

Review of the clinical development of alipogene tiparvovec gene therapy for lipoprotein lipase deficiency

Daniel Gaudet^{a,*}, Janneke de Wal^b, Karine Tremblay^a, Stéphane Déry^a, Sander van Deventer^b,
Andreas Freidig^b, Diane Brisson^a, Julie Méthot^a

^a Department of Medicine, Université de Montréal, ECOGENE-21 Clinical Research Center, Chicoutimi Hospital, Canada

^b Amsterdam Molecular Therapeutics (AMT) B.V., Amsterdam, The Netherlands

Received 19 March 2010; received in revised form 29 March 2010; accepted 31 March 2010

Abstract

Alipogene tiparvovec (AAV1-LPL^{S447X}) gene therapy is developed to prevent complications and decrease the clinical morbidity of lipoprotein lipase deficiency (LPLD). LPLD is an autosomal recessive disease associated with severe hypertriglyceridemia (hyperTG), severe chylomicronaemia, and low HDL. Acute pancreatitis, the most frequent serious clinical LPLD complication, is a complex and heterogeneous inflammatory condition having many causes including hyperTG and chylomicronaemia. In many patients, low fat diet and currently available lipid lowering drugs are ineffective to prevent hyperTG or pancreatitis in LPLD. The clinical development program of alipogene tiparvovec includes observational studies as well as phase I/II and II/III clinical trials. Pooled data are collected on safety and efficacy issues, including the incidence of pancreatitis.

© 2010 Elsevier Ireland Ltd. All rights reserved.

Keywords: Lipoprotein lipase; Gene therapy; Pancreatitis; Chylomicronaemia

1. Introduction

Lipoprotein lipase deficiency (LPLD) is characterized by the inability of affected individuals to produce functionally active lipoprotein lipase (LPL). LPL is mainly produced in skeletal muscle, fat tissue, and heart muscle [1] and has multiple key functions, among which is the catabolism of triglyceride (TG)-rich lipoproteins, chylomicrons (CM) and very low-density lipoproteins (VLDL). Off-loading TG from CM (and VLDL) normally protects against excessive post-prandial rise in chylomicron mass and TG. In LPLD, LPL is dysfunctional and more than 12 h after meals hyperTG and chylomicronaemia are still present and visible as lipemia.

LPLD is an autosomal recessive disorder caused by loss-of-function mutations in the LPL gene. The LPL gene is located on chromosome 8p22 and comprises 10 exons. To

date, more than 70 LPL gene mutations have been described, most of them associated with loss of catalytic function [2]. Patients are either homozygous for such mutations or compound heterozygous. In a few areas in the world, such as in South Africa and Eastern Quebec, the disease is more common, because of a “founder” effect, with the majority of diagnosed patients having the same few mutations [3]. The prevalence outside of founder populations is estimated to be 1–2 in a million of the population, and hence it is an orphan disease.

LPLD is the most common Mendelian cause of (hyper)chylomicronaemia. Other causes include Apolipoprotein CII (APOC2) or Glycosylphosphatidylinositol Anchored High Density Lipoprotein Binding Protein 1 (GPIHBP1) deficiency [4]. Some cases due to circulating anti-LPL antibodies have been reported [5]. As phenotypic presentation of LPLD may overlap with that of other causes of chylomicronaemia genetic diagnosis in this day and age may be the preferred way to establish the diagnosis definitively.

In LPLD, extreme concentrations of circulating large chylomicrons (chylomicronaemia) are present, which are thought to be responsible for causing most of the clinical man-

* Corresponding author at: Department of Medicine, Université de Montréal, ECOGENE-21 Clinical Research Center, Chicoutimi Hospital, Pavillon des Augustines (5th floor), 225, rue St-Vallier, Chicoutimi (QC), Canada G7H 7P2. Tel.: +1 418 541 1077; fax: +1 418 541 1116.

E-mail address: daniel.gaudet@umontreal.ca (D. Gaudet).

ifestations. LPLD may present during infancy or childhood with (repeated) severe abdominal pain episodes or failure to thrive [1,6,7]. On physical examination, eruptive xanthomas, lipaemia retinalis, and hepatosplenomegaly may be detected. The most severe manifestation is acute pancreatitis, which can be lethal. Diabetes is another complication seen frequently in LPLD, and may be due to recurrent pancreatitis, ultimately resulting in endocrine as well as exocrine pancreatic insufficiency [1,8,9] and/or by impaired 'energy metabolism and distribution' related to broad LPL dysfunction in various tissues. Premature atherosclerosis can occur [10].

Fasting lactescent plasma often is the trigger to further work up and ultimate diagnosis. The severity of the symptoms being roughly proportional to level of chylomicronaemia, usually measured as whole plasma TG; TG concentrations above 10 mmol/L have been suggested to be critical levels [1] below which the risk of pancreatitis would be substantially reduced.

Currently, no drug therapy for LPLD is available, and patients are managed by a severely fat-restricted diet, which however does not fully eliminate the risk of pancreatitis or disease progression. Alipogene tiparvovec is being developed to control the symptoms and prevent complications of LPLD.

2. Lipoprotein lipase deficiency (LPLD) and pancreatitis

Acute pancreatitis is a heterogeneous inflammatory condition, which leads to significant morbidity or mortality in 20–30% of patients [11]. There are several genes associated with susceptibility to acute pancreatitis, which include the cationic trypsinogen (*PRSSI* and *PRSS2*), chymotrypsinogen C (*CTRC*), pancreatic secretory trypsin inhibitor (*SPINK1*), cystic fibrosis transmembrane conductance regulator (*CFTR*) and calcium-sensin receptor (*CASR*) [12–15]. Although not being the only genetic cause of recurrent pancreatitis, LPLD is undoubtedly an important one since almost all severe hyperTG phenotypes increase the risk (Fig. 1). Thus, what can be learned through LPLD and its therapy could be valuable for other, more frequent, causes of pancreatitis due to severe hyperTG as well.

A number of primary factors can initiate pancreatic inflammation; these factors include, but are not limited to, gallstones or other mechanic obstruction of the pancreatic duct, alcohol abuse, toxins, hypercalcemia and hyperTG [11,15,16]. In patients with hyperTG, the risk of pancreatitis increases as a function of plasma TG, being the highest among patients with fasting values > 10.0 mmol/L (Fig. 1). HyperTG is the consequence of increased production and/or reduced or delayed catabolism of TG-rich lipoproteins: endogenous very low-density lipoproteins (VLDL) or exogenous chylomicrons (CM) [17]. In the presence of severe hyperTG and fasting chylomicronaemia due to LPLD, when LPL activity is less than 5% of normal [18], the risk of acute pancreatitis

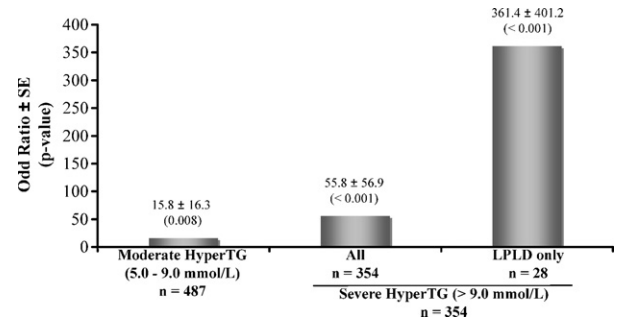


Fig. 1. Risk of acute pancreatitis associated with moderate (TG: 5.0–9.0 mmol/L) and severe (TG > 9.0 mmol/L) hypertriglyceridemia compared to normolipidemic controls. Risk of pancreatitis is reported as odds ratio ± SEM. Among subjects with severe hyperTG, the risk of pancreatitis is the highest in LPLD. All comparisons were done with a sample of 364 normolipidemic individuals using logistics regression models controlling for the effect of age, gender and alcohol consumption.

is 360 fold higher than in normotriglyceridemic individuals (Fig. 1).

3. Conventional clinical management of LPLD

Clinical management of LPLD patients currently consists of severe reduction in dietary fat to less than 20% of caloric intake and the use of medium-chain TG. It is almost impossible to always, life-long, comply with such a dietary regimen. As shown in Fig. 2, even when LPLD patients are compliant to the diet and are tightly followed in a lipid clinic by a dietician and a medical team, TG do not decrease below the threshold of increased pancreatitis risk. In the prospective observational studies that formed part of the alipogene tiparvovec development, this finding was confirmed: controlled diet alone does not eradicate chylomicronaemia or lower TG further and does not prevent pancreatitis. The adjunction of fenofibrate does not provide any incremental benefit [19]. As there is currently no drug or any specific therapy available to modulate the course of the illness, these patients are at high risk of morbidity and mortality. Enzyme replacement therapy is no option due to the short half-life of the LPL protein (approximately 15–30 min). Therefore, patients with LPLD, a serious debilitating condition, were considered very suitable candidates for gene therapy.

4. Alipogene tiparvovec

Alipogene tiparvovec [Glybera®; AMT-011; AAV1-LPL^{S447X}] contains the human *LPL* gene variant *LPL*^{S447X} (the active component). The *LPL*^{S447X} 'gain-of-function' variant is found in 20% of Caucasians and is associated with enhanced removal of proatherogenic apoB100-containing

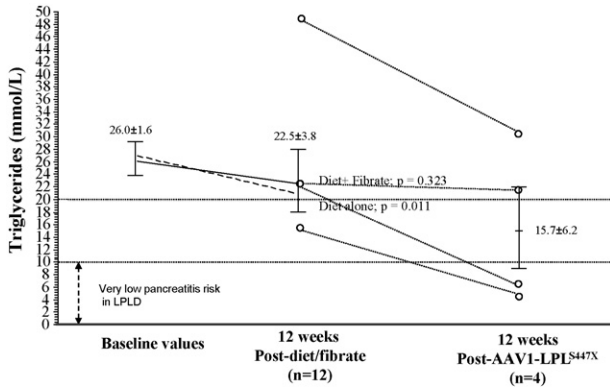


Fig. 2. Effect of severe low fat diet and adjunction of fibrates on fasting plasma triglyceride in LPL deficiency. *P*-values from paired *t*-tests. Thin dot lines represent the thresholds of pancreatitis risk. In a sample of 12 adults with LPLD, very severe diet and thigh clinical follow-up, although clinically relevant and statistically significant, was not sufficient to decrease plasma TG and chylomicronaemia under the threshold of pancreatitis risk. Adjunction of fibrates (fenofibrate 200 mg daily) for 12 weeks in a sub-sample of 6 patients did not contribute to any incremental benefit. In comparison, 4 LPLD patients having participated in the diet + fibrate trial [19] were treated with AAV1-LPL^{S447X} later on and three of them showed significant but transient TG reduction, 2 of them reaching values below 10 mmol/L. Comparable results were observed among the 8 LPLD patients treated with intramuscular administration of AAV1-LPL^{S447X} in the AMT-010 study who presented with a significant ($P < 0.007$) albeit transient decrease of median TG levels at 12 weeks compared to baseline [23]. Although transient, the decrease in TG levels was followed by long-term (>1 year) changes in acylglycerol metabolism. The observation that fibrates do not decrease plasma TG below pancreatitis risk in LPLD has been replicated in several affected patients over years.

particles, including LDL-cholesterol [20], lower plasma TG levels, higher HDL cholesterol concentrations, and lower rates of cardiovascular disease, when compared to the general population [20,21]. In addition, it may have anti-inflammatory properties [22], which could provide some advantages in preventing pancreatitis.

The vector used to deliver the therapeutic gene in alipogene tiparvovec is a non-replicating and non-integrating vector which comprises a protein shell derived from adeno-associated virus serotype 1 (AAV1), the CMV promoter, a woodchuck hepatitis virus posttranscriptional regulatory element and AAV2 derived inverted terminal repeats.

5. Alipogene tiparvovec is provided as a solution for intramuscular injection

AAV1-LPL^{S447X}, initially designated AMT-010, was previously produced in a mammalian cell system which was not appropriate for large-scale production. Hence, this method has been superseded by a more efficient production system to produce alipogene tiparvovec (AMT-011).

6. Preclinical studies

Proof of principle of the efficacy of gene therapy with AAV1-LPL^{S447X} was obtained in LPL deficient mice and cats. Intramuscular (IM) delivery of AMT-010 or AMT-011 to LPL deficient animals resulted in >95% reduction in plasma TG. In mice, the effect was long-term, lasting over a year, and was dose-dependent, with full correction of lipemia and reduction of plasma TG to near-normal levels at a dose of 1×10^{13} genome copies (gc)/kg. The safety studies of AMT-010 and AMT-011 showed both products were well tolerated.

7. Clinical development program

LPLD is a very rare condition (prevalence worldwide 1–2 per million). As with most very rare conditions, the presentation and phenotypes, natural history and evolution, morbidity and mortality, and effects of therapies and interventions are incompletely understood. Therefore, prospective observational studies to document the natural history, presentation, evolution and burden of disease were included in the clinical development program. Not only were values for TG established, to assess the efficacy of the stringent fat-restricted diet in preventing disease in LPLD were, but individual baseline data for other possible safety and efficacy parameters against which to judge the effect of therapy were collected as well. These observational studies were followed by phase I/II and II/III clinical trials performed in the Netherlands and Canada, including adults with LPLD (diagnosed by high TG, low LPL activity in post-heparin plasma AND mutation analysis) and a prior history of acute pancreatitis. In all interventional studies conducted in humans to date, AAV1-LPL^{S447X} was administered by a single series of intramuscular (IM) injections at multiple sites in the upper, and if necessary lower, limbs (range of 30–70 injections depending upon body weight). In relation to the nature of gene therapy, next to parameters measured in any clinical study, shedding of the vector DNA via body fluids (saliva, urine, semen) and immune responses against not only the LPL enzyme but also the vector (viral-derived capsid antigens) were monitored.

CT-AMT-010-01 was the first in man study of AAV1-LPL^{S447X} and was an open label, dose escalation study conducted in the Netherlands [23], with 12 weeks observation post-dosing. As mentioned above, prior to this interventional study all enrolled subjects had participated in a prospective observational study (PREP-01), in which a low fat diet was advised and controlled by a dedicated dietician. Despite this, TG levels remained high and pancreatitis events did occur. Not all subjects screened for PREP-01 were entered; one 25-year-old subject died from acute pancreatitis prior to enrollment being effected (case described by Rip et al.) [24]. During PREP-01 another male patient was lost due to sudden cardiac death.

A total of 8 LPLD subjects out of 14 followed in PREP-01 were administered AMT-010 IM at a dose of 1×10^{11} gc/kg

Table 1

Cox Proportional Hazard Regression model, adapted for historic control studies (Multiplicative Intensity Model, Lachin, 2000) [30].

(A) Time at risk and number of pancreatitis events before and after AAV1-LPL^{S447X} gene therapy administration.

Study	Medical history/PREP/run in		Main study/long-term follow-up	
	Time at risk (days)	Number of events	Time at risk (days)	Number of events
	010-01	5168	5	9897
011-01	50,684	26	8192	2
011-02	16,775	39	401	0
Overall	72,627	70	18,490	7

(B) Outcome of analysis

Analysis population	<i>P</i> value for treatment effect	Hazard ratio	80% confidence interval
Overall	0.23	0.272	0.067–1.106

(4 subjects) or 3×10^{11} gc/kg body weight (4 subjects). AMT-010 was well tolerated at doses of 1×10^{11} gc/kg and 3×10^{11} gc/kg. There were no drug related serious adverse events (SAEs) and no dose-limiting toxicity. The median TG in 1 of the 4 subjects in the low dose group (1×10^{11} gc/kg) and 2 of the 4 subjects in the second dose group (3×10^{11} gc/kg) declined to below the target level of 10 mmol/l or led to a >40% reduction in TG, the pre-defined efficacy endpoints. All patients from CT-AMT-010-01 were enrolled into a 5-year long-term follow-up study, results of which will be reported elsewhere.

Based upon the 12-week results of CT-AMT-010-01 and in view of the safety and efficacy data obtained in animal studies, it was concluded that administering a dose of 1×10^{12} gc AAV1-LPL^{S447X}/kg body weight in the second study, CT-AMT-011-01, would be appropriate. T-cell immune responses against the AAV1 capsid were detected in half of the subjects during the initial months after AMT-010 administration [25], and were at that time thought to be potentially attenuating the efficacy of AAV1-LPL^{S447X} gene therapy. Therefore, it was decided to add an immunosuppressants regime in CT-AMT-011-01. In this study a total of 22 Canadian adults with LPLD and a history of pancreatitis were enrolled and 20 of them had been followed for at least 7 months (range: 7–18 months) in a non-controlled observation study (PREP-02 Study). During the course of the observation study, they received counseling by a dietician every 6 ± 2 weeks. They were instructed to comply with a diet in which the fat content did not exceed 20% of the total daily caloric intake and contained no more than 55 g/day. The majority (21/22) of the patients had previously been followed in a lipid clinic long term. Four patients enrolled in PREP-02 had even participated in a 12 weeks open label, parallel arm study assessing the efficacy of a low fat MCT-enriched LPLD diet and fibrates to decrease plasma TG in LPLD [19]. This study and other data (Fig. 2) showed no incremental TG lowering effect of fibrates on top of a strict diet and tight follow-up; none of the LPLD subjects in this

older study reached TG values below 10 mmol/L under this dietary-fibrates regimen.

Following the PREP-02 study, eligible subjects who met all the inclusion criteria and provided an informed consent were invited to participate in an open label, dose escalation study to assess the safety and efficacy of gene therapy with alipogene tiparvec (AMT-011) in LPLD. Fourteen subjects were thus administered alipogene tiparvec.

CT-AMT-011-01 was designed to evaluate the safety of alipogene tiparvec and its efficacy on fasting TG, long-term expression of LPL^{S447X} in the muscle tissue as well as occurrence of pancreatitis and LPLD co-morbidities. Participants were randomized to one of three dose cohorts. Cohort 1 ($n=2$) were dosed with 3×10^{11} gc/kg of alipogene tiparvec, cohort 2 ($n=4$) with the same dosage in combination with immunosuppressive medication for 12 weeks after IM injections and cohort 3 ($n=8$) received 1×10^{12} gc/kg in combination with immunosuppressants. A combination of cyclosporine (3 mg/kg) and mycophenolate mofetil (2 g/day) was chosen, as it had been widely used to prevent cytotoxic T lymphocyte function in graft rejection and chronic inflammatory diseases, was well tolerated, and not associated with a significantly increased rate of infections [26–29]. Furthermore, there was no effect of these immunosuppressants on AMT-011 mediated transgene expression was found in mice, and such co-administration was well tolerated. The 12 weeks CT-AMT-011-01 was followed by a 5-year long-term follow-up study; most subjects are halfway in this period.

Following CT-AMT-011-01, a third clinical trial, the CT-AMT-011-02 study, was designed; this protocol included documenting the post-prandial effects of alipogene tiparvec and collection of additional efficacy and safety data. A dosing regimen of alipogene tiparvec at a dose 1×10^{12} gc/kg in combination with cyclosporine, mycophenolate and a pre-dose bolus of methylprednisolon was employed. Efficacy endpoints include the assessment of fasting and post-prandial TG and lipid-lipoprotein metabolism, lipoprotein kinetics and the turnover of free fatty acids (FFA),

TG-rich lipoprotein content and density, muscle expression of the LPL^{S447X} transgene and enzyme, secondary symptoms to LPL deficiency and occurrence of pancreatitis or other LPLD co-morbidities. CT-AMT-011-02 is an open label. Five eligible adults with LPLD and prior history of acute pancreatitis were included in the study.

Detailed reports on the results of studies CT-AMT-011-01 and CT-AMT-011-02 and their long-term follow-up will be published elsewhere. In summary, during PREP-02 the inability of controlled diet to lower TG and to prevent pancreatitis was confirmed. In the interventional studies alipogene tiparvovec was tolerated well, without dose-limiting toxicity; also in long-term follow-up thus far this therapy has an excellent safety profile. During the active study phases TG decreases were noted in virtually all patients. Muscle biopsies taken half a year post-dosing in CT-AMT-011-01 from both injected and non-injected muscle clearly indicate persistent expression and biologic activity, while long-term clinical follow-up is indicating continued benefit for these patients as compared to their disease state during PREP-01.

8. Additional studies and pooled data on the incidence of pancreatitis in LPLD

The clinical development of alipogene tiparvovec started in 2005. All participants to the CT-AMT-010-01, 011-01 and 011-02 trials are intended to be followed for 15 years after dosing in a registry. This long-term follow-up will allow the ongoing assessment of safety and efficacy of gene therapy with AAV1-LPL^{S447X} which includes continued collection of data on the incidence of pancreatitis. Modeling of pancreatitis data available from the entire program until October 2009, using a Cox Proportional Hazard Regression model, adapted for historic control studies (Multiplicative Intensity Model, Lachin, 2000) [30], was performed. Use of data from medical history records was limited to the period of time where it appeared upon subjective review of the data available for analysis by an independent statistical expert that pancreatitis events had been captured with sufficient detail to allow for analysis. Panel A in Table 1 provides details per study on data used. The outcome of the modeling analysis suggests that the risk of pancreatitis decreases clinically importantly after a single dose administration of AAV1-LPL^{S447X}: with a probability of approximately 80% the results indicate that the risk of pancreatitis is reduced by 70% after administration of alipogene tiparvovec, as compared to pre-administration (Table 1). The incidence of pancreatitis in LPLD following AAV1-LPL^{S447X} treatment will be followed and monitored over years in order to obtain stronger evidence on the efficacy of the treatment. Till now, the long-term clinical benefits of the therapy have not been consistently correlating with TG levels, and hence the utility of total fasting plasma TG as a surrogate marker of risk of clinical events post-gene therapy with alipogene tipar-

vovec is not as linear as expected initially. Ancillary studies are conducted to better understand acylglycerol metabolism, identify novel biomarkers of disease or drug response and to highlight the mechanisms of action of alipogene tiparvovec.

9. Risk assessment and ethical issues

In addition to long-term efficacy follow-up, specific features of risk associated with AAV1-LPL^{S447X} gene transfer will be monitored over years. The objective is to strike the most appropriate balance between efficacy (reduced risk of pancreatitis and other LPLD co-morbidities) and safety (risk of severe adverse events) [31,32]. For both efficacy and safety issues, risk is defined as the probability over time that an event is due to hazard or exposure [33]. The notion of risk is closely tied to uncertainty and unknown outcome [31–33]. AAV1-LPL^{S447X} uses active biological agent rather than chemical. It is composed of genetic material that affects gene expression and requires a viral-based delivery device (the AAV-vector). In AAV-based gene therapy, both the transgene and the vector present potential risk of latencies that should be tightly monitored and compared to long-term benefits. Due to the nature of the treatment, it is essential to assess the probability that a serious adverse event that may occur relate to the treatment, the vector, the underlying disease (LPLD) or another factor. That's why all LPLD patients receiving AAV1-LPL^{S447X} are prospectively followed for 15 years.

10. Summary

LPLD is a serious chylomicronemic disorder. LPLD is associated with increased coronary artery disease and diabetes risk, but the most debilitating complication is pancreatitis. Acute pancreatitis is a complex, heterogeneous, highly morbid and potentially fatal disease. Efficacy results from the clinical studies on AAV1-LPL^{S447X} appear promising, especially regarding reduced risk of acute pancreatitis events. In these studies, alipogene tiparvovec was in general well tolerated and safe. Further analyses and long-term follow-up are ongoing to definitively confirm the long-term benefit and safety.

Conflict of interest statement

All co-authors have been directly involved in the clinical development program of alipogene tiparvovec. JdW, AF and SvD are AMT employees. DG was the principal investigator for AMT-011-01 and AMT-011-02 studies and has received honoraria from AMT. SD was co-investigator in these studies and received honoraria for his involvement. JM was the AMT-011-01 and AMT-011-02 trials clinical pharmacist and has received honoraria for her participation. DB and KT are

involved in the execution and the scientific development of the AMT-011-01 and 02 studies.

Acknowledgements

Authors are thankful to all participants in the clinical studies and to the staff of the Academic Medical Center in Amsterdam, the ECOGENE-21 Clinical Research Center in Chicoutimi and Amsterdam Molecular Therapeutics (AMT) B.V. J. Méthot is a Université de Montréal post-doctoral fellow, received support from the Canadian Institutes of Health Research (CIHR). K. Tremblay is a Université de Montréal post-doctoral and CCRP fellow and receives support from ECOGENE-21. D. Gaudet holds the Canada Research Chair in preventive genetics and community genomics (www.chairs.gc.ca) which is also supported by a CIHR team grant (# CTP-82941).

References

- [1] Brunzell JD, Deeb SS. Familial lipoprotein lipase deficiency, Apo C-II deficiency and hepatic lipase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. *The metabolic basis of inherited disease*. 8th ed. New York: McGraw-Hill; 2000. p. 2789–816.
- [2] Goldberg IJ, Merkel M. Lipoprotein lipase: physiology, biochemistry, and molecular biology. *Front Biosci* 2001;6:D388–405.
- [3] Gagne C, Brun LD, Julien P, et al. Primary lipoprotein-lipase-activity deficiency: clinical investigation of a French Canadian population. *CMAJ* 1989;140:405–11.
- [4] Franssen R, Young SG, Peelman F, et al. Chylomicronemia with low postheparin lipoprotein lipase levels in the setting of GPIIb/IIIa defects. *Circ Cardiovasc Genet* 2010.
- [5] Pruneta-Deloche V, Marcis C, Perrot L, et al. Combination of circulating antilipoprotein lipase (Anti-LPL) antibody and heterozygous S172 fsX179 mutation of LPL gene leading to chronic hyperchylomicronemia. *J Clin Endocrinol Metab* 2005;90:3995–8.
- [6] Black DM, Sprecher DL. Dietary treatment and growth of hyperchylomicronemic children severely restricted in dietary fat. *Am J Dis Child* 1993;147:60–2.
- [7] Santamarina-Fojo S. The familial chylomicronemia syndrome. *Endocrinol Metab Clin N Am* 1998;27:551–67.
- [8] Fortson MR, Freedman SN, Webster 3rd PD. Clinical assessment of hyperlipidemic pancreatitis. *Am J Gastroenterol* 1995;90:2134–9.
- [9] Etemad B, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001;120:682–707.
- [10] Benlian P, De Gennes JL, Foubert L, et al. Premature atherosclerosis in patients with familial chylomicronemia caused by mutations in the lipoprotein lipase gene. *N Engl J Med* 1996;335:848–54.
- [11] Frossard JL, Steer ML, Pastor CM. Acute pancreatitis. *Lancet* 2008;371:143–52.
- [12] Chen JM, Ferec C. Chronic pancreatitis: genetics and pathogenesis. *Annu Rev Genomics Hum Genet* 2009;10:63–87.
- [13] Schneider A, Barmada MM, Slivka A, et al. Clinical characterization of patients with idiopathic chronic pancreatitis and SPINK1 mutations. *Scand J Gastroenterol* 2004;39:903–4.
- [14] Cohn JA, Friedman KJ, Noone PG, et al. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. *N Engl J Med* 1998;339:653–8.
- [15] Whitcomb DC. Genetic aspects of pancreatitis. *Annu Rev Med* 2010;61:413–24.
- [16] Criddle DN, McLaughlin E, Murphy JA, et al. The pancreas misled: signals to pancreatitis. *Pancreatol* 2007;7:436–46.
- [17] Ferns G, Ketji V, Griffin B. Investigation and management of hypertriglyceridaemia. *J Clin Pathol* 2008;61:1174–83.
- [18] Chait A, Brunzell JD. Chylomicronemia syndrome. *Adv Intern Med* 1992;37:249–73.
- [19] Paradis G. Effect of a nutritional supplement rich containing medium-chain triglyceride (MCT) and the adjunction of fibrates on lipid-lipoprotein profile in subjects with homozygous LPL deficiency. Master of Sciences Thesis, Université Laval, Québec; 2001.
- [20] Rip J, van Dijk KW, Sierts JA, et al. AAV1-LPL(S447X) gene therapy reduces hypertriglyceridemia in apoE2 knock in mice. *Biochim Biophys Acta* 2006;1761:1163–8.
- [21] Wittrup HH, Tybjaerg-Hansen A, Nordestgaard BG. Lipoprotein lipase mutations, plasma lipids and lipoproteins, and risk of ischemic heart disease. A meta-analysis. *Circulation* 1999;99:2901–7.
- [22] Ak K, Isbir S, Tekeli A, et al. Presence of lipoprotein lipase S447X stop codon affects the magnitude of interleukin 8 release after cardiac surgery with cardiopulmonary bypass. *J Thorac Cardiovasc Surg* 2007;134:477–83.
- [23] Stroes ES, Nierman MC, Meulenberg JJ, et al. Intramuscular administration of AAV1-lipoprotein lipase S447X lowers triglycerides in lipoprotein lipase-deficient patients. *Arterioscler Thromb Vasc Biol* 2008;28:2303–4.
- [24] Rip J, Nierman MC, Sierts JA, et al. Gene therapy for lipoprotein lipase deficiency: working toward clinical application. *Hum Gene Ther* 2005;16:1276–86.
- [25] Mingozzi F, Meulenberg JJ, Hui DJ, et al. AAV-1-mediated gene transfer to skeletal muscle in humans results in dose-dependent activation of capsid-specific T cells. *Blood* 2009;114:2077–86.
- [26] Frimat L, Cassuto-Viguié E, Charpentier B, et al. Impact of cyclosporine reduction with MMF: a randomized trial in chronic allograft dysfunction. The 'reference' study. *Am J Transplant* 2006;6:2725–34.
- [27] Vogt B, Antoniadis A, Klinger M, Vitko S. Efficacy and safety of enteric-coated mycophenolate sodium (myfortic) in de novo renal transplant recipients: results of a 12-month multicenter, open-label, prospective study. *Transplant Proc* 2006;38, 1301–1306.26.
- [28] Sadek S, Medina J, Arias M, et al. Short-term combination of mycophenolate mofetil with cyclosporine as a therapeutic option for renal transplant recipients: a prospective, multicenter, randomized study. *Transplantation* 2002;74:511–7.
- [29] Ahsan N, Johnson C, Gonwa T, et al. Randomized trial of tacrolimus plus mycophenolate mofetil or azathioprine versus cyclosporine oral solution (modified) plus mycophenolate mofetil after cadaveric kidney transplantation: results at 2 years. *Transplantation* 2001;72:245–50.
- [30] Lachin JM. *Biostatistical methods: the assessment of relative risks*. 1st ed. New York: J Wiley; 2000, 544 pp.
- [31] Kimmelman J. Recent developments in gene transfer: risk and ethics. *BMJ* 2005;330:79–82.
- [32] Deakin CT, Alexander IE, Kerridge I. Accepting risk in clinical research: is the gene therapy field becoming too risk-averse? *Mol Ther* 2009;17:1842–8.
- [33] Kaplan S, Garrick BJ. On the quantitative definition of risk. *Risk Anal* 1981;1:11–27.