Journal of Clinical Lipidology (2017) 11, 1480–1484

Journal of Clinical Lipidology

A case of severe acquired hypertriglyceridemia in a 7-year-old girl

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KEYWORDS:
Hypertriglyceridemia;
Lipoprotein lipase;
Autoantibodies;
Immunosuppression;
Disease phenocopy;
Pancreatitis;
Lipid modulators;
Lupus;
Sjögren's

Abstract: We report a case of severe type I hyperlipoproteinemia caused by autoimmunity against lipoprotein lipase (LPL) in the context of presymptomatic Sjögren's syndrome. A 7-year-old mixed race (Caucasian/African American) girl was admitted to the intensive care unit at Vanderbilt Children's Hospital with acute pancreatitis and shock. She was previously healthy aside from asthma and history of Hashimoto's thyroiditis. Admission triglycerides (TGs) were 2191 mg/dL but returned to normal during the hospital stay and in the absence of food intake. At discharge, she was placed on a low-fat, low-sugar diet. She did not respond to fibrates, prescription fish oil, metformin, or orlistat, and during the following 2 years, she was hospitalized several times with recurrent pancreatitis. Except for a heterozygous mutation in the promoter region of LPL, predicted to have no clinical significance, she had no further mutations in genes known to affect TG metabolism and to cause inherited type I hyperlipoproteinemia, such as *APOA5, APOC2*, *GPIHBP1*, or *LMF1*. When her TG levels normalized after incidental use of prednisone, an autoimmune mechanism was suspected. Immunoblot analyses showed the presence of autoantibodies to LPL in the patient's plasma. Autoantibodies to LPL decreased by 37% while patient was on prednisone, and by 68% as she subsequently transitioned to hydroxychloroquine monotherapy. While on hydroxychloroquine, she underwent a supervised high-fat meal challenge and showed normal ability to metabolize TG. For the past 3 years and 6 months, she has had TG consistently <250 mg/dL, and no symptoms of, or readmissions for, pancreatitis.

Introduction

Fredrickson's type 1 hyperlipoproteinemia (T1HLP; OMIM# 238600) is characterized by elevations in fasting plasma triglycerides (TGs) to >1000 mg/dL, with chylomicronemia and a low total cholesterol/TG ratio (<0.1).[1] Children with T1HLP are typically assumed to have inherited defects in the processing of TG-rich lipoproteins, which result in chronic severe hypertriglyceridemia, recurrent pancreatitis, and lipemia retinalis.[2] Lipoprotein lipase (LPL) is the rate-limiting enzyme for hydrolysis of TG-rich lipoproteins, and homozygous loss of LPL is a cause of severe hypertriglyceridemia presenting in childhood.[3] This extreme dyslipidemia is rare, affecting 1 in 1,000,000 individuals,[3] whereas most pediatric hypertriglyceridemia cases are due to secondary causes such as drugs

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Submitted June 12, 2017. Accepted for publication August 7, 2017.

1933-2874/
http://dx.doi.org/10.1016/j.jacl.2017.08.003

or comorbidities affecting lipids.[4] Previous studies have shown that hypertriglyceridemia can be induced by autoantibodies against TG-regulating targets, such as LPL and GPIHBP1,[5–11] a protein that binds LPL and shuttles it to its site of action in the capillary lumen. Some of the reported patients with GPIHBP1 or LPL autoantibodies responded to treatment with immunosuppressive agents leading to full or partial normalization of their plasma TG levels.[6–9]

Case report

Case presentation

A previously healthy but obese 7-year-old girl with a history of asthma and Hashimoto's thyroiditis was admitted at Vanderbilt Children's Hospital after 2 weeks of abdominal pain, vomiting, and lethargy. On examination, she had severe epigastric tenderness, an enlarged and firm thyroid, and eruptive xanthomatosis on the face and hands. There was no acanthosis nigricans, and puberty was Tanner stage I. The patient's plasma was noted to be lipemic, making initial laboratory evaluation difficult. After several attempts, a lipid panel was obtained, 12 hours after admission, and revealed TG of 2191 mg/dL (December 7, 2011) with markedly elevated amylase and lipase levels (240 and 135 U/L, respectively). Abdominal CT revealed fat stranding in the pancreas with development of a pseudocyst. During her admission, the patient was treated with intravenous hydration and bowel rest. Of note, bowel rest as an approach to pancreatitis is no longer the norm and has been shown to not improve recovery.[12] Her TG levels were reduced to the 250 to 500 mg/dL range with the introduction of nasogastric tube feeds with the low-fat formula Vivonex. TG levels decreased to 150 mg/dL after an overnight fast. She was discharged home with the presumptive diagnosis of LPL deficiency, and family was counseled by our specialized dietician to follow a very low-fat diet (<20 g/d) also restricted in simple sugars. The patient's mother had a history of obesity with mild elevation in TG (166 mg/dL, nonfasting) and low high-density lipoprotein (36 mg/dL). Her father was not available for testing but was reported to have normal lipid levels. The patient is an only child.

The patient continued to develop recurrent pancreatitis and hypertriglyceridemia requiring inpatient treatment in the following months. During each episode, her TG level would rise well above 2500 mg/dL (Fig. 1) and would drop to, and remain within, the normal range while in the hospital without oral nutrition. Her family reported strict compliance with the prescribed outpatient diet, but the patient's weight remained stable (>95th percentile for her age), rather than decreasing as expected with such dietary restriction. Because fibrate, metformin, orlistat, and high-dose omega-3 fatty acids (4 g daily) had minimal effects on her lipid profile or clinical course, they were used in cycles and eventually discontinued altogether.

In late November 2012, the patient contracted an upper respiratory infection and had an asthma exacerbation. Her pediatrician prescribed prednisone at a dose of 1 mg/kg twice daily for 5 days. Due to concern for the glucocorticoid potentially elevating her TG levels to the pancreatitis range, her plasma lipids were checked during therapy, and her TG had normalized to 105 mg/dL. The low TG levels were originally attributed to low appetite and reduced food consumption in the setting of her illness; however, TG remained low for the next 7 weeks, a phenomenon that had not been witnessed in this patient outside hospital control. After this period of excellent control, she was readmitted with abdominal pain and pancreatitis with an acute rise in the TG level to 6840 mg/dL. Her TG levels in hospital once again decreased to within the normal range.

Investigation

Given the patient's response to prednisone, the diagnosis of autoimmune response affecting LPL function was considered. The patient was found to have positive anti-Ro (SS-A) and anti-La (SS-B) antibodies. However, antibodies to double-stranded DNA, Smith antigen, and ribonucleoprotein were negative. Total immunoglobulin G levels were near the upper limit of normal at 1599 mg/dL. She had mild leukopenia (3400 white cells/μL) but no other clinical features of lupus or Sjögren's syndrome were present. The patient did not have mutations in *GPIHBP1*, lipase maturation factor 1 (*LMF1*), apolipoprotein A5 (*APOA5*), or apolipoprotein C2 (*APOC2*), the key proteins in the processing of TG-rich lipoproteins. A heterozygous mutation in the promoter region of LPL was discovered (intron 1-10delT). Splice site analysis suggested a potential creation of a leaky splice site, with no predicted effects on protein sequence or expression. This mutation has not been previously reported.

To assess whether the patient's plasma contained antibodies reacting against LPL, we used recombinant human LPL (Santa Cruz Biotechnology, Santa Cruz, CA, USA) in a Western blot assay as shown in Figure 2.

Purified human LPL (15–30 μg) was loaded in multiple lanes onto 4% to 12% Bis-Tris precast gels (Invitrogen, Waltham, MA, USA) for electrophoresis and then transferred onto a nitrocellulose membrane. Because each lane on the blot had to be incubated with different plasma samples (as "primary antibody"), we cut the membrane into vertical stripes. After the incubation of each strip with a different plasma sample (at 1:100 dilutions) or with commercially available anti-LPL antibody (Abcam, Cambridge, MA, USA) as positive control (at 1:5000 dilution), all stripes were taped back together, washed, and incubated together with horseradish peroxidase–conjugated goat anti-human IgG followed by addition of enhanced chemiluminescence solution for detection. Immunoblot analyses revealed that during her most recent hospitalization, when TG levels were 6840 mg/dL, her plasma contained antibodies against LPL (Fig. 2A and B). Because of

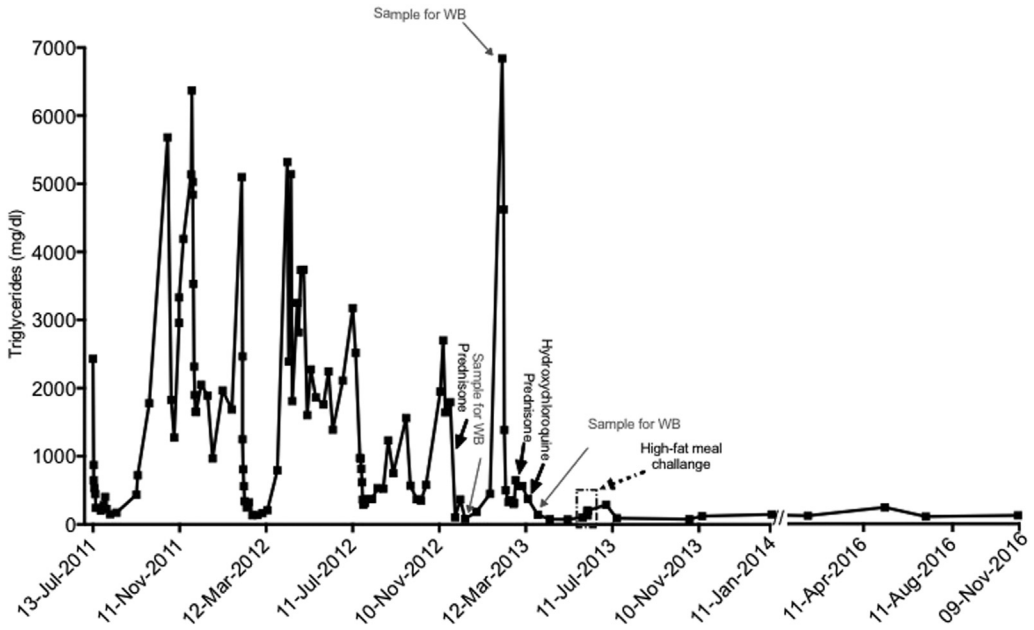

Figure 1 Plasma triglyceride levels starting from patient's first hospitalization for pancreatitis. Black arrows indicate treatment with immunosuppressant; gray arrows indicate plasma samples that were used for the immunoblot shown in Figure 2. Dashed-line rectangle and arrow indicate triglyceride levels during a supervised high-fat meal challenge. WB, Western blot (immunoblot).

technical limitations, we were not able to determine whether the patient's plasma inhibits LPL activity.

Treatment

Once high titers of anti-LPL antibody were discovered, the patient was given a 2 mg/kg prednisone loading dose followed by 1 mg/kg twice daily, for the next week; this was slowly tapered and eventually discontinued as hydroxychloroquine was introduced. Hydroxychloroquine was chosen because of its relatively mild immunosuppressive profile and long history of use in children. Immunoblot analyses show that plasma reactivity against LPL was 37% lower 2 weeks after the start of prednisone (Fig. 2A) and 68% lower a week after hydroxychloroquine administration started (Fig. 2B). The plasma sample, collected while the patient was on hydroxychloroquine, did not have detectable anti-GPIHBP1 antibodies (not shown). Chronic therapy with hydroxychloroquine has maintained the patient's TG levels within or just above the upper limit of normal. After taking hydroxychloroquine for almost 3 months and weaning off oral prednisone, the patient underwent a supervised high-fat meal challenge with the consent of her mother. The patient ate a fast food meal containing 120 g of fat and 56 g of sugars. TG levels were 132 mg/dL at baseline and rose to 195 and 203 mg/dL at 2 and 4 hours after the meal.

Outcome and follow-up

On the regimen of hydroxychloroquine, the patient did not develop further episodes of pancreatitis. Her TG levels fluctuated from within the normal range to <250 mg/dL despite liberalization of her diet. In November 2015, she was found to have nephrotic range proteinuria and serum albumin of 2.5 g/dL. Renal biopsy showed immune complex nephropathy in a predominantly membranous pattern,

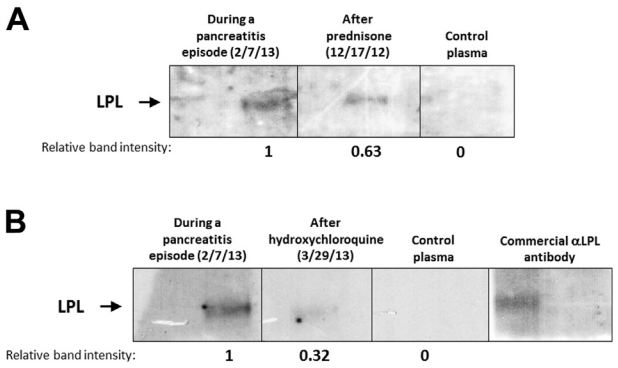

Figure 2 Immunoblot for LPL using patient's plasma as the primary antibody source. (A) Strips of nitrocellulose membrane containing 15 μg of recombinant human LPL were incubated with plasma from the patient during a pancreatitis episode (left lane) or week after prednisone administration (middle lane), and with pooled plasma from normolipidemic subjects (right lane). (B) Strips of nitrocellulose membrane containing 30 μg of recombinant human LPL were incubated with plasma from the patient during a pancreatitis episode (left lane) or 2 weeks after hydroxychloroquine administration (second lane from the left), pooled plasma from normolipidemic subjects (second lane from the right), and commercial anti(α)-LPL antibody (right lane). Plasma samples were diluted 1:100 in 3% skim milk; commercial antibody was diluted 1:500 in 3% skim milk. Semi-quantitative measurements of LPL band intensity were done using ImageJ.

consistent with membranous lupus nephropathy. Based on Systemic Lupus International Collaborating Clinics Classification Criteria,[13] she met criteria for systemic lupus erythematosus. Tacrolimus was initiated and resulted in prompt resolution of proteinuria. She eventually required mycophenolate mofetil as well for control of renal disease. She has continued hydroxychloroquine therapy in addition to tacrolimus and mycophenolate and has not had another episode of pancreatitis through June 2017.

Discussion

Children with T1HLP are typically assumed to have inherited defects in 1 or more of the proteins involved in the processing of TG-rich lipoproteins. Several proteins must work in concert to ensure normal TG processing and catabolism, ranging from circulatory (apoC2, apoA5, apoE) to capillary bound (LPL and GP1HBP1) to intracellular (LMF1) and mutations in any of these genes may disrupt function. Although the true frequency of the genetic causes of this rare autosomal recessive condition is difficult to estimate, LPL deficiency is thought to be the most common enzyme defect causing inherited chylomicronemia.[3] The presence of phenocopies is common in extreme dyslipidemia, when a phenotype does not appear to have a genetic basis and may be actually secondary to acquired causes.[4] Our patient had no mutations in any of the 5 candidate genes mentioned previously. Furthermore, her presentation was atypical for an inherited enzyme deficiency; she was asymptomatic for her first 7 years of life despite being on a high-fat, high-sugar diet resulting in obesity. This child did not have diabetes, did not take thyroid hormone replacement or any other medications, had normal thyroid function (see Supplementary Table) despite high titers of antithyroid peroxidase antibodies, and had no evidence of liver or kidney disease. There were no home remedies or supplements reported to suggest a potential environmental poison. Thus, common secondary causes of hypertriglyceridemia[4] were ruled out.

Because of her unexpected TG-normalizing response to prednisone for an asthma flare up, we discovered that the patient's plasma contained autoantibodies against LPL. Antibodies against LPL could affect its function in several ways, including blocking LPL activity or blocking its interaction with apoC2 or GPIHPB1. A similar case was reported in a 9-year-old African American girl who was later diagnosed with Sjögren's syndrome.[6] Other cases of autoimmunity-mediated hyperlipidemia were previously reported to be associated with immune thrombocytopenic purpura and Graves' disease,[5] antithyroid peroxidase or anti-striated muscle antibodies,[8] and family history of autoimmunity.[10] Although our patient had mild leukopenia, she had no other clinical features of lupus or Sjögren's syndrome such as dry eyes, dry mouth, or joint pain. Lupus patients have been shown to test positive for anti-LPL antibodies although the association with extreme hypertriglyceridemia in these patients is not clear.[14] More recently, several cases of autoantibodies against GPIHBP1 were reported to cause acquired extreme hypertriglyceridemia.[9] Interestingly, the autoantibodies against GPIHBP1 associated with severe hypertriglyceridemia were found to block the binding of LPL to GPIHBP1.[9]

It has been reported that some patients with autoantibodies against LPL or GPIHBP1 achieved clinically significant reduction in TG after immunosuppression with different agent, including azathioprine, mycophenolate mofetil, hydroxychloroquine, and prednisone.[6–9] Using immunosuppressants, our patient was able to achieve up to 68% reduction in her anti-LPL antibody reactivity, leading to long-lasting, near-normal TG levels. It is notable that she was able to achieve normal TG levels despite the continued presence of the autoantibody, suggesting that a threshold effect is likely at play. Although the patients' plasma, while on hydroxychloroquine, did not have detectable anti-GPIHBP1 antibodies, we cannot rule out the possibility that off treatment, the patient had antibodies against both GPIHBP1 and LPL.

In conclusion, autoimmunity is a cause of acquired severe hypertriglyceridemia and should be considered when other causes of genetic and secondary chylomicronemia have been excluded, even in children and even in the absence of symptoms or signs of autoimmune disease. Importantly, our case shows that autoimmune severe hypertriglyceridemia is not always and exclusively due to GPIHBP1 autoantibodies. Finally, immunosuppressant therapy can correct antibody-induced hypertriglyceridemia and achieve normalization of plasma TG levels.

Acknowledgment

This study was supported by an American Heart Association (AHA) Scientist Development Grant (16SDG27520011) to H.T. The authors thank Dr Robert Hegele of Robarts Laboratories in London Ontario for the performance of the genetic analyses, Dr Steve Young of UCLA for the measurement of anti-GPIHBP1 antibodies, and Deanna Plubell of OHSU for figures production.

Authors' contributions: J.C.S., M.F.L., J.C.K., T.B.G., and S.F. were responsible for the clinical management of the case. J.C.S., S.F., and H.T. were responsible for LPL reactivity analyses, data collection, and article production.

Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jacl.2017.08.003.

Financial disclosure

The authors J.C.S., M.F.L., J.C.K., T.B.G., S.F., and H.T. declare no conflicts of interest.

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