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HYPERTRIGLYCERIDEMIA: THE FUTURE OF GENETICS TO GUIDE INDIVIDUALIZED THERAPEUTIC STRATEGIES

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Accepted Clinical Lipidology
The use of genetic information to explore and treat diseases is ever expanding, varying from the use of classical approaches for monogenetic disorders, to the growing genome wide association studies (GWAS) in understanding more complex traits. In hypertriglyceridemia, development has rapidly progressed. We now have seen the use of genetic information by treating monogenetic disorders with gene therapy for the first time being implemented successfully in human subjects. Also, ASO therapy in mice and very recently also in humans has been shown to lower triglyceride levels. In polygenetic disease, the use of large-scale GWAS studies has changed our perception of the underlying phenotypes, showing a large overlap in common genetic determinants. This information is translated to understanding reaction to drug therapy, but also in relation to environment interaction. Finally, the use of genetics in predicting risk of cardiovascular disease is continuously being studied, although application is still a far road ahead.
EXECUTIVE SUMMARY

Clinical hypertriglyceridemia
High plasma triglyceride levels, both fasting and post-prandial, are associated with cardiovascular disease and acute pancreatitis.

TG Metabolism
Triglycerides are derived from dietary sources in the intestine or de novo hepatic synthesis. In the circulation, they are lipolyzed by LPL and used for energy or storage, finally remnants are cleared by the liver.

Classification of hypertriglyceridemia
Hypertriglyceridemia, defined as fasting plasma triglycerides > 200 mg/dl (or > 2.2 mmol/l) can be of secondary origin, due to diabetes or obesity, or primary causes due to genetic loss of LPL function.

Current treatment
A combination of diet, exercise and drugs is currently recommended as the first line treatment of hypertriglyceridemia. Drug classes used are fibrates, nicotinic acid and n3 fatty acids.

Implications of genetics in hypertriglyceridemia
The use of genetic information in hypertriglyceridemia comprises approaches to unravel and treat monogenetic disease, as well as the use of genome wide association studies (GWAS) to understand more complex traits. In the near future whole exome sequencing analysis will provide new insight in the genetic background of hypertriglyceridemia.

Genetic approaches in monogenetic disorders
Both the use of gene therapy and antisense therapy are currently undergoing swift development, leading to treatment options for monogenetic disease currently resistant to all known therapies.

Individualized therapy in polygenetic disorders
The use of genetic information in polygenetic disorders is complex, but has been aptly used to explain differences in reaction to drugs based on different phenotypes. Also, this information is at the base of developing genetic risk scores.

Conclusions
Genetic information in hypertriglyceridemia has been successfully used to explain monogenetic hyperTG disease, and gene therapy has for the first time been implemented in human subjects for this disease. Although the use in polygenetic disease is more complex, ever expanding information has led to a better understanding of these traits and will possibly lead to new approaches in prediction and treatment.
CHAPTER 6

CLINICAL HYPERTRIGLYCERIDEMIA

High plasma levels of triglycerides (TG) have been recognized as an independent risk factor for cardiovascular disease (CVD) since 1998. This association holds true for fasting triglyceride levels, but also for non-fasting levels, where the postprandial remnant lipoproteins are thought to contribute to atherogenesis. An exception are the rare cases of high and severe high triglycerides in the familial chylomicronemia syndrome, where the extreme levels are not clearly linked to an increased risk for the development of CVD, but their role in the onset of acute pancreatitis has since long been recognized and widely accepted.

TG METABOLISM

Triglyceride-rich lipoproteins originate from the intestine (apoB-48 containing chylomicrons), where they transport exogenous derived lipids into the circulation, or from the liver (apoB-100 containing very-low-density lipoprotein (VLDL)). In the intestine, TGs derived from dietary sources are hydrolyzed and the residues (2-monoacylglycerol (2-MG) and fatty acid (FA)) are taken up by enterocytes where they are re-synthesized into TGs by the enzyme acyl-CoA:diacylglycerol acyltransferase 1 (DGAT1). Hereafter, lipidation of apoB48 through microsomal triglyceride transfer protein (MTTP) is the first step into chylomicron formation and eventually, the nascent chylomicrons reach the systemic circulation through the lymphatic system. Triglycerides synthesized in the liver, deriving the required fatty acids from either de novo lipogenesis (DNL) or lipolysis in adipose tissue, are packaged into VLDL in the endoplasmic reticulum (ER), with apoB100 as their main apolipoprotein. Next they are transported to the Golgi apparatus for further lipidation, after which they are excreted by hepatocytes into the bloodstream. Circulating VLDL particles carry additional proteins such as apoCII, apoAV and apoCIII. These proteins function as co-factors for Lipoprotein Lipase (LPL), the protein responsible for the lipolysis that is required to generate free fatty acids (FFA) which can enter peripheral tissues as a source for energy and storage. LPL is synthesized in the parenchymal cells of tissues that require these fatty acids, and lipase maturation factor 1 (LMF1) is essential for folding it into a functional form. LPL is then transported to the endothelial cell surface where glycosyl-phosphatidyl-inositol anchored high-density lipoprotein binding protein 1 (GPIHBP1) serves as a binding platform for LPL to come into proximity with TG particles.

Finally, the tissues take up the newly freed fatty acids, whereas the liver eventually clears the remnant particles. Hepatic remnant clearance appears to be the result of several overlapping mechanisms of independent receptors, including the LDL receptor, the LDL receptor related protein 1 (LRP1), and heparan sulfate proteoglycans (HSPGs).
CLASSIFICATION OF HYPERTRIGLYCERIDEMIA

Hypertriglyceridemia, defined as fasting plasma triglycerides > 200 mg/dl (or > 2.2 mmol/l), can arise from increased TG production, reduced TG catabolism, or a combination hereof. A mild to moderate elevation (200-500 mg/dl or 2.2-5.5 mmol/l) can usually be attributed to secondary causes, including obesity, diabetes mellitus, pregnancy, alcohol and different drugs15, due to several different mechanisms. Adequately distinguishing these and other, primary, causes of high triglyceride plasma levels may prove difficult and understanding of the underlying mechanisms is crucial, as the origin will mainly determine the choice of treatment. For example, an increase in VLDL production may be found as a result of excess flux of fatty acids to the liver, by several causes. The hyperinsulinemia in type 2 diabetes leads to increased de novo lipogenesis20, while hepatic insulin resistance leads to a loss of suppression of VLDL production21 and insulin resistance in adipose tissue leads to a mild disruption of LPL function22. Differentiating between the origins of the triglyceride rich particles is partially possible by determining apoB100 levels. An apoB level > 0.75 g/L indicates a degree of fatty acid delivery to the liver and thus, at least to an extent, functional LPL. The TG:apoB ratio can thus distinguish between chylomicrons and VLDL. Sniderman et al. provided an comprehensive algorithm, where based on apoB100 and TG levels a clear direction is given towards the underlying genetic or secondary cause23.

Traditionally, primary causes of HTG can be divided into several disorders. Already in the 1960s, Fredrickson designed a system in which hyperlipidemias were divided based on their lipoprotein particle24. These phenotypes comprise both the rare monogenetic disorders, where complete loss of function mutations lead to severe elevations in plasma TGs >1000mg/dl (type 1)25 and 4 polygenic familial phenotypes (type II-IV)26.

The use of the Fredrickson Classification has been widely accepted since it’s adoption by the WHO in 197027, and still applies to the cases where (severe) hypertriglyceridemia is caused by a mutation leading to the loss of LPL function. In these cases, a loss of function of LPL is caused by either a loss of function mutation in the LPL gene or a mutation in genes coding for proteins directly involved in LPL activity. Known mutations have been extensively described previously in publications28 (especially see supplementary data) and25. These mutations result in severely high plasma triglyceride levels and, as illustrated above, additional low apoB100, low LDL and HDL levels that are resistant to current lipid-lowering therapies29, with strong restriction of dietary fat as the remaining treatment option30. Those cases are rare, with an estimated prevalence of 1-2·10^(-5). Even in a cohort of pre-selected patients with severe HTG from our hospital, only 54% was identified as having a rare, monogenetic variant as a cause of the HTG, whereas in 26% only common variants were found and no mutation in 21% of the patients. In total, 19 known and 16 novel disease causing mutations were indentified in the LPL, APOC2, APOA5 and GPIHBP125. However, cumulative evidence suggest that most of the different polygenic phenotypes IIB-V (phenotype 2A representing the familial hypercholesterolemia syndrome, often caused by heterozygous LDLR mutations32) share numerous
common genetic determinants. In these patients, lifestyle (dietary intervention) changes remain the initial treatment and although several drugs can be used to lower serum triglyceride levels, little evidence exists on the effectiveness of the different drug classes on reducing cardiovascular risk. Moreover, in these patients differences in genetic background are believed to be associated with the fasting plasma triglyceride response to pharmacological treatment and also with the response to lifestyle interventions.

CURRENT TREATMENT

A recent statement from the Endocrine Society recommends the combination of diet, exercise and drugs as the first line treatment of hypertriglyceridemia. Although no solid data exists on the effect of fibrate (peroxisome proliferator activated receptor agonists, PPAR-alfa) treatment on cardiovascular risk-reduction, these compounds are to be used as first-line treatment for those patients at risk of developing triglyceride induced pancreatitis. Furthermore treatment with niacin and n-3 fatty acids or any combination can be used, although the effect on cardiovascular risk reduction remains unclear.

IMPLICATIONS OF GENETICS IN HYPERTRIGLYCERIDEMIA

The use of genetic information in explaining and treating diverse disease modalities is ever expanding, varying from the use of classical approaches for monogenetic disorders, to the growing genome wide association studies (GWAS) in understanding more complex traits. The clinical relevance of large scale GWAS studies remains a topic of wide discussion and investigation, where some suggest that most common variants found in such studies will be of little biological interest because of their small effect. Still, in 2010 Teslovich et al. discovered 59 new loci associated with blood lipids contributing to 10-12% of the total variance in blood lipids, amongst which some with clear biological and clinical importance. The relevance of the ever expanding available genomic information to our biological understanding of diseases seems quite clear, however, the challenge remains to translate this epidemiological-genetic information to pathophysiology and subsequently the clinic in order to provide new therapeutic opportunities for an individual patient.

The assessment of common genetic variations on plasma TG levels is complicated by the role of gene-environment interactions (GxE). Yet, this may provide the bridge between genetic risk and actual clinical disease in many cases, where the genetic variation only becomes an actual risk when certain environmental or lifestyle factors, such as diet, pass a certain threshold. A special remark should be made to non-fasting triglyceride levels, as this is the state in which humans remain the majority of time. Little information is available on the post-prandial responses and its association with common variations in genes involved in TG metabolism. Of more concern, little validation reports have been published. Based on the available data one might predict that the effect size of genetic variation in the prediction of abnormal postprandial TG excursions will be small. More
research in this field is warranted to adapt approaches specifically to alterations in the post-prandial state.

GENETIC APPROACHES IN MONOGENETIC DISORDERS

When thinking of using genetic information for therapeutic purposes, several approaches can be considered, depending on the nature of the genetic defect. As described previously, in the case of hypertriglyceridemia due to monogenetic loss of function of LPL, current therapies are deemed to be ineffective. Treatment for these subjects would obviously be found with the successful implementation of gene therapy: introducing a competent gene to supplement the dysfunctional one. This is not a novel concept, a symposium took place on May 26 1966, at Columbia University College of Physicians and Surgeons in New York City, entitled “Reflections on Research and the Future of Medicine”\(^46\). Here Edward Tanner already mentions the use of genetic engineering, possibly using viruses, a contribution he later published separately\(^47\). Though there have also been many setbacks since the first clinical trial in 1990\(^48\), advances in this field have been great. In the case of HTG based on an LPL deficiency, an AAV\(^{LPL}\) vector has successfully been introduced in murine\(^49\) and feline\(^50\) models. In humans, the LPL\(^{s447x}\) variant is associated with lower serum TGs, higher HDL-C levels and lower incidence of cardiovascular disease, as well as enhanced ApoB48 clearance\(^51\). Subsequently, AAV\(^{LPL}\) genetherapy, using a plasmid-based\(^52\) or a baculovirus-based production\(^53\), was administrated in LPL deficient subjects with resident LPL mass. This therapy was shown to be well tolerated and (moderately) effective with regard to plasma triglyceride levels. However, even though the advances on biological level in this field are great, these invasive, therapies lack longterm beneficial clinical effects. Although self-reported, clinical improvement has been shown up to 2 years in subjects, the TG-lowering effect so far has not been reported after 12 weeks\(^52,53\). A longer lasting improvement in postprandial chylomicron metabolism has been shown, but small number of subjects makes it difficult to link these results to clinical parameters\(^54\). Also, it should be noted that LPL gene replacement is only effective for complete null mutations in LPL and can not be used for patients with mutations in other genes causing complete loss of function of LPL, such as \(APOC2\ LMF1\), \(APOA5\) or \(GPIHBP1\), and thus is only suitable for a very small group of patients.

With respect to gene therapy focusing on other causative genes, preliminary data show that ApoA5 null mice have elevated levels of triglycerides and that treatment with a sense adenoviral ApoA5 construct can rescue the hypertriglyceridemic phenotype\(^55\). Gene transfer of ApoA5 lead to a marked decrease of 50% in plasma triglycerides\(^55\). However, the mechanism by which ApoAV affects human triglyceride metabolism remains to be elucidated. SNPs in the \(APOA5\) have been linked to hypertriglyceridemia\(^56,54,54\), but controversy remains over the association between ApoAV levels and plasma triglycerides, which have rendered both positive\(^56\) and inverse\(^55\) correlations. Interestingly, in type 2 diabetes a positive correlation was found between apoAV and plasma triglyceride levels, whereas atorvastatin treatment was actually able to reduce both plasma ApoAV levels and
plasma triglycerides. Moreover, analysis in the EPIC-Norfolk cohort showed a significant positive correlation. The differences in these findings may be the direct result of gene-environment interactions, as was shown particularly for carriers of the APOA5 -1131T>C SNP, who have a strong inverse correlation between fat intake and TG levels as opposed to major allele carriers. Thus, until the mechanism by which ApoAV influences human triglyceride metabolism has been further investigated, genetic approaches aiming at altering plasma triglyceride levels seem an unlikely option.

Finally, genetics can also be used to indirectly influence expression of a gene. This can be conceived either by using antisense oligonucleotides (ASOs) or RNAi (RNA interference). One of the targets pursued is ApoCIII, which is thought to delay clearance of TG-rich lipoproteins by inhibiting LPL. Heterozygous carriers of a null mutation in APOC3 have a favorable lipid profile and lower subclinical atherosclerosis in a human population. In mice, overexpression of ApoC3 indeed leads to increased levels of plasma triglycerides, whereas silencing leads to a marked decrease. Interestingly, a recent paper from our group demonstrated a link between GALNT2 mutations and posttranslational modification of ApoCIII, thereby improving LPL mediated lipolysis. In line with these findings, preclinical data suggest that an APOC3 ASO can be used safely to reduce triglyceride levels in mice. Notably, Graham et al. revealed that a second generation APOC3 ASO was capable of lowering plasma TG and VLDL levels in mice and non-human primates. Interestingly, in healthy human subjects APOC3 ASO administration was well-tolerated and showed a dose-dependent apoCIII reductions and a significant lowering of TG levels. It should be noted that normal LPL function is necessary when aiming at apoCIII reduction.

In the case of chylomicron production, the intestinal cell would be the most likely target. Diaglycerol acyltransferase (DGAT) is responsible for the final catalyzation in triglyceride synthesis. In mice, DGAT1 inhibition by oral gavage of DGAT1i (a selective inhibitor of human DGAT1) as well as DGAT1 knockout, will inhibit chylomicron formation and thus lower postprandial triglyceride levels. Recently, the DGAT1 inhibitor LCQ908 was assessed in a very small group of LPL deficient patients, showing decreases in mean fasting triglycerides after 3 weeks of treatment. Currently a randomized, double-blind, placebo trial is running to further evaluate these findings.

INDIVIDUALIZED THERAPY IN POLYGENETIC DISORDERS

Using genetic information in the treatment of polygenetic hypertriglyceridemia seems much more complex. In this respect, two major approaches can be distinguished: Pharmacogenetics and Genetic Risk Prediction. The genetic basis of inter-individual differences in response to pharmaceutical treatment is a field of broad interest. Dating back to the 1950s, when a role for genetics in adverse drug reactions was first suggested. Brisson et al. showed that carriers of a polymorphism in APOE (ApoE2, leading to a dysfunctional ApoE protein) respond more pronounced to treatment with fibrates than non-carriers. Although the ApoE2 allele is mainly associated with type III hypertriglyceridemia, these findings were also reproduced in ApoE2 carriers of subtypes IIB, IV and V, thus suggesting that in these cases response to therapy could be dependent on the genetic
architecture\textsuperscript{29}. Yet, the debated position of triglycerides in the prevention of cardiovascular disease and the lack of therapeutic options make it difficult to implicate that pharmacogenetic findings may lead to identification of novel pathways and possibly the development of more target-specific pharmaceutical products.

Finally, when thinking of individualized medicine, genetic risk scoring has to be evaluated. The concept of using genetic information to further enhance current risk scores is a field of broad discussion with up to date no certainty about it’s feasibility. The utility of genetic risk scores in cardiovascular diseases has been discussed elaborately and is excellently summarized by Thannasoulis and Vassan\textsuperscript{76}. These authors propose that, at the current time, genetics are not ready to be used in risk prediction for cardiovascular diseases. This problem is partially driven by the fact that current major risk factors already may explain the major burden of CVD\textsuperscript{77,78} and that most likely, the risk factor ‘family history of CVD’ represents the net effect of many common risk variants\textsuperscript{76}.

Another difficulty when using combined genetic loci to explain cardiovascular disease is the notion that there may be additional effects of found variants on other causal pathways\textsuperscript{41}. In the case of TG level associated loci, another problem emerges. The extent to which elevated triglyceride levels are an active partaker in cardiovascular disease or merely ‘innocent bystanders’ still remains a topic of wide discussion. Analysis of current available data failed to identify a causal role of triglycerides in CVD\textsuperscript{77} and it has been argued that after adjustment for HDL levels, triglycerides do not significantly contribute to CVD risk\textsuperscript{79}. Thus, the debated causal role of triglycerides in cardiovascular disease in conjunction with the lack of specific genetic risk factors minimize a possible role for TG specific genes in cardiovascular risk management. Moreover, it has been shown that the discrimination in genetic risk score between the high-TG population and the normal population is small though significant, and subsequently risk allele scores are currently mainly able to discriminate for extreme values\textsuperscript{31}. For example, in hyperlipidemia, and more specific for severe hypertriglyceridemia, Teslovich et al. did show that subjects with an allelic dosage score (a score summarizing the number of TG raising alleles weighted by effect size) in the top quartile have a 44 fold increased likelihood to be hypertriglyceridemic\textsuperscript{41}. This extreme increase, especially when compared to the likelihood for LDL-C and HDL-c (respectively 13 and 4), should however be interpreted with caution. As both TG levels and the odds ratio itself are widely spread, without further phenotype characterization of the subjects the clinical implication of this epidemiological-genetic finding seems limited.

CONCLUSIONS

The ever-expanding genomic information has led to an explosion of novel approaches to unravel the pathophysiology of hypertriglyceridemia. We have highlighted the use of gene therapy, or much more pathway specific treatments in the case of monogenetic disease as well as the reclassification, novel approaches to risk assessment and treatment of polygenetic disease. Although in the case
of monogenetic disease, these novel therapies are scarce, they do seem promising and approval of these therapies would mean an enormous improvement over the current options in treating monogenetic severe hypertriglyceridemia. In the polygenic diseases, the future is less clear. Although evidence has been found that genetic differences can influence personal drug response, a translation to the use of this information to clinical practice is not yet available. Finally, the evidence for using genetics in cardiovascular risk assessment is too limited to allow application in current clinical practice.

At this time, the importance of genetic information in therapeutics for hypertriglyceridemia can be balanced between unravelling and understanding of pathophysiological pathways underlying this disease, redefining disease categories and to predict inter-individual response-differences to therapies.
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