Review Article

Genetic polymorphisms associated with diabetic foot ulcer: A review article

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Abstract

Diabetic foot ulcer (DFU) or Lower limb ulcers are one of the major complications caused by diabetes mellitus especially when patients fail to maintain tight glycemic control. DFU is linked to multiple risk factors along with the genetic factors and ethnicity which play a significant role in the development of DFUs through their effects on multiple aspects of the pathophysiological process. This narrative review aimed to summarize all the previous studies within the last ten years associating gene polymorphism and DFU. Polymorphism associated with vascular endothelial growth factor (rs699947), the G894T polymorphism of the endothelial nitric oxide synthase gene, interleukin-6–174 G>C gene polymorphism, heat shock protein 70 gene polymorphism, the apolipoprotein E gene polymorphism, Sirtuin 1 (sirt1) polymorphisms (rs12778366 and rs3758391), hypoxia-inducible factor -1 alpha exon 12 mutation, toll-like receptor gene (thr399ile polymorphism), the effect of both monocyte chemoattractant protein-1 (MCP-1) –2518A/G and the vascular endothelial growth factor (VEGF) –634g/c polymorphisms were summarized in this review. The results of all these studies indicating that screening for Polymorphisms might be helpful for early screening and prevention of DFU through their regulatory function on the transcription activity of the genes. Additional studies should be conducted in larger and different populations and ethnic regions to confirm the results of all previous studies mentioned in this review.

***Keywords*:** Diabetic foot ulcer, diabetes, single nucleotide polymorphism

Introduction

Diabetes mellitus (DM) is a group of metabolic disorders which are caused by insulin secretion and/or action defects (Association, 2010). Diabetes is associated with chronic hyperglycemia that leads to many complications, dysfunction, and even failure of several organs such as eyes, kidneys, nerves, heart, and blood vessels (Logan et al., 2018). Tight glycemic control has a pivotal role in reducing diabetic complications although many diabetic patients believe that anti-diabetic medications had poor ability to achieve good glycemic control (Hussein et al., 2017). One of the major complications associated with uncontrolled diabetes mellitus is diabetic foot ulcer (DFU) or Lower limb ulcers which may lead to amputation in up to 90% of cases (Ahmad et al., 2014). DFU is related to several risk factors which include the duration of diabetes, blood glucose levels, age of the patient, peripheral nerve damage, defect in vascular circulation, blood pressure, and smoking (Tapp et al., 2003).

Genetic factors and ethnicity also play a significant role in the development of DFUs through their effects on multiple aspects of the pathophysiological process (Lamont et al., 2013). DFUs involve complex organic processes and wound healing can be promoted or inhibited depending on a variety of molecular and genetic components (Jhamb et al., 2016).

Recently, microRNAs (miRNAs) which are non-coding small RNAs that negatively control gene expressions displayed an important role in the regulation of gene expression in various cells of the skin, including stem cells, immune cells, and keratinocytes (Xu et al., 2014). Moreover, several miRNAs were shown to be differentially expressed in diabetic skins and their expression levels varied during the wound healing process (Madhyastha et al., 2012). For instance, miRNA-126 depletion causes defective endothelial cell proliferation, migration, and angiogenesis contributed by elevated levels of miRNA-503 which is specially grown in diabetes resulting in impaired wound healing via persistent

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ischemia (Madhyastha et al., 2012).

Other proteins that play a key role in the innate immune system in diabetic patients with DFUs are toll-like receptors (TLR) which are down regulated in T2DM wounds compared to control wounds (Ey et al., 2009).

Additionally, persistent toll-like type 2 receptors (TLR2) signaling in diabetic patients may lead to hyper inflammation in the wound microenvironment and may lead to the development of high-grade chronic wounds (Dasu et al., 2014). Whereas Polymorphism of growth factors especially Vascular endothelial growth factor (VEGF) is suggested as an effective target for the management of diabetic foot complications (Mu et al., 2009). This narrative review aimed to summarize all the previous studies within the last ten years associating gene polymorphism and DFU.

Methods

The two authors survey Google scholar and PubMed sites for any previous studies within the last ten years associating between gene polymorphism and DFU.

Types of polymorphisms associated with DFU

Vascular endothelial growth factor polymorphism (rs699947)

Vascular endothelial growth factor (VEGF), also known as vascular permeability factor (VPF), is produced by many cells such as macrophages, T cells, retinal pigment epithelium, and pericytes (Tavakkoly-Bazzaz et al., 2010).

The location of the VEGF gene is on chromosome 6p21.3, and it includes 8 exons and 7 introns (Sudhesan et al., 2017). According to Ratnasari et al (2017), there are at least 25 different VEGF polymorphisms.

Two single nucleotide polymorphisms (SNPs) in VEGF gene like Rs699947 (-2578C/A) and rs13207351 (-125A) polymorphisms were located in the promoter region of VEGF gene (Sellami et al., 2018).

These polymorphisms were found to be associated with DFU formation and progression (Li et al., 2018). In addition, a significant association was found between the VEGF polymorphism (rs699947) and DFU In an Iraqi study that investigated the molecular basis of the SNP (rs699947) and its correlation with biochemical parameters, such as glycated hemoglobin (HbA1c) and glutathione (GSH). This study showed that the A/C genotype especially the C allele significantly raised the risk of DFU (Ahmed et al., 2020). According to this study; the alteration in the VEGF sequence due to polymorphism led to an increase in the oxidative stress and decrease in antioxidant glutathione (GSH) which lead to an increase in the risk for DFU (Ahmed et al., 2020). Another association was found between the VEGF polymorphism at position -2578*C/A and DFU located at the minor allele (A) and conferred a protective effect from DFU (Kuo et al., 2016). Another case-control study in Jakarta investigate the vascular endothelial growth factor gene +405 C>G and -460 C>T polymorphism revealed that there was no significant relationship between VEGF + 405 C>G and VEGF-460 C> T genes polymorphism with DFU in patients with DM (Zhuang et al., 2017). Actually, the results showed that G and T alleles have as a potential protective role against the existence of DFU in Indonesian diabetic patients (Zhuang et al., 2017).

The G894T Polymorphism of the Endothelial Nitric Oxide Synthase Gene

Two studies explained the role of Nitric Oxide (NO) in wound healing (BOYKIN and JV, 1999; Jude et al., 1999).

NO is produced by endothelial nitric oxide synthase (eNOS) which is expressed by vascular endothelium and plays a key role in local vascular homeostasis (Deng and Rapp, 1995). polymorphisms in the eNOS gene located on chromosome 7q35–36 and consists of 26 exons covering 21 kb can influence NO production(Deng and Rapp, 1995). It was found that the healing of the DFU may be delayed if the production of NO increases (Kanetsuna et al., 2007). A study by Bivalacqua et al (2003) found that the G894T polymorphism in the 7th exon of the eNOS gene can cause a structural change in eNOS protein by amino acid substitution (Glu298Asp) (Bivalacqua et al., 2003). Another study by Demet et al, that measured the association between G894T polymorphism of the eNOS gene and DFU, in Turkish diabetic patients, concluded that there was no significant association between G894T polymorphism and DFU. This finding suggested that eNOS gene polymorphisms may not be associated with early-onset diabetic microangiopathy (Corapcioglu et al., 2010).

Another study that aimed to evaluate the influence of both eNOS G894T and VEGF C936T gene polymorphism in DFU patients also considered that the gene polymorphism of eNOS G894T is not a risk factor for DFU formation (Erdogan et al., 2018). This study found that although the T allele is a risk factor for diabetes existence, but T allele is not a risk factor for DFU formation, while The gene polymorphism of VEGF C936T and T allele are not risk factors for diabetes happening or DFU development (Erdogan et al., 2018).

Interleukin-6–174 G>C gene Polymorphism

Interleukin-6 (IL-6) as Proinflammatory cytokines play an important role in the inflammatory and autoimmune processes (Polska et al., 2010). IL-6 is involved in the

pathogenesis and complication of type two diabetes (Lukic et al., 2014) and effects on microvascular blood flow (microvascular dysfunction) (Guo et al., 2014)

One study by Erdogan et al aimed to investigate the relationship between Interleukin (IL)-6 -174 G>C Gene Polymorphism and the development of diabetic foot ulcers in Turkish type 2 diabetic patients concluded that even though an IL-6 gene promoter -174 G>C polymorphism could represent a possible genetic marker to predict an individual's susceptibility to type 2 diabetes, but could not predict its association with diabetic foot ulcer development (Erdogan et al., 2017).

Heat Shock Protein 70 Gene Polymorphism

The main functions of the Heat Shock Proteins (HSPs) are acting as a carrier for immunogenic peptides that are presented on antigen-presenting cells (APC) to cytotoxic T cells in addition to its action as an activator for the innate immune system (Srivastava et al., 1994), additionally its act as a recognition structure for natural killer (NK) cells (Multhoff, 2002).

The Three members of the HSP70 gene family (HSP70-1, HSP70-2, and HSP70-hom) are located in the major histocompatibility complex (MHC) class III region in humans specifically between complement (~150 kb) and TNF genes (~300 kb) (Sargent et al., 1989; Milner and Duncan Campbell, 1990).

Two coding polymorphs have been discovered in the HSP70-hom gene (Milner and Campbell, 1990). The first one lies within a NcoI restriction site and involve T to C transition at nucleotide 2437 (resulting in a Met-493-Thr variation), (Milner and Campbell, 1992), while the second coding polymorphism is known as (Glu602Lys variation) is G to A transition at nucleotide 2763 of HSP70-hom (Jenkins et al., 2000) , located at 10 kDa domain associated with the regulation of substrate binding resulting in a glutamic acid to lysine alteration at position 602 in the C-terminal domain of HSP70-hom (Mir et al., 2009).

One prospective cohort study in North Indian populations with type2 DM was conducted to investigate the potential role of C2437T (Met493Thr) SNP of the heat shock protein (HSP) 70 in diabetic foot ulcer patients (Zubair and Ahmad, 2018). The findings of the study indicated that T/T genotype is significantly and positively associated with predispositions to foot ulcerations. The findings further explained that there was a higher plasma concentration of HSP70 in diabetic patients suffering from DFU (Zubair and Ahmad, 2018).

Monocyte chemoattractant protein-1 (MCP-1) –2518A/G and vascular endothelial growth factor (VEGF) –634G/C polymorphisms

The monocyte chemoattractant protein-1 (MCP-1) is a chemokine which is also named chemokine (C-C motif) ligand 2 (CCL2) that function as an activator for monocytes, macrophages, and lymphocytes (Dong et al., 2014).

Some diseases like clear-cell renal cell carcinoma, cerebral ischemic stroke, coronary artery disease can cause abnormal expression of MCP-1 (Akdoğan et al., 2015; Bonifačić et al., 2016; Yang et al., 2016). Hyperglycemia can cause abnormal expression in addition to an increase in the production of MCP-1 in vascular endothelial cells which contribute to the complications related to angiogenesis and vascular events among T2DM patients (Dong et al., 2014).

Numerous studies have revealed that the polymorphisms of MCP-1 –2518A/G may influence the production of MCP-1 (Jiang et al., 2016; Rovin et al., 1999), although the effects of MCP-1 –2518A/G polymorphism and its association with MCP-1 level had been rarely reported among patients with DFU (Rovin et al., 1999).

One study explored the presence of monocyte MCP-1 –2518A/G and VEGF –634G/C polymorphisms in type 2 diabetic patients with DFU. Moreover, the study evaluated the effects of these 2 polymorphisms on serum levels of MCP-1 and VEGF in all participants (Li, 2018).

The results revealed a significant but different distribution of MCP-1 –2518A/G and VEGF –634C/G polymorphisms between T2DM and DFU patients. Also, the genotypes of the 2 studied polymorphisms may influence serum levels of MCP-1 and VEGF in DFU patients (Li, 2018). The detected polymorphisms of the genes may play important roles in the occurrence and progression of DFU through their regulatory function on the transcription activity of the genes (Li, 2018).

The apolipoprotein E gene polymorphism

The gene of apolipoprotein E (ApoE or APOE), performs a crucial role in lipid metabolism (Vauhkonen et al., 1997).

The most important polymorphism in (APOE) is (e2, e3, and e4) in exon 4, which affects plasma lipoprotein concentrations. ApoE polymorphisms have been suggested as one of the risk factors for the development of diabetic complications (47). Several studies have explored the relationship between ApoE isoforms and diabetic complications. Nevertheless, the results of most of these studies were inconclusive (Araki et al., 2000).

One retrospective study on 50 Type 2 diabetic Turkish patients investigates the association between APOE gene polymorphism in the development of DFU (Mehmet et al., 2016).

The results showed that although the gene polymorphism of ApoE and specifically the E3 allele are a risk factor for diabetes, gene polymorphism of ApoE is not an independent risk factor for the diabetic foot. They attributed the lack of

association with ethnic differences (Mehmet et al., 2016).

Sirtuin 1 (SIRT1) polymorphisms (rs12778366 and rs3758391)

Sirtuin 1 protein (SIRT1), also known as NAD-dependent deacetylase sirtuin-1, in humans is encoded by the SIRT1 gene which is located at chromosome 10q21.3 (Sun et al., 2007).

SIRT1 expression is reduced in the case of high insulin resistance and results in reduced insulin sensitivity (Sun et al., 2007). Neurons' functions of neurons could be altered by SIRT1by limiting the calorie intake of these neurons (Srivastava and Haigis, 2011). SIRT1 is associated with oxidative stress and is down-regulated in patients with T2DM (Srivastava and Haigis, 2011). The suppression of SIRT1 expression accelerates the inflammation in patients with T2DM (Peng et al., 2018). Moreover, SIRT1 enhances the survival of endothelial cells (Jiang et al., 2015). Polymorphisms in the SIRT1 gene might alter the expression or function of it, then contribute to different disorders, such as neural or vascular lesions (Figarska et al., 2013).

According to a case-control study in the Chinese Han, Beijing population, two SNPs, rs12778366 and rs3758391, which located in the promoter region of the SIRT1 gene with the minor allele frequencies more than 0.1. were explored to detect their association with DFU susceptibility (Peng et al., 2018). The study concluded that the T allele of the SIRT1 gene (rs12778366) could lower individual susceptibility to DFU in the Chinese Han population. Although SIRT1 rs12778366 and rs3758391 polymorphisms had no significant impact on SIRT1 expression and DFU severity (Peng et al., 2018).

Hypoxia Inducible Factor -1 Alpha Exon 12 Mutation

Both of hypoxia-inducible factors (HIF) HIF-1α and HIF-1β are regulated under hypoxic conditions (Mole et al., 2009). HIF-1α polymorphism includes the substitution of Proline to Serine (P582S C-T, rs11549465) in DFU has been reported to be associated with DFU (Pichu et al., 2015). One study that intended to verify if the HIF-1α gene polymorphisms have an association with protein expression in 529 diabetic patients with and without DFU (Pichu et al., 2018). The results showed that there is a significant association in HIF-1α polymorphisms with DFU when compared to that of diabetes and control subjects (Pichu et al., 2018). Furthermore, the study showed that there is a diminished circulatory expression of HIF-1α on DFU when compared to that of T2DM and control subjects (Pichu et al., 2018). The reduced expression level of HIF-1α on DFU suggested a possible mechanism for the pathogenesis of diabetic complications in DFU (Pichu et al., 2018).

Toll-Like Receptor Gene (Thr399Ile polymorphism)

Toll-like receptors (TLR) are a wide group of transmembrane pattern recognition receptors (PRRs) (Kaisho and Akira, 2006). TLRs play a key role in the enhancement of the innate immune response due to their expression on sentinel cells such as macrophage and dendritic cells, which in turn identify the invading pathogen conserved molecules or endogenous damage signals and induces the innate immune response (Kaisho and Akira, 2006). TLR type 4 (TLR4) is a protein encoded by the TLR4 gene and involved in the pathogenic process of type-2 diabetes, via stimulation of a chronic sub-clinical inflammatory process, which additionally triggers dysfunction of the pancreatic B cells (Turin and Riva, 2008).

Ligand-binding capacity with TLR4 may be disturbed by SNPs in the extracellular domain of TLR4 that leads to deregulation of the TLR4 signaling modifying the risk of chronic inflammation, thus prolonging wound healing (Liu et al., 2016).

The SNP named Thr399Ile (rs4986791), affect the TLR4 mediated effector functions by different means. These SNPs can reduce the binding efficiency of TLR4 with its endogenous and exogenous ligands (Arbour et al., 2000). Furthermore, it might reduce the extracellular buildup of functional TLR4 thereby causing poor TLR4 signaling in response to microbial infection (Singh et al., 2013). The effect of TLR 4 polymorphism was studied in a case-control study included 120 Iraqi patients by Al-karawi et al. (2019) that assessed whether Thr399Ile polymorphism in TLR-4genes is related to DFU in a sample of Iraqi patients having type 2 diabetes mellitus (T2DM).

The results revealed that theThr399Ile polymorphisms were not associated with a diabetic foot ulcer in type 2 Iraqi diabetic patients (Al-Karawi et al., 2019). Asp299Gly (rs4986790) and Thr399Ile (rs4986791) SNPs affect the TLR4 mediated effector functions in a variety of ways like reducing the binding efficiency of TLR4 with its endogenous and exogenous ligands (Singh et al., 2013), or reduce the extracellular accumulation causing inadequate TLR4 signaling in response to microbial infection (Prohinar et al., 2010). Three more SNPs, namely, rs11536858 (now merged into rs10759931), rs1927911, and rs1927914 of the TLR4 gene were also reported to be associated with inflammatory diseases including cancer (Song et al., 2009).

One study evaluated the association of TLR4 SNPs (Asp299Gly rs4986790, Thr399Ile rs4986791, rs11536858 merged into rs10759931, rs1927911, and rs1927914) with DFU risk in patients with T2DM. The results illustrated that TLR4 SNPs and their haplotypes may increase the risk of impairment of wound healing in T2DM patients (Singh et al., 2013).

Vitamin D receptor gene FokI variant

Most antioxidant vitamins protect against DNA damage induced by reactive oxygen species (ROS) (Pati et al., 2015). Vitamin D antioxidant activity is related to its ability to modulate free radical formation and upregulate glutathione peroxidase and superoxide dismutase in OS (Hashemi Taheri et al., 2015).

Vitamin D nuclear receptor is transcribed from a gene located in chromosome 12q13 (Uitterlinden et al., 2004)

A single nucleotide polymorphism of the vitamin D receptor (VDR) gene is named FokI (rs2228570 CNT), in the exon 2, comprising of a T allele to C altered located in a start codon (ATG) and at the presence of the C variant this lead to a different start position resulting in a synthesis of a protein with a different size (Valdivielso and Fernandez, 2006).

FokI polymorphism is the only mutation that is particularly translated into two distinct VDR protein products. Besides; it is the only polymorphism within the VDR gene that is not linked to the other VDR variants, and thus it might have a unique role (Neyestani et al., 2013).

According to Zhong et al (Zhong et al., 2015), FokI polymorphism is significantly linked with diabetic complications. The T allele of FokI polymorphism was shown to be correlated with increased risk of Diabetic Retinopathy (DR) and has been considered as a biomarker that might predict the risk of retinopathy in Chinese patients with T2DM.

Moreover, according to a meta-analysis study in the Caucasian population FokI polymorphism has a role in the level of susceptibility to Diabetic Nephropathy (DN) (Yang et al., 2017). A single study investigated the association between vitamin D receptor (VDR) gene FokI polymorphism and DFU in the Iranian population as well as its correlation with biomarkers for oxidative stress (Soroush et al., 2017). The results showed a significantly elevated occurrence of the T allele of VDR gene FokI polymorphism among Iranian patients with DFU. Hence, this allele might be used as a candidate biomarker in preventive medicine to reduce the risk of this life-threatening complication of diabetes (Soroush et al., 2017).

Conclusions

Several risk factors that depend on a variety of molecular and genetic components can lead to DFU. Improved understanding and knowledge of the genomic basis that increases the risk for DFU or interferes with the healing process will permit the development of targeted therapies specific to each patient. Screening for Polymorphisms might be helpful for early screening and prevention of DFU through their regulatory function on the transcription activity of the genes. Additional studies should be conducted in larger and different populations and ethnic regions to confirm the results of all previous studies mentioned in this review.

References

Ahmad N, Thomas GN, Gill P, Chan C, Torella F. 2014. Lower limb amputation in England: prevalence, regional variation and relationship with revascularisation, deprivation and risk factors. A retrospective review of hospital data. Journal of the Royal Society of Medicine, 107(12):483–9. doi: 10.1177/0141076814557301.

Ahmed ST, Ewadh MJ, Jeddoa ZMA. 2020. The association of vascular endothelial growth factor polymorphism (rs699947) with diabetic foot ulcer and oxidative status. Gene Reports, 19: 100606. doi: 10.1016/j.genrep.2020.100606.

Akdoğan MF, Azak A, Denizli N, Huddam B, Koçak G, Gücün M, et al. 2015. MCP-1 and soluble TWEAK levels are independently associated with coronary artery disease severity in patients with chronic kidney disease. Renal Failure, 37(8):1297–302.

Al-Karawi FN, Al-Hasnawi ATN, Al-Kashwan TAJ. 2019. Role of toll-like receptor gene polymorphisms in patients with type 2 diabetes and diabetic foot ulcer. Indian Journal of Public Health Research and Development, 10(6):1254–1257. doi: 10.5958/0976-5506.2019.01466.9.

Araki S, Moczulski DK, Hanna L, Scott LJ, Warram JH, Krolewski AS. 2000. APOE polymorphisms and the development of diabetic nephropathy in type 1 diabetes: results of case-control and family-based studies. Diabetes, 49(12):2190-5. doi: 10.2337/diabetes.49.12.2190.

Arbour NC, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. 2000. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. Nature Genetics, 25(2):187-91. doi: 10.1038/76048.

Association AD. 2010. Diagnosis and classification of diabetes mellitus. Diabetes Care. American Diabetes Association, S62–S69. doi: 10.2337/dc10-S062.

Bivalacqua TJ, Usta MF, Champion HC, Kadowitz PJ, Hellstrom WJ. 2003. Endothelial dysfunction in erectile dysfunction: role of the endothelium in erectile physiology and disease. Journal of Andrology, 24(6 Suppl): S17-37.

Bonifačić D, Toplak A, Benjak I, Tokmadžić VS, Lekić A, Kučić N. 2016. Monocytes and monocyte chemoattractant protein 1 (MCP-1) as early predictors of disease outcome in patients with cerebral ischemic stroke. Wien Klin Wochenschr, 128(1-2):20-7. doi: 10.1007/s00508-015-0878-4.

Boykin JV, Shawler LG, Sommer VL, Crossland MC, Kalns JE 1999. Diabetes-impaired wound healing predicted by urinary nitrate assay: A preliminary, retrospective study. Wounds, 11: 62–69.

Corapcioglu D, Sahin M, Emral R, Celebi ZK, Sener O, Gedik VT. 2010. 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. Genetic Testing and Molecular Biomarkers, 14(4):483-8. doi: 10.1089/gtmb.2010.0023.

Dasu MR, Ramirez SR, La TD, Gorouhi F, Nguyen C, Lin BR, Mashburn C, Stewart H, Peavy TR, Nolta JA, Isseroff RR. 2014. Crosstalk between adrenergic and toll-like receptors in human mesenchymal stem cells and keratinocytes: a recipe for impaired wound healing. STEM CELLS Translational Medicine 3(6):745-59. doi: 10.5966/sctm.2013-0200.

Deng AY, Rapp JP. 1995. Locus for the inducible, but not a constitutive, nitric oxide synthase cosegregates with blood pressure in the Dahl salt-sensitive rat. Journal of Clinical Investigation. American Society for Clinical Investigation, 95(5): 2170–2177. doi: 10.1172/JCI117906.

Dong L, Lv XY, Wang BJ, Wang YQ, Mu H, Feng ZL, Liu P. 2014. Association of monocyte chemoattractant protein-1 (MCP-1)2518A/G polymorphism with proliferative diabetic retinopathy in northern Chinese type 2 diabetes. Graefe's Archive for Clinical and Experimental Ophthalmology, 252(12):1921-6. doi: 10.1007/s00417-014-2651-1.

Erdogan M, Kulaksizoglu M, Solmaz S, Berdeli A. 2017. The relationship of Interleukin-6 -174 G>C gene polymorphism in type 2 diabetic patients with and without diabetic foot ulcers in Turkish population. Foot (Edinb). 30:27-31. doi: 10.1016/j.foot.2017.02.001.

Erdogan M, Kulaksizoglu M, Tetik A, Solmaz S, Kucukaslan AS, Eroglu Z. 2018. The relationship of the endothelial nitric oxide synthase (eNOS) and vascular endothelial growth factor (VEGF) gene polymorphism in Turkish type 2 diabetic patients with and without diabetic foot ulcers. Foot (Edinb). 37:5-10. doi: 10.1016/j.foot.2018.06.006.

Ey B, Eyking A, Gerken G, Podolsky DK, Cario E. 2009. TLR2 mediates gap junctional intercellular communication through connexin-43 in intestinal epithelial barrier injury. Journal of Biological Chemistry, 284(33):22332-43. doi: 10.1074/jbc.M901619200.

Figarska SM, Vonk, JM, Boezen HM. 2013. SIRT1 Polymorphism, Long-Term Survival and Glucose Tolerance in the General Population', PLoS ONE. Edited by B. Mittal. Public Library of Science, 8(3):e58636. doi: 10.1371/journal.pone.0058636.

Guo F, Dong M, Ren F, Zhang C, Li J, Tao Z, Yang J, Li G. 2014. Association between local interleukin-6 levels and slow flow/microvascular dysfunction. Journal of Thrombosis and Thrombolysis, 37(4):475-82. doi: 10.1007/s11239-013-0974-0.

Hashemi Taheri AP, Radmard AR, Kooraki S, Behfar M, Pak N, Hamidieh AA, et al. 2015. Radiologic resolution of malignant infantile osteopetrosis skeletal changes following hematopoietic stem cell transplantation. Pediatric Blood & Cancer, 27;62(9):1645–9.

Hussein EA, Kadhim DJ, Al-Auqbi TF. 2017. Belief About Medications Among Type 2 Diabetic Patients Attending the National Diabetes Center in Iraq. Iraqi Journal of Pharmaceutical Sciences, 26(2).

Jenkins SC, March RE, Campbell RD, Milner CM. 2000. A novel variant of the MHC-linked hsp70, hsp70-hom, is associated with rheumatoid arthritis. Tissue Antigens, 56(1):38-44. doi: 10.1034/j.1399-0039.2000.560105.x.

Jhamb S, Vangaveti VN, Malabu UH. 2016. Genetic and molecular basis of diabetic foot ulcers: Clinical review. Journal of Tissue Viability. 25(4): 229–236. doi: 10.1016/j.jtv.2016.06.005.

Jiang B, Jen M, Perrin L, Wertheim JA, Ameer GA. 2015. SIRT1 Overexpression Maintains Cell Phenotype and Function of Endothelial Cells Derived from Induced Pluripotent Stem Cells. Stem Cells and Development, 24(23):2740-5. doi: 10.1089/scd.2015.0191.

Jiang Z, Hennein L, Xu Y, Bao N, Coh P, Tao L. 2015. Elevated serum monocyte chemoattractant protein-1 levels and its genetic polymorphism is associated with diabetic retinopathy in Chinese patients with Type 2 diabetes. Diabetic Medicine, 33(1):84–90.

Jude EB, Boulton AJ, Ferguson MW, Appleton I. 1999. The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor beta 1. Diabetologia, 42(6):748-57.

Kaisho T, Akira S. 2006. Toll-like receptor function and signaling. Journal of Allergy and Clinical Immunology. Mosby, 979–987. doi: 10.1016/j.jaci.2006.02.023.

Kanetsuna Y, Takahashi K, Nagata M, Gannon MA, Breyer MD, Harris RC. 2007. Deficiency of Endothelial Nitric-Oxide Synthase Confers Susceptibility to Diabetic Nephropathy in Nephropathy-Resistant Inbred Mice. The American Journal of Pathology, 170(5):1473–84. doi: 10.2353/ajpath.2007.060481.

Kuo Y-R, Chien C-M, Kuo M-J, Wang F-S, Huang E-Y, Wang C-J. 2016. Endothelin-1 Expression Associated with Lipid Peroxidation and Nuclear Factor-κB Activation in Type 2 Diabetes Mellitus Patients with Angiopathy and Limb Amputation. Plastic and Reconstructive Surgery, 137(1):187e–195e. http://dx.doi.org/10.1097/prs.0000000000001886.

Lamont P, Franklyn K, Rayman G, Boulton AJM. Update on the Diabetic Foot 2012. The International Journal of Lower Extremity Wounds, 12(1):71–5. http://dx.doi.org/10.1177/1534734613476519

Li X. 2018. The association between MCP-1, VEGF polymorphisms and their serum levels in patients with diabetic foot ulcer. Medicine, 97(24):e10959. http://dx.doi.org/10.1097/md.0000000000010959

Li X., Lu Y, Wei P. 2018. Association between VEGF genetic variants and diabetic foot ulcer in Chinese Han population: A case-control study. Medicine, 97(20). doi: 10.1097/MD.0000000000010672.

Liu Y, Gan LN, Qin WY, Sun SY, Zhu GQ, Wu SL, Bao WB. 2016. Differential expression of Toll-like receptor 4 signaling pathway genes in Escherichia coli F18-resistant and - sensitive Meishan piglets. Polish Journal of Veterinary Sciences, 19(2):303-8. doi: 10.1515/pjvs-2016-0037.

Logan S, Pharaoh GA, Marlin MC, Masser DR, Matsuzaki S, Wronowski B, Yeganeh A, Parks EE, Premkumar P, Farley JA, Owen DB, Humphries KM, Kinter M, Freeman WM, Szweda LI, Van Remmen H, Sonntag WE. 2018. Insulin-like growth factor receptor signaling regulates working memory, mitochondrial metabolism, and amyloid-β uptake in astrocytes. Molecular Metabolism, 9:141-155. doi: 10.1016/j.molmet.2018.01.013.

Lukic L, Lalic NM, Rajkovic N, Jotic A, Lalic K, Milicic T, Seferovic JP, Macesic M, Gajovic JS. 2014. Hypertension in obese type 2 diabetes patients is associated with increases in insulin resistance and IL-6 cytokine levels: potential targets for an efficient preventive intervention. International Journal of Environmental Research and Public Health, 11(4):3586-98. doi: 10.3390/ijerph110403586.

Madhyastha R, Madhyastha H, Nakajima Y, Omura S, Maruyama M. 2012. MicroRNA signature in diabetic wound healing: promotive role of miR-21 in fibroblast migration. International Wound Journal, 9(4):355-61. doi: 10.1111/j.1742-481X.2011.00890.x.

Mehmet E, Zuhal E, Mustafa K, Soner S, Aslı T, Sevki C. 2016. The relationship of the apolipoprotein E gene polymorphism in Turkish Type 2 Diabetic Patients with and without diabetic foot ulcers. Diabetes and Metabolic Syndrome, 10(1 Suppl 1):S30-3. doi: 10.1016/j.dsx.2015.09.006.

Milner CM, Campbell RD. 1992. Polymorphic analysis of the three MHC-linked HSP70 genes. Immunogenetics. Springer-Verlag, 36(6): 357–362. doi: 10.1007/BF00218042.

Milner CM, Duncan Campbell R. 1990. Structure and expression of the three MHC-linked HSP70 genes', Immunogenetics, 32(4): 242–251. doi: 10.1007/BF00187095.

Mir KA, Pugazhendhi S, Paul MJ, Nair A, Ramakrishna BS. 2009. Heat-shock protein 70 gene polymorphism is associated with the severity of diabetic foot ulcer and the outcome of surgical treatment. British Journal of Surgery, 96(10):1205-9. doi: 10.1002/bjs.6689.

Mole DR, Blancher C, Copley RR, Pollard PJ, Gleadle JM, Ragoussis J, Ratcliffe PJ. 2009. Genome-wide association of hypoxia-inducible factor (HIF)-1alpha and HIF-2alpha DNA binding with expression profiling of hypoxia-inducible transcripts. Journal of Biological Chemistry, 284(25):16767-75. doi: 10.1074/jbc.M901790200.

Mu H, Zhang XM, Liu JJ, Dong L, Feng ZL. 2009. Effect of high glucose concentration on VEGF and PEDF expression in cultured retinal Müller cells. Molecular Biology Reports, 36(8):2147-51. doi: 10.1007/s11033-008-9428-8.

Multhoff G. 2002. Activation of natural killer cells by heat shock protein 70', International Journal of Hyperthermia 576–585. doi: 10.1080/0265673021000017109.

Neyestani TR, Djazayery A, Shab-Bidar S, 2013. Vitamin D Receptor Fok-I polymorphism modulates diabetic host response to vitamin D intake: need for a nutrigenetic approach. Diabetes Care, 36(3):550-556. doi:10.2337/dc12-0919

Pati S, Pilia M, Grimsley JM, Karanikas AT, Oyeniyi B, Holcomb JB, Cap AP, Rasmussen TE. 2015.Cellular Therapies in Trauma and Critical Care Medicine: Forging New Frontiers. Shock. 44(6):505-23. doi: 10.1097/SHK.0000000000000482.

Peng J, Zhou Y, Deng Z, Zhang H, Wu Y, Song T, Yang Y, Wei H, Peng J. 2018. miR-221 negatively regulates inflammation and insulin sensitivity in white adipose tissue by repression of sirtuin-1 (SIRT1). Journal of Cellular Biochemistry, 119(8):6418-6428. doi: 10.1002/jcb.26589

Peng Y, Zhang G, Tang H, Dong L, Gao C, Yang X, Peng Y, Xu Y. 2018. Influence of SIRT1 polymorphisms for diabetic foot susceptibility and severity. Medicine, 97(28):e11455. doi: 10.1097/MD.0000000000011455.

Pichu S, Sathiyamoorthy J, Krishnamoorthy E, Umapathy D, Viswanathan V. 2015. Impact of the hypoxia inducible factor-1α (HIF-1α) pro582ser polymorphism and its gene expression on diabetic foot ulcers. Diabetes Research and Clinical Practice, 109(3):533-40. doi: 10.1016/j.diabres.2015.05.014.

Pichu S, Vimalraj S, Sathiyamoorthy J, Viswanathan V. 2018. Association of hypoxia inducible factor-1 alpha exon 12 mutation in diabetic patients with and without diabetic foot ulcer. International Journal of Biological Macromolecules, 119:833-837. doi: 10.1016/j.ijbiomac.2018.08.011.

Prohinar P, Rallabhandi P, Weiss JP, Gioannini TL. 2010. Expression of functional D299G.T399I polymorphic variant of TLR4 depends more on coexpression of MD-2 than does wild-type TLR4. Journal of immunology 184(8): 4362-7. doi:10.4049/jimmunol.0903142.

Ratnasari N, Nurdjanah S, Sadewa AH, Hakimi M, Yano Y. 2017. Difference of polymorphism VEGF-gene rs699947 in Indonesian chronic liver disease population. PLoS One, 12(8):e0183503.

Rovin BH, Lu, Saxena R. 1999 A novel polymorphism in the

MCP-1 gene regulatory region that influences MCP-1 expression. Biochemical and Biophysical Research Communications, 259(2): 344–348. doi: 10.1006/bbrc.1999.0796.

Sargent CA, Dunham I, Trowsdale J, Campbell RD. 1989. Human major histocompatibility complex contains genes for the major heat shock protein HSP70. Proceedings of the National Academy of Sciences of the United States of America, 86(6):1968-72. doi: 10.1073/pnas.86.6.1968.

Sellami N, Lamine LB, Turki A, Sarray S, Jailani M, Al-Ansari AK, Ghorbel M, Mahjoub T, Almawi WY. 2018. Association of VEGFA variants with altered VEGF secretion and type 2 diabetes: A case-control study. Cytokine, 106:29-34. doi: 10.1016/j.cyto.2018.03.003.

Siemińska L, Wojciechowska C, Kos-Kudła B, Marek B, Kajdaniuk D, Nowak M, Głogowska-Szelag J, Foltyn W, Strzelczyk J. 2010. Serum concentrations of leptin, adiponectin, and interleukin-6 in postmenopausal women with Hashimoto's thyroiditis. Polish Journal of Endocrinology 2010 61(1):112-6.

Singh K, Singh VK, Agrawal NK, Gupta SK, Singh K. 2013. Association of Toll-like receptor 4 polymorphisms with diabetic foot ulcers and application of artificial neural network in DFU risk assessment in type 2 diabetes patients. BioMed Research International, 2013:318686. doi: 10.1155/2013/318686.

Song J, Kim DY, Kim CS, Kim HJ, Lee DH, Lee HM, Ko W, Lee G. 2009. The association between Toll-like receptor 4 (TLR4) polymorphisms and the risk of prostate cancer in Korean men. Cancer Genetics and Cytogenetics, 190(2):88-92. doi: 10.1016/j.cancergencyto.2008.

Soroush N, Radfar M, Hamidi AK, Abdollahi M, Qorbani M, Razi F, Esfahani EN, Amoli MM. 2017. Vitamin D receptor gene FokI variant in diabetic foot ulcer and its relation with oxidative. stress. Gene. 599:87-91. doi: 10.1016/j.gene.2016.11.012.

Srivastava PK, Udono H, Blachere NE, Li Z. 1994. Heat shock proteins transfer peptides during antigen processing and CTL priming. Immunogenetics, 39(2): 93-8. doi: 10.1007/BF00188611.

Srivastava S, Haigis CM. 2011. Role of Sirtuins and Calorie Restriction in Neuroprotection: Implications in Alzheimers and Parkinsons Diseases. Current Pharmaceutical Design, 17(31): 3418–3433. doi: 10.2174/138161211798072526.

Sudhesan A, Rajappa M, Chandrashekar L, Ananthanarayanan PH, Thappa DM, Satheesh S, Chandrasekaran A. 2017. Vascular endothelial growth factor (VEGF) gene polymorphisms (rs699947, rs833061, and rs2010963) and psoriatic risk in South Indian Tamils. 78(10): 657-663. doi: 10.1016/j.humimm.2017.08.004.

Sun C, Zhang F, Ge X, Yan T, Chen X, Shi X, Zhai Q. 2007. SIRT1 improves insulin sensitivity under insulin-resistant conditions by repressing PTP1B. Cell Metabolism, 6(4):307-19. doi: 10.1016/j.cmet.2007.08.014.

Tapp RJ, Shaw JE, de Courten MP, Dunstan DW, Welborn TA, Zimmet PZ; AusDiab. 2003. Foot complications in Type 2 diabetes: an Australian population-based study. Diabetic Medicine, 20(2):105-13. doi: 10.1046/j.1464-5491.2003.00881.x.

Tavakkoly-Bazzaz J, Amoli MM, Pravica V, Chandrasecaran R, Boulton AJ, Larijani B, Hutchinson IV. 2010. VEGF gene polymorphism association with diabetic neuropathy. Molecular Biology Reports, 37(7):3625-30. doi: 10.1007/s11033-010-0013-6.

Turin, L, Riva F. 2008. Toll-like receptor family in domestic animal species, Critical Reviews in Immunology. 2008: 513–538. doi: 10.1615/critrevimmunol.v28.i6.30.

Uitterlinden AG, Fang Y, Van Meurs JB, Pols HA, Van Leeuwen JP. 2004. Genetics and biology of vitamin D receptor polymorphisms. Gene, 338(2):143-56. doi: 10.1016/j.gene.2004.05.014.

Valdivielso JM, Fernandez E. 2006. Vitamin D receptor polymorphisms and diseases. Clinica Chimica Acta, 2006: 1–12. doi: 10.1016/j.cca.2006.02.016.

Vauhkonen I, Niskanen L, Ryynänen M, et al. 1997. Divergent association of apolipoprotein E polymorphism with vascular disease in patients with NIDDM and control subjects. Diabetic Medicine, 14(9):748-756. doi: 10.1002/(SICI)1096-9136(199709)14:9<748::AID-DIA469>3.0.CO;2-N.

Xu J, Zgheib C, Hu J, Wu W, Zhang L, Liechty KW. 2014. The role of microRNA-15b in the impaired angiogenesis in diabetic wounds. Wound Repair and Regeneration, 22(5):671-7. doi: 10.1111/wrr.12217.

Yang L, Wu L, Fan Y, Ma J. 2017. Vitamin D receptor gene polymorphisms in association with diabetic nephropathy: A systematic review and meta-analysis. BMC Medical Genetics. 18(1): 1–8. doi: 10.1186/s12881-017-0458-8.

Yang Y, Zhai C, Chang Y, Zhou L, Shi T, Tan C, Xu L, Xu J. 2016. High expression of chemokine CCL2 is associated with recurrence after surgery in clear-cell renal cell carcinoma. Urologic Oncology: Seminars and Original Investigations, 34(5): 238.e19-238.e26. doi: 10.1016/j.urolonc.2015.11.026.

Zhong X, Du Y, Lei Y, Liu N, Guo Y, Pan T. 2015. Effects of vitamin D receptor gene polymorphism and clinical characteristics on risk of diabetic retinopathy in Han Chinese type 2 diabetes patients. Gene. 566(2): 212–216. doi: 10.1016/j.gene.2015.04.045.

Zhuang J, Li J, Yao Y, Jing B, Shan P, Han P, Wang H. 2017. VEGF gene promoter polymorphisms are associated with diabetic foot ulcer. Biomedical Research. 28(4): 1689-92.

Zubair M,and Ahmad J. 2018. Heat Shock Protein 70 Gene Single Nucleotide Polymorphism and Diabetic Foot Ulcer. Is There Any Relationship? Journal of Clinical Medicine, 7(8): 187. doi: 10.3390/jcm7080187.