GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 16, Number 12, 2012

Pp. 1424–1427
DOI: 10.1089/gtmb.2012.0189

Evaluation of the Association Between the *ADRA2A* Genetic Polymorphisms and Type 2 Diabetes in a Chinese Han Population

Tianjie Li,[1,*] Xilin Zhu,[1,*] Xiaopan Wu,[1] Jingyun Li,[1] Liping Pan,[1] Pengtao Li,[1] Zhenhui Xin,[1] Harvest F. Gu,[2] and Ying Liu[1]

Alpha-2-adrenergic receptor (*ADRA2A*) is involved in the sympathetic nervous system and plays a role in the regulation of insulin secretion and lipolysis. Recent studies have indicated that the *ADRA2A* polymorphisms are associated with type 2 diabetes (T2DM) in Caucasians and African Americans. The present study aimed to evaluate the association between the *ADRA2A* polymorphisms and T2DM in a Chinese Han population. Two single-nucleotide polymorphisms (SNPs) rs521674 and rs553668 in the *ADRA2A* gene were genotyped in 2094 Chinese subjects (1042 T2DM patients and 1052 nondiabetic controls) by using the TaqMan allelic discrimination technique. A single-locus analysis indicated that SNP rs553668 was associated with T2DM ($p = 0.04$). Further analysis indicated that the association of SNP rs553668 was found in T2DM patients with body mass index (BMI) $< 25\,kg/m^2$ ($p = 0.03$), but not in the patients with BMI $\geq 25\,kg/m^2$ ($p = 0.56$). This association was still significant in a recessive model ($p = 0.01$, odds ratio = 0.68, 95% confidence interval = 0.51–0.92). In conclusion, the present study provides evidence that the *ADRA2A* polymorphism, rs553668, is associated with lean T2DM patients in a Chinese Han population. Further investigation to explore the role of *ADRA2A* in the regulation of body weight has been taken into our consideration.

Introduction

Type 2 diabetes (T2DM) is a complex disease caused by both genetic and environmental factors. Identification of the susceptibility genes will provide useful information for better understanding pathomechanisms and future therapies of this disease (DeFronzo *et al.*, 1992). In recent years, genome-wide association studies (GWAS) in T2DM have been successful in identifying susceptibility genes that contribute to the causation of this complex disease. However, only ~10% of the heritability can be accounted for by these identified variants (Billings and Florez, 2010). Therefore, it is necessary to further investigate the genetic factors with detection of low-frequency variants and replication studies of the identified variants in different ethnic populations.

The Alpha-2-adrenergic receptor (*ADRA2A*) is involved in the sympathetic nervous system (SNS). Evidence has indicated that low activity of SNS is a risk factor for body weight gain and obesity (Davy and Orr, 2009). SNS modulates release of insulin and glucagon (Kurose *et al.*, 1990), and *ADRA2A* plays a role in the regulation of insulin secretion and lipolysis (Kurose *et al.*, 1990; Fagerholm *et al.*, 2004; Dupuis *et al.*, 2010; Rosengren *et al.*, 2010). It is reported that *ADRA2A* is associated with the function of β cells (Boesgaard *et al.*, 2010). The *ADRA2A* gene is located at chromosome 10q24-q26, within a linkage region to T2DM (Duggirala *et al.*, 1999). In this chromosomal region, several susceptibility genes for T2DM, including TCF7L2 and HHEX/IDE, have been identified by GWAS (Ruchat *et al.*, 2009). Furthermore, a recent genetic study has demonstrated that the *ADRA2A* polymorphism, rs553668, confers a susceptibility risk to the development of T2DM in Caucasians and African Americans (Rosengren *et al.*, 2010). This polymorphism is found to be associated with upregulation of *ADRA2A* expression and reduction of insulin secretion (Liggett, 2009; Rosengren *et al.*, 2010; Kurnik *et al.*, 2011). However, it is unknown whether the *ADRA2A* genetic polymorphisms are associated with T2DM in a Chinese population. In the present study, we have

[1]National Laboratory of Medical Molecular Biology, Peking Union Medical College, Institute of Basic Medical Sciences, Chinese Academy of Medical Sciences, School of Basic Medicine, Beijing, China.
[2]Department of Molecular Medicine and Surgery, Rolf Luft Centre for Diabetes Research, Karolinska Hospital, Karolinska Institute, Stockholm, Sweden.
*These two authors contributed equally to this study.

evaluated the association between the *ADRA2A* genetic polymorphisms and T2DM in a Chinese Han population, which may provide additional information for understanding the genetic impact of *ADRA2A* in the development of T2DM.

Materials and Methods

Subjects

A total of 2094 unrelated subjects, including 1042 T2DM patients and 1052 nondiabetic controls were enrolled in the present study. All of them were Han Chinese and selected from hospitals in Beijing city and Hebei province, China. Nondiabetic control subjects were older than 45 years old, had normal fasting plasma glucose levels, and no relative with T2DM. T2DM patients were diagnosed according to the World Health Organization criteria (WHO, 1998; fasting plasma glucose ≥7.0 mM and/or ≥11.1 mM at 2 h after a 75 g glucose load) as the same as described in our previous study (Alberti, 1998; Liu *et al.*, 2008). Clinical characteristics of all nondiabetic control subjects and T2DM patients are summarized in Table 1. The study was performed after obtaining informed consent from all subjects and was approved by the local ethics committee.

Single-nucleotide polymorphism selection and genotyping

Genomic DNA samples were isolated from the peripheral blood samples of the subjects. Three single-nucleotide polymorphism (SNPs) (rs1800544, rs521674, and rs553668) in the *ADRA2A* gene were selected for genotyping experiments based upon the information from dbSNP database and previous reports (Rosmond *et al.*, 2002; Kurnik *et al.*, 2006; Rosengren *et al.*, 2010). Briefly, these three polymorphisms are located in the 5′-promoter and 3′-untranslated regions, respectively. A perfect linkage disequilibrium ($r^2=1$) between rs1800544 and rs521674 was observed. Then, genotyping experiments on the two SNPs (rs521674 and rs553668) were performed with TaqMan SNP genotyping assays (Applied Biosystems) and the Bio-Rad iQ5 system (Bio-Rad Laboratories). For genotyping quality control, negative controls (water blanks) were added in each plate. Successful genotype calls were >98%. The randomly selected 24 samples were assessed for direct sequencing analyses and the data showed 100% concordance with the genotypes.

Table 1. Clinical Characteristics of Nondiabetic Control Subjects and Type 2 Diabetic Patients

	Cont	*T2DM*	p-*Value*
N (women/men)	1052 (614/438)	1042 (516/526)	<0.001
Age (years)	53.8±11.2	58.1±11.7	<0.001
BMI (kg/m^2)	24.8±3.4	25.3±3.5	0.001
Systolic blood pressure (mmHg)	126.0±15.8	131.4±18.7	<0.001
Diastolic blood pressure (mmHg)	77.4±11.0	80.7±10.9	<0.001
Fasting plasma glucose (mM)	5.0±0.4	8.9±3.1	<0.001
Fasting triglyceride (mM)	1.5±1.0	1.9±1.8	<0.001
Total cholesterol (mM)	5.3±0.9	5.0±1.2	<0.001

Data are shown as means±standard deviation.
Cont, nondiabetic control subjects; T2DM, type 2 diabetic patients; BMI, body mass index.

Table 2. Association Between the *ADRA2A* Genetic Polymorphisms and Type 2 Diabetes

SNP ID	*Genotype and allele*	*T2D* n *(%) 1042*	*Cont* n *(%) 1052*	p	*OR (95% CI)*
rs521674	AA	138 (13.2)	144 (13.7)	0.27	—
	AT	483 (46.4)	451 (42.9)		
	TT	421 (40.4)	457 (43.4)		
	A	759 (36.4)	739 (35.1)	0.38	1.06 (0.93–1.20)
	T	1325 (63.6)	1365 (64.9)		
rs553668	AA	205 (19.7)	249 (23.6)	0.04	—
	AG	546 (52.4)	500 (47.5)		
	GG	291 (27.9)	303 (28.9)		
	A	956 (45.9)	998 (47.4)	0.31	0.94 (0.83–1.06)
	G	1128 (54.1)	1106 (52.6)		

SNP, single-nucleotide polymorphism; OR, odds ratio; CI, confidence interval.

Statistical analysis

Allele frequencies and genotype distributions for the studied SNPs were tested for Hardy–Weinberg equilibrium (HWE) by using the χ^2 (chi-square) test. Multivariate logistic regression analysis was conducted with adjustment for age, sex, and body mass index (BMI). The association between genotypes and quantitative traits was determined by performing a Kruskal–Wallis analysis for traits with non-normal distribution or analysis of variances for normally distributed traits. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 16.0. The criterion of statistical significance was $p<0.05$.

Results

We have conducted a genetic association of the *ADRA2A* genetic polymorphisms with T2DM in a Chinese Han population. The genotype distributions of both SNPs rs521674 and rs553668 in the population were kept in HWE. We began with single-locus association analysis and the data indicated the discrepancy of genotype distributions in SNP rs553668 between the groups of cases and controls ($p=0.04$). The genotype distributions and allele frequencies of SNP rs553668 in nondiabetic control subjects and T2DM patients are summarized in

Table 3. Multivariate Logistic Regression Analysis of rs553668 in Type 2 Diabetes

Variable	β	*SE*	p-*Value*	*OR (95% CI)*
Age	0.033	0.004	<0.001	1.034 (1.026–1.042)
Sex	0.336	0.090	<0.001	1.400 (1.173–1.670)
BMI	0.049	0.013	<0.001	1.051 (1.024–1.078)
Recessive model	0.210	0.109	0.054	1.234 (0.996–1.528)
Dominant model	−0.032	0.100	0.748	0.968 (0.797–1.177)
Additive model	0.059	0.064	0.356	1.061 (0.936–1.202)

Tests are done after adjusted for age, sex, and BMI.

Table 4. Genotype Distributions and Allele Frequencies in BMI ≥ 25 and BMI < 25 Groups

SNP ID	Genotype and allele	BMI ≥ 25 (kg/m²) T2D, n (%)	BMI ≥ 25 (kg/m²) Cont, n (%)	p	p[a]	OR (95% CI)	BMI < 25 (kg/m²) T2D, n (%)	BMI < 25 (kg/m²) Cont, n (%)	p	p[a]	OR (95% CI)
rs521674	AA	70 (12.9)	76 (15.6)	0.30		—	68 (13.6)	68 (12.0)	0.35		—
	AT	255 (47.1)	209 (43.0)				228 (45.5)	242 (42.8)			
	TT	216 (40.0)	201 (41.4)				205 (40.9)	256 (45.2)			
	A	395 (36.5)	361 (37.1)	0.77	0.22	0.80	364 (36.3)	378 (33.4)	0.16	0.45	1.15
	T	687 (63.5)	611 (62.9)			(0.57–1.14)	638 (63.7)	754 (66.6)			(0.80–1.65)
rs553668	AA	111 (20.5)	106 (21.8)	0.56			94 (18.8)	143 (25.3)	0.03		—
	AG	271 (50.1)	227 (46.7)				275 (54.9)	273 (48.2)			
	GG	159 (29.4)	153 (31.5)			—	132 (26.3)	150 (26.5)			
	A	493 (45.6)	439 (45.2)	0.86	0.61	0.93	463 (46.2)	559 (49.4)	0.14	0.01	0.68
	G	589 (54.4)	533 (54.8)			(0.69–1.25)	539 (53.8)	573 (50.6)			(0.51–0.92)

[a]Means tests are done with recessive models.

Table 2. To predict the association of this polymorphism with phenotypes, such as fasting plasma glucose levels, diastolic and systolic blood pressures, body BMI, fasting triglyceride levels and total fasting cholesterol levels, comparison analyses among T2DM patients carrying three different genotypes GG, GA, and AA were conducted. No statistically significant difference of phenotypes among the carriers was observed (data not shown). Following logistic regression, analysis by adjusting age, sex, and BMI was performed to exclude false positives. The association was not significant no matter under recessive, dominant, or additive models (Table 3). *ADRA2A* is a regulator of catecholamine, which is associated with energy metabolism (Lima *et al.*, 2007). We then stratified the single-locus association analysis of SNP rs553668 with BMI < and $\geq 25\,kg/m^2$ because BMI $\geq 25\,kg/m^2$ is defined as overweight (Yang *et al.*, 2010). We compared the T2DM risk in subjects who were overweight and those who were underweight. Data indicated that SNP rs553668 is associated with T2DM with BMI $< 25\,kg/m^2$ ($p = 0.03$), but not with BMI $\geq 25\,kg/m^2$ ($p = 0.56$, Table 4). This association was still significant in a recessive model ($p = 0.01$, odds ratio [OR] = 0.68, 95% confidence interval [CI] = 0.51–0.92) in the patients with BMI $< 25\,kg/m.^2$ The same analyses for SNP rs521674 were done and no significant association of this polymorphism with T2DM was found.

Discussion

The present study shows that the SNP of rs553668 is associated with T2D in the BMI $< 25\,kg/m^2$ Chinese Han population ($p = 0.01$, OR = 0.68, 95% CI = 0.51–0.92). No associations were found between the genotypes of rs553668 and phenotypes though the SNP is located at the 3′ untranslated region (UTR), a position that is important to posttranscriptional modification of mRNA, allocation and transportation of amino acids, and, thus, to maintain the stability of mRNA and the efficiency of translation (Conklin *et al.*, 2002). There are two reasons to explain. The effect between rs553668 and phenotypes is indirect and the statistical validity declined after division into groups according to genotypes.

T2DM is often linked with obesity (Whitmore, 2010). Both T2DM and obesity are characterized by insulin resistance (IR) (Garcia-Guerra *et al.*, 2010). Previous studies have demonstrated that adrenergic receptor polymorphisms may contribute to obesity-related phenotypes, such as hyperinsulinemia, increased systolic blood pressure (Li *et al.*, 2006), and IR. In addition, genetic polymorphisms in beta 2- and beta 3-adenergic receptors have been found to be associated with obesity and T2DM (Siitonen *et al.*, 2004; Papazoglou *et al.*, 2006; Lima *et al.*, 2007). Therefore, we have a hypothesis that ADRA2A may play an important role in the link between T2DM and obesity. In the present study, we have observed the association between SNP rs553668 in the *ADRA2A* gene and lean T2DM in a Chinese Han population. This finding gets rid of the influence of overweight and directly tells us *ADRA2A* is a risk factor to T2DM. The result is in accordance with Rosengren's, which was based on the level of transcription and protein. The possible underlying mechanism is that the signals of *ADRA2A* related to T2D and overweight are distinct. Further investigation related to the regulation of *ADRA2A* on the body weight has been taken into our consideration.

In conclusion, we have evaluated the association of the *ADRA2A* genetic polymorphisms with T2DM in a Chinese Han population and found that SNP rs553668 is associated with T2DM in patients who are lean.

Acknowledgments

We thank all the patients and their families who participated in this study. This work was supported by grants from the Science Fund for Creative Research Groups (No. 30721063) and the Grant from European Foundation for the Study of Diabetes, which is supported for Collaborative Diabetes Research between China and Sweden.

Author Disclosure Statement

No competing financial interests exist.

References

Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part I: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. Diabet Med 15:539–553.

Billings LK, Florez JC (2010) The genetics of type 2 diabetes: what have we learned from GWAS? Ann N Y Acad Sci 1212:59–77.

Boesgaard TW, Grarup N, Jorgensen T, *et al.* (2010) Variants at DGKB/TMEM195, ADRA2A, GLIS3 and C2CD4B loci are associated with reduced glucose-stimulated beta cell function in middle-aged Danish people. Diabetologia 53:1647–1655.

Conklin D, Jonassen I, Aasland R, *et al.* (2002) Association of nucleotide patterns with gene function classes: application to human 3′ untranslated sequences. Bioinformatics 18: 182–189.

Davy KP, Orr JS (2009) Sympathetic nervous system behavior in human obesity. Neurosci Biobehav Rev 33:116–124.

DeFronzo RA, Bonadonna RC, Ferrannini E (1992) Pathogenesis of NIDDM. A balanced overview. Diabetes Care 15: 318–368.

Duggirala R, Blangero J, Almasy L, *et al.* (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.

Dupuis J, Langenberg C, Prokopenko I, *et al.* (2010) New genetic loci implicated in fasting glucose homeostasis and their impact on type 2 diabetes risk. Nat Genet 42:105–116.

Fagerholm V, Gronroos T, Marjamaki P, *et al.* (2004) Altered glucose homeostasis in alpha2A-adrenoceptor knockout mice. Eur J Pharmacol 505:243–252.

Garcia-Guerra L, Nieto-Vazquez I, Vila-Bedmar R, *et al.* (2010) G protein-coupled receptor kinase 2 plays a relevant role in insulin resistance and obesity. Diabetes 59:2407–2417.

Kurnik D, Muszkat M, Li C, *et al.* (2006) Variations in the alpha2A-adrenergic receptor gene and their functional effects. Clin Pharmacol Ther 79:173–185.

Kurnik D, Muszkat M, Li C, *et al.* (2011) Genetic variations in the alpha(2A)-adrenoreceptor are associated with blood pressure response to the agonist dexmedetomidine. Circ Cardiovasc Genet 4:179–187.

Kurose T, Seino Y, Nishi S, *et al.* (1990) Mechanism of sympathetic neural regulation of insulin, somatostatin, and glucagon secretion. Am J Physiol 258:E220–E227.

Li JL, Canham RM, Vongpatanasin W, *et al.* (2006) Do allelic variants in alpha2A and alpha2C adrenergic receptors predispose to hypertension in blacks? Hypertension 47:1140–1146.

Liggett SB (2009) alpha2A-adrenergic receptors in the genetics, pathogenesis, and treatment of type 2 diabetes. Sci Transl Med 1:12ps15.

Lima JJ, Feng H, Duckworth L, *et al.* (2007) Association analyses of adrenergic receptor polymorphisms with obesity and metabolic alterations. Metabolism 56:757–765.

Liu Y, Niu N, Zhu X, *et al.* (2008) Genetic variation and association analyses of the nuclear respiratory factor 1 (nRF1) gene in Chinese patients with type 2 diabetes. Diabetes 57:777–782.

Papazoglou D, Papanas N, Papatheodorou K, *et al.* (2006) An insertion/deletion polymorphism in the alpha2B adrenoceptor gene is associated with age at onset of type 2 diabetes mellitus. Exp Clin Endocrinol Diabetes 114:424–427.

Rosengren AH, Jokubka R, Tojjar D, *et al.* (2010) Overexpression of alpha2A-adrenergic receptors contributes to type 2 diabetes. Science 327:217–220.

Rosmond R, Bouchard C, Bjorntorp P (2002) A C-1291G polymorphism in the alpha2A-adrenergic receptor gene (ADRA2A) promoter is associated with cortisol escape from dexamethasone and elevated glucose levels. J Intern Med 251:252–257.

Ruchat SM, Elks CE, Loos RJ, *et al.* (2009) Association between insulin secretion, insulin sensitivity and type 2 diabetes susceptibility variants identified in genome-wide association studies. Acta Diabetol 46:217–226.

Siitonen N, Lindstrom J, Eriksson J, *et al.* (2004) Association between a deletion/insertion polymorphism in the alpha2B-adrenergic receptor gene and insulin secretion and Type 2 diabetes. The Finnish Diabetes Prevention Study. Diabetologia 47:1416–1424.

Whitmore C (2010) Type 2 diabetes and obesity in adults. Br J Nurs 19, 880, 882–886.

Yang W, Lu J, Weng J, *et al.* (2010) Prevalence of diabetes among men and women in China. N Engl J Med 362:1090–1101.

Address correspondence to:
Dr. Ying Liu
National Laboratory of Medical Molecular Biology
Peking Union Medical College
Institute of Basic Medical Sciences
Chinese Academy of Medical Sciences
School of Basic Medicine
5 Dongdan 3 Tiao
Beijing 100005
China

E-mail: liuyingpumc@yahoo.com