

Short Report

MGEA5-14 Polymorphism and Type 2 Diabetes in Mexico City

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ABSTRACT A family-based study has recently reported that a variant located in intron 10 of the gene *MGEA5* increases susceptibility to Type 2 Diabetes (T2D). We evaluated the distribution of this SNP in a sample of T2D patients ($N = 271$) and controls ($N = 244$) from Mexico City. The frequency of the T allele was higher in the cases (2.6%) than in the controls (1.8%). After adjusting for age, sex, BMI, education, and individual ancestry the odds ratio was 1.60 but the 95% confidence interval was wide and overlapped 1 (0.52–4.86, P -value : 0.404). In order to characterize the distribution of the *MGEA5*-14 polymorphism in the relevant parental populations, we genotyped this variant in European (and European Americans), West African, and Native American samples. The T-allele was present at a frequency of 2.3% in Spain, 4.2% in European Americans, and 13% in Western Africans, but was absent in two Native American samples from Mexico and Peru. Given the low frequency of the T-allele, further studies using large sample sizes will be required to confirm the role of this variant in T2D. *Am. J. Hum. Biol.* 19:593–596, 2007. © 2007 Wiley-Liss, Inc.

The meningioma expressed antigen 5 (hyaluronidase) gene (*MGEA5*), located at 10q24.1-q24.3 and encoding a β -O-linked *N*-acetylglucosaminidase (O-GlcNAcase), is a biological and positional candidate gene for Type 2 diabetes (T2D). Several studies indicate that altered regulation of O-linked protein glycosylation can cause insulin resistance, β cell dysfunction and ultimately β cell death (Arias et al., 2004; Konrad and Kudlow, 2002; Parker et al., 2003; Vosseller et al., 2002). The gene encodes two isoforms with 130 and 75 KDa (Comtesse et al., 2001). The O-GlcNAcase activity is present in the 130-KDa isoform and it is widely expressed in various tissues, including the insulin-producing β -cells of the pancreas (Farook et al., 2002; Patti et al., 1999). A previous study reported linkage of T2D to a region overlapping the gene *MGEA5* on chromosome 10q in the San Antonio Family Diabetes Study (SAFDS), an extended pedigree study consisting of 27 Mexican American families (Duggirala et al., 1999). A subsequent analysis in this sample (Lehman et al., 2005) using a measured genotype approach reported that the *MGEA5*-14 SNP located in intron 10

of the *MGEA5* gene is associated with T2D and age at diabetes onset. Individuals carrying a copy of the T-allele have 2.77 times greater T2D risk in comparison to homozygotes for the A-allele. The authors hypothesized that the presence of the intronic T-allele may decrease expression of the 130-KDa isoform that contains the O-GlcNAcase activity. However, functional studies to determine the role of the *MGEA5*-14 polymorphism are still lacking.

Our study evaluates the distribution of the *MGEA5*-14 SNP in a sample of unrelated T2D patients ($N = 271$) and controls ($N = 244$)

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from Mexico City. We also report frequencies of the *MGEA5-14* polymorphism in two Native American samples, two European samples, and one West African sample.

MATERIALS AND METHODS

Sample of T2D patients and controls

Samples from 515 unrelated individuals from Mexico City were collected at the Medical Center "Siglo XXI". T2D patients were recruited from their Primary Care clinics within the first 2 years of diagnosis, which was made according to the American Diabetes Association criteria (fasting plasma glucose ≥ 126 mg/dl). The sample consisted of 271 T2D patients (186 females and 85 males) and 244 controls (76 females and 158 males). The controls were selected based on: 1/Absence of family history of diabetes in the previous two generations and 2/Negative results for the glucose tolerance test. Data on sex, age, BMI, and education were also collected. Table 1 provides relevant details about this sample. Informed consent was obtained from each participant, and the research was approved by the Ethical Research Board of the Medical Center "Siglo XXI" and the Ethics Review Office at the University of Toronto.

Parental samples

Eighty-four individuals of Nahua, Tlapanec, and Mixtec ancestry from the State of Guerrero (Southwest Mexico, Bonilla et al., 2005), 43 from Spain (Valencia, Bonilla et al., 2005) and 69 from Nigeria (Bini from the Edo region, Kittles et al., 2001) were genotyped to reveal the distribution of the *MGEA5-14* alleles in relevant parental population samples. We also characterized this polymorphism in 65 Quechuas from Peru (Brutsaert et al., 2003) and in 214 European Americans from Pennsylvania (Wagner et al., 2002). In the sample from Mexico, self-identified mestizos were excluded from the analysis (Bonilla et al., 2005). Additional information about these samples can be found in the references indicated above.

Genotyping

Amplification of the DNA segment encompassing the *MGEA5-14* polymorphism was carried out by PCR using the primers previously described by Lehman et al. (2005). Genotyping was carried out using restriction enzyme digestion with *Tsp509I* (New England

TABLE 1. Details about the sample of T2D patients and controls from Mexico City

	Controls	T2D patients
Number	244	271
Sex (% females)	31.1%	68.6%
Age (SD)	39.60 (9.40)	55.69 (10.12)
BMI (SD)	27.53 (4.22)	29.64 (4.77)
Education ^a (SD)	2.58 (1.11)	1.63 (0.94)
Native American admixture (SD)	0.643 (0.127)	0.654 (0.110)
European admixture (SD)	0.318 (0.124)	0.302 (0.103)
West African admixture (SD)	0.040 (0.021)	0.045 (0.028)

^aPrimary school = 1, Secondary school = 2, Preparatory school = 3, University degree and/or postgraduate = 4.

Biolabs) and agarose gel electrophoresis. In addition to the *MGEA5-14* polymorphism, we also genotyped 69 autosomal ancestry informative markers (AIMs) in order to control for potential confounding due to the presence of genetic stratification (Hoggart et al., 2003). Detailed information about the 69 AIMs is available in Martinez-Marignac et al. (2006).

Statistical analysis

We used the program ADMIXMAP v2.2 to test for association of the *MGEA5-14* T variant with T2D. The parameter tested is the coefficient β for the effect of the allele under study (coded as 0, 1, or 2 copies) in a regression model that includes admixture and other covariates. Additional information about this program can be found in Hoggart et al. (2003). Departure of genotype frequencies from Hardy-Weinberg proportions was tested in cases and controls using the Fisher exact test.

RESULTS AND DISCUSSION

Brief description of the Mexico City sample

The analysis of the Mexico City sample using the panel of 69 AIMs indicated that the Native American, European, and West African genetic contributions were 65, 31, and 4%, respectively. There was evidence of substantial genetic stratification in the sample, indicating potential for confounding in association studies. Age, female sex, and BMI were significantly associated with T2D. Diabetes was significantly associated with educational status, with a strong inverse relationship between education level and T2D risk (Table 1). An extensive description of admixture history and dynamics, as well as genetic stratification

TABLE 2. Distribution of LLY-MGEA5-14 genotypes in the admixed sample from Mexico City and four samples of European, Native American, and Western African ancestry

Sample	MGEA5-14 AA	MGEA5-14 AT	MGEA5-14 TT	Allele frequencies (%)
Mexico City				
Controls	235	9	0	A = 98.2, T = 1.8
T2D patients	257	14	0	A = 97.4, T = 2.6
Native American samples				
Guerrero (Mexico)	84	0	0	A = 100.0, T = 0.0
Quechua (Peru)	65	0	0	A = 100.0, T = 0.0
European samples				
Spain	41	2	0	A = 97.7, T = 2.3
European Americans (Pennsylvania)	197	16	1	A = 95.8, T = 4.2
Western Africa				
Nigerians	51	18	0	A = 87, T = 13

in this sample is available in Martinez-Mari-gnac et al. (2006).

MGEA5-14 polymorphism in case-control samples from Mexico City and in parental population samples

The MGEA5-14 T-allele was present at a very low frequency in the sample of T2D patients and controls from Mexico City (2.6% and 1.8%, respectively, Table 2). There were no significant deviations from Hardy-Weinberg proportions in either sample. After adjusting for age, sex, BMI, education, and individual ancestry the odds ratio is 1.60 but the 95% confidence interval is wide and overlaps 1 (0.52–4.86, P -value:0.404). We also genotyped the MGEA5-14 polymorphism in five additional population samples (Table 2). We did not observe any MGEA5-14 T variant in the 79 individuals of Native American ancestry from Mexico and the Quechua from Peru (Table 2). The MGEA5-14 T-allele was present at very low frequencies in the samples of European ancestry (Spain, 2.3%, and 4.2% in European Americans,) and at a relatively higher frequency in Nigerians (13%).

MGEA5-14 polymorphism and T2D risk

After adjusting for age, sex, BMI education, and individual admixture, the difference in frequency between the sample of T2D patients and controls from Mexico City is not significant ($P = 0.404$). This is not surprising given our sample size and the low frequency of the MGEA5-14 allele in the Mexican sample. It would be necessary to genotype more than one thousand cases and the same number of controls to detect a variant conferring an odds ratio of 1.6 and present at a frequency of 2% in the population. Our estimate of the odds ratio (1.6) is substantially lower than the relative

risk (2.77) reported by Lehman et al. (2005). However, it is important to note that there are important differences in study design (family-based vs. case-control) and sample characteristics between this study and that of Lehman et al. (2005), making it difficult to directly compare both studies.

The analysis of the parental samples provides additional information regarding the distribution of the MGEA5-14 polymorphism and its potential impact on diabetes risk. This analysis indicates that the MGEA5-14 T-allele is absent in populations of Native American ancestry from Mexico and Peru. In European populations the allele is present at very low frequencies (2.3%–4.2%) and in West Africans the frequency is relatively higher, around 13%. Based on our estimate of the odds ratio and the frequency distributions observed in the admixed sample of Mexico City and the parental samples (Table 2), the population attributable risk (PAR) conferred by this variant, if causative, would be expected to be relatively low (e.g. less than 10%), except in the Nigerian population (PAR ~ 30%). Given the functional evidence indicating that MGEA5 is involved in β cell function and insulin resistance and previous reports indicating linkage of a region on chromosome 10q with T2D in several populations (reviewed in Lehman et al., 2005), it would be important to carry out additional studies of this candidate gene in large samples.

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LITERATURE CITED

- Arias EB, Kim J, Cartee GD. 2004. Prolonged incubation in PUGNAc results in increased protein O-linked glycosylation and insulin resistance in rat skeletal muscle. *Diabetes* 53:921–930.
- Bonilla C, Gutierrez G, Parra EJ, Kline C, Shriver MD. 2005. Admixture analysis of a rural population of the state of Guerrero, Mexico. *Am J Phys Anthropol* 128: 861–869.
- Brutsaert TD, Parra EJ, Shriver M, Gamboa A, Palacios JA, Rivera M, Rodriguez I, Leon-Velarde F. 2003. Spanish genetic admixture is associated with larger VO₂max decrement from sea level to 4,338 meters in Peruvian Quechua. *J Appl Physiol* 95:519–528.
- Comtesse N, Maldener E, Meese E. 2001. Identification of nuclear variant of MGEA5, a cytoplasmic hyaluronidase and a *B-N*-acetylglucosaminidase. *Biochem Biophys Res Commun* 283:634–640.
- Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell, Stern MP. 1999. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet* 64:1127–1140.
- Farook VS, Bogardus C, Prochazka M. 2002. Analysis of *MGEA5* on 10q24.1-q24.3 encoding the β -O-linked *N*-acetylglucosaminidase as a candidate gene for type 2 diabetes mellitus in Pima Indians. *Mol Genet Metab* 77:1–2.
- Hoggart CJ, Parra EJ, Shriver MD, Kittles RA, Clayton DG, McKeigue PM. 2003. Control of confounding of genetic associations in stratified populations. *Am J Hum Genet* 72:1492–1504.
- Kittles RA, Young D, Weinrich S, Hudson J, Argyropoulos G, Ukoli F, Adams-Campbell L, Dunston GM. 2001. Extent of linkage disequilibrium between the androgen receptor gene CAG and GGC repeats in human populations: Implications for prostate cancer risk. *Hum Genet* 109:253–261.
- Konrad RJ, Kudlow JE. 2002. The role of O-linked protein glycosylation in β -cell dysfunction. *Int J Mol Med* 10: 535–539.
- Lehman DM, Fu D-J, Freeman AB, Hunt KJ, Leach RJ, Johnson-Pais T, Hamlington J, Dyer TD, Arya R, Abboud H, Göring HHH, Duggirala R, Blangero J, Konrad RJ, Stern MP. 2005. A single nucleotide polymorphism in MGEA5 encoding O-GlcNAc-selective *N*-Acetyl- β -D glucosaminidase is associated with type 2 diabetes in Mexican Americans. *Diabetes* 54:1214–1221.
- Martinez-Marignac VL, Valladares A, Cameron E, Chan A, Perera A, Globus-Goldberg R, Wachter N, Kumate J, McKeigue P, O'Donnell D, Shriver MD, Cruz M, Parra EJ. 2006. Admixture in Mexico City: implications for admixture mapping of type 2 diabetes genetic risk factors. *Hum Genet* 120:807–819.
- Parker G, Lund KC, Taylor RP, McClain DA. 2003. Insulin resistance of glycogen synthase mediated by o-linked *N*-acetylglucosamine. *J Biol Chem* 278:10022–10027.
- Patti ME, Virkamaki A, Landaker EJ, Kahn CR, Yki-Jarvinen H. 1999. Activation of the hexosamine pathway by glucosamine in vivo induces insulin resistance of early postreceptor insulin signaling events in skeletal muscle. *Diabetes* 48:1562–1571.
- Vosseller K, Wells I, Lane MD, Hart GW. 2002. Elevated nucleocytoplasmic glycosylation by O-GlcNAc results in insulin resistance associated with defects in Akt activation in 3T3-L1 adipocytes. *Proc Natl Acad Sci USA* 99:5313–5318.
- Wagner JK, Jovel C, Norton HL, Parra EJ, Shriver MD. 2002. Comparing quantitative measures of erythema, pigmentation and skin response using reflectometry. *Pigment Cell Res* 15:379–384.