, and renal failure. Clinical examination revealed hepatosplenomegaly and ultrasound showed ascites. Due to multi-organ involvement she was put on MARS (Molecular Adsorbent Recirculation System) filtration and listed for urgent liver transplantation. A bone marrow aspiration excluded hemophagocytic lymphohistiocytosis (HLH). Infectious diseases work-up revealed influenza A infection treated with oseltamivir for 5 days and E. coli in the urine treated with antibiotics.

NBAS-associated RALF was suspected as an etiology due to the recurrent episodes for which she was put on intravenous glucose and lipid infusion. She recovered within days without liver transplantation. Her acute renal failure required two weeks of hemofiltration and resolved within 90 days. WES identified compound heterozygous VUS in *RINT1*.

A more detailed work-up was performed given the *RINT1* variants and a possible overlap with the multisystemic phenotype of NBAS deficiency. An ophthalmological work-up ruled out optical atrophy. Neurological exam was positive for a slight motor deficit on her left side including an inclined posture of her head to the left, which was still present at discharge. Imaging revealed anterior beaking of vertebrae and vertebral irregularities (**Figure 2C**). Skin exam revealed acrokeratosis of unknown origin in a mosaic form known to worsen during summer time. A detailed physical exam by a geneticist revealed mild epicanthus, laterally ascending axis of the eyelid, and low-set eyes. Hypoplastic alae of the nose are parentally inherited.

This individual eventually underwent elective deceased-donor liver transplantation. The rationale for transplantation was that ALF episodes seem to be worsening with age. Post-operative course was remarkable for a hemoperitoneum requiring surgical drainage, chylous ascites, and hepatic artery stenosis. She was discharged home one month post-operatively.

Supplemental Figures

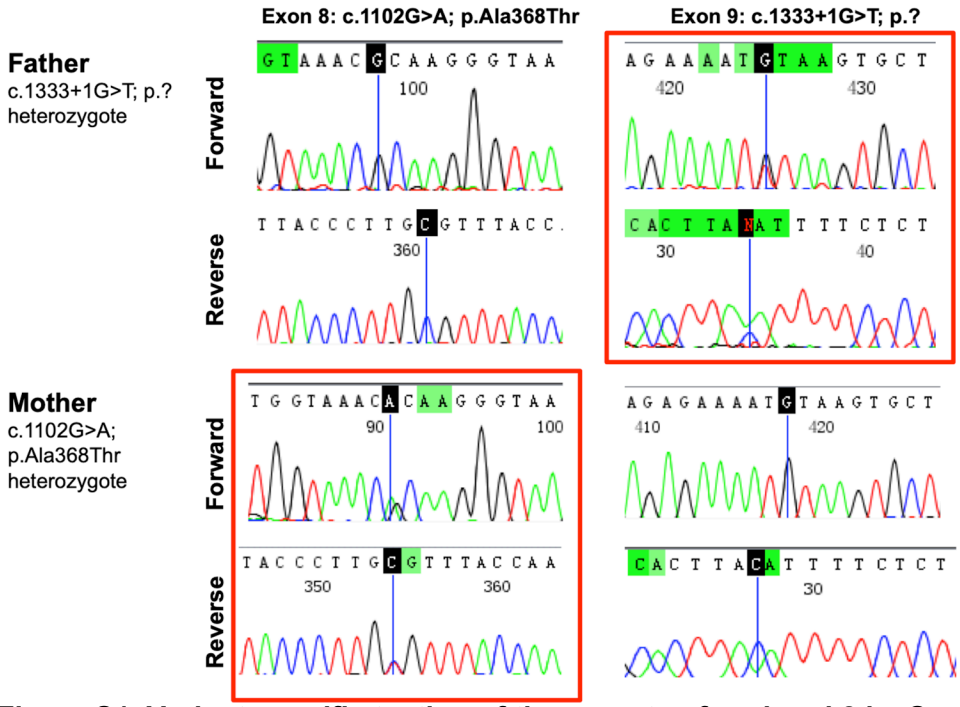
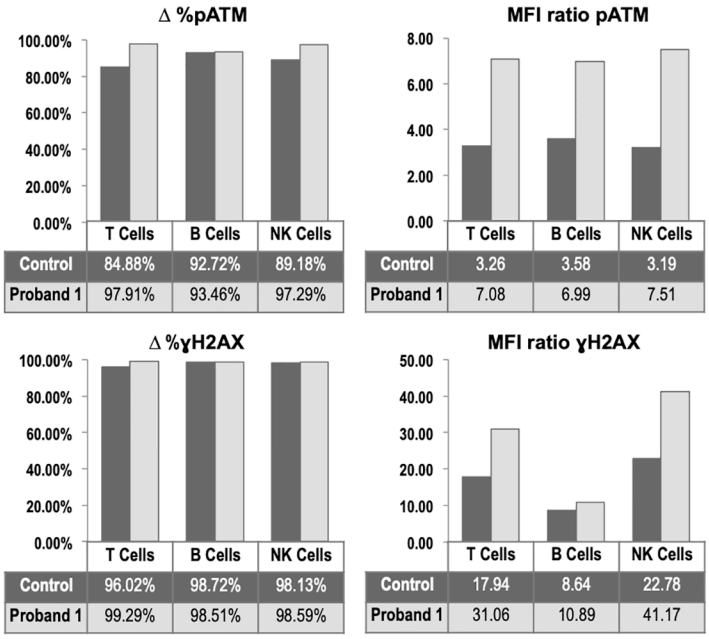


Figure S1. Variant-specific testing of the parents of proband 2 by Sanger sequencing reveals *RINT1* variants to be in trans.

A. 1 hr post-irradiation



B. 24 hr post-irradiation

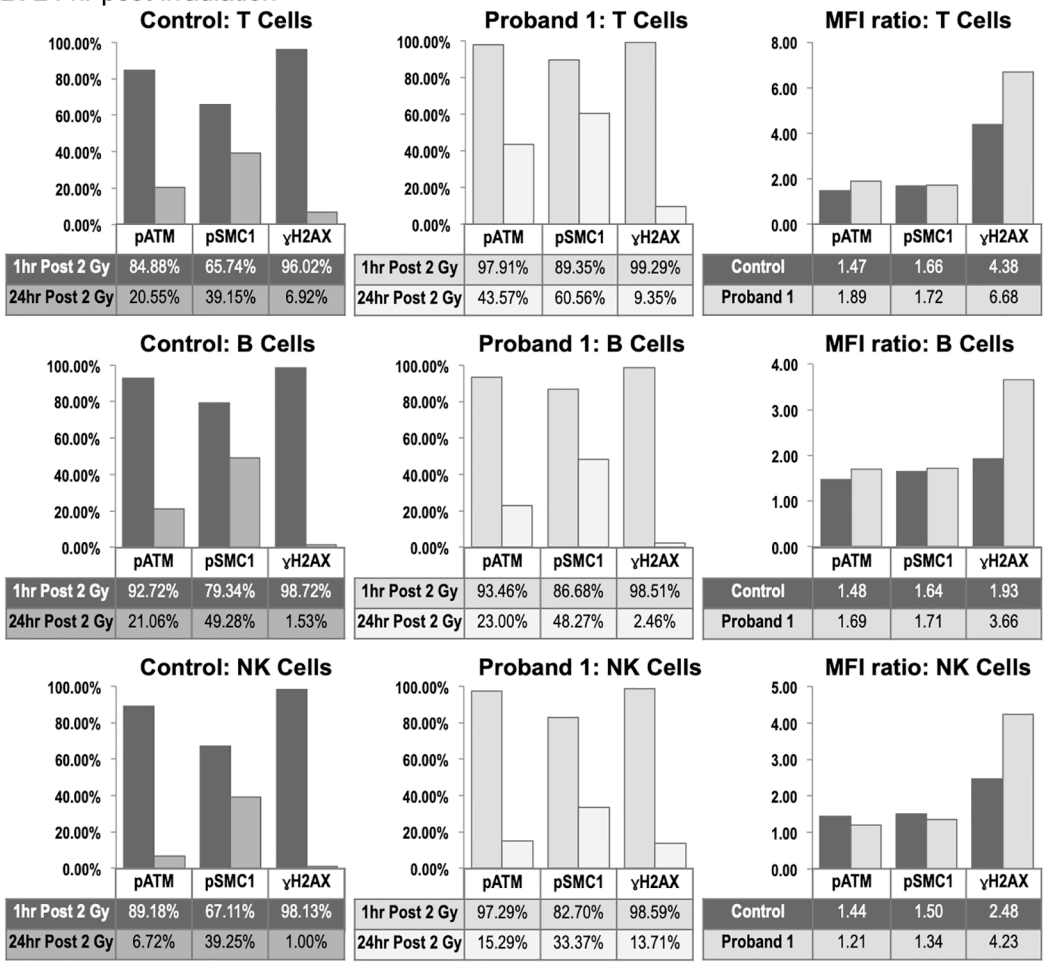


Figure S2. Proband 1 has a normal double-strand break repair pathway in lymphocytes. The DNA repair pathway was assessed in this individual by measuring phosphorylation of ATM, SMC1 (data not shown) and H2AX (γH2AX), since RINT1 associates with RAD50 in the MRN complex. PBMCs from proband 1 and a healthy control were either irradiated with low-dose irradiation (2Gy) or unirradiated, and phosphorylation (p) of these proteins was assessed by flow cytometry at 1h post-irradiation (**A**), and de-phosphorylation was measured at 24h post-irradiation (**B**). Proband 1 is able to phosphorylate ATM, SMC1 (not shown) and H2AX at 1h post-irradiation, and de-phosphorylate normally at 24h post-irradiation, in all lymphocyte subsets.