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Genetic contribution of polymorphism of the GLUT1 and GLUT4 genes to the susceptibility to type 2 (non-insulin-dependent) diabetes mellitus in different populations

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Abstract Polymorphic variation of genes encoding the glucose transporters glycoproteins (GLUT) may contribute to the genetic susceptibility to type 2 (non-insulin-dependent) diabetes. In this study we evaluated the allele and genotype frequencies of GLUT1 and GLUT4 restriction fragment length polymorphism (RFLP), revealed by digestion with *Xba*I for GLUT1 and *Kpn*I for GLUT4, in Caucasian, Chinese, Japanese, Asian Indian and American black populations. No differences of the *Kpn*I GLUT 4 RFLP were found between control and diabetic subjects in any ethnic group or when all data are combined. In contrast, positive results were found for the *Xba*I RFLP: (1) most ethnic groups showed an association of allele 1 with type 2 diabetes, and this association was maintained when all groups were analysed together; (2) after stratifying for sex and obesity, this association was significant only for overweight/obese women. This joint analysis suggests that GLUT1 polymorphism may contribute to susceptibility to type 2 diabetes in some populations, and especially in overweight/obese women.

Key words Glucose transporter glycoproteins · Non-insulin-dependent diabetes · Genetic polymorphism

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Introduction

In the last years cloning and sequencing of the genes encoding the facilitative glucose transporter glycoproteins (GLUT) have allowed the study of polymorphic variants of these genes in non-diabetic and diabetic populations [1, 2]. For the HepG2/erythrocyte gene, which encodes a high-affinity glucose transporter (GLUT1), some authors described a significant difference of x1x1 plusx x1x2 vs x2x2 genotypes between North European and Japanese non-diabetic subjects, although this was not the case in West Indians and Caucasian controls [3, 4]. Li et al. also demonstrated a significant increase of the genotype and allele 1 frequencies in diabetic North European, South European and Japanese subjects as compared with non-diabetic subjects [3]. However, these results are not consistent, as no association with type 2 diabetes was found in American blacks [5, 6] and Chinese Americans [7]. Our previous study [8] indicated no significant differences in the allele and genotype frequencies of GLUT1 in non-diabetic and diabetic subjects of an Italian population, probably because of the small sample examined; however, by stratifying this population, we observed a trend to association between GLUT1 allele 1 frequency and overweight/obese diabetic patients. Another candidate that might contribute to susceptibility to type 2 diabetes is GLUT4, the glucose transporter specific for skeletal muscle and adipose tissue. Molecular scanning has revealed one mutation and one silent polymorphism [9–11], but no strong association with type 2 diabetes.

The aims of this study were to compare allele and genotype frequencies of GLUT1 and GLUT4 in different ethnic groups, and to evaluate a possible association between polymorphism of these genes and type 2 diabetes.

Materials and methods

Subjects

Original data regarding American Chinese subjects, normal and diabetic, were made available by G.I. Bell and K. Xiang; data regarding British Caucasoid, European and Asian Indians were provided by J. U. Weaver, G. A. Hitman, V. Mohan and Dr M. Viswanathan; M. Baroni provided data on the Italian population studied in Rome. Details of the Italian population studied in Milan had already been published [8]; data regarding American blacks, Japanese and North European subjects were derived from published papers [3, 5, 12]. Criteria for the selection of diabetic patients and non-diabetic controls have already been published [3, 5, 8]. According to Foster [13] the limit between normal body weight and overweight/obesity, based on the body mass index (BMI, kg/m^2), was set at 25.0 for men and 24.0 for women. Table 1 gives the clinical details of all subjects under study.

DNA studies

DNA was extracted from whole blood, digested with endonuclease according to the manufacturer's instructions, electrophoresed in 0.8% agarose gel and blotted on nylon membranes. Filters were hybridised with specific probes that were previously radiolabeled by a random primer DNA labelling system with [^{32}P]dCTP and autoradiographed for 1–3 days at –80°C. Two cDNA probes of the transporter gene family were used. GLUT4: phJHT-3 (vector pBR327) contains a 1.7-kb *Eco*RI insert and includes 145 bp of 5′ untranslated region, 1326 bp encoding amino acids 1-442 and 267 bp of an intron following codon 442. phJHT-3 encodes the major insulin responsive glucose transporter which is expressed in skeletal muscle, heart and adipocytes. GLUT1 consists of a cDNA subclone (pGT255) isolated from the HepG2 cDNA library and extends from bp 179 to 281 (5′-cDNA). Both probes were kindly provided by G. I. Bell. The DNA sequence polymorphism, revealed by digestion of genomic DNA with the endonuclease *XBa*I and hybridisation with GLUT1, detects two alleles of 6.2 (allele 1) and 5.9 kb (allele 2) [14]. *Kpn*I digestion and hybridisation with GLUT4 detect a two-allele polymorphism with fragment sizes of 6.5 (allele 1) and 5.8 kb (allele 2) [15].

Statistical analysis

The Hardy-Weinberg equilibrium in different ethnic groups was assessed by chi-square test of observed versus expected genotype frequencies. Frequencies of alleles and genotypes of GLUT1 and GLUT4 were computed in diabetic patients and in non-diabetic controls. Statistical significance of the differences in genotype frequencies was assessed by the chi-square test, while trends were checked by the chi-square test for trends [16]. The association between allele 1 and type 2 diabetes mellitus was also measured by the odds ratios (OR) of allele frequency: the OR indicates how many times more frequent allele 1 is among diabetic patients than among controls. Individual OR were computed for each ethnic group (Oriental, North European, Italian and American black) and for each stratum of sex and obesity: common OR was computed by pooling all subjects together. Adjusted OR, according to Mantel-Haenszel [17], was also computed after stratification for ethnic group, in order to avoid any potential bias due to different proportions in cases and controls in different populations. When considering the significance of OR in the individual groups after stratification for sex and obesity, we applied Bonferroni's correction for multiple tests [18], in order to preserve experiment-wise statistical significance. We use 95% confidence intervals (CI). Age and BMI are reported as mean ±standard deviation.

Results

GLUT1

GLUT1 polymorphism was assessed in the following populations: Japanese, Chinese, North European (including British), Italian and American blacks. After grouping control subjects into three large strata (Orientals=groups 1+2; Europeans=groups 5+6+7+8; and American blacks=group 9), we observed a significant difference of allele 1 frequency (0.18, 0.30 and 0.45, respectively: χ^2=22.4, P<0.001).

Table 1 Clinical details of control and diabetic subjects considered for the joint analysis. Data regarding sex and body mass index (BMI) are missing for some ethnic groups and are not available for all patients in others. C=Control subjects; DM=subjects with type 2 diabetes; *n*=number of subjects tested. Mean ±SE for age and BMI; absolute numbers for sex, GLUT1 and GLUT4

Group	Ethnic group	C/DM	Age (years)	BMI (kg/m^2)	GLUT1 (*n*)	GLUT4 (*n*)	Sex (M, W)
1 [3]	Japanese	C	37.5±6.5		49		33, 16
		DM	57.1±10.0		45		35, 10
2	Chinese	C	42.6±1.7	22.3±0.3	73		32, 41
		DM	64.1±1.1	23.7±0.3	92		39, 53
3	Asian Indians	C	37.8±4.1	25.1±1.2		43	26, 7
		DM	51.7±1.7	25.6±0.5		47	33, 13
4	European	C	37.9±2.9	23.7±0.6		24	16, 8
		DM	64.0±1.8	27.7±1.0		35	18, 17
5 [3]	North European	C	55.9±0.9	24.8±0.2	104		104, 0
		DM	64.3±1.2	27.3±0.5	89		51, 38
6	Italian (Rome)	C	54.0±1.0	25.7±0.6	91	53	35, 56
		DM	60.1±1.1	28.1±0.4	93	42	50, 43
7 [8]	Italian (Milan)	C	57.9±1.5	25.3±0.6	66	66	38, 28
		DM	58.7±1.5	24.7±0.6	68	68	41, 27
8	British	C	31.4±0.8	33.0±1.0	84	101	0, 104
		DM	64.0±1.1	27.8±0.8	47	28	33, 41
9 [5, 12]	American blacks	C	61.3±2.2	28.1±0.9	44	42	33, 11
		DM	57.6±2.0	31.3±0.9	63	37	50, 13

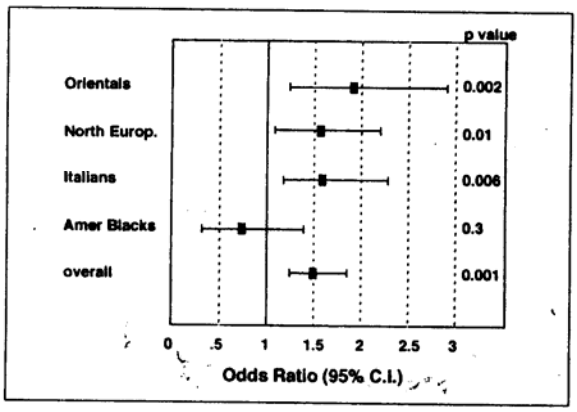

Fig. 1 Odds ratios (OR), confidence intervals (95% CI) and *P* values for allele 1 of GLUT1 frequencies in different populations and common OR

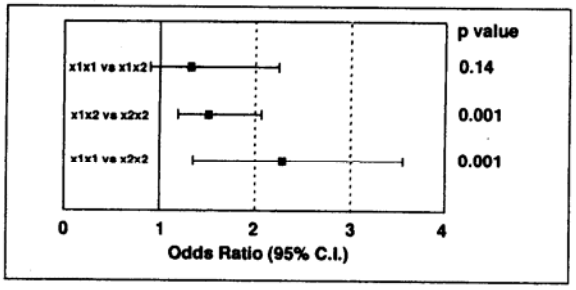

Fig. 2 OR and confidence intervals (95% CI) for genotypes of GLUT1 frequencies. Data are obtained by combining all populations

Table 2 shows allele and genotype frequencies of GLUT1 in diabetic and non-diabetic subjects among Orientals, North Europeans, Italians and American blacks; in all the populations observed, except for American blacks, the genotype frequencies were not significantly different from those expected according to the Hardy-Weinberg equilibrium. OR for allele 1 frequency are shown in Fig. 1: the frequency of allele 1 is significantly higher in type 2 diabetic patients than in controls in all groups except American blacks. The common OR is 1.508 (CI 1.248–1.823), which is highly significant ($P<0.001$). Adjusted OR according to Mantel-Haenszel is almost identical (1.526, CI 1.260–1.849).

In three of the four groups studied, a significant difference in genotype frequencies is observed in diabetic and control subjects; even more significant is the trend when genotypes are ordered according to the number of allele 1 types (x1x1, x1x2, x2x2), suggesting an increasing risk of type 2 diabetes mellitus proceeding from none to one to two copies of allele 1 in the genotype. This trend is maintained when the occurrence of type 2 diabetes is compared within single genotypes: the OR are 1.41 (CI 0.89–2.23, $P=0.14$) for x1x1 vs x1x2; 1.60 (CI 1.23–2.08, $P<0.001$) for x1x2 vs x2x2; and 2.26 (CI 1.44–3.55, $P<0.001$) for x1x1 vs x2x2, when all groups are analysed together (Fig. 2).

The diabetic sample of the American black data is not in Hardy-Weinberg equilibrium for GLUT1 genotypes. As stated above, the genotypes were obtained from a published paper, so that details about the collection of this sample are not available. This group was not taken into consideration for further statistical analysis.

Only ethnic groups with all data (Orientals, North Europeans and Italians) were analysed by stratifying for sex and BMI; the OR for allele 1 frequency was considerably higher in women with BMI>24 (OR=2.05, CI 1.32–3.18, $P=0.004$ after Bonferroni's correction) than in lean women, obese men and lean men (Fig. 3). The strong association in the obese women subgroup is maintained after controlling for population affiliation (adjusted OR=2.62, CI 1.59–4.31, $P<0.001$). Similar results are obtained when genotypes instead of allele frequencies are analysed (Table 3).

Table 2 GLUT1 genotypes in different ethnic groups

Groups		Alleles		Genotypes			χ^2	χ^2 for trend
		All 1 (%)	All 2 (%)	X1X1 (%)	X1X2 (%)	X2X2 (%)		
Orientals	C	18	82	5	33	84		
(1+2)	DM	29	71	8	64	65	$P=0.002$	$P=0.002$
North Europeans	C	29	71	11	88	89		
(5+8)	DM	39	61	18	70	48	$P=0.02$	$P=0.007$
Italians	C	31	69	13	71	73		
(6+7)	DM	41	59	29	75	57	$P=0.017$	$P=0.007$
American blacks	C	45	55	6	14	9		
(9)	DM	37	63	4	37	20	$P=0.13$	$P=0.26$
Overall	C	28	72	35	206	255		
	DM	37	63	59	246	190	$P<0.001$	$P<0.001$

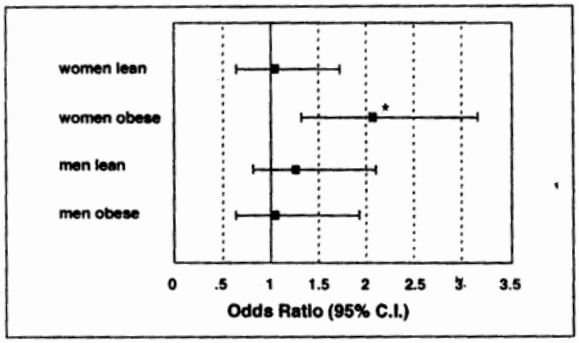

Fig. 3 OR and confidence intervals (95% CI) for allele 1 of GLUT1 frequencies according to gender and obesity strata. * *P* value after Bonferroni's correction for multiple tests=0.004

Table 3 GLUT1 genotype frequencies in different strata

		Lean (BMI <24)			Obese (BMI >24)		
Genotype:		x1x1	x1x2	x2x2	x1x1	x1x2	x2x2
Women	C	5	45	33	4	49	65
	DM	7	21	24	12	36	28
Chi-square			3.55			12.19	
P value			0.17			0.008*	
		Lean (BMI <25)			Obese (BMI >25)		
Genotype:		x1x1	x1x2	x2x2	x1x1	x1x2	x2x2
Men	C	7	23	33	4	16	18
	DM	9	31	31	4	28	24
Chi-square			1.024			0.71	
P value			0.60			0.70	

* Significance level allowing for multiple testing (Bonferroni's correction)

GLUT4

The frequency of allele 1 in the control subjects showed no significant changes, through all the populations examined. Neither in single groups nor in general is allele 1 of GLUT4 associated with a significant risk of type 2 diabetes, even after stratifying for sex and BMI (common OR for GLUT4 allele 1=0.95, χ^2=0.16, P>0.7).

Discussion

Type 2 diabetes mellitus is characterised by reduced insulin release and by reduced insulin sensitivity: the two defects lead to hyperglycaemia that in turn impairs both insulin release and insulin sensitivity. It is a heterogeneous disease in terms of pathogenesis, clinical course and chronic complications; as to pathogenesis, recent studies indicate that in lean patients the primary defect is defective insulin release, while in obese patients the primary defect is insulin resistance in the presence of maintained insulin release, even several years after diagnosis [19–21]. Among the different molecular defects considered recently to explain insulin resistance in type 2 diabetes, glucose transporters were extensively studied, in particular the isoform 4 (GLUT4). Some authors described decreased levels of mRNA GLUT4 in muscles [22, 23], but no correlation was found between insulin resistance and the total level of mRNA GLUT4 protein [24]. We evaluated in this study the allele and genotype frequencies of GLUT1 and GLUT4 in Oriental, Caucasian and American black control and diabetic subjects, in order to identify a possible genetic marker of susceptibility to type 2 diabetes. Our data indicate that GLUT4 polymorphism is not associated with type 2 diabetes in any of the populations studied, in contrast to previous reports [10–12], and the allele frequency of GLUT4 is not different either in men and women or in subjects with BMI lower or higher than 24/25.

In contrast, our results indicate that there is a different interethnical frequency of allele 1 of GLUT1, increasing from Orientals through Caucasians to American blacks; since control subjects were chosen for matching with type 2 diabetic patients, this result should be interpreted with caution, and not be considered fully representative of the different ethnic groups; in addition, the group of American blacks is too small to draw any definitive conclusion. With the exception of American blacks, the allele 1 frequency is higher in diabetic than in control subjects, and this difference is maintained when all groups are considered together. Allele 1 frequency is significantly associated with type 2 diabetes, and this association is stronger in overweight/obese women. The frequency of the three genotypes is also associated with type 2 diabetes, and the results suggest an effect dependent on the number of allele 1 copies in the genotype.

The different OR observed in sex and obesity strata emphasise the concurrent contribution of genetic and environmental background to the susceptibility to type 2 diabetes. This can explain different results previously published, showing a significantly increased frequency of allele 1 of GLUT1 in the diabetic subjects only in some populations, but not in others [3, 5–7].

In conclusion, this study indicates genetic heterogeneity of type 2 diabetes in lean and in overweight/obese patients, possibly also in men and women, and suggests that allele 1 of GLUT1, overweight/obesity, and female sex may all interact in modifying the susceptibility to type 2 diabetes.

Lastly, a speculation can be proposed: it has been shown [25, 26] that the rat obesity gene (*fa*) and the mouse diabetic gene (*db*) mutations are in a gene cluster containing the GLUT1 gene, and both present a similar obesity syndrome. If these genes (*fa* or *db*) occur in man, the most syntenic region is probably 1p 31–34, which also contains the GLUT1 gene. This would indicate in humans the existence of other loci linked to GLUT1, and it might be possible that the differences in GLUT1 frequencies in obese women

are due to the hypothetical presence of a *db* mouse homologue.

References

1. Mueckler M, Family of glucose-transporter genes. Diabetes 39: 6–11, 1990
2. Bell GI, Kajano T, Buse JB, Burant CF, Takeda J, Lin D, Fukumoto H, Seino S, Molecular biology of mammalian glucose transporters. Diabetes Care 13: 198–208, 1990
3. Li SR, Baroni MG, Oelbaum RS, Stock J, Galton DJ, Association of genetic variant of the glucose transporter with non-insulin-dependent diabetes mellitus. Lancet 2: 368–370, 1988
4. Li SR, Oelbaum RS, Boulox PMG, Stocks J, Baroni MG, Galton DJ, Restriction site polymorphisms at the human HepG2 glucose transporter gene locus in Caucasian and West Indian subjects with non-insulin dependent diabetes mellitus. Hum Hered 40: 38–44, 1990
5. Kaku K, Matsutani A, Mueckler M, Permutt A, Polymorphism of HepG2/erythrocyte glucose-transporter gene. Diabetes 39: 49–56, 1990
6. Cox NJ, Xiang KS, Bell GI, Karam JH, Glucose transporter gene and non insulin dependent diabetes. Lancet 2: 793–794, 1988
7. Xiang KS, Cox NJ, Sanz N, Huang P, Karam JH, Bell GI, Insulin-receptor and apolipoprotein genes contribute to development of NIDDM in Chinese Americans. Diabetes 37: 17–23, 1989
8. Magnani C, Capra F, Calderara A, Bonini PA, Taramelli R, Ferrari M, Pontiroli AE, Type 2 (non-insulin-dependent) diabetes mellitus and glucose transporter gene (GLUT1 and GLUT4) polymorphism. Acta Diabetol 29: 110–112, 1992
9. Bell GI, Murray JC, Nakamura J, Kayano T, Eddy RL, Fan YS, Byers MG, Shows TG, Polymorphic human insulin-responsive glucose transporter gene on chromosome 17p13. Diabetes 38: 1072–1075, 1989
10. Kusari J, Verma US, Buse JB, Henry RR, Olefsky JM, Analysis of the gene sequence of the insulin receptor and the insulin sensitive glucose transporter (GLUT4) in patients with common-type non-insulin-dependent diabetes mellitus. J Clin Invest 88: 1323–1330, 1991
11. Choi WH, O'Rahilly S, Buse JB, Morgan R, Fluer JS, Moller DE, Molecular scanning of insulin-responsive glucose transporter (GLUT4) gene in NIDDM subjects. Diabetes 40: 1712–1718, 1991
12. Matsutani A, Koranyi L, Cox N, Permutt MA, Polymorphism of GLUT2 and GLUT4 genes. Diabetes 39: 1534–1542, 1990
13. Foster DW, Eating disorders and anorexia nervosa. In: Wilson JD, Foster DW (eds) William's textbook of endocrinology, 7th edn. Saunders, Philadelphia, pp 1081–1107, 1985
14. Shows TB, Eddy RL, Byers MG, Fukushima Y, Dehaven CR, Murray JC, Bell GI, Polymorphic human glucose transporter gene (GLUT) is on chromosome 1p31.3p35. Diabetes 36: 546–549, 1987
15. Leysen N, Murray JC, Bell GI, KpnI polymorphism for the human insulin responsive glucose transporter gene (GLUT4) is on chromosome 17. Nucleic Acids Res 17: 3621, 1989
16. Armitage P, Test for linear trend in proportions and frequencies. Biometrics 11: 375–386, 1955
17. Mantel N, Haenszel W, Statistical aspects of the analysis of data from retrospective studies of disease. J Natl Cancer Inst 22: 719–748, 1959
18. Snedecor GW, Cochran WG, Statistical methods, 7th edn. Iowa State University Press, Iowa City, pp 115–117, 1989
19. Pontiroli AE, Calderara A, Maffi P, Bonisolli L, Carenini A, Piatti PM, Monti LD, Gallus G, Pozza G, Illeni MT, Secondary failure to oral hypoglycaemic agents in non-obese patients with non-insulin-dependent diabetes mellitus is related to reduced insulin release. Diabete Metab 5: 79–84, 1989
20. Arner P, Pollare T, Litthell H, Different aetiologies of type 2 (non-insulin-dependent) diabetes mellitus in obese and non-obese subjects. Diabetologia 34: 483–487, 1991
21. Banerji MA, Lebovitz HE, Insulin action in NIDDM in Black Americans. Diabetes Care 15: 1295–1302, 1992
22. Friedman JE, Pories WJ, Legget-Frazier N, Evidence for decreased insulin sensitive glucose transporters (GLUT4) in skeletal muscle from a large sample of obese and non obese-NIDDM patients. Diabetes 40: A158, 1991
23. Vogt B, Muehlbacher C, Carrascosa J, Subcellular distribution of GLUT4 in the skeletal muscle of lean type 2 (non-insulin-dependent) diabetic patients in the basal state. Diabetologia 35: 456–463, 1992
24. Friedman JE, Dohm GL, Legget-Frazier N, Restoration of insulin responsiveness in skeletal muscle of morbidly obese patients after weight loss. J Clin Invest 89: 701–705, 1992
25. Truett GA, Bahary N, Friedman JM, Leibel RL, Rat obesity gene fatty (fa) maps chromosome 5: evidence for homology. Proc Natl Acad Sci USA 88: 7806–7808, 1991
26. Bahary N, Leibel RL, Joseph L, Friedman JM, Molecular mapping of the mouse db mutation. Proc Natl Acad Sci USA 87: 8642–8646, 1990