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Disarranged Sphingolipid Metabolism From Sphingosine-1-Phosphate Lyase Deficiency Leads to Congenital Nephrotic Syndrome

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INTRODUCTION

Congenital nephrotic syndrome is a disorder characterized by severe proteinuria, hypoalbuminemia, and anasarca presenting during the first 3 months of life. The disease is typically caused by genetic defects that disrupt the integrity of the glomerular filtration barrier, with mutations in the podocyte genes *NPHS1*, *NPHS2*, *WT1*, and *LAMB2* contributing to approximately 85% of cases of congenital nephrotic syndrome.[1]

Recently, recessive mutations in the gene *sphingosine-1-phosphate lyase* (*SGPL1*) have been described in 16 families. Patients present with a syndromic form of congenital, steroid-resistant nephrotic syndrome characterized by a variable phenotype including adrenal insufficiency, neuronal dysfunction, muscular hypotonia, immunodeficiency, hypothyroidism, thrombocytopenia, and skin, skeletal, and genital abnormalities.[2–6]

Sphingosine-1-phosphate (S1P) is a ubiquitously expressed sphingolipid molecule contributing to multiple cellular responses, including cell proliferation and survival, differentiation, migration, and immune response modulation.[7] SGPL1 irreversibly degrades S1P, thus playing a key role in the regulation of this signaling molecule (Figure 1).[8] Mutations in *SGPL1* lead to decreased cell survival through accumulation of S1P.[9] Here, we describe the clinical course, renal histology, and lipidomic profile of a male infant with congenital nephrotic syndrome with immunodeficiency and neurodegeneration found to have novel *SGPL1* mutations.

CASE PRESENTATION

The patient was born as the second child of healthy, nonconsanguineous white parents after 39+3 weeks of pregnancy by spontaneous delivery (birth weight 3370 g). Prenatal ultrasound showed polyhydramnios and adrenal calcifications. He presented at 6 weeks of life with diffuse edema and was diagnosed with nephrotic syndrome. Infectious workup (cytomegalovirus, HIV, syphilis, and toxoplasmosis) was negative. He was managed at home on oral diuretics and was admitted twice for fluid management. Between 3 and 4 months of age he had rapid deterioration of kidney function with creatinine increasing from 0.32 mg/dl to 2.5 mg/dl and blood urea nitrogen increasing from 21 mg/dl to 80 mg/dl. In addition, he developed significant nephromegaly (right kidney 8.5 cm, left kidney 8.9 cm) leading to abdominal distention and feeding intolerance. The decision was made to perform bilateral nephrectomies and initiate peritoneal dialysis at 4 months of life, which remained the primary mode of renal replacement therapy.

At birth, he was found to have borderline micropenis, inguinal testes, and hypoplastic scrotum. Initial evaluation for endocrinologic abnormalities revealed normal serum electrolytes, fasting glucose, and adrenal steroids (sodium level 140 mmol/l, potassium level 5.2 mmol/l, testosterone-to-dihydrotestosterone ratio 1.1, 17-hydroxyprogestone level 30 ng/dl, and dehydroepiandrosterone level 47 ng/dl). A workup for hypogonadism revealed abnormally elevated gonadotropins (follicle-stimulating hormone 41 mIU/ml, luteinizing hormone 23 mIU/ml) and low serum testosterone

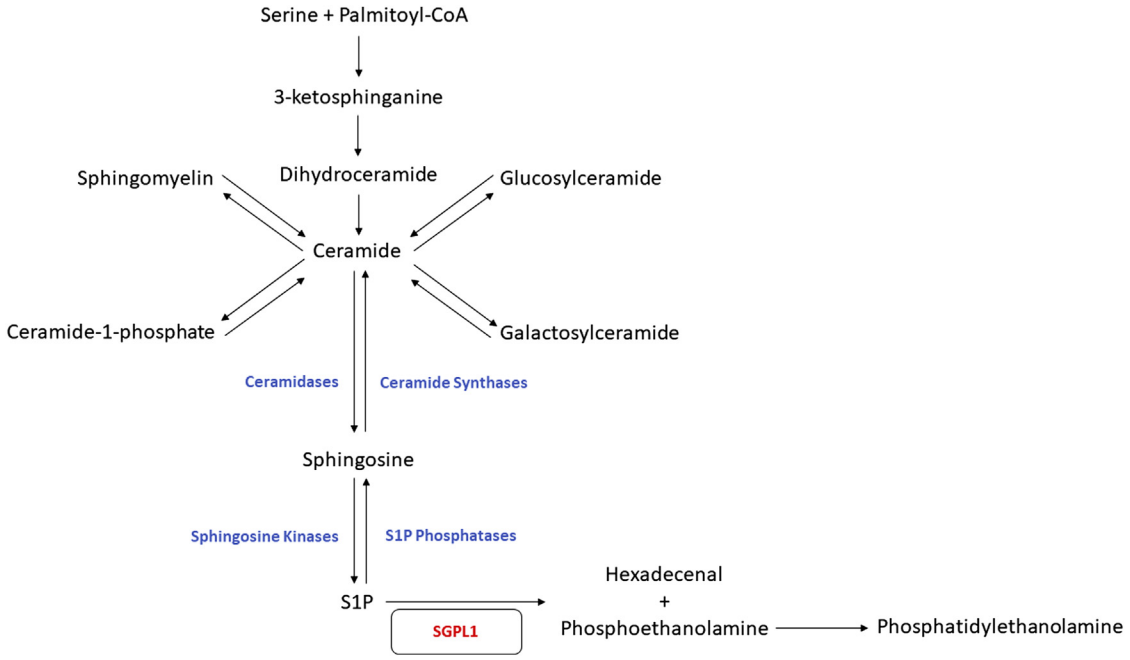

Figure 1. Sphingolipid metabolism. Sphingosine-1-phosphate lyase (SGPL1) catalyzes the final step of the sphingolipid pathway by irreversibly converting sphingosine-1-phophate (S1P) to its by-products.

(3 ng/dl). Postnatal imaging continued to show concern for calcification and enlargement of the adrenal glands. Low-dose adrenocorticotropic hormone stimulation test at 2 weeks of life was normal. Adrenocorticotropic hormone stimulation test was repeated at 14 months of age and confirmed adrenal insufficiency (baseline cortisol level 12.5 μg/dl and peak level 12.7 μg/dl). Stress dose hydrocortisone was given during illnesses and surgical procedures, but he did not require physiologic steroid administration. Additional endocrine issues included primary hypothyroidism (thyroid-stimulating hormone 13.3 mIU/l, free T4 1.4 ng/dl), for which he was started on levothyroxine at 1 month of life, and suboptimal growth with low-normal growth hormone level (4.7 ng/ml), low insulin-like growth factor–1 and insulin-like growth factor–binding protein 3 (15.7 ng/ml and 1011 ng/ml, respectively). Given his chronic kidney disease and poor growth velocity, he was started on growth hormone at 11 months of life. Dermatologic abnormalities included hyperpigmentation and hyperkeratosis.

He was found to have immunodeficiency consisting of persistent neutropenia (absolute neutrophil count $<500/mm^3$), lymphopenia (absolute lymphocyte count $<200/mm^3$) and hypogammaglobulinemia (IgA <6 mg/dl, IgG <120 mg/dl, IgM <35 mg/dl). Inflammatory markers and cytokines, including C-reactive protein, procalcitonin, and interleukin-6, were elevated. Bone marrow aspirate and flow cytometry showed B-cell progenitor hyperplasia. He was treated with filgrastim and i.v. Ig infusions. The patient was admitted multiple times for presumed infections, including sepsis, osteomyelitis, and meningitis, all of which were culture negative.

Neurologically, he was found to have sensorineural hearing loss shortly after birth. Between 12 and 14 months of age, he experienced rapid loss of developmental milestones accompanied by seizures and choreiform movements requiring treatment with antiepileptics and benzodiazepines. Magnetic resonance imaging of the brain, which was initially normal at 2 months of life, showed abnormal T2 hyperintense signal and calcifications in the basal ganglia with abnormal signal extending into the surrounding white matter. Steroids were initiated but imaging continued to show worsening of brainstem edema.

Genetic and Pathologic Findings

Renal Pathology

Light microscopy showed dysplastic-appearing kidney tissue with abnormally developed glomeruli associated with progressive degenerative changes, including globally sclerotic glomeruli, pseudo-crescents, tubular atrophy, and interstitial fibrosis. Light microscopic findings were consistent with diffuse mesangial sclerosis characterized by increased mesangial matrix and mesangial cell expansion.

The electron microscopy showed proliferative lesions with abnormal podocytes and thin basement membranes. There was no evidence of immune complex–mediated glomerulonephritis.

Cryosections of kidney tissue were obtained from the patient and age/gender-matched control.

Antibody staining was performed using goat anti-SGPL1 (R&D Systems, Minneapolis, MN), mouse anti-WT-1 (Santa Cruz Biotechnology, Dallas, TX), Ki-67 (Invitrogen, Carlsbad, CA), and 4′,6-diamidino-2-phenylindole nuclear stain. Imaging was obtained using a Nikon (Tokyo, Japan) A1 confocal microscope. Immunofluorescence intensity was measured using intensity profile on NIS Elements software version 5.20.00. Average intensity in the 561 nm was measured and a ratio of signal comparing patient with control was determined. Immunofluorescence of the patient's renal tissue showed an absence of SGPL1 staining and a 3-fold increase in S1P signal intensity in tubules compared with an age- and gender-matched control (Figure 2). Immunofluorescence staining by LC3A/B antibody was used to capture the degree of autophagy, and demonstrated reduced autophagy in the patient's renal tissue. In addition, patient kidney sections revealed increased Ki-67–positive cells, particularly in the glomeruli, suggesting increased proliferation.

Exome Sequencing

Based on high suspicion for SGPL1 deficiency, targeted gene sequencing was performed at Cincinnati Children's Hospital Medical Center. DNA was isolated from peripheral blood. Polymerase chain reaction and bidirectional sequencing were used to analyze the entire coding region and exon/intron boundaries of the gene *SGPL1*. Targeted sequencing revealed 2 novel variants consisting of a paternally inherited c.868T>C (p.Phe290Leu) and a maternally inherited c.993C>G (p.Tyr331*) variant. The first variant (c.868T>C) was not found in any publicly available databases. Multiple *in silico* prediction tools (AlignGVGD, SIFT, MutationTaster, Polyphen-2, Grantham Distance, BLOSUM45, BLOSUM62, BLOSUM80) were used to determine pathogenicity. Based on all available information, the variant was classified by our laboratory as likely pathogenic. The latter mutation (c.993>G) results in a premature stop codon in exon 11 of 15, which is thought to result in nonsense-mediated mRNA decay. This variant is not listed in the Human Gene Mutation Database or ClinVar. In gnomAD, it has a reported minor allele frequency of 0.00090% in the non-Finnish European population. Based on the available information, this variant was classified as pathogenic. As a result of these findings, we concluded that these variants were likely disease-causing in our patient.

Lipidomic Analysis

Serum and kidney tissue was obtained from our patient (nephrectomized specimen) and an age/gender-matched control. Sample preparation and lipid extraction were performed as previously described by Folch *et al.*[S1] Following tissue homogenization and lipid extraction, highly sensitive ultraperformance liquid chromatography coupled to tandem mass spectrometry was used to quantify different sphingolipid classes in each sample. Given its hydrophilic nature, levels of S1P were unable to be assessed via lipidomic analysis. Analysis revealed elevated levels of SGPL1 precursor substrates sphingosine, ceramide, and glucosylceramide in the patient's kidney tissue (Figure 3a–c) and serum (Figure 4a–c) compared with control samples. Sphingomyelin levels were elevated

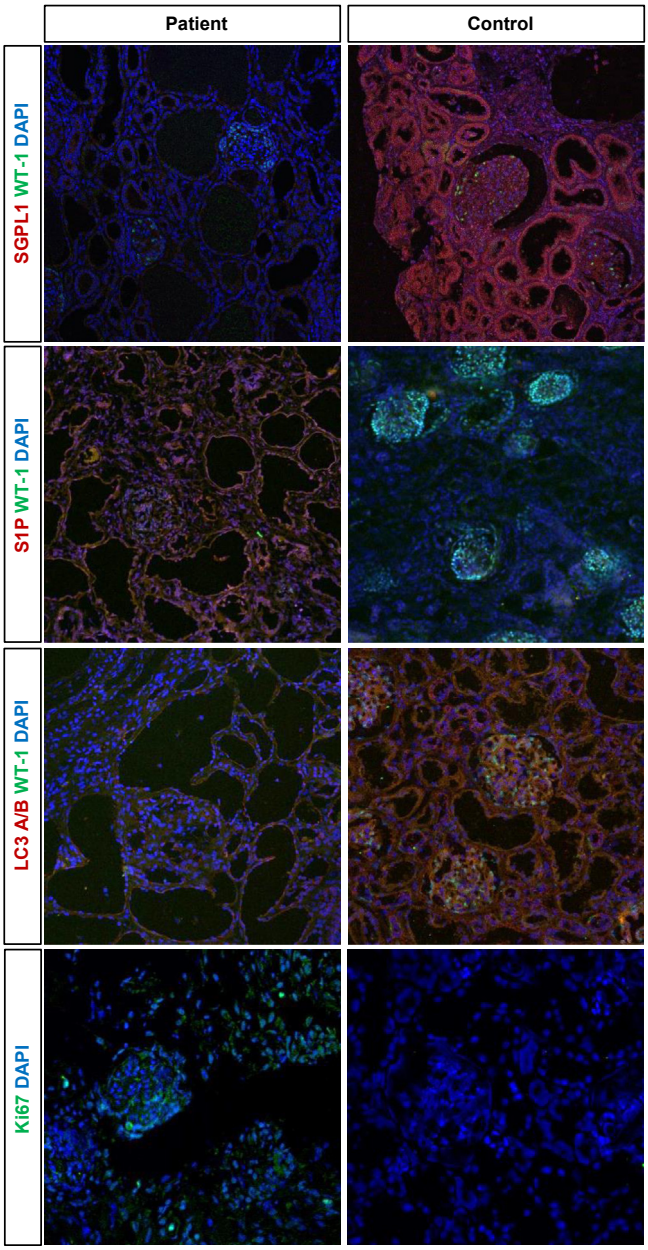

Figure 2. Immunofluorescence. Immunofluorescent microscopy of kidney tissue of a patient with sphingosine-1-phosphate lyase (SGPL1) deficiency showed absent SGPL1 staining, increased sphingosine-1-phosphate (S1P) staining, decreased autophagy (1A/1B-light chain 3 [LC3 A/B]), and increased proliferation (Ki-67 protein [Ki67]) compared with control tissue. Original magnification ×20. DAPI, 4′,6-diamidino-2-phenylindole; WT1, Wilms tumor 1.

in the patient's serum (Figure 4d), however, were decreased in kidney tissue (Figure 3d).

Follow-up

The patient was referred for transplantation; however, evaluation was put on hold to evaluate the etiology of the patient's neurodegeneration. The patient was diagnosed with SGPL1 deficiency at 12 months of age based on his clinical features and genetic testing results. In an effort to slow down the progression of the disease, he was started on soy formula containing phosphatidylethanolamine, an end product of SGPL1 metabolism. In an effort to stabilize the SGPL1 protein, he was started on high-dose pyridoxal phosphate, and later on i.v. pyridoxine when he developed feeding intolerance and concern for poor enteral absorption. Despite these efforts, his neurological status continued to worsen, and he died at 17 months of age secondary to cerebral edema.

DISCUSSION

In this study, we report a patient found to have 2 novel heterozygous variants in *SGPL1*. Including this study, 16 different variants of *SGPL1* in 16 families have been reported.[2–6] Our patient with congenital nephrotic syndrome symptoms presented with similar findings to these reported cases, including adrenal insufficiency, neurologic abnormalities, muscular hypotonia, skin and genital abnormalities, immunodeficiency, and hypothyroidism. In addition, the patient presented here had rapid enlargement of his kidneys, significant decline in kidney function by 4 months of age, and neurologic deterioration starting at 12 months of age.

Defects of sphingolipid metabolism are known to result in renal disease through the accumulation of sphingolipids. In Fabry disease, accumulation of globotriaosylceramide leads to progressive podocyte injury.[S2] *SGPL1* encodes for SGPL1, an enzyme that acts as a gatekeeper of sphingolipid metabolism by executing the only exit point for sphingolipid intermediates by converting the substrate S1P irreversibly into ethanolamine phosphate and hexadecenal (Figure 1).[9] S1P, acting through plasma membrane G-protein–coupled receptors or directly on intracellular targets, commands a complex network of cellular responses including cell proliferation, survival, differentiation, and migration.[7]

As previously identified, mutations in *SGPL1* have been shown to result in reduced or absent SGPL1 protein, enzymatic activity, or protein mislocalization. It has been hypothesized that disease may result from excess intracellular S1P or S1P receptor signaling, an imbalance of sphingoid bases, or lack of S1P by-products.[2–5] We suspect our patient had a completely nonfunctional SGPL1 enzyme, given his genetic mutation, which resulted in a premature stop codon. Further supporting this theory, lipidomic analysis showed significantly elevated upstream products, and immunofluorescence showed absence of renal SGPL1 staining. The heterogeneity and severity of symptoms in reported subjects may reflect differing amounts of functional SGPL1 protein, with our case presenting with a more severe phenotype in the absence of a functional SGPL1 protein.

Prior research has shown that serum and fibroblasts isolated from patients with SGPL1 deficiency have increased levels of SGPL1 substrates in comparison with controls.[2–4] SGPL1 has been shown to be localized to the endoplasmic reticulum of renal podocytes and mesangial and endothelial cells.[2] Accumulation of S1P, a substrate for SGPL1, induces mesangial cell proliferation via activation of endothelial differentiation gene family of G-coupled receptors.[S3] We propose that the complete absence of *SGPL1* expression coupled with increased S1P levels in renal tissue of our patient explains the extensive mesangial cell proliferation and nephromegaly. It has been shown that the balance between different sphingolipid levels, specifically S1P and ceramide, can regulate renal tubular apoptosis.[S4] In our patient, levels of proteins upstream of SGPL1, including sphingosine, ceramide, and glucosylceramide, were increased in the kidney tissue. Increased levels of ceramides cause cells to exhibit proliferation arrest, apoptosis, and autophagy, and glycosylation of ceramides arrest these processes and has been shown to be responsible for resistance to cancer treatment in tumor cells.[S5] We propose that increased glucosylceramide levels in patient kidney tissue limit apoptosis and tilt the balance toward cellular proliferation in mesangial cells. Increased levels of glucosylceramide are also seen in the lysosomal storage disease Gaucher disease, in which accumulation of this sphingolipid leads to cytopenia, splenomegaly, hepatomegaly, bone lesions, and neurological impairment.[S6]

In response to cellular growth cues, autophagy manifests itself as a cellular mechanism to limit cell proliferation and promote cell survival. In stress cell signaling response through the endoplasmic reticulum causes increase in misfolded proteins. Mouse models have demonstrated that inhibition of sphingosine kinase-1, which converts sphingosine to S1P, leads to renal fibrosis through decreased autophagy in renal tubular epithelial cells.[S7] Phosphatidylethanolamine, an end product of SGPL1 activity, modulates neuronal autophagy.[S8] We speculate that decreased autophagy due to decreased phosphatidylethanolamine levels in the presence of proliferation inhibits elimination of misfolded proteins resulting in increased cellular damage and fibrosis in SGPL-1 deficiency. Further research is needed to investigate this. It also has been hypothesized that S1P buildup may contribute to renal fibrosis through its upregulation of cyclooxygenase-2

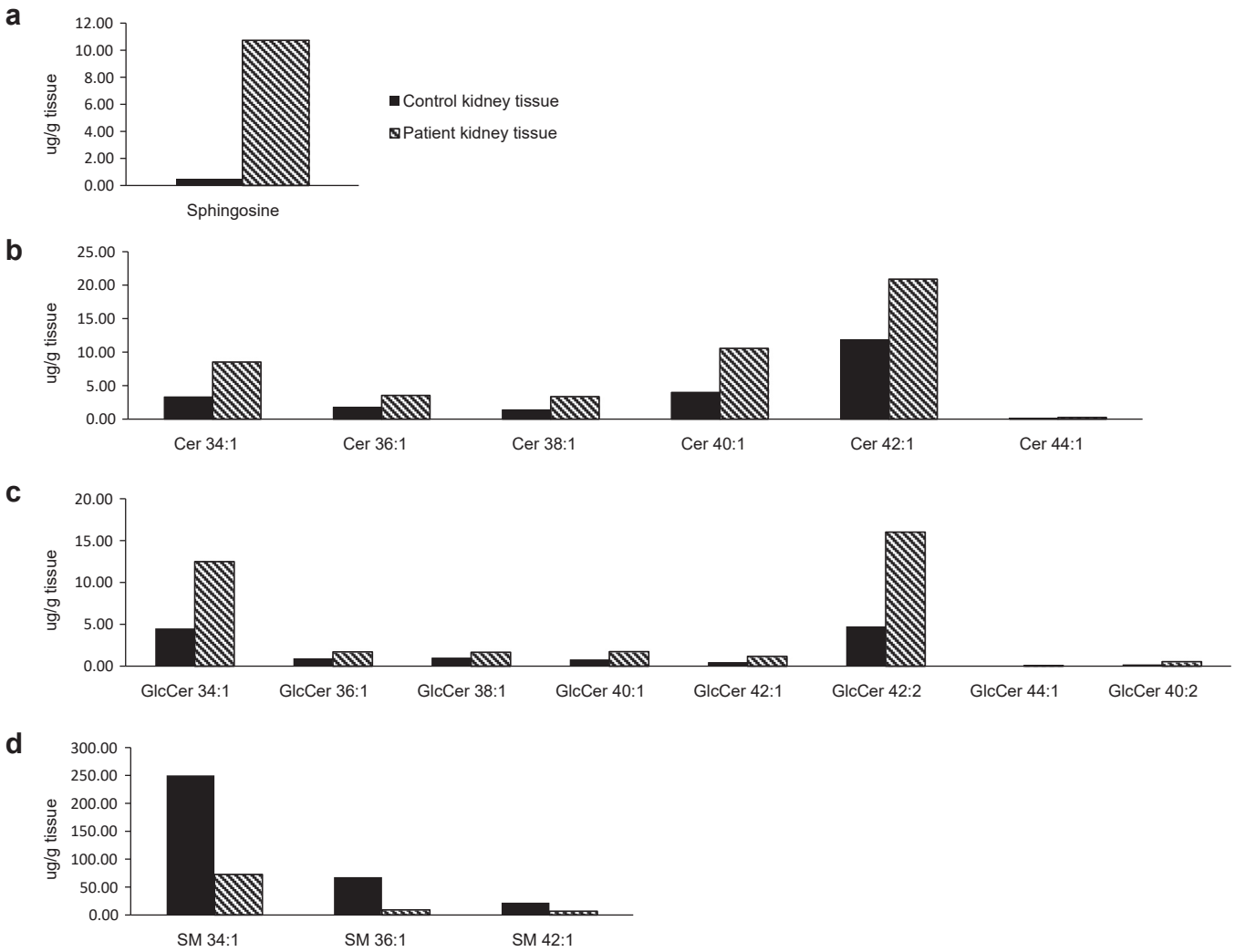

Figure 3. Kidney tissue sphingolipids. Levels (a) sphingosine, (b) ceramide (Cer), (c) glucosylceramide (GlcCer), and (d) sphinomyelin (SM) in a patient with sphingosine-1-phosphate lyase deficiency compared with control tissue. Cer and GlcCer levels were increased in patient kidney tissue, whereas SM levels were elevated in normal kidney tissue.

expression and subsequent prostaglandin E2 formation in renal mesangial cells.[S9]

In addition, lipidomic analysis of patient kidney tissue revealed decreased sphingomyelin levels, whereas serum analysis showed increased sphingomyelin levels compared with control sample. This may represent a difference in the metabolism of ceramide to sphingomyelin in different tissue types and release into the circulation; however, further research is needed. Accumulation of S1P in SGPL1-deficient neurons has been shown to induce apoptosis indicating organ-specific effect of S1P on cell cycle.[S10] Approximately half of the reported patients surviving for at least 1 month exhibit pathological neurological features ranging from mild neurodevelopmental delay, sensorineural hearing loss, and peripheral nerve paralysis to severe encephalopathic neurodegenerative disease.[2–6] Our patient had rapid neurological decline with MRI abnormalities first presenting in the basal ganglia, which may have been secondary to accumulation of S1P and other sphingolipids. The importance of sphingolipid metabolism in neuronal function also has been demonstrated by researchers identifying an atypical form of Charcot-Marie-Tooth neuropathy caused by compound heterozygous mutations in *SGPL1*.[S11] In addition, the accumulation of sphingolipid metabolites has been shown to cause neurodegenerative disease in other sphingolipidoses, including Gaucher, Fabry disease, and Niemann-Pick disease.[S6]

Our patient also had immunodeficiency characterized by neutropenia, lymphopenia, and hypogammaglobinemia. SGPL1-deficient mice exhibit a heightened inflammatory response and have impaired neutrophil migration into inflamed tissue.[S12] They also have lymphopenia from impaired egress from primary and secondary lymphoid organs, and enhanced

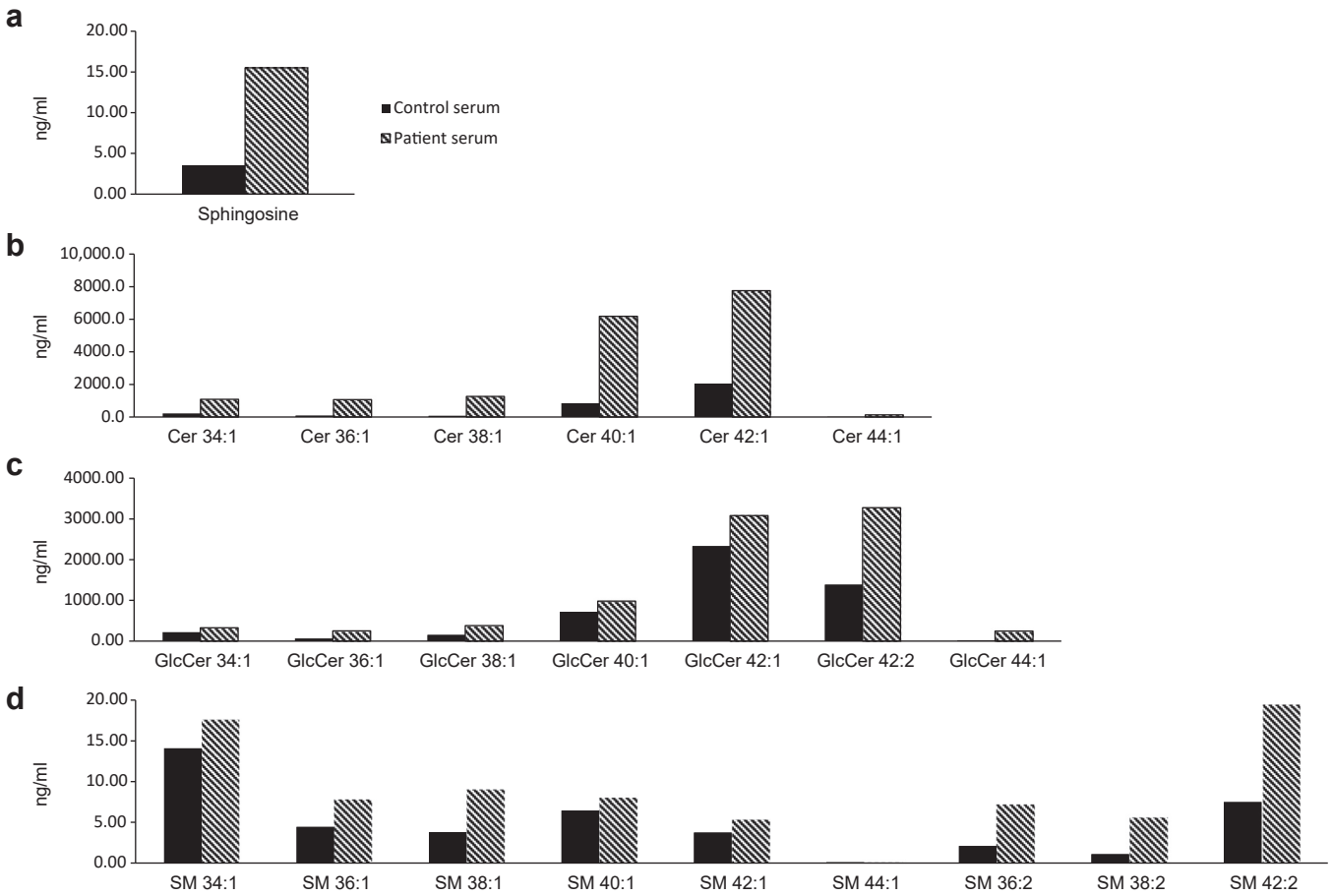

Figure 4. Serum sphingolipids. Levels of (a) sphingosine, (b) ceramide (Cer), (c) glucosylceramide (GlcCer), and (d) sphinomyelin (SM) were elevated in patient serum with sphingosine-1-phosphate lyase deficiency compared with control.

apoptosis of developing T cells from accumulation of ceramides.[S13] Our patient was hospitalized several times for culture-negative infection. Given his elevated cytokines and inflammatory markers, it was questioned whether these episodes represented true infections or the byproduct of excess inflammation. As patients with SGPL1 deficiency can have immunodeficiency, providers must be vigilant for signs of infection and also be judicious about antimicrobial use.

Patients with SGPL1 deficiency also demonstrate adrenal abnormalities, including insufficiency, calcifications, and enlargement.[2–6] Recently, others have identified patients with primary adrenal insufficiency secondary to missense mutations in *SGPL1* without other clinical manifestations.[S14] It has been suggested the adrenal issues seen in this disease may arise from disrupted adrenocortical zonation and the high presence of SGPL1 in the zona reticularis, which is responsible for adrenal androgen secretion.[2]

Currently, there is no curative treatment for SGPL1 deficiency. It has been suggested that phosphatidylethanolamine supplementation may serve a therapeutic role in treatment of the neurodegenerative disease via modulation of neuronal atrophy.[S8] Given the patient's rapid neurological decline, a trial of soy formula containing high amounts of phosphatidylethanolamine was initiated.[S15] In addition, pyridoxal phosphate and pyridoxine were given in an effort to stabilize any SGPL1 protein present as SGPL1 is a pyridoxal 5′-phosphate-dependent enzyme.[9] These treatments may have been more beneficial if the patient had some degree of enzymatic activity or if initiated earlier. Studies have shown that bacterial SGPL1 delivery reduces circulating S1P levels by 70% in mice, and therefore enzyme replacement therapy may be a potential intervention in the future.[S16]

This is the first study to examine renal sphingolipid metabolites using lipidomic analysis and immunofluorescence in a patient with SGPL1 deficiency. We showed sphingolipids upstream to the SGPL1 enzyme, including ceramide and glucosylceramide, were elevated in a patient with SGPL1 deficiency. However, we were unable to examine levels of S1P and SGPL1 by-products by lipidomic analysis. In addition, to our knowledge, we are the first to trial treatments as a way to slow disease progression. Evaluation of treatment response was

Table 1. Teaching points

1. Deficiency of the enzyme SGPL1 leads to increased accumulation of sphingolipids.
2. Renal cells deficient in this enzyme show decreased autophagy and increased proliferation.
3. Gene sequencing for mutations in *SGPL1* is recommended for patients with nephrotic syndrome presenting with extrarenal manifestations, immunodeficiency, and endocrinological and neurodevelopmental abnormalities.
4. Early diagnosis of genetic causes of nephrotic syndrome can allow for appropriate counseling.

SGPL1, sphingosine-1-phosphate lyase.

limited as we were unable to assess enzymatic activity along with phosphatidylethanolamine and S1P levels before and after treatment. Further research is needed in these areas to improve patient outcomes.

Based on our patient's presentation and clinical course, we recommend that genetic testing directed to *SGPL1* mutations be considered in patients with congenital and steroid-resistant nephrotic syndrome presenting with extrarenal manifestations, particularly with immune and neurodevelopmental abnormalities. Although there is no treatment for SGPL1 deficiency, early diagnosis can allow early identification of other comorbidities of the disease and enable clinicians to counsel patients and families appropriately (Table 1).

DISCLOSURE

All the authors declared no competing interests.

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SUPPLEMENTARY MATERIAL

Supplementary File (Word)

Supplementary References.

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