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Means and methods for modulating lipid metabolism

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(54) Title: MEANS AND METHODS FOR MODULATING LIPID METABOLISM

(57) Abstract: The invention relates to means and methods for the modulation of lipid metabolism in an individual. Among others, it relates to a novel regulator of levels of plasma lipids and hepatic lipids and the use thereof for therapeutic intervention. Provided is a modulator of the Small leucine-rich protein 1 (human) (SMLR1) gene, a SMLR1 gene product, and/or a transcriptional regulator of SMLR1. Also provided is the use of the SMLR1 gene, a SMLR1 gene product, and/or a transcriptional regulator of SMLR1, as target in a method of modulating lipid metabolism in a subject.

Title: Means and methods for modulating lipid metabolism.

5 The present invention relates to means and methods for the modulation of lipid metabolism in an individual. Among others, it relates to a novel regulator of levels of plasma lipids and hepatic lipids and the use thereof for therapeutic intervention.

Elevated plasma level of total cholesterol, low-density lipoprotein 10 (LDL) cholesterol and triglycerides are considered independent risk factors for atherosclerotic cardiovascular disease (ASCVD), the main cause of death worldwide. Importantly, plasma lipid levels are modifiable risk factors. Current insight shows that advices to improve lifestyle are insufficient to reduce plasma lipid levels to prevent the consequences of ASCVD.

15 Statins are the first line of treatment to reduce total cholesterol and LDL cholesterol which can be combined with bile acid sequestrants and/or inhibition of dietary uptake of cholesterol in the intestine. These drugs can generally reduce risk of ASCVD by 35%. A portion of the residual 65% risk can nowadays be targeted with a novel class of drugs called 20 proprotein convertase subtilisin kexin 9 (PCSK9) inhibitors. On top of statins, these drugs can reduce LDL cholesterol levels by an additional 50% and further reduce risk by 15%. PCSK9 inhibition in the clinic is currently limited to subcutaneous injection with monoclonal antibodies. Due to high costs (approximately 7k€ per year in the Netherlands), the use of these 25 drugs is currently limited to statin-intolerant patients and patients at very high risk of ASCVD despite high-intensity treatment.

The residual ASCVD risk mentioned above is considered to be not only mediated by cholesterol in LDL, but also by cholesterol in remnant lipoproteins, i.e. the remains of chylomicrons (produced by the small 30 intestine after a meal) and very low-density lipoprotein (VLDL) which are produced in the liver to supply the periphery with triglycerides for use (skeletal/heart muscle) or storage (adipose tissue). Statins and PCSK9

inhibition do not target the risk associated with remnant lipoprotein cholesterol. It is important to note that nowadays the idea that plasma triglycerides are a proxy for remnant cholesterol is gaining more strength.

Attempts in the past to strictly reduce plasma triglycerides with fibrates and niacin have been successful, but these interventions have not been proven to reduce ASCVD in clinical end point trials. New strategies are being developed to target residual risk, with a focus on bringing down triglyceride levels through targeting the hepatic production of key proteins in lipid metabolism. Specifically, phase II clinical trials are currently testing the use of antisense oligonucleotides (ASO) to silence the expression of APOC3 and ANGPTL3. While APOC3 silencing is primarily tested in familial chylomicronemia syndrome and familial partial lipodystrophy, ANGPTL3 silencing is primarily tested in a setting of hypertriglyceridemia but also persisting hypercholesterolemia. WO2017/189813 relates to the use of an ANGPTL3 inhibitor with concomitant lipid- lowering therapies to treat patients with familial hypercholesterolemia in order to achieve optimal serum lipid and lipoprotein levels. For a review on lipid-lowering medication that is currently used or tested in the clinic, see Hegele & Tsimikas (Circ Res. 2019;124:386-404). The review by Van Zwol *et al.* (J. Clin.Med. 2019, 8, 1085) summarizes the evidence and rationale to lower lipoprotein(a) and triglycerides, and provides an overview of strategies to lower Lp(a), apolipoprotein C-III and angiopoietin-like protein 3.

Importantly, all currently registered drugs to lower LDL cholesterol act via the LDL receptor pathway. Antisense ANGPTL3 inhibition (under development), may become an exception to this rule. This is important for patients that suffer from a complete loss of function of their LDL receptors (homozygotes for ‘null alleles’).

The present inventors aimed at the identification of novel genes with roles in human lipid metabolism, in particular plasma lipids and hepatic lipids. In doing so, they aimed at providing novel therapeutic targets that allow for modulation of lipid metabolism.

To that end, they have made use of 3 monogenic disorders which are associated with a loss of the capacity of the liver to package triglycerides into VLDL for subsequent secretion into the circulation. Specifically, these are genetic APOB, MTTP and ANGPTL3 deficiency. These genes are key to the production of triglyceride-containing lipoproteins (chylomicrons, VLDL) which are converted to remnant lipoproteins in the periphery. All 3 respective genes and their products are validated targets for pharmaceutical intervention to treat hypercholesterolemia and/or hypertriglyceridemia.

APOB encodes for apolipoprotein (apo) B48 in the small intestine and for apoB100 in the liver. ApoB48 and apoB100 are the main structural component of chylomicrons and (V)LDL, respectively. Damaging mutations in APOB cause hypobetalipoproteinemia (HBL), a rare autosomal dominant disorder. Homozygotes for HBL mutations present with extremely low levels of cholesterol in all lipoprotein fractions. In the clinic, Mipomersen (KYNAMRO), an APOB ASO (delivery through subcutaneous injections), is currently used to reduce apoB and thereby hepatic VLDL production for secretion into the circulation to treat severe hypercholesterolemia. Reduced VLDL production will ultimately render lower levels of LDL as end product of VLDL triglyceride lipolysis through lipoprotein lipase. This treatment, however, comes with a significant risk of hepatic lipid accumulation which results NAFLD.

MTTP, encoding for microsomal triglyceride transfer protein, is involved in the assembly of chylomicrons in the small intestine and VLDL in the liver which involves lipidation of the apoB protein at the endoplasmatic reticulum. Complete loss of MTTP, a rare recessive disorder, is associated with abetalipoproteinemia (ABL), i.e. marked reductions of cholesterol in all lipoproteins as described above for homozygous HBL. MTTP deficiency also causes malabsorption of fat and fat-soluble vitamins from food in the intestine. Lomitapide, a small molecule inhibitor of MTTP, is used to treat severe hypercholesterolemia but as for Mipomersen such use comes with an increased risk of NAFLD.

ANGPTL3 encodes for a protein that is produced in the liver and secreted into the circulation. Complete genetic ANGPTL3 deficiency in humans is associated with a reduction of cholesterol in all lipoprotein fractions. The mechanisms of actions of ANGPTL3 include inhibition of peripheral
5 hydrolysis of triglycerides by lipoprotein lipase and promoting hepatic VLDL assembly. Evinacumab, a monoclonal antibody against ANGPTL3 is currently tested in Phase II clinical trials and has been shown to effectively reduce plasma triglycerides (up to 63%). It has also been shown to reduce and LDL cholesterol by 50% in patients with homozygous familial
10 hypercholesterolemia (hoFH) indicating that its activity is LDL receptor independent.

Using gene expression data, the inventors identified novel candidate therapeutic targets having similar spatial and conditional regulation
15 patterns as these three genes, designated here as “lead genes”. This approach surprisingly resulted in the identification of a novel lipid gene, *SMLR1*, which is almost exclusively expressed in the liver and small intestine and is predicted to encode for a small (107aa) transmembrane protein Small leucine-rich protein 1 (SMLR1). The amino acid sequence of
20 the human SMLR1 protein is
MLSKGRSPRRKQVQTQRKAALVLSVTPMVPVGSVWLAMSSVLSAFMRE
LPGWFLFFGVFLPVTLLLLLLIAYFRIKLIEVNEELSQNCDRQHNPKDGS
SLYQRMKWT (NCBI Reference Sequence NP_001182526.1). The coding sequence can be accessed at NM_001195597.1 (Gene ID: 100507203).

25

SUMMARY

The invention provides a modulator of the Small leucine-rich protein 1 (human) (*SMLR1*) gene, a SMLR1 gene product, and/or a transcriptional
30 regulator of SMLR1, and the use thereof in a method of modulating lipid metabolism or treating a lipid metabolism disorder in a subject.

Also provided is the use of the Small leucine-rich protein 1 (*SMLR1*) gene, a SMLR1 gene product, and/or a transcriptional regulator of SMLR1, as target in a method of modulating lipid metabolism in a subject.
The invention also relates to a method of modulating lipid metabolism in a subject, comprising targeting the Small leucine-rich protein 1 (*SMLR1*) gene, a SMLR1 gene product, and/ or a transcriptional regulator of SMLR1. The transcriptional regulator of SMLR1 can be a transcriptional activator or repressor of SMLR1, which is suitably targeted to modulate SMLR1 expression.

In one embodiment, the invention provides an SMLR1-inhibitor, wherein the SMLR1 inhibitor is or comprises a peptide, a peptidomimetic, a small molecule inhibitor, an inhibitory (antisense) oligonucleotide, or a polypeptide molecule. In a preferred aspect, the SMLR1 modulator is an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, guide RNA (gRNA), siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense DNA or RNA.

A further embodiment relates to a pharmaceutical composition comprising a an SMLR1 modulator according to the invention, preferably an SMLR1 inhibitor, and a pharmaceutically acceptable carrier, vehicle or diluent.

DETAILED DESCRIPTION

SMRL1 is a gene that is completely new in the lipid/lipoprotein and the non-alcoholic fatty liver (NAFLD) research fields. Information in the public domain on SMLR1 is limited to its name and a prediction that it interacts with other proteins (String). As is demonstrated herein below, hepatic downregulation of *Smrl1* in mice reduces cholesterol in all lipoprotein fractions when the mice are fed a regular chow diet. This validation uncovers *SMLR1* as a lipid candidate gene with a function that is very

closely related to that of the "lead genes". The hepatic accumulation of cholesterol and triglycerides in the liver following *Smlr1* downregulation points at a role for SMLR1 in VLDL synthesis and/or secretion.
The findings demonstrate that the *Smlr1* gene or a downstream gene
5 product thereof (RNA, protein), or a transcriptional regulator of SMLR1, can be used as a novel target for modulation lipid metabolism, including plasma and hepatic lipid/lipoprotein metabolism. Targeting the *SMLR1* gene, its product(s) or regulator(s) according to the invention can provide a solution to various health problems that are related to intestinal lipid absorption
10 (uptake of lipid soluble vitamins), hepatic lipids, and plasma lipids and lipoproteins (NAFLD, atherosclerosis, pancreatitis).

Accordingly, in one embodiment, the invention provides a modulator of the *SMLR1* gene, of a SMLR1 gene product, and/or of a transcriptional
15 regulator of SMLR1. Also provided herein is a modulator of SMLR1 capable of targeting the *SMLR1* gene, gene product, or transcriptional regulator thereof, for use in a method of modulating lipid metabolism in a subject. Also provided is a modulator of the SMLR1 gene or gene product, for use as a medicament.
20 The modulator can modulate the activity and/or the expression level of the *SMLR1* gene, the activity and/or the expression level of a SMLR1 gene product and/or the activity and/or the expression level of a transcriptional regulator of SMLR1. The modulator of the invention is suitably used in a method of modulating lipid metabolism in a subject. In
25 one aspect, the invention provides a modulator of the (human) (SMLR1) gene. In another aspect, it provides a modulator of a SMLR1 gene product (RNA, protein). In yet another aspect, it provides a modulator of a transcriptional regulator of SMLR1.

A further embodiment relates to a method of modulating lipid metabolism in a subject, comprising targeting the Small leucine-rich protein 1 (SMLR1) gene, gene product or transcriptional regulator thereof. Also provided herein is the *SMLR1* gene or a gene product thereof for use as target in a method of modulating lipid metabolism in a subject.
In a preferred aspect, the targeting of the *SMLR1* gene (product) comprises organ-specific SMLR1 targeting. In one specific embodiment, the invention provides a modulator capable of liver-specific targeting of the *SMLR1* gene or a gene product thereof, and the use thereof in a method of modulating plasma and/or hepatic lipid metabolism in a subject. A further specific embodiment relates to a method of modulating plasma and/or hepatic lipid metabolism in a subject, comprising targeting the *SMLR1* gene or a gene product thereof in the liver of an individual. Also provided herein is the *SMLR1* gene or a gene product thereof for use as target in a method of modulating plasma and/or hepatic lipid metabolism in a subject.

In another specific embodiment, the invention provides a modulator capable of targeting of the *SMLR1* gene or a gene product thereof in the small intestine, and the use thereof in a method of modulating lipid metabolism in a subject. A further specific embodiment relates to a method of modulating lipid metabolism in a subject, comprising targeting the *SMLR1* gene or a gene product thereof in the small intestine of an individual. Also provided herein is the *SMLR1* gene or a gene product thereof for use as target in a method of modulating lipid metabolism in the small intestine of a subject.

Also provided herein is a method of treating a disease related to lipid metabolism, preferably wherein said disease is ASCVD, hypercholesterolemia, hypertriglyceridemia, statin-intolerance and/or cancer related to perturbed lipid metabolism, comprising administering to a subject a modulator of the *SMLR1* gene or gene product.
A further aspect relates to a modulator of the (human) *SMLR1* gene or, a *SMLR1* gene product, for use in such method of treatment.

In one embodiment, the invention finds its use in the treatment of patients at high increased risk of ASCVD despite aggressive lipid-lowering therapy. Conventional therapies to treat homozygous familial hypercholesterolemia (hoFH) can remain insufficient for this category of patients. This is related to the notion that conventional therapies are largely dependent of the presence of functional LDL receptors. Some HoFH patients, however, have no LDL receptors and are thus refractory to treatment. These patients are in some countries such as Germany treated with apheresis. Lomitapide is an option but long-term safety and efficacy data are yet to be provided (PMID: 30945578). Mipomersen is also used, but at the risk of developing NAFLD. ANGPTL3 inhibitors are the most novel LDL receptor independent experimental drugs which are currently studied in hoFH. Hepatic downregulation of SMLR1 as disclosed in the present invention can now be added to this list. In other words, it can help reducing cholesterol in hoFH. In addition, it can help further reducing LDL cholesterol in patients suffering from statin-intolerance or patients at very high risk of ASCVD despite aggressive treatment. These are the same patients that are currently targeted with PCSK9 inhibition (at very high costs; see above). In view of our current data, hepatic SMLR1 downregulation can be expected to render similar results to ANGPTL3 inhibition or MTTP inhibition. It is feasible to assume that combined ANGPTL3 and/or MTTP, SMLR1 downregulation in the liver will render synergistic effects which would allow for down titration of the respective existing and experimental drugs.

In a further embodiment, the invention finds an application in the treatment of (severe) hypertriglyceridemia. Attempts to reduce the risk of pancreatitis in patients with severe hypertriglyceridemia through LPL gene therapy have only rendered very limited success and is now halted. As indicated above, there are no evidence-based drugs that reduce ASCVD through lowering triglycerides although retrospective studies using fibrates and niacin have shown that subgroups of ASCVD patients with increased triglycerides would benefit from these interventions. So far, the use of fish

oils or derivatives to reduce plasma triglycerides have shown variable results. Only one study with Vascepa showed a 18% reduction in triglycerides and 25% in cardiovascular mortality on top of the use of statins.

5 Antibodies against ANGPTL3 (Evinacumab) have a significant impact on plasma triglycerides (60%). In addition, antisense APOC3 therapy (Volanesorsen) has in a Phase I/IIa setting been shown to reduce plasma triglycerides up to 70% with moderate reduction of LDL cholesterol (17%) and increased HDL cholesterol (56%). Taken that the function of SMLR1 is 10 closely related to the roles of APOB, MTTP, ANGPTL3 as well as APOC3 in the hepatic assembly and secretion of VLDL, the downregulation of SMLR1 can render significant reductions in plasma triglycerides.

SMLR1 can furthermore be used for targeting NAFLD. ASOs against APOB have been shown to cause NAFLD, while inhibition of MTTP 15 also comes with the risk of developing a fatty liver. In view of the close interactions with SMLR1 with APOB and MTTP at the transcriptional level, it is possible that it may act as a chaperone to help the folding of apoB as anticipated for protein disulfide isomerase which completes the heterodimeric MTTP. Targeting SMLR1, can thus be anticipated to affect 20 the development of NAFLD.

Another interesting use of SMLR1 involves the targeting of intestinal fat adsorption and fat-soluble vitamin absorption. Excess caloric intake comes with the development of obesity, insulin resistance, metabolic syndrome and type 2 diabetes. SMLR1 is anticipated to play an important 25 role in the assembly of chylomicrons in the small intestine following a meal. Reduced intestinal fat uptake through SMLR1 inhibition may provide a means to reduce body weight gain. On the other hand, increasing SMLR1 function in the small intestine (expression, activity) may on the other hand promote the uptake of lipids and fat-soluble vitamins by the intestine in 30 patients with e.g. chylomicron-retention disease (SAR1B deficiency), and patients suffering from HBL and abetalipoproteinemia.

As used herein, the term "modulating lipid metabolism" encompasses the regulation, re-balancing or normalization of the level, content or synthesis at least one species or category of lipid or lipoprotein. Modulating may comprise stimulating/enhancing or inhibiting/decreasing.

5 In one aspect, modulating lipid metabolism comprises modulating one or more of

(a) plasma total cholesterol (TC) level,
(b) plasma triglyceride (TG) level,
(c) plasma low-density lipoprotein (LDL) level,
10 (d) plasma low density lipoprotein (VLDL) level
(e) VLDL assembly, synthesis and/or secretion
(f) chylomicron assembly, synthesis and/or secretion
(g) hepatic cholesterol content
(h) hepatic triglyceride content

15

In one preferred embodiment, it comprises modulating at least the plasma TC and plasma TG level. In another preferred embodiment, it comprises modulating at least VLDL assembly, synthesis and/or secretion. In a still further embodiment, it comprises modulating chylomicron assembly,
20 synthesis and/or secretion.

As said, the invention encompasses the downregulation/inhibition as well as the upregulation/activation of the *SMLR1* gene or gene product thereof or a transcriptional regulator. When used in reference to the expression of a
25 nucleic acid molecule, such as the *SMLR1* gene, refers to any approach which results in a decrease in production of a gene product. A gene product can be RNA (such as mRNA, rRNA, tRNA, and structural RNA) or protein.

In a preferred embodiment, it comprises the downregulation/inhibition of
30 the *SMLR1* gene or gene product thereof. SMLR1 downregulation or deactivation includes processes that decrease transcription of a gene or translation of mRNA. Examples of processes that decrease transcription

include those that facilitate degradation of a transcription initiation complex, those that decrease transcription initiation rate, those that decrease transcription elongation rate, those that decrease processivity of transcription and those that increase transcriptional repression. Gene
5 downregulation can include reduction of expression above an existing level. Examples of processes that decrease translation include those that decrease translational initiation, those that decrease translational elongation and those that decrease mRNA stability.

10 Gene downregulation includes any detectable decrease in the production of a gene product. In certain embodiments, the invention comprises a gene product decrease by at least 2-fold, for example at least 3-fold or at least 4-fold, as compared to a control (such as an amount of SMLR1 gene expression in a vector-transduced or transformed cell, organ or animal).
15 Accordingly, in one aspect of the invention the SMLR1 modulator is an SMLR1-inhibitor capable of down-regulating or inhibiting SMLR1 expression or SMLR1 (protein) activity. Preferably, said downregulation/inhibition is organ-specific downregulation/inhibition. For example, SMLR1 is targeted exclusively in the liver or (small) intestine.
20

Targeting SMLR1 in the (small) intestine can be achieved using methods known in the art. For example, a recombinant viral vector such as adeno-associated virus (AAV) vector-mediated approach is suitably used for poly- or oligonucleotide delivery to intestinal epithelial cells. In particular, a
25 replication deficient AAV is used (WO1993/003769). In a specific aspect of the invention, intestinal SMLR1 is targeted, e.g. using siRNA or gRNA, using AAV pseudotype 2/1, 2/2, or 2/5. Other suitable techniques include a CRISPR based method, for example adenosine-based editing (ABE), may be used for targeting in humans (permanent silencing of targeted genes (in e.g.
30 the liver).

In a particularly preferred aspect, the invention provides a modulator of Small leucine-rich protein 1 (SMLR1) capable of liver-specific downregulation/inhibition the *SMLR1* gene or a gene product thereof, for use in a method of modulating lipid metabolism in a subject. Also provided is a method of modulating lipid metabolism in a subject, comprising liver-specific downregulation/inhibition of the *SMLR1* gene or a gene product thereof. Still further, it provides the *SMLR1* gene or a gene product thereof for use as liver-specific target in a method of modulating lipid metabolism in a subject.

Targeting of SMLR1 in the liver can be accomplished using one of the various strategies that have been proposed to improve the delivery of different drugs to liver and hepatocytes. These include passive accumulation of nanoparticle therapeutics and active targeting by surface modifications of nanoparticles with specific ligands such as carbohydrates, peptides, proteins and antibodies. Ligand-mediated approaches for targeting SMLR1 in the liver may involve the targeting of one or more of Mannose – 6 –phosphate receptor (by Mannose-6-phosphate), Type VI collagen receptor (e.g. by Cyclic RGD), the PDGF receptor (by PDGF), Scavenger receptor class A (e.g. by Human serum albumin), the Asialoglycoprotein receptor (e.g. by Galactoside or Galactosamine), the Plasma membrane fatty acid binding protein (putative) (e.g. by Linoleic acid), the Scavenger receptor class B type I (e.g. by Apolipoprotein A-I), Heparan sulfate (e.g. by Acetyl CKNEKKNKIERNNKLKQPP-amide), the IL-6-receptor and/or immunoglobulin A binding protein (Putative) (e.g. by Pre-S1) and Glycyrrhizin receptors (by Glycyrrhizin).

The (organ-specific) downregulation/inhibition of the SMLR1 gene or a gene product thereof can have various effects on lipid metabolism. In one embodiment, it results in a reduction in one or more of:

(a) plasma total cholesterol (TC) level,

(b) plasma triglyceride (TG) levels,

(c) plasma low-density lipoprotein (LDL) levels,
(d) plasma low-density lipoprotein (VLDL) levels
(e) VLDL assembly, synthesis and/or secretion
(f) chylomicron assembly, synthesis and/or secretion.

5

The SMLR1-based approach of the present invention is particularly suited to modulate lipid metabolism in a human subject. As will be recognized and appreciated by a person skilled in the art, the invention is generally applicable to diseases or disorders related to or associated with a perturbed
10 lipid metabolism.

As indicated herein above, the SMLR1-based approach herein disclosed does not rely on the presence of a functional LDL receptor. Accordingly, it is advantageously used in a human subject which is a heterozygote or a
15 homozygote for familial hypercholesterolemia. However, the invention is of benefit for a broad target population, e.g. for any subject that is at high risk of developing, or known to be suffering from severe hyperlipidaemia or atherosclerotic cardiovascular disease (ASCVD).

20 Hyperlipidaemias are the primary metabolic disease in the developed world and are associated with a range of conditions, including diabetes, obesity, cardio-vascular pathology, renal failure, nephrotic syndrome, alcohol abuse, cirrhosis of the liver and hypothyroidism. Hyperlipidaemia and other abnormalities in lipid metabolism may be identified by measuring levels of
25 one or more serum markers such as total cholesterol, LDL-cholesterol, apolipoprotein B and triglycerides. Aberrant levels of one or more of these markers in an individual are characteristic of hyperlipidaemia and other medical conditions. In one embodiment, the human subject is at high risk of developing, or known to be suffering from, hypercholesterolemia,
30 hypertriglyceridemia and/or statin-intolerance.

In a further embodiment, the disease is cancer. Lipid metabolism reprogramming is a hallmark for tumor which contributes to tumorigenesis

and progression. There is increasing evidence that the alterations in tumor metabolism, including metabolite abundance and accumulation of metabolic products, lead to local immunosuppression in the tumor microenvironment.

5 Accordingly, the invention also provides a pharmaceutical composition comprising a modulator according to the invention, and a pharmaceutically acceptable carrier, vehicle or diluent. Preferably, the pharmaceutical composition comprises an SMLR1-inhibitor, more preferably an inhibitor capable of down-regulating or inhibiting SMLR1 expression and/or SMLR1
10 (protein) activity, preferably down-regulation or inhibition of SMLR1 in the liver.

The SMLR1-inhibitor can comprise or consist of a peptide, a peptidomimetic, a small molecule inhibitor, an (antisense) poly-or oligonucleotide, or a
15 polypeptide molecule.

In accordance with the present description, the SMLR1 inhibitor reduces the accumulation of a cholesterol and/or cholesterol in the subject. In one embodiment, the subject is assessed and determined to exhibit prior to
20 treatment, elevated levels relative to a control of lipids in the liver or plasma. In one embodiment, the SMLR1 inhibitor is administered in order to reduce the accumulation of one or more lipids or lipoprotein species, e.g. TC, TG, LDL cholesterol, LDL triglycerides and/or VLDL cholesterol and VLDL triglycerides in the subject. In one embodiment, the SMLR1 inhibitor
25 is administered in order to reduce the accumulation of lipid or lipoprotein species in the plasma of a subject. In one embodiment, the SMLR1 inhibitor is in the form of a pharmaceutical or physiological composition comprising the SMLR1 inhibitor as the active ingredient. For example, the SMLR1 inhibitor is in the form of a pharmaceutical or physiological composition
30 comprising the SMLR1 inhibitor and a suitable diluent and/or carrier. The agents described may be in the form of a composition or kit comprising same.

In one embodiment, the SMLR1 inhibitor is an inhibitor of SMLR1 expression and/or SMLR1 activity. In one embodiment, SMLR1 activity is determined by evaluating the level or activity of a protein whose expression is correlated with SMLR1 expression in a liver cell as determined herein. In one embodiment, the level or activity of SMLR1 in blood, plasma, serum from a subject is determined. In one embodiment, SMLR1 activity is determined by evaluating the level of SMLR1 polypeptide in a cell or tissue including blood, plasma or serum, of a subject. In one embodiment, the peptide is a phosphopeptide or phosphomimetic. In a further aspect, the SMLR1-inhibitor is an anti-SMLR1 antibody or an antigen binding fragment thereof. In one embodiment, the SMLR1 inhibitor comprises a moiety allowing for organ-specific targeting, preferably liver-specific targeting or (small) intestinal targeting.

In a preferred embodiment, the SMLR1 modulator is a polynucleotide. Polynucleotide sequences include oligonucleotides. Oligonucleotide modulators are known in the art. In one embodiment, oligonucleotides comprise sequences complementary or substantially complementary to at least one SMLR1 nucleotide sequence. SMLR1 nucleotide and amino acid sequences are known in the field, include variants including variants for multiple species, and are identified in publically available sequence databases.

In one embodiment the SMLR1 modulator is an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense DNA or RNA. In one embodiment, the inhibitory RNA is selected from a shRNA, gRNA, siRNA, miRNA, miRNA mimic or chimeric antisense RNA. SMLR1 inhibitors may comprise or encode an antisense, siRNA, shRNA, miRNA, ribozyme, DNAzyme or other nucleic acid molecules. Such agents are typically isolates or non-naturally occurring and are made synthetically or recombinantly. The SMLR1-targeting polynucleotide may be comprised in a vector. The vector is a viral vector or a non-viral vector. Any suitable

viral or non-viral vector may be employed. An adenoviral or lentiviral vector, for example, is useful for administering to the liver.

In one embodiment, SMLR1 is targeted using antisense technology. One advantage of antisense technology in the treatment of a disease or condition that stems from a disease-causing gene is that it is a direct genetic approach that has the ability to modulate expression of specific disease-causing genes. Generally, the principle behind antisense technology is that an antisense compound hybridizes to a target nucleic acid and affects modulation of gene expression activity or function, such as transcription, translation or splicing. The modulation of gene expression can be achieved by, for example, target degradation or occupancy-based inhibition. Hence, antisense technology is an effective means for modulating lipid metabolism via reducing the expression of SMLR1 and, if desired, one or more further specific gene products. Chemically modified nucleosides are routinely used for incorporation into antisense compounds to enhance one or more properties, such as nuclease resistance, pharmacokinetics or affinity for a target RNA.

Accordingly, in one embodiment, the invention provides an antisense oligonucleotide (ASO), typically 8 to 50 nucleotides in length, which is targeted to mRNA encoding human *SMLR1* and which is capable of inhibiting SMLR1 expression. In one embodiment, the antisense oligonucleotide is about five, six, seven, eight, nine, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30 nucleotides or more in length. The ASO may be targeted to a translation initiation site, 3' untranslated region or 5' untranslated region of mRNA encoding human SMLR1. The ASO may target any portion of transcript involved in the process of translating the human SMLR1 gene into the SMLR1 protein or any of its isoforms. The ASO is suitably comprised in a pharmaceutically acceptable carrier.

Exemplary ASO’s targeting human SMLR1 according to the present invention comprise or consist of the following sequences (5’-> 3’) :

TTCTGGGGCTCCGGCC; CCAGGTCGCATCATGC;
CCCCCAGGGGCTAAAA; GCAGCTGAATGTAGGG;
CCTGGTGAAAGGCCTT;GGTCTAGGTGGAGGCC;
GCAGCAAAGTCACGGG; CTCCGGCCTTTGCTCA;
5 CAGACCCCACGGGGAC; TGTAGGGAGACTGCCG;
GGCCTGACTGTGCAGC; GTCTGAAAACCCCGGC;
ACCCCGGCAGACATTT; GGGGCTCCCACTGAAA;
GTAGGCGATGAGGAGG;GAGATGGTGGACCAGC;
GGAATGCAAAGGACCG;GTGCCTTCAGGCAGAT;
10 TGTTGGCGATCACAGT;GTCTCTTTGGGGCTCC.

In vivo and *in vitro*, all-native DNA ASOs are quickly rendered useless by nuclease activity. In vivo, though both endonucleases and exonucleases may lead to degradation, exonucleases appear to do most of 15 the damage. To be effective, the ASOs require chemical modification to resist nuclease degradation. Numerous nucleic acid analogs are available for modifying ASOs.

For example, the ASO may have at least one phosphorothioate (PS) linkage. This modification was among the few that is considered first-20 generation. PS-ASOs are nuclease resistant and therefore have longer plasma half lives compared to all-native DNA ASOs. In addition, they retain negative backbone charges, which facilitates PS-ASO entry into the cell. Interestingly, PS appears to have a bigger impact on transport and entry into the cell than it does on nuclease resistance.

25 In another embodiment, the ASO is methylated. This modification was among the few that are considered second-generation. When combined with PS in ASOs, 2'-OMe-RNA has been found to improve upon the benefits of PS alone, i.e. increased nuclease resistance, plasma half life, and tissue uptake.

30 SMLR1 inhibitors may be conjugates of the molecules described herein. For example, the inhibitor may comprise one or more targeting ligands selected from the group consisting of cholesterol, biotin, vitamins,

galactose derivatives or analogs, lactose derivatives or analogs, N-acetylgalactosamine a derivative or analog, an N-acetylglucosamine derivative or the like, and any combination thereof. In a specific aspect, the invention provides a GalNAc-conjugated siRNAs or antisense
5 oligonucleotide capable of targeting SMLR1.

In a further embodiment, SMLR1 is silenced using the RNA-guided CRISPR-Cas system, which has emerged as a promising platform for programmable targeted gene regulation.

10 The CRISPR-Cas system relies on two main components: a guide RNA (gRNA) and CRISPR-associated (Cas) nuclease. The guide RNA for use in the present invention is a specific RNA sequence that recognizes the SMLR1 target DNA region and directs the Cas nuclease there for editing. The gRNA is made up of two parts: crispr RNA (crRNA), a 17-20 nucleotide
15 sequence complementary to the SMLR1 DNA, and a tracr RNA, which serves as a binding scaffold for the Cas nuclease.

Exemplary gRNA sequences of the invention include those targeting human exon-1 of SMLR-1. For example, provided herein are the gRNA's 5'- GTGACTCCCATGGTCCCCGT *GGG* and 5'-
20 ACAGACCCCACGGGGACCAT *GGG*, and uses thereof as herein disclosed.

The CRISPR-associated protein is a non-specific endonuclease. It is directed to the specific SMLR1 DNA locus by a gRNA, where it makes a double-strand break. There are several versions of Cas nucleases isolated from
25 different bacteria. The most commonly used one is the Cas9 nuclease from *Streptococcus pyogenes*. Adeno-associated virus (AAV) vectors have been proposed for gene delivery of CRISPR-Cas9 components for in vivo studies and therapeutic applications. AAV vectors provide stable gene expression with low risk of mutagenic integration events. AAV vectors can be
30 engineered to target tissues of interest *in vivo* and are already in use in humans in clinical trials. In a specific aspect, the SMLR1 modulator is a gRNA which targets SMLR-1, such as exon-1 of human SMLR-1. Preferably,

said gRNA is comprised in a viral vector, like an adeno-associated virus (AAV) vector, for example AAV serotype 8.

The invention also provides a composition comprising an organ-specific
5 modulator of the SMLR1 gene or a gene-product thereof, preferably a liver-specific or small intestine-specific SMLR1 modulator. In one embodiment, the composition comprises an SMLR1-inhibitor, preferably an siRNA or antisense oligonucleotide targeting SMLR1. The composition may comprise one or more further agents capable of modulating lipid metabolism,
10 preferably an agent capable of down-regulating or inhibiting ANGPTL3, MTTP, APOB and/or GPR146.

In one embodiment, the invention provides a pharmaceutical composition comprising a modulator of the (human) SMLR1 gene or gene product, and a
15 pharmaceutically acceptable carrier, vehicle or diluent. Preferably, it comprises an SMLR1-inhibitor capable of down-regulating or inhibiting SMLR1 expression and/or SMLR1 activity, more preferably wherein the SMLR1-inhibitor is capable of down-regulation or inhibition of SMLR1 in the liver. For example, the pharmaceutical composition comprises a SMLR1
20 modulator being an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, guideRNA (gRNA), siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense DNA or RNA. In a specific aspect, the pharmaceutical composition comprises a siRNA or gRNA targeting SMLR-1, such as exon-1 of human SMLR-1,
25 preferably wherein said siRNA or gRNA is comprised in a viral vector, such as an adeno-associated virus (AAV) vector.

An SMLR1-inhibitor according to the invention is advantageously used in combination with one or more further active agent(s), for example further
30 modulator(s) of lipid metabolism, of vitamin absorption, and/or drugs that are used in metabolic diseases or anti-fibrotic drugs. In particular, the SMLR1-inhibitor may be used in combination with a

downregulator/inhibitor of ANGPTL3, APOB, GPR146 and/or MTTP, and/or genes that are tightly co-expressed with any one of these four genes. For example, APOC3, APOA2 and ABCG5 are strongly correlated with expression of APOB and MTTP. SLC2A2 expression is strongly correlated
5 with ANGPTL3.

As is shown herein below, hepatic downregulation of SMLR1 in mice causes increases in hepatic cholesterol and triglycerides . This implies that hepatic overexpression of SMLR1 agonism may reduce NAFLD.
10 A further aspect of the invention therefore relates to modulating lipid metabolism by increasing or up-regulating the expression and/or activity of SMLR1. In one embodiment the invention provides a modulator of Small leucine-rich protein 1 (SMLR1) capable of up-regulating SMLR1 expression and/or activity by targeting the *SMLR1* gene or a gene product thereof, and
15 the use thereof in a method of modulating lipid metabolism and intestinal vitamin uptake in a subject.

Also provided is a method of modulating lipid metabolism in a subject, comprising up-regulating SMLR1 expression and/or activity by targeting the SMLR1 gene or a gene product thereof. Preferably, up-
20 regulation of SMLR1 expression and/or activity is specifically achieved in the liver. The approach involving an up-regulation or activation of (liver) SMLR1 is suitably used in a subject that is at high risk of developing or known to be suffering from one or more of NAFLD and/or hypobetalipoproteinemia (HBL) or abetalipoproteinemia (ABL).

LEGEND TO THE FIGURES

Figure 1. **Target discovery approach used in this study**. A key component is the manual curation of public expression datasets to create a high-quality compendium of gene expression data. The Sample level data is aggregated into representative profiles for the Anatomy, Cell Lines and Perturbations (including diseases, drugs, genotypes and other perturbational conditions). Co-expression analysis can be carried out on one or more of these profiles, and results can be evaluated against LDLc GWAS hits and molecular pathways from Reactome for further evidence. In the case of *SMLR1*, no further evidence could be found in GWAS studies likely due to its very short sequence and absence of sequence polymorphisms in this gene locus, and in the literature because it's an entirely uncharacterized protein. It was selected due to its striking similarity of spatial and response to perturbations of the lead genes.

Figure 2. Effects of liver-specific downregulation of *Smlr1* on plasma lipids and lipoproteins. Ten male (Alb-Cas9) mice received either control adenovirus or adenovirus with gRNA's against *Smlr1*. Following virus delivery, mice were individually housed, kept on a chow diet, and were terminated after 8 wks. Panel A: Hepatic *Smlr1* mRNA expression. Panel B: Hepatic Smlr1 protein levels (quantitative proteomics). Data represent mean ± SD. Abbreviations: LKD, liver-specific knock-down; EV, empty vector.

Figure 3. Liver-specific downregulation of *Smlr1* and plasma lipids. Ten male (Alb-Cas9) mice received either control adenovirus or adenovirus with gRNA's against *Smlr1*. Following virus delivery, mice were individually housed, kept on a chow diet, and were terminated after 8 wks. Panel A: plasma total cholesterol. Panel B: lipoprotein cholesterol profile of pooled plasma obtained at 2 weeks following virus administration in each experimental group. Panel C: plasma triglyceride levels. Data represent

mean ± SD (n=9-10) or pooled samples (week 8 of plasma lipid levels and FPLC).

Figure 4. Liver-specific downregulation of *Smlr1*, liver weight and
5 **liver lipids.** Ten male (Alb-Cas9) mice received either control adenovirus or adenovirus with gRNA's against *Smlr1*. Following virus delivery, mice were individually housed, kept on a chow diet, and were terminated after 8 wks. Panel A: liver weight. Panel B: Liver weight/BW. Panel C: hepatic cholesterol. Panel D: hepatic triglycerides. Panel E: representative images of
10 hepatic cryosections stained with Oil-red-O. Scale bar = 100 μm. Data presented as individual values with mean ± SD. Abbreviation: LKD, liver-specific knock-down.

Figure 5. Liver-specific downregulation of *Smlr1* reduces hepatic
15 **VLDL production**. Seven female (Alb-Cas9) mice received either control adenovirus or adenovirus with gRNA's against *Smlr1*. Following virus delivery, mice were individually housed, kept on a chow diet, and were terminated after 8 wks. Panel A: total plasma cholesterol. Panel B: plasma triglycerides. Panel C: plasma triglyceride levels in LKD mice (pink line)
20 and controls (black line) following poloxamer injections at 5 weeks following virus delivery. Panel D: hepatic *Smlr1* mRNA levels at sacrifice. Black lines/boxes are controls, Pink lines/boxes are LKD mice. Data presented as pooled samples or mean ± SD (n=4-7). Abbreviation: LKD, liver-specific knock-down.

EXPERIMENTAL SECTION

EXAMPLE 1: Contextual co-expression analysis

5 This example describes the identification of new LDL genes that are contextually co-expressed with *APOB*, *APOC3* and *MTTP* ('lead genes') using transcriptome expression data aggregated from over 50.000 human samples. The corresponding microarray data were globally normalized and aggregated into matrices representing a) spatial expression ("Anatomy"), 10 and b) conditional expression ("Perturbations").

We chose to work with GENEVESTIGATOR (https://genevestigator.com), a high-quality database and search engine of expertly curated microarray and RNA-seq experiments. Its rich diversity of content across diseases, drugs, 15 tissues, cancers, cell lines and genotypes allowed generating very refined profiles for individual dimensions. As a proof of principle, we chose to work on data from the namely Affymetrix GeneChip Human Genome U133 Plus2.0 array. The choice of this platforms was motivated by the high number of studies and experimental conditions publicly available and 20 already curated in GENEVESTIGATOR.

The *Anatomy* expression matrix was created by aggregating all microarray samples originating from the same tissue into a single anatomical category. As a result, for each gene in the matrix a vector of expression across over 25 400 different tissues and cell types was used for subsequent analysis.

The *Perturbations* matrix comprised a compilation of experimental comparisons, such as "diseased versus healthy" or "treated versus untreated controls". Perturbations stand for treatment with compounds, diseases, 30 genotype comparisons, or other types of main experimental variables tested. For each gene in the matrix, a vector of log2-ratios from over 5000 perturbations was created and used for subsequent analysis.

First, for each of the lead genes a co-expression analysis was performed by correlating their *Anatomy* expression with that of all other genes measured on this microarray platform, resulting in a list of genes having the most similar anatomical expression profiles (as measured by Pearson's correlation coefficient).

Second, for each of the lead genes a co-expression analysis was performed by correlating their *Perturbations* expression with that of all other genes measured on this microarray platform, resulting in a list of genes having the most similar perturbational response profiles (as measured by Pearson's correlation coefficient). As a result, for each lead gene, we obtained a list of genes having most similar anatomical expression and a list of genes having most similar perturbational response.

Third, we compared the lists of genes obtained from each co-expression analysis and prioritized those genes having most similar profiles as our lead genes, both by anatomical and by perturbational co-expression.

The above contextual gene co-expression analysis approach is outlined in Figure 1. It resulted in the surprising finding that *SMLR1,* encoding for small leucine-rich protein, is strongly co-regulated, both spatially and perturbationally, with *APOB*, *MTTP*, and *APOC3*. With no information in the public domain, we set out to validate *SMLR1* as a novel lipid regulatory gene.

EXAMPLE 2: Effects of Smlr1 down-regulation in mice.

This example describes the generation and analysis of a murine model to investigate the role of SMLR1 as regulator of lipid metabolism. To this end, the murine ortholog *Smlr1* was downregulated in the liver of mice expressing Cas9 under the control of an albumin-specific promoter through adenoviral delivery of gRNAs directed against *Smlr1.* Control mice were injected with control ('empty') adenovirus.

Materials and Methods

Animal Studies

All studies were approved by the Institutional Animal Care and Use Committee, University of Groningen (Groningen, the Netherlands). Liver-specific *Smlr1-/-* mice were generated using CRISPR/Cas9 editing technology. Mice expressing Cas9 under an albumin promotor (in house breeding) received retro-orbital administration of either adenovirus (AV) harboring 3 single guide RNAs (gRNA's) targeting exon 1 (GGGGACCATGAGCTCCGTGC, GATTGCCAGCACGGAGCTCA, CGTGCTGGCAATCTTCTTGC) or a matched viral dose of empty AV (pAd/pX459) particles as control.

Mice were injected with a total AV dose of $1x10^{11}$ virus particles and adjusted to 100ul with sterile Phosphate-Buffered Saline (PBS). At the start of the experiments, mice were 10-12 weeks of age. All mice were individually housed, fed *ad libitum* with standard rodent chow diet (RMH-B, AB Diets, the Netherlands). In all experiments, littermates were used as controls. Body weight and food intake was measured every week in the morning. Blood was collected by retro-orbital bleeding one week before and two weeks after virus administration. Mice were sacrificed following a 4-hours morning fasting-period under anesthesia (isoflurane). Tissues were harvested and snap-frozen in liquid nitrogen and stored at -80°C until further analysis. Blood was drawn by cardiac puncture, and plasma was isolated by centrifugation at 1000 *g* for 10 min at 4°C and stored at –80°C until further analysis.

Plasma lipid measurements and lipoprotein profiling

Plasma triglyceride and total cholesterol levels were measured using Infinity Triglycerides Reagent and Cholesterol Reagent (1187771, and 11489232, Roche Molecular Biochemicals, respectively) following

manufacturer instructions. Standards for the liver lipid measurements were made in 2% Triton.

For fast protein liquid chromatography (FPLC) analysis, pooled plasma samples. In brief, the system used contained a PU-980 ternary pump with
5 an LG-980-02 linear degasser and an UV-975 UV/VIS detector (Jasco). An extra PU 2080i-plus pump (Jasco) was used for in-line cholesterol ready-to-use enzymatic reagent (Biomerieux) addition at a flow rate of 0.1 ml/min. Sample buffer mixture was loaded on a Superose 6 HR 10/30 column (GE Healthcare) for lipoprotein separation at a flow rate of 0.31 ml/min.
10 Chromatographic profiles of pooled WT mouse plasma served as reference. For cholesterol measurements, EDTA plasma was diluted 1:1 with PBS. Triglycerides measurements were conducted with undiluted EDTA plasma.

Liver lipids

15 Liver homogenates were prepared as 15% (w/v) solutions in ice-cold PBS (with the beat beater), and subjected to lipid extraction according to Bligh & Dyer method (Bligh and Dyer 1959). In short, 600 μl of demi-water was mixed with 200 μl of liver homogenate, and 3 ml of chloroform/methanol (2:1; v/v) was added and mixed followed by vigorous vortex for 30 sec. After
20 30 min of incubation, 1.2 ml of H_2O and 1 ml of chloroform were added, mixed and subsequently centrifuged (10 min, 500 *g* at room temperature). The chloroform layer was transferred into a new glass tube, and was evaporated using nitrogen at 50°C. Lipids were resolved in 1 ml of chloroform, separated into two tubes of 400 and 600μl. The 400μl was
25 evaporated again and dissolved in 500μl Triton X-100 2% in chloroform, followed by evaporation and subsequently adding 500μl demi-water. The samples were incubated for 15 min at 37°C and stored at room temperature for further analysis.

30 VLDL production

After 4 hours fasting in the morning, mice were injected intraperitoneal with 1 g/kg body weight Poloxamer-407 (Sigma) in PBS (1 g/kg body weight).

Immediately before, and at 30, 60, 120 and 240 min after injection, blood was drawn by tail vein bleeding. Plasma was isolated by centrifugation at 1000 *g* for 10 min at 4°C and stored at –80°C until further analysis.

5 Gene expression analysis

100 µg of mouse liver was homogenized in 1 ml QIAzol Lysis Reagent (Qiagen). Total RNA was isolated by chloroform extraction. Isopropanol-precipitated and ethanol-washed RNA pellets were dissolved in RNase/DNase-free water. 1 µg of RNA was used to prepare cDNA with the
10 Transcriptor Universal cDNA Master kit (Roche), according to the manufacturer's instructions. 20 ng of cDNA was used for quantitative real-time PCR (qRT-PCR) analysis using the FastStart SYBR Green Master (Universal SYBR master ROX / Roche: 04913914001) and the 7900HT Fast Real-Time PCR System (Applied Biosystems). The PCR reaction was
15 performed as follows: 2 min at 50°C, 10 min at 95°C, 40 cycles of 15 seconds at 95°C, and 1 min at 60°C. Expression data were analyzed using SDS 2.3 software (Applied Biosystems), using the ΔΔCT method of calculation. PPIA expression was used as an internal control. The sequence of the primers is available upon request.

20

Proteomic analyses

Isotopically labeled Smlr1-specific peptides were used to develop targeted liquid chromatography mass spectroscopic (MS) assays. Details of the method used are described elsewhere (Fedoseienko, 2018). Smlr1 protein
25 concentrations were measured in liver homogenates.

Liver histology

Paraffin embedded sections of tissues (4 µm) were processed for histological evaluation. Standard Oil red O staining was performed on cryosections of
30 liver tissue.

Statistical analyses

Analyses were performed using GraphPad version 8.0. Unpaired 2-tailed Student's *t* test was used to test for statistical significance between 2 groups. 2way ANOVA with multiple comparisons was used for the triglyceride secretion rate (VLDL production experiment). Data represent mean ± SD. Asterisks denote corresponding statistical significance $^*p<0.05$, $^{**}p<0.005$, $^{***}p<0.0005$.

RESULTS

Downregulation of hepatic *Smlr1* mRNA and protein following the adenoviral delivery of gRNAs targeting *Smlr1*

Eight weeks after virus administration, mice that received gRNAs against *Smlr1* (liver-specific knock down, (LKD) showed a 77% reduction of *Smlr1* mRNA, and a 66% downregulation of Smlr1 protein (Figure 2, panels A and B, respectively). Over the course of this experiment, the groups did not show differences in body weight gain and food intake (data not shown).

Hepatic downregulation of *Smlr1* reduces plasma lipids and increases liver lipids

Two weeks after virus administration, total cholesterol levels were significantly reduced in the LKD group compared to controls (Figure 3, panel A). Lipoprotein cholesterol profiling of pooled plasma samples (Figure 3, panel B) revealed reduction of cholesterol in both LDL as well as high-density lipoprotein (HDL). Figure 3 panel C shows that reductions in triglyceride levels were observed at the end of the experiment (t=8).

At sacrifice, we identified increased liver weight and liver weight over body weight (Figure 4, panels A and B) with significant increases of hepatic cholesterol (panel C; $p<0.0001$) and hepatic triglycerides (panel D; $p<0.0001$). In line, hepatic cryosections showed increased neutral lipid accumulation (Figure 4, panel E).

Hepatic downregulation of *Smlr1* attenuates hepatic VLDL production

Next, we investigated whether reductions in plasma cholesterol levels and triglycerides observed in the first experiment were a consequence
5 of reduced VLDL production. To this purpose, a similar liver-specific *Smrl1* knock-down experiment as described above was carried out in female mice (n=7 per group). As in male mice, we observed a significant reduction in plasma cholesterol at 2 weeks following virus administration (Figure 5, Panel A) but in contrast to the males at week 2 also a reduction in plasma
10 triglycerides (Figure 5, Panel B) was observed albeit in pooled plasma samples.

To block hydrolysis of plasma VLDL triglycerides, we injected poloxamer at 5 weeks following virus administration. Figure 5, panel C shows a significant 55% reduction of plasma triglycerides ($p < 0.001$) over the 4
15 hours following poloxamer injection. Figure 5, panel D validates that the effects observed were found in the context of an efficient 77% knock down of *Smlr1* mRNA levels. As in male mice, we did not identify changes in body weight gain or food intake over the course of the experiment (data not shown).

CLAIMS

1. A modulator of the Small leucine-rich protein 1 (human) (SMLR1) gene or gene product, for use as a medicament.

5

2. The modulator of claim 1, for use in a method of treating a disease related to lipid metabolism, preferably wherein said disease is ASCVD, hypercholesterolemia, hypertriglyceridemia, statin-intolerance and/or cancer related to perturbed lipid metabolism.

10

3. The modulator of claim 1 or 2, wherein the SMLR1 modulator is an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense DNA or RNA.

15

4. The use of Small leucine-rich protein 1 (SMLR1) gene, or a SMLR1 gene product, as target in a method of modulating lipid metabolism in a subject.

20 5. A method of modulating lipid metabolism in a subject, comprising targeting the Small leucine-rich protein 1 (SMLR1) gene or a SMLR1 gene product.

6. The use or method according to claim 4 or 5, wherein said

25 targeting comprises organ-specific SMLR1 targeting, preferably liver- or intestinal-specific SMLR1 targeting.

7. The modulator, use or method according to any one of claims 1-5, wherein modulating lipid metabolism comprises modulating one or more of

30 (a) plasma total cholesterol (TC) level,

(b) plasma triglyceride (TG) level,

(c) plasma low-density lipoprotein (LDL) level,

(d) plasma low density lipoprotein (VLDL) level
(e) VLDL assembly, synthesis and/or secretion
(f) chylomicron assembly, synthesis and/or secretion
(g) hepatic cholesterol
5 (h) hepatic triglycerides.

8. The modulator, use or method according to any one of the preceding claims, wherein the subject is a human subject, preferably wherein said human subject is a heterozygote or a homozygote for familial
10 hypercholesterolemia.

9. The modulator, use or method according to claim 8, wherein the human subject is at high risk of developing or known to be suffering from ASCVD, hypercholesterolemia, hypertriglyceridemia, statin-intolerance
15 and/or cancer related to perturbed lipid metabolism.

10. The modulator according to any one of claims 1-3 and 7-9, wherein the modulator is an SMLR1-inhibitor capable of down-regulating or inhibiting SMLR1 expression and/or SMLR1 (protein) activity.
20

11. The SMLR1-inhibitor according to claim 10, capable of down-regulation or inhibition of SMLR1 in the liver.

12. The SMLR1-inhibitor according to claim 10 or 11, wherein
25 administration of at least one dose of the SMLR1 inhibitor results in a reduction in one or more of:
(a) plasma total cholesterol (TC) level,
(b) plasma triglyceride (TG) levels,
(c) plasma low-density lipoprotein (LDL) levels,
30 (d) plasma low-density lipoprotein (VLDL) levels,
(e) VLDL assembly, synthesis and/or secretion,
(f) chylomicron assembly, synthesis and/or secretion.

13. The SMLR1-inhibitor according to any one of claims 10-12, wherein the SMLR1 inhibitor is or comprises a peptide, a peptidomimetic, a small molecule inhibitor, an inhibitory (antisense) oligonucleotide, or a polypeptide molecule.

5

14. The SMLR1-inhibitor according to claim 13, being an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, guide RNA (gRNA), siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense DNA or RNA.

10

15. The SMLR1-inhibitor according to claim 14, being a guide RNA (gRNA) or siRNA targeting SMLR-1, such as exon-1 of human SMLR-1, preferably wherein said gRNA or siRNA is comprised in a viral vector, more preferably in an adeno-associated virus (AAV) vector.

15

16. The SMLR1-inhibitor according to any one of claims 10-15, wherein the SMLR1 inhibitor is used in combination with one or more further modulator(s) of lipid metabolism.

20 17. The SMLR1-inhibitor according to claim 16, which is used in combination with a downregulator of ANGPTL3, APOB, GPR146 and/or MTTP.

18. The modulator, use or method according to any one of claims 1-9,
25 wherein modulating or targeting SMLR1 comprises up-regulating SMLR1 expression and/or activity in a subject, preferably up-regulating SMLR1 expression and/or activity in the liver of a subject.

19. The modulator, use or method according to claim 18, wherein the
30 subject is at high risk of developing or known to be suffering from non-alcoholic fatty liver disease (NAFLD) and/or hypobetalipoproteinemias (HBL).

20. A pharmaceutical composition comprising a modulator of the Small leucine-rich protein 1 (human) (SMLR1) gene or gene product, and a pharmaceutically acceptable carrier, vehicle or diluent.

5 21. The pharmaceutical composition according to claim 20, wherein the modulator is an SMLR1-inhibitor capable of down-regulating or inhibiting SMLR1 expression and/or SMLR1 activity, preferably wherein the SMLR1-inhibitor is capable of down-regulation or inhibition of SMLR1 in the liver.

10

22. The pharmaceutical composition according to claim 20 or 21, wherein the SMLR1 modulator is an inhibitory oligonucleotide selected from an isolated or synthetic antisense RNA or DNA, guideRNA (gRNA), siRNA or siDNA, miRNA, miRNA mimics, shRNA or DNA and chimeric antisense 15 DNA or RNA.

23. The pharmaceutical composition according to claim 22, comprising siRNA or a gRNA targeting SMLR-1, such as exon-1 of human SMLR-1, preferably wherein said siRNA or gRNA is comprised in a viral 20 vector, more preferably in an adeno-associated virus (AAV) vector.

24. The pharmaceutical composition according to any one of claims 20-23, comprising one or more further modulator(s) of lipid metabolism, preferably comprising a downregulator of ANGPTL3, APOB, GPR146 and/or 25 MTTP.

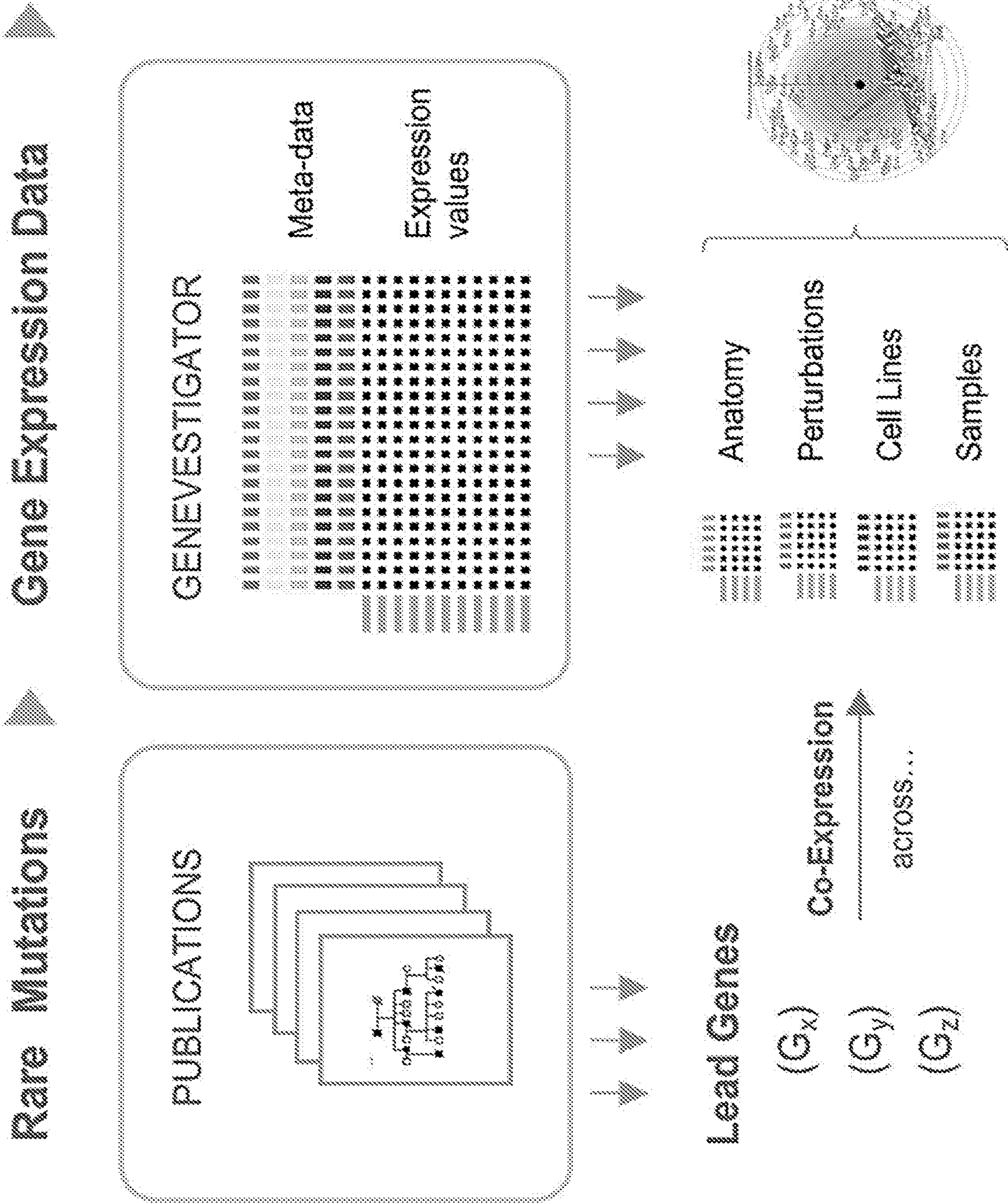

Fig. 1

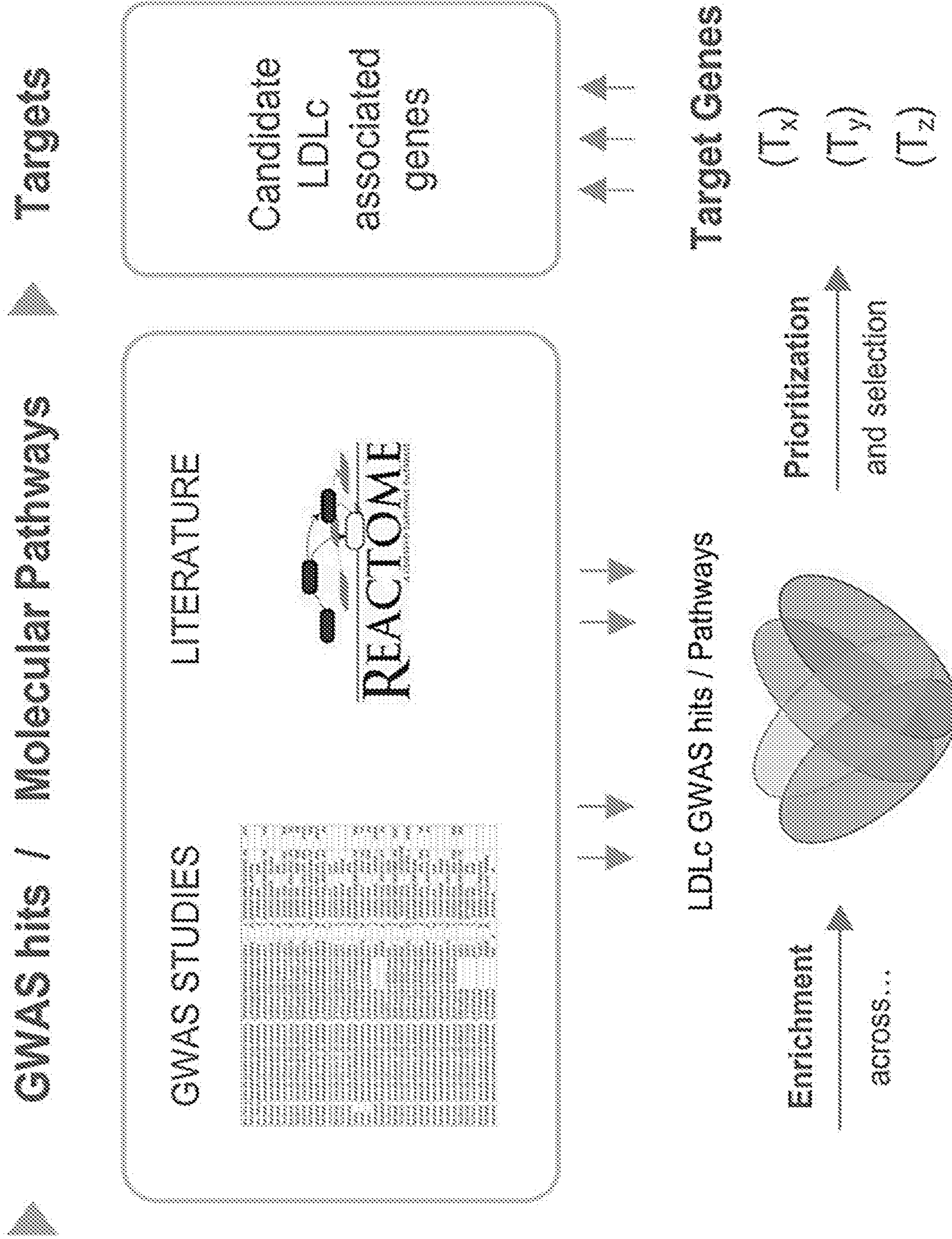

Fig. 1, Cont'd

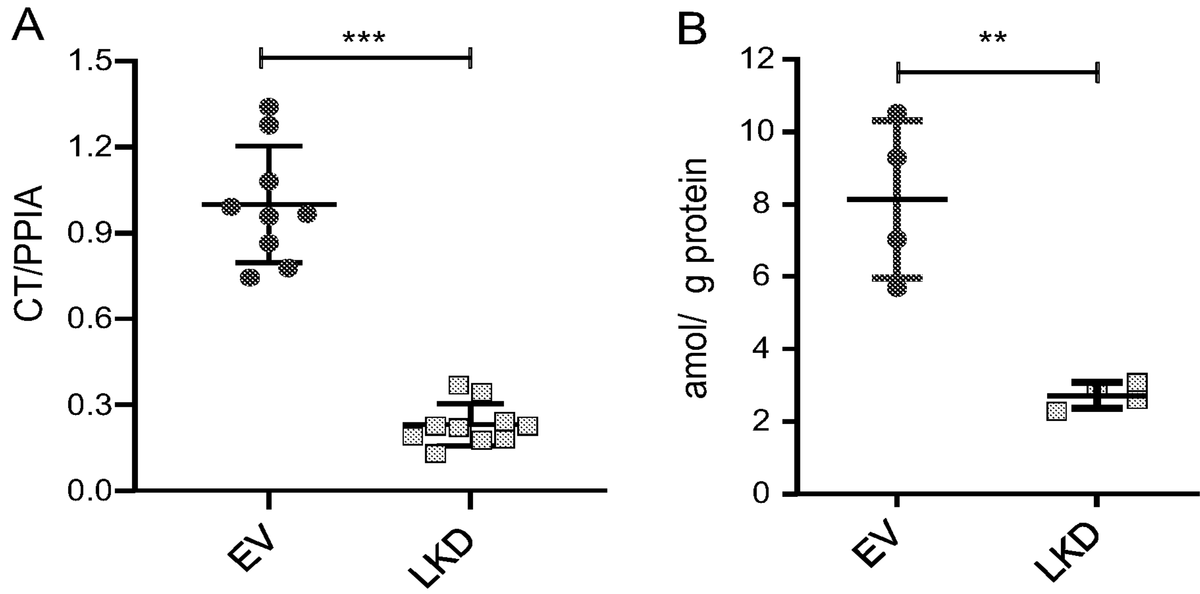

Fig. 2

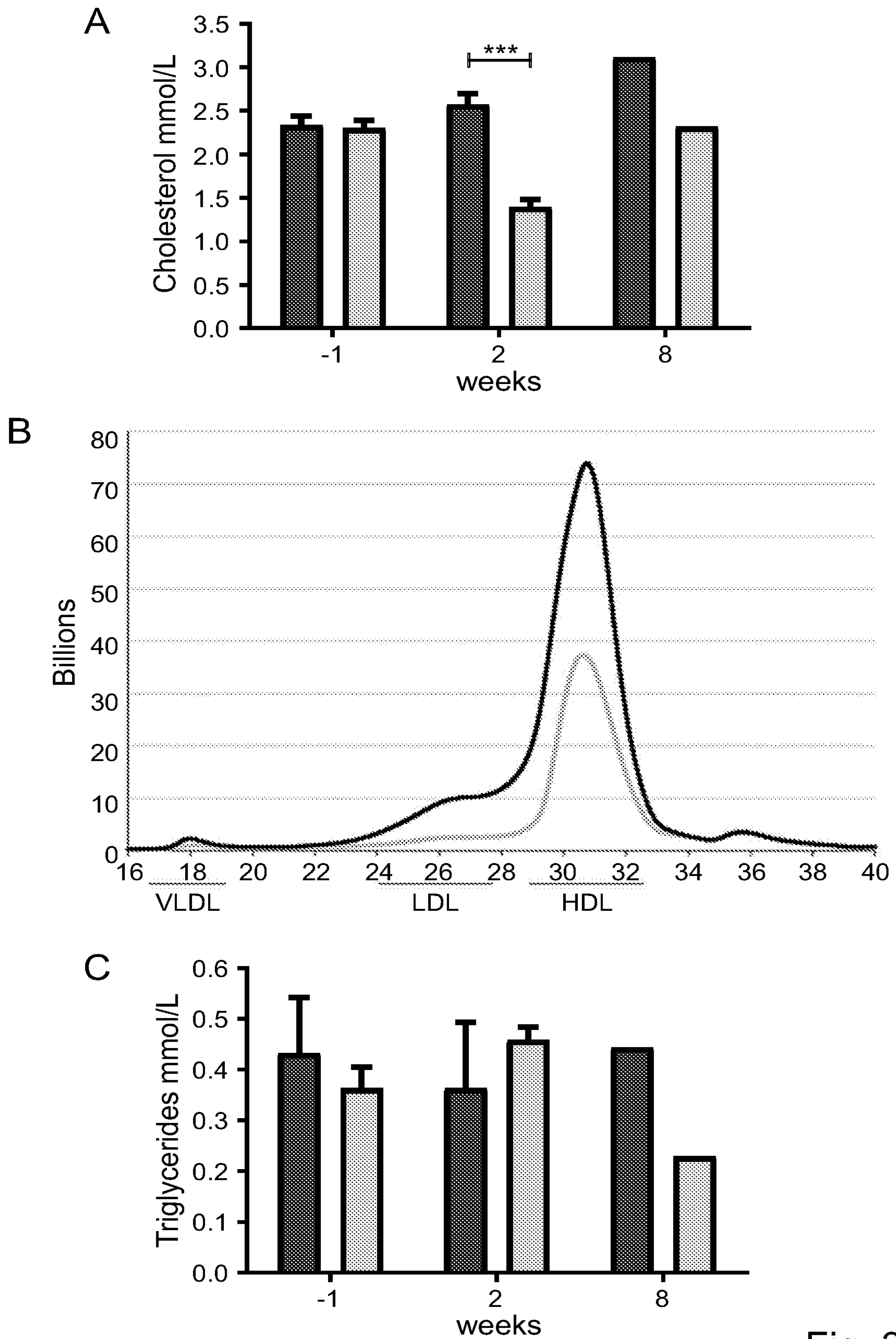

Fig. 3

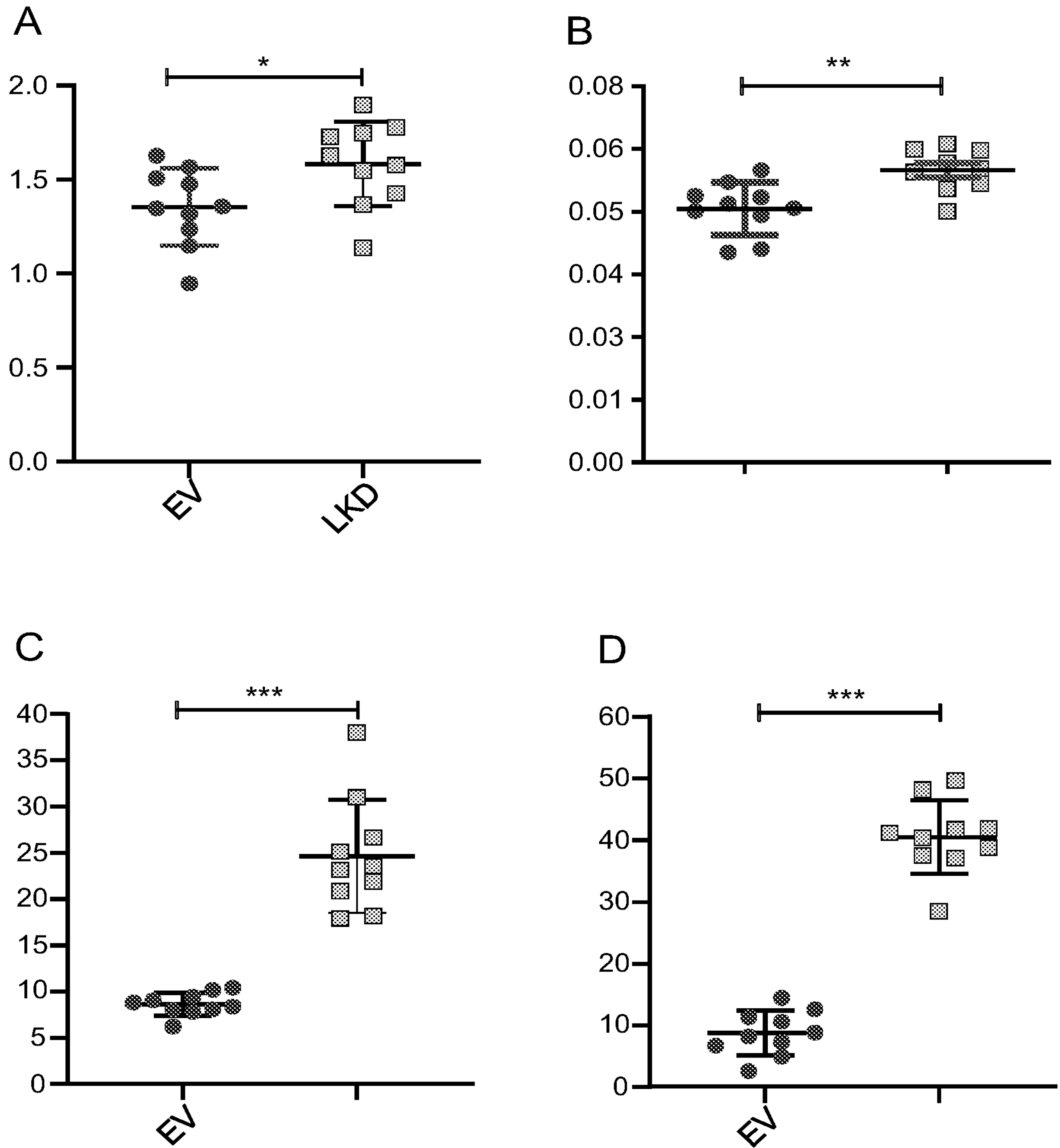

Fig. 4

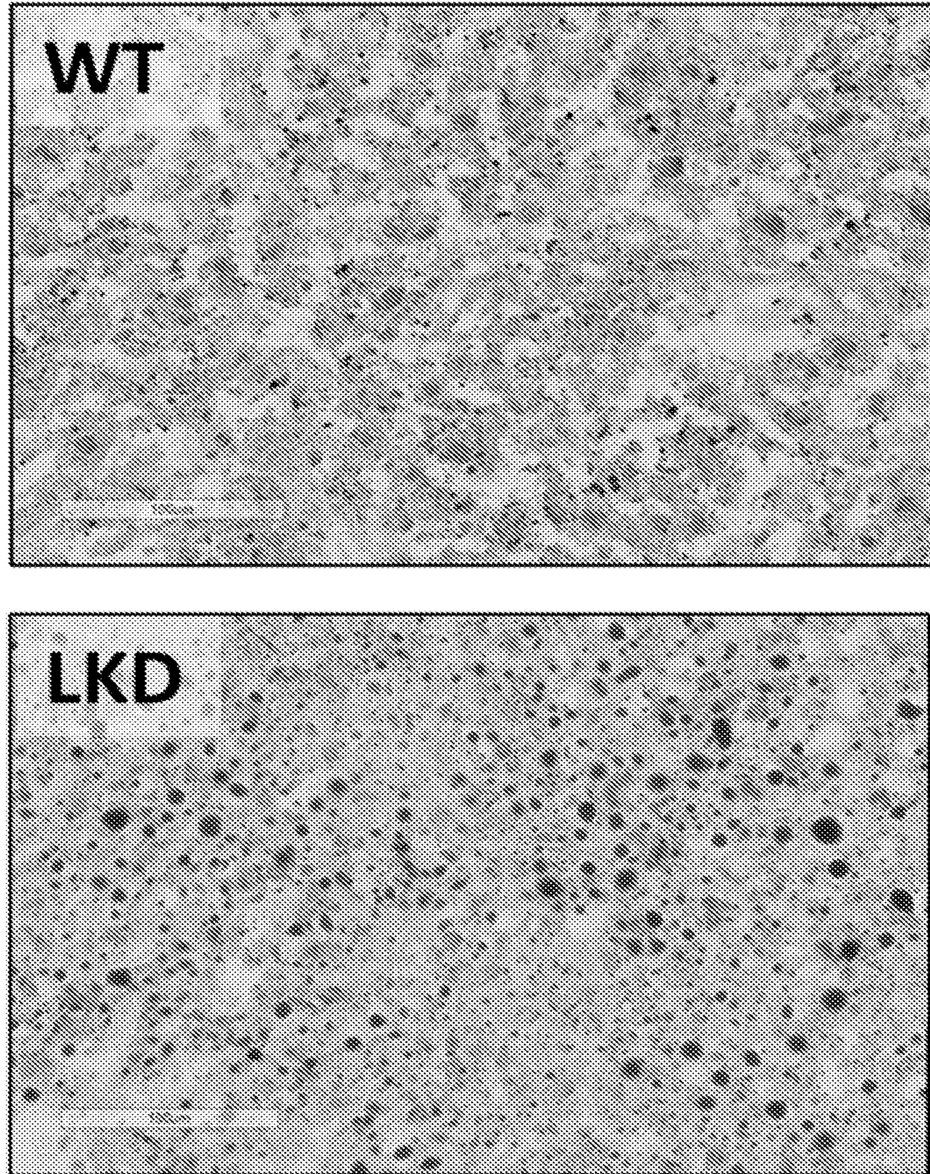

Fig. 4E

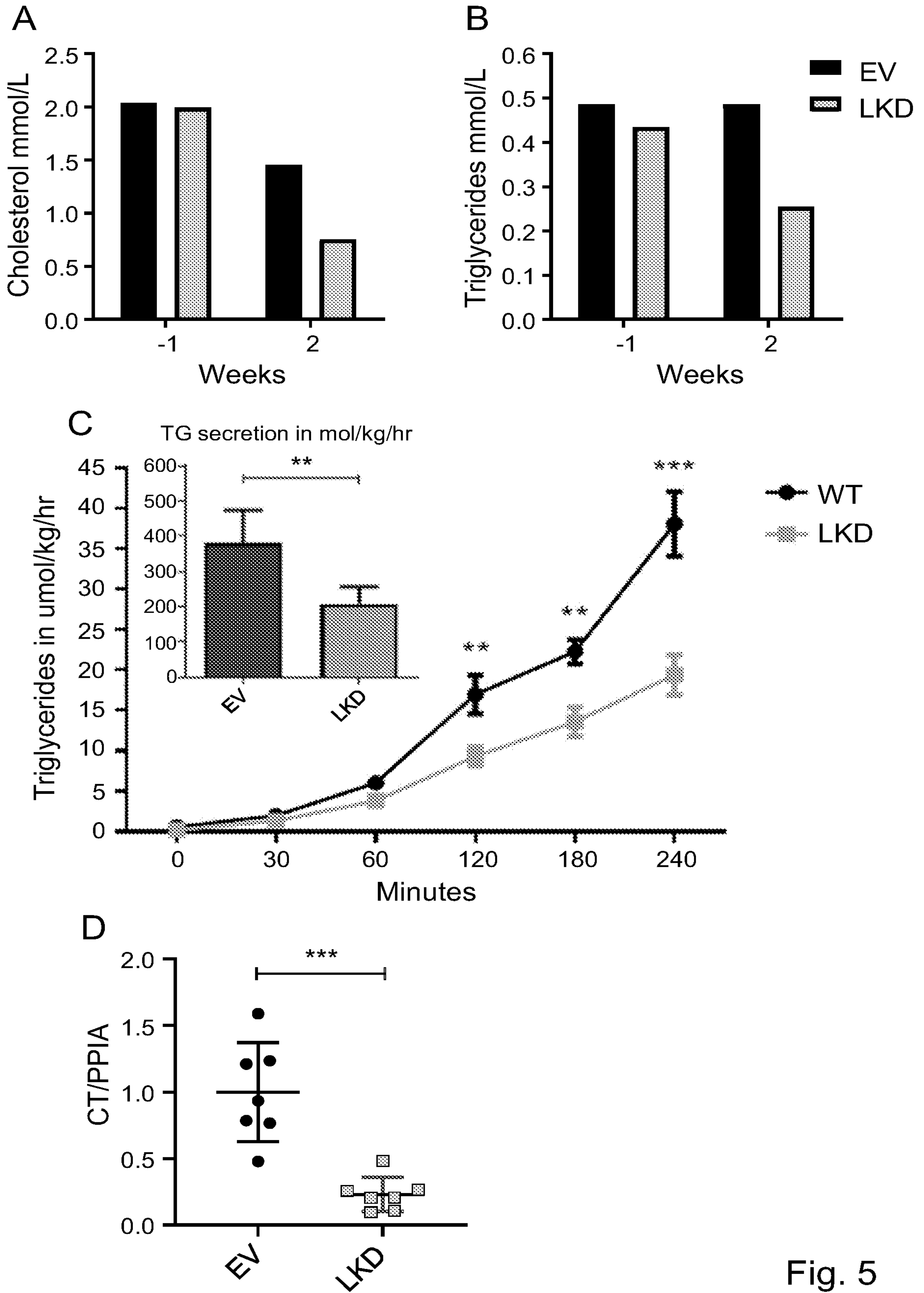

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2021/050057

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/113 A61K31/7088
ADD. A61P3/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, BIOSIS, Sequence Search, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	Anonymous: "SMLR1 - Anti-SMLR1 Antibodies, shRNA, siRNA & Gene Information", Sigma-Aldrich Catalog, 2020, XP055711127, Retrieved from the Internet: URL:https://www.sigmaaldrich.com/catalog/genes/SMLR1?lang=en ion=US [retrieved on 2020-07-02] the whole document -----	1-14, 16-22,24
A	WO 2017/189813 A1 (REGENERON PHARMA [US]) 2 November 2017 (2017-11-02) cited in the application the whole document -----	1-24

-/--

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 April 2021	06/05/2021

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Andres, Serge

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	ROBERT A. HEGELE ET AL: "Lipid-Lowering Agents : Targets Beyond PCSK9", CIRCULATION RESEARCH, vol. 124, no. 3, February 2019 (2019-02), pages 386-404, XP055711295, US ISSN: 0009-7330, DOI: 10.1161/CIRCRESAHA.118.313171 cited in the application the whole document -----	1-24
A	ZWOL ET AL: "The Future of Lipid-lowering Therapy", JOURNAL OF CLINICAL MEDICINE, vol. 8, no. 7, 23 July 2019 (2019-07-23), pages 1085/1-1085/16, XP055711000, DOI: 10.3390/jcm8071085 cited in the application the whole document -----	1-24

INTERNATIONAL SEARCH REPORT

International application No.
PCT/NL2021/050057

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2021/050057

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2017189813 A1	02-11-2017	AU 2017258105 A1	22-11-2018
		CA 3021884 A1	02-11-2017
		CN 109069641 A	21-12-2018
		EP 3448426 A1	06-03-2019
		JP 2019514907 A	06-06-2019
		KR 20190003679 A	09-01-2019
		US 2017312359 A1	02-11-2017
		US 2020061189 A1	27-02-2020
		WO 2017189813 A1	02-11-2017
		ZA 201806557 B	31-07-2019