

Polymorphisms in the gene encoding sterol regulatory element-binding factor-1c are associated with type 2 diabetes

A.-H. Harding · R. J. F. Loos · J. Luan · S. O’Rahilly ·
N. J. Wareham · I. Barroso

Received: 13 May 2006 / Accepted: 24 July 2006 / Published online: 20 September 2006
© Springer-Verlag 2006

Abstract

Aims/hypothesis The sterol regulatory element-binding factor (SREBF)-1c is a transcription factor involved in the regulation of lipid and glucose metabolism. We have previously found evidence that a common *SREBF1c* single-nucleotide polymorphism (SNP), located between exons 18c and 19c, is associated with an increased risk of type 2 diabetes. The present study aimed to replicate our previously reported association in a larger case–control study and to examine an additional five *SREBF1c* SNPs for their association with diabetes risk and plasma glucose concentrations.

Methods We genotyped six *SREBF1c* SNPs in two case–control studies ($n=1,938$) and in a large cohort study ($n=1,721$) and tested for association with type 2 diabetes and with plasma glucose concentrations (fasting and 120-min post-glucose load), respectively.

Results In the case–control studies, carriers of the minor allele of the previously reported SNP (rs11868035) had a significantly increased diabetes risk (odds ratio [OR]=1.20 [95% CI 1.04–1.38], $p=0.015$). Also, three other SNPs (rs2236513, rs6502618 and rs1889018), located in the 5’ region, were significantly associated with diabetes risk

(OR ≥ 1.21 , $p \leq 0.006$). Furthermore, two SNPs (rs2236513 and rs1889018) in the 5’ region were weakly ($p < 0.09$) associated with plasma glucose concentrations in the cohort study. Rare homozygotes had increased ($p \leq 0.05$) 120-min post-load glucose concentrations compared with carriers of the wild-type allele. Haplotype analyses showed significant ($p=0.04$) association with diabetes risk and confirmed the single SNP analyses.

Conclusions/interpretation In summary, we replicated our previous finding and found evidence for SNPs in the 5’ region of the *SREBF1c* gene to be associated with the risk of type 2 diabetes and plasma glucose concentration.

Keywords Case–control study · Cohort study · Plasma glucose concentration · Polymorphism · Population-based · Sterol regulatory element-binding factor · Type 2 diabetes

Abbreviations

EPIC	European Prospective Investigation on Cancer
HWE	Hardy–Weinberg equilibrium
LD	linkage disequilibrium
MAF	minor allele frequency
OR	odds ratio
SNP	single-nucleotide polymorphism
SREBF1c	sterol regulatory element-binding factor-1c

A.-H. Harding · R. J. F. Loos · J. Luan · N. J. Wareham
MRC Epidemiology Unit,
Cambridge, UK

S. O’Rahilly
Cambridge Institute for Medical Research,
University Department of Clinical Biochemistry,
Cambridge, UK

I. Barroso (✉)
Metabolic Disease Group, The Wellcome Trust Sanger Institute,
The Wellcome Trust Genome Campus, Hinxton,
Cambridgeshire CB10 1SA, UK
e-mail: ib1@sanger.ac.uk

Introduction

Dysregulation of fatty acid metabolism may be an underlying cause of type 2 diabetes. Lipotoxicity in peripheral tissues, which is promoted by defective adipose tissue, could be the link between obesity, insulin resistance and type 2 diabetes [1].

Sterol regulatory element-binding factors (SREBFs) are transcription factors that regulate lipid homeostasis by activation of genes involved in fatty acid and cholesterol metabolism. Two genes, *SREBF1* located on chromosome 17p11.2 and *SREBF2* on 22q13, encode three isoforms of the protein [2]. Through alternative transcription start sites *SREBF1* encodes SREBF1a and SREBF1c. Due to its longer acidic transactivation domain, SREBF1a is a more potent transcriptional activator than SREBF1c and can activate all SREBF-responsive genes, including those that mediate the synthesis of cholesterol, fatty acids and triacylglycerols [2]. SREBF1c preferentially activates transcription of genes involved in fatty acid and triacylglycerol synthesis, whereas SREBF2 mainly activates genes that control cholesterol synthesis [2–4]. SREBF1c is the main SREBF1 isoform, being highly expressed in most tissues of mice and humans, including liver, adipose tissue and skeletal muscle [5–7]. SREBF processing is controlled by the cellular sterol content; high sterol levels block the translocation of SREBF to the nucleus and SREBF-mediated gene transcription does not occur [8, 9]. SREBF1c activity is additionally regulated by fatty acids [3]. Animal feeding studies have demonstrated that polyunsaturated fatty acids suppress *SREBF1* mRNA expression and protein processing [10, 11]. Furthermore, insulin increases *SREBF1c* mRNA in skeletal muscle and SREBF1c levels in adipose tissue [7, 12–14]. Given the mediating role of SREBF1c in the nutritionally and insulin-regulated transcription of genes involved in triacylglycerol and fatty acid synthesis, SREBF1c may underlie the mechanisms that lead to lipotoxicity [1]. The *SREBF1c* gene can therefore be considered as a good candidate for type 2 diabetes.

There have been only two reports in humans that have examined the relationship between *SREBF1* gene variants and diabetes phenotypes to date [15, 16]. We have recently reported significant associations between an intronic C>T single-nucleotide polymorphism (SNP) (rs11868035), located between exons 18c and 19c, and the risk of type 2 diabetes, and plasma concentrations of total and LDL cholesterol [15]. A nearby located synonymous SNP in exon 18 (G952G, rs2297508) was found to be associated with morbid obesity and diabetes in a French population [16]. This G952G variant also influenced cholesterol synthesis in a Finnish population [17] and was associated with hypertriacylglycerolaemia in the men of the French population [16].

The present study aimed to replicate our previously reported association between the intronic C>T SNP (rs11868035) and the risk of type 2 diabetes. To identify whether this SNP is the true variant or whether other polymorphisms in the *SREBF1c* gene underlie the association with diabetes risk, we selected an additional five

SNPs, such that coverage of the genetic variation in the *SREBF1c* gene and its promoter region was ensured. In total, six SNPs were genotyped in two case–control cohorts (the Cambridgeshire Case–Control Study and the European Prospective Investigation on Cancer [EPIC] Case–Control Study) to examine the contribution of each SNP and their haplotypes to the risk of type 2 diabetes. Subsequently, we genotyped the six SNPs in a large cohort study (the Ely Study) and tested for association with plasma glucose concentrations, fasting and at 120-min post-glucose load.

Subjects and methods

Study populations

Cambridgeshire Case–Control Study The Cambridgeshire Case–Control Study consists of 552 patients with type 2 diabetes aged 45–76 years, randomly sampled from a population-based diabetes register and 552 controls, recruited from the same population and individually matched for age, sex and geographical location. The study design and methods have been described in detail elsewhere [18]. Briefly, cases were defined by onset of diabetes after the age of 30 years without insulin treatment in the first year following diagnosis. Diabetes was excluded in controls by medical record search, and by an HbA_{1c} measurement of <6%.

The cases and controls were of the same age (controls: 63.8 years [SE 0.33] vs cases: 63.5 years [SE 0.33], $p=0.54$), but cases had a higher BMI compared with controls (controls: 27.3 kg/m² [SE 0.18] vs cases: 29.7 [SE 0.22], $p<0.001$). The average age at onset of type 2 diabetes was 55.7 years (SD 9.0); 9% were treated with insulin, 64% with tablets and 27% followed a diet regimen. Of those with known family history, 32% (171 out of 529) of the cases had a family history for type 2 diabetes compared with 13% (71 out of 539) of the controls.

EPIC Case–Control Study The EPIC Case–Control Study is nested within the EPIC-Norfolk Study, a population-based cohort study of men and women aged 40–78 years. Both the case–control and full cohort [19, 20] study have been previously described in detail. Briefly, the case–control study consists of 417 incident type 2 diabetes cases and two sets of 417 controls, each matched in terms of age, sex, general practice and recruitment date, with one set additionally matched for BMI. A case was defined by a physician's diagnosis of type 2 diabetes, with no insulin prescribed within the first year following diagnosis, and/or HbA_{1c} >7% at the health check. Controls were randomly selected from the EPIC-Norfolk cohort from among those without diabetes, cancer, stroke or myocardial infarction at

baseline and who had not developed diabetes by the time of selection. Potential controls with measured HbA_{1c} levels >6% were excluded. The case and controls of the EPIC Case–Control study were of similar age (controls: 62.2 years [SE 0.3] vs cases: 62.2 years [SE 0.44], $p=0.9$). The BMI of cases was compared with controls (controls: 27.7 kg/m² [SE 0.1] vs cases: 29.8 [SE 0.3], $p<0.0001$). Of those with known family history, 21% (73 out of 354) of the cases had a family history of type 2 diabetes compared with 9.5% (70 out of 741) of the controls.

Ely Study The Ely Study is a population-based cohort study of the aetiology and pathogenesis of type 2 diabetes and related metabolic disorders. The study design and methods have been described in detail elsewhere [21]. This analysis included 1,721 men and women without diagnosed diabetes in Phase III of the Ely Study. The average age and BMI in the Ely cohort was 61.4 years (SD 9.0) and 27.3 kg/m² (SD 3.9) for men and 60.8 years (SD 9.1) and 27.2 kg/m² (SD 5.5) for women. Participants underwent standard anthropometric measurements and a 75-g OGTT having fasted since 22.00 h the previous night. Plasma samples were obtained at fasting and 120-min post-glucose load. Glucose concentrations were measured immediately in the routine laboratory using the hexokinase method.

Ethical permission

Ethical permission for the three studies was granted by their respective Local Research Ethics Committee, and study participants had provided informed consent.

SNP selection and genotyping

SNP selection First, the C>T SNP located between exon 18c and 19c (rs11868035, SNP6) was selected to replicate our previous findings [15]. Subsequently, four additional SNPs (rs2236513 [SNP1], rs6502618 [SNP2], rs4925118 [SNP3] and rs1889018 [SNP4]) were selected to ensure coverage of the genetic variation in the *SREBF1c* gene and its promoter region (up to 10 kb before the start codon of isoform 1a). Finally, we added a sixth, non-synonymous, SNP (V556M [SNP5]) that was identified by re-sequencing the *SREBF1* gene in the Severe Insulin Resistance cohort as described previously [15]. We used the data available in the International HapMap Project-Phase II (release no. 20) to estimate how much of the genetic variation in the *SREBF1c* gene and its promoter are captured by the selected SNPs [22]. The HapMap reports 13 SNPs that are polymorphic (minor allele frequency [MAF] >1%) in the CEPH population (Utah residents with northern and western European ancestry) [22]. The SNPs selected in the present

study capture the genetic variation of 11 of the 13 HapMap SNPs, whereas two rare intronic HapMap SNPs (rs1653007, with MAF=0.033, and rs1108511, with MAF=0.026) remained uncovered.

Genotyping and quality control DNA was extracted from whole blood/EDTA samples (Whatman Biosciences, Ely, UK). Samples were arrayed on 96-well plates with three replicates and one water control per plate. For the case–control populations, cases and control samples were randomly distributed across each 96-well plate, with approximately the same number of cases and controls per plate. Genotyping of samples was performed in 384-well plates at the Wellcome Trust Sanger Institute, Cambridge, using an adaptation of the homogeneous MassExtend protocol supplied by Sequenom, Inc. for the MassArray system (Sequenom, Inc.) [23]. Call rates were on average $\geq 95\%$ for SNP1, SNP2, SNP3, SNP5 and SNP6 and 87% for SNP4. Concordance rates between duplicate samples were $\geq 98\%$.

Statistical methods

A χ^2 ‘goodness-of-fit’ test was performed to assess whether the observed genotype frequencies in the controls of the case–control studies, separately and combined, and in the cohort study, were in Hardy–Weinberg equilibrium (HWE). Linkage disequilibrium (LD) between SNPs was estimated in the controls of the combined case–control studies using Haploview V3.2 (<http://www.broad.mit.edu/mpg/haploview>).

In the case–control studies, separately and combined, each SNP was tested for association with type 2 diabetes in a logistic regression analysis, adjusted for age, sex and BMI. There was no evidence of heterogeneity between the two case–control studies ($p>0.3$) and the association between the SNPs and the risk of type 2 diabetes was the same in both studies (SNP-by-cohort interaction: $p>0.33$). Therefore, we present the results of the combined case–control studies only. In the cohort study, association between fasting plasma glucose and 120-min post-load plasma glucose concentrations and the six SNPs was tested in a multiple regression model, adjusting for age and sex. We applied two genetic models: (1) an additive model, which assumes an additive effect for the presence of none (wild-type homozygotes), one (heterozygotes) or two (rare homozygotes) rare alleles, and (2) a general model, which compares the three genotypes irrespectively of their carrier status. Likelihood ratio tests indicated that the general model was not significantly better than the additive model ($p=0.35–0.99$) such that we report the results of the additive genetic model only.

Haplotype frequencies were estimated using the HAPLOTYPE procedure in SAS/Genetics 9.1 (SAS Institute Inc., Cary, NC, USA). Haplotypes were arranged in frequency order, such that haplotype 1.1.1.1 is the most frequent. Haplotypes prevalent at >5% were retained for further haplotype analysis. In the case–control studies, we tested for association between the haplotypes and type 2 diabetes using a logistic regression analysis, adjusting for age, sex and BMI. In the cohort study, we used a general linear regression model, adjusted for age and sex, and weighted by the probability for haplotype and clustered by individual ID.

Statistical analyses were conducted using Stata SE 8.2 for Windows (StataCorp, College Station, TX, USA). Power calculations were performed using Quanto v1.1.1 (<http://hydra.usc.edu/gxe>).

Results and discussion

The genotype frequencies of the six SNPs were in HWE in the controls of the case–control studies (Table 1) and in the cohort study (Table 2). Three SNPs, SNP1 (rs2236513), SNP2 (rs6502618) and SNP4 (rs1889018), were in high ($r^2>0.95$) LD with each other (Fig. 1). These three SNPs were in moderate LD ($r^2=0.59$) with the previously reported C>T SNP (SNP6, rs11868035). LD with the two remaining SNPs, SNP3 (rs4925118) and SNP5 (V556M), was low ($r^2<0.21$).

In the case–control studies, we tested for association between the six SNPs and type 2 diabetes risk using the additive logistic regression model (Table 1). The minor allele (T-allele) of SNP6 (rs11868035), for which we previously reported significant associations [15], was also in the present

study significantly associated with an increased risk of type 2 diabetes. Corroborating evidence comes from a French study that reported a significant association between the minor allele of the G952G variant (rs2297508), located 216 bp downstream of SNP6, and an increased risk of type 2 diabetes [16]. According to data from the International HapMap, LD between SNP6 and the G952G variant is high ($D'=1$ and $r^2=0.87$) [22], suggesting that the associations found in the present study, our previous study [15] and the French study [16] may be caused by SNP6, the G952G variant or a currently unidentified *SREBF1c* gene variant that is in LD with both of these SNPs. In addition to SNP6, we also found that the minor alleles of SNP1, SNP2 and SNP4 were significantly associated with an increased risk of type 2 diabetes (Table 1). It should be noted, however, that LD between these three SNPs is high and, therefore, the three association results cannot be considered as independent findings.

In the cohort study, we tested for association between the six SNPs and plasma glucose levels (fasting and at 120 min after loading) using the additive linear regression model. None of the associations reached statistical significance. However, the minor alleles of SNP1 and SNP4 tended to be associated with increased plasma glucose concentrations, supporting the observation from the case–control studies that also showed an increased diabetes risk with the minor alleles of these SNPs. When a recessive genetic model was applied to SNP1 and SNP4, i.e. comparing rare homozygotes with carriers of the wild-type allele, borderline significant associations were obtained for plasma glucose concentrations at 120 min (SNP1: $p=0.05$ and SNP4: $p=0.03$).

The case–control study is sufficiently powered (80% at $\alpha=0.05$) for detecting a genetic effect of 0.05 of odds ratios (ORs) >1.2 for SNPs with a MAF >33% (SNP1, SNP2,

Table 1 Association between the six *SREBF1c* SNPs and type 2 diabetes in the combined EPIC Case–Control Study and Cambridgeshire Case–Control Study

SNP	Location	rs number	Status	Genotype counts (<i>n</i>)			MAF	HWE <i>p</i> value	OR per minor allele (95% CI)	<i>p</i> value
				1/1	1/2	2/2				
SNP1	5'-20422G>T	rs2236513	Case	318	378	128	0.38	–	1.23 (1.07–1.41)	0.003
			Control	522	560	140	0.34	0.58	–	
SNP2	5'-19797T>C	rs6502618	Case	319	384	129	0.39	–	1.21 (1.05–1.38)	0.006
			Control	505	554	142	0.35	0.60	–	
SNP3	5'-12219G>A	rs4925118	Case	668	135	16	0.10	–	1.03 (0.83–1.28)	0.77
			Control	983	220	13	0.10	0.86	–	
SNP4	5'-7796T>C	rs1889018	Case	300	359	129	0.39	–	1.26 (1.10–1.44)	0.001
			Control	479	463	137	0.34	0.13	–	
SNP5	EX9+132G>T V556M	–	Case	801	29	1	0.02	–	1.25 (0.76–2.07)	0.38
			Control	1192	36	0	0.01	0.60	–	
SNP6	IVS18c-3C>T	rs11868035	Case	379	371	89	0.33	–	1.20 (1.04–1.38)	0.014
			Control	614	515	98	0.29	0.49	–	

The ORs and *p* values were obtained from logistic regression analysis using the additive genetic model adjusted for age, sex and BMI. The OR reflects the increased risk with each extra minor allele

Table 2 Association between the six *SREBF1c* SNPs and plasma glucose concentrations (fasting and at 120-min post-OGTT) in the Ely Study

SNP	Location	rs number	Time of sample	HWE <i>p</i> value	Plasma glucose concentration (mmol/l)			<i>p</i> value ^c
					Genotype (95% CI) ^a			
					1/1	1/2	2/2	
SNP1	5'-20422G>T	rs2236513	Fasting (<i>n</i> =714/716/196) ^b	0.42	5.0 (5.0–5.1)	5.0 (5.0–5.1)	5.1 (5.0–5.2)	0.084
			At 120 min (<i>n</i> =666/660/184) ^b	0.30	5.0 (5.8–6.1)	6.0 (5.8–6.1)	6.4 (6.1–6.7)	0.085
SNP2	5'-19797T>C	rs6502618	Fasting (<i>n</i> =682/719/191) ^b	0.94	5.0 (5.0–5.1)	5.1 (5.0–5.1)	5.1 (5.0–5.2)	0.43
			At 120 min (<i>n</i> =632/665/178) ^b	0.88	6.0 (5.8–6.1)	6.0 (5.9–6.2)	6.4 (6.1–6.7)	0.27
SNP3	5'-12219G>A	rs4925118	Fasting (<i>n</i> =1,289/321/26) ^b	0.25	5.0 (5.0–5.1)	5.1 (5.0–5.2)	5.0 (4.8–5.3)	1.00
			At 120 min (<i>n</i> =1,199/293/24) ^b	0.21	6.0 (5.9–6.1)	6.1 (5.9–6.4)	6.1 (5.4–7.0)	0.92
SNP4	5'-7796T>C	rs1889018	Fasting (<i>n</i> =630/654/188) ^b	0.37	5.0 (5.0–5.1)	5.0 (5.0–5.1)	5.1 (5.0–5.2)	0.084
			At 120 min (<i>n</i> =585/601/177) ^b	0.25	5.9 (5.8–6.1)	5.9 (5.8–6.1)	6.3 (5.9–6.6)	0.085
SNP5	EX9 +132G>T V556M	–	Fasting (<i>n</i> =1,594/501) ^b	0.35	5.0 (5.0–5.1)	5.0 (4.8–5.2)	5.8 (4.4–7.6)	0.43
			At 120 min (<i>n</i> =1,476/491) ^b	0.37	6.0 (5.9–6.2)	5.6 (5.1–6.2)	6.4 (3.3–12.5)	0.27
SNP6	IVS18c- 3C>T	rs11868035	Fasting (<i>n</i> =368/274/48) ^b	0.76	5.0 (4.9–5.1)	5.0 (4.9–5.1)	5.0 (5.0–5.2)	1.00
			At 120 min (<i>n</i> =338/248/43) ^b	0.78	5.8 (5.8–6.0)	6.1 (5.8–6.3)	5.8 (5.2–6.4)	0.92

^aData are geometric means adjusted for age and sex

^bNumbers represent genotype count of homozygotes for the common allele, heterozygotes and homozygotes for the minor allele, respectively

^cThe *p* values were obtained from regression analysis using the additive genetic model adjusted for age and sex

SNP4 and SNP6), OR >1.35 for SNP3 (MAF=10%) and OR >1.75 for SNP5 (MAF=2%). The cohort study has sufficient power (80% at $\alpha=0.05$) for detecting difference between genotypes in fasting plasma glucose concentration of >0.15 mmol/l for SNP1, SNP2, SNP4 and SNP6, of >0.25 mmol/l for SNP3 and of >0.55 mmol/l for SNP5. These power analyses show that the cohort study was not sufficiently powered, as the observed differences were below the detectable level for the given samples size and MAF. Therefore, replication in other cohort studies is required to confirm our findings.

The case–control studies and the cohort study both provide evidence that SNP1, SNP2 and SNP4 may influence plasma glucose concentrations and diabetes risk, respectively. These three SNPs are located in the 5' region, 7.8–20.4 kb before the start of exon 1c and therefore most probably too distant to be considered as promoter SNPs. However, the HapMap reports a SNP (rs8066560), possibly located in the putative promoter region at –1,099 bp of exon 1c, that is in high LD with the SNPs in the 5' region ($r^2>0.95$). The promoter region has been shown to be of importance in the transcriptional activation of the gene [24–26]. Analyses of the mouse *SREBF1c* gene have identified several functional regions in the promoter region, such as a sterol regulatory element complex, a sterol inducible region and a liver X receptor–retinoid X receptor region [24, 25]. The human *SREBF1c* promoter contains additional binding sites for pyruvate dehydrogenase com-

plex-1 and hepatocyte nuclear factor-4 that upon binding upregulate the promoter activity [26]. Functional studies will be required to identify whether SNP1, SNP2, SNP4 or another SNP in LD affect any of these promoter binding sites and/or the transcriptional activity of *SREBF1c*.

Haplotype analyses will be more powerful than single SNP analyses when none of the genotyped SNPs are associated with disease, as haplotypes may represent genetic variation that is not captured by any of the independently analysed SNPs. Therefore, we performed haplotype analyses with four haplotype-tagging SNPs (SNP2, SNP3, SNP5 and SNP6). Eight haplotypes were present in the studied populations; however, only three were common (haplotype frequency >5%) and used for further analyses (Table 3). Relative to the most common haplotype (1.1.1.1), haplotype 2.1.1.2 significantly increased the risk of type 2 diabetes in the case–control studies. These analyses show that the simultaneous presence of the rare alleles of SNP2 and SNP6 (haplotype 2.1.1.2) significantly increased the risk of type 2 diabetes, which confirms the results of the single SNP analyses. The effect size for the 2.1.1.2 haplotype is similar to that of the single SNPs, suggesting that the haplotype does not provide additional information beyond the single SNPs. In the cohort study, there was no evidence for association between the haplotypes and plasma glucose concentrations.

In summary, our findings support the hypothesis that the *SREBF1c* gene is a candidate gene for the risk of type 2

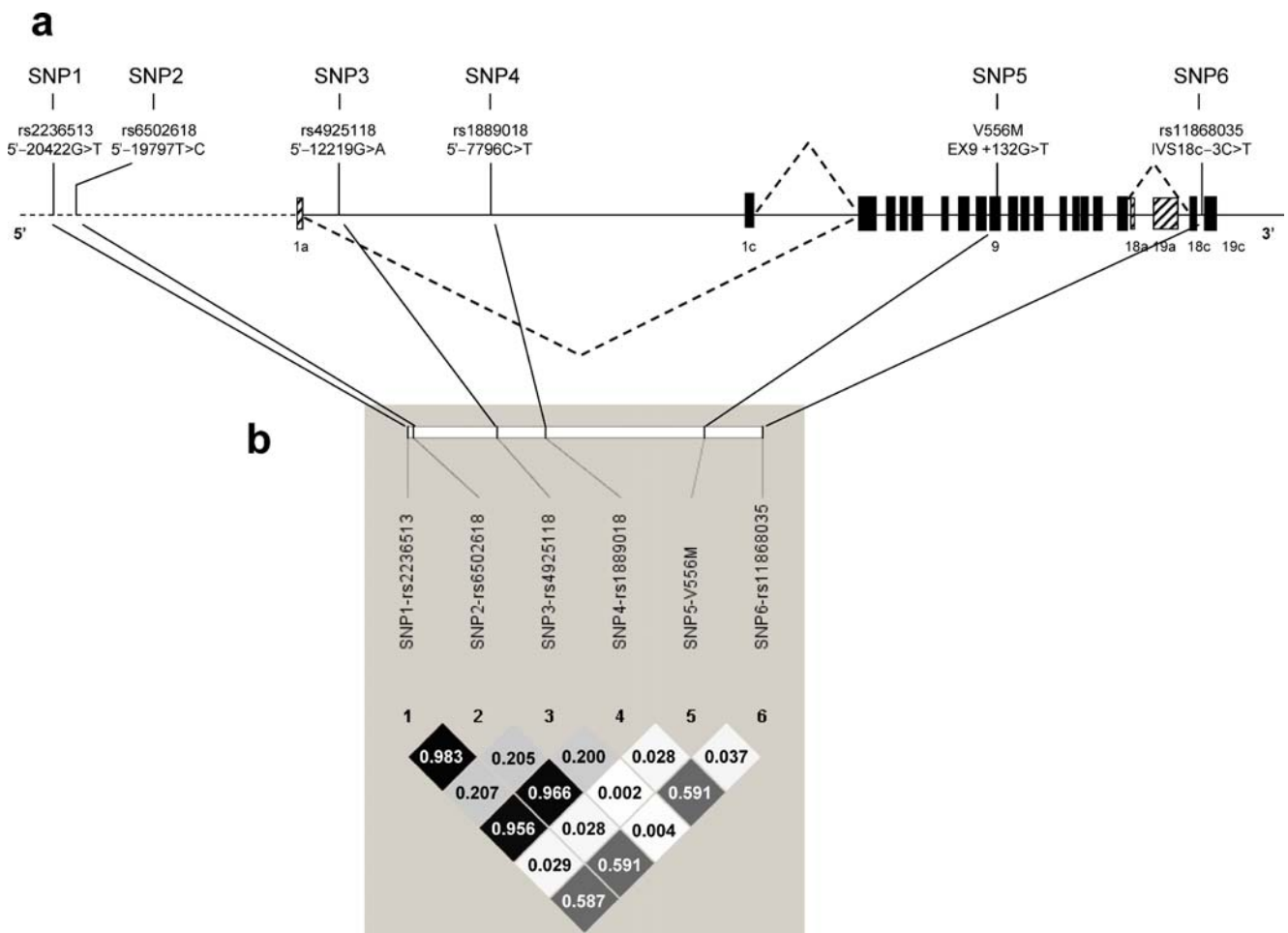


Fig. 1 **a** Location of the six *SREBF1c* SNPs. The SNP location of SNP1, SNP2, SNP3 and SNP4 reflects their relative position (in bp) to the first nucleotide of exon 1c. SNP5 is located 132 bp after first nucleotide of exon 9, and SNP6 is located three nucleotides before the

start of exon 19c. **b** LD (r^2) between the six *SREBF1c* SNPs in the controls of the combined EPIC Study and Cambridgeshire Case–Control Study

Table 3 Association between *SREBF1c* haplotypes and risk of type 2 diabetes in the case–control studies and plasma glucose concentrations (fasting and at 120 min) in the Ely Study

	Haplotype ^a			<i>p</i> value
	1.1.1.1	2.2.1.1	2.1.1.2	
Case–control studies				
Haplotype frequency, % (<i>n</i>)				
Cases	59.7 (919)	5.2 (80)	24.3 (374)	
Controls	62.9 (1,444)	5.9 (135)	21.1 (485)	
OR (95% CI)	1 (Reference)	0.93 (0.70–1.24)	1.22	0.041
Ely Study				
Haplotype frequency, %	63.4	7.5	18.8	
Fasting glucose (mmol/l) (95% CI)	5.0 (5.0–5.1)	5.0 (4.9–5.1)	5.0 (5.0–5.1)	0.79
120-min glucose (mmol/l) (95% CI)	5.9 (5.7–6.0)	6.2 (5.8–6.6)	6.1 (5.8–6.4)	0.10

Haplotypes are based on SNP2, SNP3, SNP5 and SNP6

^aData are adjusted for age and sex

diabetes and variation in plasma glucose concentrations. We replicated our previous finding suggesting that the *SREBF1c* SNP located between exon 18c and 19c (rs11868035) is associated with an increased risk of type 2 diabetes in a new case–control study. In addition, we found convincing evidence for three other SNPs, located in the 5′ region of the *SREBF1c* gene, to be associated with the risk of type 2 diabetes. Two SNPs (SNP1 and SNP4) in the 5′ region, were also weakly related to plasma glucose concentration in a large cohort, supporting the observed association with diabetes risk in the case–control studies.

Acknowledgements We are grateful to the volunteers who participated in the Cambridgeshire Case–Control Study, the EPIC-Norfolk Study and the Ely Study. We also thank S. Bumpstead for technical assistance. The Cambridgeshire Case–Control Study was funded by the Wellcome Trust, the Ely Study was funded by the Medical Research Council (MRC) and the Wellcome Trust, and the EPIC-Norfolk Study is supported by programme grants from the UK MRC and Cancer Research UK. S. O’Rahilly and I. Barosso acknowledge support from EU FP6 funding (contract no. LSHM-CT-2003-503041). Additional support was provided by the Stroke Association, British Heart Foundation, Department of Health, Food Standards Agency and the Wellcome Trust.

Duality of interest The authors declare that they have no duality of interest.

References

- Lelliott C, Vidal-Puig A (2004) Lipotoxicity, an imbalance between lipogenesis de novo and fatty acid oxidation. *Int J Obes Relat Metab Disord* 28:S22–S28
- Horton JD, Goldstein JL, Brown MS (2002) SREBPs: activators of the complete program of cholesterol and fatty acid synthesis in the liver. *J Clin Invest* 109:1125–1131
- Osborne TF (2000) Sterol regulatory element-binding proteins (SREBPs): key regulators of nutritional homeostasis and insulin action. *J Biol Chem* 275:32379–32382
- Shimano H, Horton JD, Shimomura I, Hammer RE, Brown MS, Goldstein JL (1997) Isoform 1c of sterol regulatory element binding protein is less active than isoform 1a in livers of transgenic mice and in cultured cells. *J Clin Invest* 99:846–854
- Shimomura I, Bashmakov Y, Shimano H, Horton JD, Goldstein JL, Brown MS (1997) Cholesterol feeding reduces nuclear forms of sterol regulatory element binding proteins in hamster liver. *Proc Natl Acad Sci USA* 94:12354–12359
- Ducluzeau PH, Perretti N, Laville M et al (2001) 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
- Sewter C, Berger D, Considine RV et al (2002) Human obesity and type 2 diabetes are associated with alterations in SREBP1 isoform expression that are reproduced ex vivo by tumor necrosis factor- α . *Diabetes* 51:1035–1041
- Espenshade PJ, Li WP, Yabe D (2002) Sterols block binding of COPII proteins to SCAP, thereby controlling SCAP sorting in ER. *Proc Natl Acad Sci USA* 99:11694–11699
- Brown AJ, Sun L, Feramisco JD, Brown MS, Goldstein JL (2002) Cholesterol addition to ER membranes alters conformation of SCAP, the SREBP escort protein that regulates cholesterol metabolism. *Mol Cell* 10:237–245
- Xu J, Nakamura MT, Cho HP, Clarke SD (1999) Sterol regulatory element binding protein-1 expression is suppressed by dietary polyunsaturated fatty acids: a mechanism for the coordinate suppression of lipogenic genes by polyunsaturated fats. *J Biol Chem* 274:23577–23583
- Kim HJ, Takahashi M, Ezaki O (1999) Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver: a possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J Biol Chem* 274:25892–25898
- Foretz M, Pacot C, Dugail I et al (1999) ADD1/SREBP-1c is required in the activation of hepatic lipogenic gene expression by glucose. *Mol Cell Biol* 19:3760–3768
- Kim JB, Sarraf P, Wright M et al (1998) Nutritional and insulin regulation of fatty acid synthetase and leptin gene expression through ADD1/SREBP1. *J Clin Invest* 101:1–9
- Lay SL, Lefrere I, Trautwein C, Dugail I, Krief S (2002) Insulin and sterol-regulatory element-binding protein-1c (SREBP-1C) regulation of gene expression in 3T3-L1 adipocytes. Identification of CCAAT/enhancer-binding protein beta as an SREBP-1c target. *J Biol Chem* 277:35625–35634
- Laudes M, Barroso I, Luan J et al (2004) Genetic variants in human sterol regulatory element binding protein-1c in syndromes of severe insulin resistance and type 2 diabetes. *Diabetes* 53:842–846
- Eberle D, Clement K, Meyre D et al (2004) SREBF-1 gene polymorphisms are associated with obesity and type 2 diabetes in French obese and diabetic cohorts. *Diabetes* 53:2153–2157
- Laaksonen R, Thelen KM, Paiva H et al (2006) Genetic variant of the SREBF-1 gene is significantly related to cholesterol synthesis in man. *Atherosclerosis* 185:206–209
- Rathmann W, Haastert B, Icks A et al (2001) Low faecal elastase 1 concentrations in type 2 diabetes mellitus. *Scand J Gastroenterol* 36:1056–1061
- Harding AH, Day NE, Khaw KT et al (2004) Dietary fat and the risk of clinical type 2 diabetes: The European Prospective Investigation of Cancer-Norfolk Study. *Am J Epidemiol* 159:73–82
- Day NE, Oakes S, Luben R et al (1999) EPIC-Norfolk: study design and characteristics of the cohort. *European prospective investigation of cancer. Br J Cancer* 80:95–103
- Williams DR, Wareham NJ, Brown DC et al (1995) Undiagnosed glucose intolerance in the community: the Isle of Ely diabetes project. *Diabet Med* 12:30–35
- The International HapMap Consortium (2005) A haplotype map of the human genome. *Nature* 437:1299–1320
- Whittaker P, Bumpstead S, Downes K, Ghori J, Deloukas P (2006) SNP analysis by MALDI-TOF mass spectrometry. In: Celis J, Carter N, Simons K, Small JV, Hunter T (eds) *Cell biology: a laboratory handbook*, 3rd edn. Academic Press, San Diego, (in press)
- Yoshikawa T, Shimano H, Memiya-Kudo M et al (2001) Identification of liver X receptor–retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. *Mol Cell Biol* 21:2991–3000
- Memiya-Kudo M, Shimano H, Yoshikawa T et al (2000) Promoter analysis of the mouse sterol regulatory element-binding protein-1c gene. *J Biol Chem* 275:31078–31085
- Tarling E, Salter A, Bennett A (2004) Transcriptional regulation of human SREBP-1c (sterol-regulatory-element-binding protein-1c): a key regulator of lipogenesis. *Biochem Soc Trans* 32:107–109