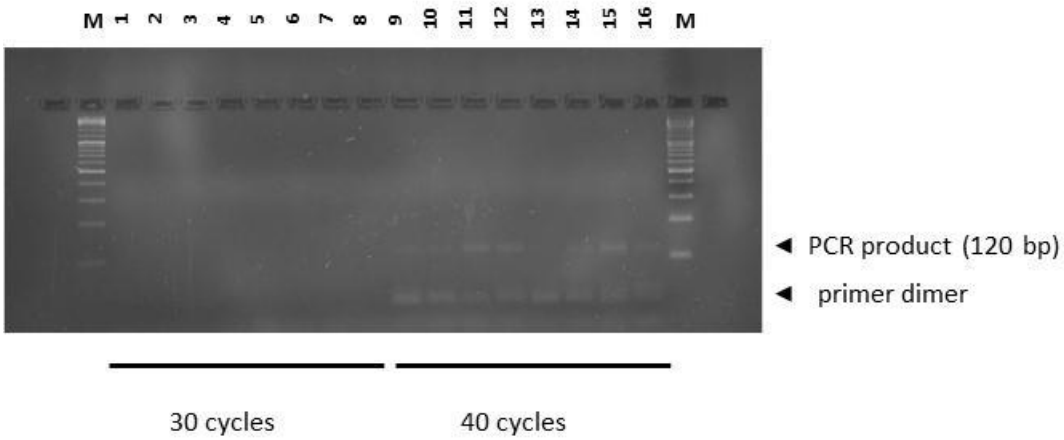
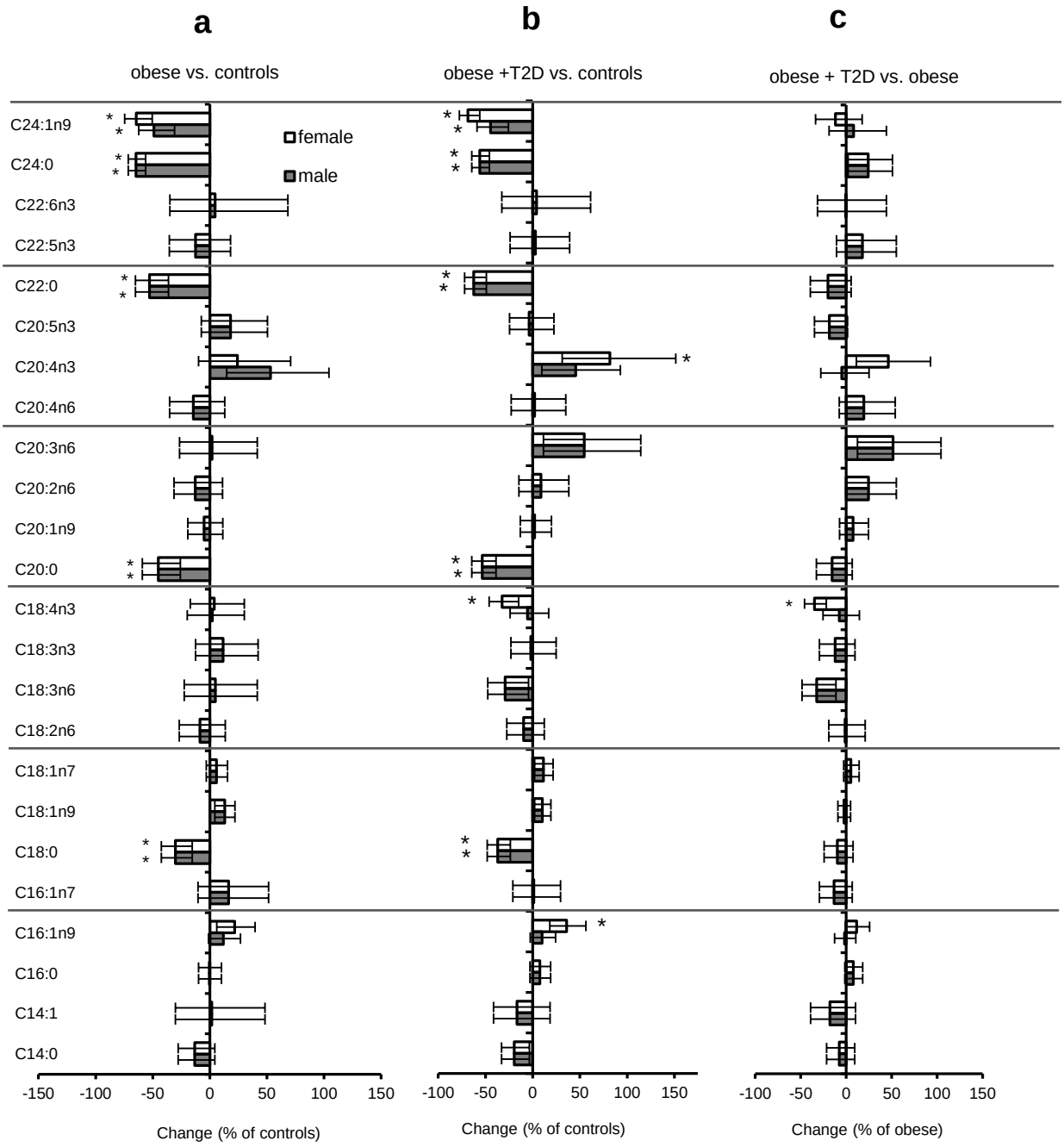
Supplementary Information

Supplementary Fig. S1. ChREBP-β expression in human SAT is too low for qPCR



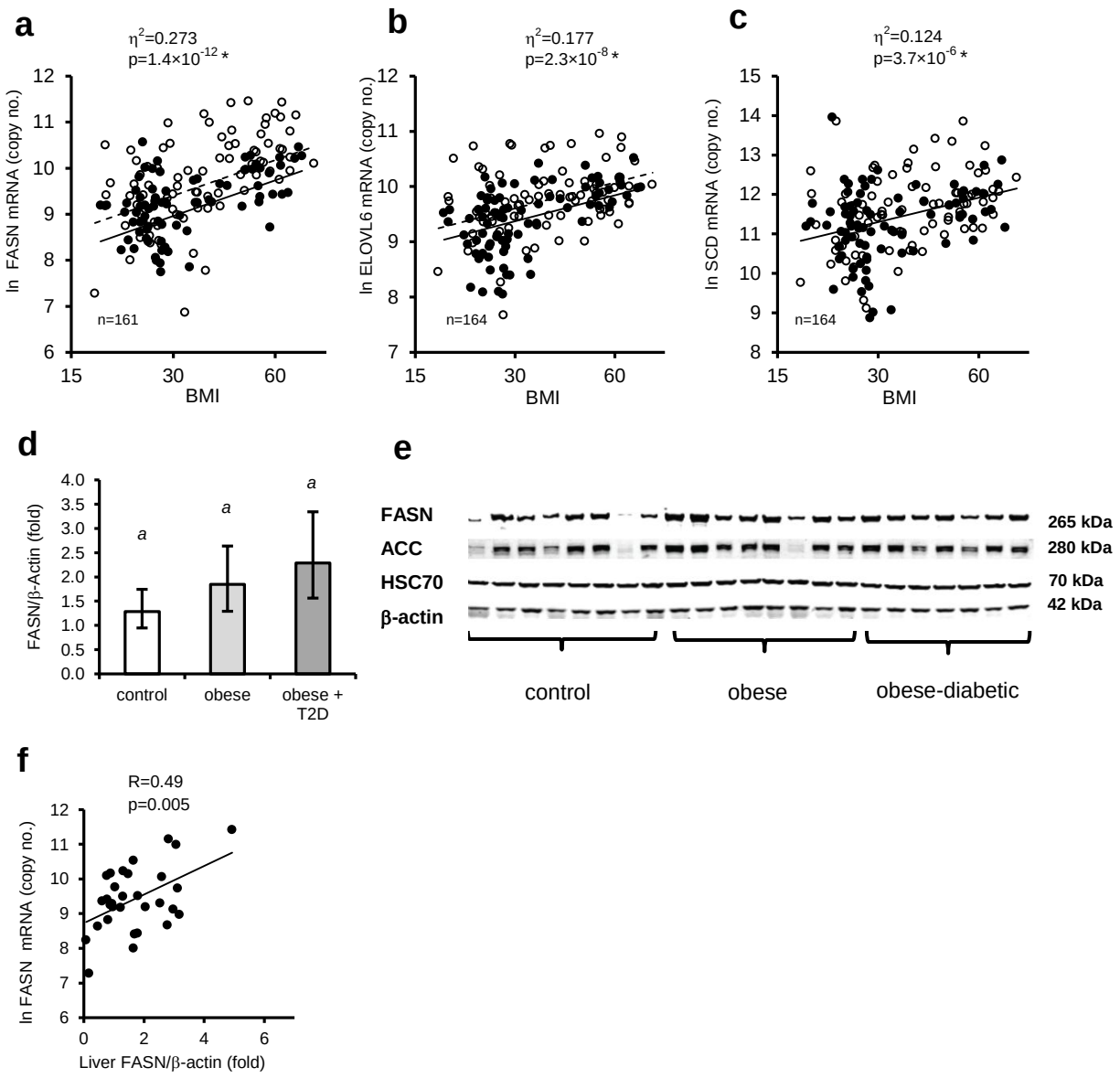
SYBR Green real-time PCR for ChREBP-β in SAT cDNA was unsuccessful in most samples, as indicated by primer-dimer formation (data from melting curve analysis, not shown). Low ChREBP-β expression as well as primer dimer formation was confirmed by PCR product analysis, using primers and conditions identical to the SYBR Green method. 8 randomly selected SAT cDNA samples were used and PCR was run for 30 cycles (lanes 1-8) and 40 cycles (lanes 9-16), respectively. M: 100 bp ladder. Expected size of the ChREBP-β PCR product is 120 bp.

Supplementary Fig. S2. Obesity and type 2 diabetes cause altered VAT fatty acid profile



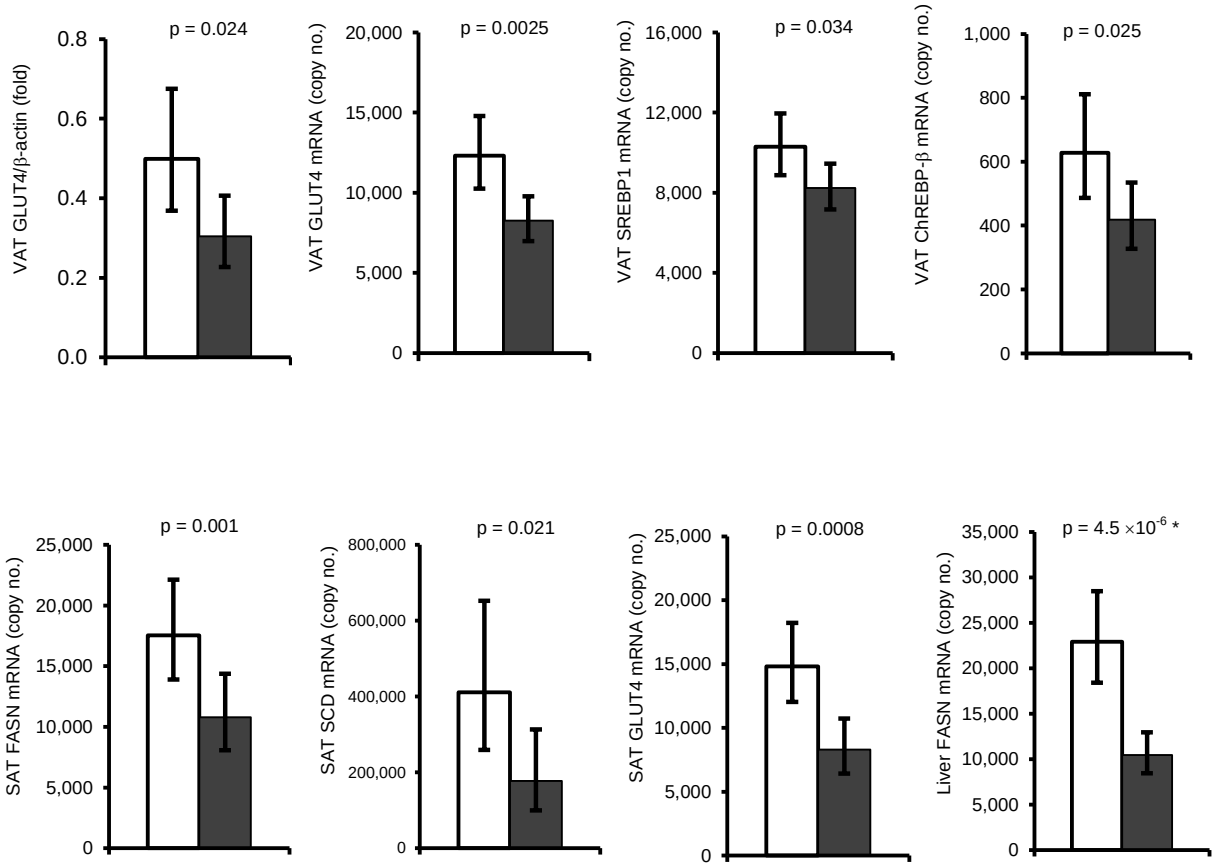
Percental change of individual fatty acids in obese compared to non-obese, (**a**), obese-diabetic compared to non-obese, (**b**), and obese-diabetic compared to obese, (**c**). Changes and 95% confidence intervals were calculated from estimated marginal means, as determined by ANCOVA. n=15-21 * statistically significant after adjustment for multiple testing ($p<0.0005$)

Supplementary Fig. S3. Liver FASN is upregulated in obese compared to non-obese subjects



Copy numbers of FASN, (**a**)**,** ELOVL6, (**b**), and SCD, (**c**)**,** mRNA in liver plotted against BMI (logarithmic scale, non-logarithmic numbers, ○ females, ● males). Trend lines (females, broken line) and partial eta-squared values (η^2) describe effect of BMI as independent variable in ANCOVA including gender as confounder (full ANCOVA, see Supplemental Table S1). Group analysis (n=7-13) comparing FASN protein (**d**), (estimated marginal means with 95% confidence intervals as determined by ANCOVA). Representative VAT Western blot (**e**). Correlation of liver FASN mRNA with FASN protein, (**f**). R, Pearson correlation coefficient. * statistically significant after adjustment for multiple testing

Supplementary Fig. S4. Gender differences in lipogenic gene expression



Expression of lipogenic mRNAs and protein in females (open bars) and males (closed bars) as determined by ANCOVA based on effects of group, gender and age (group effects see Figs. 1, 2, and 6). Only the parameters exhibiting a significant gender effect ($p<0.05$) in the ANCOVA are shown. Number of cases (n, females/males): VAT, n=23-28/24-31; SAT, n=21/16; liver, n=30/31. Estimated marginal means and 95% confidence intervals; * statistically significant after adjustment for multiple testing ($p<0.0005$)

Supplementary Table S1. Relationship of BMI, gender and age with DNL mRNA expression

dependent variable	η^2 (BMI)	η^2 (gender)	η^2 (age)	η^2 (BMI × age)	η^2 (BMI× gender)	η^2 (gender × age)
ln Liver FASN (n=161)	0.149 ↑††*	0.105 ↑††	0.070 ↑†	0.067 ↓†	-	-
ln Liver SCD (n=164)	0.124 ↑††*	-	-	-	-	-
ln Liver ELOVL6 (n=164)	0.082 ↑††	0.048 ↑†	0.033 ↑†	0.027 ↓†	-	-
ln VAT FASN (n=164)	-	-	0.036 ↓†	0.038 ↓†	-	-
ln VAT SCD (n=160)	0.151 ↑††*	-	-	-	-	-
ln VAT ELOVL6 (n=160)	-	0.033 ↓†	0.031 ↑†	0.036 ↓†	0.030 ↑†	-

ANCOVA was used to calculate the effect of BMI, gender and age on FASN, ELOVL6 and SCD mRNA expression in VAT and liver. Partial eta-squared values (η^2) describe the effect size. The direction of gender and age apparent effects on gene expression are denoted by the symbols ↑ (higher in females, increase with age) and ↓ (lower in females, decrease with age). Similarly, ↓ in the interaction terms BMI × age indicates that the apparent increasing effect of BMI on liver FASN and ELOVL6 is diminished with age and that the decreasing effect of BMI on VAT FASN and ELOVL6 is exaggerated with age. The apparent decreasing effect of BMI on VAT ELOVL6 is weaker in females, as indicated by symbol ↑ in the BMI × gender. Only effects with $p < 0.05$ are shown. †† $p < 0.001$, † $p < 0.05$ * statistically significant after adjustment for multiple testing

Supplementary Table S2. Relationship of HOMA-IR, liver steatosis, gender and age with DNL protein expression

dependent variable	η^2 (HOMA-IR)	η^2 (liver steatosis)	η^2 (gender)	η^2 (age)
ln VAT ChREBP-β mRNA (n=46)	-	0.171 ↓†	-	-
ln VAT FASN/β-actin (n=37)	0.234 ↓†	-	-	-
ln VAT GLUT4/β-actin (n=37)	0.465 ↓††*	-	-	-
ln liver ChREBP-β mRNA (n=49)	0.177 ↑†	-	-	-
ln liver FASN mRNA (n=49)	0.178 ↑†	-	0.241 ↑††	-

ANCOVA was used to calculate the association of HOMA-IR, liver steatosis, gender and age with protein and mRNA expression of ChREBP-β, FASN and GLUT4 in VAT and liver. Partial eta-squared values (η^2) describe the effect size. Direction of association is denoted by the symbols ↑ (positive association, higher in females) and ↓ (negative association). Only effects with $p<0.05$ are shown. †† $p < 0.001$, † $p < 0.05$ * statistically significant after adjustment for multiple testing

Supplementary Table S3. Clinical characterization of the subgroup used for liver Western blot

	control	obese	obese-diabetic	p
n (male/female)	(9/4)	(4/5)	(3/4)	
Age, females (yr)	55.0 (42.5-67.5) ^{a}	33.0 (21.8-44.2) ^{b}	44.3 (31.8-56.7) ^{ab}	0.325
males, (yr)	51.8 (43.5-60.1) ^{a}	58.8 (46.3-71.2) ^{a}	48.7 (34.3-63.1) ^{a}	0.325
BMI, females (kg/m^2)	21.8 (17.7-26.9) ^{a}	54.5 (45.2-65.8) ^{b}	52.1 (42.2-64.2) ^{b}	3.0×10^{-8}*
males (kg/m^2)	22.3 (19.4-25.6) ^{a}	33.5 (27.2-41.4) ^{b}	43.9 (34.4-56.0) ^{b}	3.0×10^{-8}*
CRP, females (mg/L)	4.1 (1.1-15.7) ^{a}	14.8 (3.8-57.4) ^{a}	9.9 (2.6-38.2) ^{a}	0.118
males (mg/L)	3.8 (1.5-9.3) ^{a}	0.6 (0.2-2.5) ^{a}	15.3 (3.2-72.7) ^{a}	0.118
Liver steatosis (%)	0.4 (0.0-1.9) ^{a}	10.2 (3.4-27.1) ^{ab}	20.1 (6.8-56.2) ^{b}	2.0×10^{-4}*
HbA1c (%)	5.2 (4.9-5.6) ^{a}	5.7 (5.2-6.3) ^{a}	6.9 (6.2-7.7) ^{a}	0.001
HOMA-IR	0.9 (0.5-1.6) ^{a}	3.3 (1.7-6.4) ^{ab}	11.8 (5.6-24.9) ^{b}	4.0×10^{-5}*
TG (mg/dL)	115 (94-141) ^{a}	145 (113-185) ^{a}	184 (140-244) ^{a}	0.030
HDL-C (mg/dL)	41 (33-51) ^{a}	40 (31-52) ^{a}	29 (22-38) ^{a}	0.113
LDL-C (mg/dL)	125 (107-146) ^{a}	126 (104-152) ^{a}	103 (83-127) ^{a}	0.258
SBP, females (mm Hg)	110 (102-120) ^{a}	140 (128-152) ^{a}	131 (120-142) ^{a}	0.005
males (mm Hg)	122 (116-129) ^{a}	115 (106-125) ^{a}	136 (124-149) ^{a}	0.005
DBP (mm Hg)	75 (70-79) ^{a}	81 (75-87) ^{a}	83 (76-90) ^{a}	0.096

Estimated means (95% confidence intervals) and p values for effect of group as determined by ANCOVA. BMI was significantly higher in females compared to males. SBP and LDL-C showed significant increase with age and estimated means are calculated for median age (51 yr). Age, BMI, SBP and CRP are listed by genders, as group and gender interacted significantly in the in ANCOVA model. Groups identified by the same superscript letter (*a*,*b*,*c*) are not significantly different from each other ($p \geq 0.0005$). * statistically significant after adjustment for multiple testing

Supplementary Table S4. Clinical characterization of the study cohort

Parameter	Median (min-max)
n	165
Gender (f/m)	82/83
Age (yr)	57 (17-90)
BMI (kg/m^2)	31.0 (17.6-78.0)
WC (cm)	107 (64-177)
SBP (mm Hg)	130 (90-170)
DBP (mm Hg)	80 (40-120)
CRP (µg/mL)	4.8 (0.5-66.2)
HbA1c (%)	5.7 (3.7-11.1)
Insulin (µU/mL)	10.5 (1.1-171.4)
HOMA-IR	2.9 (0.2-60.9)
Glucose (mg/dL)	98 (43-289)
TG (mg/dL)	128 (45-366)
HDL-C (mg/dL)	42 (9-71)
LDL-C (mg/dL)	114 (47-238)

Clinical parameters were determined in the fasted state, WC, waist circumference, SBP, systolic blood pressure, DBP, diastolic blood pressure, CRP, C-reactive protein, TG, plasma triglycerides, HDL-C, plasma HDL cholesterol, LDL-C, plasma LDL-cholesterol.

Supplementary Table S5. TaqMan primer-probe sets used for qPCR

Gene	abbreviation	Assay ID
Fatty acid synthase	FASN	Hs00188012_m1
Fatty acid elongase 6	ELOVL6	Hs00225412_m1
Stearoyl-CoA desaturase	SCD	Hs01682761_m1
Glucose transporter-4	GLUT4 (SLC2A4)	Hs00168966_m1
Sterol regulatory element-binding protein 1	SREBP1 (SREBF1)	Hs01088691_m1
Carbohydrate-response element-binding protein-α	ChREBP-α (MLXIPL)	Hs00263027_m1
Tumor necrosis factor α	TNFα (TNF)	Hs00174128_m1
TATA box binding protein-associated factor	TAF1	Hs00270322_m1

When not identical with name used in manuscript the official symbol given by HUGO gene nomenclature committee is provided in brackets. Assay IDs are for TaqMan assays as supplied by Applied Biosystems.