Genetic-epidemiological component of "Genomics of Lipid-Associated Disorders" (GOLD)

Principal Investigator: Florian Kronenberg

Background

The prevalence of overweight and obesity is dramatically increasing. A large fraction of people is suffering from consequences of this "obese epidemic" which are mainly of cardiovascular nature. Among other factors, one underlying cause is dyslipidosis (disruption of fat metabolism), which leads to massive deposits of triglycerides in fatty tissue and of cholesterol in the arterial wall. The goal of this project is to discover and explain the function of each gene and protein involved in the process of uptake, storage and mobilization of lipids (fats) by cells.

Goal of GOLD

GOLD is a consortium of researchers from various fields grown since 2002 and headed by our coordinator Rudolf Zechner from the University of Graz. Our group is responsible for the genetic-epidemiological component of the project that aims to identify genetic variation in candidate genes identified by structural and functional studies in other project components and to identify genetic variants that can be associated with phenotypes of interest.

The following projects are examples of our activities within GOLD.

Genetic epidemiology of Adipose Triglyceride Lipase (ATGL)

Adiponutrin, lipoprotein metabolism and liver function

Genome-wide Association Studies

Genetic epidemiology of Adipose Triglyceride Lipase (ATGL)

The influence of common variants with the *ATGL* gene

With ATGL the first promising candidate gene identified within the GOLD consortium became available and our main focus shifted to its detailed investigation. We studied more than 2400 precisely phenotyped individuals from Utah including a group of 1120 patients with a BMI between 35 and 90 kg/m². We sequenced the entire gene and its flanking regions in 48 morbid obese and 48 control subjects to find sequence variations. We identified further SNPs and an insertion/deletion polymorphism and selected 12 tagging SNPs which were finally genotyped in the 2400 patients and controls. Since ATGL is responsible for the initial step in the triacylglycerol catabolism releasing free fatty acids (FFA) into the circulation, we measured FFA as an intermediate phenotype. We observed clear associations between single SNPs as well as haplotypes of the *ATGL* gene with FFA levels (Figure 1) **[1]**. Our results are in line with functional studies of other consortium members including the finding that ATGL knock-out mice have lower FFA levels.

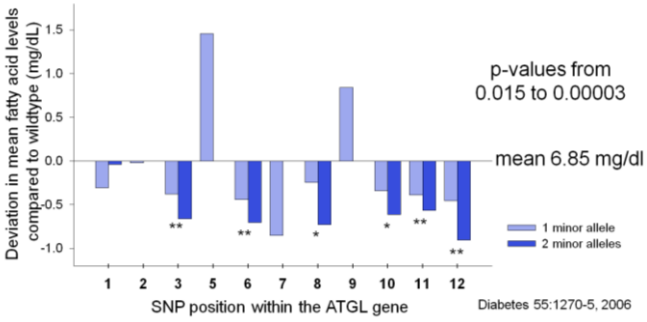

Figure 1: *SNP association analysis showing differences in mean free fatty acid levels for those subjects with one copy and two copies of the minor allele compared to zero copies (wild-type). Results are shown for each of the 11 SNPs genotyped. The mean free fatty acid level of the entire population is 6.85 mg/dL. The presented p-values assume a trend per minor allele copy (additive inheritance model).*

The influence of rare variants within the *ATGL* gene

In order to assess the frequency and role of rare variants in a healthy population, we performed a mutation analysis of the full *ATGL* gene region in 1473 individuals of the healthy working SAPHIR population using an adapted pooled Ecotilling approach **[2]**. This revealed 55 novel, mostly rare (n=20) or private (n=34) variants and showed that even in a healthy working population rare variants are collectively frequent. Indeed, 7.7% of all individuals carried a rare variant and still 2.6% carried a rare missense variant.

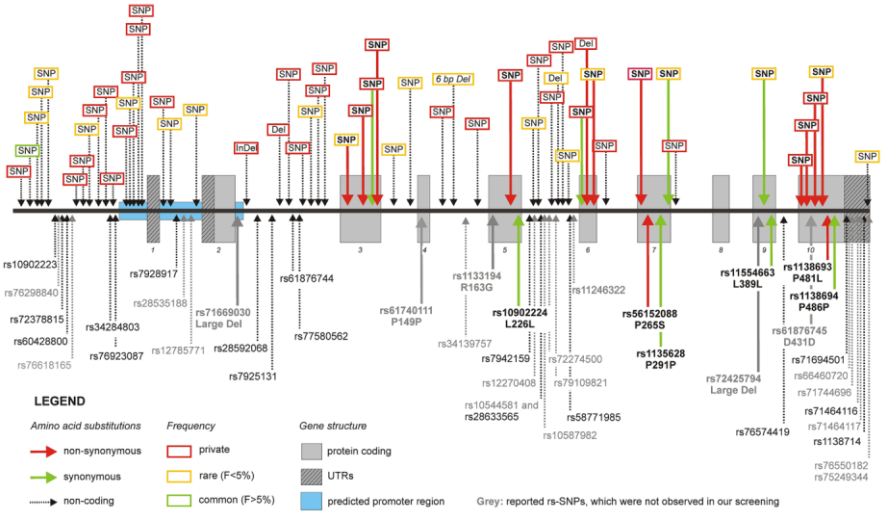

Figure 2: *Overview of the ATGL variants found in SAPHIR. This figure shows the localization of both novel and previously known ATGL variants superimposed to the gene structure. The upper part of the figure shows the 55 novel variants, while the lower part depicts previously known SNPs according to dbSNP build 131. Details about the meaning of the colour codes and arrow types are given in the legend within the figure. Nearly all genetic variants were rare (yellow boxes, n=20) or even private (red boxes, n=34). Image taken from reference [3].*

Functional investigations by cell culture studies revealed a large spectrum of residual activity ranging from virtually wild-type activity to total loss of function (Figure 3). In order to evaluate the relevance of these in-vitro observations for the FFA levels in the population, we performed association studies and observed a trend towards lower FFA levels in rare variant carriers as well as an accumulation of rare variant carriers in the lower 10th percentile of the FFA distribution. However, and very surprisingly, the overall impact of these variants on the FFA levels was rather small despite the observed functional impairments.

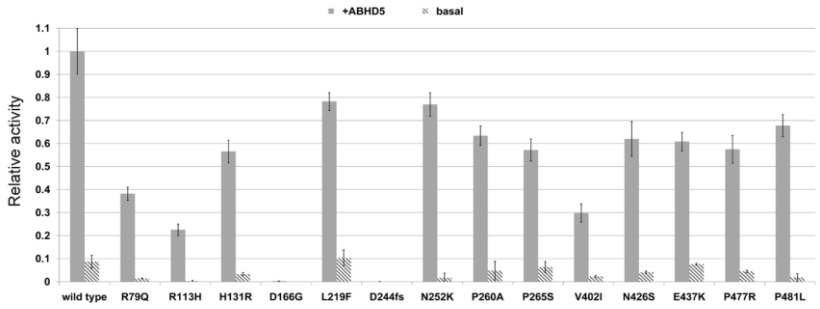

Figure 3: *Relative triglyceride hydrolase activities of the tested non-synonymous ATGL variants. Triglyceride hydrolase activities are given as relative to wild type ATGL after stimulation with murine GST-CGI58 (ABHD5) and corrected for COS7 background activity (presented as mean ± standard deviation).*

[top]

Adiponutrin, lipoprotein metabolism and liver function

Adiponutrin (*PNPLA3*) is a predominantly liver-expressed transmembrane protein with phospholipase activity that is regulated by fasting and feeding. Recent genome-wide association studies identified *PNPLA3* to be associated with hepatic fat content and liver function, thus pointing to a possible involvement in the hepatic lipoprotein metabolism. Therefore, our group examined two common variants in this gene and parameters of lipoprotein metabolism in 23,274 participants from eight independent West-Eurasian study populations. We observed for the first time a strong additive association of a common nonsynonymous variant of adiponutrin (rs738409) with lipoprotein concentrations adjusted for age, gender, and alanine-aminotransferase: each copy of the minor allele significantly decreased levels of total cholesterol, non-HDL cholesterol, and LDL cholesterol levels (Figure 4). Our results suggest that adiponutrin is involved in the metabolism of apoB-containing lipoproteins **[3]**. Furthermore, we observed that genetic variants within this gene are associated with liver function **[4]**.

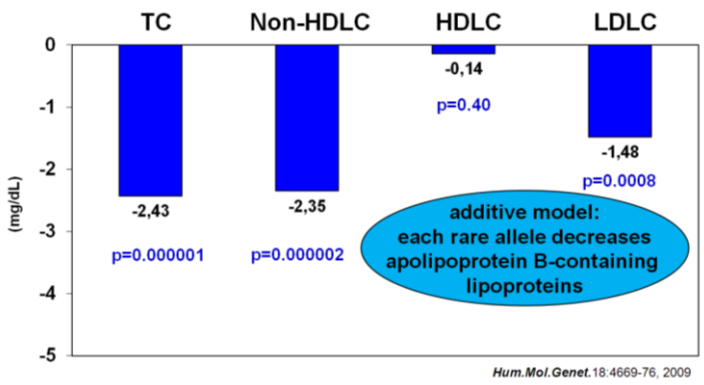

Figure 4: *Association of the SNP rs738409 of adiponutrin gene with apolipoprotein B-containing lipoproteins in 23,274 participants from eight independent West-Eurasian study populations.*

[top]

Genome-wide Association Studies

Our group is involved in several genome-wide association studies (GWAS), the most important of which is the GWA study on lipids. After carrying out a GWA study on HDL cholesterol **[5]**, we and the field came to realize that very large sample sizes are required to have the statistical power to identify new genes and pathways involved in lipid metabolism. Therefore, we joined the European ENGAGE consortium with 16 population-based cohorts including up to 22.000 study participants. This initiative observed 22 genetic loci associated with serum lipid levels, 6 of which were newly identified. A score of risk alleles for lipids found associations of these alleles with carotid intima-media thickness as well as coronary heart disease incidence **[6]**. Since these 22 genetic loci still explained only up to 4.8% of lipid levels, we joined the "Global Lipids Genetics Consortium" with far more than 100 000 individuals investigated. This study finally identified 95 (59 new) genetic loci associated with serum lipids levels. These mapped variants explained 10-12% of each trait which corresponds to about a quarter of the variance for each trait expected to be explained by genes. Many of these genes were not previously implicated in lipoprotein metabolism and they

contribute not only to normal variation in lipid traits but also to extreme lipid phenotypes and lipid traits in multiple ethnic populations **[7]**.

Further GWAS studies investigated phenotypes such as adiponectin plasma concentrations **[8]** or a large number of metabolic traits derived from metabolomic analyses **[9,10.]** For details, see project "Genome-wide association studies".

Team members:

Stefan Coassin, Barbara Kollerits, Claudia Lamina, Anita Kloss-Brandstätter, Veit Schoenborn, Margot Haun, Gertraud Erhart, Arno Lingenhel

Main collaborators:

- Rudolf Zechner, Robert Zimmermann, Martina Schweiger, (University of Graz)
- Iris M. Heid (University of Regensburg)
- Bernhard Paulweber (Paracelsus Private Medical University Salzburg)
- Steven C. Hunt, Ted Adams (University of Utah, USA)
- H.-Erich Wichmann, Thomas Illig, Norman Klopp, Annette Peters, Angela Döring, Konstantin Strauch, Christian Gieger, Karsten Suhre (Helmholtz Center Munich, D)
- KORA Study Group, SAPHIR Study, Utah Obesity Case-Control Study, Bruneck Study, CoLaus Study, SHIP Study, ERF Study, MICROS Study, Rotterdam Study

Selected Publications:

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