Brief Genetics Report

The *ATGL* Gene Is Associated With Free Fatty Acids, Triglycerides, and Type 2 Diabetes

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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, 12 polymorphisms identified via sequencing and database search were studied in 2,434 individuals of European ancestry from Utah. These polymorphisms and their haplotypes were analyzed in subjects not taking diabetes medication for association with plasma free fatty acids (FFAs) as primary analysis, as well as triglycerides and glucose as a secondary analysis (n = 1,701, 2,193, or 2,190, respectively). Furthermore, type 2 diabetes (n = 342 of 2,434) was analyzed as an outcome. FFA concentrations were significantly associated with several single nucleotide polymorphisms (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 type 2 diabetes (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. *Diabetes* 55:1270–1275, 2006

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Received for publication 15 November 2005 and accepted in revised form 30 January 2006.

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Additional information on this article can be found in an online appendix available at http://diabetes.diabetesjournals.org.

ATGL, adipose triglyceride lipase; FFA, free fatty acid; SNP, single nucleotide polymorphism.

DOI: 10.2337/db05-1498

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Understanding of pathogenetic mechanisms, including the genetic components of type 2 diabetes, is a major challenge for research. However, finding genes for a complex disease such as type 2 diabetes is often an extremely difficult and long-lasting process. One of the reasons might be the difficulty in phenotyping disease end points, to which many pathways contribute with completely different genes involved. The influence of one gene to the disease end point 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 type 2 diabetes.

FFAs play a major role in the pathogenesis of type 2 diabetes, 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 is the enzyme primarily responsible 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, and PNPLA2) (3–5). Overexpression of *ATGL* enhanced the lipolysis in adipocytes, and inhibition did the opposite (3). Villena et al. (5) observed the level of mRNA highly upregulated in fasting mice and reduced after refeeding. 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 hormone-sensitive lipase (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 type 2 diabetes.

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TABLE 1
Characteristics of 2,434 participants from the three recruited groups of subjects

	Severe obesity	Coronary artery disease	General population
n	1,101	469	864
Females	82	24	52
Age			
<30 years	13	0	0
30–40 years	22	2	3
40–50 years	30	21	38
50–60 years	27	52	34
≥60 years	8	25	24
BMI (kg/m^2)	45.8 ± 7.6	28.2 ± 5.0	27.6 ± 4.9
Cardiovascular event	1.8	100	2.1
Type 2 diabetes analyzed in 2,434 subjects*	20.1 (n=1,101)	19.0 (n=469)	7.3 (n=864)
Fasting FFAs (mg/dl)† analyzed in 1,701 subjects*	272 ± 102 (n = 898)	301 ± 181 (n = 232)	244 ± 128 (n = 571)
Fasting triglycerides (mg/dl) analyzed in 2,193 subjects*	178.5 ± 92.5 (n = 912)	206.5 ± 133.5 (n = 449)	153.1 ± 99.4 (n = 832)
Fasting glucose (mg/dl) analyzed in 2,190 subjects*	96.1 ± 22.7 (n = 913)	96.3 ± 38.7 (n = 446)	88.9 ± 13.8 (n = 831)

Data are means ± SD or percent. *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 FFAs, triglycerides, and glucose, that no antidiabetes medications were taken. †Plasma FFAs were only measured in 1,922 out of 2,434 subjects with stored plasma samples available.

RESEARCH DESIGN AND METHODS

The study is based on 2,434 individuals of European ancestry from Utah, recruited either for severe obesity (BMI between 35 and 90 kg/m^2, n = 1,101), coronary artery disease (n = 469), or as a 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 (available at http://diabetes.diabetesjounrals.org).

Briefly, from sequencing 96 subjects and database information, 12 polymorphisms were selected (Fig. 1 and online appendix 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 antidiabetes medication. The analogous analysis for the outcome type 2 diabetes 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 (Fig. 1; for PCR and primers see online appendix Tables 1 and 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, and 10), insertion/deletion polymorphism (SNP 11), location at the intron-exon boundary (SNP 6), location in the 3′ untranslated region (SNP 12), database entry at the beginning of the project (July 2004) (SNPs 3, 5, 8, and 9), or being a tagging SNP (SNPs 1 and 2) (online appendix Table 3). These 12 polymorphisms (online appendix Table 4) were genotyped in the entire group of 2,434 individuals. None of the genotyped SNPs violated Hardy-Weinberg equilibrium. One variant (SNP 4) 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 (online appendix Table 4).

The gene structure of ATGL, the frequenies of the minor alleles for each SNP, and the correlation of alleles between various SNPs are depicted in Fig. 1. There were three SNPs without notable correlation to any other (SNPs 1, 2, and 7; $r < 0.50$). The rare polymorphisms 5 and 9 were highly correlated ($r = 0.95$). SNPs 3, 6, 8, 10, 11, and 12 showed moderate to high inter-SNP correlations ($r > 0.60$) with SNP 6 and 12 ($r = 0.94$) and SNPs 3, 8, 10, and 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 FFAs. Figure 2*A* 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, SNP 6 and 12, as well as SNPs 3, 8, 10, and 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 SNP 12, with mean FFA values (retransformed mean computed on the log-scale) of 257, 241, and 225 μmol/l for 0, 1, and 2 minor allele copies (P = 0.00003), respectively. SNPs 5 and 9 showed a tendency toward 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 ~15–20% of the mean, ranging from −30 to +50 μmol/l. The mean (±SE) levels of FFAs for each genotype are provided in online appendix 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. The pattern was the same when analyzing triglyceride concentrations in our secondary analysis (Fig. 2*B* and online appendix Table 6), with P values that were less pronounced compared with FFA levels (lowest P value = 0.01 for SNP 11). No evidence for heterogeneity of the triglyceride estimates in the three subgroups was observed. Spearman correlation coefficient between FFAs and triglycerides was 0.29 and between FFAs and glucose

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TABLE 2
Odds ratio (95% CI) for type 2 diabetes (n = 342 out of 2,434) and average glucose concentrations (n = 2,190 due to restriction to subjects with no diabetes medication) for SNP5 and SNP9

	Type 2 diabetes		Fasting glucose concentrations (mg/dl)	
	SNP5 [OR (95% CI); n of MAC; P]	SNP9 [OR (95% CI); n of MAC; P]	SNP5 [means* (95% CI) for MAC vs. wild type; P]	SNP9 [means* (95% CI) for MAC vs. wild type; 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				
Female subjects	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)
Male subjects	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 disease	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 retransformed mean computed on the log-scale. †Via the logistic or linear regression model adjusted for age and sex. ‡As in † but additional 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,

$$b = \left(\sum_{i=1,2,3} b_i/s_i^2\right) \Big/ \left(\sum_{i=1,2,3} 1/s_i^2\right),\ se(b) = 1/\sqrt{\sum_{i=1,2,3} 1/s_i^2}$$

and 95% $CI(b) = b \pm 1.96\ se(b)$ with b_i being the log(OR) or the difference in glucose levels comparing minor allele carriers and wild-type by subgroup, i = 1,2,3, and s_i the corresponding standard error. MAC, minor allele carrier.

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 SNPs 5 and 9, respectively, compared with the wild type ($P < 0.00001$ for both SNPs) (Fig. 2*C*, Table 1, and online appendix Table 7). This association was consistent with the direction of the change in FFAs 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 type 2 diabetes with odds ratios of 2.65 and 2.47, respectively (Table 2). It can be seen that the type 2 diabetes odds ratios 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 nondiabetic subjects markedly alter the results (online appendix Tables 5–7).

Haplotype analysis. Haplotype analysis provided consistent findings. We statistically reconstructed haplotypes based on the 2,228 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 (online appendix 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, and 223 μmol/l; $P < 0.0001$ assuming a trend for increasing number of haplotypes different from the reference) when compared with 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 online appendix Table 8. It can be seen that only the haplotype H6 containing the minor allele of SNPs 5 and 9 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 (SNP 7 = N252K and SNP 10 = P481L), were among the most convincing. Overall, the statistical significance for four of nine haplotypes indicated a good differentiation of FFA levels by the *ATGL* haplotypes. The relationship between haplotypes can be viewed in a minimal spanning net (online appendix Fig. 1). Consistent with the associations of the rare alleles in SNPs 5 and 9 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 SNPs 5 and 9.

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 type 2 diabetes, which altogether suggests an important role of this gene in the pathways of the metabolic syndrome.

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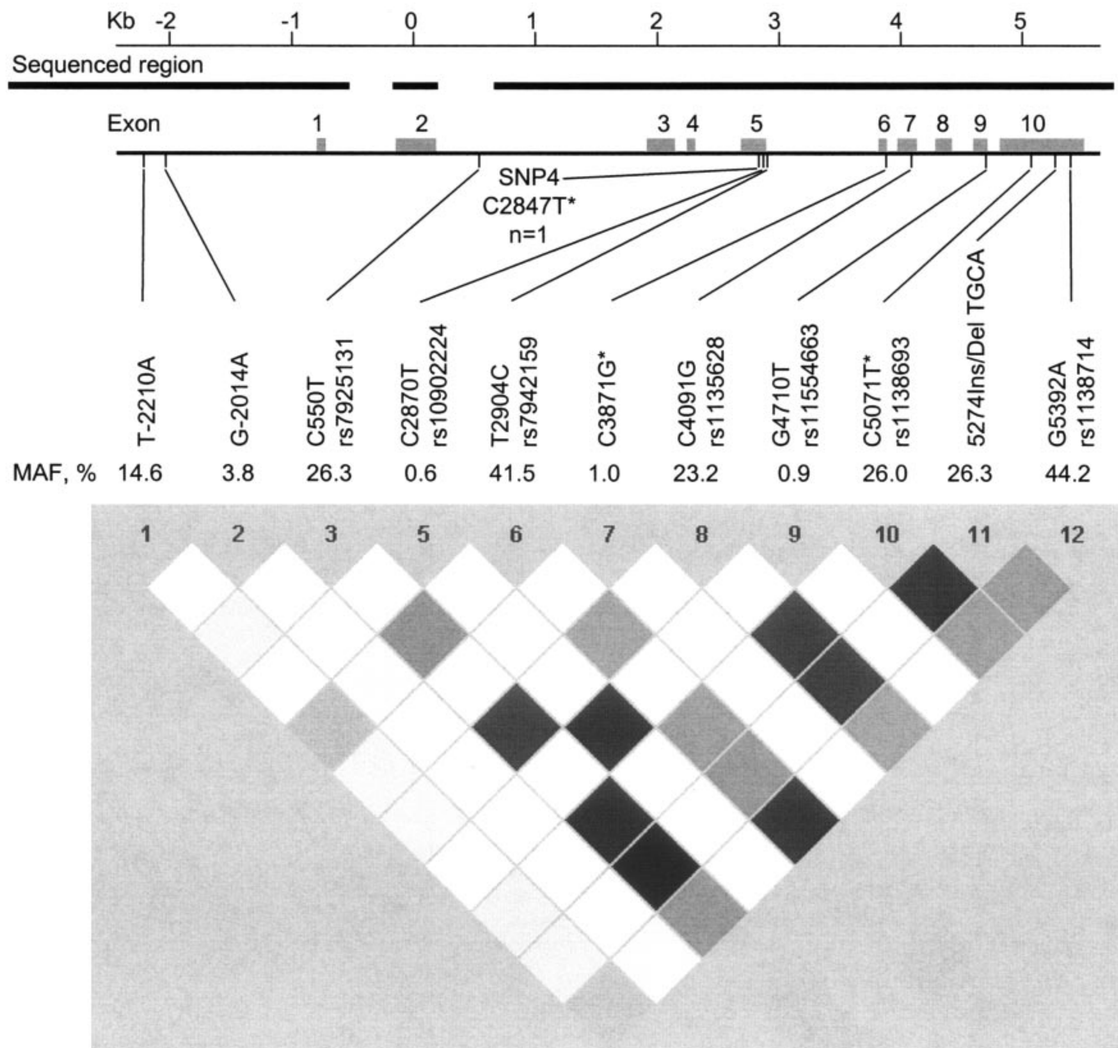

FIG. 1. Genetic structure of the *ATGL* gene, including the genotyped polymorphism. *A*: Schematic of the *ATGL* gene with the sequenced region (shown as thick line with two gaps) as well as the SNP locations. The SNPs are provided with position names as well as rs numbers. SNPs marked with an asterisk are causing a nonsynonymous amino acid exchange: SNP 4 = L219F, SNP 7 = N252K, and SNP 10 = P481L. MAF, minor allele frequency. *B*: The shade of the diamonds represents the pairwise 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 pairwise r^2, with black reflecting high r^2 (>0.8), dark gray indicating r^2 between 0.8 and 0.6, moderate gray an r^2 between 0.6 and 0.4, light gray an r^2 between 0.4 and 0.2, and white reflecting low r^2 (<0.2).

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); and reduction of mRNA in adipose tissue of genetically obese mice (5). *ATGL* also functions in nonadipocyte 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 FFAs were one of the expected intermediate phenotypes to be associated with *ATGL* variation. However, it has to be considered that the pool of FFAs is supplied not only from the hydrolysis of FFAs from triacylglycerol by *ATGL* but also from hydrolysis of diacylglycerols by hormone-sensitive lipase 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

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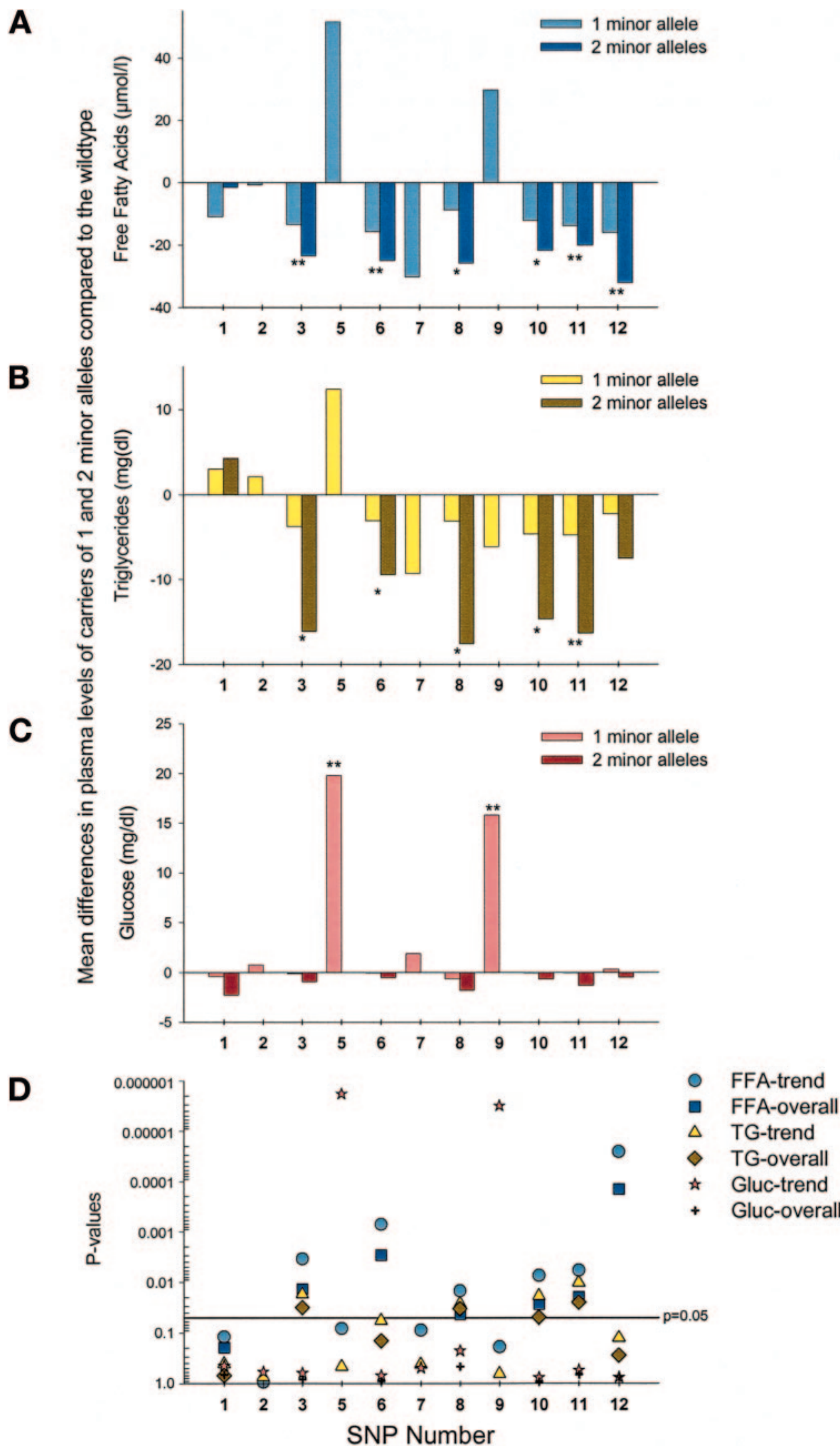

FIG. 2. *A*: Primary analysis: association of the 11 analyzed polymorphisms of the *ATGL* gene indicating mean changes in plasma concentrations of FFAs for subjects with one (light colored bars) or two (dark colored bars) copies of the minor allele compared with subjects with the wild type (SNP 4 discarded 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 asterisks mark the SNPs, for which the mean concentration changes compared with the wild type are statistically significantly different from 0 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 online appendix Tables 5–7.

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alleles of the two highly correlated SNPs 5 and 9 point into the opposite direction as the other SNPs (Fig. 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 with the wild type ($P < 0.00001$ for both SNPs). This association was so strong that it resulted in a 2.65-fold increased odds of type 2 diabetes. This finding was strengthened by the fact that the type 2 diabetes risk estimates pointed into the same direction in all analyzed

subgroups. In the light of the various pathways leading to type 2 diabetes 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 type 2 diabetes 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 (coronary artery disease). 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 (Table 2 and online appendix).

It must be considered a limitation that the number of subjects on which the association of type 2 diabetes with the rare polymorphisms is based is rather small ($n = 27$ and 39 with the minor alleles of SNPs 5 and 9, respectively). Therefore, more studies in the future will have to elucidate the role of the *ATGL* in type 2 diabetes. However, our findings were consistent in all analyzed subgroups and both sexes. 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 (FFAs, 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 type 2 diabetes 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.

ACKNOWLEDGMENTS

Parts of this work were supported by GOLD (Genomics of Lipid-Associated Disorders) of the GEN-AU (Austrian Genome Research Programme) and the German Ministry of Education and Research (BMBF)/National Genome Research Network (NGFN) and the Deutsche Forschungsgemeinschaft (SFB 386 and Wi621/12-1). It was also supported by National Institutes of Health Grants DK55006 and HL21088 and a Public Health Service Research Grant M01-RR00064 from the National Center for Research Resources.

We thank the following people for their continuous support throughout the project: Guido Fischer and Richard Gress (IT and data management), Michael Han and Michael Putz (IT, Linux analysis software), Nadja Baumgartner, Arsin Sabunchi, Anke Lämmle, and Klara Fizzi (laboratory assistance).

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