

# Association of Variants in the Sterol Regulatory Element-Binding Factor 1 (*SREBF1*) Gene With Type 2 Diabetes, Glycemia, and Insulin Resistance

## A Study of 15,734 Danish Subjects

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**OBJECTIVE**—We evaluated the association of variants in the sterol regulatory element-binding factor 1 gene (*SREBF1*) with type 2 diabetes. Due to the previous inconclusive quantitative trait associations, we also did studies of intermediate quantitative phenotypes.

**RESEARCH DESIGN AND METHODS**—We genotyped four variants in *SREBF1* in the population-based Inter99 cohort ( $n = 6,070$ ), the Danish ADDITION study ( $n = 8,662$ ), and in additional type 2 diabetic patients ( $n = 1,002$ ). The case-control studies involved 2,980 type 2 diabetic patients and 4,522 glucose-tolerant subjects.

**RESULTS**—The minor alleles of rs2297508, rs11868035, and rs1889018 (linkage disequilibrium  $R^2 = 0.6-0.8$ ) associated with a modestly increased risk of type 2 diabetes (rs2297508: OR 1.17 [95% CI 1.05–1.30],  $P = 0.003$ ), which was confirmed in meta-analyses of all published studies (rs2297508 G-allele: 1.08 [1.03–1.14] per allele,  $P = 0.001$ ). The diabetes-associated alleles also associated strongly with a higher plasma glucose at 30 and 120 min and serum insulin at 120 min during an oral glucose tolerance test (all  $P < 0.006$ ) and the minor allele of rs1889018 with a surrogate measure of insulin sensitivity ( $P = 0.03$ ). Furthermore, the diabetes-associated alleles associated with a modestly increased A1C level in the population-based Inter99 of middle-aged subjects and in the ADDITION study of high-risk individuals ( $P = 0.006$  and  $P = 0.008$ , respectively).

**CONCLUSIONS**—We associate sequence variation in *SREBF1* with a modestly increased predisposition to type 2 diabetes. In the general population, the diabetes-associated alleles are discreetly associated with hyperglycemia presumably due to decreased insulin sensitivity. Because sterol regulatory element-

binding protein-1c is a mediator of insulin action, the findings are consistent with the presence of a yet undefined subtle loss-of-function *SREBF1* variant. *Diabetes* 57:1136–1142, 2008

The sterol regulatory element-binding factor (*SREBF1*) gene encodes the transcription factors sterol regulatory element-binding protein (SREBP)-1a and -1c by differential transcription start sites (1). SREBP-1a and -1c, and the third family member SREBP-2, are implicated in regulation of cholesterol and fatty acid synthesis (rev. in 2). SREBP-1c is, in humans, expressed in most tissues including liver, adipose tissue, and skeletal muscle, while SREBP-1a is expressed mainly in the spleen and intestine (3). SREBP-1c is a mediator of insulin action in liver, adipose tissue, and skeletal muscle (4) with the ability to activate lipogenic genes, glucokinase, and hexokinase in these metabolic tissues (5,6). SREBP-1c thereby induces both glucose utilization and lipid metabolism, suggesting that a low level of SREBP-1c is a contributing factor in the pathogenesis of insulin resistance and type 2 diabetes. In the adipose tissue and skeletal muscle of type 2 diabetic patients, expression of SREBP-1c is indeed decreased (7). This is consistent with studies of SREBP-1c-specific knock-out mice showing a mild hyperglycemic phenotype (8).

In contrast, since SREBP-1c promotes fatty acid synthesis and lipogenesis, SREBP-1c overexpression could moreover be a factor responsible for insulin resistance through overaccumulation of lipids also leading to lipotoxicity (9). Interestingly, increased expression of SREBP-1c in the liver has been observed in animal models of obesity and type 2 diabetes (10).

Genome-linkage scans have linked the 17p11 region comprising *SREBF1* to type 2 diabetes (11), and case-control studies including 1,000–2,000 participants have consistently associated *SREBF1* with type 2 diabetes, although with different variants in linkage disequilibrium (12–15). Recent genome-wide association studies (GWAS) did not, however, report *SREBF1* as a type 2 diabetes locus (16–20). Furthermore, associations with obesity (12), circulating total and LDL cholesterol (13), HDL cholesterol (15), and plasma glucose levels (14) have been reported, yet none of these associations has been conclusively replicated (12,13,15).

In the present study we evaluated the association between

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GWAS, genome-wide association studies; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; SREBP, sterol regulatory element-binding protein.

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*SREBF1* variants and type 2 diabetes. Due to the prior inconsistent quantitative trait associations, we additionally aimed to establish a quantitative metabolic phenotype in statistically well-powered cohorts of middle-aged Danes. Given the biological evidence, we primarily hypothesized that *SREBF1* variants influenced peripheral insulin action.

## RESEARCH DESIGN AND METHODS

Further details and phenotypic characteristics are given in the online appendix (available at <http://dx.doi.org/10.2337/db07-1534>). Participants from the population-based Inter99 cohort (clinical trial reg. no. NCT00289237, clinicaltrials.gov) (21) involving 5,970 middle-aged subjects who were characterized by an oral glucose tolerance test (OGTT) as having normal glucose tolerance ( $n = 4,522$ ), impaired fasting glycemia ( $n = 503$ ), impaired glucose tolerance ( $n = 693$ ), or screen-detected and treatment-naïve type 2 diabetes ( $n = 252$ ) were investigated for associations between genotype and quantitative metabolic traits. Patients with treated type 2 diabetes ( $n = 100$ ) were not included.

Further studies of quantitative traits were performed in the Danish ADDITION screening cohort including 8,662 participants (clinical trial reg. no. NCT00237548, clinicaltrials.gov) (22).

The case-control studies included all type 2 diabetic case subjects and all glucose-tolerant control subjects from the Inter99 cohort ( $n_{\text{cases}} = 352$ ,  $n_{\text{controls}} = 4,522$ ) and the Danish ADDITION study ( $n_{\text{cases}} = 1,626$ ), as well as samples recruited from the outpatient clinic at Steno Diabetes Center ( $n_{\text{cases}} = 1,002$ ). All control subjects had normal fasting glycemia and were glucose tolerant following an OGTT. Diabetes was diagnosed according to the World Health Organization 1999 criteria (23).

Informed written consent was obtained from all participants. The studies were conducted in accordance with the Declaration of Helsinki II and were approved by the local ethics committees of Copenhagen and Aarhus.

**Biochemical and anthropometric measures.** Biochemical and anthropometric measures are described in the online appendix.

**Selection of gene variants.** Tag single nucleotide polymorphisms were selected based on the HapMap CEU population ([www.hapmap.org](http://www.hapmap.org), release 21a) using the Carlson greedy algorithm (24) capturing all variants in *SREBF1* including 1,000 bp up- and downstream with a minor allele frequency  $>4\%$  with  $R^2 = 0.8$ . In the analysis, we force-included three variants in *SREBF1* (rs11868035, rs2297508, and rs1889018) that have been reported to associate with metabolic traits (12–15).

**Genotyping.** Genotyping of four selected variants in *SREBF1* (rs4925118, rs1889018, rs2297508, and rs11868035) was performed by TaqMan allelic discrimination (KBiosciences, Hoddesdon, U.K.). All genotyping success rates were above 97% with a mismatch rate below 0.25% in 968 duplicate samples. The distributions of genotypes for all variants were in Hardy-Weinberg equilibrium (all  $P > 0.05$ ) (online appendix Table 2).

**Statistical analysis.** Linkage disequilibrium (LD) between markers was evaluated using Haploview version 4.0 (<http://www.broad.mit.edu/mpg/haploview/>). All other analyses were performed using RGui, version 2.2.4 (<http://www.r-project.org>). In the association studies of type 2 diabetes, logistic regression was used to examine differences in genotypes assuming an additive model with adjustment for sex, age, and BMI. Meta-analyses of the present and previously published studies and tests of homogeneity between studies were performed using the Mantel-Haenszel method applying a generalized linear model. In all meta-analyses, imputed data from the Wellcome Trust Case-Control Consortium (19) were included, and, for the rs1889018 variant, data were further obtained from the Diabetes Genetics Initiative (18) by use of a perfect proxy ( $R^2 = 1$ ) (rs9899634) based on LD in the HapMap database ([www.hapmap.org](http://www.hapmap.org)). No perfect proxies for rs2297508 and rs11868035 were available. A general linear model was used for testing quantitative traits in relation to genotype, adjusting for the effect of sex, age, and BMI, when appropriate. Quantitative traits were checked for normality of the residuals and, if appropriate, logarithmically transformed. Haplotype frequencies were estimated using the EM-algorithm and association, and effect sizes of each haplotype were estimated by modeling the haplotype-phenotype interaction (25). Haplotypes with a frequency  $>1\%$  were included. A  $P$  value  $<0.05$  was considered significant.

## RESULTS

Initially we analyzed the pattern of LD between the four genotyped variants in *SREBF1* and observed relatively high LD between rs1889018, rs2297508, and rs11868035 ( $R^2 = 0.6–0.8$ ) (Fig. 1). The four *SREBF1* variants were

assessed for potential associations with type 2 diabetes in a case-control study involving 2,980 type 2 diabetic patients and 4,522 glucose-tolerant control subjects (Table 1). The rs2297508 G-allele associated with increased susceptibility to type 2 diabetes with an odds ratio (OR) of 1.17 per risk allele (95% CI 1.05–1.30,  $P = 0.003$ ) when adjusting for the impact of age, sex, and BMI. The minor alleles of rs1889018 and rs11868035 showed similar associations with type 2 diabetes (Table 1). We performed meta-analyses of the unadjusted association with type 2 diabetes including the present study, all previously published studies (12–15), and online data from two GWASs (18,19). The minor alleles of rs2297508, rs1889018, and rs11868035 all associated with a modest increase in type 2 diabetes susceptibility (OR 1.06–1.08,  $P < 0.01$ ) (Fig. 2). Tests of between-study homogeneity showed no heterogeneity for rs1889018 and rs11868035 variants but some heterogeneity for rs2297508 ( $P = 0.02$ ).

To evaluate the metabolic phenotype of the type 2 diabetes susceptibility allele carriers, the four *SREBF1* variants were investigated in the population-based Inter99 sample involving 5,970 middle-aged treatment-naïve individuals (Table 2). At the population level, the G-allele of rs2297508 associated with a slight increase in fasting plasma glucose level ( $P = 0.02$ ) and with a slightly increased plasma glucose at 30 and 120 min during an OGTT ( $P = 0.006$  and  $P = 0.001$ , respectively). In addition, we observed a strong association with a higher 120-min serum insulin during an OGTT ( $P = 0.0002$ ) and with a slightly increased A1C level ( $P = 0.006$ ) (Table 2). The minor alleles of the rs1889018 and rs11868035 variants showed similar associations with glycemia (online appendix Table 2).

A surrogate measure of insulin resistance was reported as the BIGTT-insulin sensitivity index (BIGTT- $S_i$ ) (26) and as the homeostasis model assessment index of insulin resistance. We observed a decreased insulin sensitivity assessed by BIGTT- $S_i$  in carriers of the minor rs1889018 C-allele ( $P = 0.03$ ) (online appendix Table 2), and this association was strengthened after adjustment for the level of insulin release ( $P = 0.006$ ).

Further analyses of metabolic phenotypes were performed in the ADDITION screening cohort consisting of 8,662 subjects at high risk of type 2 diabetes. We replicated the association of the G-allele of rs2297508 with a 0.45% increase in A1C per allele ( $P = 0.008$ ) (Table 2). In the ADDITION study we also found nominal associations with decreased BMI in rs2297508 minor G-allele carriers (Table 2) and increased fasting serum cholesterol level for carriers of the minor allele of rs11868035 (online appendix Table 3). The rs4925118 variant did not associate with examined metabolic traits (Table 1 and online appendix Tables 2–3). Haplotype association analyses did not further add to single variant analyses (online appendix Table 5).

## DISCUSSION

In the present study we report associations of sequence variation in *SREBF1* with a modest increase in type 2 diabetes risk. In the well-characterized population-based Inter99 sample of middle-aged treatment-naïve individuals, we report strong associations for the diabetes risk-allele carriers with higher plasma glucose and serum insulin after an oral glucose load as well as with a slight increase in A1C. The latter was supported by association with A1C

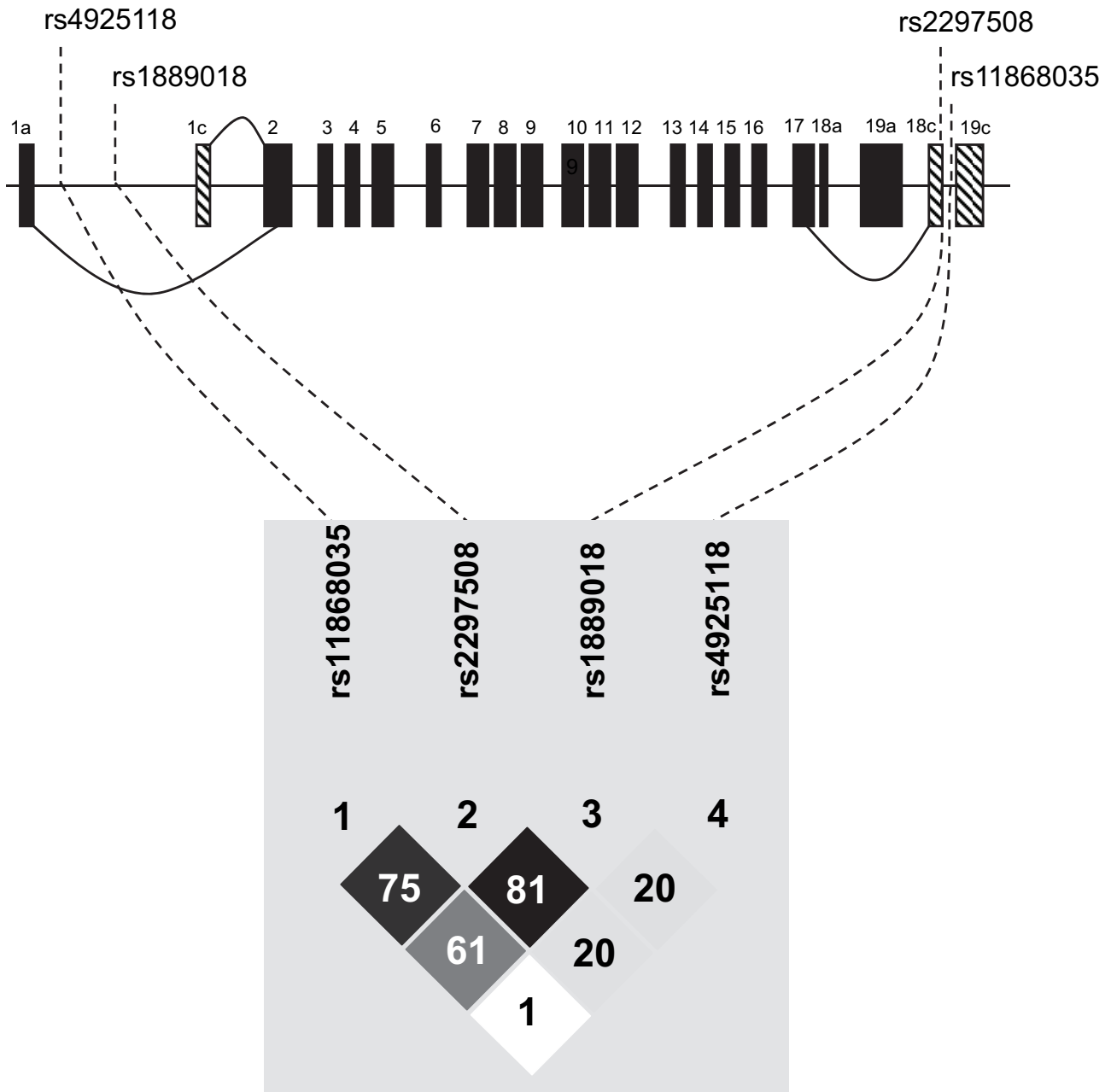


FIG. 1. Schematic structure of *SREBF1*, localization of the genotyped variants, and pairwise LD between variants estimated by  $R^2$ . Exons are numbered indicating the alternatively spliced -a and -c variants. Numbers in squares designate the degree of LD ( $R^2$ ) between any two markers. LD estimates were made using Haploview version 4.0 (<http://www.broad.mit.edu/mpg/haploview/>).

in the Danish ADDITION study. Also, variants in *SREBF1* associate with BIGTT- $S_1$ , which is a well-documented surrogate measure of insulin sensitivity (26).

Because the transcription factor SREBP-1c is a mediator of insulin action in skeletal muscle, adipose tissue, and liver, it is conceivable that a polymorphism conferring a subtle loss of function may affect the expression of SREBP-1c and could contribute to the insulin resistance phenotype. The observed effects are evident in the postprandial state. Enzymes involved in glucose metabolism are highly regulated by SREBP-1c, and if downregulated due to a mild SREBP-1c dysfunction, glucose metabolism would likely be impaired in the peripheral tissues, potentially leading to postprandial hyperglycemia and insulin resistance.

Prior association studies have shown an impact of the

rs2297508 variant (12,15), the rs11868035 variant (13,14), and rs2236513, rs6502618, rs1889018 variants (14) in *SREBF1* on risk of type 2 diabetes. All of these variants are in substantial LD (HapMap:  $R^2 = 0.67-0.95$ ). In the present report we replicate associations of rs2297508, rs11868035, and rs1889018 with type 2 diabetes. As none of the recent GWASs (16–20) reported *SREBF1* as a type 2 diabetes locus, we engaged in meta-analyses of the present data and previously published studies (12–15) as well as all online available data from GWASs (18,19). In combined analyses we showed discreet increases in type 2 diabetes risk for all three variants. However, it should be noted that meta-analysis, which in principle might be expected to provide conclusive answers, may be compromised by heterogeneity of ethnicity and outcome phenotypes in addition to publication and ascertainment bias. Moreover,

TABLE 1

Association studies of type 2 diabetes and *SREBF1* variants in 2,980 type 2 diabetes case subjects and 4,522 glucose-tolerant control subjects

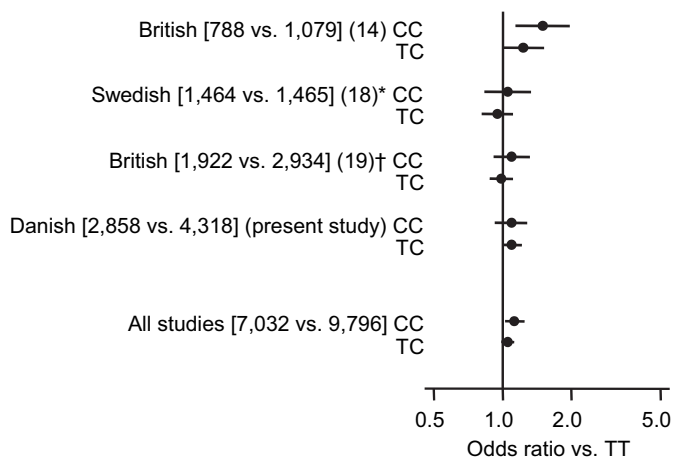
Variant		Glucose tolerant		Type 2 diabetic		OR (95% CI)	P
		Genotypes	MAF (95% CI)	Genotypes	MAF (95% CI)		
rs4925118	GG	3,537 (82)	9.4 (8.8–10.0)	2,292 (81)	10.3 (9.6–11.2)	1.12 (0.95–1.33)	0.2
	GA	734 (17)		525 (18)			
	AA	38 (1)		32 (1)			
rs1889018	TT	1,936 (45)	33.0 (32.0–34.0)	1,217 (43)	34.3 (33.1–35.6)	1.12 (1.01–1.24)	0.04
	TC	1,914 (44)		1,321 (46)			
rs2297508	CC	468 (11)	33.2 (32.2–34.2)	320 (11)	34.7 (33.5–35.9)	1.17 (1.05–1.30)	0.003
	CG	1,921 (45)		1,192 (42)			
	GG	1,874 (44)		1,328 (47)			
rs11868035	CC	481 (11)	27.2 (26.2–28.1)	322 (11)	28.0 (26.8–29.2)	1.19 (1.07–1.33)	0.002
	CT	2,304 (53)		1,473 (52)			
	TT	1,702 (39)		1,153 (40)			
		326 (8)		221 (8)			

Data are *n* of subjects with each genotype (% of each group) unless otherwise indicated. Patients having type 2 diabetes were recruited at Steno Diabetes Center (*n* = 1,002) from the population-based Inter99 cohort (*n* = 352) and from the ADDITION study (*n* = 1,626). Glucose-tolerant subjects were recruited from the Inter99 cohort (*n* = 4,522). The *P* values compare genotype distributions between type 2 diabetes case subjects and glucose-tolerant control subjects applying an additive logistic regression model, while adjusting for age, sex, and BMI. MAF, minor allele frequency.

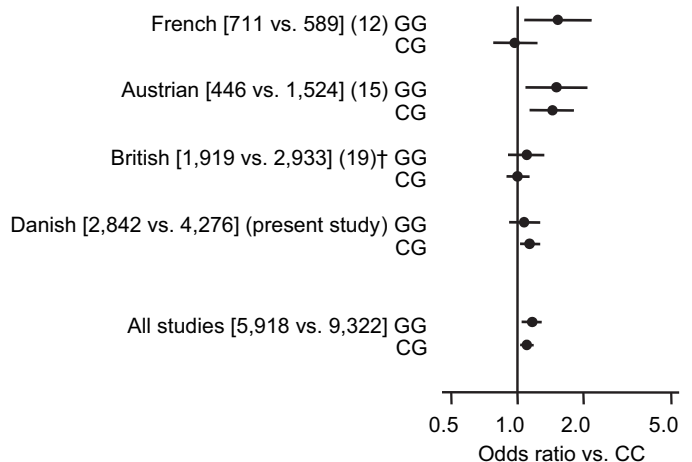
the present meta-analyses include imputed data and data based on perfect LD with another marker, yet data from three other GWASs (16,17,20) were not available. In any

case, in the meta-analyses we were not able to adjust for the effect of confounding factors such as age, sex, and BMI, which further weakens the analysis; in fact, in the

### A rs1889018 OR 1.06 (1.01-1.11) per allele, *P*=0.01



### B rs2297508 OR 1.08 (1.03-1.14) per allele, *P*=0.001



### C rs11868035 OR 1.07 (1.02-1.13) per allele, *P*=0.006

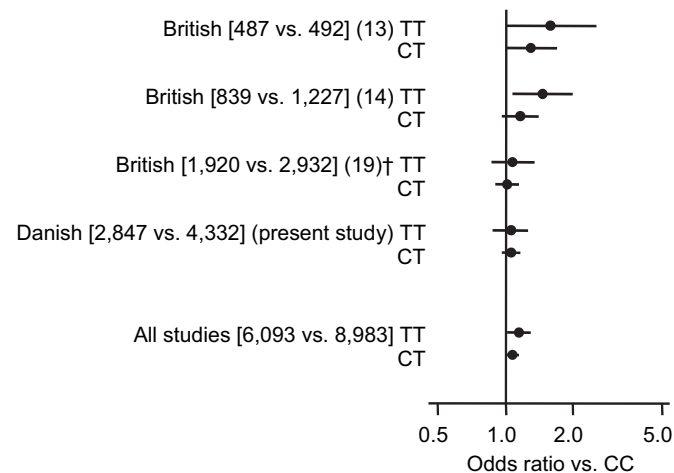


FIG. 2. Estimated ORs (95% CIs) of type 2 diabetes in minor allele carriers of rs1889018 (A), rs2297508 (B), or rs11868035 (C) of *SREBF1* in combined analyses of all currently available studies. OR (95% CI) values for type 2 diabetes are rs1889018: 1.06 (1.01–1.11), *P* = 0.01; rs2297508: 1.08 (1.03–1.14), *P* = 0.001; and rs11868035: 1.07 (1.02–1.13), *P* = 0.006. No heterogeneity between studies was observed for the rs1889018 and rs11868035 variants (*P* = 0.3 and *P* = 0.2, respectively). Some heterogeneity was found for rs2297508 (*P* = 0.02). Numbers in square brackets designate numbers of type 2 diabetic patients and control subjects. Numbers in round brackets indicate the reference number. \*Genotypes based on the rs9899634 variant, which is in perfect LD with rs1889018 (HapMap: *R*<sup>2</sup> = 1). Data were obtained at <http://www.broad.mit.edu/diabetes/>. †Genotypes based on imputation and were obtained at <http://www.wtccc.org.uk/>.

**TABLE 2**  
Quantitative metabolic traits in the population-based Inter99 cohort including 5,970 middle-aged subjects with normal glucose tolerance, impaired fasting glycaemia, impaired glucose tolerance, or screen-detected and treatment-naïve type 2 diabetes and in 8,662 subjects of the ADDITION screening cohort stratified according to genotype of the *SREBF1* rs2297508 variant

	Trait			P
	CC	CG	GG	
Inter99				
<i>n</i> (men/women)	2,510 (1,265/1,245)	2,462 (1,231/1,231)	644 (324/320)	
Age (years)	46.2 ± 7.9	46.1 ± 8.0	45.6 ± 8.0	1
BMI (kg/m <sup>2</sup> )	26.2 ± 4.5	26.2 ± 4.5	26.2 ± 4.6	0.6
Waist (cm)	86.5 ± 13.1	86.5 ± 13.2	86.6 ± 13.1	0.2
Serum triglycerides (mmol/l)	1.1 (0.8–1.5)	1.1 (0.8–1.5)	1.1 (0.8–1.6)	0.4
Serum cholesterol (mmol/l)	5.6 ± 1.1	5.5 ± 1.1	5.5 ± 1.1	0.2
Serum HDL cholesterol (mmol/l)	1.4 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.3
Fasting serum insulin (pmol/l)	35 (24–51)	34 (24–51)	35 (24–53)	0.6
Serum insulin at 30 min (pmol/l)	244 (175–353)	246 (174–355)	251 (178–358)	0.0002
Serum insulin at 120 min (pmol/l)	152 (91–247)	157 (99–261)	168 (101–262)	0.02
Fasting plasma glucose (mmol/l)	5.51 ± 0.7	5.55 ± 0.9	5.56 ± 0.9	0.006
Plasma glucose at 30 min (mmol/l)	8.65 ± 1.8	8.69 ± 1.9	8.84 ± 1.9	0.001
Plasma glucose at 120 min (mmol/l)	6.09 ± 2.0	6.26 ± 2.2	6.29 ± 2.2	0.006
AIC (%)	5.81 ± 0.45	5.83 ± 0.53	5.87 ± 0.59	0.7
Insulinogenic index <sub>insulin</sub>	24.2 (16.9–36.7)	24.7 (16.7–36.8)	25.1 (16.9–36.0)	0.2
HOMA-IR (mmol/l × pmol/l)	8.32 (5.66–12.67)	8.27 (5.65–12.94)	8.56 (5.72–13.44)	0.06
BIGTT-S <sub>i</sub>	9.32 ± 4.1	9.16 ± 4.1	9.06 ± 4.1	
ADDITION				
<i>n</i> (men/women)	3,660 (2,000/1,660)	3,784 (2,045/1,739)	983 (546/437)	
Age (years)	60.0 ± 6.8	60.0 ± 6.7	59.7 ± 7.0	0.03
BMI (kg/m <sup>2</sup> )	28.7 ± 5.0	28.5 ± 4.8	28.4 ± 4.6	0.3
Waist (cm)	97.3 ± 13.6	96.8 ± 13	97.0 ± 13	0.7
Serum cholesterol (mmol/l)	5.79 ± 1.1	5.84 ± 1.0	5.83 ± 1.1	0.09
Serum HDL cholesterol (mmol/l)	1.55 ± 0.43	1.56 ± 0.43	1.53 ± 0.41	0.1
Fasting blood glucose (mmol/l)	5.37 ± 1.2	5.38 ± 1.2	5.42 ± 1.4	0.008
AIC (%)	5.84 ± 0.73	5.86 ± 0.72	5.89 ± 0.78	

Data are means ± SD or median (interquartile range) unless otherwise indicated. Values of p-glucose, s-insulin, insulinogenic index<sub>insulin</sub> and triglycerides were logarithmically transformed before statistical analysis. Calculated *P*-values were adjusted for age, sex, and BMI, where appropriate, assuming an additive model. Insulinogenic index<sub>insulin</sub> was calculated as (serum insulin at 30 min [pmol/l] – fasting serum insulin [pmol/l])/plasma glucose at 30 min (mmol/l). Homeostasis model assessment of insulin resistance (HOMA-IR) (mmol/l × pmol/l) was calculated as fasting plasma glucose (mmol/l) multiplied by fasting serum insulin (pmol/l) and divided by 22.5. BIGTT-S<sub>i</sub> uses information on sex and BMI combined with analysis of plasma glucose and serum insulin levels at the time points 0, 30, and 120 min to provide an index for S<sub>i</sub>, which highly correlates with indexes obtained during an intravenous glucose tolerance test, and were calculated as described elsewhere (26).

present report the association with type 2 diabetes was abolished when not adjusting for the effect of age and sex. Therefore, cautious interpretations of these meta-analyses are crucial.

Previous reports regarding associations between *SREBF1* variants and quantitative metabolism have been inconclusive. One study has indicated an association of the rs11868035 variant with increased fasting total and LDL cholesterol levels (13). In contrast, two studies did not show any association of the rs2297508 variant with fasting cholesterol levels (12,15). We found nominal associations of the rs11868035 variant with increased fasting serum cholesterol levels and the rs2297508 and rs1889018 variants with decreased BMI in the ADDITION study of subjects at high risk for type 2 diabetes; however, these associations were not observed in the population-based Inter99 study. These ambiguities may be due to the diversity of the populations, e.g., accentuation of associations in the ADDITION cohort due to the high-risk selection procedure, yet could more likely be a result of statistical type I errors. Also, associations with obesity previously have been conflicting (12,13,15,18), and the present study does not support previous associations to obesity-related traits. Moreover, a recent study found borderline significant associations of the minor alleles of the rs1889018 and rs2236513 variants with higher plasma glucose level at fasting and 2 h after an oral glucose challenge (14). We substantiate these findings in the current study. Because published studies have not investigated the exact same variants in *SREBF1*, these somewhat inconsistent reports may be explained by population-specific differences in LD pattern, but could also be caused by discrepancies in the examined populations, e.g., environmental modifying effects, or by statistical type I or II errors.

Thus far the causal variant giving rise to the described associations has not been identified. The present results are not able to elucidate this, as none of the variants are generally more associated than others. None of the investigated variants are obvious functional candidates, yet a causal variant in LD with the associated variants may influence regulation or function of SREBP-1c. However, since HapMap data demonstrate that *SREBF1* is located in an extended haplotype block spanning almost 300 kb, a putative causal variant may be situated at some distance.

Although we present data supporting an association with type 2 diabetes and glycemia, we recognize that the present results may be falsely positive due to the fact that no correction for multiple testing was applied. Yet, we argue that based on previous reports a *SREBF1* effect on risk of type 2 diabetes was the primary hypothesis of this study, and as the association with glycemia, evaluated by A1C, was supported by association in an independent cohort, no correction is strictly needed.

In conclusion, we associate variants in *SREBF1* with a slight increase in type 2 diabetes risk. Of novelty, we present data suggesting an association with glycemia in the general population of middle-aged people, possibly due to a decreased SREBP-1c function in individuals carrying the as yet undefined causal variant.

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#### REFERENCES

- Hua X, Wu J, Goldstein JL, Brown MS, Hobbs HH: Structure of the human gene encoding sterol regulatory element binding protein-1 (SREBF1) and localization of SREBF1 and SREBF2 to chromosomes 17p11.2 and 22q13. *Genomics* 25:667–673, 1995
- Horton JD, Goldstein JL, Brown MS: SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109:1125–1131, 2002
- Shimomura I, Shimano H, Horton JD, Goldstein JL, Brown MS: Differential expression of exons 1a and 1c in mRNAs for sterol regulatory element binding protein-1 in human and mouse organs and cultured cells. *J Clin Invest* 99:838–845, 1997
- Duchateau PH, Perretti N, Laville M, Andreelli F, Vega N, Riou JP, Vidal H: Regulation by insulin of gene expression in human skeletal muscle and adipose tissue: evidence for specific defects in type 2 diabetes. *Diabetes* 50:1134–1142, 2001
- Guillet-Deniau I, Mieulet V, Le Lay S, Achouri Y, Carré D, Girard J, Fougelle F, Ferré P: Sterol regulatory element binding protein-1c expression and action in rat muscles: insulin-like effects on the control of glycolytic and lipogenic enzymes and UCP3 gene expression. *Diabetes* 51:1722–1728, 2002
- Foretz M, Guichard C, Ferré P, Fougelle F: Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci U S A* 96:12737–12742, 1999
- Sewter C, Berger D, Considine RV, Medina G, Rochford J, Ciaraldi T, Henry R, Dohm L, Flier JS, O'Rahilly S, Vidal-Puig AJ: Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor- $\alpha$ . *Diabetes* 51:1035–1041, 2002
- Liang G, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS: Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 277:9520–9528, 2002
- Schmitz-Peiffer C: Signalling aspects of insulin resistance in skeletal muscle: mechanisms induced by lipid oversupply. *Cell Signal* 12:583–594, 2000
- Shimomura I, Bashmakov Y, Horton JD: Increased levels of nuclear SREBP-1c associated with fatty livers in two mouse models of diabetes mellitus. *J Biol Chem* 274:30028–30032, 1999
- Demenaï F, Kanninen T, Lindgren CM, Wiltshire S, Gaget S, Dandrieux C, Almgren P, Sjögren M, Hattersley A, Dina C, Tuomi T, McCarthy MI, Froguel P, Groop LC: A meta-analysis of four European genome screens (GIFT Consortium) shows evidence for a novel region on chromosome 17p11.2-q22 linked to type 2 diabetes. *Hum Mol Genet* 12:1865–1873, 2003
- Eberlé D, Clément K, Meyre D, Sahbatou M, Vaxillaire M, Le Gall A, Ferré P, Basdevant A, Froguel P, Fougelle F: SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 53:2153–2157, 2004
- Laudes M, Barroso I, Luan J, Soos MA, Yeo G, Meirhaeghe A, Logie L, Vidal-Puig A, Schafer AJ, Wareham NJ, O'Rahilly S: Genetic variants in

- human sterol regulatory element binding protein-1c in syndromes of severe insulin resistance and type 2 diabetes. *Diabetes* 53:842–846, 2004
14. Harding AH, Loos RJ, Luan J, O'Rahilly S, Wareham NJ, Barroso I: Polymorphisms in the gene encoding sterol regulatory element-binding factor-1c are associated with type 2 diabetes. *Diabetologia* 49:2642–2648, 2006
  15. Felder TK, Oberkofler H, Weitgasser R, Mackevics V, Krempler F, Paulweber B, Patsch W: The SREBF-1 locus is associated with type 2 diabetes and plasma adiponectin levels in a middle-aged Austrian population. *Int J Obes (Lond)* 31:1099–1103, 2007
  16. Sladek R, Rocheleau G, Rung J, Dina C, Shen L, Serre D, Boutin P, Vincent D, Belisle A, Hadjadj S, Balkau B, Heude B, Charpentier G, Hudson TJ, Montpetit A, Pshzhetsky AV, Prentki M, Posner BI, Balding DJ, Meyre D, Polychronakos C, Froguel P: A genome-wide association study identifies novel risk loci for type 2 diabetes. *Nature* 445:881–885, 2007
  17. Steinthorsdottir V, Thorleifsson G, Reynisdottir I, Benediktsson R, Jonsdottir T, Walters GB, Stykarsdottir U, Gretarsdottir S, Emilsson V, Ghosh S, Baker A, Snorraddottir S, Bjarnason H, Ng MCY, Hansen T, Bagger Y, Wilensky RL, Reilly MP, Adeyemo A, Chen Y, Zhou J, Gudnason V, Chen G, Huang H, Lashley K, Doumatey A, So WY, Ma RCY, Andersen G, Borch-Johnsen K, Jorgensen T, Vliet-Ostapchouk JV, Hofker MH, Wijmenga C, Christiansen C, Rader DJ, Rotimi C, Gurney M, Chan JCN, Pedersen O, Sigurdsson G, Gulcher JR, Thorsteinsdottir U, Kong A, Stefansson K: A variant in CDKAL1 influences insulin response and risk of type 2 diabetes. *Nat Genet* 39:770–775, 2007
  18. Diabetes Genetics Initiative of Broad Institute of Harvard and MIT, Lund University, and Novartis Institutes of BioMedical Research; Saxena R, Voight BF, Lyssenko V, Burtt NP, de Bakker PIW, Chen H, Roix JJ, Kathiresan S, Hirschhorn JN, Daly MJ, Hughes TE, Groop L, Altschuler D, Almgren P, Florez JC, Meyer J, Ardlie K, Bengtsson Bostrom K, Isomaa B, Lettre G, Lindblad U, Lyon HN, Melander O, Newton-Cheh C, Nilsson P, Orho-Melander M, Rastam L, Speliotes EK, Taskinen MR, Tuomi T, Guiducci C, Berglund A, Carlson J, Gianniny L, Hackett R, Hall L, Holmkvist J, Laurila E, Sjogren M, Sterner M, Surti A, Svensson M, Svensson M, Tewhey R, Blumenstiel B, Parkin M, DeFelicis M, Barry R, Brodeur W, Camarata J, Chia N, Fava M, Gibbons J, Handsaker B, Healy C, Nguyen K, Gates C, Sougnez C, Gage D, Nizzari M, Gabriel SB, Chim GW, Ma Q, Parikh H, Richardson D, Ricke D, Purcell S: Genome-wide association analysis identifies loci for type 2 diabetes and triglyceride levels. *Science* 316:1331–1336, 2007
  19. Zeggini E, Weedon MN, Lindgren CM, Frayling TM, Elliott KS, Lango H, Timpson NJ, Perry JRB, Rayner NW, Freathy RM, Barrett JC, Shields B, Morris AP, Ellard S, Groves CJ, Harries LW, Marchini JL, Owen KR, Knight B, Cardon LR, Walker M, Hitman GA, Morris AD, Doney ASF, The Wellcome Trust Case Control Consortium, McCarthy MI, Hattersley AT: Replication of genome-wide association signals in UK samples reveals risk loci for type 2 diabetes. *Science* 316:1336–1341, 2007
  20. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, Erdos MR, Stringham HM, Chines PS, Jackson AU, Prokunina-Olsson L, Ding CJ, Swift AJ, Narisu N, Hu T, Pruim R, Xiao R, Li XY, Conneely KN, Riebow NL, Sprau AG, Tong M, White PP, Hetrick KN, Barnhart MW, Bark CW, Goldstein JL, Watkins L, Xiang F, Saramies J, Buchanan TA, Watanabe RM, Valle TT, Kinnunen L, Abecasis GR, Pugh EW, Doheny KF, Bergman RN, Tuomilehto J, Collins FS, Boehnke M: A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. *Science* 316:1341–1345, 2007
  21. Jorgensen T, Borch-Johnsen K, Thomsen TF, Ibsen H, Glumer C, Pisinger C: A randomized non-pharmacological intervention study for prevention of ischaemic heart disease: Baseline results Inter99 (1). *Eur J Cardiovasc Prev Rehab* 10:377–386, 2003
  22. Lauritzen T, Griffin S, Borch-Johnsen K, Wareham NJ, Wolfenbittel BH, Rutten G, for the ADDITION Study Group: The ADDITION study: proposed trial of the cost-effectiveness of an intensive multifactorial intervention on morbidity and mortality among people with type 2 diabetes detected by screening. *Int J Obes Relat Metab Disord* 24 (Suppl. 3):S6–S11, 2000
  23. World Health Organization Study Group: Definition, Diagnosis and Classification of Diabetes Mellitus and its Complications. Part 1. Diagnosis and Classification of Diabetes Mellitus. Geneva, World Health Org., 1999
  24. Carlson CS, Eberle MA, Rieder MJ, Yi Q, Kruglyak L, Nickerson DA: Selecting a maximally informative set of single-nucleotide polymorphisms for association analyses using linkage disequilibrium. *Am J Hum Genet* 74:106–120, 2004
  25. Lake SL, Lyon H, Tantisira K, Silverman EK, Weiss ST, Laird NM, Schaid DJ: Estimation and tests of haplotype-environment interaction when linkage phase is ambiguous. *Hum Hered* 55:56–65, 2003
  26. Hansen T, Drivsholm T, Urhammer SA, Palacios RT, Vølund A, Borch-Johnsen K, Pedersen O: The BIGTT test: a novel test for simultaneous measurement of pancreatic  $\beta$ -cell function, insulin sensitivity, and glucose tolerance. *Diabetes Care* 30:257–262, 2007