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# The association between *MCP-1*, *VEGF* polymorphisms and their serum levels in patients with diabetic foot ulcer

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**Abstract**

The purpose of the present study was to investigate distribution of monocyte chemoattractant protein-1 (*MCP-1*) –2518A/G and vascular endothelial growth factor (*VEGF*) –634G/C polymorphisms in type 2 diabetes melitus patients (T2DM) presenting diabetic foot ulcer (DFU). Additionally, we evaluated the effects of these 2 polymorphisms on serum levels of MCP-1 and VEGF in the study population.

Patients diagnosed with T2DM without or with DFU were recruited in the study. The distribution of *MCP-1* –2518A/G and *VEGF* –634G/C polymorphisms was investigated by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Enzyme-linked immunosorbent assay (ELISA) was applied to detect the protein levels of MCP-1 and VEGF. The comparisons of protein levels in DFU patients were performed by student *t* test according to their genotypes.

The frequencies of GG genotype and G allele of *MCP-1* –2518A/G was increased in DFU patients, compared with T2DM patients (odds ratio [OR] = 2.60, 95% confidence interval [CI] = 1.23–5.50, $P = .011$ and OR = 1.72, 95% CI = 1.18–2.50, $P = .005$, respectively). Moreover, the increased frequency of GG was significantly associated with up-regulated MCP-1 level in DFU patients ($P < .001$). Analysis for *VEGF* –634G/C polymorphisms indicated that the prevalence of CC genotype and C allele of the polymorphisms was decreased in DFU patients, compared with T2DM patients (OR = 0.36, 95% CI = 0.17–0.77, $P = .008$ and OR = 0.63, 95% CI = 0.43–0.91, $P = .015$, respectively). DFU patients carrying CC genotype had a higher level of VEGF than those with other genotypes ($P = .007$).

*MCP-1* –2518A/G and *VEGF* –634G/C polymorphisms may involve in occurrence and progress of DFU through regulating transcription activity of the genes.

**Abbreviations:** AGE = agarose gel electrophoresis, BMI = body mass index, BR = blood press, CCL2 = chemokine (C-C motif) ligand 2, CI = confidence interval, DFU = diabetic foot ulcers, ELISA = enzyme-linked immunosorbent assay, MCP-1 = monocyte chemoattractant protein-1, OR = odds ratio, PCR-RFLP = polymerase chain reaction-restriction fragment length polymorphism, SD = standard deviation, SNP = single nucleotide polymorphism, T2DM = type 2 diabetes melitus, VEGF = vascular endothelial growth factor, WHO = World Health Organization.

**Keywords:** diabetic foot ulcer, monocyte chemoattractant protein-1, polymorphisms, vascular endothelial growth factor

## 1. Introduction

Type 2 diabetes melitus (T2DM) is one of the most prevalent metabolic disorders and its prevalence is increasing in recent years.[1,2] T2DM can lead to various complications. Diabetic foot ulcer (DFU) is one of the common diabetic complications which is a leading cause for hospitalization and amputation among patients diagnosed with T2DM.[3,4] Until now, there are no effective treatments for DFU patients, due to the poor blood circulation at the wound sites.[5] Therefore, the factors associated with angiogenesis and vascular functions may involve in occurrence and development of DFU.

Monocyte chemoattractant protein-1 (*MCP-1*), also named chemokine (C-C motif) ligand 2 (CCL2), is a chemokine which could active monocytes, macrophages, and lymphocytes.[6] Abnormal expression of *MCP-1* has been observed in various diseases, such as clear-cell renal cell carcinoma, cerebral ischemic stroke, coronary artery disease.[7–9] Hyperglycemia can enhance the production of *MCP-1* in vascular endothelial cells and its abnormal expression may contribute to the complications related to angiogenesis and vascular functions among T2DM patients.[6] Recently, growing studies have indicated that the polymorphisms of *MCP-1* –2518A/G may influence the production of *MCP-1*.[10,11] But the effects of *MCP-1* –2518A/G polymorphism and its association with *MCP-1* level had been rarely reported among patients with DFU.

Vascular endothelial growth factor (VEGF), a potent angiogenesis and vascular functions factor, is significantly associated with occurrence and development of diabetic complications.[12] A meta-analysis including 6 related studies demonstrated that *VEGF* polymorphisms could influence individual susceptibility to

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**Table 1**

**Primers sequences and restriction enzymes used in the present study.**

| Gene | SNPs position | Primer sequences | Restriction enzymes |
|---|---|---|---|
| *MCP-1* | −2518A/G | Forward: 5′-CCGCATTCAATTTCCCTTTAT-3′,<br>Reverse: 5′-TTCCAAAGCTGCCTCCTCA-3′ | *PvuII* |
| *VEGF* | −634C/G | Forward: 5′-TTGCTTGCCATTCCCCACTTGA-3′<br>Reverse: 5′-CCGAAGCGAGAACAGCCCAGAA-3′ | *BsmFI* |

Notes: *MCP-1* = monocyte chemoattractant protein-1, SNP = single nucleotide polymorphism, *VEGF* = vascular endothelial growth factor.

diabetic retinopathy.[13] The genetic association of *VEGF* polymorphisms with risk of DFU had also been reported in the existing literature. Amoli et al[14] reported that *VEGF* polymorphism at position -2578*C/A was closely correlated with risk of DFU. In the study of Mohajeri-Tehrani et al,[5] the increased level of *VEGF* hold the capacity to improve blood flow and tissue temperature, thus, promoted wound healing for DFU patients. The expression level of *VEGF* may be influenced by its genetic variants. Sa-Nguanraksa et al[15] had found that *VEGF* expression levels were different among patients carrying different genotypes of *VEGF* –634G/C polymorphism. However, the polymorphism of *VEGF* +405C/G might not influence blood concentration of VEGF in Chinese Han population.[16] Thus, we hypothesized that *VEGF* –634G/C polymorphism might influence individual susceptibility to DFU through its regulation on VEGF production.

The purpose of the study was to investigate the genetic effects of *VEGF* –634G/C and *MCP-1* –2518A/G polymorphisms on risk of DFU in Chinese Han population. The T2DM patients without diabetic complications except DFU were recruited in this study, blood specimens were collected from the study subjects. The detected polymorphisms may be useful for targeted therapy of DFU.

## 2. Materials and methods

### 2.1. Study subjects

All the participants were collected from The First Affiliated Hospital of Shihezi University Medical College. The volunteers recruited in the present study should meet the following inclusion criterion: the Chinese Han adults population, without blood relationship; from the same geographical region; diagnosed with T2DM according to World Health Organization (WTO) criteria; without diabetic complications, except DFU. Exclusion criterion: immune diseases, cerebrovascular diseases, or other serious diseases. The patients who met the included criterion and had no excluded symptoms would be included in current study. According to the presence of DFU, the patients were divided into T2DM and DFU groups. The 2 study groups were matched in age and sex.

After an overnight fast, 6 mL peripheral blood was obtained from all the individuals using EDTA tubes. Then the blood specimens were stored in –80 °C until use.

The present study was supported by the ethic committee of The First Affiliated Hospital of Shihezi University Medical College. All the patients signed the written informed contents before blood collection. The study procedures were in accordance with the Declaration of Helsinki.

### 2.2. DNA extraction and genotyping

QiaAmp DNA Blood Mini Kit (Qiagen, Hilden, Germany) was applied for DNA extraction following the instruction of the manufacturer's. In the current study, the genotyping of *VEGF* –634G/C and *MCP-1* –2518A/G were determined by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). PCR primers were designed by Primer Premier 5.0 software and the sequences were listed in Table 1 (*MCP-1*,[10] *VEGF*[17]). DNA products were amplified in a 25 μL reaction system containing 12.5 μL PCR mastermix (2×), 1 μL DNA template, 0.5 μL specific primers, 0.5 μL $MgCl_2$, 10 μL free nuclease water. PCR programs were carried out according to the following settings: 95 °C for 1 minute, followed by 35 cycles of 95 °C for 45 second, 62 °C (for *VEGF* –634C/G) or 55 °C (for *MCP-1* –2518A/G) for 40 seconds, 72 °C for 40 seconds, then an extra extension at 72 °C for 10 minutes.

PCR products were analyzed by 1% agarose gel electrophoresis (AGE) and then submitted to enzymes digestion using the specific restriction enzymes. The enzyme-digested fragments were separated by 2% AGE.

### 2.3. MCP-1 and VEGF level evaluation

Enzyme-linked immunosorbent assay (ELISA) kits were applied to detect the protein levels of MCP-1 and VEGF in collected blood specimens. The MCP-1 levels were detected using human MCP-1 ELISA kits (R&D Systems, Minneapolis, MN),[10] while human VEGF Quantikine ELISA kit (R&D Systems, Minneapolis, MN)[17] was used for evaluation of VEGF level following the instructions of the manufacturer.

### 2.4. Statistical analysis

Statistical analysis was performed using SPSS 18.0 software. Odds ratio (OR) with corresponding 95% confidence interval (95% CI) calculated by $\chi^2$ test was applied to evaluate the different frequencies of genotypes and alleles between T2DM and DFU patients. The comparisons of VEGF and MCP-1 levels in DFU cases were performed using student *t* test according to their genotypes. *P* value <.05 was considered significant difference.

## 3. Results

### 3.1. Baseline characteristics of the study population

One hundred eight T2DM patients without DFU (male: 58, female: 50) were enrolled in the present study as T2DM group, and their average age was 57.06 ± 10.96 years old. The DFU groups included 71 men and 50 women and their mean age was 56.02 ± 9.83 years old. Analysis results indicated that the T2DM group and DFU group were age–sex matched ($P > .05$ for both). The other clinical characteristics of the study population were listed in Table 2. There were no significant differences between the 2 groups ($P > .05$ for all).

**Table 2**

**Baseline characteristics of the study population.**

| Characteristics | T2DM (n = 108) | DFU (n = 121) | P value |
|---|---|---|---|
| Age, y | 57.06 ± 10.96 | 56.02 ± 9.83 | .454 |
| Gender | | | .449 |
| Male | 58 | 71 | |
| Female | 50 | 50 | |
| BMI, kg/m$^2$ | 22.20 ± 2.07 | 21.89 ± 2.14 | .266 |
| Duration of disease, y | 8.94 ± 4.03 | 9.91 ± 4.97 | .111 |
| Systolic BP, mmHg | 131.33 ± 5.89 | 132.84 ± 6.41 | .066 |
| Diastolic BP, mmHg | 79.55 ± 4.82 | 78.80 ± 4.95 | .251 |
| $HbA_{1c}$ (%) | 8.99 ± 0.91 | 9.21 ± 1.07 | .100 |
| Cholesterol, mg/mL | 192.87 ± 9.44 | 193.45 ± 9.55 | .643 |
| Triglyceride, mg/dL | 160.12 ± 13.96 | 157.63 ± 12.03 | .148 |

BMI = body mass index, BP = blood press, DFU = diabetic foot ulcer, T2DM = type 2 diabetes mellitus.

### 3.2. Genotypes of MCP-1 –2518A/G

RFLP-PCR was applied to analyze the distributions of *MCP-1* –2518A/G polymorphism. The frequencies of AA, AG, and GG were separately 24.07%, 48.15%, and 27.78% in T2DM group. Meanwhile, their frequencies were 14.88%, 40.50%, and 44.63% in DFU group, respectively. In addition, chi-square analysis indicated that compared with AA genotype, GG genotype was significantly correlated with DFU susceptibility (OR = 2.60, 95% CI = 1.23–5.50, $P = .011$). However, there was no association between AG and DFU occurrence.

Allele distribution analysis demonstrated that the allele occurrence was 48.15% (A) and 51.85% (G) in T2DM group. In DFU group, A allele frequency was 35.12% and the frequency of G allele was 64.88%, respectively. Moreover, compared with A allele, G allele was significantly associated with risk of DFU (OR = 1.72, 95% CI = 1.18–2.50, $P = .005$) (Table 3).

### 3.3. Analysis for VEGF –634C/G polymorphism

There were 3 genotypes for *VEGF* –634C/G polymorphism, including CC, CG, and GG. The prevalence of these 3 genotypes was 35.18% (CC), 50.00% (CG), and 14.81% (GG) in T2DM group; while for DFU group, the frequencies were 23.97% (CC), 47.93% (CG), and 28.10% (GG), respectively. The distribution of CC was significantly decreased in DFU group, compared with GG ($P = .008$). CC genotype might be a protective factor for occurrence of DFU (OR = 0.36, 95% CI = 0.17–0.77).

Additionally, the frequency of C allele was separately 60.18% and 47.93%, while the prevalence of G allele was respectively 39.72% and 52.07% in T2DM and DFU group. Chi-square analysis demonstrated that compared with G allele, the frequency of C allele significantly reduced the risk of DFU (OR = 0.63, 95% CI = 0.43–0.91, $P = .015$) (Table 3).

**Table 3**

**Frequencies of alleles and genotypes of *MCP-1* and *VEGF* polymorphisms in study groups.**

| Genotype allele | T2DM (n = 108) | DFU (n = 121) | OR (95% CI) | P |
|---|---|---|---|---|
| *MCP-1* –2518A/G | | | | |
| AA | 26 (24.07%) | 18 (14.88%) | 1.00 (Reference) | – |
| AG | 52 (48.15%) | 49 (40.50%) | 1.36 (0.66–2.79) | .398 |
| GG | 30 (27.78%) | 54 (44.63%) | 2.60 (1.23–5.50) | .011 |
| A | 104 (48.15%) | 85 (35.12%) | 1.00 (Reference) | – |
| G | 112 (51.85%) | 157 (64.88%) | 1.72 (1.18–2.50) | .005 |
| *VEGF* –634C/G | | | | |
| CC | 38 (35.18%) | 29 (23.97%) | 0.36 (0.17–0.77) | .008 |
| CG | 54 (50.00%) | 58 (47.93%) | 0.51 (0.25–1.02) | .054 |
| GG | 16 (14.81%) | 34 (28.10%) | 1.00 (Reference) | – |
| C | 130 (60.18%) | 116 (47.93%) | 0.63 (0.43–0.91) | .015 |
| G | 86 (39.72%) | 122 (52.07%) | 1.00 (Reference) | – |

CI = confidence interval, DFU = diabetic foot ulcer, *MCP-1* = monocyte chemoattractant protein-1, OR = odd ratio, T2DM = type 2 diabetes mellitus, *VEGF* = vascular endothelial growth factor.

### 3.4. Serum levels of MCP-1 and VEGF

ELISA was applied to evaluate the serum concentrations of MCP-1 and VEGF in the included patients. Analysis results indicated that MCP-1 expressed was elevated in DFU group, compared with T2DM group (23.88 ± 3.72 pg/mL vs 16.63 ± 2.87 pg/mL, $P < .001$) (Fig. 1A).

VEGF concentration analysis suggested the expression level of VEGF was obviously down-regulated in patients diagnosed with DFU, compared with those in T2DM group (71.06 ± 8.80 pg/mL vs 107.77 ± 10.98 pg/mL, $P < .001$) (Fig. 1B).

### 3.5. Association between MCP-1 and VEGF level and their variants

In the present study, we analyzed the effects of *MCP-1* –2518A/G polymorphism on MCP-1 production among DFU patients. Analysis results indicated that the expression level of MCP-1 was higher in GG group than that in AA group ($P < .001$). There were no significant differences between AA group and AG group ($P > .05$) (Fig. 2A).

In addition, the comparison of VEGF expression was also performed in DFU patients according to their genotypes of *VEGF*–634C/G polymorphism. The results suggested that the DFU patients carrying CC genotype had a higher level of VEGF than those with GG genotype ($P = .007$). Moreover, there were no significant differences between DFU patients with CG genotype and GG genotype ($P > .05$) (Fig. 2B).

## 4. Discussion

DFU is a main reason for amputation and death among diabetic patients, moreover, the treatments for DFU lead to a heavy economic burden to the patients and their family.[18] Early detection and timely treatment may significantly improve life quality and outcomes of the patients.[19] There are 2 major reasons contributing to the occurrence of DFU: diabetic neuropathy and peripheral vascular diseases.[20] Therefore, the expression level and polymorphisms of *MCP-1* and *VEGF* which were related to angiogenesis and vascular functions were investigated in the study. The present study may be helpful for diagnosis and prevention of DFU in T2DM patients.

MCP-1 protein which is encoded by *MCP-1* gene is involved in various processes, such as inflammation, wound healing, fibrosis, and formation of vessels.[21] Accumulating evidences have demonstrated that *MCP-1* was involved in various diabetic complications.[18] A study carried out by Jeon et al[22] had indicated that *MCP-1* –2518A/G polymorphism was correlated with risk of proliferative diabetic retinopathy in a Korean population with T2DM. In the study of Raina et al,[23] genotypes of *MCP-1*–2518A/G polymorphism may influence the susceptibility of end stage renal disease caused by T2DM based on north-west Indian population of Punjab. In the current study, we

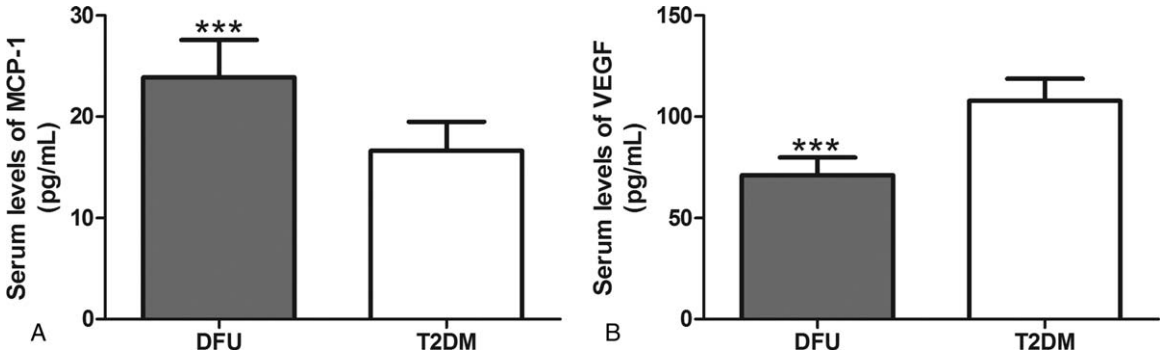


**Figure 1.** Protein levels of MCP-1 and VEGF in collected blood specimens. A: Comparison of MCP-1 level between T2DM and DFU patients. The DFU patients showed an increased level of MCP-1, compared with T2DM patients. ***: $P < .001$. B: The comparison analysis for VEGF level in the study population. Down-regulated level of VEGF was detected in DFU patients, compared with T2DM patients. ***: indicated $P$ value $< .001$. DFU = diabetic foot ulcers, MCP-1 = monocyte chemoattractant protein-1, T2DM = type 2 diabetes melitus, VEGF = vascular endothelial growth factor.

compared the distribution of *MCP-1* –2518A/G polymorphism between T2DM patients and DFU patients. PCR-RFLP results indicated that the prevalence of GG genotype was significantly different between test group and control group. The results indicated that GG genotype may increase the risk of DFU for T2DM patients. In addition, we investigated the serum level of MCP-1 in collected specimens and the results suggested that compared with T2DM patients, the DFU patients showed a high level of MCP-1. The abnormal expression of MCP-1 may involve in the progress of DFU. Kasiewicz et al[24] had proved that down-regulated of *MCP-1* could break the signal way of chronic inflammation within diabetic wound healing in an in vitro co-culture model of DFU. The results were accorded with our findings.

In the study, we evaluated the relationship between MCP-1 level and genotypes of *MCP-1*–2518A/G polymorphism in DFU patients. Analysis results indicated that patients carrying GG genotype showed a higher expression level than those with AA genotype. There was no significant difference between AA genotype and AG genotype. The results can be explained that polymorphisms in the location of -2518 may influence the transcriptional activity of the gene, thus, regulate its expression. The results were supported by the previous researches. A study carried out by Pham et al[25] had demonstrated that G allele in *MCP-1* –2518A/G polymorphism was preferentially transcribed and the donors with G allele exhibited an up-regulated level of *MCP-1*, compared with those carrying C allele. In a word, *MCP-1* –2518A/G polymorphism may take part in occurrence and progress of DFU through regulating the gene expression.

In addition, we investigated the distribution of *VEGF* –634C/G polymorphism in the study populations. Results demonstrated that the prevalence of CC genotype was significantly decreased in DFU patients, compared with T2DM patients. The results implied that T2DM patients with CC genotype of *VEGF* –634C/G polymorphism may be susceptible to DFU. *VEGF*–634C/G was a common polymorphism in the 5′-untranslated region of the gene. The polymorphism of the locations had been reported to be associated with risk of osteonecrosis of femoral head, therapeutic effects of 5-FU based chemo/radiotherapy in patients with esophageal squamous cell carcinoma, as well as diabetic complications.[26–28] These results supported the findings in the present study. In addition, low expression of VEGF was detected in DFU patients, compared with T2DM patients. The increased level of VEGF in the local wounding may contribute to wound healing, which may be a potential therapeutic target for DFU.[29] Moreover, we found that the expression of VEGF was imbalance between patients carrying different genotypes of *VEGF* –634C/G polymorphism. Analysis results indicated that DFU patients with CC genotype showed a higher level of VEGF than those carrying GG. The expression of VEGF was not significantly different between GG and GC genotypes. The effects of *VEGF* –634C/G polymorphism on VEGF

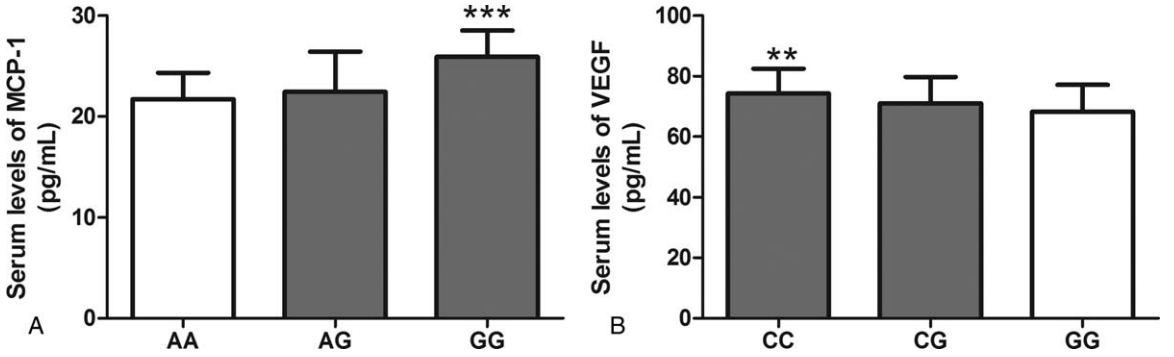


**Figure 2.** Association between gene polymorphisms and protein level in DFU patients. A: Relationship between *MCP-1* –2518A/G polymorphism and MCP-1 level. DFU patients carrying GG genotype showed a higher level of MCP-1 than those carrying AA genotype. There was no difference between AA and AG genotypes. ***: $P < .001$. B: The effects of *VEGF* –634C/G polymorphism on VEGF level in DFU patients. CC genotype was significantly associated with up-regulated level of VEGF, compared with GG genotype. No significant difference was detected between patients carrying GG and GC genotypes. **: indicated $P$ value $< .01$. DFU = diabetic foot ulcers, MCP-1 = monocyte chemoattractant protein-1, T2DM = type 2 diabetes melitus, VEGF = vascular endothelial growth factor.

expression was approved by Awata et al.[17] In their article, VEGF serum level was proved to be higher in healthy individuals with CC genotype of *VEGF* –634C/G polymorphisms than that in those carrying the other genotypes. However, some studies hold the opposite opinions. A study based on Parkinson disease population indicated that there was not association between VEGF polymorphisms and serum level of VEGF.[30] The study carried out by Ungerback et al[31] had indicated that there were not significant association between VEGF –634C/G polymorphism and expression of VEGF in colorectal cancer patients based on a Swedish population. The differences might be attributed to the different study populations and the divergences in study diseases. The issue was needed to be verified in the following researches.

In current study, we found that the production of VEGF and MCP-1 was significantly associated with genetic variants in their coding genes. The present study might be helpful in early prevention and diagnosis of DFU in T2DM patients. Moreover, to detect polymorphisms of *VEGF* and *MCP-1*, as well as their protein production, might provide guidance for treatment of DFU. However, there were still several limitations in current study. Firstly, the sample size was relatively small that might reduce the reliability of our results. Second, all the patients were collected from the same hospital. The results might be not suitable for other populations due to the regional differences. Additionally, the molecular mechanisms underlying the regulatory function of *MCP-1* and *VEGF* polymorphisms on their protein production remained poorly known. In order to improve our conclusions, further well-designed studies with a larger sample size will be required.

In conclusion, the distributions of *MCP-1* –2518A/G and *VEGF* –634C/G polymorphisms are significantly different between T2DM and DFU patients. Moreover, the genotypes of the 2 studied polymorphisms may influence serum levels of MCP-1 and VEGF in DFU patients. The detected polymorphisms of the genes may play important roles in the occurrence and progress of DFU through their regulatory function on transcription activity of the genes.

## Author contributions

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**Writing – original draft:** Xiaolei Li.
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## References

[1] Khodaeian M, Enayati S, Tabatabaei-Malazy O, et al. Association between genetic variants and diabetes mellitus in Iranian populations: a systematic review of observational studies. J Diabetes Res 2015;2015: 585917.
[2] Ramirez HA, Liang L, Pastar I, et al. Comparative genomic, MicroRNA, and tissue analyses reveal subtle differences between non-diabetic and diabetic foot skin. PLoS One 2015;10:e0137133.
[3] Uckay I, Gariani K, Dubois-Ferriere V, et al. Diabetic foot infections: recent literature and cornerstones of management. Curr Opin Infect Dis 2016;29:145–52.
[4] Li X, Xu G, Chen J. Tissue engineered skin for diabetic foot ulcers: a meta-analysis. Int J Clin Exp Med 2015;8:18191–6.
[5] Mohajeri-Tehrani MR, Nasiripoor F, Torkaman G, et al. Effect of low-intensity direct current on expression of vascular endothelial growth factor and nitric oxide in diabetic foot ulcers. J Rehabil Res Dev 2014;51:815–24.
[6] Dong L, Lv XY, Wang BJ, et al. Association of monocyte chemoattractant protein-1 (MCP-1)2518A/G polymorphism with proliferative diabetic retinopathy in northern Chinese type 2 diabetes. Graefe's Arch Clin Exp Ophthalmol 2014;252:1921–6.
[7] Yang Y, Zhai C, Chang Y, et al. High expression of chemokine CCL2 is associated with recurrence after surgery in clear-cell renal cell carcinoma. Urol Oncol 2016;34:238.e19–26.
[8] Bonifacic D, Toplak A, Benjak I, et al. Monocytes and monocyte chemoattractant protein 1 (MCP-1) as early predictors of disease outcome in patients with cerebral ischemic stroke. Wien Klin Wochenschr 2016;128:20–7.
[9] Akdogan MF, Azak A, Denizli N, et al. MCP-1 and soluble TWEAK levels are independently associated with coronary artery disease severity in patients with chronic kidney disease. Ren Fail 2015;37:1297–302.
[10] Jiang Z, Hennein L, Xu Y, et al. Elevated serum monocyte chemoattractant protein-1 levels and its genetic polymorphism is associated with diabetic retinopathy in Chinese patients with Type 2 diabetes. Diabet Med 2016;33:84–90.
[11] Rovin BH, Lu L, Saxena R. A novel polymorphism in the MCP-1 gene regulatory region that influences MCP-1 expression. Biochem Biophys Res Commun 1999;259:344–8.
[12] Schlingemann RO, Van Noorden CJ, Diekman MJ, et al. VEGF levels in plasma in relation to platelet activation, glycemic control, and microvascular complications in type 1 diabetes. Diabetes Care 2013; 36:1629–34.
[13] Xie XJ, Yang YM, Jiang JK, et al. Association between the vascular endothelial growth factor single nucleotide polymorphisms and diabetic retinopathy risk: a meta-analysis. J Diabetes 2017;9:738–53.
[14] Amoli MM, Hasani-Ranjbar S, Roohipour N, et al. VEGF gene polymorphism association with diabetic foot ulcer. Diabetes Res Clin Pract 2011;93:215–9.
[15] Sa-Nguanraksa D, Kooptiwut S, Chuangsuwanich T, et al. Vascular endothelial growth factor polymorphisms affect gene expression and tumor aggressiveness in patients with breast cancer. Mol Med Rep 2014;9:1044–8.
[16] Wang M, Zhou X, Zhang H, et al. Associations of the VEGF level, VEGF rs2010963 G/C gene polymorphism and ankylosing spondylitis risk in a Chinese Han population. Immunol Lett 2016;179:56–60.
[17] Awata T, Inoue K, Kurihara S, et al. A common polymorphism in the 5'-untranslated region of the VEGF gene is associated with diabetic retinopathy in type 2 diabetes. Diabetes 2002;51:1635–9.
[18] Karri VV, Kuppusamy G, Talluri SV, et al. Current and emerging therapies in the management of diabetic foot ulcers. Curr Med Res Opin 2016;32:519–42.
[19] Ilonzo N, Patel M, Lantis JC2nd. Managing the diabetic foot ulcer: how best practices fit the real 2018 United States. Surg Technol Int 2018;31.
[20] Viswanathan V, Rao VN. Managing diabetic foot infection in India. Int J Low Extrem Wounds 2013;12:158–66.
[21] Wang W, He M, Huang W. Association of monocyte chemoattractant protein-1 gene 2518A/G polymorphism with diabetic retinopathy in type 2 diabetes mellitus: a meta-analysis. Diabetes Res Clin Pract 2016; 120:40–6.
[22] Jeon HJ, Choi HJ, Park BH, et al. Association of monocyte chemoattractant protein-1 (MCP-1) 2518A/G polymorphism with proliferative diabetic retinopathy in Korean type 2 diabetes. Yonsei Med J 2013;54:621–5.
[23] Raina P, Matharoo K, Bhanwer AJ. Monocyte chemoattractant protein-1 (MCP-1) g.-2518A > G polymorphism and susceptibility to type 2 diabetes (T2D) and end stage renal disease (ESRD) in the North-West Indian population of Punjab. Ann Hum Biol 2015;42:276–82.
[24] Kasiewicz LN, Whitehead KA. Silencing TNFalpha with lipidoid nanoparticles downregulates both TNFalpha and MCP-1 in an in vitro co-culture model of diabetic foot ulcers. Acta Biomater 2016;32: 120–8.
[25] Pham MH, Bonello GB, Castiblanco J, et al. The rs1024611 regulatory region polymorphism is associated with CCL2 allelic expression imbalance. PLoS One 2012;7:e49498.

[26] Wang Y, Xia CJ, Wang BJ, et al. The association between VEGF -634C/G polymorphisms and osteonecrosis of femoral head: a meta-analysis. Int J Clin Exp Med 2015;8:9313–9.
[27] Tamura T, Kuwahara A, Yamamori M, et al. VEGF -634C/G genotype is predictive of long-term survival after treatment with a definitive 5-fluorouracil/cisplatin-based chemoradiotherapy in Japanese patients with esophageal squamous cell carcinoma. Int J Med Sci 2012;9:833–7.
[28] Yang Y, Andresen BT, Yang K, et al. Association of vascular endothelial growth factor -634C/G polymorphism and diabetic retinopathy in type 2 diabetic Han Chinese. Exp Biol Med (Maywood) 2010;235:1204–11.
[29] Zhang J, Guan M, Xie C, et al. Increased growth factors play a role in wound healing promoted by noninvasive oxygen-ozone therapy in diabetic patients with foot ulcers. Oxid Med Cell Longev 2014;2014:273475.
[30] Mihci E, Ozkaynak SS, Sallakci N, et al. VEGF polymorphisms and serum VEGF levels in Parkinson's disease. Neurosci Lett 2011;494:1–5.
[31] Ungerback J, Elander N, Dimberg J, et al. Analysis of VEGF polymorphisms, tumor expression of VEGF mRNA and colorectal cancer susceptibility in a Swedish population. Mol Med Rep 2009; 2:435–9.