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ORIGINAL ARTICLE

ความสัมพันธ์ของโพลิมอร์ฟิซึมของจีนแอลฟาไฟบริโนเจน (*FGA* -58G/A) กับกลุ่มอาการกล้ามเนื้อหัวใจขาดเลือดเฉียบพลันในผู้ป่วยเบาหวานชนิดที่ 2

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บทคัดย่อ

ไฟบริโนเจนเป็นหนึ่งในตัวบ่งชี้ทางกระบวนการอักเสบที่มีบทบาทสำคัญเกี่ยวกับพยาธิสรีรวิทยาของโรคหัวใจร่วมหลอดเลือด (cardiovascular disease; CVD) ปริมาณของไฟบริโนเจนที่เพิ่มขึ้นมีความสัมพันธ์กับการเกิดภาวะผนังหลอดเลือดแข็ง (atherosclerosis) และ CVD ซึ่งเป็นภาวะแทรกซ้อนของผู้ป่วยเบาหวานชนิดที่ 2 โดยโพลิมอร์ฟิซึมของจีนไฟบริโนเจนเป็นหนึ่งในปัจจัยสำคัญที่ทำให้มีการเพิ่มขึ้นของปริมาณไฟบริโนเจน ดังนั้นการศึกษานี้จึงได้ศึกษาความสัมพันธ์ระหว่างโพลิมอร์ฟิซึมของยีนแอลฟาไฟบริโนเจน (*FGA* -58G/A) กับการเกิดโรคหลอดเลือดหัวใจ (coronary artery disease; CAD) ในผู้ป่วยเบาหวานชนิดที่ 2 โดยศึกษาในกลุ่มผู้ป่วยเบาหวานที่ได้รับการวินิจฉัยว่ามีภาวะเจ็บเค้นอกคงที่ (stable CAD) หรือมีภาวะหัวใจขาดเลือดเฉียบพลัน (acute coronary syndrome; ACS) จำนวน 123 ราย และกลุ่มควบคุมที่ไม่เป็นเบาหวานและไม่มีการตีบของหลอดเลือดหัวใจหลักมากกว่าร้อยละ 50 จำนวน 86 ราย ตรวจวัดโพลิมอร์ฟิซึมของ *FGA* -58G/A ด้วยเทคนิค polymerase chain reaction-restriction fragment length polymorphism ผลการศึกษาพบว่าจีโนไทป์ AA และแอลลีล A ของ *FGA* -58G/A มีความสัมพันธ์อย่างอิสระกับเบาหวาน [adjusted OR (95% CI) = 3.3 (1.2, 8.9) และ 3.3 (1.6, 6.6) ตามลำดับ] นอกจากนี้ยังพบว่าจีโนไทป์ AA และแอลลีล A ยังมีความสัมพันธ์กับการเกิด ACS ในผู้ป่วยเบาหวาน [adjusted OR (95% CI) = 3.9 (1.3, 11.7) และ 2.0 (1.2, 3.5) ตามลำดับ] แต่ไม่พบความสัมพันธ์กับการเกิด stable CAD จากผลการศึกษานี้ อาจบ่งบอกว่าโพลิมอร์ฟิซึม *FGA* -58G/A มีความสัมพันธ์กับการลุกลามของ atherosclerosis ที่นำไปสู่การเกิด ACS ซึ่งเป็นกลุ่มอาการทางคลินิกชนิดรุนแรงของ CAD ในผู้ป่วยเบาหวานชนิดที่ 2

คำสำคัญ: ไฟบริโนเจน โพลิมอร์ฟิซึม เบาหวานชนิดที่ 2 กลุ่มอาการกล้ามเนื้อหัวใจขาดเลือดเฉียบพลัน

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ORIGINAL ARTICLE

Association of alpha fibrinogen -58G/A genetic polymorphism with acute coronary syndrome in type 2 diabetes mellitus

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Abstract

Fibrinogen is one of the inflammatory markers and plays a crucial role in pathophysiological process of cardiovascular diseases (CVD). High levels of fibrinogen are associated with atherosclerosis progression and CVD complication in type 2 diabetes mellitus (DM). Furthermore, fibrinogen genetic polymorphisms are one of the important factors affecting their levels. Therefore, this study aimed to evaluate the associations of *FGA* -58G/A polymorphism with clinical manifestations of coronary artery disease (CAD) in type 2 DM. A case-control study included 123 documented DM patients presenting with either acute coronary syndrome (ACS) or stable CAD and 86 control individuals without DM and presenting none or less than 50% stenosis of coronary artery. All subjects were genotyped for the *FGA* -58G/A polymorphism by using polymerase chain reaction-restriction fragment length polymorphism technique. The results showed that AA genotype and A allele of the *FGA* -58G/A polymorphism were independently associated with DM [adjusted OR (95% CI) = 3.3 (1.2, 8.9) and 3.3 (1.6, 6.6), respectively]. Moreover, the AA genotype and A allele were also significantly associated with ACS in diabetic patients [adjusted OR (95% CI) = 3.9 (1.3, 11.7) and 2.0 (1.2, 3.5), respectively], while the association with stable CAD was not observed. In conclusion, the results of this study may indicate the association of the *FGA* -58G/A polymorphism with the atherosclerotic progression which may in turn leads to the severe clinical manifestation of CAD in DM.

Keywords: Fibrinogen, Polymorphism, Diabetes mellitus, Acute coronary syndrome

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Introduction

Diabetes mellitus (DM) is a major risk factor for coronary artery disease (CAD). It has been known that cardiovascular disease (CVD) is the most important complication of type 2 diabetic patients. A number of studies showed that the diabetic patients had a 2-4 fold increased risk of developing CAD. Moreover, the incidence of severe clinical CVD complication in DM is higher than those in non-DM [1]. However, there is no selective agent responsible for the atherosclerotic process in diabetic patients due to its several influences. Assessment of some biomarkers and conventional risk factors has been ineffective in completely predicting the development of the atherosclerotic process suggesting that specific genetic factor should be taken into account.

Recently, many studies have suggested that endothelial dysfunction occurs in response to cardiovascular risk factors resulting in the development of atherosclerosis [2]. One of the most important causes of endothelial dysfunction is inflammation. Inflammation is implicated in the pathogenesis of type 2 diabetes and in the development of atherosclerotic plaque and their destabilization[3,4]. Inflammatory processes involve altering of endothelial and smooth muscle cells, leukocytes recruitment, as well as complement activation[5]. Fibrinogen is one of an acute phase reactant proteins which is elevated in response to inflammatory conditions. It plays a crucial role in the early stages of plaque formation and late complications of CVD[6]. In addition, fibrinogen strongly affects hemostasis, blood rheology, and platelet aggregation. Increased levels of fibrinogen have been reported to be associated with enhancing atherosclerosis, reducing blood flow, and predisposing to thrombosis [7]. Some reports demonstrated that the elevated plasma fibrinogen concentration is not only responsible for CVD but also increase in other cardiovascular risk factors such as metabolic syndrome, hypertension, obesity and diabetes mellitus [8, 9]. Thus, high plasma fibrinogen levels could contribute to the excess cardiovascular morbidity and mortality in these conditions.

Previous study suggested that approximately 50% of the total variability in fibrinogen levels is determined by the genes encoding the three fibrinogen chains; alpha, beta, and gamma (*FGA*, *FGB*, and *FGG* genes, respectively) [10]. *FGA* -58G/A (rs2070011) is a single nucleotide polymorphism (SNP) in a promoter of the *FGA* gene which has been reported to affect the levels of fibrinogen and associate with DM[11]. However, there is no study investigating the possible relationship of the polymorphism with DM and the progression of atherosclerotic plaque in coronary artery of the patients with macrovascular complication. Therefore, we decided to investigate whether the *FGA* -58G/A polymorphism influence the progression of CAD complication in type 2 DM.

Materials and methods

Study subjects

A total of 207 participants who attended to the Cardiac Catheterization Unit, Queen Sirikit Heart Center of the Northeast Hospital, Khon Kaen University, were recruited in this study. Patients with cancer, autoimmune disease, infectious diseases, *renal failure,* and immune-compromised individuals were excluded. Type 2 DM was diagnosed according to the criteria of the World Health Organization[12]. According to the coronary

angiographic results, the DM patients (n = 123) presented with either acute coronary syndrome (ACS) (n = 56) or stable CAD (n = 67) which defined as more than 50% stenosis in at least one of the three main coronary vessels. ACS was defined as unstable angina, non-ST-elevation myocardial infarction (NSTEMI) or ST-elevation myocardial infarction (STEMI) corresponding to European Society of Cardiology (ESC), American College of Cardiology Foundation (ACCF) and American Heart Association (AHA) [13]. The individuals without DM and presenting none or less than 50% stenosis of the coronary artery were classified as a control group (n = 84). Other clinical variables including age, sex, CAD risk factors, and use of medicines were obtained from medical records. Individuals presenting at least one of the following parameters including total cholesterol (TC) ≥ 240 mg/dL, triglyceride ≥ 200 mg/dL, high density lipoprotein-cholesterol (HDL-C) < 40 mg/dL, and low density lipoprotein-cholesterol (LDL-C) ≥ 160 mg/dL or use of lipid-lowering drugs were diagnosed as dyslipidemia [14]. Individuals whom had blood pressure ≥ 140/90 mmHg and/or used anti-hypertensive drugs were defined as hypertension [15]. Participants with body mass index (BMI) ≥ 25 kg/m^2 were classified as obesity [16]. Metabolic syndrome was considered if any three in five risk factors were presented: (i) waist circumference ≥ 90 cm in male and ≥ 80 cm in female, (ii) TG ≥ 150 mg/dL or on lipid-lowering drug treatment, (iii) HDL-C < 40 mg/dL in male and < 50 mg/dL in female, (iv) blood pressure ≥ 130/85 mmHg or use of anti-hypertensive medications, and (v) fasting blood glucose (FBG) ≥ 100 mg/dL or use of glucose-lowering drugs[17]. The study protocol was approved by the Khon Kaen University Ethics Committee for Human Research (HE621240) and consent forms were obtained from all participants.

Genotyping

Genomic DNA was extracted from peripheral white blood cells using a Flexi Gene DNA extraction kit (QIAGEN, Hilden, Germany). Genotyping of *FGA* -58G/A polymorphism was determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genomic DNA was amplified by PCR using specific primers including forward primer (5'GAG GGT TGA CTG TCT ACA CA 3') and reverse primer (5'CAG GCC TGG GGT CAT AAA 3'). The optimal condition for the PCR reaction was an annealing temperature of 58 °C. The PCR product was further digested with 2 units of *Aci*I restriction enzyme (New England Biolabs Inc., MA, USA). Each genotype of the polymorphism was interpreted with different sizes of amplicons as follows: GG homozygote demonstrated 236 and 83 base pair (bp) bands; GA heterozygote demonstrated 318, 236 and 83 bp bands; and AA homozygote demonstrated 318 bp band.

Statistical analysis

Statistical analysis was performed using SPSS software version 17.0 (SPSS Inc, IL, USA). Distributions of genotype and allele frequencies, categorical variables and the Hardy-Weinberg equilibrium were determined using a Chi-square test. Kolmogorov-Smirnov test was used to assess the data whether is normal distribution. Continuous variables were expressed as means ± standard deviations (SDs) and categorical variables were reported as number and percentages. Continuous

variables without normal distribution were reported as a geometric means ± SDs. Logistic regression analysis was performed to evaluate the association between the *FGA* -58G/A polymorphism with DM and severity of CAD complication in DM patients. Statistical significance was defined as a *p*-value less than 0.05.

Results

Clinical and demographic characteristics of the study individuals are shown in **Table 1**. Individuals in DM group presented higher proportions of hypertension, metabolic syndrome, as well as levels of FBG and LDL-C as compared to controls. No significant differences between both groups were observed for SBP, DBP, BMI, TC, TG, and HDL-C.

The allele and genotype frequencies of the *FGA* -58G/A polymorphism in DM patients and controls are presented in **Table 2**. The SNP was in agreement with Hardy–Weinberg equilibrium in each group. The significant differences were observed in genotype (p = 0.022) and allele (p = 0.015) frequencies between DM patients and controls. To evaluate the association of genetic variations with DM, multivariate logistic regression analysis was performed. The results demonstrated that presences of AA genotype and A allele of the *FGA* -58G/A polymorphism were independently associated with DM after adjustment for sex, age, hypertension, and metabolic syndrome **(Table 2)**.

Table 1 Demographic data of the study subjects

Variables	Controls (n=84)	DM (n=123)	*p*-value
Age (years)*	59.6±8.8	61.5±9.0	0.136
Gender			
Male, n (%)	38 (45.2)	69 (56.1)	0.125
Female, n (%)	46 (54.8)	54 (43.9)	
DS, n (%)	69 (82.1)	108 (87.8)	0.256
HT, n (%)	59 (70.2)	101 (82.1)	0.045
MET, n (%)	38 (45.2)	113 (91.9)	<0.001
Obesity, n (%)	42 (50.0)	57 (46.3)	0.605
SBP (mmHg)	127.9±18.1	135.3±22.0	0.399
DBP (mmHg)	73.1±9.5	74.7±11.3	0.115
BMI (kg/m^2)	25.3±3.7	24.8±3.6	0.316
FBG (mg/dL)*	92.5±12.3	155.2±66.2	0.002
TC (mg/dL)*	166.0±41.9	173.7±50.2	0.537
TG (mg/dL)*	133.5±82.8	154.2±113.6	0.071
LDL-C (mg/dL)*	87.4±35.4	103.4±41.4	0.010
HDL-C (mg/dL)*	43.5±14.3	38.7±10.4	0.244

Independent sample t-test and Chi-square test were used to compare continuous values and categorical variables between both groups, respectively. Category data are expressed as n (%), continuous data are expressed as mean ± SD. * Values are presented as geometric means ± SD. DM, diabetes mellitus; DS, dyslipidemia; HT, hypertension; MET, metabolic syndrome; SBP, systolic blood pressure; DBS, diastolic blood pressure; BMI, body mass index; FBG, fasting blood glucose; TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 2 Genotype distributions of the *FGA* -58G/A polymorphism in study subjects and its association with DM

Genotype/ allele	Frequency, n (%)		*p*-value	OR (95% CI)			
	Controls (n=84)	DM (n=123)		Crude OR	*p*-value	Adjusted OR*	*p*-value
GG	26 (31.0)	29 (23.6)	0.022	1.0	-	1.0	-
GA	45 (53.6)	54 (43.9)		1.1 (0.6,2.1)	0.828	1.3 (0.6,2.9)	0.573
AA	13 (15.5)	40 (32.5)		2.8 (1.2,6.3)	0.015	3.3 (1.2,8.9)	0.021
G allele	97 (57.7)	112 (45.5)	0.015	1.0	-	1.0	-
A allele	71 (42.3)	134 (54.5)		2.8 (1.5,4.9)	0.001	3.3 (1.6,6.6)	0.001

Chi-square-test was used to compare the frequencies of genotypes between DM and control groups. *Conditional logistic regression model adjustment for sex, age, hypertension and metabolic syndrome. DM, Diabetes mellitus; OR, Odds ratio; CI=Confidence interval

To assess the influence of the *FGA* -58G/A polymorphism on the severity of CAD complication, DM individuals were divided into two subgroups including DM with stable-CAD and with ACS. Significant differences were found in the frequencies of AA genotype (p = 0.003) and A allele (p = 0.004) in DM with ACS when compared to controls. However, the significant differences were not found in patients with stable-CAD when compared to controls **(Table 3)**. Multivariate logistic regression analysis was performed to investigate the relationship between the *FGA* -58G/A polymorphism and ACS in diabetic individuals **(Table 4)**. The results demonstrated that after adjustment for sex, age, hypertension, and metabolic syndrome, the AA genotype and A allele were significantly associated with an increased risk of ACS.

Table 3 Genotype distributions of the *FGA* -58G/A polymorphism in DM with CAD according to clinical manifestation

Genotype/ allele	(1) Controls (n=84)	Frequency, n (%)		*p*-value	
		DM with CAD manifestation			
		(2) stable CAD (n=67)	(3) ACS (n=56)	(2) vs. (1)	(3) vs. (1)
GG	26 (31.0)	17 (25.4)	12 (21.4)	0.304	0.003
GA	45 (53.6)	33 (49.3)	21 (37.5)		
AA	13 (15.5)	17 (25.4)	23 (41.1)		
G allele	97 (57.7)	67 (50.0)	45 (40.2)	0.180	0.004
A allele	71 (42.3)	67 (50.0)	67 (59.8)		

Chi-square test was performed for comparison of the frequencies of each genotype among the study groups. DM, Diabetes mellitus; CAD, Coronary artery disease; ACS, Acute coronary syndrome

Table 4 Association of the *FGA* -58G/A polymorphism with ACS in DM

Genotype/ allele	OR (95% CI)			
	Crude OR	*p*-value	Adjusted OR*	*p*-value
GG	1.0	-	1.0	-
GA	1.0 (0.4, 2.4)	0.980	1.0 (0.4, 2.8)	1.000
AA	3.8 (1.5, 10.1)	0.006	3.9 (1.3, 11.7)	0.014
G allele	1.0	-	1.0	-
A allele	2.0 (1.3, 3.3)	0.004	2.0 (1.2, 3.5)	0.014

*Conditional logistic regression model adjustment for sex, age, hypertension and metabolic syndrome. DM, diabetes mellitus; ACS, acute coronary syndrome; OR, Odds ratio; CI, Confidence interval

Discussion

Fibrinogen is a complex protein composed of three pairs of subunits (FGA, FGB, and FGG). In addition to its physiological role as a cofactor for platelet aggregation and a precursor of fibrin, fibrinogen is involved in many pathophysiological processes such as inflammation, atherosclerosis, and thrombosis[18]. Previous study showed that fibrinogen is a potential biomarker for prediction of future risk of CVD[19]. A high fibrinogen concentration has been reported to enhance the risk of CVD in diabetic individuals[20]. In addition, several studies also have provided evidences for the relation between plasma fibrinogen levels and DM. Actually, the elevated level of fibrinogen was observed in type 2 DM patients, and predicted the progression of CVD in diabetes. Several polymorphisms inducing overproduction of the three fibrinogen subunits might influence the high levels of plasma fibrinogen, which in turn develop type 2 DM and CAD. Therefore, the genetic variations in these genes may define susceptibility to the disease. A previous study demonstrated that the nucleotide base substitutions of the *FGA* -58G/A polymorphism in the 5' UTR (promoter) of the *FGA* gene can modulate the *FGA* gene

expression through accelerating the mRNA transcription, which may affect increasing mRNA levels and resulting in the high level of fibrinogen[21].

At present investigation demonstrated the association of the *FGA* -58G/A polymorphism with the plaque progression leading to ACS in type 2 diabetic patients. To the best of our knowledge, this study is the first report of the effect of the *FGA* -58G/A polymorphism on the severity of CAD complication in diabetes. We have observed that AA genotype and minor A allele of the *FGA* -58G/A polymorphism was associated with an increased risk of DM in this population. This result supported the previous study reported by Hwang *et al*.[11] which suggested that major G allele of this polymorphism had a protective effect on DM. Our study also found that diabetic individuals carrying the minor A allele of the polymorphism were independently associated with ACS. These results suggested that the *FGA* -58G/A polymorphism may affect the occurrence and severe progression of atherosclerosis in type 2 DM. However, the mechanism that links genetic variations of the *FGA* -58G/A polymorphism to risk of DM and severe CAD complication in diabetic individuals are remained unclear. To date, genome-wide association studies (GWAS) have identified a large number of robust associations between genetic variations and type 2 DM. Ban HJ and colleagues [22] found that the *FGA* -58G/A polymorphism combined with the rs9658173 of peroxisome proliferator-activated receptor PPAR-δ (PPARD) gene was associated with contributing risk of type 2 DM, through elevating the levels of FBG [23]. Moreover, the polymorphism was significantly related to the increased plasma levels of TG and TC [11]. Therefore, this polymorphism may have a possible role in the developing of DM via modulating plasma concentrations of lipids and glucose.

Mannila *et al*. [24] demonstrated that the *FGA* -58G/A polymorphism appeared to influence the relation between plasma fibrinogen concentration and fibrin clot porosity. They found that the presence of homozygous for the A allele leads to increase fibrinogen concentration and decrease fibrin clot porosity. In addition, prior study has shown that *FGA* -58G/A polymorphism was in a strong linkage disequilibrium with *FGA* Thr312Ala polymorphism (rs6050)[11]. As Thr312Ala polymorphism influences clot stability through increasing factor XIII cross-linking[25] leading to thicker fibrin fibers which resist to lysis [26]. This might be an evidence to explain a possible effect of the *FGA* -58G/A polymorphism on an increased ACS risk in patients with DM. However, it should be noted that the sample size was not large enough which might have reduced the statistical power of tests. Thus, further investigation with a larger sample size is needed to elucidate the effect of *FGA* -58G/A polymorphism on ACS in type 2 DM.

Conclusions

The present study has demonstrated the association of the *FGA* -58G/A polymorphism with ACS in type 2 DM. This relationship suggested the importance of genetic variations of the *FGA* -58G/A which may eventually be used as the ACS risk assessment in diabetic individuals.

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