

Tricho-hepato-enteric syndrome with novel SKIV2L gene mutations

A case report

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Abstract

Rationale: Tricho-hepato-enteric syndrome (THES) is a rare disorder caused by mutations in the TTC37 or SKIV2L genes and characterized by chronic diarrhea, liver disease, hair abnormalities, and high mortality in early childhood due to severe infection or liver cirrhosis.

Patient concerns: The patient is the second child of three siblings born to non-consanguineous healthy Japanese parents. She had intrauterine growth retardation and was delivered at 33 weeks of gestation due to placental abruption. She presented with watery diarrhea, elevated levels of liver enzymes, multiple episodes of recurrent bacterial infection, and mild mental retardation. She had facial dysmorphism, including prominent forehead and hypertelorism, and had woolly hair without trichorrhexis nodosa.

Diagnosis: Clinical features led to consideration of THES. Novel compound heterozygous nonsense mutations, c.1420G>T (p.Q474*) and c.3262G>T (p.E1088*), in the SKIV2L gene were identified in the patient, and decreased levels of SKIV2L protein expression were revealed by flow cytometry and confirmed by western blot analysis using patient peripheral blood mononuclear cells (PBMCs).

Interventions: Total parenteral nutrition was required from day 30 to day 100. Trimethoprim-sulfamethoxazole prophylaxis was started at the age of 7 years after multiple episodes of bacterial pneumonia and otitis media.

Outcomes: Chronic diarrhea persisted for more than 10 years, but the symptoms gradually improved with age. At the age of 13 years, she started a normal diet in combination with oral nutritional supplementation and her height and weight were just below the 3rd percentile for healthy individuals. She developed secondary sex characteristics, and menarche occurred at the age of 12 years. Facial dysmorphism, including prominent forehead and hypertelorism, and woolly hair without trichorrhexis nodosa became noticeable as she matured.

Lessons: Physicians must be aware of THES when they encounter a patient with infantile diarrhea, hair abnormalities, immune deficiency, mental retardation, and liver disease. Moreover, flow cytometric detection of SKIV2L protein in PBMCs may facilitate early diagnosis.

Abbreviations: ISG = interferon stimulated gene, PBMC = peripheral blood mononuclear cell, SKIV2L = superkiller viralicidic activity 2, THES = tricho-hepato-enteric syndrome, TTC37 = tetratricopeptide repeat domain-containing protein 37.

Keywords: flow cytometric analysis, SKIV2L, tricho-hepato-enteric syndrome, type i interferon signature

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Informed consent for the publication of patient data was obtained from the patient's mother.

The authors disclose no conflicts of interest.

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1. Introduction

Tricho-hepato-enteric syndrome (THES) is a rare and severe disorder associated with mutations in the tetratricopeptide repeat domain-containing protein 37 (TTC37) and superkiller viralicidic activity 2 (SKIV2L) genes. It is characterized by intractable diarrhea of infancy, hair and facial abnormalities, immunodeficiency, liver disease, intrauterine growth retardation, and developmental delay. The severity of the infantile diarrhea associated with THES ranges from mild to severe; patients with mild symptoms need only oral enteral nutrition, while those with severe malnutrition require long-term total parenteral nutrition.^[1,2] Histopathological features of small intestine biopsy are nonspecific villous atrophy, with or without mixed inflammatory cell infiltration. As some patients with THES present with inflammatory bowel disease resembling colitis, recently, increasing attention has been paid to the relationship between TTC37 or SKIV2L gene mutations and the pathogenesis of very early-onset inflammatory bowel disease.^[3] Other common features of THES include hepatic involvement, which can develop irrespective of parenteral nutrition. Fabre et al^[4] reported that 12 of 22 (55%) patients with THES had liver disease, 9 of 18 (50%) had hepatic cirrhosis, and 4 of 17 (24%) had hepatic hemosiderosis, indicating that iron overload may contribute to THES pathogenesis. It is difficult to clearly define the type of immunodeficiency associated with THES, since literature reports are inconsistent^[1,5,6]; however, low serum concentrations of immunoglobulins and absence of humoral immune responses to vaccination are most frequently reported.^[1,7]

Patients with THES usually die in early childhood due to severe infection or liver cirrhosis,^[1] and early diagnosis is vital for optimal management and to improve outcomes for THES patients. A small number of THES patients with genetic diagnoses have been reported in the literature. Only 1 case of SKIV2L deficiency from Asia has been reported, and there has been no report of a genetically confirmed Japanese THES patient.

Herein, we present the first Japanese case of THES with SKIV2L deficiency. We also suggest that flow cytometric detection of SKIV2L protein expression in peripheral blood mononuclear cells (PBMCs) could be a rapid method of screening for SKIV2L deficiency.

2. Case report

The patient is the second child of 3 siblings born to non-consanguineous parents. She had intrauterine growth retardation (birth weight, 1276 g) and was delivered by cesarean section at 33 weeks' gestation because of a placental abruption. There was no family history. Watery diarrhea 10 to 20 times a day started at the age of 14 days. Owing to failure to thrive, total parenteral nutrition was required from day 30 to day 100. Esophagogastroduodenoscopy and colonoscopy performed at 7 years old revealed villous atrophy in the duodenum and the terminal ileum. Biopsies from the duodenum and the terminal ileum showed villous atrophy with a mild lymphocytic infiltration (Fig. 1). Although chronic diarrhea persisted for more than 10 years, the symptoms gradually improved with age. Follow-up endoscopy and biopsies performed at 10 years old showed histological improvement of villous atrophy in the ileum. At the age of 13 years, she started a normal diet in combination with oral nutritional supplementation and her height and weight were just below the 3rd percentile for healthy individuals. She also developed secondary sex characteristics, and menarche occurred at the age of 12 years.

Elevated levels of liver enzymes were noticed at the age of 2 months and continued until 4 years old. Ultrasonography and magnetic resonance imaging showed hepatic nodularity and hypertrophy of the caudate suggesting the presence of liver cirrhosis. Liver biopsy performed at age 2 years showed a mild degree of portal-portal bridging fibrosis. No iron deposition was observed in the liver, and no progression of hepatic fibrosis was observed at a second liver biopsy performed at 7 years old (Fig. 2).

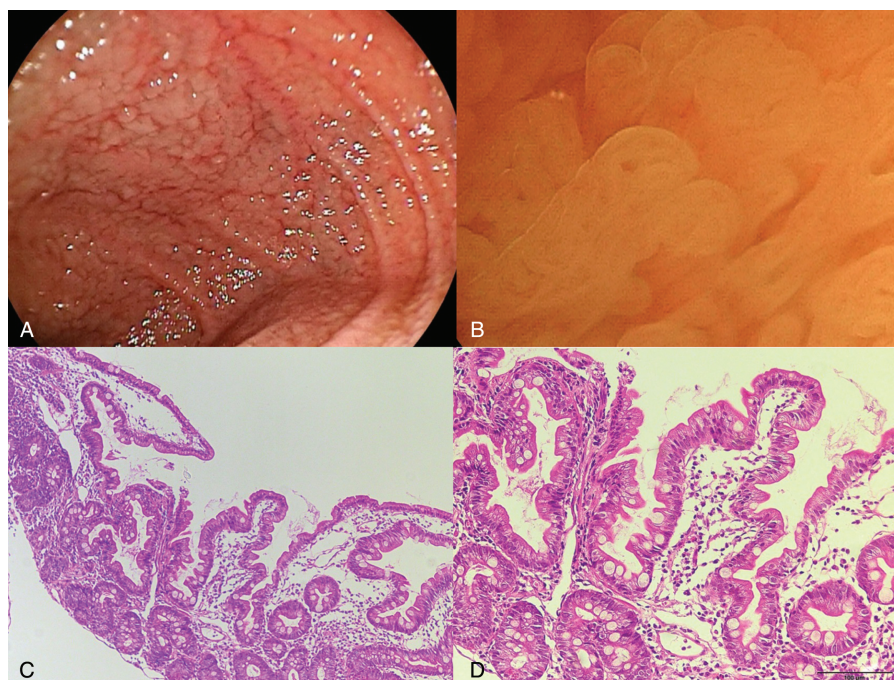


Figure 1. Duodenum endoscopy and biopsy findings, showing villous atrophy. Endoscopic findings of duodenum (A) without magnification and (B) with magnification. H&E stained duodenal biopsies (C) original magnification $\times 40$ and (D) original magnification $\times 400$. H&E = hematoxylin-eosin.

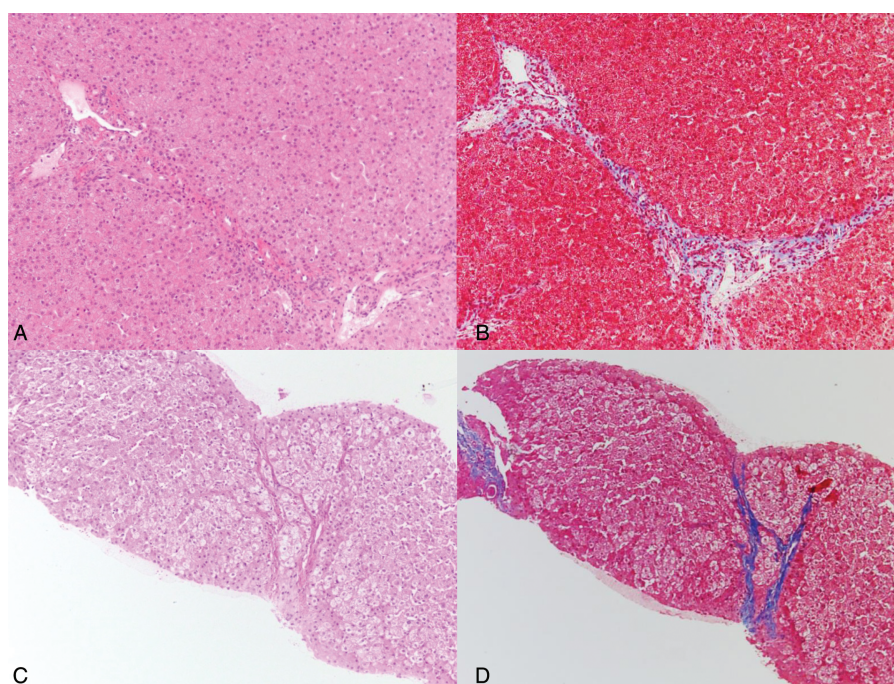


Figure 2. Liver biopsy findings showing nonprogressive mild fibrosis. A liver wedge biopsy obtained at age 2 years stained with (A) H&E and (B) Masson trichrome stain, and a liver needle biopsy obtained at age 7 years stained with (C) H&E and (D) Masson trichrome stain. H&E = hematoxylin-eosin.

The cause of liver disease could not be identified despite thorough workup, including screening for infection and autoimmune and metabolic diseases. Immunological screening performed at 2 years old showed normal serum IgG and IgA levels and robust proliferative responses to mitogens, including phytohemagglutinin and concanavalin A. Lymphocyte subset analysis showed a normal number of CD3, CD4, CD8, CD20, and CD56 positive cells. The specific antibody titers measured by enzyme immunoassay for pertussis (anti-FHA), rubella, and measles vaccination were 29, 52.7, and 2.0, respectively, suggesting that she could mount antigen-specific immune responses. She had multiple episodes of bacterial pneumonia and otitis media until the age of 7 years when trimethoprim-sulfamethoxazole prophylaxis was started. Facial dysmorphism, including prominent forehead and hypertelorism, and woolly hair without trichorrhexis nodosa became noticeable as she matured (Fig. 3). She had mild to moderate mental retardation requiring special education support. Workup did not suggest the presence of congenital heart disease or abnormally-sized platelets. These clinical features led to consideration of THES, and we performed DNA sequencing and gene expression analysis of TTC37 and SKIV2L.

Sequencing analysis showed compound heterozygous non-sense mutations, c.1420G>T (p.Q474^{*}) of the paternal allele and c.3262G>T (p.E1088^{*}) of the maternal allele, in the SKIV2L gene (Fig. 4). No mutation was found in TTC37. Flow cytometry indicated decreased expression of SKIV2L protein in PBMCs from the patient compared with that in a healthy control sample, and these results were confirmed by western blot analysis (Fig. 5).

We also investigated whether the patient had a type I interferon response signature by measuring expression levels of 4 interferon stimulated genes (ISGs) (IFI27, ISG15, RSAD2, and SIGLEC1) by quantitative real-time polymerase chain reaction. No elevation was found in the expression of these genes in a PBMC sample from the patient compared with that in a sample from a healthy control (Fig. 6).

3. Discussion

According to a previous report by Fabre et al,^[8] 48 patients who were positive for THES presented persistent infantile diarrhea with or without villous atrophy and colitis; hair abnormalities described as woolly, easily removable, unmanageable, brittle and scanty; and facial dysmorphism, including a prominent forehead, a broad flat nose, and hypertelorism. They often presented liver disease (23 of 44 patients) and immune defects (39 of 44); skin abnormalities such as café au lait spot, xerosis, and rubbery skin (18 of 36); and a small birth weight for their gestational age (31 of



Figure 3. Photographs of the patient's face and hair at the age of 12 years. Characteristic face, including prominent forehead and broad nasal root, and woolly hair (A, B). Light microscopic findings of hair shaft showed no trichorrhexis nodosa (C).

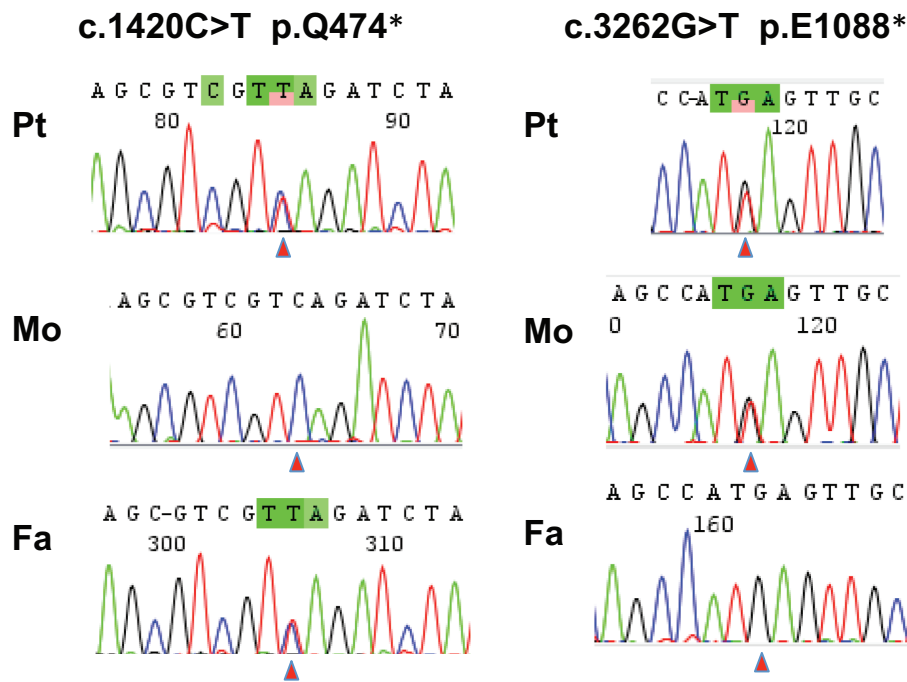


Figure 4. Sequencing chromatogram showing the compound heterozygous nonsense mutations, c.1420C>T and c.3262G>T, in the SKIV2L gene. The mutations c.1420C>T (p.Q474*) and c.3262G>T (p.E1088*) were paternally and maternally derived, respectively. Fa = father, Mo = mother, Pt = patient, SKIV2L = superkiller viralicidic activity 2.

46). Other rare symptoms included cardiac abnormalities (8 of 31) and abnormally-sized platelets (5 of 21).

The present case showed intractable diarrhea of infancy associated with intestinal villous atrophy; liver dysfunction with fibrosis; recurrent infections; intrauterine growth retardation, a prominent forehead; hypertelorism; and woolly hair without trichorrhexis nodosa. These features were consistent with THES, although we did not detect skin, cardiac, or platelet abnormalities in our patient.

In our case, liver enzyme elevation during the prolonged period of total parenteral nutrition suggested that the patient also had liver

disease, although liver damage is usually associated with iron deposition. Her presentation with recurrent bacterial infections was suggestive of immunodeficiency; however, immunological workup did not lead us to a specific classification of her immune status.

The prognosis of THES is generally poor. Death usually results from liver failure, recurrent infection, or complications associated with long-term parenteral nutrition, and can occur early in life, although a few patients can survive to the third decade. In case reports published in the 1990s, 5 of 8 THES patients without genetic testing died under 5 years of age because of sepsis or cirrhosis, despite aggressive intervention.^[1] Meanwhile, a recent study demonstrated better survival rates in THES patients with TTC37 or SKIV2L mutations.^[4,8] Advances in the management of nutrition and treatment of infection may improve survival. Moreover, increased understanding of the disease could also facilitate early diagnosis and appropriate management in its early stages. Interestingly, in our THES patient, the severity of diarrhea and intestinal villous atrophy improved and the frequency of bacterial infection decreased with age. Unlike the common clinical course of THES, her symptoms improved as she grew older.

THES is caused by homozygous or compound heterozygous mutations in the SKIV2L gene on chromosome 6p21.3 or TTC37 on chromosome 5q15.^[4,7,8] The function of these gene products in humans is not yet fully elucidated. SKIV2L and TTC37 encode orthologs of the yeast proteins SKI2 and SKI3, respectively, which form the superkiller complex, together with 2 copies of SKI8. The superkiller complex is a cofactor of the cytosolic exosome, which is involved in the degradation of aberrant mRNA molecules. The mechanism by which a defect in the mRNA degradation system leads to the specific symptoms associated with THES remains unclear. In our case, direct sequencing of the SKIV2L gene revealed novel heterozygous nonsense mutations, and flow cytometric and western blotting

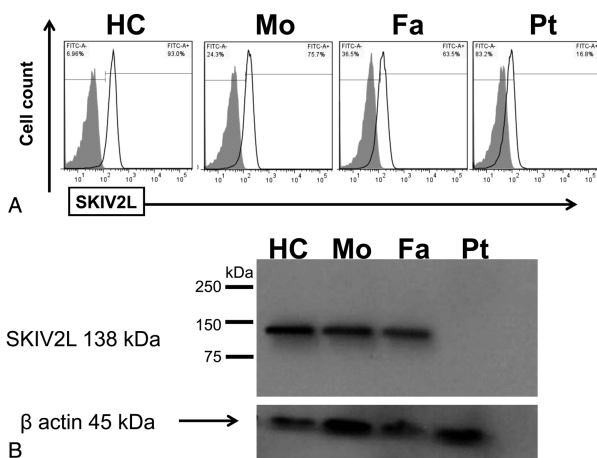


Figure 5. Decreased SKIV2L protein expression in PBMCs from the patient. Decreased levels of SKIV2L protein expression in PBMCs derived from the patient determined by (A) flow cytometry and (B) western blot analysis (β actin was used as a loading control). Fa = father, HC, healthy control, Mo = mother, PBMC = peripheral blood mononuclear cell, Pt = patient, SKIV2L = superkiller viralicidic activity 2.

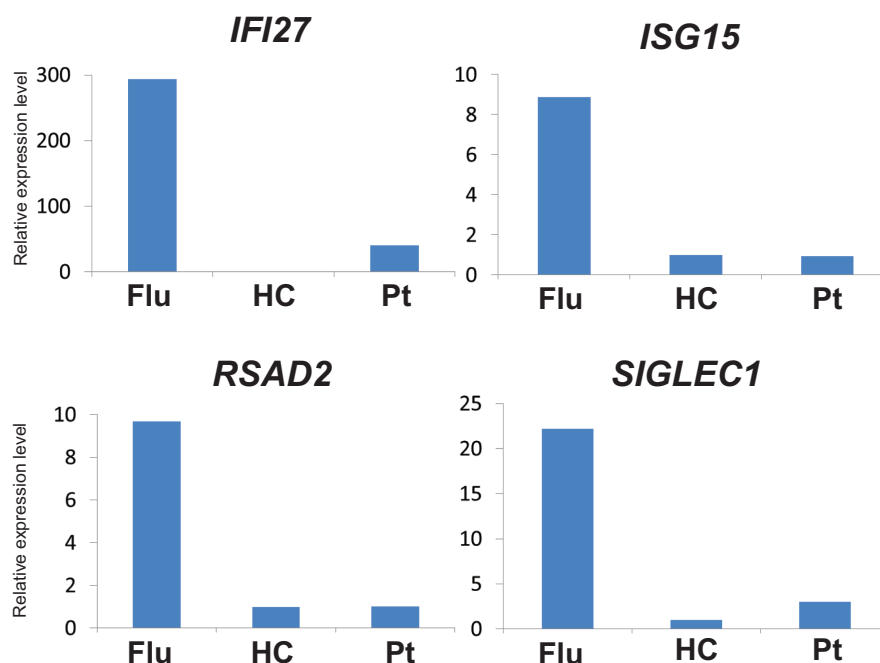


Figure 6. Transcription of ISGs was not elevated in the patient. Quantitative real-time PCR of a panel of 4 ISGs in PBMCs from the THES patient, a healthy control, and a patient infected with influenza virus (positive control). Flu = influenza virus-infected control, HC = healthy control, ISG = interferon stimulated gene, PCR = polymerase chain reaction, PBMC = peripheral blood mononuclear cell, Pt = patient, THES = tricho-hepato-enteric syndrome.

analysis using patient PBMC samples demonstrated defective SKIV2L protein expression. Only 1 case of genetically confirmed THES has previously been reported from Asia,^[2] and, to the best of our knowledge, this is the first Japanese case. Most patients with SKIV2L or TTC37 mutations are reported to have absent or significantly reduced expression levels of SKIV2L or TTC37 proteins. As these proteins are abundantly expressed in PBMCs,^[8,9] their investigation using flow cytometry could be an useful screening method to identify THES patients.

SKIV2L is a negative regulator of RIG-I-like receptors, which trigger innate immune responses against viral infection, and loss of SKIV2L function can result in type I interferon overproduction.^[9] Overwhelming type I interferon response is considered to contribute to the autoimmune nature of lesions such as chronic intestinal inflammation observed in some THES patients,^[10–12] and THES patients with SKIV2L mutations are reported to have strong type I interferon peripheral blood signatures,^[9] although the connection between excess type I interferon production and the pathogenesis of THES remains to be elucidated. However, there were no clinical findings suggestive of type I interferon overproduction in our patient, who showed a milder degree of developmental delay, malnutrition, and microscopic inflammation of the intestine than previously reported cases. We speculate that there may be a correlation between disease severity and type I interferon production, and that determining the expression of ISGs could assist in the prediction of the prognosis and severity of symptoms in patients with THES. Further research is required to determine the relationship between disease severity and the quantity of type I interferon production in THES patients.

4. Conclusions

We describe the first Japanese THES patient with causative novel compound heterozygous nonsense mutations in the SKIV2L gene. Physicians must be aware of THES when they encounter a

patient with infantile diarrhea, hair abnormalities, immune deficiency, mental retardation, and liver disease. Moreover, flow cytometric detection of SKIV2L protein in PBMCs may facilitate early diagnosis.

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