

**Association of variants in the sterol regulatory element-binding factor 1 gene (*SREBF1*) with type 2 diabetes, glycemia, and insulin resistance - A study of 15,734 Danish subjects**

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**Running Title:** *SREBF1* associates with diabetes and glycemia

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## **ABSTRACT**

*Objective:* We evaluated 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 the 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: OR 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 minutes and serum-insulin at 120 minutes during an OGTT (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 HbA<sub>1c</sub> 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. Since SREBP-1c is a mediator of insulin action the findings are consistent with the presence of a yet undefined subtle loss-of-function *SREBF1* variant.

**KEYWORDS.** Type 2 diabetes, *SREBF1*, case-control study, insulin resistance, glycemia

**ABBREVIATIONS.** BIGTT-S<sub>I</sub>, BIGTT-insulin sensitivity index; GWAS, genome-wide association studies; HOMA-IR, homeostasis model assessment index; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; OR, odds ratio; *SREBF1*, the gene encoding sterol regulatory element-binding factor 1

The sterol regulatory element-binding factor (*SREBF1*) gene encodes the transcription factors 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 [reviewed 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 the SREBP-1c-specific knock-out mice showing a mild hyperglycaemic phenotype (8).

In contrast, since SREBP-1c promotes fatty acid synthesis and lipogenesis, SREBP-1c over-expression could moreover be a factor responsible for insulin resistance through over-accumulation 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, however, not 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 have been conclusively replicated (12,13,15).

In the present study we intended to evaluate association between *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.

## **SUBJECTS, MATERIALS AND METHODS**

**Subjects.** Further details and phenotypic characteristics are given in the Appendix (available at <http://diabetes.diabetesjournals.org>). Participants from the population-based Inter99 cohort (ClinicalTrials.gov ID-no: NCT00289237) (21) involving 5,970 middle-aged subjects who were characterized by an oral glucose tolerance test (OGTT) as subjects with 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 (ClinicalTrials.gov ID-no: NCT00237548) (22).

The case-control studies included all type 2 diabetic cases 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 Ethical Committees of Copenhagen and Aarhus.

**Biochemical and anthropometric measures.** Biochemical and anthropometric measures are described in the Appendix.

**Selection of gene variants.** Tag SNPs 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 1000 bp up- and downstream with a minor allele frequency above 4% with an  $R^2$  of 0.8. In the analysis we force-included three variants in *SREBF1* (rs11868035, rs2297508 and rs1889018) which have been reported to associate with metabolic traits (12-15).

**Genotyping.** Genotyping of four selected variants in *SREBF1* (rs4925118, rs1889018, rs2297508, rs11868035) was performed by TaqMan allelic discrimination (KBiosciences, Hoddesdon, UK). 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  $P>0.05$ ) (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 generalised 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 modelling the haplotype-phenotype interaction (25). Haplotypes with a frequency above 1% were included. A  $P$ -value of less than 0.05 was considered significant.

## RESULTS

Initially we analyzed the pattern of LD between the four genotyped variants in *SREBF1* and we observed relatively high LD between rs1889018, rs2297508 and rs11868035 ( $R^2=0.6-0.8$ ) (Figure 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 GWAS (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,  $P<0.01$ ) (Figure 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 minutes during an OGTT ( $P=0.006$  and  $P=0.001$ , respectively). In addition, we observed a strong association to a higher 120-min serum-insulin during an OGTT ( $P=0.0002$ ) and with a slightly increased HbA<sub>1c</sub> level ( $P=0.006$ ) (Table 2). The minor alleles of the rs1889018 and rs11868035 variants showed similar associations with glycemia (Appendix table 2).

A surrogate measure of insulin resistance was reported as the BIGTT-insulin sensitivity index (BIGTT-S<sub>i</sub>) (26) and as the homeostasis model assessment index (HOMA-IR). We observed a decreased insulin sensitivity

assessed by BIGTT-S<sub>i</sub> in carriers of the minor rs1889018 C-allele ( $P=0.03$ ) (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 HbA<sub>1c</sub> 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 (Appendix table 3). The rs4925118 variant did not associate with examined metabolic traits (Table 1, Appendix tables 2-3).

Haplotype association analyses did not add further to single variant analyses (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 HbA<sub>1c</sub>. The latter was supported by association with HbA<sub>1c</sub> in the Danish ADDITION study. Also variants in *SREBF1* associate with BIGTT-S<sub>i</sub> which is a well-documented surrogate measure of insulin sensitivity (26).

Since 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, could contribute to the insulin resistance phenotype. The observed effects are evident in the post-prandial state. Enzymes involved in the glucose metabolism are highly regulated by SREBP-1c, and if down-regulated due to a mild SREBP-1c dysfunction the glucose metabolism would likely be impaired in the peripheral tissues potentially leading to post-prandial 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 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 GWAS (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 GWAS (18,19). In combined analyses we showed discreet increases in type 2 diabetes risk for all three variants. However, it should be pointed out that meta-analysis which in principle might be expected to provide conclusive answers, may be compromised by heterogeneity of ethnicity and outcome phenotypes besides publication and ascertainment bias. Moreover, the present meta-analyses include imputed data and data based on perfect LD with another marker, yet data from three other GWAS (16,17,20) were not available. Besides, we are in the meta-analyses 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 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 rs11868035 variant with increased fasting serum-cholesterol level and the rs2297508 and rs1889018 variants with decreased BMI in the ADDITION study of subjects in high risk of 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. In fact, association with obesity has also previously 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 hours after an oral glucose challenge (14). In the current study we substantiate these findings. Since published studies do not investigate 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.

So 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 haploblock spanning almost 300 kilobases a putative causal variant may be situated at some distance.

Even though 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 HbA<sub>1c</sub>, 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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**TABLE 1.** Association studies of type 2 diabetes and *SREBF1* variants in 2,980 type 2 diabetes cases and 4,522 glucose-tolerant control subjects

Variant	Glucose-tolerant		Type 2 diabetes		OR (95%CI)	
	Genotypes	MAF	Genotypes	MAF	<i>P</i> -value	
rs4925118	GG	3537 (82)	9.4	2292 (80)	10.3	1.12 (0.95-1.33)
	GA	734 (17)	(8.8-10.0)	525 (18)	(9.6-11.2)	<i>P</i> = 0.2
	AA	38 (0.9)		32 (1.1)		
rs1889018	TT	1936 (45)	33.0	1217 (43)	34.3	1.12 (1.01-1.24)
	TC	1914 (44)	(32.0-34.0)	1321 (46)	(33.1-35.6)	<i>P</i> = 0.04
	CC	468 (11)		320 (11)		
rs2297508	CC	1921 (45)	33.2	1192 (42)	34.7	1.17 (1.05-1.30)
	CG	1874 (44)	(32.2-34.2)	1328 (47)	(33.5-35.9)	<i>P</i> = 0.003
	GG	481 (11)		322 (11)		
rs11868035	CC	2304 (53)	27.2	1473 (52)	28.0	1.19 (1.07-1.33)
	CT	1702 (39)	(26.2-28.1)	1153 (41)	(26.8-29.2)	<i>P</i> = 0.002
	TT	326 (8)		221 (8)		

**TABLE 2.** Quantitative metabolic traits in the population-based Inter99 cohort including 5,970 middle-aged subjects with normal glucose tolerance, impaired fasting glycemia, 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	CC	CG	GG	P
<b>Inter99</b>				
<i>n</i> (men/women)	2510 (1265/1245)	2462 (1231/1231)	644 (324/320)	
Age (years)	46.2±7.9	46.1±8.0	45.6±8.0	
BMI (kg/m <sup>2</sup> )	26.2±4.5	26.2±4.5	26.2±4.6	1
Waist (cm)	86.5±13.1	86.5±13.2	86.6±13.1	0.6
s-Triglyceride (mmol/l)	1.1 (0.8-1.5)	1.1 (0.8-1.5)	1.1 (0.8-1.6)	0.2
s-Cholesterol (mmol/l)	5.6±1.1	5.5±1.1	5.5±1.1	0.4
s-HDL cholesterol (mmol/l)	1.4±0.4	1.4±0.4	1.4±0.4	0.2
Fasting s-insulin (pmol/l)	35 (24-51)	34 (24-51)	35 (24-53)	0.3
s-insulin at 30 min (pmol/l)	244 (175-353)	246 (174-355)	251 (178-358)	0.6
s-insulin at 120 min (pmol/l)	152 (91-247)	157 (99-261)	168 (101-262)	0.0002
Fasting p-glucose (mmol/l)	5.51±0.7	5.55±0.9	5.56±0.9	0.02
p-glucose at 30 min (mmol/l)	8.65±1.8	8.69±1.9	8.84±1.9	0.006
p-glucose at 120 min (mmol/l)	6.09±2.0	6.26±2.2	6.29±2.2	0.001
HbA <sub>1c</sub> (%)	5.81±0.45	5.83±0.53	5.87±0.59	0.006
Insulinogenic index <sub>insulin</sub>	24.2 (16.9-36.7)	24.7 (16.7-36.8)	25.1 (16.9-36.0)	0.7
HOMA-IR (mmol/lxpmol/l)	8.32 (5.66-12.67)	8.27 (5.65-12.94)	8.56 (5.72-13.44)	0.2
BIGTT-S <sub>i</sub>	9.32±4.1	9.16±4.1	9.06±4.1	0.06
<b>ADDITION</b>				
<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	
BMI (kg/m <sup>2</sup> )	28.7±5.0	28.5±4.8	28.4±4.6	0.03
Waist (cm)	97.3±13.6	96.8±13	97.0±13	0.3
s-Cholesterol (mmol/l)	5.79±1.1	5.84±1.0	5.83±1.1	0.7
s-HDL cholesterol (mmol/l)	1.55±0.43	1.56±0.43	1.53±0.41	0.09
Fasting b-glucose (mmol/l)	5.37±1.2	5.38±1.2	5.42±1.4	0.1
HbA <sub>1c</sub> (%)	5.84±0.73	5.86±0.72	5.89±0.78	0.008

## TABLE AND FIGURE LEGENDS

**Table 1.** Data are number of subjects with each genotype (% of each group), minor allele frequencies in percent (95% CI), and odds ratio (OR) (95% CI). 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 cases and glucose-tolerant control subjects applying an additive logistic regression model, while adjusting for age, sex, and BMI. MAF, minor allele frequency; OR, odds ratio.

**Table 2.** Data are mean  $\pm$  standard deviation or median (interquartile range) (values of s-insulin, s-triglyceride, HOMA-IR and insulinogenic index). Values of p-glucose, s-insulin, insulinogenic index<sub>insulin</sub> and triglyceride 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 (s-insulin at 30 minutes [pmol/l] - fasting s-insulin [pmol/l]) / p-glucose at 30 minutes (mmol/l). Homeostasis model assessment of insulin resistance (HOMA-IR) (mmol/l $\times$ pmol/l) was calculated as fasting plasma-glucose (mmol/l) multiplied by fasting serum-insulin (pmol/l) and divided by 22.5. BIGTT-insulin sensitivity index (BIGTT-S<sub>i</sub>) uses information on sex and BMI combined with analysis of p-glucose and s-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 IVGTT, and were calculated as described elsewhere (26). B, blood; P, plasma; s, serum.

**Figure 1.** Schematic structure of *SREBF1*, localisation 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/>).

**Figure 2.** Estimated odds ratios (95% CI) 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. Odds ratios (95% CI) of type 2 diabetes are rs1889018: 1.06 (1.01-1.11),  $P=0.01$ ; rs2297508: 1.08 (1.03-1.14),  $P=0.001$ ; 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 diabetes patients and control subjects. Numbers in round brackets indicate the reference number. \* Genotypes were based on the rs9899634 variant which is in perfect LD with rs1889018 (HapMap:  $R^2=1$ ). Data were obtained at <http://www.broad.mit.edu/diabetes/>. † Genotypes are based on imputation and were obtained at <http://www.wtccc.org.uk/>.

FIGURE 1

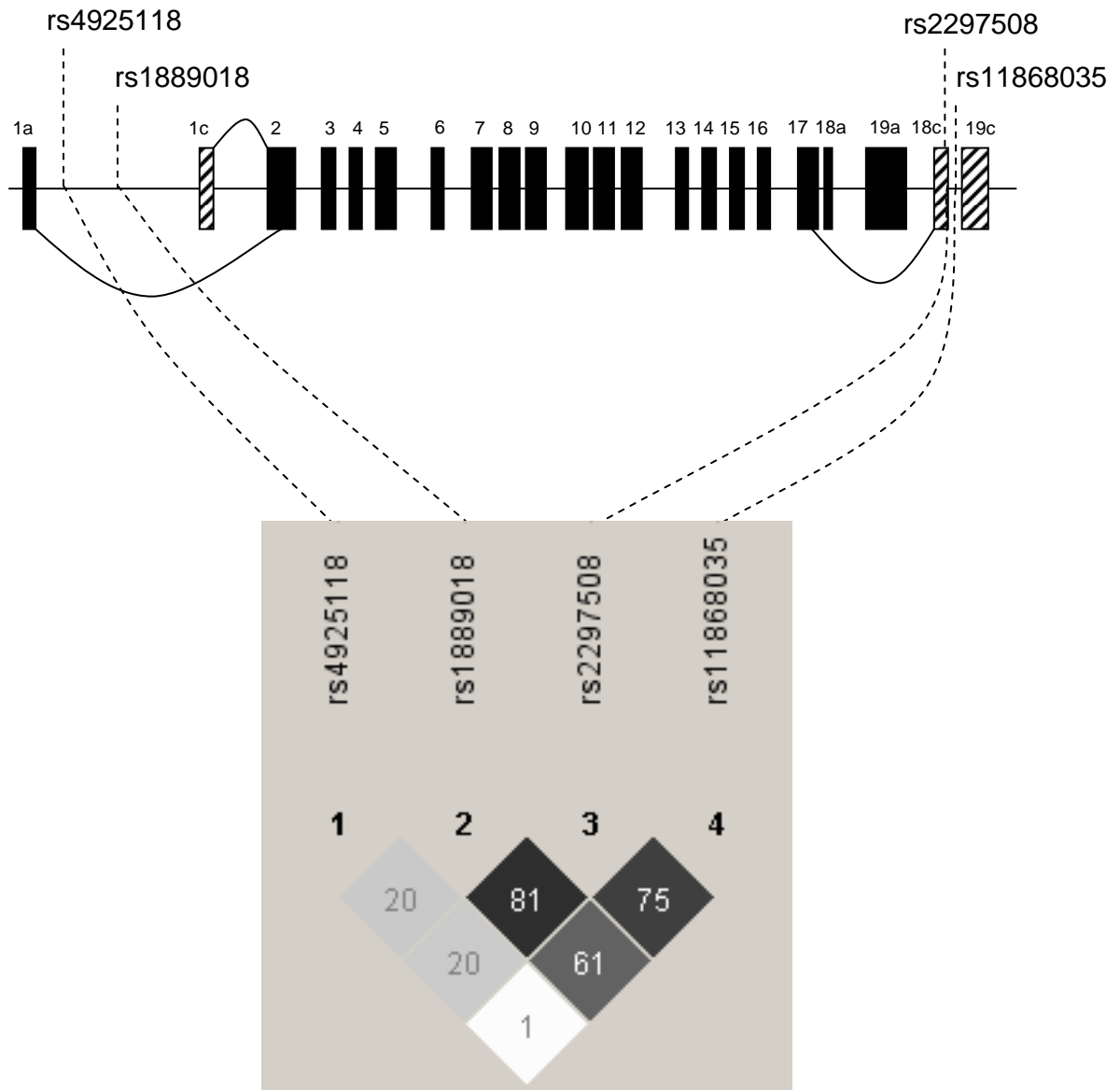


FIGURE 2

