Int J Clin Exp Med 2018;11(10):10795-10801
www.ijcem.com /ISSN:1940-5901/IJCEM0076597

Original Article

Association of *NOS3* polymorphism rs1799983 with susceptibility to diabetic foot: a meta analysis

Aihong Wang, Yuxiang Wu, Zhangrong Xu

Department of Endocrinology, The 306th Hospital of PLA, Beijing 100101, China

Received March 23, 2018; Accepted July 10, 2018; Epub October 15, 2018; Published October 30, 2018

Abstract: Objective: Previous studies have discussed the relationship between endothelial nitric oxide synthase (*NOS3*) polymorphism rs1799983 and diabetic foot (DF) occurrence, but their results were not uniform. Present research was, therefore, carried out to statistically explore association of this polymorphism with DF susceptibility through means of meta-analysis. Methods: Relevant articles were collected from online databases and other sources. Crude odds ratios (ORs) and their 95% confidence intervals (95% CIs) were computed to assess DF susceptibility in association with *NOS3* rs1799983 polymorphism. Stratification analyses by ethnicity and source of control were performed for specific estimates. Inter-study heterogeneity was investigated through Chi-square-based Q statistic test. Begg's funnel plot and Egger's test were employed to probe potential publication bias across eligible studies. Results: The present meta-analysis ultimately incorporated 4 eligible publications, containing 5 independent studies. Overall findings indicated that *NOS3* rs1799983 polymorphism significantly reduced susceptibility to DF under the comparisons of TT vs. GG (OR=0.35, 95% CI=0.18-0.69) and TT vs. GG + GT (OR=0.36, 95% CI=0.18-0.70). Additionally, a similar tendency was observed in Caucasians and hospital-based subgroups under corresponding models after stratified by ethnicity and source of control. Conclusion: *NOS3* polymorphism rs1799983 may play a protective role against development of DF, especially in Caucasians.

Keywords: Nitric oxide, *NOS3*, polymorphism, diabetic foot, meta-analysis

Introduction

Diabetic foot (DF) refers to the infection, ulceration, and deep tissue destruction of lower extremities caused by arterial disease and peripheral neuropathy in diabetes mellitus (DM) patients [1]. One of the main complications of DM, DF is a chronic and progressive disorder affected by many factors, such as metabolic disturbance, ischemia of extremity end, neuropathy, and infection [2]. Chronic ulcers, the common manifestation in DF, usually lead to amputation and even death. This disease not only impacts patient life quality but also imposes heavy burden on individuals, families, and national health care [3, 4]. Therefore, it is important to find out how to effectively prevent and avoid occurrence and development of DF. With deepened understanding of DF, scholars have suggested that incidence of this disease can be attributed to interaction between multiple factors [5]. Diabetic peripheral vasculopathy has been reported to be an independent risk factor for onset of DF, playing a crucial role in the disease etiology [6]. Nitric oxide (NO), also known as endothelium-derived relaxing factor, is very active chemically. It can dilate blood vessels, regulate blood pressure, change local blood flow, increase the permeability of vascular walls, inhibit platelet aggregation, resist the proliferation of smooth muscle cells, and adjust neurotransmitter transmitting [7]. Moreover, NO has a cytotoxic effect as well, possessing dual mechanisms of protection and damage. Endothelial nitric oxide synthase (eNOS, or NOS3) is the major kinase for NO synthesis in vessels under physiological conditions. It is also a critical precondition for NO physiological function [8, 9]. *NOS3* gene is located on chromosome 7q35-36 with a length of 21 Kb, consisting of 26 exons and 25 introns. *NOS3* gene polymorphisms may influence expression of the protein and, consequently, alter NOS activity, may cause abnormal NO synthesis, affecting the progression of diabetic microangiopathy [10]. The effects of

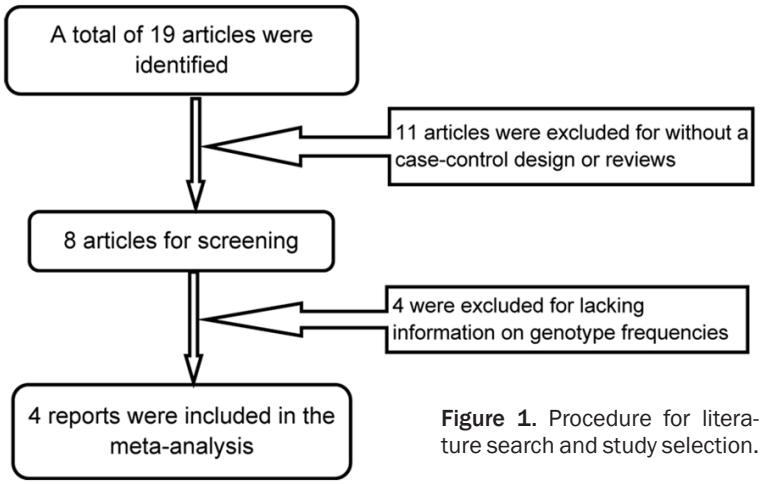

Figure 1. Procedure for literature search and study selection.

NOS3 polymorphism rs1799983 on susceptibility of DF have been explored in the last decade [11, 12]. However, previous studies have yielded conflicting results. Considering inconsistent conclusions concerning this topic, this meta-analysis was carried out to ascertain the association between *NOS3* polymorphism rs1799983 and DF occurrence.

Materials and methods

Literature sources and search strategy

Potentially relevant articles regarding *NOS3* polymorphisms rs1799983 and DF occurrence were retrieved from PubMed, EMBASE, Google Scholar Web, Chinese National Knowledge Infrastructure (CNKI), and Wanfang databases, as well as from other sources, using a combination of the following keywords: "endothelial nitric oxide synthase or *eNOS* or *NOS3* or endothelial-constitutive nitric oxide synthase or *ecNOS*", "polymorphism or variation or mutation", and "diabetic foot or diabetic foot ulcer or diabetic microangiopathy or diabetic complications". All enrolled reports were published in English or Chinese. No other restrictions were put on publication year, country of origin, ethnicity, or sample size in the literature search. Moreover, references of all included articles were manually examined for more relevant reports.

Selection criteria

Eligible studies for this meta-analysis met the following inclusion criteria: (a) Used a case-control design; (b) Evaluated correlation of *NOS3* polymorphism rs1799983 with DF susceptibility; (c) Presented sufficient information to calculate pooled odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs); and (d) Enrolled human beings as study subjects. Reviews, letters, and papers with insufficient data were excluded from the current meta-analysis.

Data extraction

Essential information was extracted from each included study by two reviewers using a standard data-collection form, including first author's name, publication year, country of origin, ethnic descent, source of control, genotyping method, numbers of cases and controls, genotype and/or allele frequencies in case and control groups, and *P* value for Hardy-Weinberg equilibrium (HWE) in controls. In case of any disagreements, the two reviewers launched a discussion until a final consensus was reached.

Statistical analysis

Crude ORs and corresponding 95% CIs were calculated to estimate the strength of association between *NOS3* polymorphism rs1799983 and DF under TT vs. GG, TT + GT vs. GG, TT vs. GG + GT, allele T vs. allele G, and GT vs. GG models. Moreover, subgroup analyses based on ethnicity and control source were further implemented. Inter-study heterogeneity was inspected through Q statistic test, with $P<0.05$ representing significant levels. When heterogeneity among included studies was significant, a random-effects model was adopted to compute OR. Otherwise, a fixed-effects model was chosen. Sensitivity analysis was undertaken to evaluate the influence of individual data sets on pooled estimates when the amount of included studies was more than 6. Potential publication bias was assessed using both Begg's funnel plot and Egger's regression test. STATA 12.0 software (Stata Corporation, College Station, TX, USA) was used for all statistical analyses in this meta-analysis and $P<0.05$ indicates statistical significance.

Results

Characteristics of identified studies

The search strategy retrieved a total of 19 potentially relevant articles, initially, with 11 of

Table 1. Principal characteristics of studies included in the meta-analysis

First author	Publication year	Country of study	Ethnicity	Source of control	Genotyping method	Case			Control			HWE
						GG	GT	TT	GG	GT	TT	
Sadati	2018	Iran	Caucasian	HB	PCR-RFLP	54	64	5	42	71	21	0.317
Corapcioglu	2010	Turkey	Caucasian	PB	PCR-RFLP	46	46	5	48	42	12	0.549
Ma	2007	China	Asian	PB	PCR-RFLP	36	6	0	86	14	0	0.452
Yang	2004	China	Asian	PB	PCR-RFLP	67	28	1	63	21	1	0.606
Yang	2004	China	Asian	HB	PCR-RFLP	67	28	1	89	25	0	0.189

Notes: HB, hospital-based; PB, population-based; PCR-RFLP, polymerase chain reaction-restriction fragment length polymorphism; HWE, Hardy-Weinberg equilibrium.

Table 2. *NOS3* rs1799983 polymorphism and DF susceptibility

Genetic comparison	Group/Subgroup		OR (95% CI)	*Ph*
TT vs. GG	Ethnicity	Caucasian	0.27 (0.13, 0.58)	0.276
		Asian	1.84 (0.24, 13.77)	0.506
	Source of control	HB	0.27 (0.11, 0.68)	0.075
		PB	0.48 (0.17, 1.35)	0.615
		Total	0.35 (0.18, 0.69)	0.239
TT + GT vs. GG	Ethnicity	Caucasian	0.74 (0.51, 1.08)	0.173
		Asian	1.32 (0.88, 2.00)	0.776
	Source of control	HB	0.93 (0.36, 2.41)*	0.018
		PB	1.08 (0.73, 1.59)	0.867
		Total	0.96 (0.73, 1.27)	0.165
TT vs. GG + GT	Ethnicity	Caucasian	0.29 (0.14, 0.61)	0.441
		Asian	1.70 (0.23, 12.73)	0.517
	Source of control	HB	0.30 (0.12, 0.75)	0.108
		PB	0.45 (0.16, 1.22)	0.612
		Total	0.36 (0.18, 0.70)	0.360
T vs. G	Ethnicity	Caucasian	0.69 (0.52, 0.91)	0.205
		Asian	1.29 (0.88, 1.88)	0.751
	Source of control	HB	0.92 (0.37, 2.29)*	0.007
		PB	0.96 (0.69, 1.33)	0.662
		Total	0.86 (0.69, 1.07)	0.060
GT vs. GG	Ethnicity	Caucasian	0.87 (0.59, 1.29)	0.222
		Asian	1.31 (0.86, 1.98)	0.821
	Source of control	HB	0.96 (0.64, 1.43)	0.071
		PB	1.16 (0.78, 1.74)	0.946
		Total	1.05 (0.79, 1.40)	0.432

Notes: HB, hospital-based; PB, population-based; *Ph*, *P*-value of heterogeneity test; *, data calculated using the random-effects model.

them removed for being reviews or lacking a case-control design (**Figure 1**). Of the remaining ones, 4 publications were further deleted due to insufficient genotype data. As a consequence, 4 articles published between the years of 2004 and 2018 were ultimately accepted into the present meta-analysis [11-14], involving 5 independent studies of 454 cases and 535 controls. There were 2 studies on Caucasians and 3 on Asians. More detailed descriptions of these studies are displayed in **Table 1**. Genotype distributions of *NOS3* polymorphism rs1799983 in the controls conformed to HWE for all of enrolled studies in this meta-analysis ($P>0.05$).

Meta-analysis results

Table 2 presents results concerning the relationship between *NOS3* polymorphism rs1799983 and DF susceptibility.

Overall estimates indicated that *NOS3* polymorphism rs1799983 significantly inhibited occurrence of DF under the contrasts of TT vs. GG [OR=0.35, 95% CI= 0.18-0.69 (**Figure 2**)] and TT vs. GG + GT [OR=0.36, 95% CI=0.18-0.70 (**Figure 3**)]. After stratification analyses by ethnicity and control source, a similar association between the polymorphism and the disease was also observed in Caucasians [TT vs. GG: OR=0.27, 95% CI=0.13-0.58 (**Figure 2**); TT vs. GG + GT: OR=0.29, 95% CI=0.14-0.61; T vs. G: OR=0.69, 95% CI=0.52-0.91] and hospital-based [TT vs. GG: OR=0.27, 95% CI=0.11-0.68; TT vs. GG + GT: OR=0.30, 95% CI=0.12-0.75 (**Figure 3**)] subgroups.

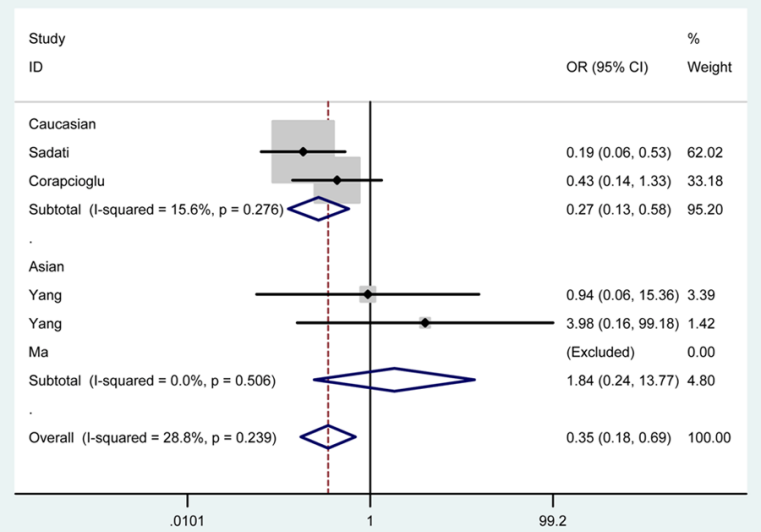

Figure 2. Forest plot for DF susceptibility associated with *NOS3* polymorphism rs1799983 under TT vs. GG model after stratified by ethnicity. Squares and horizontal lines correspond to the study-specific OR and 95% CI. Area of the squares reflects the weight (inverse of the variance). Diamond represents the summary OR and 95% CI.

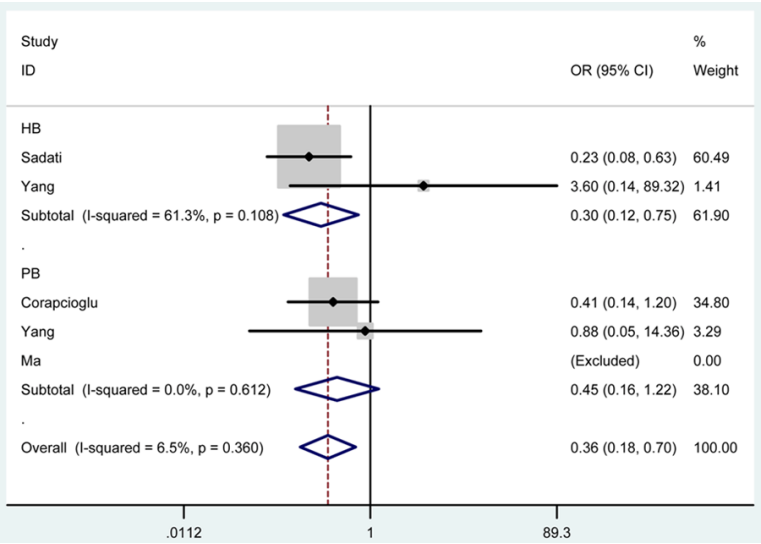

Figure 3. Forest plot for DF susceptibility associated with *NOS3* polymorphism rs1799983 under TT vs. GG + GT model after stratified by source of control. Squares and horizontal lines correspond to the study-specific OR and 95% CI. Area of the squares reflects the weight (inverse of the variance). Diamond represents the summary OR and 95% CI.

Heterogeneity analysis and sensitivity analysis

Q test detected no significant between-study heterogeneity under any genetic models (P>0.05). Thus, a fixed-effects model was selected to calculate ORs. Since the number of included studies was less than 6, sensitivity analysis was not performed.

Publication bias evaluation

Publication bias was evaluated by Begg's funnel plot and Egger's test. All funnel plots were visually symmetrical under the five genetic models, statistically validated by data from Egger's test [TT vs. GG: P=0.629; TT + GT vs. GG: P=0.141; TT vs. GG + GT: P=0.740; T vs. G: P=0.212 (**Figure 4**); GT vs. GG: P= 0.250]. Therefore, there was no apparent publication bias among eligible studies in the present meta-analysis.

Discussion

With an aging population and changes in lifestyles, prevalence rates of DM and its chronic complications have increased rapidly, especially in China and other developing countries in Asia. These major non-infectious diseases severely threaten public health [15, 16]. Chronic complications of DM, such as diabetic nephropathy, diabetic retinopathy, and DF have become the main causes of kidney failure, blindness, and amputation, respectively [17, 18]. DF is characterized by a long course and high disability, gravely reducing the life quality of patients [19]. Lower extremity arterial disease and/or neuropathy caused by long-term poor control over blood glucose in DM patients may be a key in the pathogenic mechanisms of DF. Despite rapid advancement in therapeutic methods, diabetic foot-related amputation still represents a leading cause of disability and even death in DM patients. Consequently, it is of great significance to ascertain the etiology of DF, thus reducing morbidity.

NO is a simple small molecule with a series of physiological regulating activity. It plays a vital

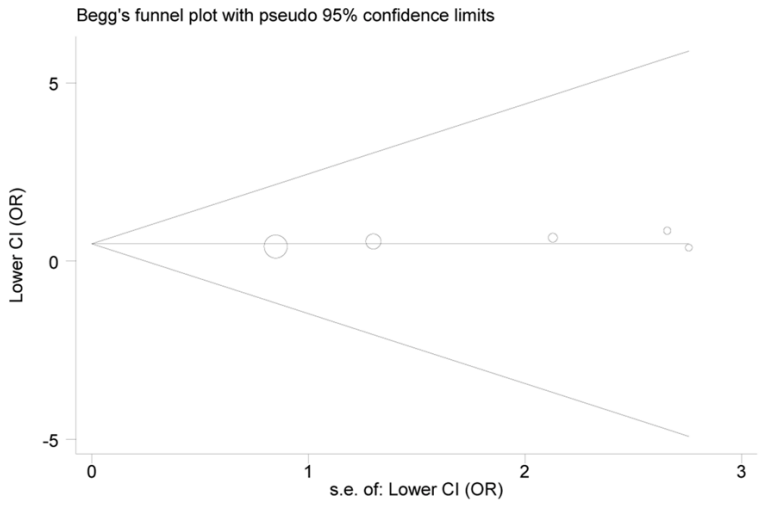

Figure 4. Begg's funnel plot for publication bias under the model of T vs. G. Each point represents a separate study for the indicated association. Log (OR), natural logarithm of OR. Horizontal line, mean effect size.

role in maintaining vascular tension and regulating blood pressure, which are endothelium-dependent. NO released by endothelial cells not only has strong effects on vasodilatation, but also inhibits platelet aggregation, proliferation of smooth muscle cells, monocyte adhesion, and adhesion molecule expression, preventing atherosclerosis and thrombosis [20]. NOS is the initial enzyme and rate-limiting enzyme for the synthesis of NO. Existing studies have shown that NO could not exert its normal physiological functions at advanced stages of DM, due to deficiency. This situation further contributes to vasomotor dysfunction and abnormal increase of platelet activity, leading to extensive microvascular lesions in certain tissues and aggravating diabetic complications [21]. In addition, decreased expression of *NOS3* has been found to be an important cause of endothelial dysfunction and neuropathy due to decreased blood flow to feet.

Previous investigations have demonstrated that single nucleotide polymorphisms (SNPs) in *NOS3* gene are closely related to occurrence of multiple diseases, such as coronary artery disease [22], myocardial infarction [23], hypertension [24], atherosclerosis [25], and diabetic nephropathy [26]. In recent years, researchers have explored the role of *NOS3* polymorphism rs1799983 in DF risk, but no consistent results have been obtained. For instance, Sadati et al. found that the T allele of this polymorphism might possess protective effects against DF in an Iranian population [11]. Conversely, a study by Yang et al. supported that SNP might be a risk factor for DF in Northern Chinese Han population [13]. However, another study involving 97 Turkish patients with diabetic foot ulcers and 102 controls reported that the polymorphism had no significant association with foot ulcers [12].

Multiple factors might have led to such discrepancies in these results. For example, the duration of DM might be significantly different between these studies. Besides, various genetic backgrounds may also be major contributors to these discrepancies. Additionally, these studies possessed uneven sample sizes, some of which only contained a relatively small number. Hence, it was essential to conduct a meta-analysis, obtaining a clearer perspective on the relationship between *NOS3* polymorphism rs1799983 and DF susceptibility. In the present research, 4 available publications from 2004 to 2018 were eventually embraced, containing a total of 454 cases and 535 controls in 5 independent studies. Overall results uncovered that *NOS3* polymorphism rs1799983 was noticeably associated with decreased risk of DF under TT vs. GG and TT vs. GG + GT. A similar correlation was observed in Caucasians and hospital-based subgroups under corresponding genetic comparisons after stratification analyses by ethnicity and source of control.

In the present meta-analysis, no significant heterogeneity existed between enrolled studies under any genetic models. Examination of publication bias revealed that the effects of bias on ultimate summarizes was negligible. However, some limitations to this work should be noted. First, this study only selected articles published in English or Chinese. Consequently, certain publication bias might have been introduced, though it was not statistically significant. Second, included data sets were only towards Caucasian and Asian populations. Thus, final results may have been less than representative of other ethnic groups. Third, further subgroup

analyses based on disease duration and other potentially relevant factors were not implemented due to the lack of original information.

In conclusion, the present meta-analysis offers statistical evidence supporting the protective effects of *NOS3* polymorphism rs1799983 against DF incidence, especially in Caucasians. Noting the shortcomings of this meta-analysis, present findings should be further verified by larger-scale studies involving multiple ethnic groups.

Acknowledgements

This work was supported by Beijing Municipal Science & Technology Commission No. Z141107002514181.

Disclosure of conflict of interest

None.

Address correspondence to: Zhangrong Xu, Department of Endocrinology, The 306th Hospital of PLA, Beijing 100101, China. E-mail: xzr1021@vip.sina.com

References

[1] Sinwar PD. The diabetic foot management - recent advance. Int J Surg 2015; 15: 27-30.
[2] Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA and Kirsner RS. Diabetic foot ulcers: part I. Pathophysiology and prevention. J Am Acad Dermatol 2014; 70: 1, e1-18.
[3] van Acker K, Leger P, Hartemann A, Chawla A and Siddiqui MK. Burden of diabetic foot disorders, guidelines for management and disparities in implementation in Europe: a systematic literature review. Diabetes Metab Res Rev 2014; 30: 635-645.
[4] Sekhar MS, Thomas RR, Unnikrishnan MK, Vijayanarayana K and Rodrigues GS. Impact of diabetic foot ulcer on health-related quality of life: a cross-sectional study. Semin Vasc Surg 2015; 28: 165-171.
[5] Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, Armstrong DG, Woo K, Boeni T, Ayello EA and Kirsner RS. Diabetic foot ulcers: part II. management. J Am Acad Dermatol 2014; 70: 21.e21-24.
[6] Noor S, Zubair M and Ahmad J. Diabetic foot ulcer-A review on pathophysiology, classification and microbial etiology. Diabetes Metab Syndr 2015; 9: 192-199.
[7] Craig J and Martin W. Dominance of flow-mediated constriction over flow-mediated dilatation in the rat carotid artery. Br J Pharmacol 2012; 167: 527-536.
[8] Li H, Horke S and Forstermann U. Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis 2014; 237: 208-219.
[9] Ning WH and Zhao K. Propionyl-L-carnitine induces eNOS activation and nitric oxide synthesis in endothelial cells via PI3 and Akt kinases. Vascul Pharmacol 2013; 59: 76-82.
[10] Grutzmacher C, Park S, Zhao Y, Morrison ME, Sheibani N and Sorenson CM. Aberrant production of extracellular matrix proteins and dysfunction in kidney endothelial cells with a short duration of diabetes. Am J Physiol Renal Physiol 2013; 304: F19-30.
[11] Sadati SM, Radfar M, Hamidi AK, Abdollahi M, Qorbani M, Esfahani EN and Amoli MM. Association between the polymorphism of Glu-298Asp in Exon 7 of the eNOS gene with foot ulcer and oxidative stress in adult patients with type 2 diabetes. Can J Diabetes 2018; 42: 18-22.
[12] Corapcioglu D, Sahin M, Emral R, Celebi ZK, Sener O and Gedik VT. Association of the G894T polymorphism of the endothelial nitric oxide synthase gene with diabetic foot syndrome foot ulcer, diabetic complications, and comorbid vascular diseases: a Turkish case-control study. Genet Test Mol Biomarkers 2010; 14: 483-488.
[13] Yang N, Wang J and Yang A. The relationship between eNOS gene polymorphisms and diabetic foot in Northern Chinese Han population. Shandong Medical Journal 2004; 44: 46-48.
[14] Ma J, Yu D and Liu D. The relationship between the G894T mutation of the endothelial- constitutive nitric oxide synthase (ec-NOS) and type 2 diabetic microangiopathies. Chin J Diabetes 2007; 15: 471-473.
[15] Unnikrishnan R, Anjana RM and Mohan V. Diabetes mellitus and its complications in India. Nat Rev Endocrinol 2016; 12: 357-370.
[16] Demicheva TP and Shilova SP. [The epidemiologic characteristic of diabetes mellitus and its complications]. Probl Sotsialnoi Gig Zdravookhranenniiai Istor Med 2013; 19-21.
[17] Hippisley-Cox J and Coupland C. Diabetes treatments and risk of amputation, blindness, severe kidney failure, hyperglycaemia, and hypoglycaemia: open cohort study in primary care. BMJ 2016; 352: i1450.
[18] Kelly PJ, Clarke PM, Hayes AJ, Gerdtham UG, Cederholm J, Nilsson P, Eliasson B and Gudbjornsdottir S. Predicting mortality in people with type 2 diabetes mellitus after major complications: a study using swedish national diabetes register data. Diabet Med 2014; 31: 954-962.
[19] Morey-Vargas OL and Smith SA. Be smart: strategies for foot care and prevention of foot

complications in patients with diabetes. Prosthet Orthot Int 2015; 39: 48-60.
[20] Jensen BO, Kleppe R, Kopperud R, Nygaard G, Doskeland SO, Holmsen H and Selheim F. Dipyridamole synergizes with nitric oxide to prolong inhibition of thrombin-induced platelet shape change. Platelets 2011; 22: 8-19.
[21] Tessari P. Nitric oxide in the normal kidney and in patients with diabetic nephropathy. J Nephrol 2015; 28: 257-268.
[22] Yang Y, Du K, Liu Z and Lu X. Endothelial nitric oxide synthase (eNOS) 4b/a gene polymorphisms and coronary artery disease: evidence from a meta-analysis. Int J Mol Sci 2014; 15: 7987-8003.
[23] Zigra AM, Rallidis LS, Anastasiou G, Merkouri E and Gialeraki A. eNOS gene variants and the risk of premature myocardial infarction. Dis Markers 2013; 34: 431-436.
[24] Ali A, Alghasham A, Ismail H, Dowaidar M and Settin A. ACE I/D and eNOS E298D gene polymorphisms in Saudi subjects with hypertension. J Renin Angiotensin Aldosterone Syst 2013; 14: 348-353.
[25] Heidari MM, Khatami M, Hadadzadeh M, Kazemi M, Mahamed S, Malekzadeh P and Mirjalili M. Polymorphisms in NOS3, mthfr, APOB and TNF-alpha genes and risk of coronary atherosclerotic lesions in iranian patients. Res Cardiovasc Med 2016; 5: e29134.
[26] Chen F, Li YM, Yang LQ, Zhong CG and Zhuang ZX. Association of NOS2 and NOS3 gene polymorphisms with susceptibility to type 2 diabetes mellitus and diabetic nephropathy in the Chinese Han population. IUBMB Life 2016; 68: 516-525.