The Association of Genetic Polymorphisms Of Plasminogen Activator Inhibitor-1 4G/5G And Tissue Plasminogen Activator With Type 2 Diabetes Mellitus Is Influenced By Ethnicity

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Abstract

Introduction: The prevalence of type 2 diabetes mellitus (T2DM) is widely different across different races and the genetic linkage of diabetes could be varies from population to other. The aim of this study was to investigate the influence of ethnicity on the association of genetic polymorphisms of plasminogen activator inhibitor-1 (PAI-1) 4G/5G and tissue plasminogen activator (tPA) Alu repeat I/D with T2DM in Malaysian subjects. Methods: The genetic polymorphisms were genotyped by allele specific PCR in 231 normal subjects without diabetes (114 Malays, 71 Chinese and 46 Indians) and 303 T2DM (149 Malays, 51 Chinese and 103 Indians). Results: The PAI-1 4G/5G polymorphism was strongly associated with T2DM among Chinese (the dominant and additive genetic models odds ratio are 6.45, P = 0.005; 2.26, P = 0.02 respectively), whereas the tPA Alu repeat I/D polymorphism was associated with T2DM among Indian subjects (dominant genetic model odds ratio is 3.27, P = 0.03). Conclusions: The ethnicity significantly impacted the association of PAI-1 4G/5G and tPA Alu repeat I/D polymorphisms with T2DM.

Keywords: **plasminogen activator inhibitor-1, tissue plasminogen activator, type 2 diabetes mellitus.**

I. INTRODUCTION

Diabetes is the most common endocrine disorder that affects 366 million people worldwide. The International Diabetes Federation (IDF) predicts that the total number of people living with diabetes will increase up to 552 million within twenty years [1]. Diabetes, mostly type 2 diabetes mellitus (T2DM), now affects 5.9% of the world's adult population with almost 80% of patients coming from developing countries [2]. The prevalence of T2DM presents a wide spectrum in different ethnic groups. As an example of an estimate by the IDF, show 1.1% of the population in Myanmar to 30% in the Nauru population are susceptible [3]. More than 2.03 million of Malaysians have diabetes mellitus and IDF predicts that this number will increase up to 3.3 million by 2030 [1]. There is strong evidence that predisposition to T2DM is a genetic disease and the genetic causes could be different between populations. Among the SNPs of PAI-1 gene, the 4G/5G polymorphism that located in the promoter region −675 bp upstream from the mRNA synthesis initiation point has been studied. Alu-repeat I/D polymorphism was found in intron 8 of the tPA gene [4], and a number of populations have been found to be dimorphic for its presence or absence of repeats [5]. Our previous studies showed that, the PAI-1 4G/5G polymorphism was associated with metabolic syndrome (MetS) parameters, PAI-1 and tPA activities [6]. Increased fibrinolytic PAI-1 and decreased tPA activities were associated with an increased risk of developing T2DM and cardiovascular diseases (CVD) in Malaysian subjects [7]. Recently, we reported that, the tPA Alu repeat I/D polymorphism showed a risk factor for T2DM. On the other hand, the PAI-1 4G/5G polymorphism was a weakly associated with T2DM among Malaysian subjects [8]. The weak association may be attributed to the variations of ethnicity in Malaysian subjects. The aim of this study was to investigate the influence of ethnicity on the association of genetic polymorphisms of PAI-1 4G/5G and tPA Alu repeat I/D with T2DM in three main ethnic groups in Malaysian subjects.

II. MATERIALS AND METHODS

Subjects and data collection

This study involved three main ethnic groups in Malaysia. Diabetic patients receiving treatment at the University Malaya Medical Centre (UMMC), Kuala Lumpur were recruited. Normal subjects without diabetes (the control group) in the Klang Valley were also recruited. The study was approved by the Medical Ethics Committee of University Malaya Medical Centre. Written informed consent was obtained from each subject. Patients with acute or chronic infections, severe medical conditions (malignancy, renal failure, liver cirrhosis, connective tissue disease, and chronic congestive heart failure) and pregnant women were excluded from the study. Fasting venous

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blood (6ml) was collected from each subject. The collected blood was immediately taken into three labelled vacutainers: sodium fluoride (for glucose concentrations), plain (for fasting insulin and lipid profile) and EDTA (for genetic analysis)

Biochemical Analysis

Serum triglyceride (TG), HDL-c and plasma glucose were measured by an automated analyzer integrated chemistry system (Siemens Healthcare Diagnostics Inc. Deerfield, USA) by Division of Laboratory Medicine (LMC) of the UMMC, Kuala Lumpur.

Genetic Analysis

Leukocyte genomic DNA was extracted from whole EDTA blood using a genomic DNA purification kit (Promega Corporation, Madison, WI, USA) according to the manufacturer's instructions. Allele specific PCR was used to detect the genotypes of PAI-1 4G/5G. Two specific forward allele primers (5G, 5'-GAGTCTGGACACGTGGGGG-3' in which at 3 end is G and 4G, 5'-GAGTCTGGACACGTGGGGA-3 at 3 end the 5th G was replaced by A) were designed to detect the PAI-1 4G/5G SNP. An internal control was included with each PCR reaction to ensure that there was PCR amplification in each reaction. Therefore, common forward (upstream of allele specific primers 5'- TGGTCCCGTTCAGCCACCA-3') and reverse primers (downstream of allele specific primers 5'- ATGCAGCCAGCCACGTGAT-3') were used as PCR amplification controls with each PCR reaction. The reverse primer was also a reverse primer for the two allele specific primers. I/D polymorphism resulting from the presence or absence of Alu repeats in intron 8 of the tPA gene was assessed by PCR using the following primers (9). Sense primer: 5'-GTGAAAAGCAAGGTCTACCAG-3' and non-sense primer: 5'-GACACCGAGTTCATCTTGAC-3'. The procedures of PCR amplification and genotyping of the SNPs were as previously described (6). To validate results, samples of PCR products (64 of PAI-1 and 46 of tPA) were sent to Bioneer Corporation (Daejeon South Korea) for sequencing. The sequencing results were in agreement with the PCR results.

Statistical Analysis

Calculations to determine whether observed genotype frequencies were consistent with Hardy-Weinberg equilibrium (HW) was done by the method of Court (2005, 2008). All other statistical analyses were done using Social Package of Statistical Science (SPSS) 11.5 (LEAD Technologies; Inc. USA). The missing data were listwise deleted - when any of the variables were missing, the entire observation was omitted from the analysis. The associations of the PAI-1 and tPA polymorphisms with T2DM in the recessive model, dominant model or additive model among Malay, Chinese and Indian subjects were evaluated by hierarchical logistic regression controlled for age, gender, history of diabetes, BMI and HDL-c as covariates. The difference between means was considered significant when p-values were less than 0.05.

III. RESULTS

In this study 231 normal (non-diabetic) subjects were recruited for the study (114 Malay, 71 Chinese and 46 Indian races). Three hundred and three subjects who were diagnosed with T2DM at UMMC participated in this study (149 Malay, 51 Chinese and 103 Indian races). The demographic and biochemical parameters of the subjects are shown in Table 1.

Table 1 Demographic and biochemical parameters among normal and diabetics in three main Malaysian ethnic groups

Parameters	Non-diabetics			Type 2 diabetes mellitus		
	Malay (n=114)	Chinese (n=71)	Indian (n=46)	Malay (n=149)	Chinese (n=51)	Indian (n=103
Age (years)	46.0 ± 12.6	56.4 ± 7.86	54.4 ± 8.37	50.9 ± 7.58	52.2 ± 8.25	52.3 ± 6.97
Body Mass Index (kg/m^2)	25.8 ± 4.86	23.3 ± 3.89	25.2 ± 3.93	29.1 ± 5.07	26.5 ± 4.95	28.4 ± 4.60
Waist Circumference (cm)	86.7 ± 13.9	82.4 ± 10.7	92.1 ± 9.52	95.5 ± 10.6	89.8 ± 12.6	97.0 ± 10.6
Diastolic Blood Pressure (mmHg)	83.0 ± 10.4	82.5 ± 9.76	83.1 ± 8.69	83.9 ± 10.0	81.4 ± 11.2	81.7 ± 9.80
Systolic Blood Pressure (mmHg)	134 ± 19.2	138 ± 18.4	135 ± 16.1	139 ± 18.7	133 ± 18.4	132 ± 17.2
Fasting Blood Sugar (mmol/l)	1.40 ± 0.76	1.32 ± 0.62	1.43 ± 0.65	2.04 ± 1.38	1.80 ± 1.15	1.65 ± 1.107

Triglycerides(mmol/l)	1.45 ± 0.39	1.52 ± 0.35	1.28 ± 0.28	1.18 ± 0.27	1.31 ± 0.31	1.13 ±0.26
HDL-cholesterol (mmol/l)	1.42 ± 0.16	1.50 ± 0.14	1.26 ± 0.12	1.160 ± 0.13	1.29 ± 0.14	1.1 ± 0.13
Plasminogen Activator Inhibitor1 activity (IU/ml)	18.63 ± 0.94	16.86 ± 1.00	19.18 ± 1.18	19.54 ± 1.33	20.39 ± 1.38	22.54 ± 1.17
Plasminogen Activator Inhibitor1 antigen (ng/ml)	32.85 ± 0.65	28.94 ± 0.18	30.59 ± 0.59	23.78 ± 0.77	26.05 ± 0.76	24.53 ± 0.80
Tissue Plasminogen Activator antigen (ng/ml)	4.35 ± 0.94	3.91 ± 0.79	4.51 ± 0.96	8.22 ± 0.72	8.28 ± 0.77	9.56 ± 0.68
Tissue Plasminogen Activator activity (U/ml)	2.62 ± 1.00	2.24 ± 0.82	2.55 ± 0.108	2.26 ± 0.64	1.92 ± 0.49	2.00 ± 0.46

Results were presented as mean ± standard deviation

The dominant and additive models of PAI-1 polymorphism showed risk for T2DM among Chinese subjects (OR=6.45, P=0.005; OR=2.26, P=0.02) respectively. However, among Malay and Indian subjects, this polymorphism was not a risk factor for T2DM (Table 2).

Table 2 Impact of ethnicity on the association of Plasminogen activator inhibitor-1 4G/5G polymorphism with type 2 diabetes mellitus

PAI-1 4G/5G polymorphisms	**Non-diabetics**			**Type 2 diabetes mellitus**		
	Malay (n=113)	**Chinese (n=71)**	**Indian (n=46)**	**Malay (n=149)**	**Chinese (n= 51)**	**Indian (n=103)**
Risk allele frequency (4G)	0.42	0.54	0.45	0.45	0.61	0.51
4G4G/4G5G/5G5G (frequency)	0.18/0.48/0.34	0.31/0.45/0.24	0.24/0.41/0.35	0.21/0.48/0.31	0.37/0.47/0.16	0.25/0.53/0.22
Recessive Model	Odds ratio (95% CI)			0.70(0.30-1.59)	2.11(0.76-5.85)	0.90(0.32-2.52)
	P-Value			0.40	0.15	0.84
Dominant Model	Odds ratio (95% CI)			1.21(0.58-2.49)	6.45(1.76-23.6)	1.45(0.55-3.80)
	P-Value			0.60	***0.005***	0.46
Additive Model	Odds ratio (95% CI)			0.97(0.58-1.53)	2.26(1.12-4.54)	1.11(0.60-2.05)
	P-Value			1.0	***0.02***	0.73

In the additive model, genotype of homozygote for the non-risk allele 5G/5G (0/0), heterozygote 4G/5G (1/0) and homozygote for the risk allele 4G/4G (1/1) were coded as 0, 1 and 2 respectively. The recessive model was defined as

4G/4G vs. (4G/5G+5G/5G), dominant model as (4G/4G+4G/5G) vs. 5G/5G and additive model as 4G/4G vs. 4G/5G vs. 5G/5G. The results presented as frequency and corresponding odds ratio, 95% confidence interval and P-value adjusted for age, gender, history of diabetes, BMI, HDL-c which were evaluated by hierarchical logistic regression.

Table 3 Impact of ethnicity on the association of tissue Plasminogen activator Alu repeat I/D polymorphism with type 2 diabetes mellitus

tPA Alu repeat I/D polymorphism	**Non-diabetics**			**Type 2 diabetes mellitus**		
	Malay (n=113)	**Chinese (n=71)**	**Indian (n=46)**	**Malay (n=149)**	**Chinese (n= 51)**	**Indian (n=103)**
Risk Allele frequency (Insertion)	0.47	0.51	0.55	0.46	0.51	0.57
DD/ID/II (frequency)	0.30/0.45/0.25	0.27/0.43/0.30	0.24/0.41/0.35	0.31/0.46/0.23	0.25/0.47/0.28	0.17/0.52/0.31
Recessive Model	Odds ratio (95% CI)			1.54(0.73-3.24)	0.94(0.32-2.72)	0.83(0.28-2.46)
	P-Value			0.26	0.91	0.73
Dominant Model	Odds ratio (95% CI)			1.25(0.56-2.74)	1.38(0.47-4.11)	3.27(1.17-9.45)
	P-Value			0.58	0.56	***0.026***
Additive Model	Odds ratio (95% CI)			1.32(0.82-2.12)	1.1(0.57-2.11)	1.32(0.71-2.45)
	P-Value			0.24	0.78	0.39

In the additive model, genotype of homozygote for the non-risk allele D/D (0/0), heterozygote I/D (1/0) and homozygote for the risk allele I/I (1/1) were coded as 0, 1 and 2 respectively. The recessive model was defined as I/I vs. (I/D + D/D), dominant model as (I/I + I/D) vs. D/D and additive model as D/D vs. I/D vs. I/I. The results presented as frequency and corresponding odds ratio, 95% confidence interval and P-value adjusted for age, gender, history of diabetes, BMI, HDL-c which were evaluated by hierarchical logistic regression. The dominant and additive models of PAI-1 polymorphism showed risk for T2DM among Chinese subjects (OR=6.45, P=0.005; OR=2.26, P=0.02) respectively. However, among Malay and Indian subjects, this polymorphism was not a risk factor for T2DM (Table 2).

In the dominant model, tPA polymorphism was associated with T2DM among Indian individuals (OR=3.27, P=0.02) but not associated with T2DM among Malay and Chinese subjects. The additive and recessive models of tPA polymorphism showed no association with T2DM among the three ethnic groups (Table 3).

IV. Discussion

In this study, the impact of ethnicity (Malay, Chinese and Indian) on the association of PAI-1 4G/5G and tPA Alu repeat I/D polymorphisms with T2DM was evaluated. Chinese ethnicity significantly influenced the association of PAI-1 4G/5G insertion/deletion polymorphism with T2DM while Indian ethnicity increased the risk of association of tPA I/D polymorphism with T2DM. Previous studies had shown significant association with T2DM among the Chinese [10], Tunisian [11] and Pima Indian subjects [12]. However, other studies on Caucasian [13-15], Japanese [16] and Framingham subjects [17] have reported that PAI-1 4G/5G polymorphism is not associated with T2DM. This variability of genetic association can be explained, in part, by differences in the ethnicity and environmental factors that can alter the phenotypic expression of the genes. Other studies had reported a similar association between the PAI-1 4G variant and increased risk of T2DM and metabolic syndrome (MetS) among Caucasian subjects [18,19]. Furthermore, these results are in concordance with our previous findings, the PAI-1 4G genotype was significantly associated with parameters of diabetes and MetS [6]. In that study we showed that, the risk of MetS was showed higher in 4G carriers who were associated with elevated plasma PAI-1 and reduced tPA activities [6]. Further, we demonstrated, there was a clear trend toward association of increasing PAI-1 activity with increased insulin resistance, BMI, waist circumference, and decreased HDL-c. This explained the association of PAI-1 activity with MetS [7], which may be reflected by the effect of 4G/5G polymorphism on PAI-1 activity. All of these findings indicate that the 4G variant of PAI-1 could increase the risk of T2DM and CVD by their influence on the risk factors mentioned above.

The regulation of PAI-1 gene expression has been shown to be stimulated by insulin and proinsulin in Hep G2 cells and in human hepatocytes [20], and also by hyperglycaemia in aortic endothelial cells [21]. These findings suggest that hyperglycaemia and hyperinsulinemia per se may directly stimulate PAI-1 production [22,23]. The PAI-1 4G/4G genotype is more prevalent in the patients with atherothrombosis. Numerous meta-analyses have been published about the contribution of the 4G/5G polymorphism to risk of cardiovascular, ischemic stroke and venous thromboembolic disease [2426]. Furthermore, Boekholdt et al. [27] showed a 20% increased risk of myocardial infarction (MI) that could be attributed to the PAI-1 4G/4G genotype. There is limited data on the association of tPA Alu-repeat I/D polymorphism with diseases, while there is no available data on its association with T2DM. However, its association with other diseases such as MI [28], multiple sclerosis [29] and periodontitis [30] has been described.

V. Conclusion

Ethnicity plays an important role on the association of T2DM with certain genetic polymorphisms in the fibrinolytic system. The PAI-1 4G/5G polymorphism is a strong risk factor for T2DM among Chinese, whereas the tPA Alu repeat I/D polymorphism is a risk factor for T2DM among Indian subjects.

Competing interests None declared.

VI. Acknowledgement

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VII. Authors' contributions

Zaid Al-Hamodi collected data, performed practical and statistical analysis and drafted the manuscript; Riyadh Saif-Ali performed statistical analysis, helped drafting and edited the manuscript; Ikram S. Ismail helped in data collection and editing the manuscript. Sekaran Muniandy guided, supervised and edited the manuscript.

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