

Association of *MCP-1* rs1024611 polymorphism with diabetic foot ulcers

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Abstract

Monocyte chemoattractant protein-1 (*MCP-1*), a pro-inflammatory cytokine, plays an important role in inflammatory process. In present study, we evaluated the association of *MCP-1* gene rs1024611 polymorphism with risk and clinical characteristics of diabetic foot ulcers (DFUs).

This study recruited 116 patients with DFUs, 135 patients with diabetes mellitus (DM) without complications (non-DFU), and 149 healthy controls (HCs). *MCP-1* gene rs1024611 polymorphism was genotyped by direct sequencing. The expression of *MCP-1* was analyzed using quantitative real-time polymerase chain reaction. Odds ratios (ORs) and 95% confidence intervals (CIs) were used to assume the association strength.

Individuals with rs1024611 AG and GG genotypes exhibited significantly higher susceptibility to DFUs, in the comparison with HCs (AG vs AA, OR=2.364, 95% CI=1.021–5.470; GG vs AA, OR=2.686, 95% CI=1.154–6.255). Meanwhile, G allele was associated with increased DFUs susceptibility (OR=1.457, 95% CI=1.014–2.093). Besides, rs1024611 SNP was slightly correlated with increased DFUs susceptibility in patients with DM. GG genotype of rs1024611 was significantly correlated with higher epidermal thickness and lower dermis thickness in patients with DFUs ($P < .01$). Patients with DFU exhibited upregulation of *MCP-1* mRNA, and GG genotype was correlated with enhanced *MCP-1* expression in DFU and non-DFU groups.

Rs1024611 polymorphism was significantly associated with *MCP-1* expression and individual susceptibility to DFUs.

Abbreviations: CIs = confidence intervals, DFU = diabetic foot ulcer, DM = diabetes mellitus, HC = healthy control, HWE = Hardy–Weinberg equilibrium, *MCP-1* = monocyte chemoattractant protein-1, ORs = odds ratios, QRT-PCR = quantitative real-time polymerase chain reaction, SNP = single-nucleotide polymorphism, T1DM = type I diabetes mellitus, T2DM = type II diabetes mellitus.

Keywords: clinical feature, diabetic foot ulcers, *MCP-1* gene, polymorphism

1. Introduction

Diabetes mellitus (DM) is a type of metabolic diseases, which is characterized by high blood sugar levels. DM can be divided into type I and type II DM (T1DM and T2DM). Without timely treatment, DM can cause many complications. Diabetic foot ulcers (DFUs) is one of the serious long term complications.^[1] DFUs usually caused by the vascular lesions, nervous lesion, and infection in the lower limbs of patients with DM.^[2] DFU could influence nerve, blood vessel, skin, tendon, and even bone. With the increasing of DM incidence, the morbidity of DFUs exhibits increasing trend.^[3] Treatment of DFUs is difficult and costly. DFUs can cause swingeing pain, even amputation that brings heavy burden for family and society.^[4,5] In addition, the long-

term inflammatory phase in DM wound will cause the dysfunction of macrophage and cytokine, delay of granulation tissue formation, and the reduction of wound tensile strength.^[6–8] Despite unclear pathogenesis of DFUs, it is generally considered that hereditary factor, especially the genes related to inflammatory responses, play an important role in the development of DFUs.^[9–12]

Monocyte chemoattractant protein-1 (*MCP-1*), also known as C-C motif chemokine ligand 2, belongs to CC chemokine family and participates in immunoregulatory and inflammatory processes. *MCP-1* could regulate vasculature inflammation in the wound site.^[13] The expression of *MCP-1* protein was significantly upregulated in diabetic rats.^[14] Van Asten and colleagues suggested that the *MCP-1* expression level was increased in patients with diabetic foot osteomyelitis.^[15] Serum concentration of *MCP-1* was strongly correlated with DFUs.^[16] However, the genetic association of *MCP-1* gene with DFUs still remained unclear.

In present study, we analyzed the association of *MCP-1* gene rs1024611 single-nucleotide polymorphism (SNP) with DFUs susceptibility in a Chinese Han population. Meanwhile, the influences of rs1024611 SNP for DFUs clinical features were also detected.

2. Materials and methods

2.1. Study subjects

Approval was afforded by ethnic committee of Cangzhou Central Hospital. Written informed consent was signed by each patient. Process of the study was accorded with the declaration of

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Table 1**Basic features for participants.**

| Features | DFU, n=116, % | Non-DFU, n=135, % | HC, n=149, % | P1 | P2 | P3 |
|--------------|---------------|-------------------|--------------|------|------|------|
| Age, mean±SD | 64.01±8.42 | 64.53±8.81 | 65.36±9.16 | .217 | .435 | .636 |
| Gender | | | | .821 | .920 | .752 |
| Male | 63 (54.31) | 76 (56.30) | 83 (55.70) | | | |
| Female | 53 (45.69) | 59 (43.70) | 66 (44.30) | | | |
| SBP, mm Hg | 141.07±14.55 | 135.56±12.98 | 126.49±12.54 | .000 | .000 | .002 |
| DBP, mm Hg | 86.05±8.88 | 82.78±8.20 | 79.81±11.15 | .000 | .012 | .003 |
| TC, mmol/L | 4.85±1.07 | 4.49±1.11 | 4.41±1.05 | .001 | .526 | .009 |
| TG, mmol/L | 1.86±1.02 | 1.69±0.98 | 1.36±0.68 | .000 | .001 | .201 |
| LDL, mmol/L | 65.73±23.82 | 78.50±28.47 | 84.50±25.95 | .000 | .064 | .000 |
| HDL, mmol/L | 41.42±15.87 | 40.11±14.15 | 39.11±11.62 | .173 | .515 | .490 |
| FPG, mmol/L | 10.03±3.19 | 9.97±3.04 | 5.28±0.72 | .000 | .000 | .871 |

DBP = diastolic blood pressure, DFU = diabetic foot ulcers, FPG = fasting plasma glucose, HC = healthy control, HDL = high-density lipoprotein, LDL = low-density lipoprotein, P1 = P value for DFU vs HC, P2 = P value for non-DFU vs HC, P3 = P value for DFU vs non-DFU, SBP = systolic blood pressure, SD = standard deviation, TC = total cholesterol, TG = triglycerides.

Helsinki. Questionnaire was used to collect the basic information of the participants. All of the patients were Chinese Han population. Clinical features were confirmed and recorded by professionally trained nurses (Tables 1 and 2).

Patients with T2DM with DFUs were diagnosed by 2 pathologists in Cangzhou Central Hospital. The diagnosis of T2DM was performed according to the previous criteria^[17] and checked by recent criteria.^[18] Patients with DM without DFUs but with other complications were excluded from this study. Then patients with T2DM without any complications were randomly selected as the non-DFU group. Healthy individuals were randomly selected from the healthy checkup center of the same hospital, severing as healthy control (HC) group. Frequencies of age and gender were matched among these groups. Participants had no history of other clinical system diseases. HCs had no evidences for any inflammatory diseases or systemic diseases.

2.2. Genotyping method

After a 12-hour fasting, 5 mL peripheral blood was collected from elbow venous of each patient in the morning. Blood samples were used for genomic DNA extraction via DNA extraction kit (Tiangen, Beijing, China). The amplification of rs1024611 SNP^[19] was accorded with the previous study using polymerase chain reaction (PCR). Finally, PCR products were sequenced by ABI 3730XL analyzer (Applied Biosystems, Foster City, CA).

2.3. RNA extraction and quantitative real-time PCR

Total RNA samples were isolated from the blood samples using Trizol reagent (Invitrogen, Carlsbad, CA) according to the instruction of manufacturer. The concentration and purity of RNA were measured via NanoDrop 2000 spectrophotometer (NanoDrop Technologies, Wilmington, DE). PrimeScript first strand cDNA synthesis kit (Takara, Beijing, China) was utilized to synthesize the first strand of cDNA. The relative expression of *MCP-1* mRNA in the collected blood samples were evaluated using quantitative real-time PCR (qRT-PCR) method which was performed by SYBR Premix Ex Taq (Takara). The primer sequences were designed by Primer Premier 5.0 software as follows: *MCP-1*: 5'-CTCGCCTCCAGCATGAAAGT-3' and 5'-GGTGACTGGGGCATTGATTG-3'; *GAPDH*: 5'-TCCTACCCCAATGTATCCG-3' and 5'-CCTTTAGTGGGCCCTCGG-3'. *GAPDH* served as internal control. The relative expression of *MCP-1* was calculated using $2^{-\Delta\Delta C_t}$ method. Each test was performed 3 times.

2.4. Statistic analysis

The representativeness of study patients was evaluated by Hardy–Weinberg equilibrium (HWE) test via PLINK. Quantitative variables were presented by mean±standard deviation, and analyzed using *t* test or Mann–Whitney *U* test. Qualitative variables were assessed by Chi-squared test. Association strength between *MCP-1* polymorphism and DFUs susceptibility was estimated using Chi-squared test, and the results were assumed by odds ratios (ORs) and 95% confidence intervals (CIs). All the statistical analyses were performed by PASW18.0. All the analyses were 2-tailed, and the results with $P < .05$ were considered significant. The significant level for multiple test was adjusted by Bonferroni method.

3. Results

3.1. Basic and clinical features

Age and gender had no significant difference among DFU, non-DFU, and HC groups (Table 1, $P > .05$). Except high-density lipoprotein concentration, other features were significantly different among these groups (Table 1, $P < .05$). Clinical features for patients with DFUs were shown in Table 2. Epidermal thickness and dermis thickness for patients with DFUs were 1.87 ± 0.11 and 1.44 ± 0.07 mm, respectively. Among these patients, 64 were neuropathic DFU, 39 were neuroischemic DFU, 2 were ischemic DFU, and 11 were non-neuropathic, nonischemic DFU. The site of ulcer included forefoot (87), midfoot (16), and hindfoot (13).

Table 2**Clinical features for patients with diabetic foot ulcers (DFUs).**

| Features | DFU, n=116, % |
|------------------------------|---------------|
| Epidermal thickness, mm | 1.87±0.11 |
| Dermis thickness, mm | 1.44±0.07 |
| Type of ulcer, n (%) | |
| Neuropathic | 64 (55.17) |
| Neuroischemic | 39 (33.62) |
| Ischemic | 2 (1.72) |
| Non-neuropathic, nonischemic | 11 (9.48) |
| Site of ulcer, n (%) | |
| Forefoot | 87 (75.00) |
| Midfoot | 16 (13.79) |
| Hindfoot | 13 (11.21) |

Table 3
Association of MCP-1 rs1024611 polymorphism with DFU susceptibility.

| Genotype/allele | DFU, n=116 (%) | Non-DFU, n=135 (%) | HC, n=149 (%) | DFU vs HC | | Non-DFU vs HC | | DFU vs non-DFU | |
|------------------|----------------|--------------------|---------------|-----------|---------------------|---------------|---------------------|----------------|---------------------|
| | | | | P | OR (95% CI) | P | OR (95% CI) | P | OR (95% CI) |
| AA | 9 (7.76) | 18 (13.33) | 26 (17.45) | – | – | – | – | – | – |
| AG | 54 (46.55) | 62 (45.93) | 66 (44.30) | .041* | 2.364 (1.021–5.470) | .388 | 1.357 (0.678–2.716) | .268 | 1.636 (0.681–3.934) |
| GG | 53 (45.69) | 55 (40.74) | 57 (38.25) | .019* | 2.686 (1.154–6.255) | .356 | 1.394 (0.688–2.824) | .142 | 1.927 (0.796–4.668) |
| A | 72 (31.03) | 98 (36.30) | 118 (39.60) | – | – | – | – | – | – |
| G | 160 (68.97) | 172 (63.70) | 180 (60.40) | .041* | 1.457 (1.014–2.093) | .418 | 1.151 (0.819–1.616) | .214 | 1.266 (0.872–1.838) |
| P _{HWE} | 0.346 | 0.936 | 0.366 | | | | | | |

CI=confidence interval, DFU=diabetic foot ulcers, HC=healthy control, HWE=Hardy–Weinberg equilibrium, MCP-1=monocyte chemoattractant protein-1, OR=odds ratio.
 *Represented P values less than 0.05.

3.2. Association of MCP-1 polymorphism with DFUs susceptibility

Genotype distribution of rs1024611 SNP did not depart from the HWE test respectively in DFU, non-DFU, and HC groups (Table 3, P>.05), revealing the well goodness of the patients.

Compared with the HCs, rs1024611 AG and GG genotypes were significantly associated with the increased susceptibility of DFUs (Table 3, AG vs AA, P=.041, OR=2.364, 95% CI=1.021–5.470; GG vs AA, P=.019, OR=2.686, 95% CI=1.154–6.255). Similar result was observed between G allele and DFUs susceptibility (P=.041, OR=1.457, 95% CI=1.014–2.093). Variant allele carriers of rs1024611 SNP had higher frequencies in non-DFU group than that in HC group, but the difference was not significant (Table 3, P>.05). Meanwhile, higher frequencies of AG and GG genotypes were discovered in DFU group, compared with non-DFU group. But the difference was insight (Table 3, P>.05). Thus, we suggested that rs1024611 SNP was slightly associated with increased DFUs susceptibility in patients with DM.

3.3. Influence of rs1024611 SNP for DFU clinical features

We analyzed the differences of DFUs’ basic and clinical features according to their genotypes of rs1024611 polymorphism. We failed to find any significant results between the basic features and rs1024611 genotypes (P>.05, data not shown). Meanwhile, rs1024611 genotypes had no significant influence on the type and site of ulcer (Table 4, P>.05). The epidermal thickness of the individuals carrying AA, AG, and GG genotypes were 1.80±0.08, 1.93±0.09 and 1.95±0.10 mm, respectively. The epidermal

thickness of GG genotype carriers was significantly higher than that of AA and AG carriers (Table 4, P<.01). In AA, AG, and GG genotype carriers, the dermis thicknesses were 1.50±0.04, 1.45±0.08, and 1.42±0.05 mm, respectively. GG genotype carriers had significantly lower dermis thickness, compared with other 2 genotypes (Table 4, P<.01). Therefore, we considered that rs1024611 GG genotype was significantly associated with the epidermal and dermis thickness in patients with DFUs.

3.4. The expression of MCP-1 mRNA in the study population

In the present study, qRT-PCR was performed to investigate the expression profiles of MCP-1 mRNA in DFU, non-DFU, and HC groups. Compared with HC group, the relative expression of MCP-1 mRNA was significantly increased in non-DFU group (P=.008) and DFU group (P<.001). Moreover, patients with DFU exhibited obviously higher levels of MCP-1 than the individuals in non-DFU group (P=.010) (Fig. 1A).

3.5. Effects of rs1024611 SNP on expression of MCP-1

In addition, we compared the expression of MCP-1 in the study groups according to genotypes of rs1024611 SNP. In HC group, the individuals who carried different genotypes of rs1024611 polymorphism did not exhibit different expression patterns of MCP-1 (P>.05 for all) (Fig. 1B). In non-DFU group, the cases carrying GG genotype showed significantly increased levels of MCP-1 than those carrying AA genotype (P=.020). However, the levels of MCP-1 were similar between AG and AA carriers in non-DFU group (P=.404) (Fig. 1C). In DFU group, we found

Table 4
Influence of rs1024611 single-nucleotide polymorphism for diabetic foot ulcer (DFU) clinical features.

| Features | Total | Genotype | | |
|------------------------------|------------|-----------|------------|--------------------------|
| | | AA | AG | GG |
| Epidermal thickness, mm | 1.87±0.11 | 1.80±0.08 | 1.93±0.09 | 1.95±0.10 ^{*,†} |
| Dermis thickness, mm | 1.44±0.07 | 1.50±0.04 | 1.45±0.08 | 1.42±0.05 ^{*,†} |
| Type of ulcer, n (%) | | | | |
| Neuropathic | 64 (55.17) | 5 (55.56) | 30 (55.56) | 29 (54.72) |
| Neuroischemic | 39 (33.62) | 3 (33.33) | 18 (33.33) | 18 (33.96) |
| Ischemic | 2 (1.72) | 0 | 1 (1.85) | 1 (1.89) |
| Non-neuropathic, nonischemic | 11 (9.48) | 1 (11.11) | 5 (9.26) | 5 (9.43) |
| Site of ulcer, n (%) | | | | |
| Forefoot | 87 (75.00) | 7 (77.78) | 41 (75.93) | 39 (73.58) |
| Midfoot | 16 (13.79) | 1 (11.11) | 7 (12.96) | 8 (15.09) |
| Hindfoot | 13 (11.21) | 1 (11.11) | 6 (11.11) | 6 (11.32) |

Significant level was adjusted by Bonferroni method, P_a=.016.

* Compared with AA genotype carriers, P<.016.

† Compared with AG genotype carriers, P<.016.

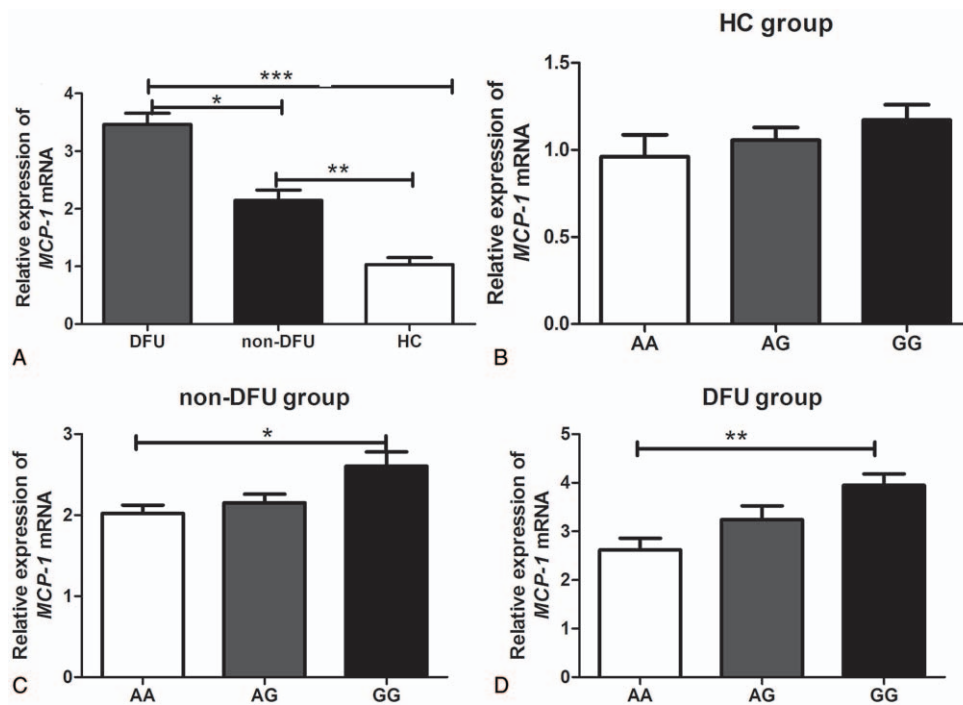


Figure 1. The expression profiles of monocyte chemoattractant protein-1 (*MCP-1*) mRNA in the study population. (A) The relative expression of *MCP-1* in healthy control (HC), non-diabetic foot ulcer (DFU), and DFU groups. Compared with HC group, the expression of *MCP-1* was significantly higher in DFU and non-DFU groups. Moreover, the cases in DFU group showed significantly increased levels of *MCP-1*. (B) The expression patterns of *MCP-1* mRNA in HC group according to their genotypes of rs1024611 genotype. The individuals carrying different genotypes of rs1024611 SNP did not show significant differences in *MCP-1* expression. (C) The comparison of *MCP-1* expression in non-DFU group according to their genotypes of rs1024611 polymorphism. Compared to AA genotype carriers, the individuals with AG genotype exhibited higher levels of *MCP-1* mRNA. (D) The expression profile of *MCP-1* mRNA in DFU group. The levels of *MCP-1* mRNA were significantly different between DFU cases carrying AA and AG genotypes. * $P < .05$; ** $P < .01$; *** $P < .001$.

that patients carrying GG genotype exhibited obviously higher levels of *MCP-1* than those carrying AA genotype ($P = .004$). The individuals carrying AG genotype did not show differences in *MCP-1* expression with AA genotype carriers ($P = .134$) (Fig. 1D).

4. Discussion

In the present study, we investigated the genetic association of *MCP-1* rs1024611 polymorphism with DFU susceptibility in a Chinese Han population. We found that the individuals carrying AG and GG genotypes were more likely to develop DFU. Furthermore, GG genotype was significantly associated with the epidermal and dermis thickness in patients with DFUs. Compared with HC group, DFU and non-DFU groups had high levels of *MCP-1*. GG genotype was significantly associated with increased levels of *MCP-1* mRNA. All the data revealed the important role of *MCP-1* polymorphism in etiology of DFUs.

The DFUs could develop from both T1DM and T2DM. It is attributed by the abnormal inflammatory responses which caused by wound healing or other conditions. Immune cells and cytokines are important components in inflammatory responses. *MCP-1* holds the capacity to modulate monocyte chemotaxis, activate endothelial cell, and regulate inflammatory progression and pro-inflammatory cytokines production. In DM, the patients with high levels of *MCP-1* were more likely to develop diabetic complications, such as diabetic nephropathy.^[20,21] Furthermore, the upregulation of *MCP-1* in patients with DM may lead to abnormal leukocyte infiltration in ulcerative tissue.^[16] In human,

MCP-1 gene is located at 17q12 and contains 3 exons. Accumulating evidences have demonstrated that the polymorphisms in *MCP-1* gene may influence genetic predisposition to the complications of DM.^[20–22] Rs1024611 is a frequently studied polymorphism in promote region of *MCP-1* gene, which has been reported to be involved in various immune and inflammatory diseases.^[23–25] He and colleagues found that G allele of rs1024611 SNP was associated with enhanced *MCP-1* expression.^[26] Consequently, we speculated that rs1024611 SNP might be involved in the development of DFUs.

In the present study, AG genotype of rs1024611 SNP predicted 2.364 times increased susceptibility for DFUs in HCs, but had no significant association with the increased DFUs susceptibility in patients with DM. About 2.686 times enhanced susceptibility of DFUs was brought by rs1024611 GG genotype in the healthy individuals. Rs1024611 G allele was significantly associated with elevated susceptibility of DFUs approximately 1.457 times in total patients. A recent meta-analysis study indicated that rs1024611 SNP was positively associated with the increased susceptibility to diabetic retinopathy in patients with T2DM.^[22] Besides, rs1024611 SNP was distinctly associated with the end-stage renal disease in patients with T2DM.^[27]

Basic features, ulcer type and site had no significant differences in the 3 different genotypes of rs1024611 SNP in patients with DFUs. GG genotype was distinctly related to increased epidermal thickness and reduced dermis thickness in patients with DFUs. In addition, we analyzed the expression patterns of *MCP-1* mRNA in HC, non-DFU, and DFU groups. The results demonstrated that compared with HC group, the patients with DM with or without

DFU exhibited increased levels of *MCP-1*. Moreover, patients with DFU had significantly higher levels of *MCP-1* than the DM cases without DFU. The conclusion was consistent with the previous report.^[16] We also compared the expression of *MCP-1* in the study population according to their genotypes of rs1024611 polymorphism. Analysis results suggested that both in DFU and non-DFU groups, the individuals carrying GG genotype exhibited obviously increased *MCP-1* expression. GG genotype might enhance the expression of *MCP-1*. The conclusion was in line with the study of He et al.^[26] Rs1024611 polymorphism is located at promoter region -2518 site of *MCP-1* gene, an important regulatory region for *MCP-1* transfection. Genetic mutations in this region might alter transfection of *MCP-1* gene, and the gene function, thus leading to abnormalities in a series of inflammatory cascade response, and inflammatory-related diseases. However, the molecular mechanisms underlying the function of *MCP-1* in DFU had not been explored in our study. Further investigations will be required.

Based on above results, we suggested that rs1024611 SNP might promote the onset and development of DFUs via regulate the gene transfection. The individuals carrying GG genotype of rs1024611 SNP were more likely to have high expression of *MCP-1*, and develop DFU. Some limitations in the current study should be stated. First of all, due to the short term of study period, the sample size was not large enough to provide a high statistical power. Secondly, patients were recruited from a single hospital that might limit the applicable scope of the present results. Thirdly, association strength was not adjusted by confounding factors that might affect the stability of current result. Therefore, well-designed studies with enlarged sample size are necessary in the future.

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