

Association between intercellular adhesion molecule 1 (*ICAM1*) polymorphisms and diabetic foot susceptibility

A case–control study

Xue-Xia Cao, MD^{a,*}, Jin-Kui Yang, MD^b, Li Wang, MD^a

Abstract

The objective of the present study was to explore the association between intercellular adhesion molecule 1 (*ICAM1*) polymorphisms (rs5498 and rs3093030) and diabetic foot (DF) susceptibility in a Chinese Han population.

128 type 2 diabetes mellitus (T2DM) patients with DF, 147 T2DM patients without DF, and 155 healthy individuals were enrolled in this study. *ICAM1* polymorphisms were genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The genotypes and alleles of the polymorphisms were compared by χ^2 test between the 2 groups. Association between *ICAM1* polymorphisms and DF susceptibility was expressed through odds ratio (OR) with corresponding 95% confidence interval (95%CI). Effects of *ICAM1* polymorphisms on DF clinical characteristics were analyzed by *t* test.

GG genotype of rs5498 polymorphism was distinctly correlated with decreased T2DM risk (OR=0.369, 95%CI=0.152–0.895) and reduced susceptibility to DF among healthy controls (OR=0.316, 95%CI=0.119–0.837). Similar results were discovered between rs5498 G allele and decreased risk of T2DM (OR=0.676, 95%CI=0.475–0.963) and DF (OR=0.656, 95%CI=0.453–0.950) among healthy controls. Individuals carrying rs3093030 T allele had low susceptibility to DF developed from T2DM (OR=0.634, 95%CI=0.412–0.974). DF patients carrying rs5498 AA genotype had significantly higher serum creatinine levels than GG genotype carriers (*P*=.003).

ICAM1 rs3093030 polymorphism may act as a protective factor against DF developed from T2DM, moreover, rs5498 may be involved in onset of T2DM.

Clinical trial number: ChiCTR-INR-18010231.

Abbreviations: 95%CI = 95% confidence interval, APN = acute pyelonephritis, BMI = body mass index, CCC = coronary collateral circulation, DBP = diastolic blood pressure, DF = diabetic foot, DM = diabetes mellitus, DMI = diabetic microvascular complications, DN = diabetic nephropathy, DR = diabetic retinopathy, FBG = fasting plasma glucose, HDL = high density lipoprotein, HWE = Hardy–Weinberg equilibrium, *ICAM1* = intercellular adhesion molecule 1, LDL = low density lipoprotein, OR = Odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SBP = systolic blood pressure, SCr = serum creatinine, SNPs = single nucleotide polymorphisms, T1DM = type 1 DM, T2DM = type 2 DM, TC = total serum cholesterol, TG = triglycerides.

Keywords: diabetic foot, *ICAM1*, polymorphisms

Editor: Robert Chen.

Funding support: None.

The authors have no conflicts of interest to disclose.

Supplemental Digital Content is available for this article.

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How to cite this article: Cao XX, Yang JK, Wang L. Association between intercellular adhesion molecule 1 (*ICAM1*) polymorphisms and diabetic foot susceptibility: A case–control study. *Medicine* 2020;99:11(e18052).

Received: 3 December 2018 / Received in final form: 9 October 2019 /

Accepted: 22 October 2019

<http://dx.doi.org/10.1097/MD.00000000000018052>

1. Introduction

Diabetes mellitus (DM) is a metabolic disorder and could be divided into type 1 DM (T1DM) and type 2 DM (T2DM) in clinic. Long-term DM usually leads to different complications.^[1] Diabetic foot (DF) is a common complication which is caused by the combination of neuropathy and different levels of vascular lesions.^[2] It shows high prevalence and also represents the main cause of disability.^[3] Its occurrence significantly reduces the patients' life quality and brings heavy economic burden to the patients and family members.^[4] DF is usually caused by combined effects of infection and ulceration in lower limbs or vascular lesions in deep tissues.^[5–7] Peripheral neuropathy in limb abates pain feeling, and leads to the loss of pain protection mechanism.^[8] Therefore, even if foot lesions occur, patients could not find it in time. While infection will trigger nonspecific immune response, thus slowing down the ulceration.^[9] Previous study indicated that various factors participate in the development of DF.^[10] Genetic factors determine individual responses to DF risk factors.

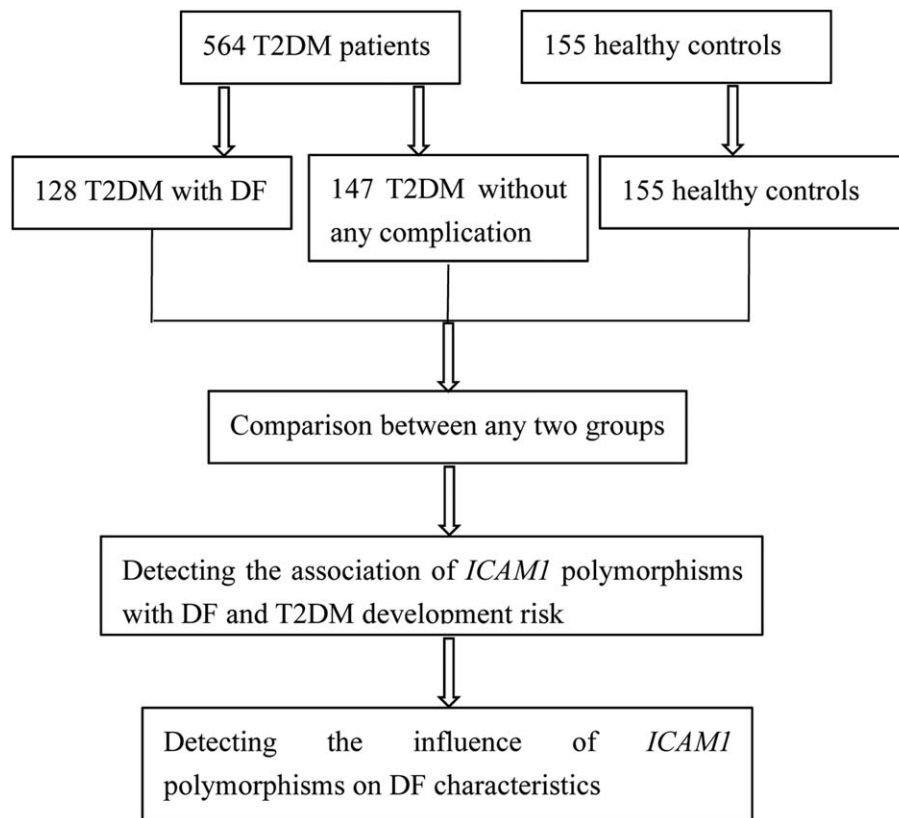


Figure 1. The flow diagram of this case-control study.

Intercellular adhesion molecule 1 (ICAM1) is a cell surface glycoprotein and expressed in immune and endothelial cells.^[11] ICAM1 could mediate interactions between cell-cell and cell-cellular matrix.^[12] ICAM1 concentration is elevated in cardiovascular and inflammatory patients.^[13] In peripheral nervous system, ICAM1 expression is induced by nerve injury.^[14] ICAM1 is an important regulator for cardiovascular disorders and peripheral neuropathy in diabetic patients.^[15] ICAM1 gene is located at 19p13.2 and its single nucleotide polymorphisms (SNPs) in exon regions may influence the expression or function of the protein. Minor allele frequencies of ICAM1 gene rs5498 (exon 6) and rs3093030 (non-coding exon c.-286C>T) SNPs were more than 0.1% in CHB (Chinese Han in Beijing) population. These 2 SNPs have been explored in various diseases.^[16,17] Then we suggested that ICAM1 SNPs might be associated with DF onset.

However, no previous study is focused on such association. Therefore, we selected rs5498 (exon 6) and rs3093030 (non-coding exon c.-286C>T) polymorphisms to explore their association with DF susceptibility in a Chinese Han population (Fig. 1).

2. Materials and methods

2.1. Study population

This study adopted a case-control design, and was ratified by the Ethic Committee of Xuanwu Hospital of Capital Medical University (approval number: SN201704302). Study subjects were informed the study process and signed written informed

consents. Study process conformed to the declaration of Helsinki. The controls were matched with cases in age and gender.

564 T2DM patients were diagnosed in Xuanwu Hospital of Capital Medical University from April, 2017 to October, 2018 based on previous guideline.^[18] 128 T2DM patients with DF were selected as case group (DF group), while 147 T2DM patients without any complications were recruited as T2DM group. Besides, 155 healthy persons with normal glucose tolerance and receiving regular healthy checkup were randomly enrolled as control group. All participants suffered no severe systemic diseases, vascular diseases, neurogenic diseases, or tumors. Basic characteristics, including age, gender, body mass index (BMI), smoking, drinking, systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting plasma glucose (FBG), serum creatinine (SCr), total serum cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) were collected by fully-fledged nurses through questionnaire.

2.2. Sample collection and genotyping method

Blood samples were collected from cubital vein for every subject in early morning and put into blood-collecting tubes with EDTA. Genomic DNA was extracted from whole blood using TIANamp Blood DNA Kit (TIANGEN Biotech, Beijing), and then stored at -20°C .

Primer sequences for ICAM1 gene rs5498 and rs3093030 polymorphisms were designed by Primer Premier 5.0 and Primer-BLAST based on information from GenBank database. Forward primer for rs5498 SNP was 5'-TGTGCCAGGCTTGGGGA-3',

while reverse primer was 5'-ACATTCACGGTCACCTTGCGG-3'. Primer sequences for rs3093030 were 5'-GGACCATAGGCT-CACAACACCACA-3' (forward) and 5'-GATTCGCTCCC-CAGGTCAAG-3' (reverse). PCR application utilized a 25 μ L system, including 2 μ L template, 2.5 μ L 10 \times buffer, 2.5 μ L dNTPs, 0.8 μ L each of forward and reverse primers, 0.5 μ L DNA polymerase, and 15.9 μ L ddH₂O. PCR reaction process was as follows: initial denaturation at 94°C for 10 min, 35 cycles of denaturation at 94°C for 55 s, annealing at 60°C for 50 s, extension at 72°C for 60 s, and final extension at 72°C for 10 min. PCR products were digested by restriction enzymes, BstUI for rs5498 (A: 169 bp, G: 151+18 bp) and MscI for rs3093030 (T: 373 bp, C: 173+200 bp), at 37°C for 4 hours. Then the products were separated through 3% agarose gel electrophoresis.

2.3. Statistical analysis

Hardy-Weinberg equilibrium (HWE) examination detected the representativeness of participants in healthy control group. Quantitative variables were evaluated by *t* test. Genotype and allele distributions were compared between the case and control groups by χ^2 test. Association strength between *ICAM1* polymorphisms and DF risk was expressed by odds ratio (OR) with 95% confidence interval (95%CI). SPSS 18.0 was used to perform data syntheses. Two-sided *P* < .05 represented statistical significant level.

3. Results

3.1. Basic characteristics of study subjects

Mean age for healthy controls, T2DM patients, and DF patients were 58.13 \pm 11.59, 57.35 \pm 11.21, and 58.70 \pm 11.04 years old, respectively. There were 71 males and 57 females in DF group, 85 males and 62 females in T2DM group, and 85 males and

70 females in control group. While, no significant difference was discovered among 3 groups (Table 1, *P* > .05). Besides, smoking and drinking status were not significantly associated with DF or T2DM development (*P* > .05). SBP, FBG, TC, SCr, and TG levels were significantly different among DF, T2DM patients, and healthy controls (Table 1, *P* < .05). However, DBP, HDL, and LDL levels had no significant difference between 3 groups (*P* > .05).

3.2. Association of *ICAM1* polymorphisms with DF susceptibility

Genotype distributions of rs5498 and rs3093030 SNPs in healthy controls did not deviate from HWE (Table 2, *P* > .05). It suggested that the study subjects could represent general population.

AA, AG, and GG genotype frequencies of *ICAM1* rs5498 polymorphism were 55.47%, 39.84%, 4.69% in DF patients, 55.10%, 39.46%, 5.44% in T2DM patients, and 45.81%, 41.93%, 12.26% in healthy controls. Frequencies of rs5498 A and G alleles were 75.39% and 24.61% in DF patients, 74.83% and 25.17% in T2DM patients, and 66.77% and 33.23% in healthy controls (Table 2). Allele distributions among 3 groups had statistically significant difference (*P* = .033).

Compared with AA genotype, rs5498 GG genotype significantly decreased susceptibility to T2DM (*P* = .023, OR = 0.369, 95%CI = 0.152–0.895) and DF (*P* = .016, OR = 0.316, 95%CI = 0.119–0.837) among healthy controls (Table 3). Meanwhile, individuals with rs5498 G allele faced reduced risk of T2DM (*P* = .030, OR = 0.676, 95%CI = 0.475–0.963) and DF (*P* = .025, OR = 0.656, 95%CI = 0.453–0.950) among controls, compared with A allele. However, genotypes and alleles of rs5498 had no significant association with susceptibility to DF developed from T2DM (*P* > .05).

Table 1

Characteristics of the subjects.

Characteristics	Groups			All <i>P</i>	Pairwise comparisons		
	DF, n=128 (%)	T2DM, n=147 (%)	Control, n=155 (%)		<i>P</i> ₁	<i>P</i> ₂	<i>P</i> ₃
Age	58.70 \pm 11.04	57.35 \pm 11.21	58.13 \pm 11.59	.579	0.317	0.676	0.552
BMI (kg/m ²)	25.73 \pm 2.92	25.77 \pm 3.12	26.05 \pm 3.14	.649	0.908	0.372	0.433
Gender							
Male	71 (55.47)	85 (57.82)	85 (54.84)	.862	0.694	0.916	0.601
Female	57 (44.53)	62 (42.18)	70 (45.16)				
Smoking							
No	82 (64.06)	101 (68.71)	113 (72.90)	.279	0.416	0.110	0.423
Yes	46 (35.94)	46 (31.29)	42 (27.10)				
Drinking							
No	96 (75.00)	113 (76.87)	127 (81.94)	.335	0.717	0.155	0.276
Yes	32 (25.00)	34 (23.13)	28 (18.06)				
SBP (mm Hg)	131.49 \pm 16.72	130.14 \pm 17.40	119.35 \pm 17.89	<.001	0.514	<0.001	<0.001
DBP (mm Hg)	82.75 \pm 10.33	83.33 \pm 11.61	81.41 \pm 10.17	.406	0.662	0.273	0.126
Disease duration (yr)	9.55 \pm 4.08	8.64 \pm 4.22			0.070		
Laboratory results							
FBG (mg/dL)	157.71 \pm 33.05	144.14 \pm 35.71	84.11 \pm 16.33	<.001	0.001	<0.001	<0.001
TC (mg/dL)	166.51 \pm 44.12	175.47 \pm 45.06	154.49 \pm 39.48	.001	0.098	0.016	<0.001
SCr (mg/dL)	1.38 \pm 0.42	1.17 \pm 0.43	0.92 \pm 0.23	<.001	<0.001	<0.001	<0.001
TG (mg/dL)	155.63 \pm 31.14	138.27 \pm 28.97	133.14 \pm 26.08	<.001	<0.001	<0.001	0.106
HDL (mg/dL)	35.47 \pm 10.15	35.81 \pm 13.68	33.62 \pm 11.42	.144	0.817	0.155	0.131
LDL (mg/dL)	104.03 \pm 28.75	106.68 \pm 29.92	109.10 \pm 22.24	.417	0.456	0.096	0.425

BMI=body mass index, DBP=diastolic blood pressure, FBG=fasting plasma glucose, HDL=high density lipoprotein, LDL=low density lipoprotein, SBP=systolic blood pressure, SCr=serum creatinine, TC=total serum cholesterol, TG=triglycerides; *P*₁, DF vs T2DM; *P*₂, DF vs control; *P*₃, T2DM vs control.

Table 2
Genotype and allele distributions of *ICAM1* polymorphisms.

Genotype/allele	DF, n = 128 (%)	T2DM, n = 147 (%)	Control, n = 155 (%)	P
rs5498				
AA	71 (55.47)	81 (55.10)	71 (45.81)	.082
AG	51 (39.84)	58 (39.46)	65 (41.93)	
GG	6 (4.69)	8 (5.44)	19 (12.26)	
A	193 (75.39)	220 (74.83)	207 (66.77)	.033
G	63 (24.61)	74 (25.17)	103 (33.23)	
P_{HWE}			0.494	
rs3093030				
CC	89 (69.53)	87 (59.18)	89 (57.42)	.013
CT	37 (28.91)	52 (35.37)	57 (36.77)	
TT	2 (1.56)	8 (5.44)	9 (5.81)	
C	215 (83.98)	226 (76.87)	235 (75.81)	.041
T	41 (16.02)	68 (23.13)	75 (24.19)	
P_{HWE}			0.975	

DF = diabetes foot, HWE = Hardy–Weinberg equilibrium, T2DM = type 2 diabetes mellitus.

Rs3093030 CC, CT, and TT genotype frequencies were 69.53%, 28.91%, 1.56% in DF group, 59.18%, 35.37%, 5.44% in T2DM patients, and 57.42%, 36.77%, 5.81% in healthy controls. Genotype distributions were significantly different between 3 groups (Table 2, $P = .013$). However, there were no significant associations between rs3093030 genotypes and susceptibility to T2DM or DF (Table 3, $P > .05$). Meanwhile, C and T allele frequencies were 83.98% and 16.02% in DF group, 76.87% and 23.13% in T2DM group, and 75.81% and 24.19% in healthy controls. Significant differences were also discovered among these 3 groups (Table 2, $P = .041$). T allele of rs3093030 SNP was significantly correlated with decreased DF risk based on healthy controls ($P = .016$, OR = 0.598, 95%CI = 0.391–0.912) and T2DM cases ($P = .037$, OR = 0.634, 95%CI = 0.412–0.974) (Table 3).

3.3. Effects of *ICAM1* SNP genotypes on DF characteristics

In this study, we also detected the effects of *ICAM1* polymorphisms on the characteristics of DF patients. SCr level was significantly higher in rs5498 GG genotype carriers than in AA genotype carriers (Table 4, $P = .003$). However, no significant

difference was discovered for rs5498 genotypes with SBP, DBP, disease duration, FBG, TC, TG, HDL, or LDL levels (Table 4, $P > .05$). Meanwhile, these basic characteristics were not significant difference between rs3093030 genotype carriers (Supplement Table 1, <http://links.lww.com/MD/D945>).

4. Discussion

As a biomarker of cell–cell interaction, *ICAM1* plays an important role in diabetic nephropathy.^[19,20] *ICAM1* level is elevated in inflammatory and malignant disorders.^[21] Plasma *ICAM1* levels were higher in diabetic nephropathy (DN) patients and T2DM patients than in healthy controls. Meanwhile, plasma *ICAM1* level was significantly higher in healthy individuals with rs5498 AA genotype than persons with AG or GG genotypes.^[22] Serum level of *ICAM1* was influenced by rs3093030 SNP.^[23] Therefore, we speculated that *ICAM1* polymorphisms might contribute to individuals susceptibility to DF onset. However, no previous study focused on the association of *ICAM1* polymorphisms with DF risk.

In the current study, we found that in comparison with healthy controls, rs5498 GG genotype and G allele were significantly correlated with reduced susceptibility to T2DM and DF, but the

Table 3
Association of *ICAM1* polymorphisms with DF susceptibility.

Genotype/allele	T2DM vs control		DF vs control		DF vs T2DM	
	P	OR (95%CI)	P	OR (95%CI)	P	OR (95%CI)
rs5498						
AA	–	Reference	–	Reference	–	Reference
AG	.312	0.782 (0.486–1.259)	.334	0.785 (0.479–1.284)	.782	0.856 (0.283–2.584)
GG	.023	0.369 (0.152–0.895)	.016	0.316 (0.119–0.837)	.990	1.003 (0.613–1.642)
A	–	Reference	–	Reference	–	Reference
G	.030	0.676 (0.475–0.963)	.025	0.656 (0.453–0.950)	.879	0.970 (0.659–1.430)
rs3093030						
CC	–	Reference	–	Reference	–	Reference
CT	.777	0.933 (0.579–1.505)	.094	0.649 (0.391–1.078)	.166	0.696 (0.416–1.164)
TT	.852	0.909 (0.335–2.465)	.060	0.222 (0.047–1.058)	.101	0.244 (0.050–1.183)
C	–	Reference	–	Reference	–	Reference
T	.758	0.943 (0.648–1.373)	.016	0.598 (0.391–0.912)	.037	0.634 (0.412–0.974)

DF = diabetes foot, T2DM = type 2 diabetes mellitus.

The bold values represented P values less than 0.05, and the data had statistical significance.

Table 4
Effects of rs5498 genotypes for DF characteristics.

Characteristics	rs5498 genotype		
	AA	AG	GG
SBP (mm Hg)	130.14 ± 17.42	132.75 ± 16.32	136.83 ± 14.22
DBP (mm Hg)	82.45 ± 10.30	83.06 ± 10.68	83.67 ± 8.98
Disease duration	9.14 ± 3.83	10.00 ± 4.35	10.67 ± 4.63
FBG (mg/dL)	163.10 ± 30.32	151.90 ± 36.17	147.67 ± 30.25
TC (mg/dL)	161.94 ± 44.64	172.20 ± 45.30	172.17 ± 18.98
SCr (mg/dL)	1.33 ± 0.39	1.39 ± 0.44	1.83 ± 0.19*
TG (mg/dL)	157.01 ± 32.57	153.51 ± 29.94	157.17 ± 27.18
HDL (mg/dL)	35.23 ± 10.31	35.29 ± 9.88	39.83 ± 11.37
LDL (mg/dL)	102.82 ± 27.83	106.92 ± 31.09	93.83 ± 16.02

DBP = diastolic blood pressure, FBG = fasting plasma glucose, HDL = high density lipoprotein, LDL = low density lipoprotein, SBP = systolic blood pressure, SCr = serum creatinine, TC = total serum cholesterol, TG = triglycerides.

* GG vs AA, $P = .003$.

presence of GG genotype and G allele had no obvious association with DF onset among T2DM cases. That meant, rs5498 might be only involved in T2DM occurrence. Rs5498 polymorphism is located at exon region of *ICAM1* gene, and it is involved in transcription and translation of the gene. Thus, rs5498 SNP could influence individual susceptibility to T2DM. The SNP has been explored in several diabetic complications. A meta-analysis demonstrated that rs5498 A allele was positively correlated with susceptibility to diabetic microvascular complications (DMI).^[16] Lv et al found that rs5498 SNP had no significant association with diabetic retinopathy (DR) risk in Chinese population.^[24] Popović and colleagues suggested that rs5498 SNP was not significantly correlated with T2DM susceptibility, but its AA genotype was positively associated with annual plaques growth in T2DM patients.^[25] However, a meta-analysis performed by Fan and Liu indicated that rs5498 G allele carriers had lower susceptibility to DR in Asians than those with A allele.^[26] This SNP was also detected in other diseases. GG genotype of rs5498 was negatively correlated with susceptibility to myocardial infarction.^[27] AA genotype of rs5498 SNP was more frequent in patients with acute pyelonephritis (APN).^[28] However, another study found that rs5498 GG genotype might induce multiple sclerosis.^[29] While rs5498 had no significant association with endometriosis susceptibility.^[30]

For rs3093030, the presence of T allele indicated low risk of DF based on T2DM and healthy individuals. Rs3093030 polymorphism was located at the non-coding exon region of *ICAM1* gene, and its variants might have the ability to regulate the expression of genetic information, thus influencing the function of *ICAM1*. The article published by Zhang et al found that rs3093030 CC genotype carriers had high susceptibility to cancers.^[31] Their conclusion supported our results. In order to explore the effects of *ICAM1* polymorphisms on DF susceptibility, we checked their association with basic characteristics of DF patients. Then we found that rs5498 GG genotype carriers had significantly higher SCr level than AA genotype carriers. While, rs3093030 SNP genotypes had no significant effects on other clinical characteristics.

Some limitations in the present study should be noted. First of all, the sample size was relatively small that reduced the accuracy of final results. Secondly, 1 nationality may not represent the whole Chinese Han population. Thirdly, a variety of factors are involved in the development of DF, but potential interactions between these factors were not explored in the current study.

Finally, the exact mechanism of *ICAM1* polymorphisms on DF development was not illustrated in current study. Therefore, further studies with enlarged sample size and ethnicity number should be conducted to verify our results and investigate the mechanism of *ICAM1* polymorphisms functioning in DF etiology.

In conclusion, *ICAM1* rs3093030 polymorphism may act as a protective factor against DF occurrence developed from T2DM. Furthermore, rs5498 polymorphism may be significantly associated with the decreased risk of T2DM development.

Author contributions

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