Diabetologia (2006) 49: 1214–1221
DOI 10.1007/s00125-006-0218-3

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Association study of genetic polymorphisms of *SLC2A10* gene and type 2 diabetes in the Taiwanese population

Received: 18 September 2005 / Accepted: 30 January 2006 / Published online: 4 April 2006

Abstract *Aims/hypothesis:* The gene encoding solute carrier family 2, facilitated glucose transporter, member 10 (SLC2A10, previously known as glucose transporter 10 [GLUT10]) is a promising candidate gene for type 2 diabetes since it is highly expressed in liver and pancreas and is located on human chromosome region 20q12–q13.1, a region previously shown to harbour type 2 diabetes susceptibility genes. We investigated whether the *SLC2A10* gene could be a type 2 diabetes susceptibility gene in the Taiwanese population. *Subjects and methods:* Sequencing of *SLC2A10* gene from 48 diabetic subjects detected short tandem repeat polymorphisms in the promoter region, but did not detect any other sequence variants or new single-nucleotide polymorphisms (SNPs) other than those already in the SNPper database (http://snpper.chip.org) (30 June 2005). *Results:* Using these genetic polymorphisms, we divided the *SLC2A10* gene into four distinct linkage disequilibrium blocks and performed a case-control association study in a group of type 2 diabetes subjects (n=375) and normoglycaemic individuals (n=377). The HapD (A-G-T-C) haplotype in block 3, a rare haplotype, which consisted of four SNPs (rs3092412, rs2235491, rs2425904 and rs1059217), was modestly associated with type 2 diabetes with a haplotype score of −2.95567 (p=0.012 with the haplotype-specific test). *Conclusions/interpretation:* Our results suggest that *SLC2A10* genetic variations do not appear to be major determinants for type 2 diabetes susceptibility in the Taiwanese population.

Keywords GLUT10 · Haplotype · Linkage disequilibrium · Single-nucleotide polymorphism · *SLC2A10* · Type 2 diabetes

Abbreviations GLUT10: glucose transporter 10 · LD: linkage disequilibrium · SLC2A10: solute carrier family 2, facilitated glucose transporter, member 10 · SNP: single-nucleotide polymorphism · STRP: short tandem repeat polymorphism

Introduction

Type 2 diabetes is the most common metabolic disorder, affecting at least 5% of the world's population. Around 150 million people are affected by type 2 diabetes worldwide, and the prevalence is expected to reach 211 million by 2010 [1]. The disease is a result of complex interactions between genetic and environmental factors that regulate insulin sensitivity and/or beta cell function [2, 3]. Although type 2 diabetes is considered as a polygenic genetic disorder, the number and relative contribution of each of the genetic factors remain to be elucidated [4–6]. Two general approaches are used to search for genetic factors of complex diseases: one is the whole-genome scan to identify chromosomal regions that harbour the genes contributing to type 2 diabetes [7–11], and the other is the case-control association study with biological or positional candidate genes. One such region, 20q13.12, has been mapped for type 2 diabetes in previous studies on Finnish and Ashkenazi Jewish populations and French diabetic families [12–14]. It is believed that a candidate gene non-insulin-dependent diabetes mellitus 3 (*NIDDM3*) contributes to the development of type 2 diabetes in the 20q13.12 region. More interestingly, this region has also been mapped for type 2 diabetes in Han Chinese [15, 16].

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Table 1 Clinical characteristics (means±SD) of type 2 diabetic patients and normoglycaemic controls

	Controls (*n*=377)	Cases (*n*=375)	*p* value[a]
Age (year)	60.2±13.3	61.0±8.7	0.4326
Sex (male:female)	216:160	184:192	0.0154
BMI (kg/m^2)	23.22±3.17	24.77±3.30	<0.0001
Triglycerides (mmol/l)	1.30±0.77	1.72±0.45	<0.0001
Cholesterol (mmol/l)	5.12±1.02	5.23±1.07	0.1698
HbA_{1c} (%)	5.55±1.04	8.11±1.59	<0.0001

[a]Student's *t* test

One candidate gene, *HNF4A*, is localised in this *NIDDM3* region and is associated with type 2 diabetes in Finnish and Ashkenazi Jewish populations [17, 18]. However, the genetic variation of *HNF4A* in promoter 2 region is not associated with type 2 diabetes in the French Caucasian population [19]. It is possible that some genes other than *HNF4A* within the 20q13.12 region contribute to the development of type 2 diabetes.

The human facilitative glucose transporters play an important role in maintaining a constant supply of circulating glucose for the body. Defects in facilitative glucose transport have been implicated in the pathogenesis of type 2 diabetes [20–22]. The gene encoding the glucose transporter protein solute carrier family 2, facilitated glucose transporter, member 10 (SLC2A10, previously known as glucose transporter 10 [GLUT10]), lies within the *NIDDM3* susceptibility region, and is a novel facilitative glucose transporter that is highly expressed in the liver and pancreas, the two major organs involved in maintaining blood glucose homeostasis [23]. This gene contains five exons and spans at least 28 kb of genomic DNA [23, 24]. The SLC2A10 protein contains 541 amino acids and shares approximately 20 and 32% sequence identity with other members (SLC2A3 and SLC2A12, respectively) of the facilitative glucose transporter family. SLC2A10, like other facilitative glucose transporters, contains 12 transmembrane domains, a hydrophilic intracellular loop between helices 6 and 7, and a large extracellular loop containing a potential *N*-linked glycosylation site between helices 9 and 10 [24, 25]. Recently, a nucleotide variant Ala206Thr in the *SLC2A10* gene has been reported to be associated with fasting and post-glucose-load serum insulin concentrations. However, the association of genetic polymorphisms of *SLC2A10* with type 2 diabetes has been lacking [26]. In this association study in the Taiwanese population, we aimed to identify the genetic variants and linkage disequilibrium (LD) structure of the *SLC2A10* gene and investigate the role of this gene in type 2 diabetes.

Subjects and methods

Subjects and phenotype measurements

This study recruited 377 normoglycaemic control subjects whose 75-g OGTTs were normal in a routine physical check-up, and 375 patients with type 2 diabetes diagnosed with the WHO criteria 1998 [27] from the Metabolic Clinic of the National Taiwan University Hospital, Taipei. Both control and case study groups were unrelated. Characteristics of the study subjects, including BMI, triglyceride and cholesterol levels, are summarised in Table 1. Informed consent was obtained from each study subject, and the study protocol was approved by the Institutional Review Board. The concentrations of plasma glucose, total cholesterol and triglyceride were measured in fasting samples by an autoanalyzer (Hitachi 7250 special, Tokyo, Japan). The average HbA_{1c} was 8.11±1.59% for the

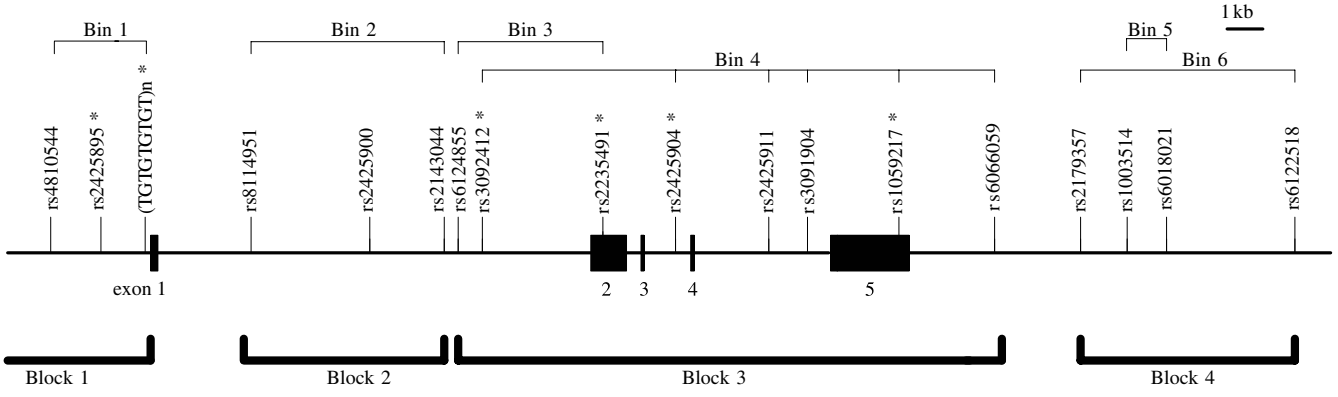

Fig. 1 Graphical representation of SNPs and the STRP (TGTGTGTGT repeat) in relation to the exon–intron structure of the human *SLC2A10* gene. The coding regions are shown with *filled rectangles*. Haplotype blocks 1, 2, 3 and 4 with their boundaries are shown beneath the SNPs. LD bins 1–6 estimated from the data of 94 normoglycaemic subjects are also shown above the SNPs. *SNPs selected for haplotype analysis

patients with type 2 diabetes and 5.55±1.04% for the normal controls.

DNA sequencing and single-nucleotide polymorphism (SNP) genotyping

Genomic DNA was isolated using the PUREGENE DNA purification system (Gentra Systems, Minneapolis, MN, USA). *SLC2A10* gene sequence variants were first determined by direct sequencing (Applied Biosystems 3730 DNA Analyzer; PE Applied Biosystems, Foster City, CA, USA) in 48 diabetes subjects. Primers were specifically designed for intron–exon junctions, exons (including all 5′ and 3′ untranslated regions), and a region 1.7 kbp upstream of the transcription start site for the *SLC2A10* gene. Primers were designed by the Primer3 PCR primer program (http://fokker.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) (30 June 2004). PCRs were performed in a final volume of 40 μl, containing 0.2 μmol/l of each primer, 50 mmol/l Tris–HCl (pH 9.1), 16 mmol/l ammonium sulphate, 3.5 mmol/l $MgCl_2$, 0.2 mmol/l dNTPs, 4.5 μg BSA, 10 ng genomic DNA, and 1.2 units of KlenTaq polymerase (Ab Peptides, St Louis, MO, USA).

Amplification conditions consisted of an initial denaturation of 2 min at 94°C, followed by 35 PCR cycles for 40 s at 94°C, 30 s at 65°C, 40 s at 72°C, and a final 10-min extension at 72°C. We then selected 25 SNPs (i.e. rs2425893, rs4810544, rs2425895, rs2425897, rs3092211, rs8114951, rs3092439, rs2425900, rs2143044, rs6124855, rs3092412, rs6094438, rs2235491, rs2425904, rs2425911, rs3091904, rs7348121, rs6094446, rs6094447, rs1059217, rs6066059, rs2179357, rs1003514, rs6018021 and rs6122518) from the public SNPper database (http://snpper.chip.org) (30 June 2005) for genotyping in 94 normoglycaemic individuals, to check allele frequency, and performed LD analysis for haplotype block identification.

SNP genotyping was performed using high-throughput matrix-assisted laser desorption and ionisation time-of-flight (MALDI-TOF) mass spectrometry (SEQUENOM MassARRAY system; Sequenom, San Diego, CA, USA) [28–30]. On average, 98.2% of attempted genotypes were successful with MALDI-TOF mass spectrometry. The failure of genotyping was further certified by direct PCR sequencing. Overall, the accuracy of genotyping was >98% by more than 3,000 re-sequencing analyses. For genotyping of SNP rs8114951 and rs2425900, PCR products were amplified with the appropriate primers 3233f (5′-TTTCACCGTGTTACCCAGGCTGA-3′)/3233r (5′-TAAAATTCAACCTCCCTGTAGCATC-3′) and 7699f (5′-AACCCACCCCATAATTGATCCATC-3′)/7699r (5′-GCCAACATGGCAAAACCCATCTC-3′), and then sequenced using primer 3233f and 7699r, respectively.

For genotyping of (TGTGTGTGT)*n* microsatellite polymorphism in the promoter region, PCR was performed with an FAM-labelled sense primer, GLUT10P1f (5′-GTGGTGTCCAAACCCAAG-3′), and antisense primer, GLU10P2r (5′-GGAAGAGGTGCCCCAATCACT-3′), and then the products were electrophoresed on an ABI Gene Analyzer 3700 system (PE Applied Biosystems). The respective sizes of the (TGTGTGTGT)*n* repeats were each calculated using GeneScan Analysis software (PE Applied Biosystems). A PCR product size of 172 and 181 suggested the presence of two or three (TGTGTGTGT)*n* repeats. To confirm the number of $(TGTGTGTGT)_n$ repeats further, some of the PCR products were subcloned into pGEM-T vector (Promega, Madison, WI, USA), and purified plasmid DNAs were subjected to sequence analysis.

Statistical analysis

A Hardy–Weinberg equilibrium proportion test was carried out for cases and controls separately before marker-trait association analysis. An SNP not obeying Hardy–Weinberg equilibrium might be subject to genotype errors. Re-sequencing experiments were then undertaken to verify (or correct) the genotyping results. After re-sequencing correction of genotyping results, all of the SNPs were consistent with Hardy–Weinberg equilibrium in all the subjects except one SNP, rs1003514, which deviated from Hardy–Weinberg equilibrium in the diabetic population. Any significant departure from the Hardy–Weinberg equilibrium proportion test indicates that the assumption of independence between the parental and maternal alleles does not hold and therefore a regular alleletype-based marker-trait association test was replaced by a genotype-based marker-trait association test. All of the above statistics analyses were carried out using SAS/Genetics (2002; SAS Institute, Cary, NC, USA) and were adjusted for age, sex and BMI covariates.

For haplotype construction, genotype data from controls were used to estimate inter-marker LD and define LD blocks. Two inter-marker LD measures, D′ and r^2, for pairs of SNPs were estimated with an expectation–maximisation algorithm implemented in GOLD program [31, 32]. In this study, an LD block was defined by a subset of consecutive SNPs if the measures of inter-marker LD of every pair of those SNPs reached 0.80 or above. A likelihood ratio test implemented in the EH program was performed to test the overall haplotype frequency difference between cases and controls. The Haplo.Score program (http://www.biostat.wustl.edu/genetics/geneticssoft) (20 January 2006) [33] was used to test the global association between the disease status (type 2 diabetes) and haplotypes, and to test the association between the disease status and each haplotype. Simulation *p* values were computed to avoid the inadequacy of the χ^2 distribution for rare haplotypes. Only haplotypes with frequency greater than 2% were considered in the analysis. Since the above analyses were conducted with each of the haplotypes, a corrected *p* value was calculated using Bonferroni correction to account for multiple comparisons. A *p* value of<0.05 was considered statistically significant.

Table 2 *SLC2A10* sequence variants and association with type 2 diabetes

SNP name	Position[a]	Distance (bp)[b]	Major/minor allele	Minor allele frequency		Odds ratio (95% CI)	p_{AF}	Genotype distribution[c]		Odds ratio (95% CI)	p_{GF}
				Diabetes	Control			Diabetes	Control		
rs4810544	−3.338	1,549	T/C	0.2533	0.2821	0.873 (0.695, 1.098)	0.2451	(212:136:27)	(193:157:27)	1.175 (0.921, 1.499)	0.1944
rs2425895	−1.789	1,581	G/A	0.0667	0.0508	1.275 (0.831, 1.957)	0.2656	(327:46:2)	(339:36:2)	0.797 (0.511, 1.244)	0.3177
(TGTGTGTGT)*n*	−0.208	10,569	3/2	0.2440	0.2821	0.831 (0.660, 1.046)	0.1140	(213:141:21)	(192:159:26)	0.807 (0.629, 1.035)	0.0910
rs2143044	10.341	485	C/T	0.3200	0.3182	1.014 (0.816, 1.260)	0.8998	(174:162:39)	(173:169:35)	1.005 (0.798, 1.266)	0.9659
rs6124855	10.826	894	C/T	0.0813	0.0963	0.826 (0.579, 1.179)	0.2919	(318:53:4)	(308:65:4)	0.857 (0.594, 1.237)	0.4100
rs3092412	11.720	4,195	T/A	0.4000	0.4131	0.950 (0.773, 1.167)	0.6226	(134:182:59)	(126:191:60)	1.047 (0.839, 1.306)	0.6840
rs2235491	15.915	2,585	G/A	0.0787	0.0936	0.821 (0.572, 1.179)	0.2849	(319:53:3)	(309:65:3)	1.173 (0.804, 1.713)	0.4074
rs2425904	18.500	3,343	T/C	0.3880	0.4118	0.908 (0.739, 1.116)	0.3596	(136:187:52)	(123:198:56)	1.088 (0.868, 1.365)	0.4626
rs2425911	21.843	1,236	G/C	0.3947	0.4078	0.949 (0.772, 1.167)	0.6210	(135:184:56)	(126:195:56)	1.027 (0.821, 1.284)	0.8177
rs3091904	23.079	3,194	C/T	0.3933	0.4118	0.929 (0.756, 1.141)	0.4813	(139:177:59)	(125:194:58)	0.930 (0.745, 1.161)	0.5209
rs1059217	26.273	3,505	C/T	0.3853	0.4091	0.908 (0.738, 1.116)	0.3588	(140:181:54)	(127:192:58)	0.926 (0.741, 1.157)	0.4976
rs6066059	29.778	2,930	C/T	0.3813	0.4051	0.907 (0.738, 1.116)	0.3575	(141:182:52)	(127:195:55)	0.922 (0.736, 1.154)	0.4768
rs2179357	32.708	1,673	C/T	0.3467	0.3850	0.849 (0.688, 1.048)	0.1265	(165:160:50)	(143:178:56)	0.860 (0.691, 1.072)	0.1794
rs1003514	34.381	1,504	G/T	0.4000	0.0201	2.053 (1.095, 3.848)	0.0221	(349:22:4)	(362:15:0)	1.948 (1.066, 3.560)	0.0302
rs6018021	35.885	4,358	T/C	0.0373	0.0201	1.911 (1.012, 3.607)	0.0424	(349:24:2)	(362:15:0)	0.532 (0.283, 1.001)	0.0505
rs6122518	40.243		G/T	0.3573	0.381	0.905 (0.734, 1.116)	0.3490	(162:158:55)	(142:183:52)	0.929 (0.746, 1.158)	0.5151

Statistical analyses were adjusted for age, sex and BMI by trend test; p_{AF} allele frequency, and p_{GF} genotype frequency

[a]kb position indicates position relative to the transcription start site

[b]Distance is the number of base pair to the next polymorphism

[c]Genotype distributions are shown as number (AA:AB:BB): AA, AB and BB represent homozygotes for the common allele, and heterozygotes and homozygotes for the rare allele, respectively

Table 3 Standardised pairwise linkage disequilibrium coefficients D' (below diagonal of empty cells) and r^2 (above diagonal) of polymorphic sites at the *SLC2A10* gene locus

	SNP	$r^2 \rightarrow$																	
		rs4810544	rs2425895	TG repeat	rs8114951	rs2425900	rs2143044	rs6124855	rs3092412	rs2235491	rs2425904	rs2425911	rs3091904	rs1059217	rs6066059	rs2179357	rs1003514	rs6018021	rs6122518
$D' \downarrow$	rs4810544	–	0.016	1.000[a]	0.210	0.367	0.130	0.153	0.404	0.153	0.440	0.453	0.414	0.414	0.404	0.449	0.008	0.008	0.491
	rs2425895	1	–	0.016	0.070	0.043	0.071	0.004	0.043	0.004	0.043	0.044	0.041	0.041	0.043	0.048	0.001	0.001	0.005
	TG repeat	1	1	–	0.210	0.367	0.130	0.153	0.404	0.153	0.440	0.453	0.414	0.414	0.404	0.449	0.001	0.001	0.491
	rs8114951	0.481	1	0.481	–	0.458	0.836[a]	0.056	0.534	0.056	0.495	0.508	0.510	0.510	0.458	0.386	0.008	0.008	0.352
	rs2425900	0.738	1	0.738	0.864	–	0.481	0.162	0.835[a]	0.162	0.794	0.814[a]	0.795	0.795	0.754	0.718	0.008	0.008	0.663
	rs2143044	0.383	1	0.383	0.925	0.896	–	0.054	0.559	0.054	0.520	0.532	0.535	0.535	0.481	0.407	0.034	0.034	0.372
	rs6124855	0.798	1	0.798	1	1	1	–	0.162	1.000[a]	0.162	0.166	0.156	0.156	0.162	0.143	0.004	0.004	0.153
	rs3092412	0.774	1	0.774	0.933	0.914	0.966	1	–	0.162	0.917[a]	0.937[a]	0.917[a]	0.917[a]	0.875[a]	0.798	0.035	0.035	0.743
	rs2235491	0.798	1	0.798	1	1	1	1	1	–	0.162	0.166	0.156	0.156	0.162	0.143	0.002	0.002	0.153
	rs2425904	0.808	1	0.808	0.899	0.891	0.932	1	0.957	1	–	0.979[a]	0.959[a]	0.959[a]	0.958[a]	0.880[a]	0.021	0.021	0.825[a]
	rs2425911	0.812	1	0.812	0.901	0.912	0.933	1	0.978	1	1	–	0.939[a]	0.939[a]	0.937[a]	0.857[a]	0.002	0.002	0.801[a]
	rs3091904	0.801	1	0.801	0.931	0.911	0.965	1	0.978	1	1	1	–	0.958[a]	0.917[a]	0.844[a]	0.021	0.021	0.791
	rs1059217	0.801	1	0.801	0.931	0.911	0.965	1	0.978	1	1	1	0.979	–	0.917[a]	0.844[a]	0.021	0.021	0.791
	rs6066059	0.774	1	0.774	0.864	0.868	0.896	1	0.936	1	0.979	0.978	0.978	0.978	–	0.880[a]	0.020	0.020	0.825
	rs2179357	0.765	1	0.765	0.744	0.903	0.773	0.879	0.952	0.879	1	0.977	1	1	1	–	0.020	0.020	0.937[a]
	rs1003514	1	1	1	1	0.417	1	1	1	1	1	1	1	1	1	0.903	–	1.000[a]	0.004
	rs6018021	1	1	1	1	0.417	1	1	1	1	1	1	1	1	1	0.903	1	–	0.004
	rs6122518	0.776	0.318	0.776	0.688	0.897	0.715	0.883	0.949	0.883	1	0.975	1	1	1	1	0.656	0.656	–

The SNPs and STRPs were determined in 94 normoglycaemic subjects

[a]LD coefficients (r^2) >0.8

Results and discussion

To identify sequence variants including SNPs or microsatellite variants in the *SLC2A10* gene locus, we initially sequenced the promoter region (1.7 kb upstream of exon 1) and all five exons and their flanking intronic sequences in 48 diabetic individuals recruited from the Han Chinese population of Taiwan. The Han Chinese is the largest ethnic group in Taiwan, making up 98% of the population. We found a tandem repeat sequence variant (TGT GTGTGT)$_n$ in the promoter region −291 bp upstream of the translation initiation site. No other sequence variants or new SNPs other than those listed in the SNPper database were found in this re-sequencing effort. We selected 25 SNPs (located between the 5′- and 3′-ends of the *SLC2A10* gene) from the SNPper database, studied the allele frequency in 94 normoglycaemic subjects, and constructed an LD block structure. Among the 25 SNPs, eight (rs2425893, rs2425897, rs3092211, rs3092439, rs6094438, rs7348121, rs6094446 and rs6094447) were non-polymorphic in our population and the remaining 17 SNPs and one TGTGTGTGT short tandem repeat polymorphism (STRP) (spanning 43 kb from the promoter region to 3′ downstream area of this gene [Fig. 1]) were polymorphic with a minor allele frequency of >0.002. The average distance between two consecutive SNPs was 2,598 bp ranging from 487 to 4,358 bp. The position of all variants (rs4810544–rs6122518 and TG repeat variant) relative to the structure of *SLC2A10* gene is illustrated in Fig. 1.

We next genotyped 15 SNPs and one STRP in type 2 diabetes patients (n=375) and normoglycaemic controls (n=377). The characteristics of the study subjects, including BMI, triglyceride and cholesterol levels, are summarised in Table 1. The age and cholesterol levels were comparable in cases and controls; however, triglyceride levels and BMI were significantly higher in the diabetic patients than the controls, a characteristic of the diabetes population. There was no significant difference in SNP allele and genotype frequencies between cases and controls and no correlation of the promoter STRP with type 2 diabetes (Table 2). After Bonferroni correction for multiple comparisons, all p values were still insignificant (data not shown). Furthermore, when the promoter activity for different repeat numbers of the TGTGTGTGT sequence was determined, there was no significant difference between two and three repeats of the TGTGTGTGT sequence by an in vitro promoter activity assay (data not shown). These findings were consistent with the result of a recent study in the Finnish population [34]. However, as suggested by the authors, it is possible that some undetected common or rare variants in and around the *SLC2A10* gene contribute to the pathogenesis of type 2 diabetes in the examined population. This is supported by the previous report showing that haplotypes produced from rare variants in the promoter and coding regions of angiotensinogen contribute to variation in angiotensinogen levels [35]. We therefore examined the LD structure of the *SLC2A10* gene.

To determine the extent of LD in the *SLC2A10* gene, we analysed pairwise D′ and r^2 between the 16 polymorphisms with >5% frequency for the minor allele in 94 control samples (Table 3). On the basis of linkage analysis using the GOLD program, the *SLC2A10* gene appeared to contain four distinct LD blocks extending from the promoter to the 3′-nongenic area (Fig. 1). Block 1 spans about 3.5 kb from the SNP rs4810544 (−3,334 bp) in the promoter region to the TGTGTGTGT-repeat variant (−291 bp). Block 2 spans 7 kb in intron 1 starting from SNP rs8114951 to SNP rs2143044. Block 3 spans about 18 kb in the *SLC2A10* gene from SNP rs3092412 in intron 1 to SNP rs6066059 in the 3′-nongenic area. This block region contains almost all the mRNA coding sequence from exon 2 to exon 5. Block 4 of the *SLC2A10* gene spans about 7.5 kb from SNP rs2179357 to SNP rs6122518. Furthermore, we defined several LD bins in the blocks because their genotype data are highly concordant (Fig. 1).

Because of the LD structure, we further examined the association between SNP haplotypes in the different major LD blocks with type 2 diabetes. Only one significant SNP haplotype, the rare haplotype HapD (A-G-T-C) in block 3, exhibited a modest protective effect against type 2 diabetes (HAP-Score=−2.9557, p=0.012 with haplotype-specific test) (Table 4). For the complex traits or complex disorders, the rare haplotypes have been recently shown to play a significant role in influencing disease susceptibility and disease endophenotypes [35, 36]. In haplotype-based association analysis, more attention needs to be paid to

Table 4 Association analysis of *SLC2A10* haplotypes with type 2 diabetes

Block 1 haplotypes						Block 3 haplotypes					
Haplotype	Composition	Frequencies		HAP-Score	p*	Haplotype	Composition	Frequencies		HAP-Score	p*
		Control	Case					Control	Case		
A	G-3	0.6653	0.6894	1.0625	0.576	A	T-G-T-C	0.5399	0.5905	1.8442	0.261
B	G-2	0.2804	0.2440	−1.5806	0.228	B	A-G-C-T	0.2909	0.3016	0.4290	1.000
C	A-3	0.0542	0.0666	0.8256	0.818	C	A-A-C-T	0.0692	0.0687	0.0262	1.000
						D	A-G-T-C	0.0447	0.0147	−2.9557	0.012

Haplotype analysis in block 1 using rs2425895 and TG analysis in block 3 using rs3092412, rs2235491, rs2425904 and rs1059217
Haplotypes of >2% frequency were tested for association with type 2 diabetes in our patient sample and analysed using the Haplo · Score program
*Statistical analyses were adjusted for age, sex and BMI and all of the p values were corrected by Bonferroni correction

rare haplotypes, so as to avoid underdetection of a true positive association. Thus, the significant difference in frequency of HapD (A-G-T-C) between cases and controls should be stressed even if the haplotype is not a common one. The association of one of the SNPs (rs2235491) in the HapD haplotype with fasting and oral glucose-induced serum insulin level has been reported [26]. This SNP, located in exon 2, created a non-synonymous change from Ala to Thr at codon 206. However, we could not detect any significant association between the HapD haplotype (or rs2235491 SNP alone) with various quantitative endophenotypes, such as the homeostasis model assessment of insulin resistance, fasting insulin level and the insulin level after glucose loading (data not shown). Thus, the effect of Ala206Thr might be indirectly related to the clinical outcomes, although the exact cause of this discrepancy is not known at present. Another explanation of this discrepancy might be a relatively small sample size as exemplified in the previous study showing a positive association by Andersen et al. [26], which was later shown to be uncorrelated with fasting and glucose-induced insulin levels in the Danish population [37]. Further study will be required to clarify this discrepancy.

In conclusion, we identified four LD blocks distributed in the *SLC2A10* gene spanning 43 kb from the promoter to the 3′-nongenic region. The data show that *SLC2A10* is not a major contributor to type 2 diabetes susceptibility in the Taiwanese population, although we found that one haplotype within LD block 3 showed a protective effect on type 2 diabetes. The variants identified in this study will be useful for future investigations of the role of *SLC2A10* in type 2 diabetes susceptibility.

Acknowledgements We thank all the patients for their cooperation in this study. This research project was supported by grants from the National Science & Technology Program for Genomic Medicine, National Science Council, Taiwan (National Clinical Core and National Genotyping Core), and the Genomics and Proteomics Program, Academia Sinica, Taiwan (to Y. T. Chen), and a grant from the National Science Council, Taiwan (NSC-93-3112-B-002-005 to L. M. Chuang).

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