

Gene therapy for genetic lipoprotein lipase deficiency: from promise to practice

M.C. Nierman¹, J. Rip¹, J. Twisk², J.J.M. Meulenberg², J.J.P. Kastelein¹, E.S.G. Stroes¹, J.A. Kuivenhoven^{1*}

¹Department of Vascular Medicine (G1-113), Academic Medical Centre, University of Amsterdam, the Netherlands, tel.: +31 (0)20-566 65 20, fax: +31 (0)20-566 92 32, e-mail: j.a.kuivenhoven@amc.uva.nl, ²Amsterdam Molecular Therapeutics (AMT) B.V., Amsterdam, the Netherlands, *corresponding author

ABSTRACT

Lipoprotein lipase (LPL) deficiency is a rare, hereditary disorder of lipoprotein metabolism characterised by severely increased triglyceride levels, and associated with an increased risk for pancreatitis. Since no adequate treatment modality is available for this disorder, we set out to develop an LPL gene therapy protocol. This paper focuses on the clinical presentation of LPL deficiency, summarises the preclinical investigations in animal models and describes the rationale to evaluate gene therapy for this monogenetic disorder of lipid metabolism in humans.

LPL exerts antiatherogenic effects. Of note, subendothelially located LPL has been described to have proatherogenic effects by increasing oxidative susceptibility of LDL facilitating the uptake of TRLs by macrophages.⁵ The latter promotes foam cell formation⁶ i.e. the hallmark of atherogenesis.⁷ In view of these heterogeneous effects, the exact role of LPL in atherogenesis is still a matter of debate.⁸ The delicate balance between proatherogenic and anti-atherogenic properties of LPL has been shown to depend in part on the exact location of this enzyme.⁸

LIPOPROTEIN LIPASE

Lipoprotein lipase (LPL) is one of the key enzymes in the metabolism of triglyceride-rich lipoproteins (TRLs) and is produced in fat tissue, skeletal muscle and heart muscle. Activated by its cofactor apolipoprotein (apo) CII,¹ LPL mediates the hydrolysis of triglycerides (TG) in chylomicrons (CM) and very-low-density lipoproteins (VLDL) at the luminal side of the endothelium. The generated free fatty acids (FFA) are subsequently used for energy production in muscle tissue or stored as fat in adipose tissue. LPL also contributes to the high-density lipoprotein (HDL) pool by shedding of phospholipids and apolipoproteins during the hydrolysis of these lipoproteins.² Besides the enzymatic activity, LPL also enhances hepatic clearance of triglyceride-rich lipoproteins (TRL) by mediating receptor-mediated uptake of these atherogenic lipoprotein particles ('ligand' or 'bridging' function).^{3,4} Through these actions

LPL MUTATIONS

More than 100 mutations in the LPL gene have been described to date. While some mutations result in total loss of function, others only exert a moderate effect on LPL activity such as the D9N, N291S, and S447X mutations. The latter are frequently found in the general population and have provided valuable insight into the relationship between LPL and the progression of atherosclerosis. Carriers of LPL^{N291S} and LPL^{D9N} with a combined frequency of 5% in the general population are characterised by low HDL cholesterol and increased TG with a concomitant increased risk of cardiovascular disease.^{9,10} Conversely, carriers of LPL^{S447X} with a frequency of 18 to 22% in the general population are characterised by increased levels of HDL cholesterol and lower TG levels. In line, this mutation has been reported to have a protective effect against cardiovascular disease (CVD).^{9,15}

GENETIC LPL DEFICIENCY

Clinical presentation and diagnosis of genetic LPL deficiency

LPL deficiency is an autosomal recessive hereditary disease caused by mutations in the LPL gene. Homozygosity or compound heterozygosity for mutations in the LPL gene, resulting in loss of catalytically active LPL, is the basis of genetic LPL deficiency. The resulting clinical chylomicronaemia syndrome (see further in text) was first described by Bürger and Grütz in 1932 and 56 years later, in 1989, the first LPL mutation responsible for this phenotype was revealed.¹⁶

LPL deficiency typically manifests itself in early childhood with a variety of symptoms including severe abdominal pain, repetitive colicky pain, hepatosplenomegaly, failure-to-thrive and acute pancreatitis.^{17,18} Increased irritability, diarrhoea and intestinal bleeding can occur even shortly after birth. Although the clinical presentation is non-specific, especially at a younger age,¹⁹ the plasma of the patients is always milky white or lipaemic (*figure 1*), even under fasting conditions. On physical examination, eruptive xanthomas (*figure 2A* and *2B*) are frequently present. These xanthomas consist of small erythematous-based yellow papules ranging in size from one to several millimetres in diameter. Eruptive xanthomas frequently exhibit the Koebner phenomenon, also called the isomorphic response, which refers to the appearance of lesions at a site of injury or pressure. Therefore, eruptive xanthomas are usually formed on the buttocks, elbows, back, and knees, but they can also occur on any cutaneous surface including the oral mucosa. These lesions generally recede with reduction of the TG levels. In addition to these skin lesions, lipaemia retinalis and hepatosplenomegaly can be observed. In clinical practice, this combination of symptoms is often not recognised to be directly related to the hyperchylomicronaemia syndrome,²⁰ and the diagnosis often becomes clear only after the first occurrence of pancreatitis. Lipid analysis reveals 10 to 100 times increased plasma TG while HDL cholesterol levels are markedly decreased. In addition, LPL deficiency is characterised by reduced LDL cholesterol levels. In line, levels of apoB100, the main structural apolipoprotein of LDL and VLDL, are reduced. The increased TG concentration increases the risk of pancreatitis,²¹ which can occur from TG concentrations of 10 mmol/l onwards.²² This clinical complication, often recurrent in LPL-deficient patients, can be lethal. It is noteworthy that in these patients, pancreatitis cannot be excluded on the basis of normal plasma amylase concentrations, since high TG concentrations can interfere with the analytical method resulting in false-negative results.²³⁻²⁵ Assessment of urine amylase excretion is more reliable as a diagnostic test for pancreatitis in a hypertriglyceridaemic patient.^{26,27} Other laboratory investigations can also be disturbed as a result of increased TG levels, such as sodium

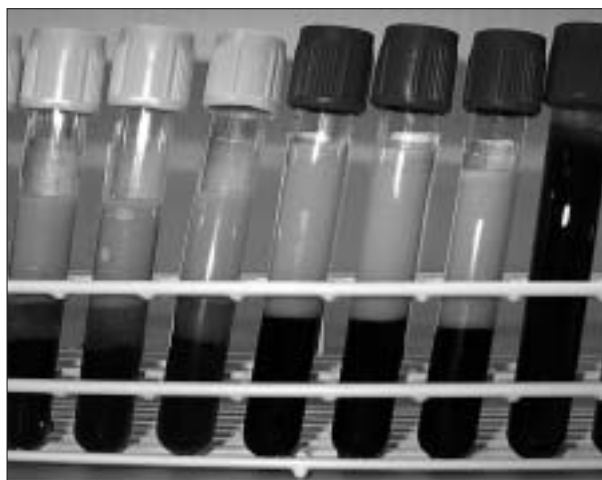


Figure 1
Lipaemic plasma from a patient with severe hypertriglyceridaemia (fasting TG = 46 mmol/l)



Figure 2
Eruptive xanthomas found on the upper legs (A) and knees (B) of a patient with severe hypertriglyceridaemia (fasting TG = 46 mmol/l)

(artificially low),²⁸ haemoglobin (artificially increased),²⁹ HbA_{1c} (artificially low)³⁰ and bilirubin (artificially increased),^{31,32}

Prevalence of genetic LPL deficiency

Exact data on the prevalence of LPL deficiency are not available. The reported prevalences for genetic LPL deficiency vary between 1:1,000,000³³ and 1:5000 in French Quebec (caused by a so-called 'founder effect').^{21,34} Based on extensive efforts to track down all LPL-deficient patients in the Netherlands, we estimate a prevalence of approximately 1:500,000.

LPL deficiency and clinical complications

The main clinical risk for LPL-deficient patients is the development of pancreatitis.²² The exact aetiology of pancreatitis in hypertriglyceridaemia is unclear but it is believed that the high concentrations of CM in the pancreatic microcirculation result in increased 'free radical' activity, which in turn can result in episodes of pancreatic ischaemia. Inflammation of the pancreas is supposed to be the result of local fatty acid generation due to small amounts of free lipases in the microcirculation of the pancreas. A disrupted microcirculation, caused by hyperchylomicronaemia, is suggested to damage pancreatic cells with ensuing increased release of lipolytic enzymes. The latter causes hydrolysis of abundantly present CM contributing to a strong increase in local FFA, followed by local pancreatic inflammation. This cascade of events is thought to eventually cause pancreatitis.³²

Elevated TG levels are a strong independent risk factor for CVD.³⁵ It is unclear, however, whether LPL deficiency is associated with an increased CVD risk.³³ Whereas two publications have reported premature atherosclerosis in LPL-deficient patients,^{36,37} LPL deficiency has also been described to be not associated with a dramatic increase in CVD.³³ The reported lack of atherosclerosis³⁸ has even been described to relate to the low concentrations of LDL cholesterol in these patients (a direct consequence of a disturbed catabolism of the precursor of LDL, i.e. VLDL). The latter phenomenon was nicely illustrated by a homozygous LPL-deficient patient suffering from familial hypercholesterolaemia (FH) with clearly lower LDL cholesterol levels compared with FH siblings and the absence of signs of CVD during follow-up.³⁹ Another potential mechanism antagonising atherogenesis is the inability of CM to penetrate in the vascular wall.⁴⁰ In line, the accumulation of TRLs in macrophages in the vascular wall has been shown to be reduced in patients with LPL deficiency.⁴¹

Therapeutic options in LPL deficiency

The primary objective of treating LPL-deficient patients is reducing the risk for pancreatitis. To reduce this risk, TG lowering below 10 mmol/l is desired.⁴²

Diet

The intake of dietary fats has to be lowered to 20 to 25% of the total daily caloric intake, i.e. 40 to 50 grams dietary fat per day. If this has insufficient effect on TG, part of the fat can be replaced by medium-chain triglycerides. These TGs are transported to the organs for hydrolysis without the need for CM packaging, thus excluding the need for LPL. In our Western society, characterised by dietary fat intakes of approximately 120 grams per day, maintaining these strict dietary regimes has been proven to be most difficult, resulting in poor adherence. Consequently, the prevention of pancreatitis is often unsuccessful in LPL-deficient patients and additional therapeutic modalities are mandatory. Of note, it should be emphasised that consequent and strict adherence to a stringent low-fat diet is likely to be associated with effective lowering of the hypertriglyceridaemia with ensuing decrease in risk for pancreatitis.

Fibrates

Fibric acids normally affect TG metabolism by reducing the hepatic production of VLDL and enhancing VLDL clearance from the circulation.⁴³ Fibric acids are agonists of a family of transcription factors, i.e. peroxisome proliferators activated receptors (PPARs). These factors have been shown to reduce the production of hepatic apoCIII (an inhibitor of LPL activity) and thereby increase LPL-mediated lipolysis. Also, via direct stimulation of the LPL gene promoter LPL synthesis is upregulated. However, in LPL deficiency, upregulation of defective LPL will not render the desired effects. In addition, a decreased VLDL synthesis may help in managing TG levels; the primary problem of these patients is the lack of lipolytic activity. As a consequence, plasma TG levels in LPL-deficient patients are generally unaffected upon fibrate therapy.^{33,44}

Statins

Statins inhibit HMG-CoA reductase, leading to a reduced hepatic cholesterol production and upregulation of LDL receptors. This results in enhanced hepatic uptake of LDL and TRL (VLDL and IDL),^{45,46} reducing the concentrations of plasma LDL cholesterol and plasma TG. LPL-deficient patients, however, are characterised by reduced concentrations of LDL cholesterol through decreased turnover of VLDL to LDL⁴⁷ as well as increased LDL catabolism.⁴⁸ As a consequence, neither TG nor LDL levels are lowered by statin therapy.^{33,44}

Nicotinic acid derivatives

Nicotinic acid derivatives (vitamin B₃) normally inhibit the hepatic synthesis and esterification of FFA, resulting in a reduced hepatic VLDL production.^{49,50} Nicotinic acid derivatives also induce accelerated intracellular degradation of apoB⁵¹ whereas a reduced hepatic clearance of apoAI

results in an increase in HDL cholesterol.⁵² In LPL-deficient patients, the response to nicotinic acid derivatives has been shown to be marginal.^{33,44}

Omega-3 fatty acids

Daily use of a high dose of omega-3 fatty acids (4 grams/day) leads to enhanced clearance of plasma CM⁵³ in combination with a reduced production of hepatic VLDL⁵⁴ without affecting LPL activity.⁵⁵ In primary hypertriglyceridaemia by causes other than LPL deficiency, the effect of this treatment varies from a TG reduction of 29 to 50%.⁵⁶⁻⁶⁰ Treatment with omega-3 fatty acids in genetic LPL-deficient patients has never been published and therefore may warrant further investigation.

Due to the lack of effective pharmacological interventions, modern treatment options are currently restricted to intensive dietary modifications. These strict dietary regimes have been proven to be most difficult, resulting in poor adherence. Consequently, the prevention of pancreatitis is unsuccessful in LPL-deficient patients and additional, effective therapeutic modalities are needed.

L P L G E N E T H E R A P Y

Rationale

Several facts have contributed to the development of gene therapy for LPL-deficient patients. First, as described above, LPL deficiency currently lacks an effective and successful therapy. Second, the diagnosis of genetic LPL deficiency can be accurately made. Third, the LPL gene is rather small, which allows the incorporation of the gene into a wide range of viral vectors. Fourth, appropriate animal models for the extensive testing of this gene therapy are available (LPL 'knock-out' mice and LPL-deficient kittens). Fifth, LPL is naturally produced in skeletal muscle. Not only can this tissue be easily reached via intramuscular injections, but it can also be targeted with vectors with a natural tropism for this tissue. Sixth, most patients present with detectable levels of inactive LPL protein in the circulation. This strongly diminishes the risk of a significant immune response against the transgenic LPL upon effective gene therapy. Finally, increases of LPL activity in the human circulation are only associated with beneficial effects. Not only does increased LPL activity result in significant lowering of both fasting and postprandial TG levels, it will likely increase antiatherogenic HDL cholesterol levels.

LPL gene therapy, choice of virus and preclinical experiments

Effectiveness of LPL gene therapy using adenovirus has long been established in animal models.⁶¹ Since the duration of transgene expression upon adenoviral infection is limited, the nonpathological adeno-associated virus (AAV)

has been put forward, a virus that has been used in several gene therapy studies in men.⁶² As transgene, we have chosen a naturally occurring LPL variant (LPL^{S447X}) that has been shown to exhibit a beneficial effect on lipids profiles and a concomitant decreased CVD risk.⁹⁻¹⁵

In murine LPL-deficient models, a single intramuscular injection of AAV₁-LPL^{S447X} (dosage: 8 x 10¹² AAV genome copies/kg body weight) resulted in a highly significant TG reduction of 97% for more than 12 months.⁶³ We have recently been able to confirm these promising results in LPL-deficient cats (dosage: 1 x 10¹¹ AAV genome copies/kg body weight; unpublished). The result of biodistribution and toxicity studies with the recombinant virus are excellent and have paved the way for further development.

LPL-deficient patients

Awaiting the initiation of the AAV₁-LPL^{S447X} gene therapy trial, the first six LPL-deficient patients have been thoroughly investigated. All patients were characterised by TG levels >10 mmol/l, despite compliance to dietary restrictions. In addition, all patients had suffered from (recurrent) pancreatitis. The patients showed complete loss of enzymatic LPL activity, whereas circulating inactive LPL protein could be demonstrated in all (protein concentration 19 to 103% of normal). We furthermore cultured myoblasts from needle biopsies of the right upper leg of all six patients (Pro-mag 2.2 automatic biopsy system, N14GA/10 cm needle; MDTECH, USA). These myoblasts were infected with AAV₁-LPL^{S447X} after which all myocytes were shown to secrete catalytically active LPL (unpublished data).

C O N C L U S I O N

LPL deficiency is a rare hereditary condition characterised by high TG levels that correlate with an increased risk for potentially lethal (recurrent) pancreatitis. In view of the lack of effective pharmacological agents, TG levels remain seriously elevated. We report here the successful implementation of an LPL gene therapy protocol using an AAV₁-LPL^{S447X} vector in both murine and feline models of LPL deficiency. In addition, we have demonstrated that the myocytes of our LPL-deficient patients are able to produce and secrete catalytically active LPL into culture media upon infection with AAV₁-LPL^{S447X}. Based on these promising results, the initiation of the first human LPL gene therapy trial in the Netherlands is expected soon. Other patient populations that may benefit from LPL gene therapy include heterozygote LPL-deficient patients with a clinical phenotype of the chylomicronaemia syndrome, patients with therapy-resistant hypertriglyceridaemia and maybe patients with hypertriglyceridaemia formerly characterised as (Fredrickson) type V hyperlipidaemia. For now, we will first evaluate the effectiveness in LPL-deficient patients.

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