

# A transition of C to G (rs1800796) at promoter region of IL6 gene is associated with lowered risk of elevated blood glucose in rural Thais

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## Research article

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# Abstract

Background Inflammation has been associated with increased risk of type 2 diabetes mellitus (T2DM). Thus, polymorphisms of genes encoding pro-inflammatory cytokines may influence the condition. This study aimed to determine the association between genetic variations in inflammation-related genes, including rs2794521 of the CRP gene, rs1800796 of the IL6 gene, and rs1799964 of the TNF gene, and risk of elevated blood glucose.

Methods A total of 296 subjects were allocated to either the control group (n=135) or elevated blood glucose group (n=161). The studied single nucleotide polymorphisms were investigated among 296 subjects by real-time PCR technique. Logistic regression model was used to evaluate the association between the genetic variations and risk of elevated blood glucose.

Results Both dietary intake and concentrations of inflammatory cytokines (CRP, IL6, and TNF- $\alpha$ ) in the subjects with different genotypes were analogous. AG carriers of rs2794521 CRP showed the highest levels of obesity indices (BMI, waist circumference, and body fat;  $p < 0.05$  for all) compared to wild type. Homozygous variants of rs1800796 (GG) and rs1799964 (CC) were associated with significantly lower HbA1c level ( $p = 0.041$ ) and fasting blood glucose concentration ( $p = 0.043$ ), respectively. An association with decreased risk of elevated blood glucose was found among GG female carriers of rs1800796 [OR 0.23 (0.06-0.87),  $p = 0.031$ ]. A tendency towards lowered blood glucose was also seen in AGT carriers [OR 0.49 (0.024-1.02),  $p = 0.058$ ].

Conclusions The polymorphisms rs2794521 CRP, rs1800796 IL6, and rs1799964 TNF were associated with risk of T2DM. Individuals who carried GG of rs1800796 or -717A-572G-1031T displayed a lower risk of T2DM.

## Background

The prevalence of type 2 diabetes mellitus (T2DM) has continually increased around the world. In 2017, there were approximately 425 million diabetic patients, which is expected to rise to 627 million in 2045 [1]. T2DM has been reported to be associated with inflammation [2, 3], and obesity is hypothesized to trigger this inflammation. The expansion of adipose tissue eventually activates chronic low-grade inflammation [3–4]. This results in increased secretion of an acute phase reactant protein, namely C-reactive protein (CRP) [5–6], as well as inflammatory cytokines such as interleukin 6 (IL6) [6–7] and tumor necrosis factor alpha (TNF- $\alpha$ ) [8], which are known to affect glucose homeostasis. Since the expression of cytokines and the degree of inflammation can be modulated by genetic polymorphisms, it is thought that the polymorphisms of genes encoding for these cytokines may also influence the development of T2DM.

Polymorphisms located in promoter regions, including rs2794521 (–717 A/G) of CRP gene, –572 C/G (rs1800796) of IL6 gene and –1031 T/C (rs1799964) of TNF gene, have been associated with T2DM risk [7, 9–13]. Several studies demonstrated a notable influence of the rs2794521 polymorphism on CRP

secretion [14–16]. Alteration of CRP concentration may modify T2DM risk [5–6, 9]. Similarly, the rs1800796 IL6 polymorphism was postulated to affect the expression of interleukin 6 [11, 17] which can initiate insulin resistance and eventually T2DM [3]. In addition, previous studies have produced related evidence [7, 11, 18]. Consistent with rs2794521 and rs1800796, the exchange of T to C allele at –1031 locus in the TNF gene was associated with T2DM [12–13]. Nevertheless, study outcomes regarding the relationship between selected genetic polymorphisms and T2DM risk are in disagreement. Additionally, to the authors' knowledge, evidence of the effect of a combination of these polymorphisms in enhancing T2DM is inconclusive. Therefore, this study aimed to investigate whether specific polymorphisms, namely rs2794521, rs1800796 and rs1799964, increase the risk of elevated blood glucose among Thais, and whether the haplotypes of the studied polymorphisms relate to the condition.

## Methods

### Subjects

Thais who were aged between 35 and 66 and living in Sun Noen district, Nakhon Ratchasima province, Thailand, were randomly recruited into the study. All subjects had been previously screened for T2DM at health promoting hospitals. Pregnant and lactating women and people who had been medically diagnosed with chronic diseases or had any type of infection were omitted. After written informed consent had been obtained, anthropometric data (weight, height, body mass index, waist circumference, percentage of body fat, percentage of visceral fat, and muscle mass), biochemical data (fasting blood glucose, 2-hour blood glucose, HbA1c, total-cholesterol, triglyceride, HDL-C, LDL-C, C-reactive protein, interleukin 6, and tumor necrosis factor- $\alpha$ ) and dietary data were determined and presented in a previous publication [6]. Leftover blood samples were obtained from this previous study, and used meet the aim of the current study. The current study procedure was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TMEC 19–050). For the purposes of the current study, the samples from subjects with normal blood glucose concentrations (fasting blood glucose <100 mg/dl, 2-hour blood glucose <140 mg/dl, and HbA1c <5.7%) were allocated to the control group (n = 135 [45 males and 90 females]) and samples from subjects who had elevated blood glucose concentrations (fasting blood glucose  $\geq$ 100 mg/dl, 2-hour blood glucose  $\geq$ 140 mg/dl, and HbA1c  $\geq$ 5.7%) were assigned to the elevated blood glucose (EBG) group (n = 161 subjects [55 males and 106 females]).

### Genetic variations analysis

All leftover blood samples were stored at –80 °C in a laboratory of the department of Tropical Nutrition and Food Science, Mahidol University prior to analysis. Genomic DNA extraction was performed following the instructions of the FlexiGene DNA kit (Qiagen, Germany) using whole blood samples. The extracts were quantified and qualified by a Nanodrop ND–1000 spectrophotometer. The selected genetic polymorphisms, which included –717 A/G (rs2794521) of the CRP gene, –572 C/G (rs1800796) of the IL6 gene and –1031 T/C (rs1799964) of the TNF gene, were analyzed using the LightCycler FastStart

DNA Master HybProbe kit (Roche Diagnostics GmbH, Germany) based on the real-time polymerase chain reaction (q-PCR) method.

## Statistical analysis

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS), version 18. The distribution of data was examined using the Kolmogorov-Smirnov test. The Mann-Whitney U test was used to determine differences between the control group and EBG group, while the differences between genotypic groups were assessed using the Kruskal-Wallis test. The distribution of genetic variations was tested by the Hardy-Weinberg equilibrium equation. Odds ratio (ORs) with 95% confidence interval (CI) were computed through binary logistic regression, to investigate the association between genetic variations and risk of elevated blood glucose. A  $p$ -value less than 0.05 was considered statistically significant.

## Results

Genetic polymorphisms including –717 A/G (rs2794521) of the CRP gene, –572 C/G (rs1800796) of the IL6 gene, and –1031 T/C (rs1799964) of the TNF gene were examined in 296 subjects. The frequencies of the selected single nucleotide polymorphisms are presented in Table 1. The studied genetic variations were compatible with Hardy-Weinberg equilibrium ( $p > 0.05$  for all). Most of the subjects (N = 296) had wild type genotypes, AA in CRP gene (n = 154, 52%), CC in IL6 gene (n = 163, 55%) and TT in TNF gene (n = 137, 46%). Genetic distribution patterns were similar in the control group and the EBG group.

Table 2 compares the characteristics of subjects with different genotypic variants. Subjects with the heterozygous variant of the CRP gene (AG) had significantly higher BMI, WC and body fat than those with the wild type (AA) or homozygous variant (GG),  $p < 0.025$  for all. There was no significant difference in blood glucose indices or lipid profiles. Regarding rs1800796, a difference was only found in HbA1c. CC carriers had higher HbA1c levels compared to GG carriers,  $p < 0.025$ . A noteworthy difference in a blood glucose index, FBG, was also present when analyzed within the TNF variant (rs1799964) groups. Subjects with the homozygous variant (CC) had lower concentrations of FBG compared to wild types (TT),  $p < 0.025$ . Moreover, a significant difference was observed in the lipid index, triglyceride. Subjects with the heterozygous variant (TC) had greater triglyceride concentrations compared to wild types (TT),  $p < 0.025$ . When investigating concentrations of inflammatory cytokines (CRP, IL6, and TNF- $\alpha$ ), no notable differences were detected in subjects,  $p > 0.05$  for all. Dietary intake was similar among subjects with different genetic variants,  $p > 0.05$  for all.

The associations between the selected genetic variants and risk of elevated blood glucose among the subjects (N = 296) and within each sex (females: n = 196; males: n = 90) are shown in Table 3. Associations were determined in three genetic models including codominant, dominant, and recessive models. There was no significant association between rs2794521 or rs1799964 versus risk of elevated blood glucose. On the contrary, regarding rs1800796 in the IL6 gene, the codominant model (C/C vs. C/G

vs. G/G) and the recessive model (C/C+C/G vs. G/G) revealed noteworthy associations in females. Those with a GG genotype were less likely to have an increased risk of elevated blood glucose [codominant model: OR 0.23 (0.06–0.88),  $p = 0.032$ ; recessive model: OR 0.23 (0.06–0.87),  $p = 0.031$ ] compared to those with other genotypes.

The relationship with risk of elevated blood glucose was also investigated within different haploid genotypes as displayed in Table 4. The most frequent pattern found in the study was ACT (37%), which was subsequently used as a reference haplotype to analyze the association. As a result, only the AGT pattern tended to have a protective effect on risk of elevated blood glucose [OR 0.49 (0.24–1.02),  $p = 0.058$ ].

## Discussion

Genetic variants of inflammation-related genes are believed to play roles in the development of elevated blood glucose, due to their ability to modulate concentrations of inflammatory cytokines [9, 11–12] which may consequently affect the glucose pathway and eventually result in T2DM [2–3]. In this study, we investigated whether polymorphisms rs2794521 of the CRP gene, rs1800796 of the IL6 gene and rs1799964 of the TNF gene are related to elevated blood glucose among the Thai population. Regarding the results, the distribution of each variant was consistent with previous studies [9, 12, 15, 19–20]. The wild type of each of the selected gene polymorphisms was most frequent, followed by the heterozygous variant, and finally the homozygous variant.

Subjects with different genotypes of the selected gene polymorphisms were likely to have comparable consumption patterns, as there was no difference in dietary intake. Nevertheless, there was remarkable dissimilarity in body fat and blood glucose indices. The body mass index, waist circumference and body fat percentage of subjects with an AG genotype of the rs2794521 CRP polymorphism were notably greater than the others. Similarly, Martínez et al. revealed a significant association between the increase in BMI and rs2794521 at the haplotype level (rs1130864T-rs1205G-rs2794521G-rs3093062G) [9]. Thus, this genetic variant possibly has a substantial influence on obesity, which is an important risk factor for T2DM. However, in the present study, the association of rs2794521 with either CRP concentration or T2DM was vague. Likewise, Sheu et al. did not detect an association between an A to G transition at rs2794521 and either CRP or blood glucose concentrations in Taiwanese subjects [21]. The CRP concentration of subjects from India with different genotypes of rs2794521 also did not differ [15]. In contrast, a study conducted in Spain reported a notable relationship between rs2794521 and CRP concentration. Subjects with the major A allele had significantly higher CRP concentrations than those with the G allele [14]. In addition, there was a significant increase in T2DM prevalence reported in Polish subjects with the AG+GG genotype [22] and black American subjects had a higher tendency to develop the disease [10]. Accordingly, the controversial association may be a consequence of differences in ethnicity.

In relation to the rs1800796 IL6 polymorphism, HbA1c level of subjects with the homozygous variant (GG genotype) was significantly lower than in those with the wild type (CC genotype). With regards to inflammatory cytokines, the transition of C to G at rs1800796 of the IL6 gene was not associated with CRP, IL6 or TNF level in this study. Similarly, Karaman et al. also reported no significant difference between the rs1800796 IL6 polymorphism and either IL6 or CRP plasma levels in a Turkish population [23]. However, studies conducted in China revealed a relationship [7, 17, 24–25]. Lu et al. showed that rs1800796 GG was associated with elevated IL6 level and, additionally, might point towards a relatively high risk for diabetic patients suffering from proliferative diabetic retinopathy [7], while Fang et al. and Cheung et al. discovered notably lower IL6 levels in subjects with –572GG of CG compared to those with the wild type [24–25]. This study demonstrated a noteworthy association between the rs1800796 homozygous variant and T2DM risk when examined within the female subgroup. Among females, carriers of the GG genotype were less liable to develop T2DM compared to carriers of the CC genotype or CC+CG genotype. On the other hand, a prospective cohort study conducted in Taiwan suggested that the GG genotype of rs1800796 may confer a greater risk of developing diabetic nephropathy [20]. Comparably, a meta-analysis of 11,681 participants of case-control studies conducted from 9 Asian studies and one European study, noted that the G allele was associated with an increased risk of T2DM [18].

Consistent with the GG genotype of rs1800796, study subjects who carried the homozygous variant (CC genotype) of rs1799964 TNF polymorphism revealed a lower level of fasting blood glucose compared to TT carriers. Thus, people who carry the homozygous variants may be less likely to develop high blood glucose levels. Nevertheless, the relationship between rs1799964 and the studied inflammatory cytokines or T2DM was unclear in the present study. Likewise, Chan et al. determined the influence of rs1799964 genetic variants among postmenopausal women [26]. They found that the variants were not significantly associated with risk of T2DM. On the contrary, in a study of north Indian subjects, the C allele at the –1031 locus of the TNF gene was associated with diabetic nephropathy, a complication of T2DM [12]. Likewise, the study of Xu et al. also revealed the effect of –1031C–863C–857C of the TNF gene in elevating the risk of T2DM among Chinese subjects [13]. In addition, we evaluated the effect of the selected polymorphisms at the haplotype level. Subjects with AGT haplotype, a combination of rs2794521, rs1800796, and rs1799964, tended to exhibit a reduced risk of T2DM, compared to the reference haplotype ACT.

## Conclusion

In conclusion, the studied polymorphisms, rs2794521 and rs1799964, tended to be associated with obesity and blood glucose indicators, both of which are substantial risk factors for the development of T2DM. The G allele of rs1800796 IL6 polymorphism strongly promoted a relatively low risk for developing T2DM. Subjects with similar dietary intakes, who carried the GG genotype of rs1800796 or AGT haplotype, were less likely to have increased T2DM risk factors, and the association was clearly seen in the females. This study suggested that rs2794521, rs1799964 and rs1800796 are involved in T2DM development in the Thai population. Thus, these polymorphisms may be an effective prognostic gene

marker for the disease. As the association among males and the difference between males and females cannot be evaluated in this study, a larger study should be conducted to verify the results.

## Abbreviations

T2DM: Type 2 diabetes mellitus; PCR: Polymerase Chain Reaction; CRP: C-reactive protein; IL6: Interleukin 6; TNF- $\alpha$ : Tumor necrosis factor alpha; BMI: Body mass index; HbA1c: Glycated hemoglobin; HDL-c: High density lipoprotein cholesterol; LDL-c: Low density lipoprotein cholesterol; WC: Waist circumference; Systolic BP: systolic blood pressure; Diastolic BP: diastolic blood pressure; FBG: Fasting blood glucose; 2hBG: 2-hour blood glucose; CHO intake: Carbohydrate intake; TG: Triglyceride.

## Declarations

### Ethics approval and consent to participate

Consent was obtained from all participants. The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (TMEC 19–050).

### Consent for publication

Not applicable.

### Availability of data and material

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

CP, PP, KK designed the study. CP, PP, KK obtained the data. CP and KK analyzed and interpreted the data. PP, HH, KK provided advice. CP wrote the first draft. All authors read and approved the final manuscript.

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## Tables

Table 1 Distribution of genetic variations

	Total (N=296)		Control (n=135)		EBG (n=161)	
	N	Frequency	N	Frequency	N	Frequency
<b>-717 A/G (rs2794521) in CRP</b>						
AA	154	0.52	69	0.51	85	0.53
AG	126	0.43	59	0.44	67	0.42
GG	16	0.05	7	0.05	9	0.06
A	434	0.73	197	0.73	237	0.74
G	158	0.27	73	0.27	85	0.26
<i>P</i> for HWE		0.18		0.28		0.42
<b>-572 C/G (rs1800796) in IL6</b>						
C/C	163	0.55	71	0.53	92	0.57
C/G	118	0.40	54	0.40	64	0.40
G/G	15	0.05	10	0.07	5	0.03
C	444	0.75	196	0.73	248	0.77
G	148	0.25	74	0.27	74	0.23
<i>P</i> for HWE		0.35		1		0.18
<b>-1031T/C (rs1799964) in TNF</b>						
T/T	137	0.46	63	0.47	74	0.46
C/T	132	0.45	60	0.44	72	0.45
C/C	27	0.09	12	0.09	15	0.09
T	406	0.69	186	0.69	220	0.68
C	186	0.31	84	0.31	102	0.32
<i>P</i> for HWE		0.59		0.84		0.86

HWE: Hardy-Weinberg Equilibrium

Table 2 Characteristics of the subjects with different genotypic variants

	CRP -717 A/G (rs2794521)			
	AA	AG	GG	<i>P</i>
BMI (kg/m <sup>2</sup> )	24.9 (16.6, 37.0)	26.5 (17.3, 43.0)	24.3 (19.1, 43.0)	0.008 <sup>*,a</sup>
WC (cm)	83 (62, 113)	87 (63, 130)	81 (64, 120)	0.016 <sup>*,a</sup>
% Body fat	30.1 (11.0, 98.0)	34.0 (10.0, 44.0)	27.0 (11.0, 44.0)	0.002 <sup>*,a,c</sup>
% Muscle	25.4 (21.0, 93.0)	24.3 (20.0, 35.0)	28.4 (21.0, 33.0)	0.002 <sup>*,a,c</sup>
FBG (mg/dl)	93 (64, 261)	91 (72, 239)	94.5 (84, 145)	0.276
% HbA1c	5.3 (4.1, 12.4)	5.3 (3.3, 11.2)	5.5 (4.6, 6.0)	0.627
2hBG (mg/dl)	119 (41, 558)	122 (48, 599)	135 (71, 451)	0.677
CRP (mg/dl)	1.52 (0.01, 96)	1.86 (0.01, 115.86)	1.03 (0.14, 8.65)	0.188
IL6 (pg/ml)	10.68 (1.13, 3988.99)	12.73 (2.02, 3185.9)	132.71 (2.5, 3301.28)	0.120
TNF-α (pg/ml)	48.26 (4.11, 401.28)	55.23 (4.72, 293.09)	57.87 (27.56, 220.54)	0.511
Energy intake (kcal/d)	2216.5 (640.5, 4983.0)	2065.7 (551.4, 4917.8)	1992.7 (792.2, 2895.3)	0.300
Protein intake (g/d)	61.1 (10.3, 309.7)	58.2 (10.5, 292.8)	52.3 (15.1, 220.0)	0.310
Fat intake (g/d)	54.2 (6.6, 328.0)	45.0 (8.1, 250.2)	51.5 (22.1, 186.8)	0.195
CHO intake (g/d)	380.5 (46.0, 1138.8)	366.6 (77.3, 842.5)	299.2 (124.6, 1028.6)	0.360
-572C/G (rs1800796) in IL6 gene				
	CC	CG	GG	<i>P</i>
FBG (mg/dl)	92 (64, 261)	93 (72, 224)	90 (69, 128)	0.642
% HbA1c	5.3 (4.1, 12.4)	5.3 (3.3, 9.7)	5.1 (4.5, 5.8)	0.041 <sup>*,b</sup>
2hBG (mg/dl)	123 (41, 599)	118.5 (61, 472)	118 (85, 295)	0.840
CRP (mg/dl)	1.61 (0.03, 115.86)	1.65 (0.01, 96)	1.62 (0.1, 6.53)	0.935
IL6 (pg/ml)	10.91 (1.18, 3301.28)	13.19 (1.13, 3988.99)	5.43 (2.41, 1941.91)	0.095
TNF-α (pg/ml)	44.73 (4.11, 220.54)	57.81 (4.72, 401.28)	68.43 (5.4, 165.77)	0.108
Energy intake (kcal/d)	2045.8 (551.4, 4983.0)	2297.0 (713.8, 4975.4)	1951.4 (817.2, 4422.1)	0.256
Protein intake (g/d)	54.7 (10.5, 309.7)	62.8 (10.3, 301.7)	47.2 (20.9, 140.4)	0.282
Fat intake (g/d)	49.9 (6.6, 328.0)	52.6 (8.5, 295.9)	34.5 (12.8, 185.5)	0.350
CHO intake (g/d)	355.9 (46.0, 1138.8)	383.8 (77.5, 842.5)	391.2 (107.6, 1028.6)	0.592

	1138.8)	838.4)	716.7)	
	TNF -1031T/C (rs1799964)			
	TT	TC	CC	<i>P</i>
TG (mg/dl)	117 (34, 838)	141 (59, 601)	105 (63, 273)	0.028 <sup>*,a</sup>
FBG (mg/dl)	93 (70, 261)	93 (64, 239)	88 (69, 252)	0.043 <sup>*,b</sup>
% HbA1c	5.3 (3.3, 12.4)	5.3 (4.3, 11.2)	5.3 (4.6, 10.8)	0.411
2hBG (mg/dl)	121 (5, 558)	122 (41, 599)	119 (63, 486)	0.987
CRP (mg/dl)	1.63 (0.01, 94.82)	1.62 (0.03, 115.86)	1.56 (0.1, 96)	0.966
IL6 (pg/ml)	11.25 (1.13, 3185.9)	12.49 (1.18, 3988.99)	16.63 (1.3, 2481.51)	0.667
TNF- $\alpha$ (pg/ml)	54.8 (4.11, 293.09)	55.06 (4.72, 401.28)	36.46 (4.9, 166.75)	0.355
Energy intake (kcal/d)	2211.4 (698.4, 4975.4)	2047.6 (551.4, 4983.0)	2002.8 (817.2, 3502.8)	0.636
Protein intake (g/d)	61.6 (10.5, 309.7)	55.4 (10.3, 301.7)	60.4 (11.7, 176.8)	0.630
Fat intake (g/d)	50.3 (6.6, 255.8)	50.6 (6.9, 328.0)	45.2 (11.7, 160.6)	0.371
CHO intake (g/d)	371.9 (46.0, 1060.7)	361.5 (77.5, 1138.8)	335.7 (64.3, 797.8)	0.741

Data presented as median (minimum, maximum).

\**P* was calculated by Kruskal Wallis test. *P*<0.05 was considered statistically significant.

<sup>a</sup>, <sup>b</sup>, <sup>c</sup> *P* was calculated by Mann Whitney U test.

<sup>a</sup> *P*< 0.025 for wild type vs. hetero variant

<sup>b</sup> *P*<0.025 for wild type vs. homo variant

<sup>c</sup> *P*<0.025 for hetero variant vs. homo variant

Table 3 Association between the genetic polymorphisms and risk of T2DM

	Total		Female		Male	
	OR* (95%CI)	<i>P</i>	OR** (95%CI)	<i>P</i>	OR** (95%CI)	<i>P</i>
rs2794521 CRP						
A/A	1		1		1	
A/G	0.91 (0.57-1.47)	0.697	1.00 (0.56-1.78)	0.996	0.72 (0.30-1.70)	0.448
G/G	1.01 (0.35-2.85)	0.992	0.63 (0.13-2.96)	0.554	1.44 (0.33-6.36)	0.628
A/A	1		1		1	
A/G+G/G	0.92 (0.58-1.46)	0.724	0.97 (0.55-1.69)	0.906	0.83 (0.37-1.85)	0.643
A/A+A/G	1		1		1	
G/G	1.05 (0.38-2.91)	0.930	0.63 (0.14-2.88)	0.548	1.64 (0.39-6.96)	0.503
rs1800796 IL6						
C/C	1		1		1	
C/G	0.89 (0.55-1.41)	0.608	0.96 (0.53-1.74)	0.904	0.77 (0.35-1.66)	0.497
G/G	0.38 (0.12-1.16)	0.090	0.23 (0.06-0.88)	0.032*	Data not available	
C/C	1		1		1	
C/G+G/G	0.81 (0.45-1.47)	0.356	0.79 (0.45-1.39)	0.419	0.83 (0.39-1.79)	0.636
C/C+C/G	1		1		1	
G/G	0.40 (0.13-1.2)	0.102	0.23 (0.06-0.87)	0.031*	Data not available	
rs1799964 TNF						
T/T	1		1		1	
C/T	0.99 (0.61-1.60)	0.969	1.08 (0.59-1.96)	0.814	0.85 (0.38-1.90)	0.692
C/C	0.97 (0.42-2.27)	0.944	0.96 (0.37-2.52)	0.938	1.11 (0.17-7.17)	0.915
T/T	1		1		1	
C/T+C/C	0.99 (0.62-1.57)	0.956	1.05 (0.59-1.87)	0.861	0.87 (0.40-1.91)	0.734
T/T+T/C	1		1		1	
C/C	0.98 (0.43-2.20)	0.951	0.93 (0.37-2.30)	0.870	1.19 (0.19-7.44)	0.853

Odds ratio (OR) and 95% confidential Interval (CI) calculated by binary logistic regression.

\*OR adjusted by age and gender.

\*\*OR adjusted by age.

*P*<0.05 was considered statistically significant.

Table 4 Haplotype frequency estimation and their association with risk of T2DM

CRP	IL6	TNF	Total	control	ABG	OR (95%CI)	<i>P</i>
A	C	T	0.37	0.35	0.40	1.00	---
A	C	C	0.16	0.16	0.17	0.91 (0.46 - 1.80)	0.79
A	G	T	0.15	0.18	0.12	0.49 (0.24 - 1.02)	0.058
G	C	T	0.14	0.14	0.14	0.84 (0.43 - 1.66)	0.61
G	C	C	0.07	0.09	0.06	0.52 (0.20 - 1.35)	0.18
A	G	C	0.05	0.05	0.05	0.85 (0.27 - 2.64)	0.78
G	G	C	0.03	0.02	0.04	1.36 (0.32 - 5.79)	0.68
G	G	T	0.03	0.02	0.03	1.29 (0.25 - 6.52)	0.76

Odds ratio (OR) and 95% confidential Interval (CI) calculated by binary logistic regression.

OR adjusted by age and gender.

$P < 0.05$  was considered statistically significant.