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Molecular Genetics and Metabolism 85 (2005) 323–327
Molecular Genetics and Metabolism
www.elsevier.com/locate/ymgme

Brief communication

Evaluation of *SLC2A10* (*GLUT10*) as a candidate gene for type 2 diabetes and related traits in Finns

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Received 10 February 2005; received in revised form 16 April 2005; accepted 19 April 2005
Available online 3 June 2005

Abstract

The *SLC2A10* gene encodes a glucose transporter and is located on chromosome 20q13, where evidence has been found for linkage to type 2 diabetes (T2D) in multiple studies. We investigated *SLC2A10* as a T2D candidate gene in Finns. We did not confirm the previously reported association between Ala206Thr and fasting insulin and we observed no statistically significant evidence for T2D association with any single marker. We tested haplotypes for association with diabetes-related traits and observed no excess of significant results.
Published by Elsevier Inc.

Keywords: *SLC2A10*; *GLUT10*; Type 2 diabetes; Single nucleotide polymorphism; Association analysis; Finnish; 20q

Introduction

Susceptibility to type 2 diabetes mellitus (T2D) is complex with significant evidence for a genetic component [1]. In the Finland United States Investigation of NIDDM Genetics (FUSION) study (fusion.sph.umich.edu) we performed a genome-wide linkage analysis study and observed evidence for linkage on chromosome 20q13 [2]. Evidence for T2D linkage to chromosome 20q has been observed in at least 10 samples [3]. One chromosome 20q positional candidate gene, *HNF4A*, harbors common variants that we and others have previously reported to be associated with T2D [4–7], but other chromosome 20q diabetes susceptibility genes are likely to exist.

Another candidate gene on chromosome 20q13 is *SLC2A10*, encoding solute carrier family 2, member 10, also known as the facilitative glucose transporter *GLUT10* [OMIM #606145]. Functional analysis has demonstrated that SLC2A10 transports glucose with relatively high affinity [8]. The gene is expressed most strongly in liver and pancreas and is also expressed in adipose tissue, skeletal muscle, heart, lung, brain, placenta, and kidney [8–10]. Previously, a coding variant Ala206Thr was shown to be associated with reduced fasting insulin levels in a Danish population [11]. No previous study has evaluated markers spanning the larger *SLC2A10* genomic region, including potential

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1096-7192/$ - see front matter. Published by Elsevier Inc.
doi:10.1016/j.ymgme.2005.04.011

regulatory regions. We analyzed 16 single nucleotide polymorphisms (SNPs) spanning 159 kb and observed no statistically significant evidence for association with any marker.

Research methods and procedures

Subjects

Cases were ascertained through affected sibling pairs as described [12,13]. In this study, we analyzed a single T2D case from each of 795 families originally designated as FUSION 1 and FUSION 2 [13]. Controls consist of 189 normoglycemic spouses of FUSION 1 cases and 225 elderly individuals who had normal glucose tolerance by oral glucose tolerance test at 65 and 70 years. The physiological characteristics of all participants have been described [12,13].

Variant detection and genotyping

We performed denaturing high performance liquid chromatography using a Wave DNA Fragment Analysis system (Transgenomic, Omaha, NE), followed by direct sequencing of PCR products to screen for variants in all five exons, splice junctions, and 0.5 kb of promoter sequence. Of nine variants identified, four were redundant and one was located in repetitive sequence. One novel variant, rs17861024, was submitted to dbSNP (www.ncbi.nlm.nih.gov/SNP/).

We also obtained genotype data (www.sanger.ac.uk/HGP/Chr20/ld-hmg/) for 33 SNPs in a 101 kb interval spanning *SLC2A10*, having minor allele frequency >0.10, and previously genotyped in the founders of 12 CEPH families and a UK sample [14]. We defined blocks using the method of Dawson et al. [15], which adds a SNP to a block if the SNP has a linkage disequilibrium (LD) value $D' > 0.85$ with the current haplotypes, requires that the five most common predicted haplotypes account for >75% of all haplotypes, and allows up to five intervening markers to be excluded per block. The 33 SNPs clustered into five overlapping blocks of limited haplotype diversity (see Supplementary data Fig. 1). We selected haplotype-tagging SNPs that were able to predict the most common haplotypes using the method of Johnson et al. [16] as implemented in Stata. Ten SNPs accounted for 95% of the predicted haplotype diversity within the region.

Most SNPs were genotyped by homogeneous Mass-EXTEND reaction using the MassARRAY System (Sequenom, San Diego, CA) as described [17]. Marker rs17861024, an insertion of nine nucleotides, was genotyped by HPLC (Transgenomic, Omaha, NE) using denaturing conditions, which clearly distinguished all three genotypes. We observed 97.7% genotype success and an estimated 99.75% genotyping reproducibility (6 discrepancies among 1223 blinded duplicate genotype pairs). Marker data were consistent with Hardy–Weinberg equilibrium ($p > 0.05$).

Statistical analyses

Allelic association with disease status and odds ratio (OR) calculations were performed using logistic regression where the number of alleles served as predictors (multiplicative on an OR scale). We also evaluated diabetes-related quantitative traits, which were derived from clinical measures, OGTTs, and frequently sampled intravenous glucose tolerance tests with minimal model analysis [12]. Trait values were transformed to approximate univariate normality. Tests for trait differences using dominant, recessive, and additive models were performed by analysis of variance within cases, unaffected spouses and elderly controls, with adjustment for age (except for age at diagnosis and duration), gender, and BMI (except for weight related variables and waist-to-hip ratio). For each SNP, significance was assessed by determining if the number of tests expected to exceed the 0.05 level when no association exists between the variant and any of the traits was exceeded. Details of how this method is implemented can be found in Conneely et al. [18].

To evaluate association between *SLC2A10* variants and all traits with a limited number of tests, we constructed haplotypes for each of the five blocks that showed significant LD. For each block, population haplotype frequencies were estimated from the combined case and control samples via the expectation-maximization algorithm as implemented in FUGUE, under development by Goncalo Abecasis (http://www.sph.umich.edu/csg/abecasis/fugue/). Haplogenotypes could be inferred with probability $\geqslant 0.90$ given the estimated population haplotype frequencies, therefore haplogenotypes were assigned to all individuals. Each haplotype was tested individually by using simple linear regression to test for additive, recessive, and dominant genetic models when quantitative traits were analyzed. The logistic regression model described above was used to test for T2D-haplotype associations. Evidence for a diabetes-related variant being in LD with one or more of the haplotypes within a block was determined as described above.

Results and discussion

To identify SNPs in and around *SLC2A10*, we used both molecular methods and information from databases. We included four SNPs identified by screening for variants in the *SLC2A10* exons and upstream regulatory sequence using 23 Finnish individuals, 12 of whom had T2D. Using genotype data from UK Caucasian

Table 1
Genotype counts, allele frequencies, and evidence for association in case and control study samples for *SLC2A10* SNPs

Position[a]	SNP	Genotype	*n* cases	*n* controls	Minor allele frequency cases/controls	*p* value	OR[b](95% CI)
−60207	rs1004571	GG	373	190	0.314/0.319	0.84	0.98 (0.82,1.18)
		GA	329	161			
		AA	82	46			
−49923	rs6066043	AA	14	8	0.134/0.133	0.97	1.00 (0.78,1.29)
		AG	182	91			
		GG	590	303			
−20307	rs2664574	CC	608	297	0.117/0.137	0.16	0.83 (0.65,1.08)
		CG	155	100			
		GG	13	5			
−13616	rs3091619	TT	114	51	0.375/0.361	0.53	1.06 (0.89,1.26)
		TC	348	184			
		CC	307	161			
−650	rs6094435	TT	1	0	0.036/0.030	0.48	1.19 (0.73,1.92)
		TC	54	25			
		CC	725	386			
−477	rs17861024[c]	II	404	302	0.127/0.138	0.49	0.91 (0.69,1.19)
		I-	114	103			
		—	10	5			
15915	rs2235491	GG	695	347	0.059/0.070	0.29	0.83 (0.59,1.18)
		GA	86	52			
		AA	3	2			
21032	rs998422	AA	257	130	0.429/0.432	0.88	0.99 (0.83,1.18)
		AG	380	194			
		GG	146	76			
25390	rs707507	CC	151	76	0.429/0.428	0.96	0.98 (0.82,1.18)
		CT	370	193			
		TT	262	134			
28866	rs6012027	CC	171	89	0.454/0.450	0.87	1.02 (0.86,1.20)
		CT	376	192			
		TT	244	130			
40243	rs6122518	TT	422	215	0.270/0.284	0.46	0.93 (0.78,1.12)
		TG	307	156			
		GG	59	38			
42563	rs6063020	CC	375	194	0.314/0.319	0.82	0.98 (0.82,1.18)
		CT	335	172			
		TT	81	45			
47401	rs4810551	TT	208	107	0.476/0.491	0.49	0.94 (0.79,1.11)
		TG	406	199			
		GG	171	100			
54696	rs760873	TT	371	179	0.304/0.327	0.26	0.90 (0.75,1.09)
		TG	346	186			
		GG	65	39			
80736	rs3092707	AA	318	146	0.352/0.387	0.10	0.86 (0.72,1.03)
		AG	356	190			
		GG	92	57			
98934	rs1072220	CC	83	44	0.338/0.332	0.74	1.03 (0.86,1.24)
		CT	362	168			
		TT	335	174			

I, insertion of TGTGTGTGT.

[a] Nucleotide positions are relative to the *SLC2A10* translation initiation site at chr 20, position 44,771,783 of the human reference sequence (hg17, UCSC Genome Browser, May 2004).

[b] Multiplicative odds ratios are calculated for the minor allele in controls. CI = 95% confidence interval.

[c] Submitted to dbSNP.

Table 2
Phenotype associations with Ala206Thr and fasting insulin

Group	Genotype	*N*	Mean (SD) pmol/L	*p* value[a]		
				Recessive	Dominant	Additive
T2D cases	GG	599	115.3 (98.6)	0.69	0.73	0.69
	AG	70	113.1 (72.7)			
	AA	2	111.0 (30.0)			
Unaffected spouses	GG	155	76.5 (50.7)	—	0.75	0.75
	AG	20	78.0 (48.5)			
	AA	0	—			
Unaffected elderly controls	GG	184	65.1 (32.2)	0.66	0.66	0.61
	AG	30	65.0 (30.3)			
	AA	2	69.0 (4.2)			

Trait values are unadjusted mean and standard deviations for untransformed phenotypes.
[a] *p* values are adjusted for age, gender, and BMI.

individuals and CEPH/Utah samples [14] we identified 10 likely nonredundant common variants in unscreened regions located within 80 kb of the *SLC2A10* exons and also included two SNPs from an early SNP database release. These 16 SNPs were genotyped on 795 Finnish T2D cases and 414 controls from the FUSION study. Evidence for pairwise LD is similar between cases and controls (see Supplementary data Fig. 1), and two of the SNPs detected in our screening are in almost perfect LD with other selected SNPs: rs2664574 with rs17861024 ($r^2 = 0.98$) and rs998422 with rs707507 ($r^2 = 0.99$).

No variant shows a significant allelic or genotypic association with T2D ($p \geqslant 0.10$, Table 1), and no individual haplotype was strongly associated with T2D ($p \geqslant 0.10$, data not shown). We determined that we had power of 0.80 to detect a multiplicative genotype relative risk of ≈1.26 (type I error = 0.005) [19], hence our study is well powered to detect disease predisposing variants of moderate effect.

We also looked for associations between variants and haplotypes and diabetes-related traits by testing association with each haplotype within each of the five blocks that showed significant LD (see Research methods and procedures). We found no overall excess of significant results, nor did we find more significant tests than expected within any individual block (data not shown). We estimate that we have power of 0.80 to detect a QTL that accounts for approximately 3.3% of the trait variance, using the power calculation of Purcell et al. [19]. This estimate is based on a type I error rate of 0.00005 which ensures an experiment-wise error rate of 0.05 given the sample size and effective number of SNPs and traits in our study.

SNP rs2235491, encoding Ala206Thr, was previously described to be associated with reduced fasting insulin under a dominant model [11]. In our study, the mean fasting insulin levels between carriers and non-carriers of the Thr206 allele were not significantly different from one another (Table 2). This SNP also did not show evidence for allelic association with T2D in our sample ($p = 0.29$). Based on the sample size, genotype frequencies, standard deviations, and significance of the SLC2A10 Ala/Thr variant result reported [11], we estimated the this variant accounted for 2.4% of the phenotypic variance observed in the fasting serum insulin measurements. Our study should have had power of approximately 0.98 (type I error = 0.05) to detect a variant with an equivalent effect. While the gene region has limited evidence of recombination (see Supplementary data Fig. 1), evidence for recombination in the surrounding region suggests that other nearby variants may exist that affect T2D susceptibility. Given the current data, we conclude that common variants in or near *SLC2A10* do not appear to be strongly associated with T2D in Finns, although we recognized that undetected common or rare variants may still influence susceptibility.

Acknowledgments

We thank Dr. Panos Deloukas for early access to SNP genotype data on chromosome 20, Dr. Donald Bowden for communicating results prior to publication, and Nancy Riebow for technical assistance. The FUSION study was funded by intramural funds from the NHGRI project OH95-C-N030, and by NIH Grants DK62370 and HG00376 to M.B. J.T. has been partially supported by the Academy of Finland (38387, 46558). R.N.B. was supported by NIH Grants DK27619 and DK29867. K.L.M. is the recipient of a Burroughs Wellcome Career Award in the Biomedical Sciences.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ymgme.2005.04.011.

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