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SULF2 Strongly Predisposes to Fasting and Postprandial Triglycerides in Patients with Obesity and Type 2 Diabetes Mellitus

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Abstract

Objective—Hepatic overexpression of sulfatase-2 (SULF2), a heparan sulfate remodelling enzyme, strongly contributes to high triglyceride (TG) levels in obese, type 2 diabetic (T2DM) *db/db* mice. Nevertheless, data in humans are lacking. Here we sought to investigate the association of human hepatic *SULF2* expression and *SULF2* gene variants with TG metabolism in patients with obesity and/or T2DM.

Design and Methods—Liver biopsies from 121 obese subjects were analyzed for relations between hepatic *SULF2* mRNA levels and plasma TG. Associations between seven *SULF2* tagSNPs and TG levels were assessed in 210 obese T2DM subjects with dyslipidemia. Replication of positive findings was performed in 1316 independent obese T2DM patients. Postprandial TRL clearance was evaluated in 29 obese T2DM subjects stratified by *SULF2* genotype.

Results—Liver *SULF2* expression was significantly associated with fasting plasma TG ($r = 0.271$; $p=0.003$) in obese subjects. The *SULF2* rs2281279(A>G) SNP was reproducibly associated

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with lower fasting plasma TG levels in obese T2DM subjects ($p < 0.05$). Carriership of the minor G allele was associated with lower levels of postprandial plasma TG ($P < 0.05$) and retinyl esters (RE) levels ($P < 0.001$).

Conclusions—These findings implicate SULF2 as potential therapeutic target in the atherogenic dyslipidemia of obesity and T2DM.

Keywords

Triglycerides; SULF2; obesity; diabetes; postprandial

Introduction

The prevalence of obesity, the metabolic syndrome, type 2 diabetes mellitus (T2DM) and their sequelae, particularly cardiovascular events, is increasing worldwide.(1, 2) The accelerated risk for atherosclerosis in these subjects results in large part from their atherogenic dyslipidemia, which includes increased fasting levels of very low density lipoprotein (VLDL) and its major component triglyceride (TG), in combination with impaired clearance of postprandial triglyceride-rich lipoprotein (TRL) remnants.(3–5) In particular, subendothelial retention of VLDL and particularly postprandial TRL-remnants have been linked to cardiovascular events in these individuals.(6–10)

TRL metabolism involves a series of steps that culminate in the uptake of TRL-remnants by hepatocytes.(8, 11, 12) During the past decades, hepatic heparan sulfate proteoglycans (HSPGs)(13–16) and in particular the syndecan-1 HSPG, have been implicated in TRL removal.(17–21) The syndecan-1 HSPG consists of a single-pass transmembrane core protein onto which three heparan sulfate (HS) side-chains are covalently attached. These side-chains bind several important ligands, including TRLs, resulting in hepatic uptake and clearance of these particles. About 50 genes are involved in HSPG assembly and disassembly, affecting core protein expression, HS side-chain length, epimerization of glucuronyl residues, and sulfation patterns.(22) It was recently shown that expression of glucosamine-6-*O*-endosulfatase-2 (*Sulf2* encoding for sulfatase 2, an HSPG remodelling enzyme) is substantially increased in livers of obese, T2DM *db/db* mice compared to lean controls.(23) In these mice, hepatic *Sulf2* mRNA expression was strongly and positively related to plasma TG levels and *Sulf2* protein inhibited the ability of syndecan-1 to clear model remnants by cultured liver cells. Recently, we showed that suppression of hepatic *Sulf2* expression in obese, T2DM *db/db* mice by targeted allele-specific antisense administration normalized fasting plasma TG levels and, most strikingly, eliminated postprandial hypertriglyceridemia.(24) However, human data on the biology of hepatic SULF2 and its role in triglyceride metabolism remain to be elucidated.

In the present study, we sought to answer three questions. First, is there evidence in humans for dysregulation of hepatic *SULF2* mRNA levels as part of the metabolic disturbances of obesity, particularly dyslipidemia? Second, are there *SULF2* SNPs that associate with fasting dyslipidemia in cohorts of obese, T2DM subjects? Third, does any *SULF2* SNP affect postprandial lipoprotein metabolism in obese subjects with T2DM, as assessed by oral fat tolerance studies? To address the first two questions, we used pre-existing cohorts, which

have the advantage of large numbers of subjects, but the disadvantage that fasting - not postprandial – lipoprotein values are available. Because fat tolerance studies are laborious, they are practical only in a subset of individuals, not in population-wide cohorts. Our results implicate human *SULF2* as an important participant in deranged TRL metabolism in obesity and T2DM.

Methods

The local Institutional Review Boards approved the studies in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study was registered at the Dutch Trial Register (NTR 2641).

SULF2 expression in human liver specimen of obese subjects

The Obesity Cohort comprises 121 obese patients (BMI ≥ 30 kg/m²) who had presented at the Obesity clinic of the Antwerp University Hospital, and underwent a liver biopsy before bariatric surgery or dietary intervention as recently described. (25, 26). These subjects underwent a series of additional examinations, including a fasting plasma lipid profile, and glucose and insulin measurements. Insulin resistance for handling glucose was estimated using homeostasis model assessment (HOMA).(27) Upon biopsy, liver material was immediately snap-frozen and stored at -80°C for RNA isolation. RNA was isolated from human liver by guanidinium thiocyanate/phenol/chloroform extraction.(28) Reverse transcription was performed using the High Capacity Reverse Transcription kit (Applied Biosystems, Life Technologies, Carlsbad, USA). Quantitative PCR was performed with Brilliant II SYBR Green QPCR Master Mix (Agilent Technologies, Santa Clara, USA) on a Stratagene Mx3005P system (Agilent Technologies). Specific primers for *SULF2* were designed using Primer3 software (5'-CCT TTG CCG TGT ACC TCA AT-3' and 5'-GCA CGT AGG AGC CGT TGT AT-3'). mRNA levels were normalized to those of transcription factor Iib (*TfIib*) by calculating the difference in cycle threshold (Ct).

SULF2 SNPs and fasting plasma triglycerides in cohorts of obese, T2DM patients

Associations between *SULF2* SNPs and plasma triglyceride levels were assessed in participants in the Diabetes Atorvastatin Lipid Intervention (DALI) study and subsequently validated in the Diabetes Care System cohort, both described immediately below. All analysis were performed using the baseline data from either the DALI cohort or the Diabetes Care System Cohort.

Diabetes Atorvastatin Lipid Intervention (DALI) study—This double-blind randomized placebo-controlled multicenter study evaluated the effect of atorvastatin 10 mg versus 80 mg on lipid metabolism in 217 unrelated Dutch men and women with T2DM and diabetic dyslipidemia (fasting triglyceride levels between 1.5 and 6.0 mmol/l).(29) Male and post-menopausal female patients, aged 45 to 75 years, with type 2 diabetes mellitus (HbA1c $<10\%$, i.e. <86 mmol/mol), with no history of manifest cardiovascular disease, were included. All patients refrained from lipid lowering therapy for the 4 weeks just before the start of the study. Buffy coats were used to isolate DNA according to standard procedures

Diabetes Care System Cohort—Subjects were included from this prospective dynamic cohort of patients with T2DM followed since 1998 in the Netherlands.(30) For the present study we selected 1,323 T2DM patients who had agreed to genetic analyses, and had an elevated fasting plasma triglyceride level (> 1.5 mmol/l) and the availability of a DNA sample. Buffy coats were used to isolate DNA according to standard procedures

Selection of SULF2 tagSNPs—Tagging SNPs for *SULF2* were selected with the HapMap database (31) and the TAGGER algorithm using a pairwise tagging approach.(32) Briefly, we selected from HapMap all common genetic variants (minor allele frequency >0.1) in the *SULF2* locus for a population of European ancestry. A tagging SNP approach uses the knowledge of associations between genetic variants (linkage disequilibrium (LD) structure) to limit the number of SNPs that needs to be genotyped. TagSNPs are those SNPs that most effectively represent (or “tag”) all the SNPs in a particular locus. We selected tagSNPs using an r^2 cut off level >0.8 (see supplementary Figure 1 for the genomic context of *SULF2*). Allele-specific primers were obtained from Applied Biosystems (Life Technology, Rotterdam, The Netherlands). Genotyping was performed using allelic discrimination with FAM and VIC as fluorophores. PCR conditions were denaturation for 10 min at 95°C, followed by 40 cycles (30 sec 92°C, 45 sec 60°C) run on a CFX PCR system (BioRad). Tagman PCR assay mix was obtained from Applied Biosystems.

Oral fat tolerance in obese T2DM patients

To study postprandial TRL metabolism, we performed an oral fat tolerance test in a subset of $n=29$ obese T2DM subjects, stratified by *SULF2* genotype (rs2281279 c.2494+267 A>G) from the DALI and Diabetes Care System cohorts. Exclusion criteria were a history of manifest coronary artery disease, the use of any lipid-lowering medication in the 8 weeks preceding the fat tolerance test, clinical signs of malabsorption (eg diarrhea), or exogenous insulin treatment. Participants were asked to refrain from alcohol intake the day before. Participants fasted overnight and then were admitted at 7:30 am to the ward. Cream [consisting of 40% fat (wt/vol) with a polyunsaturated fat to saturated fat ratio of 0.06, 0.001% cholesterol (wt/vol), and carbohydrates (refined sugar) were administered to achieve a dosage of 35 gram fat and 50 gram sugar per m^2 body surface. This mixture was supplemented with 150 ml water and 100,000 IU of vitamin A (Retinyl palmitate, AMC Clinical Pharmacy). The cream drink was consumed within 10 minutes. Postprandial blood samples were drawn at 0, 1, 2, 3, 4, 5 and 7 hours from a peripheral vein into EDTA-containing tubes, which were placed on ice and protected from light. Plasma was separated within 30 minutes by centrifugation at 1700 g for 20 min. at 4°C. Aliquots of plasma were frozen at -80°C for subsequent analysis of TG and retinyl esters (RE).

Biochemical analyses

Total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides were measured by standard enzymatic methods on a Cobas Mira system (Roche Diagnostics, Basel, Switzerland). Glucose was assessed using the hexokinase method (Gluco-quant, Hitachi 917; Hitachi). Plasma insulin was measured by an immunoluminimetric assay (Immulite insuline) on the Immulite 2000 autoanalyzer (Diagnostic Products). HbA1c was measured by HPLC (Reagens Bio-Rad Laboratories, Veenendaal, the Netherlands) on a Variant II

(Bio-Rad Laboratories). Homeostatic model assessment for insulin resistance for glucose handling (HOMA-IR) was calculated by the widely-used formula: $[\text{glucose (mmol/L)} * \text{insulin (mU/L)}] / 22.5$. (27) Retinyl esters (RE) were analysed in 200 μl plasma after extraction of retinyl esters using chloroform/methanol/water as described. (33, 34) In short, retinyl propionate (Sigma Chemicals; St Louis, USA), a form that does not occur in humans, was used as internal standard; methanol was used as mobile phase at a flow rate of 1 ml/min and the effluent was monitored at 330 nm. A standard curve of retinyl palmitate in pooled plasma was used as reference. Peak heights were measured and used for calculations of the absolute RE values.

Statistical analyses

Clinical parameters passed tests for normality and are expressed as mean $\pm$ standard deviation unless stated otherwise. Effects of single SNPs on triglyceride levels were examined using one way analysis of variances (ANOVA). Variables with skewed distribution were log-transformed before being used as continuous variables in statistical analyses. Effects of SNPs on baseline characteristics were analysed by ANOVA for continuous variables and with chi-square for categorical variables. Postprandial TG and RE were calculated as total area under the curve (AUC) calculated by the trapezoid rule. Incremental area under the curve (iAUC) was obtained by subtracting the fasting plasma level from each postprandial time point. Comparisons between groups were analysed using one-way ANOVA, followed by pair-wise comparison using the Kruskal-Wallis test for multiple comparisons. Two-sided probability values of less than 0.05 were considered statistical significant. Statistical analyses were performed using SPSS version 18.0.

Results

Characteristic metabolic abnormalities in obese subjects correlate with disturbed hepatic SULF2 expression

We searched for evidence in humans that hepatic *SULF2* mRNA expression is dysregulated as a part of the metabolic disturbances of obesity, particularly dyslipidemia. We studied a large consecutively recruited cohort of obese patients (n=121) who had undergone a liver biopsy before bariatric surgery or dietary intervention. Main characteristics of this cohort are presented in Table 1. Liver *SULF2* expression was significantly associated with fasting plasma triglycerides ($r = 0.271$; $p=0.003$), fasting glucose ($r = 0.252$; $p=0.005$) and HOMA-IR ($r = 0.186$; $p=0.043$; Figure 1), and with anthropometric parameters reflected by waist ($p<0.002$) and waist-to-hip ratio ($p<0.001$, Table 2)

Common genetic variants in SULF2 and fasting plasma TG levels in two independent cohorts of obese T2DM subjects

To investigate whether genetic variation in *SULF2* is associated with fasting plasma TG levels, we selected seven tagSNPs: rs2281279; c.2494+267A>G, rs6090717: c.416-3507T>C, rs6090714: c.567+4691G>A; rs6094818: -100-7141T>C, rs13044051: c.416-8793G>C, rs6122615: c.101+1307T>C and rs2235734:2227+48T>G; (supplemental Figure 1). All pairwise metrics of LD between rs2281279 and other SNPs were <0.5 this

SNP was considered as being independent of the other SNPs. (supplementary Figure 2). Each tagSNP was in Hardy-Weinberg equilibrium in each cohort.

In supplemental Table 1 we display the clinical characteristics of the DALI study patients stratified by genotype. Relationships between each of the *SULF2* tagSNPs and plasma TG levels are shown in Table 2. We observed a significant association between fasting plasma TG levels and rs2281279 in the DALI study (AA: 2.63 [IQR 2.00–3.51 mmol/l, AG: 2.55 [IQR 2.09–3.52] mmol/l, GG: 2.20 [IQR 1.89–2.83] mmol/l; $p=0.031$ for trend; Table 3).

We replicated our findings with rs2281279 in a large cohort of 1319 patients with obesity and type 2 diabetes mellitus (Diabetes Care System cohort). Their clinical characteristics are presented in supplemental Table 3. We observed a significant association between *SULF2* rs2281279 and fasting plasma TG in the Diabetes care System cohort: homozygous carriers of the major allele (AA; 2.30 mmol/l (IQR 1.90–2.83), heterozygous (AG; 2.40 mmol/l (IQR 2.00–3.03 mmol/l) and homozygote carriers of the minor allele (GG; 2.19 mmol/l (IQR 1.90–3.00 mmol/l). This association was statistically significant with $p=0.049$ for trend (Table 3).

The minor *SULF2* rs2281279 allele predisposes to lower postprandial triglyceride-rich lipoprotein levels in obese T2DM patients

Finally we sought to determine if rs2281279 SNP identified from our cohort studies affects postprandial lipoprotein metabolism, as assessed by oral fat tolerance studies. We selected 29 T2DM patients who met the inclusion and exclusion criteria listed in the Methods, and agreed upon an oral fat tolerance test. We stratified them according to their rs2281279 genotype (AA; $n=11$, AG; $n=11$ and GG; $n=7$). Baseline characteristics of T2DM patients participating in the oral fat tolerance test are listed in Table 4. Patients who were heterozygous for this allele (AG) generally were younger, with higher fasting glucose, and HbA1c levels. Homozygotes for the minor allele (GG) had the lowest fasting TG levels. HOMA-IR values, which assess insulin resistance for glucose, were statistically indistinguishable amongst the three genotype groups.

Following the oral fat tolerance test, postprandial TG levels were lower in AG and GG carriers compared to AA carriers (Figure 2A). The area under the curve (AUC) of plasma TG was 29 ± 10 (mmol/l)*h in AA individuals, 21 ± 7 (mmol/l)*h in AG individuals and 17 ± 8 (mmol/l)*h in GG individuals (Figure 2B, $p<0.05$). After adjustment for baseline TG levels, the incremental AUC (iAUC) remained significant smaller for both AG and GG individuals [TG iAUC of 6.4 ± 3.8 (mmol/l)*h in AA, 2.9 ± 2.9 (mmol/l)*h in AG and 4.1 ± 1.9 (mmol/l)*h in GG, $p<0.05$, Figure 2C]. With respect to postprandial plasma RE excursions, RE- AUCs were significantly decreased in both AG and GG individuals [101 ± 43 (mg/l)*h for AA, 63 ± 40 (mg/l)*h for AG and 15 ± 6 (mg/l)*h for GG, $p<0.001$, Figure 2D–E] again indicating a faster TRL clearance capacity in G allele individuals.

Discussion

In the present study we found significant dysregulation of hepatic *SULF2* mRNA levels as part of the metabolic disturbances of obesity in humans. Moreover, we identified a single

SULF2 SNP that associates with improved fasting dyslipidemia in two independent cohorts of obese T2DM subjects, and this same *SULF2* variant had a striking positive effect on postprandial lipoprotein metabolism in obese T2DM patients. These are the first data to implicate *SULF2* in abnormal postprandial TRL handling in humans with obesity or T2DM and are in line with previously published data from obese, T2DM db/db mice.(23, 24) The strength of our study is that we performed a systematic survey of *SULF2* tagSNPs in a small cohort of patients with T2DM, and replicated these findings in a second large independent T2DM cohort. Interestingly, Matikainen et al, like us, found that the G-allele of rs2281279 was associated with lower fasting and postprandial TG metabolism and lower *SULF2* mRNA levels in livers in healthy individuals.(35)

Potential biological mechanism and clinical implications

The *SULF2* gene encodes for the heparan sulfate glucosamine-6-*O*-endosulfatase (*SULF2*), a secreted enzyme that removes 6-*O* sulfate groups from cell-surface and extracellular heparan sulfate proteoglycans (HSPG).(36) Syndecan-1 HSPGs are important mediators of hepatic uptake of TG-rich lipoproteins.(16, 18, 21) Previously, we showed that *db/db* mice have highly increased hepatic *Sulf2* expression leading to increased plasma TG levels and impaired hepatic lipoprotein clearance of postprandial TG-rich lipoproteins.(23, 24) Inhibition of hepatic *Sulf2* expression by targeted allele-specific oligonucleotide antisense (ASO) treatment normalized (postprandial) plasma TG levels in obese T2DM *db/db* mice and increased the binding of TG-rich lipoproteins to their isolated hepatocytes. (24) These findings underscore the important role of *Sulf2* in regulating hepatic clearance of postprandial remnant lipoproteins in mice. Our current study is the first to address whether *SULF2* has a comparable function in TRL metabolism in humans. In the present study, a functional role of *SULF2* in postprandial clearance of TG-rich lipoproteins in humans is supported by the observed correlation between a genetic alteration in *SULF2* and postprandial triglycerides in obese T2DM subjects.

SULF2 modifies the sulfation grade of the HSPG chain and affects its affinity for binding TG-rich lipoproteins.(24) Increased hepatic *Sulf2* expression, which is found in T2DM mouse models, is significantly associated with higher plasma TG levels. Mechanistically, advanced glycation end-products (AGEs) stimulate *Sulf2* mRNA expression and protein; adiponectin suppresses *Sulf2* mRNA and protein; and insulin suppresses *SULF2* protein in normal livers posttranscriptionally.(23, 37) These findings provide a compelling pathophysiologic model in which AGEs, hypoadiponectinemia, and pathway-selective insulin resistance in obesity and T2DM induce abnormal up-regulation of *SULF2* in the liver, thereby impairing syndecan-1 HSPG function in remnant clearance.

Study limitations

First, hepatic *SULF2* mRNA expression was only determined in specimens from obese subjects. Moreover, the associations we found of hepatic *SULF2* mRNA levels with fasting plasma glucose and HOMA-IR in the obesity cohort cannot demonstrate causality. A likely explanation is that more severe metabolic disturbances in obesity cause higher hepatic *SULF2* levels, consistent with the pathophysiologic model, above. Alternatively, *SULF2*

might play a role in glycaemic regulation. Further evaluation of this intriguing finding is warranted and currently performed at our department.

The second limitation comes from our use of common tagSNPs to ascertain the relation between *SULF2* and plasma TG levels. Plasma concentrations of this secreted enzyme could not be measured due to a lack of a high quality antibody. On the other hand, plasma values are unlikely to reflect functionality of *SULF2* in the liver.

Third, we evaluated the association between genetic variations in *SULF2* and plasma TG levels in obese T2DM subjects with diabetic dyslipidemia. We expected that an environment of increased plasma TG levels and the presence of insulin resistance for glucose would lead to abnormalities in TG homeostasis, possibly magnifying the effects of common genetic variation in *SULF2* on hepatic remnant clearance pathways.

Finally, we measured plasma RE and TG excursions as surrogate markers for postprandial lipid handling and not plasma apoB48. In this respect, plasma apoB48 only measures the number of intestinal-derived TG-rich lipoprotein particles during the postprandial phase but not their lipid composition. Plasma RE measurement, on the other hand, better reflects the lipid excursions during the postprandial phase, although it can be disputed that in the later time points also exchange of RE will occur from apoB48-containing lipoprotein particles towards apoB-100 containing lipoproteins. (38)

In conclusion, our results provide the first human evidence for a novel mechanism, in which *SULF2* contributes to postprandial dyslipidemia of obesity and T2DM. In conjunction with our prior data from *Sulf2* antisense treatment of obese T2DM *db/db* mice, these findings indicate that *SULF2* inhibition may be an attractive target to normalize dyslipidemia in obese and/or T2DM patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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What is already known about this subject?

1. plasma TG are a main risk factor for metabolic abnormalities in obesity and type 2 diabetes mellitus
2. SULF2, a heparan sulfate remodeling enzyme, strongly contributes to high plasma TG in obese, diabetic db/db mice
3. ASO-SULF2 treatment normalizes fasting and postprandial plasma TG in obese, diabetic db/db mice

What does this study add?

1. Our analyses show, for the first time, that in obese subjects elevated hepatic SULF2 mRNA expression is significantly associated with plasma TG.
2. We show that a genetic variant in SULF2 predisposes to fasting plasma TG in two independent cohorts of obese patients with type 2 diabetes mellitus and atherogenic dyslipidemia.
3. Finally we show that the SULF2 genetic variant is associated with accelerated postprandial TG-rich lipoprotein metabolism.

Figure 1. The association of hepatic SULF2 expression with plasma TG concentrations and HOMA-IR in obese subjects
Hepatic *SULF2* expression in obese subjects was significantly associated with fasting plasma TG, and HOMA-IR. Correlations were tested for significance using the Spearman Rank Test.

Figure 2. The minor *SULF2* rs2281279 allele predisposes to lower postprandial triglyceride-rich lipoprotein levels in obese T2DM patients

A. Postprandial triglyceride curves during an oral fat load. Obese, T2DM subjects were selected according to the *SULF2* rs2281279 genotype. AA=homozygous for the common allele; AG=heterozygous for the rare allele, GG= homozygous for the rare allele. Data are presented as mean $\pm$ S.E.M.

B. area under the curve (AUC) of plasma triglycerides during the oral fat tolerance test.

C. incremental AUC of triglycerides during the oral fat tolerance test.

D. postprandial retinyl ester excursion during the oral fat tolerance test

E. AUC of RE according to rs2281279 genotypes AA, AG and GG.

Data were analyzed using ANOVA and analyses for trend. Post-hoc test were performed for testing the differences between the groups. * P<0.05; ** P<0.01; ***P<0.005

Table 1

Characteristics of the Obesity Cohort

Parameters	
N	121
Female/male	83/38
Age [years]	45 ± 12
Weight (kg)	111 ± 23
BMI kg/m ²	38.7 ± 6.7
Waist circumference (cm)	117 ± 13
Waist-to-hip ratio	0.97 ± 0.10
Cholesterol mmol/L	5.3 ± 1.0
HDL cholesterol mmol/L	1.29 ± 0.36
LDL cholesterol mmol/L	3.2 ± 0.9
Triglycerides mmol/L	1.7 ± 0.9
Glucose mmol/L	4.6 (4.3 – 5.05)
Insulin mIU/L	14 (10 – 22)
HOMA-IR	2.94 [2.04 – 4.40]
HbA1c mmol/mol	38 [34 – 40]
AST U/L	28 (24 – 36)
ALT U/L	40 (32 – 53)

Data are presented as mean ± SD for normally distributed variables or as median (interquartile range) when distribution of variable is skewed. AST: aspartate aminotransferase; ALT: alanine aminotransferase. LDL cholesterol was calculated by the Friedewald formula (cholesterol – HDL cholesterol – (triglycerides/5))(39) HOMA-IR was calculated as (glucose (mmol/l) × insulin (mIU/L))/22.5.(27)

Table 2Correlations between hepatic *SULF2* expression and anthropometric markers

Parameter	Correlation with CT relative to <i>TFIIb</i>	P-value
Waist circumference (cm)	0.280	0.002
Waist-to-hip ratio	0.310	0.001
Weight (kg)	0.175	0.055
BMI (kg/m ²)	0.072	0.430

Data are presented as mean $\pm$ SD. Statistical significance was tested using non-parametric Spearman correlation statistics. A $P < 0.05$ is considered as statistically significant.

Table 3

Association of *SULF2* tagSNPs with fasting plasma TG levels in the DALI study and the replication of rs2281279 SNP with plasma TG in the Diabetes Care System cohort

SNP	Wild type	Heterozygous mutant	Homozygous mutant	P-value
rs2281279	AA	AG	GG	
N	90	95	25	
TG (mmol/L)	2.63 [2.00–3.51]	2.55 [2.09–3.52]	2.20 [1.89–2.83]	0.031
rs6090717	TT	TC	CC	
N	71	94	28	
TG (mmol/L)	2.60 [2.03–3.57]	2.37 [2.00–2.95]	3.09 [2.19–4.24]	0.138
rs6090714	GG	GA	AA	
N	57	102	46	
TG (mmol/L)	2.72 [2.10–3.68]	2.40 [1.90–3.13]	2.60 [2.03–3.63]	0.933
rs6094818	TT	TC	CC	
N	122	56	16	
TG (mmol/L)	2.57 [2.06–3.43]	2.29 [1.90–3.35]	2.66 [2.23–3.67]	0.428
rs13044051	GG	GC	CC	
N	131	65	13	
TG (mmol/L)	2.50 [2.06–3.40]	2.55 [2.00–3.62]	2.86 [2.24–3.64]	0.465
rs6122615	TT	TC	CC	
N	141	60	7	
TG (mmol/L)	2.60 [2.01–3.41]	2.35 [1.99–3.58]	3.20 [2.30–4.25]	0.373
rs2235734	TT	TG	GG	
N	81	97	27	
TG (mmol/L)	2.60 [1.92–3.58]	2.61 [2.09–3.50]	2.29 [1.70–2.83]	0.071
Replication Diabetes Care System Cohort				
rs2281279	AA	AG	GG	P-value
N	629	557	122	
TG mmol/L	2.30 [1.90–2.83]	2.40 [2.00–3.03]	2.19 [1.90–3.00]	0.049

Values are given as median (inter quartile range). Differences between genotype groups were tested for significance using ANOVA and analysis for trend. $P < 0.05$ was considered statistically significant.

Table 4

Baseline characteristics of obese T2DM subjects participating in the oral fat tolerance test.

	rs2281279 genotypes			P-value
	AA (n=11)	AG (n=11)	GG (n=7)	
Female/Male	6/5	5/6	4/3	0.870
Age, years	60.6±6.9	57.1±7.6	67.6±5.0	0.014
Weight (kg)	88 ± 11	92 ± 14	92 ± 13	0.764
BMI, kg/m ²	30.4±4.7	32.2±4.6	29.7±4.8	0.486
Waist (cm)	110 ± 13	112 ± 13	103 ± 10	0.295
Waist-to-hip ratio	1.05 ± 0.09	1.02 ± 0.08	0.93 ± 0.08	0.029
Glucose, mmol/L	9.2±1.6	11.2±3.4	7.5±1.3	0.012
HbA1c mmol/mol	64±8	71±12	52±11	0.003
HOMA-IR	7.7±5.2	8.0±6.3	7.3±12.8	0.986
Total cholesterol, mmol/L	6.1±0.7	6.0±0.9	5.8±0.9	0.716
LDL cholesterol, mmol/L	3.4±0.9	3.6±0.9	3.7±0.6	0.860
HDL cholesterol, mmol/L	0.96±0.20	1.05±0.17	1.20±0.36	0.133
Triglycerides, mmol/L	3.5 (3.1–5.2)	2.7 (2.4–3.9)	1.5 (1.3–3.7)	0.039

Data are presented as mean (±SD), number (percentage) or median (IQR). P-value for gender was calculated by Pearson chi-square. P-value for triglycerides was calculated using Kruskal-Wallis test. Other p-values are calculated by one-way ANOVA.

Abbreviations: HOMA-IR = homeostasis model assessment of insulin resistance for glucose; LDL = low-density lipoprotein; HDL = high-density lipoprotein; BMI = body mass index.