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Type 2 Diabetes in Han Chinese in Hubei

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Abstract: The aim of this study was to investigate the association between Pro12Ala polymorphism in the PPARγ2 gene and type 2 diabetes mellitus in Han Chinese in Hubei. Peroxisome proliferator activated receptorγ2 (PPARγ2) is a nuclear receptor plays a key role in regulation of adipocyte differentiation, lipid metabolism, insulin sensitivity and the development of type 2 diabetes mellitus (T2DM). There are various studies have provided evidence for the association between common Pro12Ala polymorphism in the PPARγ2 gene and type 2 diabetes mellitus, but the results are controversial and depend on ethnicity. So we conducted a case-control association study among 330 T2DM patients and 212 controls with family-based and random case-control designs. The genotypes of the PPARγ2 Pro12Ala polymorphism were detected by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFL) method. The result indicated that the Pro12 allele was associated with type 2 diabetes in this study population.

Key words: Peroxisome proliferator activated receptor gamma2 (PPARγ2), type2 diabetes mellitus (T2DM), polymorphism

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a multifactorial, heterogeneous disorder characterized by chronic hyperglycemia resulting from pancreatic β-cell dysfunction and insulin resistance. The prevalence of the disease is increasing and the World Health Organization (WHO) estimates suggest that by 2025 there will be 300 million affected people worldwide (King *et al*., 1998). Type 2 diabetes, one of the world's most common inherited diseases and a serious international health risk accounting for ~90-95% of all diabetes syndromes. Despite numerous reports suggested a substantial genetic contribution to the susceptibility of type 2 diabetes, no major susceptibility genes have been identified so far (McCarthy and Menzel, 2001). In 1990, the first Peroxisome Proliferator Activated Receptor (PPAR) was isolated as a member of the nuclear hormone receptor superfamily of ligand-activated transcription factors (classII) (Issemann and Green, 1990). PPAR is consists of three subtypes: PPAR-α, PPAR-β and PPAR-γ. It has been shown that PPAR-γ has an important role in adipocyte differentiation and that it regulates lipid metabolism and sensitivity to insulin (Latruffe and Vamecq, 1997). The human PPARγ gene, which has nine exons and extends over more than 100 kb of genomic DNA on chromosome 3p25 (Fajas *et al*., 1997; Beamer *et al*., 1997), there are four PPARγ isoforms have been identified: PPARγ1, PPARγ2, PPARγ3 and PPARγ4, which result from either alternate transcription starts or alternate splicing (Fajas *et al*., 1997). PPARγ1, PPARγ3 and PPARγ4 proteins are identical and are encoded by exons 1 to 6, whereas PPARγ2 has an additional 30 amino acids at the N-terminus, encoded by the PPARγ2-specific exon B (Fajas *et al*., 1997, 1998). PPARγ1 is ubiquitously expressed, PPARγ2 is restricted to adipose tissue and PPARγ3 seems mainly confined to macrophages, adipose tissue and colon (Fajas *et al*., 1998). The tissue distribution of PPARγ4 has not been explored yet (Sundvold and Lien, 2001). The most common variant of the PPARγ2 is a C → G missense mutation causing an alanine substitution for proline at codon 12 of exon B of the PPARγ gene. This exon encodes the N-terminal residue that defines the adipocyte-specific PPARγ2 isoform (Yen *et al*., 1997). And the rare allele frequencies are ~12% in Caucasians, 10% in Native Americans, 8% in Samoans, 4% in Japanese, 3% in African-Americans, 2% in Nauruans and 1% in Chinese (Vigouroux *et al*., 1998; Mori *et al*., 2001). In Caucasians, the ethnic group with the highest frequency, this translates into a carrier prevalence of the polymorphism of almost 25%. The first evidence for an association between the Pro12Ala

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polymorphism in PPARγ2 and type 2 diabetes came from Japanese-Americans, in which a frequency of the rare Ala allele of 9.3% in subjects with normal glucose tolerance versus only 2.2% in patients with type 2 diabetes was observed, the Ala allele of the common Pro12 Ala polymorphism in the isoform PPARγ2 is associated with reduced (75%) risk for type 2 diabetes (Deeb *et al*., 1998).

This study was conducted to investigate the association between Pro12Ala polymorphism of PPARγ2 gene and type2 diabetes in Han Chinese in Hubei.

MATERIALS AND METHODS

Patients' selection and clinical investigation: This study was conducted from Nov 2006 to Oct 2007 and used two experimental designs: family-based and random case-control designs.

Family-based case-control: We selected 140 patient-unaffected sibling pairs. The case group consists of 140 T2DM patients (82 males, 58 females), the mean of age was 55.116±11.162 years, the controls group consists of 140 nondiabetic (76 males, 64 females), the mean of age was 56.667±14.742 years. The T2DM entry criteria, in accordance with the 1997 American diabetes association (ADA) announced new diabetes diagnostic criteria: fasting blood glucose (FPG ≥7.0) mmol L^{-1} (126 mg dL^{-1}), 2 h postprandial blood glucose ≥11.1 mmol L^{-1} (200 mg dL^{-1}) for diabetes patients and FPG<6.1 mmol L^{-1} (110 mg dL^{-1}) normal (no diabetic).

Random case-control: Random case-control consist of 387 subject, the case group consist of 190 T2DM patients (98 males, 92 females), the mean of age was 56.759±13.879 years, the controls group consists of 197 nondiabetic (107 males, 90 females), the mean of age was 58.811±14.136 years. The all subjects were from the Han population in Hubei.

Phenotyping: The weight, height, waist and hip circumference were measured, the body mass index (BMI) [BMI = weight (kg)/height (m^2)] and waist to hip ratio (WHR) [WHR = waist (cm)/hip (cm)] were calculated. Determination fasting blood glucose and 2 h postprandial blood glucose by used enzymatic methods; Blood pressure measurement using the recommended guideline for hypertension prevention and control methods; systolic blood pressure (SBP) ≥140 mmHg (1 mmHg = 0.133 kpa) and Diastolic Blood Pressure (DBP) ≥90 mmHg standards for hypertension. This information was completed by the cooperation Hospital.

Genotyping: Genomic DNA was extracted from peripheral blood leukocytes by proteinase K and the phenol chloroform method. To determine the PPARγ2 gene Pro12Ala polymorphism genotypes, a genomic DNA fragment of PPARγ2 gene was amplified by using polymerase chain reaction (PCR) method with a pair of oligonucleotide primers: The upstream of primer sequence was: 5'-GCCAATTCAAGCCCAGTC-3'and the downstream was: 5'-GATATGTTTGCAGA CAGTGTATCAGTGAAGGAATCGCTTTCCG-3' (Shanghai Sangon Biological Engineering Technology and services Co., Ltd.). The PCR amplification products were obtained using 25 μL reaction system containing; 50 ng μL^{-1} genomic DNA, ddH_2O 14.7 μL, 10 x buffer 2.5 μL, dNTP (10 mmol L^{-1}) 0.5 μL, each of primer sequences (10 μmol L^{-1}) 0.5 μL, $MgCl_2$ (25 mmol L^{-1}) 3 μL and 1U Taq enzyme. On PTC-200 thermal cycler (MJ research company). After an initial denaturation at 94°C for 5 min, the DNA was amplified by 35 PCR cycles of denaturation at 94°C for 40 sec, annealing at 58°C for 40 sec and elongation at 72°C for 40 sec, followed by a final reelongation at 72°C for 7 min. The amplification products were restricted with the 8U restriction endonuclease enzymes HpaII at 37°C water bath overnight. The products were detected by electrophoresis on a 2% agarose gel containing ethidium bromide. DNA Marker used Diamond™ 250bp DNA marker, Voltage 110V, for 55 min and visualized by Gene Genius Gel imaging system.

The PCR-RFLP products were three fragments' lengths; 267, 224 and 43 bp, but the 43 bp fragments are not visualized. The homozygous individuals for Pro12 allele (PP genotype) were identified by the presence of a single 224 bp product. The homozygous individuals for Ala12 allele (AA genotype) were identified by the presence of a single 267 bp product. And the heterozygous individuals' Pro12, Ala12 (PA genotype) were identified by the presence of both 224 and 267 bp products (Fig. 1).

Statistical analyses: Data are given as the means±standard deviation (SD); the means between two groups were compared by using independent-sample t-test, whereas paired-sample t-test was used for sib-pairs means comparison. The statistical significances of the differences of clinical characteristics between genotypes were compared by analysis of variance (ANOVA). The differences of genotypes distribution and allelic frequencies between two groups were compared with Chi-square analysis. Association analyses were

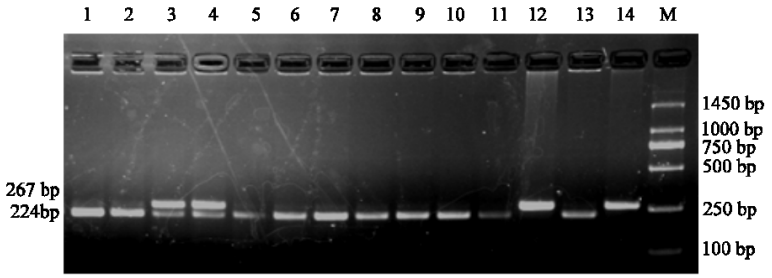

Fig. 1: PPARγ2 gene Pro12Ala polymorphism
Lane M: Diamond™250 bp DNA ladder; Lane 1, 2, 5, 6, 7, 8, 9, 10, 11 and 13: PP; Lane 3 and 4: PA; Lane 12 and 14: AA

completed by Multiple Logistic regression analysis. A p-value <0.05 was considered statistically significant. All statistical analyses were performed with SPSS11.5 for windows.

RESULTS

Clinical characteristics of T2DM group and the control group: In all subjects, the results of independent t-test analysis between T2DM patients and the controls group, showed that the sex, height, waist and hip circumference were well matched and no significant difference (p>0.05), however, the age, weight, fasting and postprandial blood glucose, BMI and WHR of the patients group were significantly higher than that of the controls group (p = 0.01, 0.000, 0.000, 0.000, 0.000, 0.001, respectively), on the other hand the systolic and diastolic blood pressure of the controls group were higher than that of T2DM patients (p = 0.028, 0.038, respectively) (Table 1). In family-based case control sample the paired-sample t-test, showed that the sex, age, height, hip circumference and WHR were also matched, as well as no significant difference (p>0.05), however, weight, waist circumference, BMI, fasting and postprandial blood glucose of T2DM patients group were significantly higher than that of controls group (p = 0.004, 0.009, 0.015. 0.000, 0.000, respectively), on the other hand the systolic and diastolic blood pressure of the controls group were higher than that of T2DM patients (p = 0.034, 0.012). Moreover, in unrelated cases control sample, the sex, age, height, waist circumference, systolic and diastolic blood pressure were matched and no significant difference (p>0.05), however, the weight, hip circumference, fasting and postprandial blood glucose, BMI and WHR of T2DM patients were significantly higher than that of controls group (p<0.01). This result suggested that the age, weight, high BMI (body fat) and high WHR (abdominal obesity) were independent risk factors for type 2 diabetes in Han Chinese in Hubei. Also for the association between Prol2Ala genotypes and the clinical characteristics in T2DM patients and controls group, the result of ANOVA test showed that, in both T2DM patients and controls group, there was no significant association between Pro12Ala polymorphism and the clinical characteristics, except in T2DM patients, the diastolic blood pressure and WHR of the Pro12 Ala12 (PA genotype individuals) were significantly higher than that of both PP genotype and AA genotypes individuals (p<0.01), which indicated that the PA genotype was associated with risk for hypertension and high WHR (abdominal obesity) in patients with type 2 diabetes in the study population.

PPARγ2 gene Pro12Ala genotypes distribution and allelic frequencies: The genotypic and allelic frequencies distributions of the Pro12Ala polymorphism were found to be in Hardy-Weinberg equilibrium in the patients and controls groups (p>0.05). The Chi-square test showed, that there was no significant difference of the genotypic frequencies of the Pro12Ala polymorphism between T2DM patients and controls group in all subjects sample (p>0.05), also no significant differences of the genotypic and allelic frequencies between T2DM patients and controls group in family-based case control sample (p>0.05) (Table 2, 3). However the allelic frequencies in all subjects sample were significantly different, that the Pro12 allele's frequency of T2DM patients was significantly higher than that of controls group (p = 0.040) (Table 2), as well as in unrelated case control sample the genotypic and allelic frequencies distributions of the Pro12Ala polymorphism between T2DM patients and controls group were significantly different, that the PP genotype and Pro12 (P) allele's frequencies in T2DM patients were significantly higher than that of controls group (p = 0. 0.025, 0.009, respectively) (Table 4).

Multivariate logistic regression analysis: Multivariate logistic regression analysis for all subjects showed that the age, weight and high WHR (abdominal obesity) were

Table 1: Clinical characteristics of all subjects

Clinical characteristics	Case group (n = 330)	Control group (n = 212)	p-value
Sex (M/F)	179/151	113/99	0.802
Age (years)	55.990±12.859	58.024±14.339	0.01*
Height (cm)	163.220±9.303	162.829±7.505	0.608
Weight (kg)	64.674±12.086	60.808±11.185	0.000**
Waist circumference(cm)	80.405±21.774	78.384±15.740	0.226
Hip circumference (cm)	89.253±22.983	90.415±16.387	0.508
Systolic blood pressure (mmHg)	134.620±22.888	139.699±26.082	0.028*
Diastolic blood pressure (mmHg)	82.547±15.334	85.498±15.164	0.038*
Fasting blood glucose (mmol L^{-1})	10.829±4.160	5.459±1.746	0.000**
Postprandial blood glucose (mmol L^{-1})	15.931±5.725	8.219±3.952	0.000**
BMI (kg m^{-2})	24.086±3.538	22.869±3.576	0.000**
WHR	0.910±0.1569	0.871±0.0869	0.001**

Note: *p<0.05, **p<0.01

Table 2: Genotypic and allelic frequencies for the Pro12Ala polymorphism in all subjects

	Genotypic frequencies (%)			Allelic frequencies (%)	
Groups	PP	PA	AA	P	A
Cases	289 (87.6)	38 (11.5)	3 (0.9)	616 (93.3)	44 (6.7)
Controls	172 (81.1)	37 (17.5)	3 (1.4)	381 (89.9)	43 (10.1)
		$\chi^2 = 4.217$, p = 0.121		$\chi^2 = 4.223$, p = 0.040*	

Note: *p<0.05

Table 3: Genotypic and allelic frequencies for the Pro12Ala polymorphism in family-based case controls groups

	Genotypic frequencies (%)			Allelic frequencies (%)	
Groups	PP	PA	AA	P	A
Cases	118 (84.3)	21 (15.0)	1 (0.7)	257 (91.8)	23 (8.2)
Controls	114 (81.4)	24 (17.1)	2 (1.4)	252 (90.0)	28 (10.0)
		$\chi^2 = 0.602$, p = 0. 740		$\chi^2 = 0.539$, p = 0.463	

Table 4: Genotypic and allelic frequencies for the Pro12Ala polymorphism in unrelated case control groups

	Genotypic frequencies (%)			Allelic frequencies (%)	
Groups	PP	PA	AA	P	A
Cases	171 (90.0)	17 (8.9)	2 (1.1)	359 (94.5)	21 (5.5)
Controls	158 (80.2)	36 (18.3)	3 (1.5)	352 (89.3)	42 (10.7)
		$\chi^2 = 7.401$, p = 0.025*		$\chi^2 = 6.818$, p = 0.009**	

Note: *p<0.05, **p<0.01

Table 5: Multivariate Logistic regressions of risk factors of T2DM

Risk factors	β-value	SE	Wald value	p-value	OR	95% CI for OR
Age	0.020	0.008	6.107	0.013*	1.021	1.004-1.037
Weight	-0.028	0.010	8.090	0.004**	0.973	0.954-0.991
DBP	0.013	0.007	3.489	0.062	1.013	0.999-1.026
WHR	-4.787	1.545	9.599	0.002**	0.008	0.000-0.172
Prol2Ala	0.428	0.267	2.583	0.108	1.535	0.910-5.588
Constant	2.844	1.458	3.805	0.051	17.176	

Note: *p<0.05, **p<0.01

an independent risk factors for type 2 diabetes (p = 0.013, 0.004, 0.002, respectively), but the Pro12Ala polymorphism was not an independent risk factor for type2 diabetes in the study population (Table 5).

DISCUSSION

PPARs are members of the nuclear receptor subfamily of transcription factors. The PPARγ subtype is involved in adipocyte differentiation and is the target for the thiazolidinedione class of antidiabetic drugs (Spiegelman, 1998), which appear to act primarily by increasing peripheral insulin sensitivity. The association between the substitution of alanine for proline at codon 12 of PPARG and the risk for T2DM has been widely studied since (Yen *et al*., 1997) first reported this polymorphism. Nevertheless, the results of these studies vary considerably. Most have found that carriers of the Ala-12 allele had a lower risk for T2DM and insulin resistance (Deeb *et al*., 1998; Frederiksen *et al*., 2002;

Gonzalez Sanchez *et al*., 2002; Soriguer *et al*., 2006; Tavares *et al*., 2005) although not all studies have found this association (Mancini *et al*., 1999; Ringel *et al*., 1999; Oh *et al*., 2000). Indeed, some studies have even found an increased risk for T2DM in subjects with the Ala-12 variant (Hegele *et al*., 2000; Lindi *et al*., 2002), but no association has so far been reported between the polymorphism and greater insulin resistance (Meirhaeghe and Amouyel, 2004). Contradictory results have been reported within the same study elsewhere, as was the cases of (Mori *et al*., 2001; Altshuler *et al*., 2000; Ghoussaini *et al*., 2005), whom found that the Ala-12 variant was associated with a reduced risk for the development of diabetes, as well as the (Douglas *et al*., 2001) reported that the Pro12Ala variant of the PPARγ2 gene was associated with protection against type 2 diabetes in Finnish subjects. In the present study the PCR-RFLP method and two experimental designs: family-based and random case-control were applied to investigate the association between Pro12Ala polymorphism and type 2 diabetes in Han Chinese in Hubei.

The study demonstrated that in all subject sample, There was no significant difference of the genotypic frequencies of the Pro12Ala polymorphism between T2DM patients and controls group, also no significant differences of the genotypic and allelic frequencies between T2DM patients and controls group in family-based case control sample ($p>0.05$). However, in all subjects sample the allelic frequencies of the Pro12Ala polymorphism between T2DM patients and controls group was significantly different, that the Pro12 allele's frequency in T2DM patients was significantly higher than that of control group (P: 93.3% VS 89.9% $p = 0.040$), as well as in unrelated case control sample the genotypic and allelic frequencies distributions between T2DM patients and controls group were significantly different, that the PP genotype and Pro12 allele's frequencies in T2DM patients were significantly higher than that of controls group (PP: 90.0% VS 80.2%, $p = 0.025$; P: 94.5% VS 89.3% $p = 0.009$). Thus, the PP genotype and Pro12 allele were associated with risk for type 2 diabetes in this study population. The result of ANOVA test showed that, in both T2DM patients and controls group, there was no significant association between Pro12Ala polymorphism and the clinical characteristics ($p>0.05$), except in T2DM patients, the diastolic blood pressure and Waist-Hip Ratio (WHR) of the Pro12 Ala12 (PA genotype individuals) were significantly higher than that of both PP and AA genotypes individuals, which indicates that the PA genotype is associated with risk for hypertension ($p = 0.004$) and high WHR (abdominal obesity) ($p = 0.004$) in patients with type 2 diabetes in the study population. Multivariate logistic regression analysis of all subjects indicated that the age, weight and high WHR (abdominal obesity) (not the Pro12Ala polymorphism) were an independent risk factors for type 2 diabetes in Han Chinese in Hubei ($p = 0.013$, 0.004, 0.002, respectively).

CONCLUSION

This study suggested that the genotypes of the Pro12Ala polymorphism were not associated with type 2 diabetes mellitus. However the Pro12 allele was observed to be associated with risk for type 2 diabetes in the study population, so it's reasonable to expect that the Pro12 allele of Pro12Ala polymorphism may be associated with type 2 diabetes in Han Chinese in Hubei. This study also demonstrated that age, weight and obesity are independent risk factors for type 2 diabetes in the study population. Further studies will be required to confirm and elucidate these results.

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