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Severe Familial Hypertriglyceridemia: Successful Treatment With Insulin and a Modified Meal Plan

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Context: Mutations in genes encoding the lipoprotein lipase enzyme, its cofactor, or transport proteins can cause severe familial hypertriglyceridemia, resulting in serious complications, such as severe pancreatitis, hepatosplenomegaly, lipid encephalopathy, and failure to thrive. Current treatment includes a low-saturated-fat formula enriched with high medium-chain triglyceride (TGs), oral fibrates, omega-3 fatty acids, or plasmapheresis.

Case Description: A 71-day-old infant with very severe hypertriglyceridemia and recurrent pancreatitis associated with a likely pathogenic variant in the *LPL* gene was treated successfully with insulin infusion and a locally prepared low-fat formula feed after stopping breast milk. Subcutaneous insulin was administered daily from 9 to 30 months of age. His serum TG level was markedly lower, although higher than normal. No episodes of hypoglycemia were noted. Fenofibrate and omega-3 fatty acids were ineffective in this infant. At the last follow-up visit, he was 36 months old and growing normally. He was consuming a special meal plan and receiving insulin injections during high-fat meals. Two other young infants with severe hypertriglyceridemia were growing normally after a short course of insulin infusion and the same modified reduced long chain fat diet.

Conclusions: Insulin is an unusual and affordable therapeutic option for some patients with severe hypertriglyceridemia and can be helpful in the prevention of acute and chronic complications. Locally available cereals and millets with high crude fiber and a low glycemic index, along with medium chain TGs, was used to prepare an economical special formula at home to maintain TG concentrations in the acceptable limits.

Freeform/Key Words: Hypertriglyceridemia, Pancreatitis, Insulin, Medium chain triglyceride

The accumulation of circulating triglycerides (TGs) results in hypertriglyceridemia (HTG) [1]. Dietary TGs are assembled into chylomicrons (large lipoprotein molecules) in the gut [2]. The enzyme lipoprotein lipase (LPL) and its cofactor apolipoprotein-CII are responsible for the clearance of chylomicrons that appear in circulation after the absorption of dietary fat [3]. LPL is produced by myocytes and adipocytes and transported to the luminal surface of

Abbreviations: HTG, hypertriglyceridemia; LPL, lipoprotein lipase enzyme; MCT, medium chain triglyceride; T2DM, type 2 diabetes mellitus; TG, triglyceride.

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capillaries for release of free fatty acids from TGs in chylomicrons and hepatic very-low-density lipoproteins [1, 4].

Factors contributing to elevated TGs include overweight status, insulin resistance, type 2 diabetes mellitus (T2DM), physical inactivity, and genetic disorders [2]. Using the serum TG levels, childhood HTG has been classified as mild (150 to 199 mg/dL), moderate (200 to 999 mg/dL), severe (1000 to 1999 mg/dL), and very severe (>2000 mg/dL) [2].

Primary LPL deficiency is a rare autosomal recessive disorder of lipoprotein metabolism, with a prevalence of ~1 per million [5, 6]. It is caused by biallelic pathogenic or likely pathogenic variants (or mutations) in the *LPL* or *APOC2* gene. Familial chylomicronemia can also be caused by mutations in *APOA5*, *GPIHBP1*, and *LMF1* genes, the proteins of which interact with LPL and lead to low LPL activity [6, 7]. *APOCIII* is an inhibitor of LPL and can increase TGs through a non-LPL mechanism [8].

The clinical features associated with LPL deficiency include recurrent abdominal pain, failure to thrive, xanthomatosis, hepatosplenomegaly, and lipemic plasma [6]. Severe complications such as recurrent pancreatitis and lipid encephalopathy have been reported [2, 6, 9]. Available treatment options include dietary fat restriction, use of medium chain TGs (MCTs), fibrates, n-3 fatty acids, plasmapheresis, apolipoprotein-CIII inhibitor and alipogene tiparvovec (AAV1) gene therapy [8, 10]. Alipogene tiparvovec (*AAV1-LPL*^{S447X} gene variant in an adeno-associated viral vector of serotype 1) gene therapy for LPL deficiency has been associated with ≥40% reduction in fasting median serum TGs at 3 to 12 weeks in one-half of the patients participating in a trial [10]. These subjects might require immunosuppression to prevent potential capsid-related immune events [10].

Secondary HTG seen in patients with T2DM is known to improve with insulin, dietary fat restriction, fibrates, or niacin [11]. Heparin and insulin infusions have been used to treat adult patients with HTG-induced acute pancreatitis by stimulating LPL activity [12]. Acquired LPL deficiency can be observed during insulin deficiency and will be improved with insulin therapy [13]. Hence, insulin was used to treat a young child from early infancy with very severe HTG and recurrent pancreatitis associated with a homozygous likely pathogenic variant in the *LPL* gene. Two other children with severe HTG, who had not undergone genetics analysis, were also treated with insulin infusion.

1. Case Scenario

A. Patient 1

A first-born 71-day-old infant boy of consanguineous parents presented with fever and vomiting on day 15 of life. The mother's antenatal history included two earlier spontaneous abortions. Grossly lipemic plasma was noted at the initial blood collection. The infant had several episodes of vomiting, refusal of feeds, and an incessant cry and was subsequently admitted. Serum amylase and lipase levels could not be estimated from the initial sample owing to the markedly lipemic plasma (TG level, 37,230 mg/dL; normal <150 mg/dL). Four similar episodes had been recorded from the 15th day of life, before his presentation at 71 days old. The child had been evaluated for septicemia at various hospitals with no pathogenic growth in the cultures of various body fluids.

The infant was maintained with no oral feeding for 48 hours after each episode and treated with maintenance IV dextrose and normal saline. These recurrent episodes of fever and vomiting were retrospectively considered to be recurrent pancreatitis, because all cultures were negative during each hospitalization. The child experienced repeated episodes, because no treatment options were available. He was losing weight from these episodes. Once his temperature had decreased to the normal range and the absence of emesis had been confirmed, the infant was discharged from the hospital. His TG concentrations continued to be >10,000 mg/dL after each of these previous episodes and never decreased to an acceptable range. The serum TG levels had decreased to 9413 mg/dL at 71 days of age when the infant was admitted with an incessant cry. The serum lipase was 781 U/L (normal, <160 U/L), which

was suggestive of pancreatitis. Breast feeding was stopped. The infant was treated with a dextrose–insulin infusion. Thus, 5% dextrose with a 0.45% saline solution was infused through one line at a maintenance rate. Insulin was infused through another line at a rate of 0.005 U/kg/h, with hourly blood glucose evaluations. The TGs had decreased to 1191 mg/dL and lipase to 157 U/L within 36 hours, with no episodes of hypoglycemia (Table 1). The insulin infusion was useful for acute reduction of the TG concentration and prevention of complications such as pancreatitis.

A special formula was prepared with Sagar (AMUL India; price equivalent to US $1.7/500 g) skimmed milk powder containing 1 g of fat per 100 g of milk powder, coconut oil (rich in MCTs), and thin rice gruel. The TGs decreased to 258 mg/dL during the next 8 days with this formula. The proportion of energy acquired from the special formula included 63% from carbohydrates, 32.6% from proteins, 4.3% from fats (54% of which consisted of MCTs). Formula was given to the infant on demand for the initial 8 days, for 150 calories/kg/d.

The infant increased in weight and length appropriately, and the TG concentration was maintained at 250 to 350 mg/dL. However, when weaning foods were introduced at 6 months of age, the serum TG increased to 1765 mg/dL. Fenofibrate at 10 mg/kg/d [14] was tried with no response and was stopped after 1 month. Omega-3 fatty acids were also tried for 1 month without any success. Some improvement in the TG level (610 mg/dL) was noted with food made with millet (high-fiber grain) replaced the carbohydrates from other cereals [15]. The formula composed of millet, low long chain fat (coconut oil), and with skimmed milk powder was useful in maintaining the TG concentrations in this child.

At 9 months of age, the infant required another admission. His serum TG had increased to 1816 mg/dL but decreased to 413 mg/dL with a dextrose–insulin infusion. When his mother had inadvertently challenged him with a high-fat sweet diet, his serum TG increased to 2086 mg/dL within 2 hours. A subsequent insulin infusion administered for 12 hours lowered the serum TGs to 223 mg/dL. On discharge, he was advised to be given 1 U of regular insulin SC on alternate days (0.1 U/kg/d), which was gradually increased to daily dosing with regular blood glucose evaluations. The insulin dose was titrated to maintain the TGs at <1000 mg/dL, because pancreatitis is more common at a TG level >1000 mg/dL [1]. No episode of hypoglycemia was noted.

At ~18 months of age, the boy began glargine injections (0.18 U/kg/d). The dosage was dynamically adjusted according to the diet and TG concentrations. The TG concentration was maintained at <1000 mg/dL. Extra doses of regular insulin were given for high-fat diet consumption such as cakes or when the TGs had increased to >2000 mg/dL. The lipid profile and pancreatic enzymes are presented in Table 1. At the last follow-up examination, the child was 36 months old with appropriate growth and development. He was consuming skimmed milk powder, MCT-rich coconut oil, a diet of various high-fiber complex carbohydrates from millet [15], and high fiber cereals, along with reduced fat intake. Daily insulin therapy had been replaced by insulin for a high-fat diet for the previous 6 months.

Table 1. Lipid Profiles and Pancreatic Enzymes of Patient 1

	Age								
Variable	**10 d**	**71 d**	**73 d**	**81 d**	**5 mo**	**7 mo**	**9 mo**	**9 mo**	**19 mo**
TC, mg/dL (normal, 85–163)	806	368	180	445	127	138	127	94	121
HDL, mg/dL (normal 29–81)	8	8	10	25	20	10	14	12	15
LDL, mg/dL (normal, 25–89)	369	104	39	299	52	26	28	46	57
TG, mg/dL (normal, 50–202)	37230	9413	1191	258	517	1765	2086	233	392
VLDL, mg/dL (normal, 12–28)	7446	1883	238	52				45	78
Amylase, U/L (normal, 40–140)	5	5	9		16				
Lipase, U/L (normal, 0–50)	104	781	157		26				

HDL, high-density lipoprotein; LDL, low-density lipoprotein; TC, total cholesterol; VLDL, very-low-density lipoprotein.

A genetic analysis on DNA extracted from the patient's peripheral blood was performed using target capture-based next generation sequencing (Illumina TruSight One) and further confirmed by targeted capillary sequencing. The findings identified the presence of a potentially relevant variant NM_000237:c.904T>C (NP_000228.1:p.Cys302Arg) in exon 6 of the *LPL* gene in a homozygous state (Fig. 1). This variant was located in a mutational hot spot (exon 6) and/or a critical and well-established functional domain. Multiple *in silico* mutation prediction tools, including SIFT, Polyphen, and Mutation Taster, predicted this variant as pathogenic, deleterious, or disease causing. Both the parents were a heterozygous carrier for this variant. With these findings and the clinical presentation findings, this variant was classified as likely pathogenic and was reported in the ClinVar database (SCV000574538.1).

B. Patient 2

A 30-day-old infant with TGs of 1758 mg/dL and lipemic serum was treated for 28 hours with a dextrose–insulin infusion. A mutation analysis was not been performed for this infant. At the last follow-up examination, the child was 18 months old and had been maintaining the TG level at <1000 mg/dL with dietary manipulation. Insulin had not been needed again.

C. Patient 3

A third infant (age, 60 days) with a TG concentration of 5084 mg/dL was treated with an insulin infusion of 0.005 U/kg/h. The insulin concentration was gradually increased, and hypoglycemia was noted when the rate had reached 0.01 U/kg/h, which was managed by increasing the concentration of glucose to 7.5% in the IV fluids. The insulin infusion was stopped when the rate had reached 0.02 U/kg/h because the blood glucose was 55 to 65 mg/dL. The infusion was restarted at 0.005 U/kg/h 2 hours later. The TG level had decreased to 178 mg/dL within 72 hours. At the last follow-up examination, the infant was 7 months old and was growing appropriately for his age with the modified diet plan and has not again required insulin.

2. Discussion

Regular insulin therapy has been useful in the treatment of severe HTG due to LPL deficiency in patient 1. The recurrent pancreatitis in the first child with extremely high TG levels had to be treated urgently, because mortality due to pancreatitis in patients with HTG has been as great as 20% [1]. Lipid encephalopathy is another recognized complication [9]. No effective treatment modality has been reported to reduce TG concentrations rapidly at such a young age.

Even the currently available treatment of AAV1 gene therapy, priced at 1 million dollars in 2015, requires weeks to be effective. We needed to find an urgent therapeutic option for our

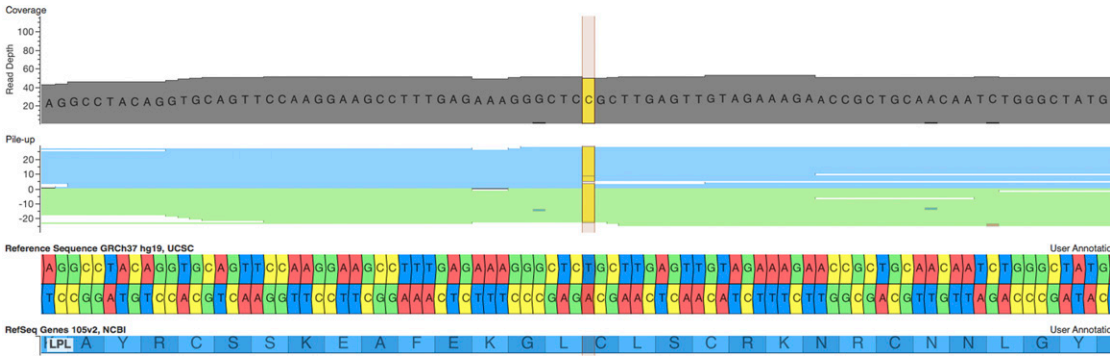

Figure 1. Homozygous substitution in exon 6 of the *LPL* gene in the .bam file aligned to GRCH37/hg19 version of human reference genome and visualized in GenomeBrowse (Golden Helix).

patient. The Monogen (Nutricia) formula with a low content of long chain TG and high amounts of MCTs has been used as feed for infants with severe infantile HTG worldwide. This formulation is unavailable in India and had to be imported, which was prohibitively expensive for the family's financial status (US $279 for four tins). The family had limited resources to buy the Monogen formula regularly. A more practical and economical dietary replacement with high-fiber complex carbohydrates enriched with MCT oil was identified for patient 1.

With the advent of pancreatitis, a substantial decrease in serum TGs had been reported previously [1]. A similar decrease in serum TGs from 37,230 mg/dL to 9413 mg/dL was also observed in our patient on day 71 before the initiation of insulin therapy. Subsequently, the serum TG level decreased rapidly with insulin infusion for 36 hours in this young infant to a manageable level that could avert complications.

To the best of our knowledge, this is the first documented successful use of insulin in a young infant given for the treatment of very severe HTG associated with a likely pathogenic variant in the *LPL* gene. The mechanism involved in the activation of LPL activity in mutated LPL is currently unexplained. Several studies have reported on the use of insulin in secondary LPL deficiency in patients with T2DM. Insulin activates LPL, which works ineffectively in the presence of T2DM owing to the insulin deficiency [11]. Few studies have reported on the treatment of heterozygous HTG with insulin [16]. To the best of our knowledge, long-term regular use of insulin in the treatment of congenital LPL deficiency has not been previously evaluated. Although insulin therapy is able to activate LPL in those with secondary LPL deficiency in the presence of T2DM, no answer is available regarding the mode of activation of LPL in patient 1 with a genetic mutation resulting in LPL deficiency.

APOCIII plays an important role in TG metabolism by inhibiting the activity of APOCII-mediated LPL activity and increasing TG concentrations. It also inhibits the activity of hepatic lipase [8]. Certain sequence variants of *APOC3* linked to HTG have been associated with increased hepatic fat and insulin resistance [17]. Some alleles of *APOC3* are related to insulin responsive elements and can reduce the responsiveness to insulin [18]. Future studies exploring this pathway could possibly help in explaining the reduction of the TG concentrations with insulin therapy in these children with inherited severe HTG.

In conclusion, the treatment plan we used should be evaluated further with a larger number of patients. However, it might serve as a useful, practical, and economical option for children with severe HTG.

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