

Heterogeneity of the *Stearoyl-CoA desaturase-1 (SCD1)* Gene and Metabolic Risk Factors in the EPIC-Potsdam Study

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Abstract

Background: Stearoyl-CoA desaturase-1 (SCD1) is an enzyme involved in lipid metabolism. In mice and humans its activity has been associated with traits of the metabolic syndrome, but also with the prevention of saturated fatty acids accumulation and subsequent inflammation, whereas for liver fat content inconsistent results have been reported. Thus, variants of the gene encoding SCD1 (*SCD1*) could potentially modify metabolic risk factors, but few human studies have addressed this question.

Methods: In a sample of 2157 middle-aged men and women randomly drawn from the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition, we investigated the impact of 7 *SCD1* tagging-single nucleotide polymorphisms (rs1502593, rs522951, rs11190480, rs3071, rs3793767, rs10883463 and rs508384) and 5 inferred haplotypes with frequency >5% describing 90.9% of the genotype combinations in our population, on triglycerides, body mass index (BMI), waist circumference (WC), glycated haemoglobin (HbA1c), high-sensitivity C-reactive protein (hs-CRP), gamma-glutamyltransferase (GGT), alanine aminotransferase (ALT) and fetuin-A.

Results: No significant associations between any of the SNPs or haplotypes and BMI, WC, fetuin-A and hs-CRP were observed. Associations of rs10883463 with triglycerides, GGT and HbA1c as well as of rs11190480 with ALT activity, were weak and became non-significant after multiple-testing correction. Also associations of the haplotype harbouring the minor allele of rs1502593 with HbA1c levels, the haplotype harbouring the minor alleles of rs11190480 and rs508384 with activity of ALT, and the haplotype harbouring the minor alleles of rs522951, rs10883463 and rs508384 with triglyceride and HbA1C levels and GGT activities did not withstand multiple-testing correction.

Conclusion: These findings suggest that there are no associations between common variants of *SCD1* or its inferred haplotypes and the investigated metabolic risk factors. However, given the results from animal models, heterogeneity of human *SCD1* warrants further investigation, in particular with regard to rare variants.

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Introduction

The human stearoyl-CoA desaturase-1 (*SCD1*) gene maps to chromosome 10q24.31, has 6 exons and is remarkably expressed in adipose tissue and the liver. It encodes the endoplasmic reticulum enzyme SCD1, which catalyses the conversion of the

saturated fatty acids (SFAs) palmitic and stearic, into the monounsaturated fatty acids (MUFAs) palmitoleic and oleic respectively [1]. These MUFAs are the major components of triglycerides, likely due to its production within the environs of the enzyme diacylglycerol acyltransferase (DGAT) [1]. Accordingly, it has been suggested that the increased activity of SCD1 in the liver,

could result in an excess assembly and accumulation of triglycerides and subsequent development of hepatic steatosis [2]. Further, the overflow of triglycerides could also be incorporated into very low density lipoprotein (VLDL) particles and transported to adipose tissue and other sites, contributing to the development of obesity [3]. Both these conditions have been associated with insulin resistance [4]. Additionally, *SCD1* deficiency has been associated with the reduced expression of fatty acid synthesis genes [5] and the up-regulation of genes involved in fatty acid β -oxidation [5,6]. In fact mice with a natural or a targeted deletion of the *SCD1* gene, have shown to be protected against hypertriglyceridemia [7], hepatic steatosis [8–10], obesity [3,5,8,11,12] and insulin resistance [11,13,14]. Conversely, there is also evidence that by channelling SFA, into triglyceride pools, increased *SCD1* activity may prevent from lipopoptosis [15], steatohepatitis [2] and inflammation [6,15–17]. Therefore, *SCD1* seems to convey both, positive and negative roles in the development of metabolic risk factors of cardiovascular diseases.

Despite the strong scientific interest in *SCD1*, most of the existing knowledge on its function comes from mice models [18]. Some human studies have provided indirect evidence of the role of its activity (approximated as fatty acids product-to-precursor ratios measured in serum, plasma, erythrocytes or adipose tissue), and have proposed that its elevation might be associated with harmful effects such as elevated plasma triglycerides levels [19,20], liver fat [21–23], obesity [19,24], diabetes [25], high-sensitivity C-reactive protein (hs-CRP) levels [26] and even with cardiovascular mortality [27]. Conversely the scarce human studies investigating the tissue-specific activity and expression of *SCD1*, suggest that elevated *SCD1* activity may protect from liver fat accumulation [28,29]. Further, the impact of *SCD1* heterogeneity on metabolic risk factors, has so far only been investigated in four human studies with focus on diabetes and obesity [30,31], metabolic syndrome (MetS) [32] or inflammation [33]. Thus, a case control study in men and women from the United Kingdom, found no associations between 6 *SCD1* single nucleotide polymorphisms (SNPs) or its inferred haplotypes and diabetes, body mass index (BMI) or waist-to-hip ratio [30]; a cross-sectional study in Swedish elderly men reported that 4 out of 8 *SCD1* tagging SNPs (tag-SNPs) related to decreased BMI and waist circumference (WC), and increased insulin sensitivity [31]; the haplotype consisting of the rare alleles of these SNPs was also associated with decreased WC; a cross-sectional study in Costa Rican middle-aged men and women reported that 1 out of 7 *SCD1* tag-SNPs was associated with an increased prevalence of MetS, and among women, also with elevated systolic blood pressure and fasting blood glucose levels [32]. Also 2 haplotypes carrying the minor allele of this SNP were associated with elevated prevalence of MetS; finally a cross-sectional study in European and Asian young adults found 1 out of 10 tag-SNPs to be associated with CRP levels [33].

In the Potsdam cohort of the European Prospective Investigation into Cancer and Nutrition (EPIC-Potsdam), we investigated the impact of common genetic variation in *SCD1*, captured by means of 7 tag-SNPs and their inferred haplotypes, on the modulation of 8 metabolic risk factors related to the activity of *SCD1*. The investigated traits were plasma triglyceride levels, traits related to obesity (BMI and waist circumference (WC)), glucose metabolism (glycated haemoglobin (HbA1c)) and chronic inflammation (hs-CRP), and, for the first time, crude estimates of the presence of liver fat (the liver enzymes gamma-glutamyltransferase (GGT) and alanine amino transferase (ALT) [34,35]), and fetuin-A, a biomarker which has been associated with fat

accumulation in the liver [36–40] as well as with insulin resistance, type 2 diabetes and cardiovascular events [40–42].

Methods

Ethics Statement

Written informed consent was obtained from all study participants, and approval was given by the Ethics Committee of the Medical Association of the State of Brandenburg, Germany.

Study Population

EPIC-Potsdam comprises 27548 individuals (10904 men and 16644 women) from the general population of the Potsdam area in Germany. Men were mainly aged 40–65 and women 35–65 years old at recruitment, which took place between 1994 and 1998 [43]. The baseline examination included a personal interview and a questionnaire on sociodemographic and lifestyle characteristics and prevalent diseases as well as anthropometric measurements [44]. The associations of 7 *SCD1* tag-SNPs and their inferred haplotypes with anthropometric and metabolic markers were investigated in a random sample of 2500 individuals (subcohort) drawn from the participants in the total cohort who had provided blood samples at baseline, following a cross-sectional design. After exclusion of individuals with missing covariates or genotype data, the final study population comprised 2157 participants. Fasting was not required at the time of blood draw, however, 615 participants were in fasting state for at least 8 h.

Laboratory Analyses

From all the study participants a 30 mL sample of venous blood was collected, fractionated into serum, plasma, buffy coat and erythrocytes, and stored in liquid nitrogen until the time of analysis. Plasma levels of triglycerides, HbA1c, GGT, ALT, fetuin-A, hs-CRP, total cholesterol and high density lipoprotein (HDL)-cholesterol were determined with the automatic ADVIA 1650 analyser (Siemens Medical Solutions, Erlangen, Germany) at the Department of Internal Medicine of the University of Tübingen, Germany, in 2007.

SNP Selection and Genotyping

Seven *SCD1* tag-SNPs (ordered according to chromosomal location: rs1502593, rs522951, rs11190480, rs3071, rs3793767, rs10883463 and rs508384) were identified in the HapMap 22/phaseII CEU population data (Utah residents with ancestry from northern and western Europe) [45] using stringent criteria (minor allele frequency (MAF) >0.05 and pairwise $r^2 \geq 0.8$) by means of the Tagger software [46] implemented in the version 4.2 of Haploview [47]. The tagged region comprised the coding region of *SCD1* as well as a 4.1 Kb upstream (promoter) and 4.3 Kb downstream (3' untranslated) region of the gene. Six SNPs were located in intronic sites and one in the 3' untranslated region (rs508384). Genotyping of whole genome amplified DNA samples was performed with a 7900HT Sequence Detection System with TaqMan assays (Applied Biosystems, Foster City, CA, USA) at the Max Delbrück Centre for Molecular Medicine, Berlin, Germany, in 2009. The average genotyping success rate in the 7 SNPs was $>98\%$.

Statistical Analyses

Normality of variables was tested by estimating their skewness and kurtosis, by comparing their means and median values and by plotting their distributions in histograms. To better reach normality of their distributions, triglycerides, GGT, ALT and hs-CRP were natural log-transformed and HbA1c inverse-

transformed, and were used like that in all analyses. Hardy–Weinberg equilibrium (HWE) of the SNPs was tested using the χ^2 test. Linkage disequilibrium between SNPs was assessed with the r^2 measure using Haploview 4.2 [47]. Each SNP was coded as 0, 1

and 2 according to the number of minor alleles a participant carried. Analysis of covariance considering the additive, dominant and recessive genetic models was used to assess the associations between the SNPs (independent variables) and triglycerides, BMI,

Table 1. Baseline characteristics of the EPIC-Potsdam subcohort and separately for men and women.

Characteristic	Subcohort (N = 2157)	Men (N = 819; 38%)	Women (N = 1338; 62%)	P value ^a
Age (years)	50.3 ± 9.0	52.4 (51.8–53.0)	49.0 (48.5–49.4)	<0.0001
Educational attainment (%):				
Vocational school or less	37.4	30.8	41.5	<0.0001
Technical school	24.9	15.9	30.4	<0.0001
University degree	37.7	53.4	28.1	<0.0001
Physical activity (%):				
<2 hours/week	75.9	74.7	76.7	0.3
≥2 hours/week	24.1	25.3	23.3	0.3
Smoking status (%):				
Current ≥20 cigarettes/d	6.3	11.3	3.2	<0.0001
Current <20 cigarettes/d	14.9	16.7	13.8	0.07
Former ≤5 years	7.5	9.5	6.3	0.006
Former >5 years	24.4	35.3	17.7	<0.0001
Never	46.9	27.2	59.0	<0.0001
Alcohol intake (%):				
Men: = 0 g/d; women: = 0 g/d	0.05	0.1	0.0	0.3
Men: >0–12 g/d; women: >0–6 g/d	47.2	36.4	53.9	<0.0001
Men: >12–24 g/d; women: >6–12 g/d	24.7	26.1	23.9	0.3
Men: >24 g/d; women: >12 g/d	28.0	37.5	22.2	<0.0001
Use of medication (%):				
Antidiabetic	2.6	3.5	2.0	0.03
Antihypertensive	19.3	19.6	19.2	0.8
Lipid lowering medication	5.1	5.7	4.7	0.3
Body mass index (kg/m ²)	26.1 ± 4.3	26.6 (26.3–26.8)	25.8 (25.6–26.1)	0.0002
Waist circumference (cm)	85.8 ± 12.9	93.4 (92.6–94.1)	81.2 (80.6–81.7)	<0.0001
Triglycerides^b (mg/dL)	90.2 (64.9–126.5)	109.3 (102.1–117.0)	82.1 (77.6–86.7)	<0.0001
Gamma-glutamyltransferase (U/L)	16.8 (11.0–30.8)	28.3 (26.8–29.9)	14.4 (13.8–15.0)	<0.0001
Glutamic-pyruvate transaminase (U/L)	18.7 (14.3–26.4)	25.9(25.1–26.7)	16.8 (16.4–17.3)	<0.0001
Fetuin-A^c (mg/dL)	0.25 ± 0.06	0.25 (0.24–0.25)	0.25 (0.25–0.26)	0.03
Glycated haemoglobin (%)	6.4 (6.1–6.8)	6.5 (6.4–6.6)	6.4 (6.4–6.5)	0.002
Hs-C-reactive protein (mg/L)	0.8 (0.2–2.1)	0.6 (0.6–0.7)	0.8 (0.6–0.7)	<0.0001
Minor allele frequency^d (%):				
rs1502593 (C>T)	44	45	43	0.2
rs522951 (G>C)	46	46	47	0.6
rs11190480 (A>G)	9	9	9	0.6
rs3071 (T>G)	35	35	34	0.8
rs3793767 (T>C)	38	36	39	0.2
rs10883463 (T>C)	8	8	8	0.7
rs508384 (C>A)	17	17	17	1.0

Subcohort: Mean ± SD, %, or median (25th percentile; 75th percentile), all such values. **Men and women:** mean and 95% confidence interval (CI) or %. Results obtained using analysis of covariance, all variables other than age are adjusted for age.

^aP value for the difference between men and women.

^bbased on the 615 participants fasting at blood draw.

^cbased on 2077 participants due to missing biomarker data.

^dAlleles given in brackets (most >less frequent allele).

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Table 2. Age- and sex-adjusted association analyses between the 7 *SCD1* tag-SNPs and the 8 investigated metabolic traits: the EPIC-Potsdam Study.

	Triglycerides ^{a, b} (mg/dL)	BMI ^c (kg/m ²)	WC ^c (cm)	HbA1c ^d (%)	GGT ^b (U/L)	ALT ^b (U/L)	Fetuin-A ^{c, e} (mg/dL)	hs-CRP ^b (mg/L)
rs1502593 (n)								
0 (679)	96.45 (89.23–104.26)	26.22±0.16	87.47±0.41	6.48 (6.43–6.53)	20.13 (18.97–21.35)	20.52 (19.81–21.25)	0.25±0.002	0.73 (0.66–0.81)
1 (1072)	92.49 (86.98–98.34)	26.25±0.13	87.36±0.33	6.50 (6.46–6.55)	20.24 (19.31–21.21)	20.85 (20.28–21.44)	0.25±0.002	0.72 (0.67–0.79)
2 (406)	96.46 (87.67–106.14)	26.00±0.21	86.63±0.53	6.45 (6.39–6.52)	20.27 (18.79–21.87)	21.52 (20.57–22.50)	0.26±0.003	0.73 (0.63–0.83)
P _{add}	0.89	0.47	0.25	0.63	0.87	0.11	0.19	0.88
P _{dom}	0.53	0.83	0.53	0.80	0.87	0.24	0.75	0.86
P _{rec}	0.63	0.30	0.18	0.25	0.93	0.14	0.06	0.96
rs522951 (n)								
0 (607)	94.06 (86.85–101.88)	26.03±0.17	86.79±0.44	6.47 (6.41–6.52)	19.93 (18.73–21.21)	20.96 (20.20–21.75)	0.25±0.002	0.72 (0.64–0.80)
1 (1095)	95.22 (89.54–101.26)	26.23±0.13	87.26±0.32	6.50 (6.46–6.54)	20.46 (19.53–21.43)	20.88 (20.31–21.46)	0.25±0.002	0.73 (0.67–0.79)
2 (455)	93.32 (85.14–102.30)	26.31±0.20	87.87±0.50	6.49 (6.43–6.55)	19.98 (18.59–21.47)	20.73 (19.87–21.64)	0.25±0.003	0.73 (0.64–0.83)
P _{add}	0.93	0.28	0.10	0.50	0.90	0.70	0.46	0.82
P _{dom}	0.90	0.27	0.20	0.35	0.60	0.78	0.20	0.80
P _{rec}	0.77	0.52	0.17	0.90	0.72	0.73	0.87	0.91
rs11190480 (n)								
0 (1787)	93.97 (89.60–98.56)	26.23±0.10	87.38±0.26	6.49 (6.46–6.52)	20.30 (19.57–21.06)	21.08 (20.62–21.54)	0.25±0.001	0.73 (0.68–0.78)
1 (357)	96.89 (87.11–107.77)	26.05±0.22	86.66±0.57	6.46 (6.39–6.53)	19.93 (18.39–21.61)	19.85 (18.92–20.82)	0.25±0.003	0.72 (0.63–0.83)
2 (13)	114.86 (39.83–331.24)	25.60±1.16	86.71±2.95	6.66 (6.29–7.06)	15.46 (10.14–23.57)	21.67 (16.88–27.81)	0.24±0.015	0.59 (0.28–1.25)
P _{add}	0.56	0.39	0.25	0.63	0.41	0.05	0.35	0.78
P _{dom}	0.58	0.42	0.24	0.49	0.54	0.03	0.36	0.85
P _{rec}	0.72	0.61	0.85	0.38	0.21	0.77	0.73	0.59
rs3071 (n)								
0 (944)	95.09 (89.07–101.53)	26.36±0.14	87.65±0.35	6.47 (6.43–6.52)	20.64 (19.63–21.70)	20.75 (20.15–21.38)	0.25±0.002	0.77(0.71–0.84)
1 (936)	94.84 (88.81–101.29)	25.96±0.14	86.79±0.35	6.50 (6.45–6.54)	19.76 (18.79–20.78)	20.86 (20.25–21.49)	0.25±0.002	0.68(0.63–0.75)
2 (277)	91.32 (81.28–102.61)	26.42±0.25	87.49±0.64	6.52 (6.44–6.60)	20.30 (18.53–22.25)	21.30 (20.17–22.48)	0.25±0.003	0.73(0.63–0.86)
P _{add}	0.62	0.47	0.36	0.28	0.46	0.46	0.92	0.22
P _{dom}	0.79	0.10	0.13	0.32	0.27	0.62	0.86	0.08
P _{rec}	0.54	0.34	0.69	0.46	0.92	0.43	0.64	0.90
rs3793767 (n)								
0 (845)	95.94 (89.64–102.68)	26.17±0.15	87.21±0.37	6.47 (6.42–6.51)	20.50 (19.45–21.61)	21.05 (20.41–21.72)	0.25±0.002	0.71 (0.65–0.78)
1 (998)	94.63 (88.81–100.83)	26.15±0.13	87.15±0.34	6.51 (6.47–6.55)	20.40 (19.43–21.41)	20.83 (20.23–21.44)	0.25±0.002	0.74 (0.68–0.81)
2 (314)	89.68 (79.88–100.67)	26.41±0.24	87.74±0.61	6.48 (6.41–6.56)	18.86 (17.30–20.57)	20.51 (19.49–21.59)	0.25±0.003	0.73 (0.62–0.84)
P _{add}	0.37	0.50	0.57	0.50	0.17	0.38	0.97	0.65
P _{dom}	0.56	0.81	0.86	0.26	0.49	0.48	0.50	0.50
P _{rec}	0.34	0.33	0.38	0.81	0.09	0.47	0.32	0.97
rs10883463 (n)								
0 (1840)	94.64 (90.34–99.15)	26.18±0.10	87.21±0.25	6.49 (6.46–6.52)	19.88 (19.17–20.61)	20.78 (20.34–21.23)	0.25±0.001	0.73 (0.68–0.78)
1 (304)	89.89 (79.74–101.33)	26.24±0.24	87.39±0.61	6.49 (6.41–6.56)	22.43 (20.56–24.48)	21.35 (20.27–22.48)	0.25±0.003	0.72 (0.62–0.85)
2 (13)	160.99 (100.50–257.90)	27.49±1.16	90.77±2.95	6.10 (5.80–6.44)	17.85 (11.72–27.19)	22.48(17.52–28.85)	0.23±0.017	0.61 (0.29–1.28)
P _{add}	0.76	0.50	0.48	0.36	0.04	0.27	0.85	0.81
P _{dom}	0.80	0.64	0.63	0.62	0.02	0.30	0.99	0.88
P _{rec}	0.03	0.26	0.23	0.03	0.56	0.56	0.32	0.63
rs508384 (n)								
0 (1489)	93.94 (89.21–98.93)	26.18±0.11	87.27±0.28	6.50 (6.46–6.53)	20.01 (19.22–20.83)	21.01 (20.51–21.51)	0.25±0.002	0.73 (0.68–0.78)
1 (610)	93.61 (86.21–101.65)	26.24±0.17	87.16±0.43	6.47 (6.42–6.53)	20.66 (19.42–21.98)	20.55 (19.81–21.31)	0.25±0.002	0.74 (0.66–0.82)
2 (58)	119.44 (92.43–154.35)	26.18±0.55	87.90±1.40	6.44 (6.27–6.62)	20.67 (16.92–25.25)	20.85 (18.52–23.48)	0.25±0.008	0.64 (0.45–0.91)
P _{add}	0.36	0.81	0.96	0.36	0.40	0.39	0.27	0.86
P _{dom}	0.69	0.78	0.92	0.39	0.38	0.33	0.31	0.95

Table 2. Cont.

	Triglycerides ^{a, b} (mg/dL)	BMI ^c (kg/m ²)	WC ^c (cm)	HbA1c ^d (%)	GGT ^b (U/L)	ALT ^b (U/L)	Fetuin-A ^{c, e} (mg/dL)	hs-CRP ^b (mg/L)
P _{rec}	0.07	0.98	0.64	0.58	0.82	0.99	0.49	0.46

Each SNP is coded as 0, 1 and 2 according to the number of minor alleles a participant carries.

^abased on the 615 participants fasting at blood draw.

^bgeometric means and (95% CI);

^cmeans and standard error;

^dinverse and (95% CI),

^ebased on 2077 participants due to missing biomarker data. All the reported significance levels are nominal P values and are not adjusted for multiple comparisons.

P_{add}, P for trend or P for the additive model; **P_{dom}**, P value for the dominant model. **P_{rec}**, P value for the recessive model.

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WC, HbA1c, GGT, ALT, fetuin-A and hs-CRP (dependent variables). Haplotypes were constructed to test whether multiple genetic variants of *SCD1* or a possible unobserved risk variant captured by the haplotypes, modulated the investigated traits. Haplotype frequencies were estimated based on the observed unphased genotypes by the expectation-maximization algorithm [48]. The effects of a particular haplotype load (0, 1 or 2 copies) were tested also by means of a regression-based analysis (ANCOVA) as suggested by Zaykin et al. [49]. Analyses were restricted to participants with a probability of 49–50% to carry one copy of the haplotype, or 100% probability to carry either none or two copies. Only haplotypes with frequencies >5% were considered. The additive, dominant and recessive models were examined. Analyses for triglycerides were performed only in participants who were fasting at the time of blood draw. Data are reported as means and standard errors, geometric means and 95% confidence intervals (CI) or inverse and 95% CI as appropriate. Regression coefficients and standard errors (SE) were also estimated. All analyses were adjusted for age and sex. Further (mutual) adjustment for known cardiovascular risk factors including smoking status (never smoker, former smoker, current smoker <20 cigarettes per day, current smoker ≥20 cigarettes per day), sports activity (<2 h/wk versus ≥2 h/wk), educational attainment (vocational school or less, technical school, university), BMI (continuous), WC (continuous), alcohol consumption (men: = 0 g/d, >0 to 12 g/d, >12 to 24 g/d; >24 g/d; women: = 0 g/d, >0 to 6 g/d, >6 to 12 g/d; >12 g/d), prevalent diabetes, prevalent hypertension, total cholesterol, HDL cholesterol and hs-CRP) were also explored for the tag-SNPs. Effect modification by sex was evaluated by modeling the cross product term sex times genotype or haplotype, along with main effects (in the age-adjusted general linear model). All statistical analyses were performed using SAS Enterprise Guide 4.3 (SAS Institute Inc., Cary, NC, USA).

Power calculations were performed with Quanto [50] considering an additive model, a desirable power of 80%, a two-sided α of 0.05, the means and standard deviations of the traits in the subcohort (non-normal variables were transformed to normality) and the genotype frequencies of the least (rs10883463, MAF = 8%) and most (rs522951, MAF = 46%) common tag-SNPs. The detectable differences in our study ranged between 0.1 and 0.2 SD for BMI, WC, GGT, ALT, fetuin-A, hs-CRP and HbA1c and between 0.2 and 0.3 SD for triglycerides. Conservative Bonferroni correction for multiple comparisons was performed ($P_{\text{Bonferroni}} = \alpha / (n \text{ individual hypothesis tested}) = 0.05 / ((7 \text{ SNPs} + 5 \text{ haplotypes}) \times 3 \text{ genetic models per SNP} \times 8 \text{ traits investigated})$). The corrected significance threshold was $P_{\text{Bonferroni}} = 0.0002$.

Results

Characteristics of the Study Population

Demographic, lifestyle, clinical, biochemical and genetic characteristics of the study population are given in **Table 1**, both for the subcohort and separately for men and women. Men (38%) were older than women due to sampling strategy. After adjusting for age, they also showed to smoke and drink more and to take more often antidiabetic medication. Further their BMI, WC and their activities of GGT and ALT were higher. They were more likely to be higher educated and also had lower hs-CRP levels than women. The genotype frequency of all *SCD1* tag-SNPs followed HWE ($P > 0.05$), their allele frequencies were comparable to those observed in HapMap 22/phaseII CEU population data [45] and did not differ among sexes. **Table S1** presents further information regarding genotype and allelic frequencies of the tag-SNPs across the EPIC-Potsdam subcohort and separately for men and women.

Linkage disequilibrium values (r^2) between each pair of tag-SNPs ranged from 0 to 0.66. Five haplotypes inferred from the genotyped SNPs, with frequency >5% described 90.9% of the genotype combinations in our population: A-B-A-A-B-A-A (34.1%); B-A-A-B-A-A-A (29.8%), B-A-A-A-A-A-A (11.0%), A-A-B-A-A-A-B (8.6%) and A-B-A-A-A-B-B (7.4%) (Haplotypes are composed of variants rs1502593 (C>T), rs522951 (G>C), rs11190480 (A>G), rs3071 (T>G), rs3793767 (T>C), rs10883463 (T>C), rs508384 (C>A) in that order; A indicates common allele, B indicates rare allele).

Associations between *SCD1* Tag-SNPs and Inferred Haplotypes and the Investigated Traits

No significant effect modifications by sex were found for any of the tag-SNPs or inferred haplotypes on the investigated traits, thus results are presented combined for men and women.

Table 2 shows results for age- and sex-adjusted mean values of the 8 investigated metabolic traits for each of the 7 *SCD1* tag-SNPs. No significant associations were found between rs1502593, rs522951, rs3071, rs3793767 or rs508384 and any of the investigated traits. Also no significant associations were found between any of the investigated SNPs and fetuin-A, BMI, WC or hs-CRP. However, carriers of the rs11190480 rare allele, presented slightly lower activities of ALT in a dominant fashion (19.91 vs. 21.07 U/L, $P = 0.03$). Carriers of the rs10883463 rare allele, showed higher triglycerides (160.97 vs. 94.02 mg/dL, $P = 0.03$) and lower HbA1c levels (6.10 vs. 6.49%, $P = 0.03$) in a recessive fashion, and slightly higher activities of GGT (22.22 vs. 19.88 U/L, $P = 0.02$) in a dominant fashion. Results were weak in precision, as shown by the wide confidence intervals, and after applying the Bonferroni correction for the multiple hypothesis tested ($P_{\text{Bonferroni}} = 0.0002$), none of them remained significant.

Table 3. Age- and sex-adjusted association analyses between the 5 SCD1 inferred haplotypes with frequency >5% and the 8 investigated metabolic traits in the EPIC-Potsdam study.

Haplotype (%)	N (%)	Triglycerides ^{a, b} (mg/dL)	BMI ^c (kg/m ²)	WC ^c (cm)	HbA1c ^d (%)	GGT ^b (U/L)	ALT ^b (U/L)	Fetuin-A ^{c, e} (mg/dL)	hs-CRP ^b (mg/L)
A-B-A-B-A-A (34.1)									
0 copies	919 (43.13)	95.76 (89.70–102.24)	26.18±0.14	87.20±0.35	6.47 (6.43–6.52)	20.59 (19.58–21.66)	21.07 (20.45–21.71)	0.25±0.002	0.71 (0.65–0.77)
1 copy	962 (45.14)	93.61 (87.77–99.85)	26.18±0.14	87.23±0.35	6.50 (6.46–6.55)	20.17 (19.20–21.20)	20.83 (20.23–21.45)	0.25±0.002	0.74 (0.68–0.81)
2 copies	250 (11.73)	91.55 (80.82–103.70)	26.37±0.27	87.84±0.68	6.48 (6.40–6.56)	18.88 (17.14–20.81)	20.43 (19.29–21.64)	0.25±0.004	0.72 (0.61–0.85)
P _{add}		0.49	0.63	0.51	0.58	0.15	0.34	0.70	0.59
P _{dom}		0.54	0.83	0.74	0.37	0.31	0.44	0.54	0.44
P _{rec}		0.62	0.51	0.39	0.82	0.14	0.42	0.89	0.94
B-A-A-B-A-A (29.8)									
0 copies	1041 (48.94)	94.50 (88.75–100.64)	26.29±0.13	87.58±0.33	6.48 (6.44–6.52)	20.73 (19.76–21.74)	20.73 (20.15–21.33)	0.25±0.002	0.76 (0.70–0.82)
1 copy	885 (41.61)	95.12 (88.91–101.77)	26.07±0.14	86.98±0.36	6.51 (6.46–6.55)	19.64 (18.65–20.69)	20.91 (20.27–21.56)	0.25±0.002	0.69 (0.63–0.76)
2 copies	201 (9.45)	86.77 (75.63–99.56)	26.15±0.30	86.59±0.75	6.46 (6.37–6.55)	20.02 (17.98–22.30)	21.35 (20.03–22.75)	0.25±0.004	0.71 (0.59–0.86)
P _{add}		0.46	0.35	0.13	0.75	0.23	0.42	0.93	0.25
P _{dom}		0.80	0.25	0.14	0.44	0.14	0.54	0.82	0.16
P _{rec}		0.23	0.88	0.36	0.55	0.87	0.46	0.56	0.86
B-A-A-A-A-A (11.0)									
0 copies	1213 (73.34)	92.97 (87.36–98.93)	26.22±0.13	87.20±0.32	6.48 (6.45–6.52)	19.99 (19.11–20.91)	20.76 (20.22–21.32)	0.25±0.002	0.72 (0.66–0.77)
1 copy	425 (25.7)	97.72 (88.58–107.81)	26.23±0.21	87.69±0.52	6.49 (6.43–6.55)	21.45 (19.92–23.10)	21.03 (20.14–21.97)	0.25±0.003	0.77 (0.68–0.88)
2 copies	16 (0.97)	96.50 (63.43–146.81)	25.35±1.07	84.24±2.69	6.03 (5.76–6.33)	23.32 (15.94–34.12)	24.69 (19.74–30.88)	0.27±0.015	0.91 (0.47–1.79)
P _{add}		0.42	0.79	0.74	0.33	0.08	0.31	0.36	0.26
P _{dom}		0.40	0.92	0.55	0.69	0.09	0.46	0.53	0.30
P _{rec}		0.91	0.41	0.25	0.003	0.48	0.14	0.11	0.51
A-A-B-A-A-B (8.6)									
0 copies	1795 (83.29)	93.99 (89.62–98.57)	26.23±0.10	87.38±0.26	6.49 (6.46–6.52)	20.30 (19.56–21.06)	21.08 (20.63–21.55)	0.25±0.001	0.73 (0.68–0.78)
1 copy	348 (16.15)	96.83 (87.00–107.76)	26.07±0.23	86.71±0.57	6.46 (6.39–6.53)	20.01 (18.44–21.72)	19.79 (18.85–20.77)	0.25±0.003	0.73 (0.63–0.85)
2 copies	12 (0.56)	114.87 (39.83–331.27)	26.10±1.21	87.90±3.07	6.68 (6.30–7.11)	17.22 (11.10–26.72)	21.96 (16.94–28.48)	0.25±0.017	0.57 (0.26–1.23)
P _{add}		0.57	0.54	0.35	0.70	0.59	0.04	0.32	0.88
P _{dom}		0.60	0.52	0.31	0.55	0.67	0.02	0.29	0.97
P _{rec}		0.72	0.94	0.84	0.33	0.47	0.70	0.87	0.53
A-B-A-A-B-B (7.4)									
0 copies	1855 (86.6)	94.51 (90.23–99.00)	26.17±0.10	87.21±0.25	6.49 (6.46–6.52)	19.93 (19.22–20.66)	20.80 (20.36–21.25)	0.25±0.001	0.73 (0.68–0.77)
1 copy	275 (12.84)	90.86 (80.18–102.97)	26.30±0.25	87.54±0.64	6.51 (6.43–6.59)	22.43 (20.47–24.59)	21.40 (20.27–22.60)	0.25±0.004	0.71 (0.61–0.84)
2 copies	12 (0.56)	179.79 (106.09–304.69)	27.90±1.20	92.07±3.07	6.09 (5.78–6.45)	18.23 (11.76–28.26)	22.96 (17.70–29.78)	0.23±0.017	0.66 (0.30–1.42)
P _{add}		0.62	0.32	0.30	0.63	0.05	0.25	0.75	0.76
P _{dom}		0.96	0.45	0.44	0.97	0.03	0.28	0.91	0.79

Table 3. Cont.

Haplotype (%)	N (%)	Triglycerides ^{a, b} (mg/dL)	BMI ^c (kg/m ²)	WC ^c (cm)	HbA1c ^d (%)	GGT ^b (U/L)	ALT ^b (U/L)	Fetuin-A ^{c, e} (mg/dL)	hs-CRP ^b (mg/L)
		0.02	0.16	0.12	0.03	0.64	0.48	0.30	0.80
P _{rec}									

Haplotypes are composed of variants rs1502593 (C>T), rs522951 (G>C), rs11190480 (A>G), rs3071 (T>G), rs3793767 (T>C), rs10883463 (T>C), rs508384 (C>A) in that order. A indicates common allele, B indicates rare allele.
^abased on the 615 participants fasting at blood draw.
^bgeometric means and (95% CI);
^cmeans and standard error;
^dinverse and (95% CI);
^ebased on 2077 participants due to missing biomarker data. All the reported significance levels are nominal P values and are not adjusted for multiple comparisons.
P_{add}: P for trend or P for the additive model; **P_{dom}**: P value for the dominant model; **P_{rec}**: P value for the recessive model.
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After further (mutual) adjustment of the statistical models for known cardiovascular risk factors, results remained essentially similar (**table S2**). Also they were not modified by adjustment for fasting status or exclusion of participants taking lipid-lowering or antidiabetic medication.

Table 3 shows results for age- and sex-adjusted mean values of the investigated metabolic traits for each of the 5 *SCD1* haplotypes. No associations between the two most common haplotypes and the investigated variables became apparent. Homozygotes for haplotype B-A-A-A-A-A harbouring the minor allele of the SNP rs1502593 presented lower HbA1c levels (6.03 vs. 6.49%, $P=0.003$). Carriers of haplotype A-A-B-A-A-B harbouring the minor alleles of the SNPs rs11190480 and rs508384 exhibited slightly lower activities of ALT in a dominant fashion (19.85 vs. 21.08 U/L, $P=0.02$). Carriers of haplotype A-B-A-A-B-B harbouring the minor alleles of the SNPs rs522951, rs10883463 and rs508384 showed higher triglyceride values 179.77 vs. 94.08 mg/dL and lower HbA1C levels (6.09 vs. 6.49%, $P=0.03$) in a recessive fashion, and higher GGT activities (22.24 vs. 19.93 U/L, $P=0.03$) in a dominant fashion. However, after correction for multiple testing, also none of these results remained significant. **Table S3** summarizes all of these results in the form of age- and sex-adjusted regression coefficients.

Discussion

In the present study of a middle-aged sample of German men and women, we evaluated the impact of 7 *SCD1* tag-SNPs and 5 inferred haplotypes on MetS related traits on suggested crude estimates of the presence of liver fat and on inflammation. Our study is, so far, the largest performed in a European population, and also the first to report association results between *SCD1* genetic variants and liver parameters. We hypothesized that any functional variant affecting the activity of *SCD1* would possibly result in the modulation of one or more of the traits. At most, we found some associations weak in magnitude, precision, and statistical significance, which after conservative Bonferroni-correction for the number of traits, SNPs and haplotypes tested, did not remain significant, thus being suggestive of chance findings.

Four previous studies in humans have investigated the association of *SCD1* polymorphisms with different metabolic traits [30–33]. In a UK case-control study of 608 cases and 600 controls, Liew et al. [30] reported upon the association of 6 *SCD1* SNPs with type 2 diabetes, BMI and waist-to-hip ratio. Three of these SNPs were in common or highly linked to SNPs of our study: rs670213 (a good proxy for rs522951, $r^2=0.87$ according to HapMap data for Caucasians of European origin [45], rs3071 and rs11598233 (a perfect proxy for rs3793767, $r^2=1$ [45]). Consistent with our results, they also reported no significant associations. Warensjö et al. [31] investigated associations of 8 *SCD1* tag-SNPs with obesity and insulin sensitivity in 1143 Swedish elderly men. Five of these SNPs, were in common or highly linked to SNPs of our study, rs3870747 (linked with rs1119040, $r^2=0.94$ [45]), rs3071, rs3793767, rs10883463 and rs508384. In line with our results, they found a tendency of rs10883463 carriers towards increased insulin sensitivity, and no significant associations for rs3870747 and rs3793767. In contrast, they reported lower WC in carriers of rs10883463, lower WC and higher insulin sensitivity in homozygotes for rs508384 rare allele and lower insulin sensitivity in heterozygotes for rs3071. This last association was also inconsistent with the results from Liew et al. [30]. Compared with our study population, the study of Warensjö et al. [31] was smaller and included only elderly men. However, we found no significant sex interaction for any of the SNPs with any of the

studied phenotypes, and further all our association analyses were adjusted for age, what makes the overall evidence for an association of *SCD1* genetic variability with WC and insulin sensitivity less consistent. Recently, Gong et al. [32] analysed the association of 7 *SCD1* tag-SNPs with MetS prevalence in 2152 Costa Rican adult men and women. Only one SNP, rs1502593, was found to be associated with MetS. This SNP was also analysed in our population. In line with our results, they did not observe significant associations between rs1502593 and triglyceride levels or WC. They did point out a borderline association of rs1502593 with elevated fasting blood glucose levels among women. In our population we found no significant associations for this SNP. Homozygotes for the haplotype harbouring its minor allele showed lower HbA1c levels, measure of the average plasma glucose levels over prolonged periods of time, but this association did not withhold after multiple-testing correction. We cannot discard the possibility that the different ethnic origin may explain the different findings. Finally, Stryjecki et al. [33] examined the relationships between 10 *SCD1* tag-SNPs and CRP levels in 279 European and 249 Asian young adults. Only one SNP located 9 Kb upstream *SCD1*, and thus not included in our study, was associated with CRP levels, and only among females of both groups.

Limitations of our study should be mentioned. It is possible that, due to sample size limitations we were not able to detect minor contributions of the alleles. This limitation was stronger in the case of the association analyses for triglycerides levels, as fasting data was available only for about one third of the population. Thus while some of our results could represent a replication for certain relationships inspected in the four association studies that precede ours [30–33], to evaluate the outcome of our work, further studies in independent cohorts analysing the same SNPs, or those in perfect linkage disequilibrium, are necessary [51]. Also, we cannot exclude the possibility that a rare causal variant exists within the typed region but was not picked up by the chosen markers.

In summary, our findings suggest that common variants of *SCD1* do not modulate the investigated metabolic factors in this

European population. However, given its biological relevance, the still scarce number of studies available and the inconsistency of their results, genetic heterogeneity of human *SCD1* in relation to impaired metabolism rewards further investigation in independent study populations, in particular with regard to rare variants of *SCD1*.

Supporting Information

Table S1 Genotype and allelic frequencies of the *SCD1* tag-SNPs across the EPIC-Potsdam subcohort and separately for men and women.

(PDF)

Table S2 Association analysis between the 7 *SCD1* tag-SNPs and the 8 investigated metabolic traits in the EPIC-Potsdam Study, (mutually) adjusted for known cardiovascular risk factors.

(PDF)

Table S3 β regression coefficients for the age- and sex-adjusted association analysis between the *SCD1* tag-SNPs and inferred haplotypes and the 8 investigated metabolic traits in the EPIC-Potsdam study.

(PDF)

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Author Contributions

Conceived and designed the experiments: MA EF CW. Performed the experiments: MA. Analyzed the data: MA BB. Wrote the paper: MA. Acquired the data: HB. Acquired biochemical data: NS. Interpreted the data: MA BB CW. Critical revision of the article for important intellectual content: BB NS DC EF RdG OC SK KA HGJ HB CW.

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