The *ATGL* gene is associated with free fatty acids, triglycerides and type 2 diabetes

Short running title: *ATGL,* free fatty acids, TG and T2DM

Veit Schoenborn [1*], Iris M. Heid [2,3*], Caren Vollmert [2*], Arno Lingenhel [1],

Ted D. Adams [4], Paul N. Hopkins [4], Thomas Illig [2], Robert Zimmermann [5],

Rudolf Zechner [5], Steven C. Hunt [4], Florian Kronenberg [1]

* authors contributed equally

[1] Division for Genetic Epidemiology; Department of Medical Genetics, Molecular and Clinical Pharmacology, Innsbruck Medical University, Innsbruck, Austria

[2] GSF-National Research Center for Environment and Health, Institute of Epidemiology, Neuherberg, Germany

[3] Institute of Biostatistics and Epidemiology, Ludwig-Maximilian-Universität München, Munich, Germany

[4] Cardiovascular Genetics, University of Utah School of Medicine, Salt Lake City, UT, USA

[5] Institute of Molecular Biosciences, Karl-Franzens-University, Graz, Austria

Address of correspondence:
Florian Kronenberg, MD
Division of Genetic Epidemiology
Department of Medical Genetics, Molecular and Clinical Pharmacology
Innsbruck Medical University
Schöpfstr. 41, A-6020 Innsbruck, AUSTRIA
Tel. (+43) 512-507-3490, Fax (+43) 512-507-9804
e-mail: Florian.Kronenberg@i-med.ac.at

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Abstract

Adipose triglyceride lipase (*ATGL*) was recently described to predominantly perform the initial step in triglyceride hydrolysis and therefore seems to play a pivotal role in the lipolytic catabolism of stored fat in adipose tissue. In the first study investigating genetic variations within the *ATGL* gene in humans, twelve polymorphisms identified via sequencing and database search were studied in 2434 individuals of European ancestry from Utah. These polymorphisms and their haplotypes were analyzed in subjects not taking diabetic medication for association with plasma free fatty acids (FFA) as primary analysis, as well as triglycerides and glucose as a secondary analysis (n=1701, 2193 or 2190 respectively). Furthermore, type 2 diabetes (T2DM, n=342 out of 2434) was analyzed as an outcome. FFA concentrations were significantly associated with several SNPs of *ATGL* (p-values from 0.015 to 0.00003), consistent with additive inheritance. The pattern was similar when considering triglyceride concentrations. Furthermore, two SNPs showed associations with glucose levels ($p<0.00001$) and risk of T2DM ($p<0.05$). Haplotype analysis supported and extended the shown SNP association analyses.

These results complement previous findings of functional studies in mammals and elucidate a potential role of *ATGL* in pathways involved in components of the metabolic syndrome.

Understanding of pathogenetic mechanisms, including the genetic components of type 2 diabetes mellitus (T2DM), is a major challenge for research. However, finding genes for a complex disease such as T2DM is often an extremely difficult and long-lasting process. One of the reasons might be the difficulty in phenotyping disease endpoints, to which many pathways contribute with completely different genes involved. The influence of one gene to the disease endpoint can therefore be extremely small. However, for precisely measurable intermediate phenotypes in the disease pathway, the contribution of a single gene can be substantial and studying their association with the gene might be more fruitful than concentrating on phenotypes too far away from the gene product. Plasma level of free fatty acids (FFAs) is such an intermediate phenotype in the pathway of T2DM.

FFAs play a major role in the pathogenesis of T2DM which is underlined by a strong link between increased plasma FFAs and insulin resistance (*1*). FFAs are released from lipid storages in fasting or energy-demanding states by lipolytic enzymes. It was believed until recently that the hormone-sensitive lipase (HSL) is the primarily responsible enzyme for the hydrolysis of triacylglycerols and diacylglycerols which releases FFAs (*2*). Recently, our research consortium and two other groups independently identified a new lipase called adipose triglyceride lipase (*ATGL*, alternative names: desnutrin, TTS2.2, iPLA2ξ, PNPLA2) (*3-5*). Overexpression of *ATGL* enhanced the lipolysis in adipocytes and inhibition did the opposite (*3*). Villena and colleagues observed the level of mRNA highly upregulated in fasting mice and reduced after refeeding (*5*). This lipase was demonstrated to catalyze the initial step of the breakdown of triacylglycerol molecules with high substrate specificity by selectively hydrolyzing the primary ester bond in adipose tissue of mammals (*3*). The hydrolyzing of the second ester bond is the domain of HSL (6). Therefore, both enzymes might substantially contribute to the pool of FFAs (*2*).

The present study thus aimed to determine whether genetic variation within the *ATGL* gene significantly influences FFA levels in humans. It was a secondary goal to elucidate the impact of this gene on parameters related to FFA metabolism such as triglyceride and blood glucose levels as well as T2DM.

Research Design and Methods

The study is based on 2434 individuals of European ancestry from Utah, USA, recruited either for severe obesity (BMI between 35 and 90 kg/m², n=1101), coronary artery disease

(n=469) or as general population sample from the same ethnicity (n=864) (Table 1). All subjects were in a fasting state at the time of blood withdrawal. Detailed information on these groups, laboratory phenotyping, sequencing, single nucleotide polymorphism (SNP) selection, genotyping and statistical methods are provided in the Online Appendix.

Briefly, from sequencing 96 subjects and data base information, 12 polymorphisms were selected (Figure 1 and Suppl.Table 4), genotyped and analyzed in the full sample. The primary analysis of these SNPs and their statistically reconstructed haplotypes with the outcome FFA as well as secondary analyses with triglycerides and glucose levels were performed via linear regression adjusting for age and sex. These analyses were restricted to subjects not taking any anti-diabetic medication. The analogous analysis for the outcome T2DM was done by logistic regression.

Results

Genetic variations within the ATGL gene

We resequenced the entire *ATGL* gene in 48 normal-weight and 48 obese subjects (Figure 1; for PCR reactions and primers see Suppl.Tables 1-2). From the SNPs detected, 12 SNPs were selected mostly guided by potential functional considerations. Selection criteria by decreasing priority were: resulting amino acid exchange (SNPs 4,7,10), insertion/deletion polymorphism (SNP11), location at the intron-exon boundary (SNP6), location in the untranslated 3'UTR region (SNP12), database entry at the beginning of the project (July 2004) (SNP3,5,8,9) or being a tagging SNP (SNP1,2) (for more details, see Online Appendix and Suppl.Table 3). These 12 polymorphisms (Suppl.Table 4) were genotyped in the entire group of 2434 individuals. None of the genotyped SNPs violated Hardy-Weinberg equilibrium. One variant (SNP4) was a very rare mutation (n=1 with minor allele) and thus discarded from analysis. The genotype and allele frequencies did not differ between the three subgroups (Suppl.Table 4).

The gene structure of ATGL, the frequenies of the minor alleles for each SNP as well as the correlation of alleles between various SNPs are depicted in Figure 1. There were three SNPs without notable correlation to any other (SNP1,2,7; $r<0.50$). The rare polymorphisms 5 and 9 were highly correlated ($r=0.95$). SNP3,6,8,10,11,12 showed moderate to high interSNP correlations ($r>0.60$) with SNP6 and 12 ($r=0.94$) and SNP3,8,10,11 ($r>0.90$) being

particularly close. Lewontin's D' was high throughout the gene with D' above 0.90 for all pairs of consecutive SNPs.

Primary analysis: association of ATGL variations with plasma free fatty acids

Figure 2a summarizes the results of our primary analysis of the SNP association with FFA concentrations. All parameters were tested on the log-scale to assure normally distributed residuals. It can be seen that the two correlated SNPs, SNP6 and 12, as well as SNP3, 8, 10, 11 were significantly associated with decreased FFA levels showing a clear trend per copy of the minor allele (p-values between 0.015 and 0.00003 for an additive inheritance model). The most pronounced association was observed for SNP12 with mean FFA values (re-transformed mean computed on the log-scale) of 257, 241 and 225 µmol/l for 0, 1 and 2 minor allele copies (p=0.00003). SNP5 and SNP9 showed a tendency towards significance with higher rather than with lower FFA levels. Considering that the mean plasma value of FFAs is 242 µmol/l, the changes in mean levels were approximately 15-20% of the mean, ranging from -30 µmol/l to +50 µmol/l. The means±SE levels of FFA for each genotype are provided in Suppl.Table 5. Computing the number of effective loci as 9.1 by the Nyholt approach (7) yielded a significance level of 0.006, which accounts for the multiple testing of the 11 SNPs. Initial FFA analysis including interaction of the genotypes with the three subgroups showed overall no evidence for heterogeneity.

Secondary analysis: association of ATGL variations with plasma triglycerides, blood glucose and type 2 diabetes mellitus

The pattern was the same when analyzing triglyceride concentrations in our secondary analysis (Figure 2b and Suppl.Table 6) with p-values less pronounced compared to FFA levels (lowest p-value=0.01 for SNP11). No evidence for heterogeneity of the triglyceride estimates in the three subgroups was observed. Spearman correlation coefficient between FFAs and triglycerides was 0.29; between FFAs and glucose it was 0.17. We have not found any significant association of any of the SNPs with BMI.

There was a highly significant association with a substantial increase in fasting glucose concentrations of 20 and 16 mg/dl in minor allele carriers of SNP5 and SNP9, respectively, compared to the wildtype (p<0.00001 for both SNPs) (Figure 2c, Table 1 and Suppl.Table 7). This association was consistent with the direction of the change in FFA for these two SNPs. None of the other SNPs revealed an association with glucose levels. Consistently,

these two SNPs showed an association with an increased risk for T2DM with an OR of 2.65 and 2.47, respectively (Table 2). It can be seen that the T2DM ORs pointed into the same direction for the three subgroups as well as for men or women separately. Also the change in mean glucose levels increased in all subgroups. Several sensitivity analyses were performed showing no effect from additional adjustment for BMI nor for the subgroups, nor did restriction of the quantitative outcome analysis to non-diabetic subjects alter the results markedly (for details see Online Appendix and Suppl.Tables 5-7).

Haplotype analysis

Haplotype analysis provided consistent findings. We statistically reconstructed haplotypes based on the 2228 subjects with all 11 SNPs successfully typed. We identified three common haplotypes (51%, 22% and 13%) and six rarer haplotypes (0.5-5%), which together constituted almost the whole spectrum of haplotype diversity (97.2%) in the *ATGL* gene (Suppl.Table 8). Haplotype reconstruction error was negligible as the percentage of unambiguously defined haplotypes was 99%. Subjects with one or two haplotypes other than reference showed significantly lower FFA levels (257, 244, 223 µmol/l, $p<0.0001$ assuming a trend for increasing number of haplotypes different from the reference) when compared to the group of subjects with two copies of the reference haplotype. The results of testing FFA levels for subjects with a certain haplotype against the subjects carrying two copies of reference are summarized in Suppl.Table 8. It can be seen that only the haplotype H6 containing the minor allele of SNP5 and SNP9 was associated with increased levels of FFAs, whereas all others were associated with decreased levels. Interestingly, the FFA associations of the haplotypes H2, H8 and H9, which carry a minor allele of either of the two SNPs involving an amino acid exchange (SNP7=N252K and SNP10=P481L), were among the most convincing. Overall, the statistical significance for 4 of the 9 haplotypes indicated a good differentiation of FFA levels by the *ATGL* haplotypes. The relationship between haplotypes can be viewed in a minimal spanning net (Suppl.Figure 1). Consistent with the associations of the rare alleles in SNP5 and SNP9 with blood glucose levels, we observed a pronounced increase of blood glucose levels of 14 mg/dl ($p<0.0001$) for carriers of haplotype H6, the only haplotype containing the rare alleles of SNP5 and SNP9.

Discussion

Our study is the first describing polymorphisms of the *ATGL* gene in humans and a clear association on closely related intermediate phenotypes such as FFA and triglyceride concentrations. We even observed an association of two rare polymorphisms of the *ATGL* gene with glucose levels and T2DM which altogether suggests an important role of this gene in the pathways of the metabolic syndrome.

The identification of *ATGL* as the key enzyme for the breakdown of triacylglycerol molecules in adipose tissue of mammals by selectively hydrolyzing the primary ester bond (3) seems to be one of the keys for a more detailed understanding of the FFA metabolism. Several functional observations underscore the importance of this enzyme: overexpression of *ATGL* enhances lipolysis in 3T3-L1 adipocytes which can be inhibited by antisense technologies against *ATGL* (3); pronounced upregulation of *ATGL* in fasting mice and a downregulation after refeeding (5;8); reduction of mRNA in adipose tissue of genetically obese mice (5); *ATGL* also functions in non-adipocyte cells having an important role in lipid droplet turnover in these cells (9). An homologue with similar functions was even found in Drosophila (10).

These promising functional studies guided us to search for naturally occurring polymorphisms within the *ATGL* gene and to investigate whether these genetic variations had an influence on the network of closely related intermediate phenotypes. Plasma levels of FFA were one of the expected intermediate phenotypes to be associated with *ATGL* variation. However, it has to be considered that the pool of FFA is supplied not only from the hydrolysis of FFA from triacylglycerol by *ATGL* but also from hydrolysis of diacylglycerols by HSL and monoacylgycerols by monoglyceride lipase. Nevertheless, we found a strong association of polymorphisms within the *ATGL* gene and FFA levels which underscores the rate-limiting function of this gene in that pathway. A weaker but still detectable association of the *ATGL* gene was observed with the more downstream-located intermediate phenotype of triglycerides. This is in line with the physiological observation that only a limited amount of FFAs is oxidized in the liver and the majority is reesterified to triglycerides, which are again transported to adipose tissue for storage (11). In addition to triglycerides being derived from nutritional intake, this process contributes to the circulating triglyceride plasma concentrations. From the physiology of this pathway and from our data, triglycerides thus appear to have a larger distance from the *ATGL* gene than FFAs in that pathway.

It is interesting that the FFA associations of the minor alleles of the two highly correlated SNP5 and SNP9 point into the opposite direction as the other SNPs (Figure 2). These two SNPs further differ from most others by their rare frequency. And they showed a very strong association with fasting glucose concentrations in carriers of the minor allele compared to the wildtype ($p<0.00001$ for both SNPs). This association was so strong that it resulted in a 2.65-fold increased odds of T2DM. This finding was strengthened by the fact that the T2DM risk estimates pointed into the same direction in all analyzed subgroups. In the light of the various pathways leading to T2DM and the numerous genes involved in these pathways, it is noteworthy that these two *ATGL* variants affecting 0.5% of the studied population and accounting for almost 1% of T2DM cases (population attributable risk) seem to play an important role. Our findings are in line with observations that FFAs induce hepatic insulin resistance and increase gluconeogenesis, which results in an increased hepatic glucose production in case of a defective hepatic autoregulation as seen in diabetogenic conditions (12).

Limitations of the study

It might be a limitation that the entire sample is a pooled sample of three subgroups. This was done to increase the sample size but also to extend the range of the quantitative phenotypes from healthy to severely obese subjects and to a group of patients known to have a wide range of the metabolic parameters (CAD). The design was accounted for in the initial analysis including interaction between SNP and subgroup to test heterogeneity, by subgroup analyses and by providing pooled estimates if necessary. Furthermore, the allele frequencies of the investigated SNPs for the three groups were very similar and there was thus no major effect from adjusting for the subgroup. For details, see Table 2 and Online Appendix.

It must be considered a limitation that the number of subjects on which the association of T2DM with the rare polymorphisms is based is rather small (n=27 and 39 with the minor alleles of SNP5 and SNP9, respectively). Therefore, more studies in the future will have to elucidate the role of the *ATGL* in T2DM. However, our findings were consistent in all analyzed subgroups and both genders. This is more than would be expected from a purely random signal.

Finally, it is a well known limitation of all association analyses that the functional SNPs cannot be pinpointed, which only functional studies can provide. Still, the association

signals may provide some indication and guide future functional studies. Another limitation of association studies is the possible occurrence of false positive findings. However, the reliability in our findings is underscored by the fact that different phenotypes within the same pathway - FFA, triglycerides, and glucose levels - showed associations in the same direction despite a rather low correlation between these phenotypes.

Conclusions

Our results show an influence of genetic variation within the human *ATGL* gene on fasting FFA and glucose levels as well as T2DM risk. Less pronounced associations were detected with triglyceride levels. These results complement previous findings of functional studies in mammals and elucidate a potential role of the *ATGL* gene in pathways involved in components of the metabolic syndrome underscoring the attractive value of *ATGL* as new drug target.

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Table 1: Characteristics of 2434 participants from the three recruited groups of subjects. Values are given as percentage or mean ± SD.

	Severe obesity (n=1101)	Coronary artery disease (n=469)	General population (n=864)
Females (%)	82%	24%	52%
Age < 30 yrs (%)	13%	0%	0%
30-40 (%)	22%	2%	3%
40-50 (%)	30%	21%	38%
50-60 (%)	27%	52%	34%
60+ (%)	8%	25%	24%
BMI (kg/m²)	45.8±7.6	28.2±5.0	27.6±4.9
Cardiovascular event (%)	1.8%	100%	2.1%
Type 2 diabetes, (%)	20.1%	19.0%	7.3%
analyzed in 2434 subjects *	n=1101	n=469	n=864
Fasting free fatty acids (mg/dL) †	272±102	301±181	244±128
analyzed in 1701 subjects *	n=898	n=232	n=571
Fasting triglycerides (mg/dL)	178.5±92.5	206.5±133.5	153.1±99.4
analyzed in 2193 subjects *	n=912	n=449	n=832
Fasting glucose (mg/dL)	96.1±22.7	96.3±38.7	88.9±13.8
analyzed in 2190 subjects *	n=913	n =446	n=831

* n indicates the number of subjects included in the SNP analysis. Inclusion criteria were that age, sex and phenotype was available, that more than six SNPs were successfully genotyped, and - additionally for free fatty acids, triglycerides, glucose - that no anti-diabetic medications were taken.

† Plasma free fatty acids were only measured in 1922 out of 2434 subjects with stored plasma samples available.

Table 2: Odds ratio (95% CI) for type 2 diabetes mellitus (n=342 out of 2434) and average glucose concentrations (n=2190 due to restriction to subjects with no diabetic medication) for SNP5 and SNP9.

	Type 2 diabetes mellitus						Fasting glucose concentrations [mg/dl]			
	SNP5			SNP9			SNP5		SNP9	
	OR (95% CI)	n of MAC	P	OR (95% CI)	n of MAC	P	Means* (95% CI) for MAC vs. wildtype	P	Means* (95% CI) for MAC vs. wildtype	P
Entire group										
Standard data analysis†	2.65 (1.14-6.14)	27	0.02	2.47 (1.21-5.03)	39	0.01	111 (102-120) vs. 91 (90-92)	0.0000018	107 (100-114) vs. 91 (90-92)	0.0000031
Adjusted for subgroup‡	3.14 (1.32-7.46)	27	0.01	2.38 (0.97-5.80)	39	0.06	110 (102-119) vs. 91 (90-91)	0.0000008	106 (99-113) vs. 91 (90-91)	0.000003
Test heterogeneity§			0.58			0.82		0.001		0.11
Pooled estimates‖	3.24 (1.33-7.88)	27	0.009	2.81 (1.35-5.89)	39	0.006	108 (101-116) vs. 91 (90-92)	0.0000008	105 (99-111) vs. 91 (90-92)	0.000001
Subgroups										
Females	1.95 (0.52-7.27)	13		2.04 (0.73 –5.69)	18		122 (111-136) vs. 90 (90-91)		112 (103-121) vs. 90 (90-91)	
Males	3.33 (1.10-10.1)	14		3.00 (1.11- 8.15)	21		100 (88-113) vs. 92 (91-93)		102 (91-113) vs. 92 (91-93)	
Severe obesity	1.69 (0.34-8.39)	9		1.75 (0.55-5.57)	16		140 (123-158) vs. 94 (93-95)		120 (108-132) vs. 94 (93-95)	
Coronary artery dis.	4.87 (1.18-20.09)	8		3.86 (1.00-14.84)	9		104 (84-128) vs. 91 (89-94)		105 (86-128) vs. 91 (89-94)	
General population	3.72 (0.74-18.8)	10		3.94 (1.02-15.3)	14		94 (86-103) vs. 88 (87-89)		96 (89-103) vs. 88 (87-89)	

* The re-transformed mean computed on the log-scale.

† via the logistic or linear regression model adjusted for age and sex.

‡ as in † but additionally adjusting for the subgroup

§ tested via including an interaction between the subgroup and the genotype

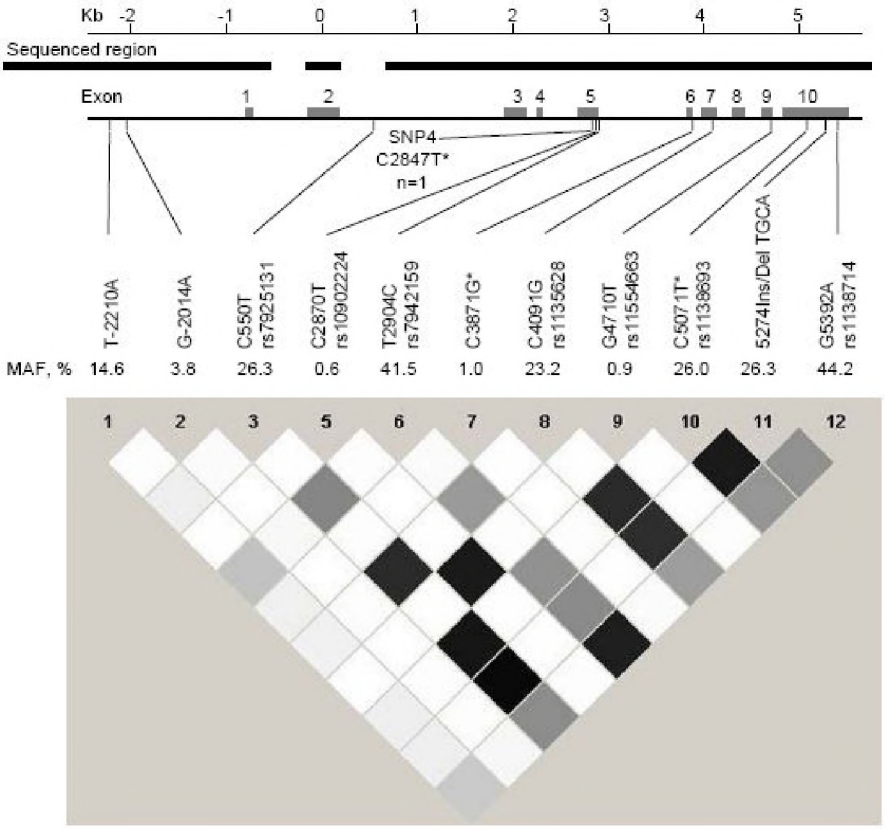
‖ computed as the inverse variance weighted mean of the estimates per subgroup, $b = (\sum_{i=1,2,3} b_i / s_i^2)/(\sum_{i=1,2,3} 1/s_i^2)$, $se(b) = 1/\sqrt{\sum_{i=1,2,3} 1/s_i^2}$ and $95\%CI(b) = b \pm 1.96se(b)$ with b_i being the log(OR) or the difference in glucose levels comparing minor allele carriers and wildtype by subgroup, i=1,2,3, and s_i the corresponding standard error.

OR, odds ratio; CI, confidence interval; MAC, minor allele carrier

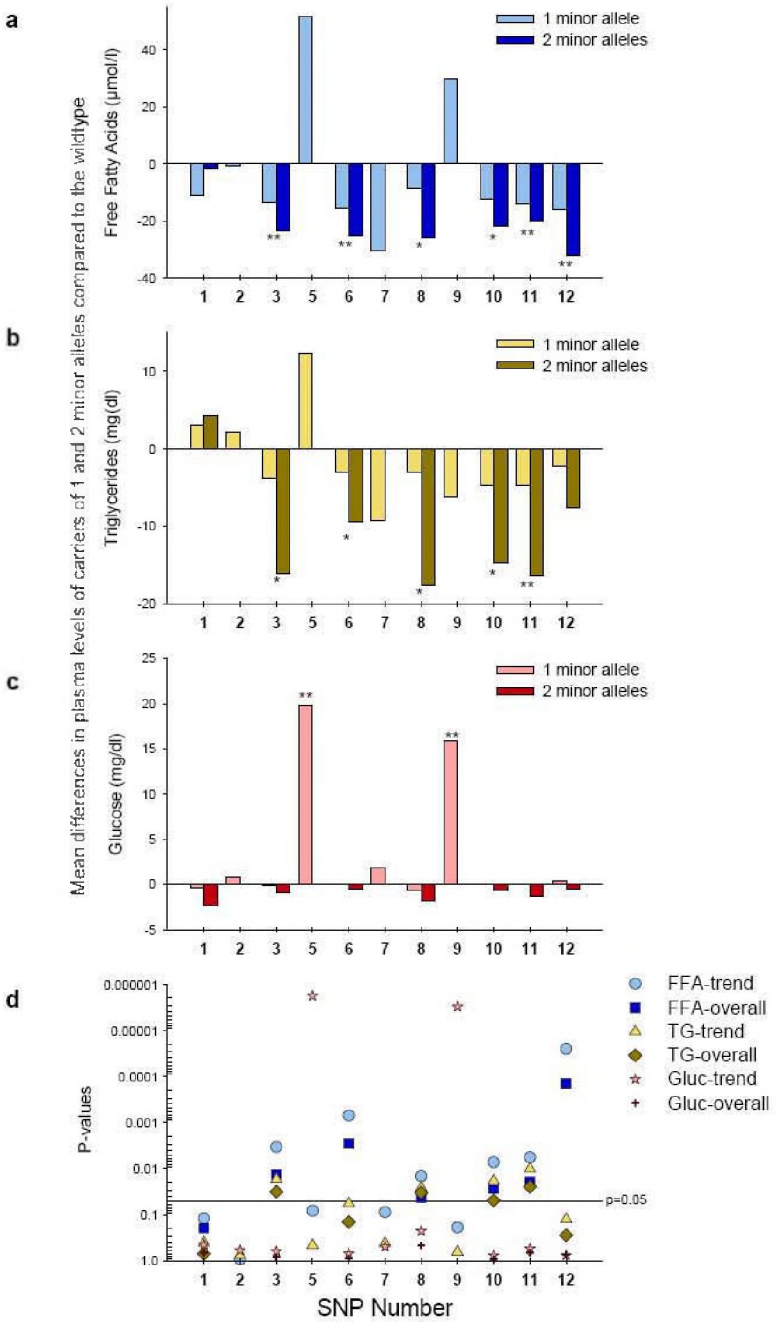
Figure legends

Figure 1. Genetic structure of the *ATGL* gene including the genotyped polymorphism. The top is a schematic of the *ATGL* gene with the sequenced region (shown as thick line with two gaps) as well as the SNP locations. The SNPs are provided with position names as well as rs-numbers. SNPs marked with an asterisk are causing a non-synonymous amino acid exchange: SNP4=L219F, SNP7=N252K, SNP10=P481L. MAF means the minor allele frequency for each SNP. The shade of the diamonds in the lower part of the figure represents the pair-wise r^2 between the two SNPs defined by the top left and the top right sides of the diamond. Shading represents magnitude and significance of the pair-wise r^2, with black reflecting high r^2 (>0.8), dark gray indicating r^2 between 0.8-0.6, moderate gray an r^2 between 0.6-0.4, light gray an r^2 between 0.4-0.2, and white reflecting low r^2 (<0.2).

Figure 2. (a) Primary analysis: association of the 11 analyzed polymorphisms of the *ATGL* gene indicating mean changes in plasma concentrations of free fatty acids (FFAs) for subjects with one (light colored bars) or two copies (dark colored bars) of the minor allele compared to subjects with the wildtype (SNP 4 discarded as observed only in one individual). Results are derived from a linear regression model adjusting for age and sex and a log-transformed outcome. Assuming an additive inheritance the stars mark the SNPs, for which the mean concentration changes compared to the wildtype are statistically significantly different from zero to the 0.05 level (*) or to a level of 0.006 corrected for 9.1 effective loci (**). (b) and (c) Secondary analyses: same as (a) for triglyceride and glucose plasma concentrations, respectively. (d) Summary of p-values testing the association with and without assuming an additive inheritance model (i.e. with p-values assuming a trend per copy of the minor allele or overall p-values). The means±SE of each of the three traits for the genotypes of each SNP are provided in the Suppl.Tables 5-7.



Schoenborn, Heid, Vollmert et al.: Figure 1



Schoenborn, Heid, Vollmert et al.: Figure 2

Online Appendix (Supplementary Material) to:

The *ATGL* gene is associated with free fatty acids, triglycerides and type 2 diabetes

by Veit Schoenborn [1*], Iris M. Heid [2*], Caren Vollmert [2*], Arno Lingenhel [1], Ted D. Adams [3], Paul N. Hopkins [3], Thomas Illig [2], Robert Zimmermann [4], Rudolf Zechner [4], Steven C. Hunt [3], Florian Kronenberg [1]

* authors contributed equally

Material and Methods

Study population

The entire analyzed study population (n=2434) consisted of three groups of subjects of European ancestry recruited in the same geographical region of Utah. The characteristics of these subjects are described in Table 1 of the main document.

First, 1101 subjects with severe obesity (BMI >35 kg/m²) were studied who were either seeking gastric bypass surgery or were randomly chosen from a population-based sample of severely obese participants not seeking gastric bypass surgery. The examination of patients undergoing gastric bypass surgery was done prior to the intervention.

Second, 469 premature, familial coronary artery disease (CAD) cases were studied who had survived a myocardial infarction, percutaneous transluminal angioplasty, or coronary artery bypass grafting before age 55 for men or age 65 for women. To minimize artefactual effects of the acute coronary syndrome on lipid or FFA levels, patients were seen at least 6 months after their acute event. Each of the CAD cases was from a family in which at least one additional first-degree relative had early CAD by this definition.

Third, the general population sample consisted of 864 individuals from the same geographical region and found to be representative of the Utah population (1). They were selected from two almost equally sized sources. The first source consisted of a random

sampling of family members participating in the Utah Health Family Tree Program (2). Briefly, high school students in health classes throughout Utah collected health information on family members with the help of their parents. The data were computerized and feedback provided to students and their families regarding familial disease tendencies. Prior samples identified from Health Family Trees have been studied and found to be representative of the Utah population (1) and are therefore considered as a general population sample. The second source was spouse pairs participating in a study of psychological factors related to CAD. This group was recruited by advertising in newspapers, at conventions, and from the University of Utah community. All three groups of subjects were described in detail elsewhere as part of recent studies (3;4).

Phenotyping

Diabetes was considered present if a prior physician diagnosis had been made or if the fasting blood glucose upon screening was ≥126 mg/dl or when insulin sensitizing agents or diabetes medications were taken by the respective individual. Blood samples were collected after an overnight fasting period. Subjects were only allowed to drink water for 12 hours prior to their clinic visit. Plasma free fatty acids (FFAs) were measured using an enzymatic colorimetric assay from Wako (Richmond, VA). Triglyceride concentrations were measured with a Roche FARA II (Roche Diagnostics, Indianapolis, Indiana) automated analyzer.

Sequencing

Polymorphism detection in the human *ATGL* gene was done by sequence analysis of defined PCR products in the promoter region and in all exons and introns (except 368 bp of intron1/2 and 440 bp of intron 2/3) in 48 normal weight (BM=20-25 kg/m²) and 48 obese individuals (BMI=35-90 kg/m²). The sequenced region is shown in Figure 1 of the main document. Thirteen overlapping PCR products covered the promoter region, and the whole gene from -3290 bp up to 5642 bp according to NM_020376 of the Ensembl database. All PCR and sequencing primers were designed using the Vector NTI suite 9.0 [www.informaxinc.com] (Supplementary Tables 1 and 2). Cycle sequencing of the purified PCR products was performed on the ABI prism 3730 and ABI prism 3130 Genetic Analyser following a standardized protocol (Applied Biosystems, Foster City, CA, USA).

SNP selection

Supplementary Table 3 shows an overview on the SNPs in the ATGL gene from database search and sequencing and how we selected the SNPs for genotyping in the entire group. This selection was mostly guided by potential functional considerations. Thereby, we selected 10 polymorphisms for the following reasons ordered by decreasing priority: resulting amino acid exchange (SNP4, 7, 10), insertion/deletion polymorphism (SNP11), location at the intron-exon boundary (SNP6), location in the untranslated 3'UTR region (SNP12), database entry at the begin of the project (July 2004) (SNP3, 5, 8, 9). For the tagging process we forced-in these ten SNPs into the tagSNP selection program due to the above mentioned considerations and we further selected five out of seven remaining SNPs (positions -2432, -2210, -2014, -1932, -254, -215, 3104) as tagging SNPs using the tagSNP program by Stram and colleagues (5), which minimizes the uncertainty in the prediction of the common haplotypes. With these five additionally selected SNPs, the common haplotypes (frequency>5%) were reconstructed with an r^2 larger than 95%, that is the haplotype variance explained by the genotypes exceeded 95%. The assays of three (-2432, -254, -215) out of the five tagging SNPs turned out to be unstable yielding 12 SNPs for the final analysis.

Genotyping of the SNPs

Supplementary Table 4 describes the 12 polymorphisms selected for genotyping. All SNPs were genotyped by MALDI TOF MS system, (Sequenom, Mass EXTEND, San Diego, USA) as described earlier (6). The insertion/deletion polymorphism (+5274) was genotyped by TaqMan using an ABI Prism 7900 HT (Applied Biosystems, Foster City, CA, USA) and TaqMan MGB "assay by design" reagents. The genotyping success rate of all 12 polymorphisms was above 98%.

Analyzed sample

The original group included 2459 individuals. Twenty-five subjects were excluded from the analysis due to missing age (n=12) or if at least 6 of the 12 SNPs were unsuccessfully genotyped (n=13) leaving 2434 subjects in the analysis. Association analysis of genetic variations in the *ATGL* gene with T2DM was done in these study participants. Fasting triglyceride and glucose levels were available for 2426 and 2429 subjects, respectively. Plasma

for measurement of FFA levels sampled and stored at -80°C in appropriate conditions was available in 1922 individuals. The sample for the analysis of these three plasma levels was further restricted to subjects not taking any anti-diabetic medication suspected to modulate FFA, triglycerides and glucose concentrations leaving 1701, 2193 and 2190 subjects in the analysis. Insulin, metformin, sulfonylurea medications, rosiglitazone and pioglitazone were considered anti-diabetic medications. Statistical haplotype reconstruction was performed in 2228 subjects in whom genotypes of all 11 polymorphisms were available. The haplotype association analysis with T2DM, FFA, triglyceride, and glucose concentrations was performed in 2015, 1563, 2010, and 2008 subjects, respectively. For a detailed description of study participants and the measurements of single variables, see Table 1 of the main document.

Statistical analysis

Genotype and allele frequencies were derived and a test of Hardy-Weinberg equilibrium was performed. The relationship between the SNPs was summarized by the correlation coefficient and Lewontin's D`. The haplotypes were estimated based on the 11 SNPs available for all 2228 subjects via the expectation-maximization algorithm by the SAS® procedure PROC HAPLOTYPE (version 9.0, release 2004) and by using PHASE (7). Both algorithms yielded the same results and the portion of unambiguously defined haplotypes was very high (99%). Rare haplotypes with frequency <0.5% were summarized into one group. SNP4 was observed only in one subject and was not considered in any of the statistical analyses.

The primary association analysis was a linear regression model adjusted for age and gender with logarithm of FFAs as the outcome. The difference of the mean FFA levels (on the log-scale) for subjects with 0, 1 or 2 copies of the minor allele of a SNP was tested for each SNP separately with and without assuming a trend per copy of the minor allele. Haplotype association analysis was carried out with the same statistical model, testing the change of log FFA levels for each copy of a haplotype against the reference (i.e. the group of subjects with two copies of the most common haplotype). We performed a pooled data analysis of all three subgroups (severe obesity, coronary artery disease, general population sample) of study participants via the generalized linear regression model $g^{-1}(Y)=\alpha + \beta 1$ SNP + $\beta 2$ AGE + $\beta 3$ SEX with g=ID for Y=log(glucose) (i.e. $\beta 1$=mean difference of glucose levels between minor allele

carriers and wildtype) and g=logit for Y=prob(T2DM=yes) (i.e. OR=exp($\beta 1$)). A possible heterogeneity of the estimates across the three subgroups was accounted for by including the subgroups as a covariate (i.e. adding + $\beta 4$ CAD + $\beta 5$ GENERAL_POP with CAD=1 if subjects from CAD sample and =0 otherwise and GENERAL_POP analogously) as well as an interaction between subgroup and SNP into the regression model for the initial analysis (i.e. adding + $\beta 6$ SUBGROUP*SNP into the model with SUBGROUB=1 if subject is from severe obese; =2, if from CAD; =3 if from general population sample). In the case of heterogeneity, pooled estimates and corresponding 95% confidence intervals were computed as described (8).

In a secondary analysis, the same statistical procedure as described above for FFA was applied for triglyceride and glucose levels which are expected to be related to FFA metabolism. Association of the SNPs with occurrence of T2DM was tested via logistic regression analysis.

Results of Sensitivity analyses

No heterogeneity of FFA and triglyceride estimates across the three subgroups was observed. Thus, the association analysis was presented as a pooled data analysis. As there was evidence for heterogeneity of the glucose estimates ($p< 0.15$ for all SNPs but SNP1), the pooled estimates of the three subgroups were provided as well. For T2DM ORs, a slight heterogeneity was given ($p<0.1$ for SNP2, 3, 8, 11); the pooled OR estimates were thus added into Table 1 of the main document.

Several sensitivity analyses were performed. Firstly, additional adjustment for BMI was included into the model, but did not show considerable changes (data not shown). Secondly, there was no confounding effect of the three subgroups as no change in the estimates was shown when additionally adjusting for the subgroup (data not shown), which was not surprising given the consistent genotype frequencies across the subgroups (see Suppl.Table 4). Thirdly, subgroup analyses were conducted by group of sample origin (severe obesity, coronary artery disease, general population) and by gender. The direction of the associations of FFA with SNP3, 5, 6, 9, 10, 11, and 12 were consistent in all analyzed subgroups. Due to the small sample size in the subgroups, some of the significance levels were compromised (see more details in the Online Appendix and in Suppl.Tables 5-7). Particularly the T2DM and the glucose

association analysis with the rare SNP5 and SNP9 gave consistent results (Table 2 of the main document). Finally, quantitative phenotype analysis was further restricted to non-diabetic subject to assure that diabetic metabolic conditions did not distort our results. This again showed no major changes (Suppl.Tables 5-7).

Supplementary Table 1: PCR reactions used for SNP screening

Primer ID	1st PCR Primer	2nd PCR Primer	Amplification Product [bp]	Q-Solution	Annealing Temperature	Annealing Time	Extension Time
PCR1	TGACGAGCCCCTGGACTTCCAT	GTTGAGGTGAGTGGATCACGAGG	-3315 - -2299	yes	67°	45s	1:30m
PCR 2	GACCTCGTGATCCACTCACC	TAGTGCACGCATGTAGTCCTAG	-2223 - -1716	yes	69°	45s	1:30m
PCR 3	CCCCACACGTGGTTACCTAC	CCAAGCTGAGTTAGGGAAGC	-1917 - -1110	no	61°	30s	1:00m
PCR 4	ATGTAAATGCCTACCGGCC	GACCCATAAACATCCAAGGC	-1267 - -617	yes	57°	45s	1:30m
PCR 5	CTGGGGAGACAGAATCTCATTG	CGAGATGTTCCACGTCTTCTC	-1017 - 33	yes	57°	45s	1:30m
PCR 6	TAGCTTCTTCGCCTCCGCCA	GCTCACCCAGGCAGACCCCG	-148 - 193	yes	69.5°	45	1:00m
PCR 7	CATGGGGGTAGCCTCTTGAG	CAAACACTCCTCTTGGGGC	605 - 1425	no	64°	30s	1:00m
PCR 8	CACTCCTGACCAGGGTGATG	ATGAGCAGAGGCCTTGAGG	1300 - 2211	no	63°	30s	1:00m
PCR 9	AGTGGGAACCTCAAGGCCTC	AGAGAGAGACTGGACAGGGAGC	2185 - 3101	yes	68°	45s	1:30m
PCR10	TTCCCAGCCACTCCTCACTG	TCTCAGTTCATCAGCGCGGC	2916 - 3955	no	66°	30s	1:00m
PCR 11	GGCCGCGCTGATGAACTGAGAATC	CAGCATGTTGGAGAGGGTGGTCAG	3935 - 4341	yes	68°	45s	1:30m
PCR 12	GAAAGAGAGAGAGGAGAGGACG	CACGTTGGTGCAGAAGAGG	4184 - 4988	yes	62°	45s	1:30m
PCR 13	CGAGGACATCCGGTGGATGA	AAATGCCAGGCCCAGGAGTG	4626 - 5643	yes	68.5°	45s	1:30m

Supplementary Table 2: Sequencing primer for SNP Screening

Primer ID	Sequence
Seq1_-3308	CCCCTGGACTTCCATCCCTA
Seq1_-2966	TGCTGAGACTATAGGTGCGC
Seq1_-2607	AAGTGCTGGGATTACAGGAG
Seq2_-2287	CTGGGATTACAGGTGTGAGC
Seq2_-1936	CTCTAGAACCCCTTAGGTGC
Seq3_-1910	CGTGGTTACCTACCTCCTCTC
Seq3_-1512	TCAGCTCACTGCAACCTCCG
Seq3_-1289	GGCCTCCTCTCATTTTGAGC
Seq4_-1267	ATGTAAATGCCTACCGGCC
Seq4_-940	AGGGATCCTGGATCTACTCC
Seq5_-616	TGTCTGTGGGTGAGCCTGTG
Seq5_-222	ATTGGTCTTCGTGTGCCGGC
Seq6_-148	TAGCTTCTTCGCCTCCGCCA
Seq6_193	GCTCACCCAGGCAGACCCCG
Seq7_606	ATGGGGGTAGCCTCTTGAGAGG
Seq7_992	AACTTTGTCCTGGGAGGGAGGG
Seq8_1301	ACTCCTGACCAGGGTGATGC
Seq8_1602	GCCGGTCAGAGTTGAGTTTC
Seq8_1876	AGCATGGGCCCCTAACCTTG
Seq9_2185	AGTGGGAACCTCAAGGCCTC
Seq9_2443	AACAGCTGTGGCAACGCACG
Seq9_2790	ACCAACATCCACGAGCTGCG
Seq10_2916	TTCCCAGCCACTCCTCACTG
Seq10_3258	TGCCACCATGCCTGGCTAC
Seq10_3616	ACAGGTGTAAGCCACTGCGC
Seq11_3935	GGCCGCGCTGATGAACTGAGAATC
Seq12_4187	AGAGAGAGAGGAGAGGACGGTGAGC
Seq12_4590	TGTCCCCAGCTTGCTGGAG
Seq13_4626	CGAGGACATCCGGTGGATGA
Seq13_5643	AAATGCCAGGCCCAGGAGTG

Supplementary Table 3: Polymorphisms at the *ATGL* locus found in dbSNP database and by own sequencing efforts

Number	Position *	Ref SNP ID	Variation	Location	SNP detection and selection †
1	-2964	rs7950489	C/T	Promoter	dbSNP, not detected
2	-2624	rs12278792	T/C	Promoter	dbSNP, not detected
3	-2432	rs6597978	G/C	Promoter	tagSNP, assay unstable
4	-2210		T/A	Promoter	**SNP 1**, tagSNP
5	-2014		G/A	Promoter	**SNP 2**, tagSNP
6	-1932	rs10902223	C/T	Promoter	offered to tagSNP programme
7	-254	rs7928917	T/G	Intron1	tagSNP, assay unstable
8	-232	rs28535188	T/C	Intron1	dbSNP, not detected
9	-215	rs12785771	C/A	Intron1	tagSNP, assay unstable
10	+409	rs28592068	T/G	Intron2	dbSNP, not detected
11	+550	rs7925131	C/T	Intron2	**SNP 3**
12	+2679	rs1133194	C/G	Exon5 R163G	dbSNP, not detected
13	+2847		C/T	Exon5 L219F	**SNP 4**
14	+2870	rs10902224	C/T	Exon5 L226L	**SNP 5**
15	+2904	rs7942159	T/C	Intron5	**SNP 6**
16	+2979	rs12270408	G/C	Intron5	dbSNP, not detected
17	+3104	rs10544581	- /CTT	Intron5	offered to tagSNP programme
18	+3111	rs10587982	-/TTT	Intron5	dbSNP, not detected
19	+3623	rs11246322	G/C	Intron5	dbSNP, not detected
20	+3871		C/G	Exon6 N252K	**SNP 7**
22	+4091	rs1135628 = rs17851511	C/G	Exon7 P291P	**SNP 8**
23	+4710	rs11554663	G/T	Exon9 L389L	**SNP 9**
24	+5071	rs1138693 = rs17851512	C/T	Exon10 P481L	**SNP 10**
25	+5087	rs17155650	C/T	Exon10 P486P	dbSNP, not detected
26	+5274		TGCA/*	3`UTR	**SNP 11**
27	+5392	rs1138714	G/A	3`UTR	**SNP 12**

* Relating to the first position of the translation starting point ATG (according to dbSNP Build 125 from September 29, 2005)

† Legend to SNP detection and selection:

- **SNP1-12**: bold marked SNPs were genotyped in the entire sample
- offered to tagSNP programme: SNPs offered to the programme but not selected
- tagSNP, assay unstable: SNPs selected as tagging SNPs, but assays were unstable
- dbSNP, not detected: SNPs described in the dbSNP database but not found in our sample

Supplementary Table 4: Genotyped polymorphisms at the *ATGL* locus and their minor allele frequencies (MAF).

					Minor allele frequency (MAF), %			
Number	Position *	Ref SNP ID	Variation	Location	Severe obesity	Coronary artery disease	General population sample	Entire group
1	-2210		T/A	Promoter	15.1	15.1	13.8	14.6
2	-2014		G/A	Promoter	3.4	4.4	3.9	3.8
3	+550	rs7925131	C/T	Intron2	25.8	26.4	27.1	26.3
4	+2847		C/T	Exon5 L219F	0.05 (n=1)	-	-	0.02 (n=1)
5	+2870	rs10902224	C/T	Exon5 L226L	0.5	0.9	0.6	0.6
6	+2904	rs7942159	T/C	Intron5	41.5	41.0	41.8	41.5
7	+3871		C/G	Exon6 N252K	0.9	0.6	1.3	1.0
8	+4091	rs1135628	C/G	Exon7 P291P	22.4	23.4	24.0	23.2
9	+4710	rs11554663	G/T	Exon9 L389L	0.8	1.0	0.8	0.9
10	+5071	rs1138693	C/T	Exon10 P481L	25.6	26.3	26.4	26.0
11	+5274		TGCA/*	3`UTR	26.0	25.6	27.0	26.3
12	+5392	rs1138714	G/A	3`UTR	44.7	43.0	44.3	44.2

* Relating to the first position of the translation starting point ATG (according to dbSNP Build 125 from September 29, 2005)

Supplementary Table 5: Mean(SE)* of fasting free fatty acid levels (µmol/l) and number of subjects for each genotype of each SNP for the entire group or stratified for females and males, severe obesity, coronary artery disease, general population sample as well as nondiabetic subjects.

SNP	Genotype	Entire Group		Males		Females		Severe obesity		Cor.art.dis.		Gen.Pop.Samp.		Nondiabetics	
SNP1	1/1	245(1.01)	1207	217(1.02)	434	261(1.02)	773	260(1.02)	635	273(1.04)	155	214(1.02)	417	243(1.01)	1165
	1/2	234(1.02)	441	203(1.04)	153	252(1.03)	288	236(1.03)	226	244(1.07)	73	225(1.04)	142	230(1.02)	423
	2/2	243(1.08)	32	221(1.17)	9	258(1.10)	23	249(1.10)	18	216(1.37)	3	240(1.16)	11	243(1.08)	32
SNP2	1/1	242(1.01)	1564	214(1.02)	549	259(1.01)	1015	254(1.01)	834	264(1.04)	209	216(1.02)	521	240(1.01)	1512
	1/1+2/2	241(1.04)	129	220(1.07)	48	254(1.05)	81	238(1.05)	59	251(1.12)	23	239(1.07)	47	237(1.04)	121
SNP3	1/1	249(1.01)	930	221(1.03)	306	266(1.02)	624	257(1.02)	492	268(1.05)	136	228(1.03)	302	247(1.02)	894
	1/2	236(1.02)	634	212(1.03)	231	249(1.02)	403	249(1.02)	342	260(1.06)	86	207(1.03)	206	233(1.02)	611
	2/2	226(1.04)	118	186(1.07)	51	255(1.06)	67	256(1.05)	56	227(1.19)	10	201(1.07)	52	226(1.04)	117
SNP5	1/1	242(1.01)	1653	214(1.02)	575	258(1.01)	1078	253(1.01)	877	262(1.04)	227	218(1.02)	549	240(1.01)	1598
	1/2+2/2	293(1.12)	17	248(1.20)	7	323(1.15)	10	318(1.15)	8	313(1.28)	5	220(1.28)	4	249(1.13)	13
SNP6	1/1	254(1.02)	580	226(1.03)	198	271(1.02)	382	262(1.02)	313	287(1.06)	80	228(1.04)	187	253(1.02)	561
	1/2	238(1.02)	805	212(1.03)	276	254(1.02)	529	251(1.02)	410	260(1.05)	122	211(1.03)	273	235(1.02)	771
	2/2	229(1.03)	293	201(1.04)	117	247(1.03)	176	243(1.03)	158	219(1.10)	30	216(1.05)	105	228(1.03)	288
SNP7	1/1	242(1.01)	1644	214(1.02)	579	259(1.01)	1065	254(1.01)	862	263(1.04)	226	218(1.02)	556	240(1.01)	1588
	1/2+2/2	212(1.08)	35	177(1.15)	11	233(1.10)	24	207(1.10)	18	268(1.48)	2	217(1.13)	15	208(1.08)	33
SNP8	1/1	246(1.01)	1017	218(1.03)	340	263(1.02)	677	257(1.02)	537	262(1.05)	146	223(1.03)	334	244(1.01)	977
	1/2	238(1.02)	578	214(1.03)	216	251(1.02)	362	246(1.02)	306	271(1.06)	77	214(1.04)	195	235(1.02)	560
	2/2	221(1.05)	93	184(1.08)	39	247(1.06)	54	250(1.06)	42	220(1.20)	9	196(1.08)	42	219(1.05)	91
SNP9	1/1	241(1.01)	1657	214(1.02)	584	258(1.01)	1073	253(1.01)	874	262(1.04)	223	218(1.02)	560	240(1.01)	1602
	1/2+2/2	271(1.09)	27	218(1.15)	11	310(1.12)	16	300(1.11)	13	305(1.25)	6	208(1.19)	8	246(1.10)	22
SNP10	1/1	249(1.01)	932	219(1.03)	312	266(1.02)	620	258(1.02)	494	272(1.05)	134	225(1.03)	304	246(1.02)	895
	1/2	236(1.02)	627	214(1.03)	229	249(1.02)	398	248(1.02)	333	255(1.06)	89	212(1.03)	205	234(1.02)	606
	2/2	227(1.04)	117	185(1.07)	45	255(1.05)	72	257(1.05)	59	219(1.20)	9	199(1.07)	49	226(1.04)	115
SNP11	1/1	249(1.01)	937	220(1.03)	312	265(1.02)	625	257(1.02)	491	270(1.05)	139	226(1.03)	307	247(1.02)	899
	1/2	235(1.02)	627	211(1.03)	226	248(1.02)	401	247(1.02)	346	265(1.07)	73	207(1.03)	208	232(1.02)	607
	2/2	229(1.04)	119	190(1.07)	48	255(1.05)	71	257(1.05)	58	241(1.20)	9	202(1.07)	52	228(1.04)	117
SNP12	1/1	257(1.02)	520	227(1.04)	179	275(1.02)	341	266(1.02)	278	284(1.06)	77	231(1.04)	165	255(1.02)	503
	1/2	241(1.02)	827	218(1.03)	284	254(1.02)	543	251(1.02)	427	262(1.05)	125	215(1.03)	275	238(1.02)	791
	2/2	225(1.03)	328	190(1.04)	124	247(1.03)	204	244(1.03)	181	218(1.10)	30	203(1.05)	117	223(1.03)	321

* The means given are the exponentiated means computed on the log-scale (i.e. the geometric mean) and the corresponding exponentiated standard error. The standard error is thus a multiplicative standard error, and corresponding 95% confidence intervals can be computed by mean*(s.e.)$^{1.96}$ and mean/(s.e.)$^{1.96}$.

Supplementary Table 6: Mean(SE)* of fasting triglyceride levels (mg/dl) and number of subjects for each genotype of each SNP for the entire group or stratified for females and males, severe obesity, coronary artery disease, general population sample as well as nondiabetic subjects.

SNP	Genotype	Entire Group		Males		Females		Severe obesity		Cor.art.dis.		Gen.Pop.Samp.		Nondiabetics	
SNP1	1/1	151.3(1.01)	1581	157.9(1.02)	655	146.9(1.02)	926	159.3(1.02)	648	176.6(1.03)	321	132.0(1.02)	612	148.6(1.01)	1504
	1/2	154.3(1.02)	549	159.7(1.04)	213	150.5(1.03)	336	160.1(1.03)	226	176.9(1.05)	116	137.3(1.04)	207	151.9(1.02)	522
	2/2	155.6(1.08)	42	176.3(1.15)	14	145.7(1.10)	28	170.7(1.11)	19	167.8(1.18)	11	126.8(1.16)	12	154.8(1.08)	40
SNP2	1/1	152.5(1.01)	2020	157.7(1.02)	815	149.0(1.01)	1205	161.1(1.02)	843	175.1(1.03)	410	133.1(1.02)	767	150.3(1.01)	1928
	1/1+2/2	154.6(1.04)	164	174.7(1.07)	68	142.5(1.05)	96	152.5(1.06)	63	195.0(1.09)	39	137.5(1.07)	62	147.6(1.04)	150
SNP3	1/1	155.5(1.01)	1187	160.2(1.03)	449	152.3(1.02)	738	163.9(1.02)	497	178.1(1.04)	246	136.3(1.02)	444	153.2(1.01)	1130
	1/2	151.8(1.02)	824	164.5(1.03)	344	143.3(1.02)	480	157.5(1.02)	349	179.2(1.04)	169	132.4(1.03)	306	148.4(1.02)	778
	2/2	139.4(1.04)	160	140.3(1.06)	79	141.7(1.05)	81	149.1(1.06)	58	155.9(1.10)	33	124.8(1.06)	69	139.5(1.04)	157
SNP5	1/1	153.0(1.01)	2137	160.7(1.02)	855	148.0(1.01)	1282	160.4(1.02)	891	176.5(1.03)	442	134.1(1.02)	804	150.6(1.01)	2036
	1/2+2/2	165.3(1.11)	23	151.8(1.17)	11	181.1(1.15)	12	187.9(1.17)	8	194.2(1.23)	7	127.6(1.19)	8	140.3(1.12)	19
SNP6	1/1	155.6(1.02)	753	160.0(1.03)	293	152.6(1.02)	460	163.1(1.03)	319	181.0(1.05)	150	136.1(1.03)	284	153.6(1.02)	720
	1/2	152.5(1.02)	1035	162.8(1.03)	415	145.9(1.02)	620	158.7(1.02)	414	181.7(1.04)	228	132.8(1.03)	393	149.0(1.02)	977
	2/2	146.2(1.03)	382	149.1(1.04)	169	144.9(1.03)	213	158.0(1.04)	162	154.1(1.07)	71	129.7(1.04)	149	145.5(1.03)	369
SNP7	1/1	152.4(1.01)	2126	158.6(1.02)	857	148.4(1.01)	1269	160.1(1.02)	876	176.8(1.03)	440	133.3(1.02)	810	149.9(1.01)	2024
	1/2+2/2	143.2(1.08)	44	149.0(1.14)	18	139.5(1.10)	26	150.0(1.11)	18	169.2(1.28)	5	131.9(1.12)	21	141.9(1.08)	42
SNP8	1/1	154.5(1.01)	1296	159.8(1.02)	499	150.9(1.02)	797	162.4(1.02)	540	178.5(1.03)	267	135.2(1.02)	489	152.0(1.01)	1234
	1/2	151.4(1.02)	755	162.2(1.03)	319	144.2(1.02)	436	157.4(1.03)	316	178.6(1.04)	154	132.5(1.03)	285	148.3(1.02)	715
	2/2	136.9(1.05)	128	136.7(1.07)	62	139.7(1.06)	66	151.2(1.07)	43	149.5(1.11)	27	122.2(1.07)	58	136.6(1.05)	124
SNP9	1/1	152.5(1.01)	2142	159.6(1.02)	865	147.8(1.01)	1277	160.1(1.02)	888	176.3(1.03)	437	133.8(1.02)	817	150.0(1.01)	2041
	1/2+2/2	146.3(1.09)	33	130.1(1.15)	15	163.1(1.12)	18	154.4(1.13)	13	206.1(1.21)	8	109.3(1.16)	12	134.4(1.10)	28
SNP10	1/1	155.7(1.01)	1193	160.2(1.03)	457	152.6(1.02)	736	163.8(1.02)	499	179.9(1.04)	244	136.3(1.02)	450	153.4(1.01)	1135
	1/2	151.1(1.02)	816	164.7(1.03)	342	142.1(1.02)	474	156.6(1.02)	341	176.1(1.04)	173	132.7(1.03)	302	147.8(1.02)	771
	2/2	141.1(1.04)	157	139.4(1.06)	72	144.9(1.05)	85	156.2(1.06)	60	157.0(1.10)	31	122.0(1.06)	66	141.0(1.04)	154
SNP11	1/1	155.3(1.01)	1175	159.7(1.03)	445	152.3(1.02)	730	164.0(1.02)	496	180.0(1.04)	237	135.4(1.02)	442	153.2(1.01)	1119
	1/2	150.6(1.02)	802	163.1(1.03)	329	142.5(1.02)	473	156.8(1.02)	354	179.0(1.04)	150	131.0(1.03)	298	147.4(1.02)	761
	2/2	139.0(1.04)	160	138.8(1.06)	76	141.6(1.05)	84	151.4(1.06)	59	157.8(1.10)	31	122.3(1.06)	70	138.6(1.04)	156
SNP12	1/1	155.5(1.02)	679	160.7(1.03)	265	152.3(1.02)	414	162.6(1.03)	284	181.7(1.05)	139	135.9(1.03)	256	153.2(1.02)	649
	1/2	153.3(1.02)	1059	163.8(1.03)	425	146.4(1.02)	634	159.9(1.02)	430	179.8(1.04)	233	133.9(1.03)	396	149.9(1.02)	998
	2/2	148.0(1.02)	427	151.0(1.04)	181	146.6(1.03)	246	158.9(1.03)	186	159.8(1.06)	77	130.7(1.04)	164	147.3(1.02)	412

* The means given are the exponentiated means computed on the log-scale (i.e. the geometric mean) and the corresponding exponentiated standard error. The standard error is thus a multiplicative standard error, and corresponding 95% confidence intervals can be computed by mean*(s.e.)$^{1.96}$ and mean/(s.e.)$^{1.96}$.

Supplementary Table 7: Mean(SE)* of fasting glucose levels (mg/dl) and number of subjects for each genotype of each SNP for the entire group or stratified for females and males, severe obesity, coronary artery disease, general population sample as well as nondiabetic subjects.

SNP	Genotype	Entire Group		Males		Females		Severe obesity		Cor.art.dis.		Gen.Pop.Samp.		Nondiabetics	
SNP1	1/1	91.4(1.00)	1579	92.3(1.01)	653	90.9(1.01)	926	94.0(1.01)	649	92.9(1.02)	319	88.0(1.01)	611	89.9(1.00)	1504
	1/2	91.1(1.01)	548	92.2(1.01)	212	90.3(1.01)	336	94.6(1.01)	226	89.1(1.03)	115	88.4(1.01)	207	89.3(1.01)	521
	2/2	89.2(1.03)	42	90.2(1.06)	14	88.5(1.04)	28	93.7(1.04)	19	85.8(1.09)	11	85.9(1.04)	12	88.9(1.03)	40
SNP2	1/1	91.3(1.00)	2019	92.0(1.01)	813	90.7(1.01)	1206	94.5(1.01)	845	90.7(1.01)	408	88.1(1.00)	766	89.8(1.00)	1929
	1/1+2/2	92.0(1.02)	162	94.6(1.03)	67	90.3(1.02)	95	90.8(1.02)	62	102.3(1.05)	38	87.8(1.02)	62	88.9(1.01)	148
SNP3	1/1	91.5(1.01)	1185	92.1(1.01)	447	91.0(1.01)	738	94.5(1.01)	498	91.6(1.02)	244	88.3(1.01)	443	89.8(1.00)	1129
	1/2	91.4(1.01)	823	93.1(1.01)	343	90.2(1.01)	480	93.6(1.01)	349	93.2(1.02)	168	87.8(1.01)	306	89.6(1.01)	778
	2/2	90.5(1.02)	160	89.3(1.02)	79	92.0(1.02)	81	96.3(1.02)	58	85.6(1.05)	33	87.9(1.02)	69	90.5(1.01)	157
SNP5	1/1	91.1(1.00)	2135	92.1(1.01)	852	90.5(1.01)	1283	93.9(1.01)	893	91.5(1.01)	439	87.9(1.00)	803	89.6(1.00)	2036
	1/2+2/2	110.9(1.04)	23	99.8(1.07)	11	122.5(1.05)	12	139.6(1.07)	8	104.0(1.11)	7	94.2(1.05)	8	102.7(1.04)	19
SNP6	1/1	91.3(1.01)	750	92.0(1.01)	291	90.8(1.01)	459	93.2(1.01)	319	93.9(1.02)	148	87.8(1.01)	283	89.8(1.01)	719
	1/2	91.3(1.01)	1035	92.8(1.01)	414	90.3(1.01)	621	94.1(1.01)	415	91.6(1.02)	227	88.3(1.01)	393	89.3(1.01)	977
	2/2	90.8(1.01)	382	90.9(1.02)	169	90.8(1.01)	213	94.8(1.01)	162	87.4(1.03)	71	87.8(1.01)	149	90.1(1.01)	369
SNP7	1/1	91.2(1.00)	2123	92.1(1.01)	854	90.7(1.01)	1269	94.2(1.01)	877	91.6(1.01)	437	88.0(1.00)	809	89.7(1.00)	2023
	1/2+2/2	93.1(1.03)	44	92.5(1.05)	18	93.6(1.04)	26	96.7(1.04)	18	79.9(1.13)	5	92.9(1.03)	21	91.6(1.03)	42
SNP8	1/1	91.5(1.01)	1295	92.4(1.01)	497	90.9(1.01)	798	94.5(1.01)	542	91.8(1.02)	265	88.3(1.01)	488	89.9(1.00)	1234
	1/2	90.9(1.01)	753	92.4(1.01)	318	89.9(1.01)	435	92.9(1.01)	315	92.8(1.02)	153	87.7(1.01)	285	89.2(1.01)	714
	2/2	89.7(1.02)	128	88.8(1.03)	62	90.8(1.02)	66	95.8(1.03)	43	83.9(1.06)	27	87.8(1.02)	58	89.7(1.01)	124
SNP9	1/1	91.1(1.00)	2139	92.1(1.01)	862	90.5(1.01)	1277	93.9(1.01)	889	91.5(1.01)	434	88.0(1.00)	816	89.6(1.00)	2040
	1/2+2/2	106.9(1.03)	33	101.7(1.06)	15	111.7(1.04)	18	119.7(1.05)	13	104.8(1.11)	8	95.9(1.04)	12	100.4(1.03)	28
SNP10	1/1	91.4(1.01)	1192	92.0(1.01)	456	91.0(1.01)	736	94.3(1.01)	500	91.8(1.02)	243	88.2(1.01)	449	89.7(1.00)	1135
	1/2	91.4(1.01)	815	93.3(1.01)	340	90.0(1.01)	475	93.7(1.01)	342	92.9(1.02)	171	87.8(1.01)	302	89.6(1.01)	771
	2/2	90.7(1.02)	157	88.9(1.03)	72	92.5(1.02)	85	97.0(1.02)	60	84.9(1.05)	31	87.8(1.02)	66	90.7(1.01)	154
SNP11	1/1	91.3(1.01)	1172	91.7(1.01)	443	91.0(1.01)	729	94.6(1.01)	496	90.8(1.02)	235	88.3(1.01)	441	89.8(1.00)	1117
	1/2	91.3(1.01)	802	93.5(1.01)	328	89.9(1.01)	474	93.5(1.01)	355	93.5(1.02)	149	87.5(1.01)	298	89.7(1.01)	762
	2/2	90.0(1.02)	160	89.2(1.03)	76	91.0(1.02)	84	94.9(1.02)	59	85.4(1.05)	31	87.8(1.01)	70	90.0(1.01)	156
SNP12	1/1	91.3(1.01)	677	91.6(1.01)	264	91.0(1.01)	413	93.2(1.01)	284	94.3(1.02)	138	87.7(1.01)	255	89.7(1.01)	648
	1/2	91.6(1.01)	1059	93.4(1.01)	423	90.4(1.01)	636	94.5(1.01)	432	92.1(1.02)	231	88.3(1.01)	396	89.7(1.01)	999
	2/2	90.8(1.01)	427	90.6(1.02)	181	91.0(1.01)	246	95.2(1.01)	186	85.8(1.03)	77	87.9(1.01)	164	90.1(1.01)	412

* The means given are the exponentiated means computed on the log-scale (i.e. the geometric mean) and the corresponding exponentiated standard error. The standard error is thus a multiplicative standard error, and corresponding 95% confidence intervals can be computed by mean*(s.e.)$^{1.96}$ and mean/(s.e.)$^{1.96}$.

Supplementary Table 8: Haplotypes, haplotype frequencies, average free fatty acid levels, 95% confidence intervals (95% CI), and p-values.

Haplotypes		Frequency ± SE*	Average (95%CI) FFA levels (mg/dl) for carriers of 0, 1, or 2 copies of the haplotype			P-value
			no copy	1 copy	2 copies (if occurring)	
SNP-Number	1 2 3 5 6 7 8 9 0 1 2					
H1	2-1-1-1-2-1-1-1-1-1-2	0.134 ± 0.005	246 (239-253) n=1158	228 (218-239) n=377	238 (200-282) n=28	0.03†
H2	2-1-1-1-2-2-1-1-1-1-2	0.010 ± 0.001	242 (237-248) n=1529	204 (175-239) n=34	-	0.02
H3	1-2-1-1-1-1-1-1-1-1-1	0.034 ± 0.003	242 (237-248) n=1459	230 (210-251) n=104	-	0.22
H4	1-1-1-1-1-1-1-1-1-1-2	0.024 ± 0.002	243 (237-248) n=1484	219 (198-243) n=79	-	0.03
H5	1-1-1-1-1-1-1-1-1-1-1	0.514 ± 0.007	-			
H6	1-1-1-2-2-1-1-2-1-1-2	0.005 ± 0.001	241 (236-247) n=1549	283 (222-360) n=14	-	0.20
H7	1-1-2-1-2-1-1-1-1-2-2	0.007 ± 0.001	242 (236-247) n=1546	204 (164-255) n=17	-	0.33
H8	1-1-2-1-2-1-1-1-2-2-2	0.024 ± 0.002	243 (237-248) n=1489	216 (194-240) n=74	-	0.04
H9	1-1-2-1-2-1-2-1-2-2-2	0.221 ± 0.006	248 (241-256) n=967	233 (224-243) n=514	213 (193-236) n=82	0.0009‡

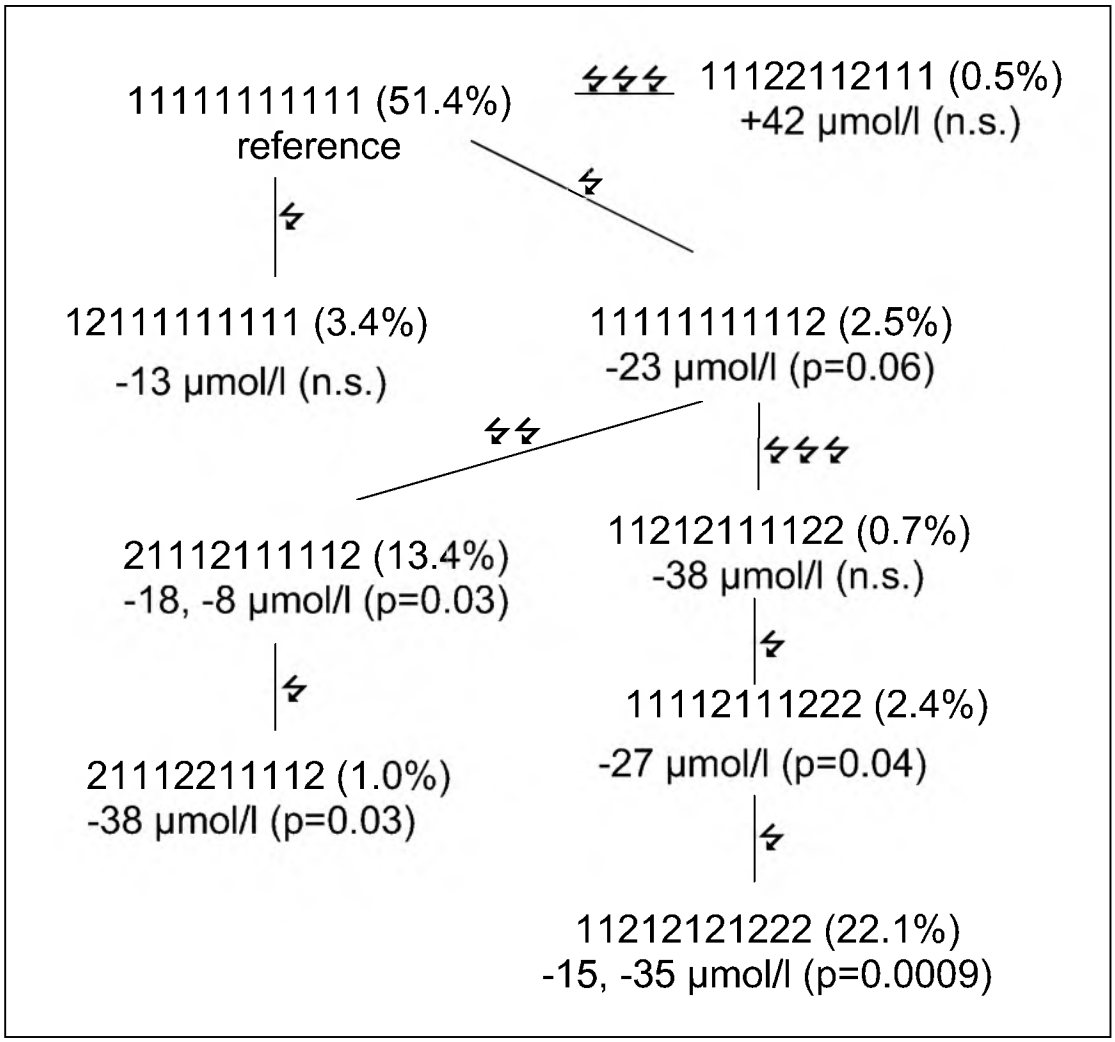
The "average FFA levels" are the exponentiated means computed on the log-scale of the free fatty acid (FFA) levels from haplotype association analysis via linear regression model adjusted for age and sex. The confidence intervals are the exponentiated confidence intervals computed on the log-scale. The stated p-values are for the test whether the average FFA levels for subjects with zero, one, or – where possible – two copies of the haplotype are significantly different while adjusting for age and sex and adjusting for all other haplotypes except the reference (i.e. the most common haplotype, H5).

* expected haplotype frequency and standard error;

† not assuming a trend per copy, ‡ assuming a trend per copy

Supplementary Figure 1

Minimal spanning net for haplotypes: starting from the most common haplotype, the cascade indicates distance of each haplotype in terms of number of mutations away from the most common haplotype. The number of flash signs indicate the number of mutations away from the haplotype before. Also stated are the frequencies of the haplotypes, the average change (i.e. exponentiated mean computed on the log-scale) in FFAs (in µmol/l) for subjects carrying one or two haplotypes compared to the reference, and the p-value testing for significance (for log FFAs) assuming a trend per haplotype copy where appropriate.



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