**SCYL1** disease in two siblings with severe liver phenotype and neurological phenotype: diagnosed by reanalysis of whole exome sequencing and direct Sanger sequencing

**Running title:** **SCYL1** disease in two siblings

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Abstract

Key words: *SCYL1* disease; liver insufficiency, liver transplantation, global developmental delay; tremor; whole exome sequencing
Introduction

SCYL1 disease (OMIM 616719) results from a biallelic pathogenic variants in SCYL1 and was first reported in 2015 (Schmidt 2015). SCYL1 encodes a ubiquitously expressed SCY1-like pseudokinase 1, which is an evolutionarily conserved and catalytically inactive protein kinase (Liu et al 2000, Schmidt et al 2015). This ubiquitous expression is in keeping with the wide-range organ involvement. Phenotypes range from autosomal recessive spinocerebellar ataxia to acute liver failure and neurodegeneration (Chavany et al 2020; Li 2019; Lenz 2018; Schmidt 2015; Shohet 2019). There are less than 20 patients reported so far since its first description. Here, we report two siblings that presented with severe hepatic phenotype leading to liver failure and requiring liver transplantation in infancy. Both siblings underwent extensive metabolic investigations pre-transplant with no identifiable cause. Their whole exome sequencing (WES) identified a single variant in SCYL1. Phenotypic similarities with SCYL1 deficiency patients reported in the literature led us to request deletion/duplication analysis of SCYL1. We identified the second variant in SCYL1 and confirmed the diagnosis in both siblings. We performed and presented the results through western blot of cultured skin fibroblasts. In addition, we present herein a literature review and summary of all reported cases of patients diagnosed with SCYL1 mutation.

Methods

The study was approved by the Hospital for Sick Children Institutional Research Ethics Board (REB#1000071421). Informed consent for both siblings were signed by the parents. Patients and parent’s DNA samples were utilized for WES according to the methods of clinical molecular genetic laboratories. Direct Sanger sequencing and deletion/duplication analysis of SCYL1 was performed using patients’ DNA samples according to the methods of clinical molecular genetic
laboratories. Review of archival histopathology slides and electron microscopic images of liver biopsy and explant specimens was performed by an expert liver pathologist. Western blot of cultured skin fibroblasts to test SCYL1 protein expression was performed using previously reported antibody and method (Kuliyev et al 2018). Briefly, protein extracts obtained from human dermal fibroblasts (Invitrogen, ), skin fibroblasts from Patient 1 and Patient 2, Hek293T (ATCC® CRL-3216™) cells and SCYL1-deficient Hek293T cells (Gingras et al., 2017) were resolved by SDS-PAGE and analysed by western blot using antibodies against SCYL1 (Pelletier et al., 2012) and β-actin (Sigma-Aldrich, A1978). Band intensities were quantified by using Image Lab version 6.1.0, build 7 (BioRad Laboratories, Inc.). Literature was reviewed using SCYL1 keyword in PubMed and in all published articles. All patients reported in the literature were entered into an Excel Database.

Results

Patients

Patient 1: This 13-year-old boy was born at term following an unremarkable pregnancy and delivery (birth weight 6 lbs, 9oz). His family history was negative at first presentation, but his younger sister presented later with a similar liver phenotype (described below).

Liver Phenotype

Following a febrile upper respiratory tract infection at 5 months of age, the patient presented with cholestatic liver disease characterized by conjugated hyperbilirubinemia (conjugated bilirubin - 266 mmol/L), mildly elevated GGT (58), elevated transaminases (ALT 1514, AST 461) and liver synthetic dysfunction leading to the diagnosis of acute liver failure (INR 2.5 after Vitamin K administration). He also presented with thrombocytopenia (60) and hypoalbuminemia (30 g/L). Abdominal ultrasound showed hepatosplenomegaly and on palpation the liver felt soft.
Extensive work-up including detailed investigations for metabolic liver diseases did not reveal an etiology. Liver biopsy done during this first episode of acute liver failure revealed severe canalicular and hepatocellular cholestasis with associated marked hepatocyte feathery degeneration, in the absence of significant portal or lobular inflammation. Extensive perivenular and periportal sinusoidal fibrosis, with central-central and central-portal bridging septa was noted, (Figure 1a, 1b).

The patient had a second admission two months later for INR of 2.1 that corrected with vitamin K. Transaminases, bilirubin, INR, platelets and albumin normalized after these two episodes of liver dysfunction. The liver became firm and after a few months began to soften again. A third similar episode of cholestatic hepatitis with liver dysfunction (conjugated bilirubin 47, ALT 1760, AST 4025, GGT 83, INR 2.7) occurred at 17 months of age after fever and clinical signs of a common cold. Ultrasound showed hepatosplenomegaly, with no ascites. At 19 month of age following a rotavirus infection with fever, vomiting and diarrhea a fourth episode of cholestasis and liver failure led to another urgent admission. The patient presented this time with increased conjugated bilirubin (96 mmol/L), mildly increased GGT (83), increased transaminases (ALT 139) and INR up to 2.8. The patient had been referred to a children’s hospital with a liver transplant program for assessment due to those recurrent episodes and persistent elevation of transaminases, thrombocytopenia and hepatosplenomegaly that developed after the third episode. Liver transplant was recommended due to recurrent severe life-threatening episodes of liver failure and symptoms of chronic liver disease between those episodes. The patient had a related live donor liver transplant (mother) at 21 months of age. Compared to the initial liver biopsy performed during his first liver failure episode, histopathology of the liver explant showed improved hepatocyte injury, incomplete fibrous septa and lesser degree of sinusoidal fibrosis.
Ultrastructural findings on electron microscopy of the liver biopsy and the explant, confirmed presence of bile and associated variable hepatocyte injury in the form of intracytoplasmic small lipid droplets, mitochondrial hyperplasia and autophagosomes. The features were non-specific to a certain etiological entity.

Graft function has remained normal since liver transplant in 2009, with no episodes of rejection or other post-transplant complications. The patient is maintained on delayed release Tacrolimus (Advagraf) for immunosuppression.

**Neurological Phenotype**

Over the course of his disease the patient developed mild hypotonia and mild subtle motor weakness. Tremor was noted at 18 months of age. Brain magnetic resonance imaging (MRI) at 2.5 years of age showed cerebral atrophy, and cavum septum pellucidum. Other neurological pathologies included stutter, cognitive dysfunction, learning disabilities, motor coordination disabilities, attention deficit hyperactivity disorder (ADHD) and behavioural problems characterized by anxiety and emotional outbursts with inconsolable crying and anger. Physical examination demonstrated fine bilateral hand tremor, stutter, abnormal smooth pursuit, dysmetria, and dysdiadochnesia. The patient was able to attend school in a regular classroom with assistance (integrated class). Developmental psychologist assessment diagnosed the patient as an exceptional learner - multiple exceptionalities: physical and mild intellectual disability. He continues to be assessed for possible ADHD and is followed by a behavioral therapist and psychiatrist for emotional regulation and anxiety.

**Skeletal Phenotype**

On assessment at age 12 years of age the patient had bell-shaped appearance of his chest and abdomen, joint laxity at the elbows and knees, hyperlordosis of the lumbar spine, lordosis,
kyphosis, scoliosis and asymmetry of the scapulae. He had reduced muscle bulk and power and difficulty rising from the floor to a standing position (modified Gower’s sign). He was not able to perform tandem gait. X-ray showed 12-degree thoracic levoscoliosis and 17-degree lower thoracic lumbar dextroscoliosis.

*Other relevant clinical history*

Past medical history was also remarkable for a right inguinal hernia, recurrent otitis media, and poor weight gain. On physical examination at age 12 years, weight and height were at the 2\textsuperscript{nd} centile for age.

**Patient 2:** This 9-year-old girl, sister of Patient 1, was born at 36 weeks’ gestation following a pregnancy complicated by intrauterine growth retardation (IUGR). She spent one week in the neonatal intensive care unit with jaundice. She had two lung infections at age 4 months and 5 months.

*Liver Phenotype*

During the second lung infection at 5 months of age, the patient was admitted for dehydration and was noted to be jaundiced. Laboratory investigations showed conjugated hyperbilirubinemia (conjugated bilirubin of 128 mmol/L), elevated liver enzymes (ALT 171, AST 579), normal GGT (32), with INR of 1.3 and albumin 38 g/L. No history of acholia. Abdominal ultrasound showed echogenic wall thickening of gallbladder and increased periportal echoes throughout the liver. In contrast to patient 1, although patient 2 did not present initially with acute liver failure, her liver function deteriorated progressively in the following weeks after admission, with hypoalbuminemia and an INR of 2 after vitamin K, meeting criteria for acute liver failure 2 weeks after initial presentation. The patient also developed hepatosplenomegaly, thrombocytopenia and ascites. Liver biopsy revealed similar cholestatic pattern of injury but of
milder degree compared to his sibling’s initial liver biopsy. Mild hepatocellular cholestasis with associated patchy hepatocyte feathery degeneration, without significant portal or lobular inflammation and variable mild to moderate periportal and perivenular sinusoidal fibrosis was present, (Figure 2a, 2b). Due to progression of liver disease to acute liver failure with no improvement (INR-1.9 at the day of transplant) and the family history, the patient underwent a related live donor liver transplantation (father) at 7 months of age. Her liver explant showed marked hepatocyte injury and parenchymal loss involving most centrilobular regions, extending into zones 2, with extensive sinusoidal fibrosis, (Figure 2c, 2d). Electron microscopic examination demonstrated similar non-specific findings related to cholestasis and hepatocyte injury as seen in sibling’s liver parenchyma. Graft function has remained normal since liver transplant in 2011, with no episodes of rejection. Post-transplant complications included incisional hernia that required repair, feeding difficulties with oral aversion requiring G-tube feeds, and CMV colitis, and mild intermittent microalbuminuria secondary to Tacrolimus use. The patient remains on delayed release Tacrolimus (Advagraf) for immunosuppression.

Neurological Phenotype

Over the course of the disease, the patient was diagnosed with global developmental delay, cognitive dysfunction, learning disabilities, generalized motor strength disability and an intention tremor. On her last assessment, she had a bilateral intention tremor of her hands, dysdiadochinesia and mildly reduced power with a modified Gower’s sign and inability to perform a tandem gait.

Respiratory Phenotype

Multiple episodes of RSV bronchiolitis, parainfluenza, pneumonia, and upper respiratory tract infections, some of which required hospitalizations (up to eight episodes per year) were noted
since birth. The patient was also diagnosed with asthma. There has been a progression in the respiratory presentation, with the symptoms initially being more severe (one admission to ICU and frequent emergency admissions requiring ventolin nebulizers) to more recently being associated with chest X-ray changes and only minimal symptoms.

Other relevant clinical history

Oral aversion and failure to thrive developed in infancy, requiring G-tube feeding since 16 months of age. Currently the patient is receiving most of her calories by mouth, with minimal G-tube requirements. On physical examination at 8 years of age, height and weight were at the 0.18th and 0.35th percentiles, respectively.

Molecular genetic investigations

Trio WES revealed two variants in two genes: 1) a likely pathogenic variant (c.399delC; p.N133KfsX136) in SCYL1 (inherited from father). Due to phenotypic similarities between our patient and patients reported in the literature, Patient 1 underwent direct Sanger sequencing and deletion/duplication analysis of SCYL1, which identified previously reported likely pathogenic variant and deletions of exons 7-8 (maternally inherited). Patient 2 had both variants in SCYL1 by targeted variant testing. The diagnosis of SCYL1 disease was confirmed in both patients.

Western Blot of cultured skin fibroblasts

Western blot analysis for SCYL1 expression in fibroblast showed that SCYL1 is not expressed in fibroblasts from patient 1 (HF SK1) or patient 2 (HF SK2). There might be a shorter version of SCYL1 expressed at very low levels in HF SK1 and HF SK2 fibroblasts compared to normal Human dermal fibroblasts (HDF) (indicated as SCYL1-De7-e8). This shorter version could correspond to SCYL1 lacking HEAT repeats 1 and 2, which are expressed by Exon 7 and 8.
Note the absence of this SCYL1 form in SCYL1-deficient Hek293T cells supporting the idea that the shorter form may be expressed from the SCYL1 allele lacking exon 7 and 8.

**Literature review**

Seven studies reporting 16 patients from 12 unrelated families were identified (Schmidt et al 2015; Smith et al 2017; Lenz et al 2018; Shohet et al 2019; Spagnoli et al 2019; Li et al 2019; Chavany et al 2020). Demographics, clinical features, neuroimaging and genotypes of all patients are summarized in Supplemental Table 1. Frequency of clinical features of all patients, including our two new patients, are summarized in Table 2.

There was one patient who had liver transplantation due to acute liver insufficiency (Lenz 2018). The remainder of the patients presented with self-limiting liver disease. Recurrent respiratory infections and insufficiency was reported in one patient only (Spagnoli et al 2019). Neurological features were present in 94% of the patients. Skeletal features were present in more than half of the patients.

**Discussion**

We report two new patients with *SCYL1* disease. Although their phenotypes are similar, the degree and range of organ system involvement and age of onset are variable in both siblings including 1) Early infantile onset cholestatic hepatitis rapidly progressing to liver failure requiring liver transplantation in the younger sibling, compared to recurrent episodes of acute liver failure leading to chronic liver dysfunction requiring liver transplantation at an older age in the older sibling; 2) Recurrent severe upper and lower respiratory tract infections in the younger sibling, whereas rare upper and lower respiratory tract infections in the older sibling. The severity and chronicity of the liver disease and the need for early liver transplantation in our patients suggest a severe phenotype with chronic liver disease features that were not reported
before. In both of our patients, onset of liver disease appeared to correlate with the onset of viral illnesses. No other organ systems appear to be adversely affected by infectious episodes, as evidenced by the younger sibling having repeated respiratory illnesses in the years after transplant with no apparent lasting organ damage to date. The mechanism for the induction of liver disease to the exclusion of other organs is unclear.

Pediatric acute liver failure (PALF) is a dynamic clinical condition manifested by an abrupt onset of a liver-based coagulopathy and biochemical evidence of hepatocellular injury resulting from rapid deterioration in liver cell function. It can lead to liver transplantation and death. Main aetiologies of PALF include infections, inherited metabolic disorders, genetic disorders, drug toxicity, and autoimmune hepatitis, with up to 50% of cases of unknown etiology after extensive investigations [Squires et al. 2018]. With advancements of genetic testing, including whole exome sequencing, new monogenic etiologies of PALF have been identified in recent years. SCYL1 disease should be included in this expanding differential diagnosis of PALF.

There have been no deaths reported following episodes of liver failure in SCYL1 disease. Prior to this publication, only one individual with SCYL1-related disorders who underwent a liver transplant at 23 months of age (Lenz 2018). Here, we report two additional individuals requiring liver transplantation due to recurrent episodes of severe liver failure leading to chronic liver dysfunction in one sibling and due to cholestatic liver disease rapidly progressing to acute liver failure in the younger sibling. Our two cases suggest a more aggressive liver phenotype than previously reported, which could be associated with a more aggressive pathogenic variant in SCYL1. Most patients described previously recovered spontaneously between liver failure episodes, as early as two weeks after the episode of onset and most patients improved over time
with reduction in acute liver failure episodes (Chavany 2020). However, severe liver crisis due to SCYL1 mutations have been reported at 8 years of age (Lenz 2018). This raises concerns about potential irreversible neurological consequences and life threatening complications due to coagulopathy if severe liver failure episodes continue to occur in the future. There is also concern about progression of liver disease to chronic liver dysfunction, portal hypertension and advanced fibrosis over time, as reported in our patients, despite initial clinical improvement in between liver crisis for one of our patients. We believe that with the available limited data on the natural history of this disease, liver transplantation should be considered in cases of severe recurrent liver failure episodes and/or when there is evidence of progression to chronic liver dysfunction, taking into account family history and paternal preferences.

In terms of liver histopathological findings, non-specific findings have been previously described including cholestasis, microvesicular steatosis and fibrosis (Lenz 2018). Other reported findings include post hepatitic pattern with some features of resolving giant cell hepatitis and no evidence of fatty change (Lenz 2018). Liver biopsies and explant of both our patients did not show macro- or microvesicular steatosis, as reported previously (Lenz 2018). Although patterns of fibrosis have not been previously described in these cases, different degrees of fibrosis seem to be consistent amongst most histopathology reports, ranging from mild fibrosis to focal nodularity suggesting early cirrhosis (Lenz 2018). Our liver biopsies prior to transplantation and histopathology of liver explant for both patients showed cholestasis and hepatocyte feathery degeneration, without significant portal or lobular inflammation; as well as periportal and perivenular sinusoidal pattern of fibrosis.
Neurological phenotype is common in *SCYL1* disease. Knock-out *scyl1* mouse model presented with an early-onset motor neuron disorder (Schmidt et al 2007, Pelletier et al 2012). In human *SCYL1* disease, almost all patients had neurological features, although they were nonspecific. We report for the first-time multiple exon deletion, which was not identified by WES or by direct sequencing requiring deletion/duplication analysis. Our report emphasizes that even most sophisticated genetic investigations may miss the diagnosis and detailed literature review as well as phenotyping of patients can help physicians to arrive at a diagnosis.

**Conclusion**

In conclusion, we expand the spectrum of reported *SCYL1* disease by reporting two new cases of siblings that presented with different types of severe liver phenotype leading to liver transplantation in infancy, characterized by either early onset cholestatic liver disease rapidly progressing to liver failure or recurrent episodes of severe liver failure leading to chronic liver disease. Although both siblings presented developmental delay, motor and learning disabilities, the neurological, skeletal and respiratory phenotypes varied between them.

**REFERENCES**


### Tables

**Table 1**: Demographics, and clinical features of Patient 1 and 2 are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at last assessment</strong></td>
<td>12 years</td>
<td>9 years</td>
</tr>
<tr>
<td><strong>Sex</strong></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td><strong>Age of presentation</strong></td>
<td>5 months with ALF</td>
<td>4 months with bronchiolitis</td>
</tr>
<tr>
<td><strong>Age at first episode of acute liver failure</strong></td>
<td>5 months</td>
<td>5 months</td>
</tr>
<tr>
<td><strong>Number of liver failure episodes</strong></td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>Hepatosplenomegaly</strong></td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Elevation of GGT</strong></td>
<td>Mildly elevated GGT</td>
<td>Normal GGT</td>
</tr>
<tr>
<td><strong>Liver transplantation</strong></td>
<td>At 21 months of age</td>
<td>At 7 months of age</td>
</tr>
<tr>
<td><strong>Neurological features</strong></td>
<td>Tremor, dysdiadochinesia, modified Gower’s sign, stutter, abnormal sacchadic eye movements, cognitive dysfunction</td>
<td>Tremor, dysdiadochinesia, modified Gower’s sign, cognitive dysfunction, global developmental delay</td>
</tr>
<tr>
<td><strong>Behavioral disorder</strong></td>
<td>ADHD, anxiety, emotional outbursts</td>
<td>Oral aversion</td>
</tr>
<tr>
<td><strong>Respiratory features</strong></td>
<td>None</td>
<td>Recurrent upper and lower respiratory tract infections</td>
</tr>
<tr>
<td><strong>Skeletal features</strong></td>
<td>Scoliosis, short stature</td>
<td>Short stature</td>
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<tr>
<td><strong>Genitourinary features</strong></td>
<td>Unilateral inguinal hernia</td>
<td>None</td>
</tr>
<tr>
<td>Endocrinological features</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Table 2: Demographics and clinical features of 18 patients (including two new patients reported in this study) with SCYL1 disease were summarized in Table 2.

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>SCYL1 disease (n=18)</th>
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<tr>
<td>Sex (F/M)</td>
<td>10 females / 8 males</td>
</tr>
<tr>
<td>Liver phenotype</td>
<td>18/18</td>
</tr>
<tr>
<td>Acute liver failure</td>
<td>17/18</td>
</tr>
<tr>
<td>Age at first episode of A</td>
<td>13.4 months (n=15)</td>
</tr>
<tr>
<td>Normal or mildly elevated GGT</td>
<td>18/18</td>
</tr>
<tr>
<td>Proportion who had a liver transplant</td>
<td>3/18</td>
</tr>
<tr>
<td>Neuro phenotype</td>
<td>17/18</td>
</tr>
<tr>
<td>Skeletal phenotype</td>
<td>11/18</td>
</tr>
<tr>
<td>Genitourinary phenotype</td>
<td>3/18</td>
</tr>
</tbody>
</table>
Figure Legends

**Figure 1. Histopathology findings for patient 1.** a) hematoxylin and eosin stain of initial liver biopsy; magnification x 200, showing cholestasis and hepatocyte feathery degeneration, without significant portal or lobular inflammation; b) Masson trichrome stain for initial liver biopsy; magnification x 100, showing extensive severe periportal and perivenular sinusoidal fibrosis; c) hematoxylin and eosin stain of liver explant; magnification x 100, showing improved hepatocyte injury; d) Masson trichrome stain of liver explant; magnification x 100, showing incomplete fibrous septa and lesser degree of sinusoidal fibrosis.

**Figure 2. Histopathology findings for patient 2.** a) hematoxylin and eosin stain of initial liver biopsy; magnification x 200, showing mild cholestasis and hepatocyte feathery degeneration, without significant portal or lobular inflammation; b) Masson trichrome stain of initial liver biopsy; magnification x 100, showing variable and patchy mild to moderate periportal and perivenular sinusoidal fibrosis; c) hematoxylin and eosin stain of liver explant; magnification x 100, showing marked hepatocyte injury and parenchymal loss, predominantly in zones 2 and 3; d) Masson trichrome stain of liver explant; magnification x 100, showing parenchymal loss and extensive sinusoidal fibrosis.

**Figure 3.** Western Blot analysis for SCYL1 expression in patients’ fibroblasts. Protein extracts obtained from human dermal fibroblasts, fibroblasts from Patient 1 (HF SK1 and Patient 2 (HF
SK2) as well as hek293T cells and SCYL1-deficient Hek293T cells were analysed by western blotting using antibodies against SCYL1 (upper panel) or β-Actin (lower panel). Note the absence of full length SCYL1 in Patients fibroblasts compared to normal Human dermal fibroblast and hek293T cells. Also note the presence of a shorter form of SCYL1 in Patients fibroblasts which is absent in SCYL1-deficient Hek293T cells. This shorter form is likely produced from the SCYL1 allele missing exon 7 and 8 (SCYL1-Δe7-e8). The right panel shows the quantification of the western blot data presented in the left panel.
Figure 1: Histopathology findings for patient 1. a) hematoxylin and eosin stain of initial liver biopsy; magnification x 200, showing cholestasis and hepatocyte feathery degeneration, without significant portal or lobular inflammation; b) Masson trichrome stain for initial liver biopsy; magnification x 100, showing extensive severe periportal and perivenular sinusoidal fibrosis; c) hematoxylin and eosin stain of liver explant; magnification x 100, showing improved hepatocyte injury; d) Masson trichrome stain of liver explant; magnification x 100, showing incomplete fibrous septa and lesser degree of sinusoidal fibrosis.
Figure 2: Histopathology findings for patient 2. a) hematoxylin and eosin stain of initial liver biopsy; magnification x 200, showing mild cholestasis and hepatocyte feathery degeneration, without significant portal or lobular inflammation; b) Masson trichrome stain of initial liver biopsy; magnification x 100, showing variable and patchy mild to moderate periportal and perivenular sinusoidal fibrosis; c) hematoxylin and eosin stain of liver explant; magnification x 100, showing marked hepatocyte injury and parenchymal loss, predominantly in zones 2 and 3; d) Masson trichrome stain of liver explant; magnification x 100, showing parenchymal loss and extensive sinusoidal fibrosis.