Genetic Variation in the 11β-hydroxysteroid-dehydrogenase 1 Gene determines NAFLD and Visceral Obesity

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Context/Objective: Acute pharmacological inhibition of 11β-hydroxysteroid-dehydrogenase 1 (11β-HSD1), which converts cortisone into the much more potent cortisol in peripheral tissues, results in reduction of total-, visceral- and liver fat, but not of insulin resistance. We now investigated whether lifelong alterations of 11β-HSD1 activity similarly affect these cardiometabolic risk parameters, by studying single nucleotide polymorphisms (SNPs) in the 11β-HSD1-coding gene (*HSD11B1*).

Design/Methods: Liver fat content was measured by ^{1}H-magnetic resonance (MR) spectroscopy and total- and visceral fat mass by MR tomography in 327 subjects. Insulin sensitivity (IS) was estimated during an oral glucose tolerance test and the euglycemic, hyperinsulinemic clamp (n=219). Nine SNPs covering the whole *HSD11B1* gene were genotyped.

Results: After correction for multiple testing, liver fat content strongly correlated with 3 SNPs, rs2235543, rs12565406 and rs4844880 (p=0.0002, p=0.001, p=0.0009), independently of gender and age. There was a nominal association of these SNPs with hepatic IS, but only of rs4844880 with whole-body IS. Subjects homozygous for the major allele had an adjusted odds ratio of 2.16 (95% CI 1.23–3.90) for rs2235543, 2.06 (1.08–4.13) for rs12565406, and 1.95 (1.13–3.49) for rs4844880 for having nonalcoholic fatty liver disease compared to carriers of the minor allele. Less strong associations of these SNPs with visceral fat mass were observed. In liver biopsies, carriers of the minor alleles of rs2235543 and rs12565406 had significantly lower *HSD11B1* mRNA expression (n=105, p=0.034 and p=0.0086).

Conclusions: 11β-HSD1 may be an important enzyme in the pathogenesis of fatty liver and visceral obesity and a promising target for their treatment.

Cushing's syndrome, a state of high circulating cortisol levels, and the metabolic syndrome share several common clinical manifestations. Furthermore, abnormalities of both the hypothalamic-pituitary-adrenal axis and cortisol levels in the metabolic syndrome have been repeatedly reported in the literature. However, other studies

Abbreviations:

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failed to show an association of either increased circulating cortisol concentrations or its metabolites with manifestations of the metabolic syndrome, including fatty liver, in humans (1–4). This led to the hypothesis that increased local amounts of cortisol may additionally contribute to the pathogenesis of the features of the metabolic syndrome (1–4). Cortisol levels in metabolic active tissues are at least in part determined by the activity of the enzyme 11β-hydroxysteroid-dehydrogenase 1 (11β-HSD1), which converts cortisone to the much more potent cortisol (1–4). We recently studied the effect of 12 weeks pharmacological inhibition of 11β-HSD1 on liver fat content, body fat distribution and metabolic parameters in humans. The inhibition of 11β-HSD1 led to a significant reduction of liver fat content, and total- and visceral fat mass, but not of hepatic or whole-body insulin resistance (5).

It is unknown whether during chronic inhibition of 11β-HSD1 compensatory mechanisms may become active, abrogating these effects. In the present study, we, therefore, investigated the possible long-term effect of alteration of 11β-HSD1 activity by studying the relationships of common single nucleotide polymorphisms (SNPs) of the 11β-HSD1-coding gene (*HSD11B1*) with liver fat content, body fat mass and distribution, and insulin sensitivity. To this aim, we genotyped the 9 SNPs covering the whole *HSD11B1* gene in a cohort of subjects at risk for type 2 diabetes, who were carefully phenotyped for these parameters and determined the relationships of the SNPs with *HSD11B1* gene expression in liver biopsies.

Research Design and Methods

Subjects

We studied Caucasians from the southern part of Germany who participated in an ongoing study on the pathophysiology and prevention of type 2 diabetes (6–9). From that study, we investigated data from 330 subjects who had measurements of liver fat content and of body fat mass and distribution, and in whom we previously investigated the impact of the rs738409 C>G p.I148M variant in the patatin-like phospholipase domain containing 3 (PNPLA3) gene on liver fat content, body fat distribution and glucose metabolism (7). Individuals fulfilled at least one of the following criteria: a family history of type 2 diabetes, a BMI > 27 kg/m^2, previous diagnosis of impaired glucose tolerance or gestational diabetes. They were considered healthy according to a physical examination and routine laboratory tests. The participants had no history of liver disease and did not consume more than 2 alcoholic drinks per day. Plasma aminotransferase levels were lower than 2 times the upper limit of normal. In 3 of these 330 individuals genotyping of two SNPs (see below) was not successful. Thus, 327 individuals, 199 women and 128 men, were included in the present analysis.

Informed written consent was obtained from all participants and the Ethics Committee of the University of Tübingen had approved the protocol according to the Helsinki Declaration.

Body fat mass and body fat distribution

Body mass index (BMI) was calculated as weight divided by the square of height (kg/m^2). Waist circumference was measured at the midpoint between the lateral iliac crest and lowest rib. Total (TAT), subcutaneous (SAT) and visceral adipose tissue (VAT) mass were measured by magnetic resonance (MR) tomography, with an axial T1-weighed fast spin echo technique with a 1.5 T whole-body imager (Magnetom Sonata, Siemens Healthcare) (5).

Liver fat content

Liver fat content was measured by localized ^{1}HMR spectroscopy, as previously described (5). Nonalcoholic Fatty Liver Disease (NAFLD) was defined as liver fat content > 5.56% (10).

Oral glucose tolerance test

All individuals underwent a 75 g oral glucose tolerance test (OGTT). Venous plasma samples were obtained at 0, 30, 60, 90 and 120 minutes for determination of plasma glucose and insulin. Blood glucose was determined using a bedside glucose analyzer (glucose-oxidase method; YSI, Yellow Springs Instruments, Yellow Springs, OH, USA). Plasma insulin was determined by a chemiluminescent immunoassay (ADVIA Centaur XP, Siemens Healthcare Diagnostics, Eschborn, Germany). Insulin sensitivity from the OGTT was calculated as $10.000/\sqrt{(Ins_{mean}Gluc_{mean}Ins_0Gluc_0)}$. Furthermore, the homeostasis model insulin resistance index (HOMA-IR)=Ins_0 [in μU/mL]$Gluc_0$ [in mmol/L]/22.5 was calculated.

Euglycemic, hyperinsulinemic clamp

A subgroup of subjects (n = 219) was eligible (mostly due to appropriate peripheral veins), gave additional consent for and underwent a euglycemic, hyperinsulinemic clamp. Insulin sensitivity was determined with a primed insulin infusion at a rate of 40 mU·m^{-2}·min^{-1} for 2 hours as previously described (11).

Hepatic and adipose tissue insulin resistance indexes

Hepatic insulin resistance index was calculated as the product of total area under curve (AUC) for glucose and insulin during the first 30 minutes of the OGTT ([AUC glucose(0–30)] x [AUC insulin(0–30)] (12). Adipose tissue insulin resistance index was calculated as fasting plasma insulin x fasting free fatty acid concentrations (13).

Analytical procedures

Plasma insulin and C-peptide concentrations were determined using a chemiluminescent immunoassay (ADVIA Centaur XP, Siemens Healthcare Diagnostics, Eschborn, Germany). Clinical chemical parameters (total-, HDL- and LDL-cholesterol, triglycerides, ALT, AST and GGT) were measured on the ADVIA 1800 Clinical Chemistry System (Siemens Healthcare Diagnostics, Eschborn, Germany). Plasma free fatty acid (FFA) concentrations were measured with an enzymatic method (WAKO Chemicals, Neuss, Germany).

Selection of tagging SNPs

Based on publicly available phase II data of the International HapMap Project derived from Utah residents with Central European ancestry (release #24 November 2008, http//hapmap-

.ncbi.nlm.nih. gov/index.html.en), we screened *in silico* the complete *HSD11B1* gene spanning 209 859 510–209 908 295 (48,785 kb) on human chromosome 1 as well as the 2 kb flanking region (promoter region). In this genomic locus, eleven SNPs with MAFs ≥ 0.05 were present: rs2235543, rs12565406, rs4844880, rs2282738, rs11119328, rs846906, rs4844488, rs3753519, rs10082248, rs11808690, and rs11807619. Since rs11808690 and rs2282738, and also rs11807619 and rs11119328 were in linkage disequilibrium ($r^2=0.84$ and $r^2=1$, respectively) according to HapMap, only the SNPs rs2235543, rs12565406, rs4844880, rs2282738, rs11119328, rs846906, rs4844488, rs3753519, rs10082248 were genotyped and further analyzed. The HapMap linkage disequilibrium (r^2) data of these nine common SNPs are schematically shown in figure 1.

Genotyping

DNA was isolated from whole blood using a commercial DNA isolation kit (NucleoSpin, Macherey & Nagel, Düren, Germany). The nine *HSD11B1* SNPs were genotyped using the Sequenom mass ARRAY system with iPLEX software (Sequenom, Hamburg, Germany). Genotyping for rs2282738 and rs11119328 was not successful in 3 subjects. The mean genotyping success rate was 99.7%. The Sequenom results were validated by bidirectional sequencing in 50 randomly selected subjects, and both methods gave 100% identical results.

RNA isolation, RT-PCR, and real-time quantitative PCR analysis of hepatic mRNA expression

For liver *HSD11B1* gene expression analysis, a total of 105 Caucasians (38 women and 67 men, age 63 ± 12 years, BMI 25.4 ± 4.1 kg/m^2, liver fat content 2.6 ± 2.8%) undergoing liver surgery, in most cases because of hepatocellular carcinoma or liver metastasis, were included in the present study. Patients were fasted overnight prior to collection of the liver biopsies, and tested negative for viral hepatitis and had no liver cirrhosis. Liver samples were taken from normal, nondiseased tissue during surgery, immediately frozen in liquid nitrogen and stored at −80°C.

Tissue samples were homogenized in phosphate-buffered-saline containing 1% Triton X-100 with a TissueLyser (Qiagen, Hilden, Germany). Triglycerides and total protein concentrations in the homogenate were quantified using the ADVIA 1800 clinical chemistry analyzer (Siemens Healthcare Diagnostics, Eschborn, Germany) and the results were calculated as mg/100 mg tissue (%) (8). RNA was extracted with the RNeasy Tissue Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Total RNA treated with RNase-free DNase I was transcribed into cDNA using first-strand cDNA kit and PCRs were performed in duplicates on a LightCycler480 (Roche Diagnostics, Mannheim, Germany). Data are presented relative to the housekeeping gene Rps13 using the ΔΔCt method. Primers used for PCR and real time PCR (Roche Diagnostics, Basel, Switzerland): Rps13, upstream primer: 5′-CCCCACTTGGTTGAAGTTGA-3′; downstream primer: 5′-ACACCATGTGAATCTCTCAGGA-3′; HSD11B1, upstream primer: 5′-CAATGGAAGCATTGTTGTCG-3′; downstream primer: 5′-GGCAGCAACCATTGGATAAG-3′.

Statistical analyses

Data are given as means ± SD (standard deviation) for normally distributed parameters and medians [interquartile range] for non-normally distributed parameters. Data that were not normally distributed (Shapiro-Wilk *W* test) were logarithmically transformed. To identify independent associations of the SNPs, we performed multiple linear regression analyses with the relevant metabolic parameters set as dependent variables. BMI, waist circumference, TAT, SAT, VAT, liver fat content and NAFLD were adjusted for age and gender. The other parameters were additionally adjusted for TAT mass. The genotype was included in the analyses as an independent continuous variable. For each dependent variable two models were applied. In the additive model the effects of all possible genotypes on the dependent variable were compared and allele-dose effects were tested; in the dominant model subjects homozygous for the major allele were compared to subjects heterozygous and homozygous for the minor allele. Logistic regression with adjustment for covariates, as described above, was applied to determine the odds ratio (OR) of the subjects with the different genotypes for having fatty liver. The Hardy-Weinberg equilibrium was tested using a χ2-test. The statistical software package JMP 11.0 (SAS Institute Inc, Cary, NC, USA) was used. A p-value ≤ 0.05, and $P \leq .0057$ after correcting for the 9 SNPs (Bonferroni), was considered statistically significant.

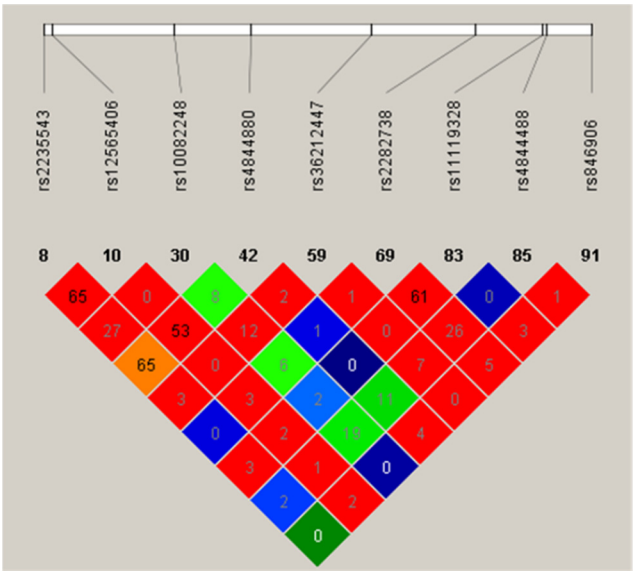

Figure 1. Genomic region of human chromosome 1 harboring the *HSD11B1* gene and HapMap linkage disequilibrium (r2) data of common (MAF ≥ 0.05) SNPs within this region. The *HSD11B1* gene consists of 7 exons and 6 introns and spans 48 785 kb from nucleotide 209 859 510 to nucleotide 209 908 295 (HapMap coordinates). In the diamonds below the SNPs, r^2 values are given.

Results

Demographic, anthropometric and metabolic characteristics of the subjects

The characteristics of the 327 subjects are shown in Table 1. All parameters covered a wide range, eg, age 18–69 years, body weight 52–156 kg, TAT mass 3.96–69.55 kg, VAT mass 0.22–10.12 kg, liver fat content 0.16–30.88%, and insulin sensitivity 1.59–33.36 arbitrary units (1 arbitrary unit = $10^{19}\cdot l^2\cdot mol^{-2}$) as assessed by

Table 1. Characteristics of the study population

	Overall population	Subgroup with Clamp
n (females/males)	327 (199/128)	219 (124/95)
Age (years)	46.0 [38.0–54.0]	48.0 [39.0–54.0]
BMI ($kg \bullet m^{-2}$)	28.99 [26.52–32.42]	28.89 [26.47–32.08]
Waist circumference (cm)	96.49 ± 13.15	96.16 ± 12.85
Total body fat mass $_{MRT}$ (kg)	24.92 [18.24–32.89]	24.84 [18.58–31.98]
Subcutaneous abdominal fat mass (kg)	10.61 [7.46–14.35]	10.52 [7.47–13.74]
Visceral fat mass $_{MRT}$ (kg)	2.62 [1.47–4.03]	2.70 [1.48–4.14]
Liver fat $_{MRS}$ (%)	3.47 [1.51–7.71]	3.42 [1.45–7.96]
NAFLD (n)	105 (32.1%)	68 (31.1%)
ALT (U/liter)	24.0 [18.0–34.0]	24.0 [18.0–34.0]
AST (U/liter)	22.0 [19.0–28.3]	23.0 [19.0–29.0]
γGT (U/liter)	18.0 [12.0–32.0]	18.0 [12.0–32.0]
Fasting glucose (mM)	5.17 [4.83–5.61]	5.11 [4.83–5.44]
2 h glucose $_{OGTT}$ (mM)	6.67 [5.83–7.89]	6.61 [5.83–7.89]
Fasting free fatty acids (μM)	644 [495–800]	632 [470–800]
Hepatic IR index	400 [278–632]	400 [278–631]
Adipose tissue IR index (x 10^3)	31.14 [20.46–51.51]	28.20 [19.96–48.43]
HOMA-IR index ($mmol \bullet mU/liter^2$)	1.56 [1.04–2.52]	1.47 [1.03–2.42]
Insulin sensitivity $_{OGTT}$ (AU)	11.42 [7.46–16.72]	11.80 [7.80–17.12]
ISI_{clamp}($\mu mol \bullet kg^{-1} \bullet min^{-1} \bullet pM^{-1}$)	0.058 [0.041–0.079]	0.058 [0.041–0.079]

Data are given as counts, percentages, mean ± SD (standard deviation) for the normally distributed parameter waist circumference, or medians [interquartile range] for the other, non-normally distributed parameters. BMI, body mass index; ALT, alanine aminotransferase; AST, aspartate aminotransferase; γGT, γ glutamyltranspeptidase; OGTT, oral glucose tolerance test; IR, insulin resistance; HOMA-IR, homeostasis model assessment of insulin resistance; AU, arbitrary units; ISI, insulin sensitivity index.

the OGTT. A total of 105 subjects (32%) had fatty liver (liver fat > 5.56%) (10). The known inverse correlation of liver fat content with insulin sensitivity was confirmed in this cohort (OGTT, r=-0.54, $P < .0001$; clamp, r=-0.58, $P < .0001$).

Genotyping of *HSD11B1* tagging SNPs

All study participants were genotyped for the 9 SNPs as described in the methods section (figure 1). All SNPs were in Hardy-Weinberg equilibrium (all $P > .05$). The observed MAFs were 0.153 (rs2235543), 0.106 (rs12565406), 0.182 (rs4844880), 0.106 (rs3753519), 0.235 (rs2282738), 0.05 (rs10082248), 0.159 (rs11119328), 0.153 (rs846906), and 0.076 (rs4844488), and were comparable to those provided by HapMap for the Central European population (0.150, 0.085, 0.167, 0.097, 0.243, 0.05, 0.174, 0.136, and 0.075, respectively). The observed genetic linkage between the nine SNPs was modest with minimal $r^2=0.05$ and maximal $r^2=0.235$ (figure 1).

Associations of *HSD11B1* SNPs with body fat compartments and plasma lipids

After adjustment for age and gender, none of the 9 SNPs associated with TAT mass or SAT mass, with the exception of a modest nominal association of the SNP rs2235543 with TAT mass ($P = .033$ in the additive, $P = .042$ in the dominant model, Table 2). However, the major C-allele of rs2235543 and the major G allele of rs12565406 were significantly associated with increased VAT mass. These associations were significant after Bonferroni correction in the additive model for rs2235543 ($P = .0047$) and in the dominant model for rs12565406 ($P = .0017$), but they almost reached significance after Bonferroni correction also in the dominant model for rs2235543 ($P = .0085$) and the additive model for rs12565406 ($P = .0064$). There was also a nominally significant association of the major T-allele of SNP rs4844880 with VAT mass ($P = .023$ in the additive and $P = .031$ in the dominant model). None of the SNPs showed significant associations with fasting FFA (Table 2 and Supplemental Tables 1–2) or fasting triglycerides (all $P > .13$), total- (all $P > .68$), HDL- (all $P > .10$) and LDL-cholesterol (all $P > .50$).

Associations of *HSD11B1* SNPs with liver fat content, NAFLD and liver enzymes

In contrast to the rather modest associations of the *HSD11B1* SNPs with body fat compartments, a robust association was observed for three SNPs, rs2235543, rs12565406, and rs4844880, with liver fat content. After adjustment for age and gender, the minor T-allele of rs2235543 was significantly associated with lower liver fat content in both, the additive and the dominant models ($P = .0002$ and $P = .0005$, respectively), withstanding Bonferroni correction. Significant associations were also found for the minor T-allele of rs12565406 ($P = .001$ in

Table 2. Associations of the SNPs rs2235543, rs12565406 and rs4844880 in the *HSD11B1* gene locus with demographic and metabolic characteristics

	rs2235543					rs12565406					rs4844880				
Parameter	CC	CT	TT	p add	p dom	GG	GT	TT	p add	p dom	TT	TA	AA	p add	p dom
Gender (females / males)	141 / 92	53 / 34	5 / 2	0.73#	0.84#	159 / 104	38 / 24	2 / 0	0.62#	0.76#	139 / 93	51 / 30	9 / 5	0.59#	0.58#
Age (years)	47.0 [37.0–54.0]	49.0 [39.0–56.0]	36.0 [25.0–43.0]	0.59	0.16	46.0 [37.0–54.0]	48.0 [40.0–55.0]	30.5 [25.0–36.0]	0.40	0.21	47.0 [37.0–54.0]	44.0 [39.0–55.0]	43.0 [34.3–55.5]	0.50	0.26
Body mass index ($kg{\cdot}m^{-2}$)	29.41 [27.01–32.46]	28.33 [24.82–32.11]	27.03 [24.98–28.73]	0.09	0.11	29.40 [26.93–32.46]	27.55 [24.48–31.43]	31.67 [27.02–36.33]	0.12	0.07	29.28 [26.93–32.45]	28.33 [25.10–32.58]	27.02 [23.47–29.54]	0.16	0.28
Waist circumference (cm)	97.37 ± 13.15	94.60 ± 13.04	90.79 ± 12.37	0.027	0.022	97.07 ± 13.05	93.88 ± 13.45	101.50 ± 13.44	0.12	0.055	97.38 ± 13.20	94.69 ± 12.16	92.25 ± 16.68	0.03	0.0402
Total body fat mass MRT (kg)	25.47 [19.28–32.92]	22.36 [17.17–33.11]	18.20 [16.65–24.60]	0.033	0.042	25.37 [18.74–33.41]	21.80 [16.62–29.28]	30.95 [23.79–38.12]	0.11	0.08	25.41 [19.22–32.66]	23.49 [17.47–34.06]	17.87 [13.14–28.20]	0.07	0.13
Sc. abd. fat mass MRT (kg)	10.92 [8.14–14.30]	9.37 [6.90–14.89]	8.82 [6.74–13.26]	0.06	0.064	10.92 [7.74–14.44]	9.27 [6.74–13.45]	14.84 [10.23–19.46]	0.11	0.07	10.92 [8.06–14.21]	9.44 [7.11–14.92]	7.73 [5.77–13.77]	0.07	0.14
Visceral fat mass MRT (kg)	2.74 [1.49–4.14]	2.52 [1.37–3.95]	1.91 [0.44–2.58]	0.0047	0.0085	2.72 [1.49–4.14]	2.30 [1.15–3.67]	2.24 [1.91–2.58]	0.0064	0.0017	2.81 [1.50–4.15]	2.47 [1.34–3.68]	2.31 [0.74–3.67]	0.023	0.031
Liver fat MRS (%)	3.93 [1.70–8.18]	2.35 [1.18–5.49]	1.26 [0.63–2.49]	0.0002	0.0005	3.82 [1.64–8.09]	2.27 [1.17–5.14]	1.20 [0.88–1.51]	0.001	0.001	3.93 [1.69–8.08]	2.78 [1.18–6.78]	1.70 [0.96–3.95]	0.0009	0.0028
NAFLD	84 (36.1%)	21 (24.1%)	0 (0%)	0.0034	0.0066	91 (34,6%)	14 (22.6%)	0 (0%)	0.024	0.027	83 (35.8%)	21 (25.9%)	1 (7.1%)	0.0057	0.017
ALT (U/liter)	25.0 [19.0–34.0]	22.0 [16.0–33.0]	20.0 [13.0–22.0]	0.055	0.13	25.0 [18.0–34.0]	21.0 [16.0–29.0]	13.0 [10.0–16.0]	0.004	0.009	25.0 [18.0–34.0]	23.0 [18.0–33.0]	20.5 [13.8–22.5]	0.06	0.22
AST (U/liter)	23.0 [19.0–29.0]	22.0 [19.0–27.0]	21.0 [19.0–36.0]	0.28	0.16	23.0 [19.0–29.0]	21.0 [19.0–26.0]	21.5 [19.0–24.0]	0.039	0.028	23.0 [19.0–29.0]	22.0 [19.0–26.0]	20.5 [18.0–27.3]	0.16	0.15
γGT (U/liter)	19.0 [13.0–32.0]	18.0 [11.0–32.3]	12.0 [11.0–18.0]	0.38	0.45	19.0 [13.0–33.0]	17.0 [11.0–30.5]	11.5 [11.0–12.0]	0.23	0.24	19.0 [13.0–32.0]	18.0 [12.0–32.0]	14.0 [9.8–20.8]	0.56	0.76
Fasting glucose (mM)	5.17 [4.83–5.61]	5.17 [4.89–5.61]	4.72 [4.44–5.67]	0.39	0.44	5.17 [4.83–5.61]	5.12 [4.83–5.63]	5.22 [4.54–5.89]	0.43	0.34	5.20 [4.83–5.61]	5.11 [4.86–5.56]	5.14 [4.64–5.63]	0.18	0.23
2 h glucose (mM)	6.61 [5.78–7.78]-	6.89 [6.06–8.06]-	6.28 [6.06–7.72]-	0.66	0.65	6.61 [5.83–7.72]	7.03 [6.06–8.18]	6.34 [6.28–6.39]	0.72	0.69	6.59 [5.72–7.69]	6.89 [6.09–8.20]	6.34 [6.05–7.92]	0.51	0.42
Fasting free fatty acids (μM)	645 [496–782]	635 [489–821]	773 [446–1178]	0.49	0.76	643 [487–787]	651 [507–824]	607*	0.43	0.39	644 [487–795]	641 [508–805]	607 [444–913]	0.69	0.84
Hepatic IR index	416 [283–657]	360 [234–507]	383 [292–805]	0.034	0.029	414 [282–647]	356 [232–527]	392 [383–400]	0.044	0.043	414 [280–665]	381 [257–524]	329 [225–501]	0.022	0.028
Adipose tissue IR index(x 10^{-3})	33.33 [21.86–52.65]	26.01 [18.14–48.11]	36.87 [15.23–63.35]	0.58	0.47	31.58 [21.18–51.40]	28.31 [18.55–51.76]	30.35*	0.76	0.78	32.28 [22.36–52.17]	26.47 [18.83–50.42]	25.16 [15.66–47.52]	0.16	0.16
HOMA-IR index	1.64 [1.11–2.55]	1.25 [0.96–2.55]	1.56 [0.86–1.91]	0.16	0.18	1.61 [1.07–2.56]	1.25 [0.91–2.52]	1.84 [1.77–1.91]	0.27	0.25	1.62 [1.13–2.56]	1.25 [0.91–2.56]	1.06 [0.97–1.80]	0.027	0.037
Insulin sensitivity OGTT (AU)	10.72 [7.28–15.82]	12.50 [7.87–18.28]	12.05 [9.81–26.10]	0.17	0.20	11.18 [7.39–16.09]	12.88 [7.71–18.84]	10.93 [9.81–12.05]	0.27	0.25	10.83 [7.28–15.78]	12.14 [7.81–18.31]	13.97 [10.03–22.29]	0.044	0.06
ISIclamp ($\mu mol{\cdot}kg^{-1}{\cdot}min^{-1}{\cdot}pM^{-1}$)§	0.057 [0.040–0.074]	0.059 [0.044–0.095]	0.085 [0.057–0.156]	0.18	0.30	0.057 [0.040–0.077]	0.062 [0.045–0.096]	0.051	0.21	0.19	0.057 [0.038–0.074]	0.062 [0.045–0.093]	0.075 [0.057–0.125]	0.019	0.022

Values represent mean ± SD (standard deviation) for the normally distributed parameter waist circumference and median [interquartile range] for the other, non-normally distributed parameters. For statistical analyses, non-normally distributed parameters were log transformed. The genotype effect was tested using an additive and a dominant model. BMI, waist circumference, total body fat mass, sc. abd. fat mass, visceral fat mass, liver fat and NAFLD were adjusted for age and gender. The other parameters were additionally adjusted for total body fat mass. #χ2-test. Sc. abd., subcutaneous abdominal; AU, arbitrary units; ISI, insulin sensitivity index, §available in 219 subjects. *available in one subject. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IR, insulin resistance.

both models) and the A-allele of SNP rs4844880 (P = .0009 and P = .0028 in the additive and the dominant model, respectively), depicting an allele-dose effect. The associations remained nominally significant even after additional adjustments for VAT mass, implying that the effect of the SNPs on liver fat content is not exclusively mediated by VAT mass (rs2235543, P = .0084 and 0.0145 for the additive and the dominant model, respectively; rs12565406, P = .0032 and P = .061; rs4844880, P = .013 and P = .031). Moreover, in logistic regression models, rs2235543 and rs4844880 were related to the presence of NAFLD even after Bonferroni correction in the additive model (P = .0034 and P = .0057, respectively). Nominally significant relationships to NAFLD were found for these SNPs in the dominant model (P = .0066 and P = .017 respectively) and for rs12565406 in both, the additive (P = .024) and the dominant (P = .027) models. Subjects homozygous for the major allele of these 3 SNPs had an adjusted odds ratio (AOR) of 2.16 (95% CI 1.23–3.90) for rs2235543, 2.06 (1.08–4.13) for rs12565406, and 1.95 (1.13–3.49) for rs4844880 for having NAFLD, compared to carriers of the minor allele. Of note, there was also a nominal association of a fourth SNP, rs3753519, with liver fat content (Supplemental Table 1) and a trend for an association of this SNP with NAFLD. With the exception of rs12565406, which was significantly related to serum transaminases (with AST levels only nominally, with ALT levels even after Bonferroni correction, Table 2), no other SNP displayed significant associations with liver enzymes (Table 2 and Supplemental Tables 1–2).

Associations of *HSD11B1* SNPs with insulin sensitivity

Given the known close relationship of liver fat content with insulin sensitivity, we further investigated the possible effect of the *HSD11B1* SNPs on insulin sensitivity. Interestingly, we observed no consistently significant associations of the 9 SNPs with whole body insulin sensitivity after adjustment for age, gender and TAT mass, as estimated from the OGTT or measured by the clamp, indicating no considerable impact of the SNPs on whole-body insulin sensitivity (Table 2 and Supplemental Tables 1–2). Nonetheless, nominally significant associations of rs4844880 with all measures of insulin sensitivity and of rs3753519 and rs2282738 with insulin sensitivity measured by the clamp were found. There was also an allele-dose effect of rs2235543 on insulin sensitivity, although the association was not statistically significant. Moreover, nominal associations of rs2235543, rs12565406 and rs4844880 with the hepatic insulin resistance index were found.

HSD11B1 mRNA expression in human liver biopsies

The relationship of rs2235543, rs4844880 and rs12565406 with *HSD11B1* mRNA expression was an-

alyzed in 105 human liver biopsies. The three SNPs were not associated with age and BMI of the donors ($P > .6$) and had an equal gender distribution ($P > .49$, $\chi 2$-test). Furthermore, *HSD11B1* mRNA expression was not associated with age, BMI and sex ($P > .3$). The minor alleles of rs2235543 and rs12565406 were associated with lower hepatic mRNA expression of *HSD11B1* (figure 2 A, C). Minor allele carriers of rs4844880 tended to have lower hepatic *HSD11B1* mRNA expression (figure 2 B). Hepatic mRNA expression of *HSD11B1* tended to correlate positively with liver fat content ($P = .126$, $r^2=0.023$). After adjustment for age, gender and BMI, this correlation almost reached statistical significance ($P = .067$, standard $\beta=0.175$).

Discussion

We recently found that 12 weeks pharmacological inhibition of 11β-HSD1 led to a significant reduction of TAT and VAT mass, as well as of liver fat content, but not of hepatic or whole-body insulin resistance (5). Moreover, several studies in rodents and humans (14–21) have suggested that genetic variation in the gene coding for 11β-HSD1 or its activity associated with features of the metabolic syndrome. In the present study, we, therefore, investigated whether common genetic variation in *HSD11B1*, which may have a lifelong effect on 11β-HSD1 activity, is related to liver fat content, body fat mass and distribution, and insulin resistance in humans.

We found a strong association of 3 SNPs in *HSD11B1* with liver fat content. After adjustment for age and gender, and correction for multiple testing, the major alleles of all 3 SNPs were related to a higher liver fat content, and subjects homozygous for these alleles had approximately double the risk for having NAFLD, compared to carriers of the minor alleles. Furthermore, we studied whether these SNPs may be functional. Indeed, homozygous carriers of the major alleles of the same SNPs had elevated *HSD11B1* gene expression levels in human liver biopsies. In addition, we found a positive trend between hepatic mRNA expression and liver fat content. These data suggest that hepatic 11β-HSD1 activity may be involved in the pathophysiology of fat accumulation in the liver. Interestingly, 11β-HSD1 activity was also reported to positively correlate with the histological stage of nonalcoholic steatohepatitis (22). The causative relationship between *HSD11B1* mRNA expression and fatty liver (rather than vice versa) could be proven by a Mendelian Randomization analysis. Based on the assumption of random allocation of the *HSD11B1* alleles by conception and even distribution of factors that possibly confound a true association among those who carry and those who do not carry the predisposing genotype (23), this statistical method calculates the effect size of the association based on the effect sizes of the genotype (allele-dose effects) on hepatic *HSD11B1* mRNA expression and fatty liver, and tests whether the calculated and actually found effect sizes are similar (24). Unfortunately, the small number of available biopsies did not allow us to perform this analysis.

To our knowledge, this is the first study investigating a relationship of SNPs of *HSD11B1* with liver fat content in humans. Our results are consistent with the findings of our previous study in humans (5). Moreover, our findings are also in line with data from animal studies: adipose- or liver-specific transgenic mice overexpressing *HSD11B1* displayed increased liver fat content (14–16, 18), while global or adipose-selective *HSD11B1* knockout (KO) mice were found to be protected from hepatic steatosis (17–19).

In the present study we also noticed significant associ-

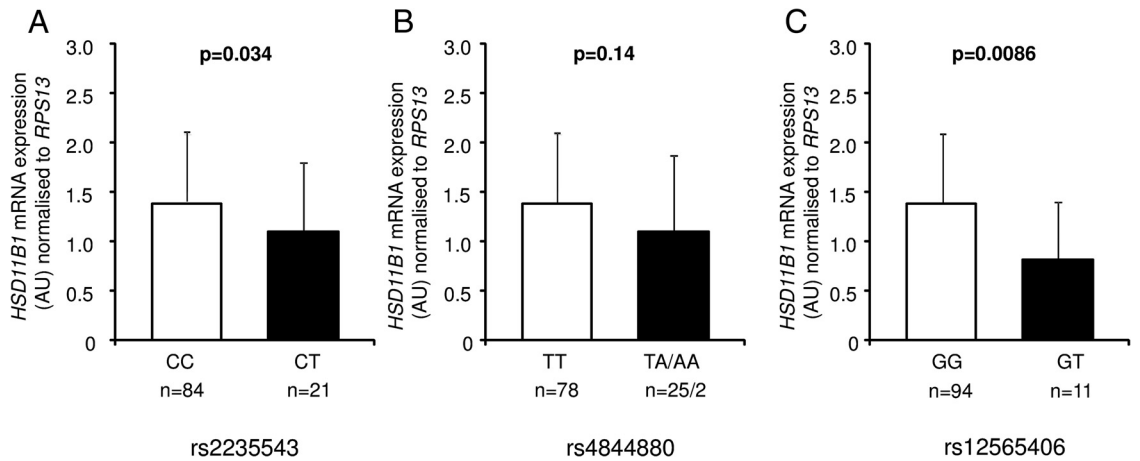

Figure 2. Associations of hepatic *HSD11B1* mRNA expression with 11β-HSD1 genotypes in 105 subjects are displayed as means ± SE for 3 SNPs (A) rs2235543 (B) rs4844880 and (C) rs12565406. *HSD11B1* mRNA expression is normalized to the *RPS13* expression. *HSD11B1* mRNA expression was log transformed for statistical analysis. *P*-values are for unadjusted parameters.

ations of the 3 aforementioned SNPs with VAT mass. Nominal associations were noted between the 3 SNPs and TAT mass, whereas only a trend for a relationship between the SNPs and SAT mass was found. These findings are again in line with the results of our pharmacological study (5), as well as with the results of studies with selective pharmacological inhibition of 11β-HSD1 in animal models of obesity (1, 2, 4).

However, the question remains whether increased expression of *HSD11B1* or activity of the enzyme in adipose tissue or in the liver is more important for the accumulation of fat in the liver. Data from other animal studies point to a key role of 11β-HSD1 in adipose tissue (25). While adipose-specific *HSD11B1* KO mice are protected from hepatic steatosis, liver-specific *HSD11B1* KO mice develop a phenotype of the metabolic syndrome including hepatic steatosis under a high fat diet (17, 18, 26). In addition, some human studies suggest an important role of 11β-HSD1 activity in SAT (27) or VAT (25, 28) in the pathogenesis of obesity and diabetes. In the present study, however, the association between the 3 SNPs and liver fat content was partly independent from VAT mass. Thus, particularly 11β-HSD1 activity in the liver may be critical for the accumulation of fat in this organ. This is in line with data suggesting that splanchnic cortisol production takes place primarily in the liver (29, 30).

Considering the close relationship of liver fat accumulation with insulin resistance (31–33), an interesting finding of our study is the absence of a clearly significant association of the 3 SNPs, that associate with liver fat content, with insulin sensitivity. Nevertheless, there was a nominal association of all 3 SNPs with the hepatic insulin resistance index. Whole-body insulin sensitivity was only nominally related with rs4844880. However, there were also nominally significant associations of rs3753519 and rs2282738 with insulin sensitivity measured by the clamp. These findings suggest that there may be an effect of the SNPs on insulin sensitivity, but this effect is weak and involves mainly hepatic insulin sensitivity. A small effect of the SNPs on muscle insulin sensitivity cannot be excluded, but, according to our results, an effect of the SNPs on adipose tissue insulin sensitivity is unlikely.

Studies that investigated potential associations of the variation in the *HSD11B1* gene with insulin sensitivity, the metabolic syndrome or type 2 diabetes in humans have provided conflicting results. In Pima Indians, rs846910 and rs3334312, which were in 100% linkage disequilibrium, were found to be associated with insulin sensitivity, insulin concentrations and type 2 diabetes, but not with obesity (20). The same SNP rs846910, and the SNP rs12086634 were associated with the metabolic syndrome in another study (21), but only with glucose levels and HDL-cholesterol, and only in women in a third study (34). Other SNPs showed no associations with obesity, the metabolic syndrome or type 2 diabetes in other studies (35–40). Possibly, the different results can be explained by the different populations examined, eg, in terms of ethnicity. We did not genotype the SNP rs846910, because of a very low MAF (0.083%) in the CEU population, according to HapMap. The SNP rs12086634 was in linkage disequilibrium with the SNP rs2282738. Thus, both, the aforementioned studies and the present analysis indicate that, at least in humans, 11β-HSD1 expression and activity only moderately affect whole-body insulin sensitivity. This would differentiate the SNPs of *HSD11B1* from specific SNPs in *DGAT2* (acyl-CoA:diacylglycerol acyltransferase 2 gene) (41), *PNPLA3* (patatin-like phospholipase 3 gene) (7), and possibly *TM6SF2* (transmembrane 6 superfamily member 2 gene) (42), which are known to confer an increased risk of fatty liver, as well as a more advanced histological stage of NAFLD, but not of insulin resistance.

Our study has certain limitations. First, it is unclear whether our findings and conclusions can be applied to other populations. Since there is no other study in the literature investigating the effect of *HSD11B1* SNPs on liver fat content, our results need replication in other ethnic groups. Second, our sample consisted of a cohort covering a wide range of age, adiposity, and insulin sensitivity. Thus, an impact of the SNPs on insulin sensitivity in a more obese or more insulin resistant population cannot be excluded. Third, in contrast to genome-wide association studies comprising hundreds of thousands of individuals to identify possible effects of common genetic variants, our study included only 327 subjects. Despite correction for multiple testing, this small sample size does not allow to ultimately exclude remaining statistical uncertainties. However, our population was precisely phenotyped, which is not feasible in very large study populations. This approach allowed us to study the possible effects of the genetic variants on multiple anthropometric and metabolic characteristics and also to adjust for confounders. Last, as a consequence of the size of the population, the number of the homozygotes for the minor allele was relatively small. Since we considered only variants with MAFs ≥ 0.05, other SNPs in *HSD11B1* affecting liver fat content and insulin sensitivity may exist, but they would be extremely rare.

To summarize, in the present study we found that genetic variation in *HSD11B1* was associated with visceral and particularly with liver fat accumulation, but not substantially with whole-body insulin resistance. These data indicate that chronic inhibition of 11β-hydroxysteroid-dehydrogenase 1 may be a valuable approach to treat NAFLD and visceral obesity.

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References

1. **Anagnostis P, Athyros VG, Tziomalos K, Karagiannis A, Mikhailidis DP.** Clinical review: The pathogenetic role of cortisol in the metabolic syndrome: a hypothesis. *J Clin Endocrinol Metab*. 2009; 94(8):2692–2701.
2. **Morton NM, Seckl JR.** 11beta-hydroxysteroid dehydrogenase type 1 and obesity. *Front Horm Res*. 2008;36:146–164.
3. **Walker BR.** Cortisol–cause and cure for metabolic syndrome? *Diabet Med*. 2006;23(12):1281–1288.
4. **Gathercole LL, Lavery GG, Morgan SA et al.** 11beta-Hydroxysteroid dehydrogenase 1: translational and therapeutic aspects. *Endocr Rev*. 2013;34(4):525–555.
5. **Stefan N, Ramsauer M, Jordan P et al.** Inhibition of 11beta-HSD1 with RO5093151 for non-alcoholic fatty liver disease: a multicentre, randomised, double-blind, placebo-controlled trial. *Lancet Diabetes Endocrinol*. 2014;2(5):406–416.
6. **Kantartzis K, Thamer C, Peter A et al.** High cardiorespiratory fitness is an independent predictor of the reduction in liver fat during a lifestyle intervention in non-alcoholic fatty liver disease. *Gut*. 2009; 58(9):1281–1288.
7. **Kantartzis K, Peter A, Machicao F et al.** Dissociation between fatty liver and insulin resistance in humans carrying a variant of the patatin-like phospholipase 3 gene. *Diabetes*. 2009;58(11):2616–2623.
8. **Peter A, Kantartzis K, Machicao F et al.** Visceral obesity modulates the impact of apolipoprotein C3 gene variants on liver fat content. *Int J Obes (Lond)*. 2012;36(6):774–782.
9. **Stefan N, Fritsche A, Schick F, Haring HU.** Phenotypes of prediabetes and stratification of cardiometabolic risk. *Lancet Diabetes Endocrinol*. 2016;4(9):789–798.
10. **Szczepaniak LS, Nurenberg P, Leonard D et al.** Magnetic resonance spectroscopy to measure hepatic triglyceride content: prevalence of hepatic steatosis in the general population. *Am J Physiol Endocrinol Metab*. 2005;288(2):E462–E468.
11. **Kantartzis K, Fritsche A, Machicao F et al.** Upstream transcription factor 1 gene polymorphisms are associated with high antilipolytic insulin sensitivity and show gene-gene interactions. *J Mol Med*. 2007;85(1):55–61.
12. **Abdul-Ghani MA, Matsuda M, Balas B, DeFronzo RA.** Muscle and liver insulin resistance indexes derived from the oral glucose tolerance test. *Diabetes Care*. 2007;30(1):89–94.
13. **Portillo-Sanchez P, Bril F, Maximos M et al.** High Prevalence of Nonalcoholic Fatty Liver Disease in Patients With Type 2 Diabetes Mellitus and Normal Plasma Aminotransferase Levels. *J Clin Endocrinol Metab*. 2015;100(6):2231–2238.
14. **Masuzaki H, Paterson J, Shinyama H et al.** A transgenic model of visceral obesity and the metabolic syndrome. *Science*. 2001; 294(5549):2166–2170.
15. **Paterson JM, Morton NM, Fievet C et al.** Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. *Proc Natl Acad Sci U S A*. 2004;101(18):7088–7093.
16. **Masuzaki H, Yamamoto H, Kenyon CJ et al.** Transgenic amplification of glucocorticoid action in adipose tissue causes high blood pressure in mice. *J Clin Invest*. 2003;112(1):83–90.
17. **Morton NM, Paterson JM, Masuzaki H et al.** Novel adipose tissue-mediated resistance to diet-induced visceral obesity in 11 beta-hydroxysteroid dehydrogenase type 1-deficient mice. *Diabetes*. 2004; 53(4):931–938.
18. **Morgan SA, McCabe EL, Gathercole LL et al.** 11beta-HSD1 is the major regulator of the tissue-specific effects of circulating glucocorticoid excess. *Proc Natl Acad Sci U S A*. 2014;111(24):E2482–E2491.
19. **Morton NM, Holmes MC, Fievet C et al.** Improved lipid and lipoprotein profile, hepatic insulin sensitivity, and glucose tolerance in 11beta-hydroxysteroid dehydrogenase type 1 null mice. *J Biol Chem*. 2001;276(44):41293–41300.
20. **Nair S, Lee YH, Lindsay RS et al.** 11beta-Hydroxysteroid dehydrogenase Type 1: genetic polymorphisms are associated with Type 2 diabetes in Pima Indians independently of obesity and expression in adipocyte and muscle. *Diabetologia*. 2004;47(6):1088–1095.
21. **Gambineri A, Tomassoni F, Munarini A et al.** A combination of polymorphisms in HSD11B1 associates with in vivo 11{beta}-HSD1 activity and metabolic syndrome in women with and without polycystic ovary syndrome. *Eur J Endocrinol*. 2011;165(2):283–292.
22. **Ahmed A, Rabbitt E, Brady T et al.** A switch in hepatic cortisol metabolism across the spectrum of non alcoholic fatty liver disease. *PLoS ONE*. 2012;7(2):e29531.
23. **Smith GD, Ebrahim S.** 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32(1):1–22.
24. **Minelli C, Thompson JR, Tobin MD, Abrams KR.** An integrated approach to the meta-analysis of genetic association studies using Mendelian randomization. *Am J Epidemiol*. 2004;160(5):445–452.
25. **Candia R, Riquelme A, Baudrand R et al.** Overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in visceral adipose tissue and portal hypercortisolism in non-alcoholic fatty liver disease. *Liver Int*. 2012;32(3):392–399.
26. **Lavery GG, Zielinska AE, Gathercole LL et al.** Lack of significant metabolic abnormalities in mice with liver-specific disruption of 11beta-hydroxysteroid dehydrogenase type 1. *Endocrinology*. 2012;153(7):3236–3248.
27. **Dube S, Norby BJ, Pattan V, Carter RE, Basu A, Basu R.** 11beta-hydroxysteroid dehydrogenase types 1 and 2 activity in subcutaneous adipose tissue in humans: implications in obesity and diabetes. *J Clin Endocrinol Metab*. 2015;100(1):E70–E76.
28. **Walker BR.** Is "Cushing's disease of the omentum" an affliction of mouse and men? *Diabetologia*. 2004;47(5):767–769.
29. **Basu R, Basu A, Grudzien M et al.** Liver is the site of splanchnic cortisol production in obese nondiabetic humans. *Diabetes*. 2009; 58(1):39–45.
30. **Basu R, Edgerton DS, Singh RJ, Cherrington A, Rizza RA.** Splanchnic cortisol production in dogs occurs primarily in the liver: evidence for substantial hepatic specific 11beta hydroxysteroid dehydrogenase type 1 activity. *Diabetes*. 2006;55(11):3013–3019.
31. **Ortiz-Lopez C, Lomonaco R, Orsak B et al.** Prevalence of prediabetes and diabetes and metabolic profile of patients with nonalcoholic fatty liver disease (NAFLD). *Diabetes Care*. 2012;35(4):873–878.
32. **Stefan N, Haring HU.** The role of hepatokines in metabolism. *Nat Rev Endocrinol*. 2013;9(3):144–152.
33. **Gastaldelli A, Kozakova M, Hojlund K et al.** Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early

atherosclerosis in a large European population. *Hepatology*. 2009; 49(5):1537–1544.
34. **Turek LV, Leite N, Rodrigues Souza RL et al.** Gender-dependent association of HSD11B1 single nucleotide polymorphisms with glucose and HDL-C levels. *Genet Mol Biol*. 2014;37(3):490–495.
35. **Franks PW, Knowler WC, Nair S et al.** Interaction between an 11betaHSD1 gene variant and birth era modifies the risk of hypertension in Pima Indians. *Hypertension*. 2004;44(5):681–688.
36. **do Nascimento FV, Piccoli V, Beer MA, von Frankenberg AD, Crispim D, Gerchman F.** Association of HSD11B1 polymorphic variants and adipose tissue gene expression with metabolic syndrome, obesity and type 2 diabetes mellitus: a systematic review. *Diabetol Metab Syndr*. 2015;7:38.
37. **Ku YH, Koo BK, Kwak SH et al.** Regulatory effect of common promoter polymorphisms on the expression of the 11beta-hydroxysteroid dehydrogenase type 1 gene. *Horm Res*. 2009;72(1):25–32.
38. **Miyamoto Y, Morisaki H, Yamanaka I et al.** Association study of 11beta-hydroxysteroid dehydrogenase type 1 gene polymorphisms and metabolic syndrome in urban Japanese cohort. *Diabetes Res Clin Pract*. 2009;85(2):132–138.
39. **Moon SS, Lee YS, Kim JG et al.** Relationship of 11beta-hydroxysteroid dehydrogenase type 1 and hexose-6-phosphate dehydrogenase gene polymorphisms with metabolic syndrome and type 2 diabetes. *Endocr J*. 2011;58(11):949–959.
40. **Robitaille J, Brouillette C, Houde A, Despres JP, Tchernof A, Vohl MC.** Molecular screening of the 11beta-HSD1 gene in men characterized by the metabolic syndrome. *Obes Res*. 2004;12(10):1570–1575.
41. **Kantartzis K, Machicao F, Machann J et al.** The DGAT2 gene is a candidate for the dissociation between fatty liver and insulin resistance in humans. *Clin Sci (Lond)*. 2009;116(6):531–537.
42. **Pirola CJ, Sookoian S.** The dual and opposite role of the TM6SF2-rs58542926 variant in protecting against cardiovascular disease and conferring risk for nonalcoholic fatty liver: A meta-analysis. *Hepatology*. 2015;62(6):1742–1756.