Clinical heterogeneity in monogenic chylomicronaemia

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SUMMARY
Chylomicronaemia accompanies hypertriglyceridaemia, usually due to a polygenic predisposition in combination with secondary risk factors. Monogenic chylomicronaemia represents a small subgroup of patients with hypertriglyceridaemia. This article describes three patients and illustrates the heterogeneity in the presentation of monogenic chylomicronaemia. The first case is a man with mild hypertriglyceridaemia who is a compound heterozygote for two variants in the LMF1 gene, without relevant medical history. The second case is a woman who is a double heterozygote of variants in the LPL and APOA5 genes. She experienced pancreatitis. The third case is a man, with recurrent pancreatitis attributed to severe hypertriglyceridaemia and homozygous for a variant in the APOC2 gene. This article highlights that in patients with hypertriglyceridaemia, the absence of pancreatitis or the presence of mild hypertriglyceridaemia does not exclude monogenic chylomicronaemia. Genetic screening should be considered in patients with unexplained or severe hypertriglyceridaemia, to determine appropriate treatment and follow-up.

BACKGROUND
Triglycerides (TGs) are transported through the blood in lipoproteins for distribution to muscle and adipose tissue. Hypertriglyceridaemia is defined as fasting plasma TGs >2.0 mmol/L and severe hypertriglyceridaemia as fasting TGs >10 mmol/L.1 TGs from the diet are incorporated in chylomicrons and the liver secretes TG in very low-density lipoproteins (VLDLs). These lipoproteins and their lipolytic remnants that vary in size and density are collectively called triglyceride-rich lipoproteins (TRLs). Chylomicrons are the largest lipoproteins with the highest TG content, but are cleared from the circulation rapidly because TGs are efficiently removed by lipolysis that processes chylomicrons and VLDL to remnant lipoproteins. Severe hypertriglyceridaemia is usually caused by the pathological presence of chylomicrons in the fasting state.2 Both mild to moderately (2.0–9.9 mmol/L) and severely increased TGs are in most cases caused by a polygenic predisposition in combination with common secondary causes of increased TG such as insulin resistance, uncontrolled diabetes mellitus, hypothyroidism, alcohol use and nephrotic syndrome.1 3 Although the vast majority of patients with severe hypertriglyceridaemia have a polygenic background, a monogenic cause resulting in a deficiency in lipolysis explains about 1%-2% of cases.

Lipolysis is performed by lipoprotein lipase (LPL) and related factors. Monogenic chylomicronaemia (formerly known as hyperlipoproteinaemia type I or familial chylomicronaemia syndrome) is caused by homozygous or compound heterozygous loss-of-function variants in each of genes coding for proteins involved in the lipolysis of TGs.

CASE PRESENTATION
Case 1
A man in his 50s was referred to the vascular medicine outpatient clinic for evaluation of hypertriglyceridaemia. The patient contacted his general practitioner because of concern about his cardiovascular risk profile. His medical history was unremarkable, he had no symptoms and took no medication. He had been smoking for 8 years and consumed five alcoholic beverages per week. Physical examination revealed no abnormalities, except for a body mass index (BMI) of 28.6 kg/m² and mild hypertension. There were no eruptive xanthomata. The patient’s fasting lipid profile was: TG 10.2 mmol/L, total cholesterol (TC) 7.6 mmol/L, low-density lipoprotein cholesterol (LDL-C) 4.5 mmol/L and high-density lipoprotein cholesterol (HDL-C) 1.0 mmol/L.

Case 2
The case of a woman in her 40s was published previously.4 In short, she had no remarkable medical history, had a BMI of 29.7 kg/m² and used an oral contraceptive (ethinyloestradiol/drospirenone 20 µg/3 mg) and ezetimibe 10 mg once daily. She did not consume alcohol. She presented to the emergency unit with pancreatitis. Because of haemodynamic instability, she was admitted to the intensive care unit (ICU), where she developed pneumonia and epiglottitis. A biliary cause of pancreatitis was excluded by abdominal ultrasound. Her maximum TG level was 28 mmol/L.

Case 3
A man in his 20s, known to have visual impairment and consanguinity (his parents are cousins), was hospitalised six times with recurrent pancreatitis during a period of 2 years, two of which led to admission to the ICU. He was not using any medication and never consumed alcohol. His BMI was 25.8 kg/m². On ultrasound, his bile duct system was normal. Imaging during the second episode showed a severe necrotising pancreatitis with disruption of the pancreatic duct, which most likely also contributed to the recurrent episodes. On first admission,
INVESTIGATIONS

Case 1
Secondary factors of hypertriglyceridaemia were excluded, including type 2 diabetes mellitus (T2DM) (glucose 5.8 mmol/L and HbA1c 34 mmol/mmol), hypothyroidism (thyroid-stimulating hormone (TSH) level 0.58 mU/L) and nephrotic syndrome (no proteinuria). Familial dysbeta1ipoproteinemia (FD) was evaluated by a non-HDL-C/Apolipoprotein B (ApoB) ratio of 4.23 mmol/g (>3.69 is suggestive of FD), but FD was ruled out by sequencing his APOE gene, which revealed an ε2ε3 genotype without any other pathogenic variants in his APOE gene. Next-generation sequencing (NGS) showed two missense variants in the LMF1 gene: (c.1351C>T; p.Arg451Trp) and (c.41C>G; p.Ser14Trp), confirming the diagnosis of monogenic chylomicronaemia. Furthermore, a post-heparin lipase test was performed. The intravenous injection of heparin leads to the release of LPL from the endothelium. In the normal situation, all LPL molecules become highly active leading to increased lipolysis and consequently a reduction in TG. A post-heparin test for the evaluation of LPL activity showed a TG reduction of 22% (figure 1). The patient underwent preventive cardiovascular screening. CT imaging revealed no coronary calcifications (Agatston score 0). Sonography of the carotid arteries and abdomen as well as an ECG was normal.

Case 2
Besides being overweight and taking the oral contraceptive, there were no other relevant potential secondary causes of hypertriglyceridaemia. She had an ε2ε3 genotype, ruling out FD. Genetic testing showed a pathogenic heterozygous variant in her LPL gene (c.173C>G, p.Pro58Arg) and a pathogenic heterozygous variant in her APOA5 gene (c.161+5G>C). A post-heparin LPL test showed a reduction in TG of only 6% (figure 1).

Case 3
There were no secondary causes of hypertriglyceridaemia: TSH was normal (1.7 mU/L), there was no proteinuria, the non-fasting glucose concentration was 7.4 mmol/L but insulin resistance was unlikely (HbA1c levels 38 mmol/mol). A genetic cause was suspected because secondary causes were absent. NGS showed a homozygous variant in his APOC2 gene (c.245T>G, p.Met82Arg). A post-heparin LPL test while on lipid-lowering medication including fibrates showed a reasonable reduction in TG of 20% (figure 1).

DIFFERENTIAL DIAGNOSIS

Typically, hypertriglyceridaemia is caused by a polygenic background in combination with secondary factors including T2DM, metabolic syndrome, abdominal obesity, polycystic ovary syndrome, nephrotic syndrome, end-stage kidney disease or haemodialysis, alcohol use, pregnancy, hypothyroidism or specific medication such as steroids (oestrogens and glucocorticoids), antipsychotic medication or antiretroviral medication. A very fatty diet may also unmask impaired lipolysis. Hypertriglyceridaemia may also result from genetic disorders, including FD and monogenic chylomicronaemia, which is defined by the presence of variants in genes related to TG lipolysis.

TREATMENT

Case 1
At first, rosuvastatin 10 mg once daily was started, but because of side effects (muscle complaints), the dose was lowered to 5 mg once daily. The patient stopped smoking and a low-fat diet was advised by a dietitian.

Case 2
A fat-free diet was advised by a dietitian and gemfibrozil 600 mg two times per day was prescribed. Also, oral contraceptives were discontinued.

Case 3
A very low-fat diet, bezafibrate 400 mg once daily and rosuvastatin 20 mg once daily were prescribed to keep TGs below 8.0 mmol/L.

OUTCOME AND FOLLOW-UP

Case 1
One year later, still on treatment with 5 mg of rosuvastatin, his lipid profile was: TG 4.5 mmol/L, TC 5.3 mmol/L, LDL-C 1.8 mmol/L, HDL-C 1.47 mmol/L, ApoB 1.12 g/L, Lp(a) <30 mg/L. His BMI remained around 29 kg/m². Cascade screening of his father without cardiovascular disease (CVD) until he was in his 80s showed a normal lipid profile (TG
0.91 mmol/L, LDL-C 1.69 mmol/L, HDL-C 1.82 mmol/L, ApoB 0.7 g/L, Lp(a) 171 mg/L). Genetic testing revealed the p.Ser14Trp variant in the father’s LMF1 gene. Approximately 1 year after the genetic screening, the father died due to complications of Parkinson’s disease. The mother of the patient had died from a brain tumour in her 60s, but had no CVD. Her lipid values were unknown. Due to heterozygosity for the variant in the LMF1 gene in the father, the mother was most likely a carrier of the p.Arg451Trp variant. Genetic testing in the patient’s brother, who had no history of CVD or dyslipidaemia (TG 1.7 mmol/L, TC 6.0 mmol/L, LDL-C 3.5 mmol/L, HDL-C 1.68 mmol/L), showed none of the variants in the LMF1 gene. The pedigree of the family is shown in figure 2.

**Case 2**

Gemfibrozil was discontinued due to hair loss (a known side effect of fibrates). With a strict diet alone, her TGs were stable around 4.0–5.0 mmol/L. The mother of the index patient was referred to the vascular department for assessment as she had dyslipidaemia for about 25 years, for which she received several statins and gemfibrozil of which all caused severe muscle complaints. She had no other relevant medical history and took barnidipine 10 mg once daily for hypertension. Her BMI was 30.9 kg/m² and no clinical stigmata of dyslipidaemia were found on physical examination. Her lipid profile showed a mixed hyperlipidaemia: TG 9.4 mmol/L, TC 9.9 mmol/L, HDL-C 0.7 mmol/L, non-HDL-C 9.2 mmol/L and directly measured LDL-C of 3.2 mmol/L. Genetic analysis found both variants in her LPL and APOA5 genes. She was already following a low-fat diet. Ezetimibe 10 mg once daily was initiated, she started using fish oil (over the counter) and continued her low-fat diet with the help of a dietitian. After this, her lipid levels were: TG 4.2 mmol/L, TC 5.7 mmol/L, HDL-C 0.8 mmol/L, non-HDL-C 4.9 mmol/L and LDL-C 3.0 mmol/L.

**Case 3**

With the interventions, his TGs stabilised between 5.0 and 6.0 mmol/L. Other lipids were remarkably low (TC 2.4 mmol/L, ApoB 0.39 g/L, HDL-C <0.5 mmol/L, direct LDL-C 0.3 mmol/L).

**DISCUSSION**

Severe hypertriglyceridaemia is often associated with impaired lipolysis, which is the process in which TGs are lipolysed to free-fatty acids and glycerol. The key protein responsible for intravascular lipolysis is LPL, with its lipolytic function being co-regulated by other proteins, such as apolipoprotein C2 (ApoC2), apolipoprotein A5 (ApoA5), glycosylphosphatidylinositol-anchored HDL-binding protein 1 (GPIHBP1) and lipase maturation factor 1 (LMF1). Variants in genes coding for these proteins influence plasma TG concentration powerfully and critically when both alleles carry pathogenic variants.

The cause of chylomicronaemia is in most cases polygenic, usually caused by clustering of common genetic variants, or heterozygosity for one of the genes involved in LPL-mediated lipolysis, in combination with lifestyle factors. Monogenic chylomicronaemia is rare with an estimated prevalence of 1–10 per 1 million persons in the general population.

LPL is expressed and located in tissues that oxidise free-fatty acids as an energy source (heart and skeletal muscle) or store fatty acids (brown and white adipose tissue). ApoC2, encoded by the APOC2 gene, is present on TRLDs and HDL and acts as an activator for LPL activity. Biallelic variants in APOC2 cause a lipoprotein phenotype indistinguishable from homozygous LPL deficiency. ApoA5, encoded by the APOA5 gene, stabilises the
LPL enzyme complex and thereby promotes lipolysis.\(^8\) GPIHBP1, codes for the endothelial protein GPIHBP1, transports secreted LPL from the parenchymal cells to the endothelial cell surface, where lipolysis takes place.\(^2,10\) Finally, the LMF1 gene encodes for the LMF1 protein, which assists maturation of LPL and hepatic lipase (HL). The LMF1 protein is a membrane-bound chaperone protein located in the endoplasmic reticulum and responsible for the post-translational maturation of nascent lipase polypeptides.\(^3\) Proper lipase maturation involves the glycosylation, folding and assembly of these polypeptides and stabilisation of the active dimeric lipases to fully active enzymes.\(^3,12\) LMF1 deficiency is therefore associated with a lipase deficiency that affects both LPL and HL function.\(^3\) Other proteins that are involved in TG metabolism are angiopoietin-like protein (ANGPTL) 3 and apolipoprotein C3 (ApoC3). Both inhibit LPL activity and thereby lipolysis of TGs.\(^2\)

Almost 95% of patients with monogenic chylomicronaemia have pathogenic variants in the LPL gene, leading to partial or complete loss of LPL activity and a small minority have pathogenic variants in the other four genes.\(^3\) Monogenic chylomicronemias are primarily associated with accumulation of TGs in large chylomicrons, as deficiency in LPL-mediated lipolysis of TGs prevents conversion of chylomicrons to smaller lipoproteins. Severe chylomicronaemia can be asymptomatic but manifestations may begin at an early age and include failure to thrive, eruptive xanthomas, lipemia retinalis and gastrointestinal manifestations such as hepatosplenomegaly, and in particular acute pancreatitis, which can be life-threatening.\(^3,12\) Hypertriglyceridaemia-related pancreatitis is thought to be initiated by the release of free-fatty acids after partial lipolysis of lipoproteins that prematurely activate trypsinogen, leading to auto-digestion of the pancreas.\(^2,14\) Hypertriglyceridaemia from a monogenic cause is usually not associated with CVD since chylomicrons contain little cholesterol and do not penetrate the arterial wall to cause atherosclerosis.\(^2,13\) In contrast, if the same degree of hypertriglyceridaemia would have been due to polygenic causes, smaller, cholesterol-rich, pro-atherogenic TRLs would be present because lipolysis is not completely disrupted.\(^13,15\) Consequently, polygenic hypertriglyceridaemia is associated with atherosclerosis and CVD, in contrast to monogenic hypertriglyceridaemia. The most effective and important therapy for severe hypertriglyceridaemia is strict restriction of dietary fat intake, preferably with less than 10% of calories from fat. However, compliance with this type of diet is generally very difficult. Optimal management of lifestyle factors such as obesity and diabetes, and no use of alcohol or medication known to increase TG (such as oestrogens, steroids or atypical antipsychotic drugs) is also essential.\(^2,13\) In addition, statins, fibrates and high dose of omega-3 fatty acids are often used in polygenic hypertriglyceridaemia. However, these drugs are generally not effective enough to reduce TGs to safe levels in patients with monogenic chylomicronaemia, because their effectiveness depends primarily on the presence of a lipolytic pathway. Statins do not add any value in the treatment of monogenic chylomicronaemia, since they generally only lower LDL-C concentration. In addition, in specific subgroups, the pancreatic lipase inhibitor orlistat, lomitapide or plasmapheresis could be an option.\(^13\) New therapies targeting ApoC3 and ANGPTL3 are being developed with the aim of specifically lowering TGs in patients without LPL activity.\(^2,1\) Also, transfusion of human plasma can provide normal ApoC2 to improve lipolysis of TRL to expedite control of hypertriglyceridaemia.

As illustrated from the three cases presented, there is heterogeneity in the clinical presentation of monogenic chylomicronaemia. The patient in case 3, with a homozygous variant in APOC2, had a severe clinical presentation with severe hypertriglyceridaemia and life-threatening pancreatitis compared with the patient in case 1, who had a mild hypertriglyceridaemia and compound heterozygous variants in LMF1. This is in line with other case reports about chylomicronaemia in which patients with variants in the APOC2 gene are generally younger at diagnosis, due to serious clinical manifestations such as failure to thrive or pancreatitis, than patients with pathogenic variants in their LMF1 gene, who are generally diagnosed later in adulthood.\(^5\) The dietary fat intake was not specifically evaluated in three cases but could have an influence on the risk of pancreatitis.

Another explanation for the difference in clinical presentation is the fact that the patient from case 3 was homozygous for the variant, the patient from case 2 was heterozygous for two different LPL-related genes and the patient from case 1 was a compound heterozygote. Homozygous patients usually have the most severe phenotype, while (compound) heterozygous variants usually lead to a milder phenotype, because in the latter some lipolysis is still possible.

Not all patients with monogenic chylomicronaemia develop or present with pancreatitis. Case 3 (TG up to 66 mmol/L) and case 2 (TG up to 28 mmol/L) developed severe pancreatitis. The risk of pancreatitis increases when TG levels exceed 10 mmol/L, and increases strongly when TG levels exceed 20 mmol/L,\(^13\) which was the case in both patients.

Regarding CVD risk, none of the patients from the three family cases had CVD or other classical signs of atherosclerosis. As mentioned previously, monogenic chylomicronaemia is generally not associated with CVD, in contrast to polygenic chylomicronaemia. The fact that patient 1 himself was free of atherosclerosis up to this point could be explained by the presence of another pathogenic variant in his LMF1 gene leading to (almost) complete loss of LPL activity and therefore to larger lipoproteins that are generally less atherogenic. The extra pathogenic variant could therefore be protective of CVD, although exposing the patient to a high pancreatitis risk.

In summary, monogenic chylomicronaemia is a group of rare genetic disorders associated with (severe) hypertriglyceridaemia caused by variants in several genes associated with LPL metabolism. Clinical presentation and prognosis can vary widely among patients depending on the gene involved, the number of variants (ie, homozygous, compound heterozygous, heterozygous) and the presence of other risk factors. This article highlights that in patients with hypertriglyceridaemia, the absence of pancreatitis or the presence of mild hypertriglyceridaemia does not exclude

Learning points

- Hypertriglyceridaemia is most often polygenic in nature in combination with lifestyle factors.
- A small proportion of cases is due to monogenic chylomicronaemia, which is caused by specific variants in genes associated with triglyceride metabolism.
- The clinical presentation of monogenic chylomicronaemia can vary significantly, even within families, as a consequence of genetic and non-genetic factors.
- In patients with hypertriglyceridaemia, the absence of pancreatitis or the presence of mild hypertriglyceridaemia does not exclude monogenic chylomicronaemia.
- Genetic screening should be considered in patients with unexplained or severe hypertriglyceridaemia, to determine appropriate treatment and follow-up.
monogenic chylomicronaemia. Owing to the high risk of pancreatitis, the good response to dietary fat restriction and the relative ineffectiveness of standard TG-lowering medication in monogenic chylomicronaemia, it is important to determine the aetiology of hypertriglyceridaemia. Genetic screening should be considered in patients with unexplained or severe hypertriglyceridaemia, to determine appropriate treatment and follow-up.

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Case reports provide a valuable learning resource for the scientific community and can indicate areas of interest for future research. They should not be used in isolation to guide treatment choices or public health policy.

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