Genes affecting triglyceride metabolism: from steatosis to lipodystrophy
Monajemi, H.

Citation for published version (APA):

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Discussion
The focus of this thesis is on genes affecting triglyceride (TG) metabolism. Fredrickson and his colleagues were one of the first pioneers in the field of human lipid metabolism who started to investigate the hyperlipidemias by using techniques available at the time (1). By performing paper electrophoresis, they were able to separate different lipoprotein particles according to their size and electric charge. By doing so they defined two major lipoprotein particles, which were designated as α- and β mobility particles corresponding to high density lipoprotein (HDL) and low density lipoprotein (LDL) particles. Accordingly the main protein constituents of these particles are designated as apolipoprotein A and B, respectively.

During the past decades our knowledge of lipoprotein metabolism has grown substantially as the alphabet of lipoproteins. The latest characterized apolipoprotein is apolipoprotein M. Many of these proteins have also several isoforms showing the diversity of proteins on lipoprotein particles. Beside the analysis of plasma lipoproteins, the evolution of techniques in genetic field has been the ground for a tremendous leap forward in understanding the lipid metabolism. Knockouts as well as transgenic animal models have been used to study the effect of a single gene in this complex pathway. Many genes have been sequenced and characterized in de past years and the number of players are increasing. Recently, Gue et al elegantly showed that about 1,5 % of all genes of Drosophila are involved in lipid-droplet formation, demonstrating not only the importance of this organelle inside the cells but also the complexity of intracellular lipid metabolism (2). It is reasonable to assume, knowing the complexity of human lipid metabolism intra- as well as extracellularly, that even more genes are involved in human lipid metabolism.

Phenotypic as well genotypic characterization of human subjects with lipid abnormalities is essential to gain insight in this process, especially because of the difference in lipid metabolism among species. This necessitates an integrative approach to discern these lipid abnormalities where, both clinicians and fundamental research scientists should be involved. Translation of basic research results into daily clinic (from bench to bedside) is as important as the opposite direction (from bedside to bench). For instance, observing lipid abnormalities in well defined pedigrees has made it possible to pinpoint the locus of the mutated gene. Maybe the best example of this kind of research is the finding of ABC-transporter A1 mutation in Tangier disease (3). Understanding the exact role of each gene involved in lipid metabolism, will eventually lead to novel therapeutic strategies.

The first part of my thesis is about unraveling the function of apoL genes and translating it to human diseases (from bench to bedside), whereas in the second part, I described a clinical phenotype and defined the mutation in the PPARγ gene, causing the disease (from bedside to bench). The necessity of bidirectional flow of information is exemplified in chapter 5 where a novel gene, Lipase H (LIPH) is described. We show that this gene, which is at the protein level homologous to triglyceride lipase family, is mainly expressed in intestine, lung, and pancreas (4). LIPH is a secreted protein with an apparent molecular weight of 63 kDa. Because of its structural resemblance to that of other triglyceride lipases, we were almost sure that it should have an effect on lipid metabolism. Although we thoroughly searched...
for the lipolytic activity of this protein, we were unable to identify its substrate. Recently, two separate groups found mutations in LIPH in families who show an inherited form of hair loss (5;6). Hereditary hypotrichosis is a rare autosomal recessive disorder characterized by sparse hair on scalp and rest of the body of affected individuals. Both groups suggest that LIPH is involved in hair growth and showed also expression in hair follicles of normal subjects. Although, these results are very convincing in the role of LIPH in hair growth, the exact function of this secreted protein remains unresolved. We found high expression of LIPH in the colon, suggesting that this protein must have other functions than merely hair growth. It would be interesting to characterize the phenotype of these patients further in detail, especially the lipid metabolism, to find a clue on the exact function of LIPH. This example shows clearly that fundamental research and clinical research can mutually affect our understanding of biological processes.

Evolutionary changes in lipid metabolism

Although mice have been used extensively to analyze lipid metabolism, the extrapolation of the data to human lipid metabolism has been very difficult. Genetic differences among species are becoming more obvious due to of high throughput sequencing of species. Phylogenetic analysis of specific genes can help us understand how the lipid metabolism has changed during the evolution and has adapted to the environmental demands. One of the essential differences in the lipid metabolism between mice and humans is the fact that mice lack Cholesterol Ester Transfer Protein (CETP) (7). In humans, CETP is a major determinant of plasma HDL-cholesterol levels. TG is transferred from TG-rich lipoprotein particles such as VLDL to cholesteryl ester-rich lipoproteins such as HDL with cholesteryl ester moving in the opposite direction. In doing so, CETP effectively lowers HDL-cholesterol levels. There are many other differences in lipid metabolism between men and mice, such as the evolution of apoB and its editing enzyme as well as microsomal triglyceride transfer protein (MTP) (see for details chapter one).

The Apolipoprotein L gene cluster is a gene family, arisen recently during evolution, that only exists in primates. Apolipoprotein L1 is the only secreted protein in this family and in plasma it is associated with HDL particles and has trypanosome lytic activity (8;9). We have shown that ApoL1-3 genes are all TNF-responsive genes that suggest a role for these genes during inflammation (chapter 3). In fact, apoL1 deficiency was associated with susceptibility to Trypanosoma Evansi infection. The serum of the infected patient was found to have no trypanolytic activity, and the finding was linked to the lack of apoL1, which was due to frame-shift mutations in both apoL1 alleles. Trypanolytic activity was restored by the addition of recombinant apoL1 (10). Somehow, during evolution these genes are duplicated and have gained different functions.

Is there an evolutionary benefit of apoLs for primates? The only non-homologous region in ApoL gene cluster is the first intron of ApoL3, which also contains many additional Alu repeats. These repetitive DNA elements were used by Nishio et al. to analyze genomic
expansion of the albumin gene family on human chromosome 4. They found that the earliest
gene during the course of the evolution of this gene family had the highest number of Alu
repeats (11). Because ApoL3 is both the least homologous of the family members and has a
much higher number of Alu repeats, we hypothesize that ApoL3 may be the ancestor gene
in this gene cluster. Introduction and rapid duplication of a gene is indicative of pressure on
gene dosage. Because the eukaryotic cell has only two alleles, the only way to upregulate
the expression of a gene is gene duplication. This is best demonstrated by the ribosomal RNA (rRNA) duplication. The ribosome is a complex of rRNAs and proteins that is involved
in translation of mRNA. Ribosomal proteins are the product of their mRNA where each
mRNA molecule gives rise into a multiple protein molecules. Because the rRNA is the end
product and thereby lacks the exponential increase of protein synthesis, the only way to
keep up with the ribosomal protein supply is gene duplication of rRNA. Therefore, rRNA
genes are organized in tandem repeats in five clusters on chromosomes 13, 14, 15, 21 and
22. It is conceivable that ApoL3 gene has been introduced into the animal genome and is
duplicated later in the evolution. This duplication must have occurred due to inability of both
apoL3 alleles to cope with the environmental demands. The normal evolution of duplicated
genes is concerted evolution, silencing or gaining new functions. It has been suggested that
selection and environmental pressure is likely to be one of the main factors determining
the duration of concerted evolution of duplicated genes (12). Another evolutionary moment
must have been the time when pressure on this gene family was dropped and allowed each
specific gene to evolve separately and gain novel functions. ApoL4 has most probably lost its
function because we were not able to find its expression in different human tissues. ApoL1
has changed dramatically and has become an extracellular protein associated with HDL
particles. It is reasonable to assume that apoL1-3 genes are beneficial to primates, because
they are still expressed and are not silenced.

Is there a role for apolipoprotein L2 and L3 in lipid metabolism? We have shown that apoL2
is an intracellular protein that co-localizes with PDI in the ER, where most of the enzymes in
the TG metabolism reside. ApoL2 overexpression induced large TG droplets in cells capable
of lipid loading, such as adipocytes, hepatocytes and macrophages but not in endothelial
cells (chapter 4). Perilipin is a major lipid droplet protein that is also a target gene for PPARγ
and has a PPARγ-responsive element in the promoter region. During foam-cell formation
PPARγ is upregulated and induces the expression of many other genes, such as perilipin,
involved in adipogenesis. ApoL2 has no responsive element for PPARγ, but its expression
was coordinated in the same fashion as PPARγ, indicating its involvement in intracellular TG
storage.

ApoL3 had the opposite effect on intracellular TG and was associated with the Golgi. While
apoL2 and PPARγ were upregulated during foam-cell formation, the expression of apoL3
was completely repressed. Two highly homologous genes, both responsive to TNFα, with
different intracellular localization thus have opposite effects on TG storage. The fact that
apoL proteins are primate specific indicates that their role in TG storage is of importance
for these species but not vital. Obviously, other mammals are capable of fat storage and breakdown without apoLs. Therefore, apoLs might be involved in the fine tuning of lipid metabolism during inflammation.

Is there a role for apoL proteins in lipid metabolism during inflammation? TNF-α has been shown to affect hepatic lipogenesis due to reducing the expression and activity of different nuclear hormone receptors such as PPARγ (13;14). TNF-α is an inflammatory cytokine that is mainly produced by macrophages. Adipocytes also express TNF-α and adipose tissue has been shown to be a major source of TNF-α locally and systemically. TNF-α was discovered not only as a soluble protein that induces the death of tumor cells but also as a molecule (cachectin) that causes hypertriglyceridemia and wasting of muscle and fat tissue (15). Inhibition of nuclear factor κB (NF-κB), the key transcription factor in TNFα-induced inflammatory response in monocytes, causes a decreased lipid loading after differentiation into macrophages. This is accompanied by increased expression of the transcription factor PPARγ (16). It seems that TNFα and PPARγ have opposing effects in TG metabolism. This effect is nicely demonstrated for an important protein in lipid metabolism, i.e. apolipoprotein E. ApoE is expressed in adipocytes and, besides its role in clearance of lipid particles in plasma, has an effect on TG storage inside the cells. Adipocytes from apoE−/− mice are smaller than those from wild-type mice and contain less TG (17). Adipocytes isolated from apoE−/− mice also accumulate significantly less TG after stimulation with PPARγ agonists, consistent with an important role for adipocyte apoE in mediating the effect of PPARγ agonists on adipocyte lipid metabolism. Furthermore, TNFα reduces the expression of apoE whereas, PPARγ induces its expression (18). The differential expression of apoL2 and apoL3 during foam-cell formation indicates their importance in TG storage. Although TNFα increases the expression of both apoLs in endothelial cells (chapter 3), in HepG2 cells, TNFα had no effect on apoL2 expression, whereas apoL3 mRNA expression was increased (unpublished data). Why would the apoL2 response to TNFα differ in different cell types? The liver has to cope with the tremendous energy demand that is required for the synthesis of acute phase proteins during inflammatory response. That might explain why “there is no place” for apoL2 in liver cells during inflammation and that other regulatory agents probably interfere with its expression to ensure sufficient energy supply. Future investigations are needed to understand the interplay between PPARγ and TNFα regarding the expression of apoLs. It would be interesting to investigate the effect of PPARγ activators on apoL2 and apoL3 expression in the presence of TNFα in different cell types. Obviously, such experiments should be performed in human cell lines, since these genes are primate specific. Although TG are essential for normal physiology, excess TG accumulation results in obesity and, particularly when it occurs in non-adipose tissues, is associated with insulin resistance. Obesity, type 2 diabetes and hyperlipidemia are coexisting conditions frequently associated with non-alcoholic fatty liver disease (NAFLD) (19). Because of the tremendous increase in the prevalence of obesity worldwide and the lack of efficacy of current medical therapies, efforts to develop novel therapies for obesity are needed. Medications currently available
for the treatment of obesity primarily act by decreasing energy input, by either suppressing appetite or interfering with lipid absorption in the gut. Other potential therapeutic strategies are inhibiting TG synthesis/storage and increasing energy usage by β-oxidation. In order to design novel therapeutic strategies, we need to understand the TG metabolism at the molecular level. In the past several years, pharmacological inhibition of Dacylglycerol:acyl-CoA acyltransferase 1 (DGAT1) has emerged as a potential strategy for inhibiting TG synthesis in obesity (20). We have shown that apoL2 overexpression causes steatosis only in cells capable of lipid loading. Maybe in steatotic human liver, the balance of gene expression is distorted and there is a higher apoL2 expression. Inhibition of apoL2, either by interfering with upstream regulatory mechanisms or direct gene silencing with anti-sense therapy, could reverse the steatotic process. Vice versa, apoL3 overexpression could reduce intracellular TG substantially. Therefore, apoL3 overexpression with adenoviral gene therapy could also reverse the steatotic process. Currently, we are investigating the endogenous expression of apoL2 and apoL3 in human liver samples and compare the expression profile in steatotic versus non-steatotic livers. If they are differentially regulated during inflammation, then either apoL3 overexpression or apoL2 inhibition could be used as a therapeutic strategy in the treatment of NAFLD or obesity.

**TG, PPARγ and atherosclerosis**

Atherosclerosis, the major cause of death from cardiovascular disease (CVD) in industrialized countries, is characterized by the progressive accumulation of lipid and fibrous depositions in the vessel wall of large arteries. There is substantial evidence that high LDL-cholesterol and low HDL-cholesterol are both associated with CVD. Likewise, although debates continue regarding TG as an independent risk factor for CVD, the presence of hypertriglyceridemia confers a considerable increase in risk among subjects with otherwise similar ratios of LDL and HDL (21;22). Fibrates are drugs that specifically activate PPARα and thereby reduce plasma TG levels. These drugs have been used for the treatment of dyslipidemia and have been shown to reduce the risk of CVD (23). Patients with metabolic syndrome due to obesity and diabetes have multiple risk factors for developing CVD. The term metabolic syndrome has been introduced to describe this cluster of metabolically related cardiovascular risk factors i.e obesity, insulin resistance, dyslipidemia and hypertension. During the past decades many novel drugs have been manufactured for each of these risk factors such as Rimonabant for treating obesity, Thiazolidinediones (TZDs) as anti-diabetic drugs and different fibrates as hypolipidemic drugs. TZDs are drugs that activate PPARγ and have been used for treatment of diabetes. Two widely used TZDs are Rosiglitazone and Pioglitazone. These compounds bind to the ligand binding domain of PPARγ and cause a conformational change that allows PPARγ to bind to DNA. Thereby, TZDs induce expression of array of genes that are involved in adipocytes differentiation and glycemic control. Both drugs have been used successfully in diabetic patients with improved metabolic parameters. Because of its beneficial effects on diabetes, it was expected to be also beneficial in preventing CVD. While information about the cardiovascular effects of these agents has gradually become available from a series
of randomized controlled trials, it has required meta-analyses to better characterize their risks. Two separate meta-analysis on the effects of Rosiglitazone on cardiovascular endpoints showed that this drug was, however, associated with increased risk of myocardial infarction (24;25).

How could we explain this paradox that Rosiglitazone improves glycemic control but increases the CVD risk? May be the best way to answer these questions is to look at a naturally occurring PPARγ insufficiency in patients with familial partial lipodystrophy. Lipodystrophies represent a heterogeneous group of diseases characterized by an abnormal, subcutaneous fat distribution that are associated with metabolic abnormalities comparable to the metabolic syndrome. Familial partial lipodystrophy type 3 (FPLD3) is a monogenic disorders that is caused by mutations in PPARγ gene (chapter 6 and 7 of this thesis). FPLD3 patients have a reduced expression of PPARγ due to haploinsufficiency of the PPARγ gene. From the currently described patients in the literature, none of them has been described to suffer from CVD. Conversely, FPLD2 patients with mutation in Lamin A (LMNA) gene have comparable clinical manifestations but do have CVD (26). Although, the number of patients is too small to draw any conclusion, it seems that patients with PPARγ deficiency, despite their metabolic abnormalities, have a lower risk of CVD compared to FPLD2. Comparing these data with the clinical data on Rosiglitazone, showing that activation of PPARγ increases the cardiovascular events one can hypothesize that PPARγ expression level is set to be in a specific range and that either low level of expression or too much activation is detrimental to the organism (Figure 1). PPARγ activation leads to metabolic improvement but increased CVD, whereas low expression of PPARγ in FPLD3 leads to metabolic abnormalities and protection from CVD. If the level of PPARγ should indeed be at a fixed biological range, then its activation with another TZD should have the same effect on CVD risk. Interestingly, in the same issue of JAMA, where a meta-analysis on Rosiglitazone was presented, Lincoff et al presented a meta-analysis on Pioglitazone (27). They showed that Pioglitazone was associated with a significantly lower risk of death, myocardial infarction, or stroke among a diverse population of patients with diabetes. How could one PPARγ activator (Rosiglitazone) be disadvantageous while another (Pioglitazone) is apparently beneficial in CVD risk lowering? There are two possibilities to explain this phenomenon. Either Rosiglitazone has additional detrimental side effects or Pioglitazone has additional positive effect. There is some evidence for the latter option. It has been shown that Rosiglitazone at concentrations used in the clinics is almost entirely restricted to activate PPARγ, whereas Pioglitazone has also PPARα activation capability (28). As already mentioned before, PPARα activation has been shown to reduce cardiovascular risk. Therefore, it is plausible that the CVD risk reduction with Pioglitazone is due to its PPARα-activating activity and the improved lipid profile and not for its PPARγ activity. To explore the above mentioned hypothesis, it would be interesting to define the atherogenic profile of FPLD2 and FPLD3 patients and compare it to obesity associated metabolic syndrome. We are planning to perform intima-media thickness (IMT) measurements, as a surrogate marker for atherosclerosis, in these patients. If the hypothesis is correct, we would find higher IMT measurements in obese subjects and FPLD2 patients.
when compared to PPARγ-deficient FPLD3 subjects. By comparing these two monogenic disorders, we can dissect the metabolic syndrome risk profile from PPARγ activation risk profile. Monogenic disorders are perfect human models to study the effect of one gene on the total body. Just a decade ago all lipodystrophies were referred as FPLD, until genetic mutations in these patients were determined by different groups. The terms FPLD2 and FPLD3 were introduced to define the genetic background of these patients. Later, it became clear that the phenotype of these two monogenic disorders were quite different that were not noticed before. For instance, the degree of lipoatrophy was shown to be significantly more in FPLD2 patients when compared to FPLD3 subject by means of MRI (29). Therefore, the term phenomics has been introduced to emphasize the importance of thorough phenotypical characterization of patients. Phenomics has been defined as systematic application of clinical, biochemical, and imaging methodologies/tools that are familiar to practicing clinicians and clinician investigators (30). Currently, we are examining patients with type 2 diabetes with extreme insulin-resistance to detect lipodystrophic patients that might have been overlooked because of the subtle clinical differences with the common type 2 diabetics. If, after a thorough examination, lipodystrophic features are present in these patients we will perform sequencing of the PPARγ- and LMNA genes to detect potentially novel mutations.

*Figure 1: level of PPARγ activity and its effect on metabolic parameters and CVD risk*

PPARγ is a nuclear transcription factor that has a specific physiological range. Either increased or decreased activity of PPARγ could be detrimental for the organism. In FPLD3 patients with low expression of PPARγ, many metabolic abnormalities such as those in metabolic syndrome are observed, but so far there is no evidence for increased CVD risk. On the other hand PPARγ activation with TZD leads to improved metabolic parameters but also increases the risk of CVD.
In summary, in this thesis, a subset of genes involved in TG metabolism has been characterized that enhances our understanding of this complex pathway. Obviously, future investigations are needed to characterize the remaining genes for further understanding of the (patho)physiology of TG metabolism in order to define novel therapeutic targets for common diseases such as obesity and steatohepatitis. Furthermore, applying phenomics to subjects with familial disorders that might have an inherited disorder is very important to pinpoint the genetic defect. Therefore, intensive collaboration between fundamental research scientists and clinicians is crucial.
Reference List


