


# Genetic variation in *TCF7L2* rs7903146 correlating with peripheral arterial disease in long-standing type 2 diabetes

Diabetes & Vascular Disease Research  
 January-February 2020: 1–4  
 © The Author(s) 2020  
 Article reuse guidelines:  
[sagepub.com/journals-permissions](http://sagepub.com/journals-permissions)  
 DOI: 10.1177/1479164119888475  
[journals.sagepub.com/home/dvr](http://journals.sagepub.com/home/dvr)  


Eu Jeong Ku<sup>1,2</sup> , Gun Woo Won<sup>3</sup>, Yong Hee Lee<sup>3</sup>,  
 Dong-Hwa Lee<sup>1,2</sup>, Hyun Jeong Jeon<sup>1,2</sup> and Tae Keun Oh<sup>1,2</sup>

## Abstract

**Aim:** The aim of this study was to investigate the association between the transcription factor 7-like 2 gene (*TCF7L2*) rs7903146 polymorphism and peripheral arterial disease in type 2 diabetes.

**Methods:** In total, 1818 Korean type 2 diabetes patients were enrolled from January 2013 to December 2017. Subjects were categorized into two groups according to their duration of type 2 diabetes: long ( $\geq 10$  years,  $n = 771$ ) and short ( $< 10$  years,  $n = 1047$ ) durations. A multivariate logistic regression model was used for assuming an additive effect on peripheral arterial disease for the presence of a variant allele in *TCF7L2* rs7903146.

**Results:** The frequency of the minor T-allele was 7.6% ( $n = 139$ ), and this allele was significantly associated with a 2.6-fold higher risk of peripheral arterial disease (odds ratio = 2.595, 95% confidence interval = 1.177–5.722,  $p = 0.018$ ) in patients exhibiting a long duration of type 2 diabetes ( $\geq 10$  years). This result was significant after adjusting for age, sex, body mass index, familial history of diabetes, smoking, duration of diabetes and laboratory measurements, which included glycated haemoglobin, fasting plasma glucose and lipid profiles. In patients with diabetes  $< 10$  years, there was no significant association between *TCF7L2* rs7903146 and peripheral arterial disease (odds ratio = 1.233, 95% confidence interval = 0.492–3.093,  $p = 0.655$ ).

**Conclusion:** Our results provide evidence that genetic variation in *TCF7L2* rs7903146 could increase risk for peripheral arterial disease in patients exhibiting long-standing type 2 diabetes.

## Keywords

Peripheral arterial disease, transcription factor 7-like 2 (*TCF7L2*), type 2 diabetes, genetic polymorphism

## Introduction

Peripheral arterial disease (PAD) contributes to reduced quality of life derived from intermittent claudication, morbidity from non-healing ulcers and a potential major amputation leading to significant disability.<sup>1</sup> Beyond local lower-extremity complications, PAD is strongly associated with higher risk of incident cardiovascular and cerebrovascular diseases, or even higher all-cause mortality.<sup>2</sup> The burden of PAD is significantly higher among patients with type 2 diabetes (T2D) as shown in the UK Prospective Diabetes Study [28% increased risk of PAD per 1% increase in glycated A1c (HbA1c) level].<sup>3</sup> Still, there is a relatively lack of active interventions to treat PAD compared to other cardiac or cerebral complications in T2D.

In a recently published genome-wide association study, 126 variants at four loci associated with risk of T2D were reported.<sup>4</sup> Among many T2D-related genes, the genetic variant of the transcription factor 7-like 2 (*TCF7L2*) gene

has been shown to be reproducibly associated with T2D in various ethnicities.<sup>5</sup>

To date, there is limited information regarding the association between *TCF7L2* rs7903146 polymorphism and PAD in patients with T2D. Therefore, the objective of this study was to evaluate the association between gene variant *TCF7L2* rs7903146 and prevalent PAD in the patients with long-standing T2D.

<sup>1</sup>Department of Internal Medicine, Chungbuk National University Hospital, Cheongju, Republic of Korea

<sup>2</sup>Department of Internal Medicine, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea

<sup>3</sup>Department of Biochemistry, College of Medicine, Chungbuk National University, Cheongju, Republic of Korea

### Corresponding author:

Tae Keun Oh, Department of Internal Medicine, College of Medicine, Chungbuk National University, Cheongju 28644, Republic of Korea.  
 Email: [tgohkjs@chungbuk.ac.kr](mailto:tgohkjs@chungbuk.ac.kr)



## Methods

### Subjects

In total, 1818 subjects with T2D who visited the Diabetes Centre at Chungbuk National University Hospital from January 2013 to December 2017 were recruited. T2D were diagnosed according to the criteria from the American Diabetes Association.<sup>6</sup> Exclusion criteria were a diagnosed type 1 diabetes, gestational diabetes, diabetes due to other causes and malignancies. For a better prediction of the risk for diabetic complications in subjects with T2D, we further classified all subjects into two groups: long duration of diabetes group ( $\geq 10$  years,  $n=771$ ) and short duration of diabetes group ( $< 10$  years,  $n=1047$ ) according to their age at diabetes diagnosis. PAD was defined as ankle-brachial index (ABI)  $\leq 0.9$  or previous history of lower extremity bypass and/or endovascular therapy. Details of the obtained clinical, laboratory data and measurement of ABI are presented in the Supplementary Methods. The study was conducted according to the principles of the revised Declaration of Helsinki, a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. All participants provided their written informed consent. All samples derived from the National Biobank of Korea were obtained with informed consent under institutional review board of Chungbuk National University Hospital-approved protocols (IRB No. 2019-04-026).

### TCF7L2 genotyping

Genomic DNA was isolated and stored at  $-20^{\circ}\text{C}$ . Genotyping master mix and fluorescent probes were used for variant detection by real-time polymerase chain reaction (PCR) system (Supplementary Methods). Frequency of the genotype was calculated to be in Hardy-Weinberg equilibrium ( $p=0.644$ ).

### Statistical analysis

We analysed the association between *TCF7L2* rs7903146 variant and PAD using following multivariate logistic regression models: model 1 unadjusted; model 2 adjusted for age, sex and body mass index (BMI); model 3 further adjusted for familial history of diabetes, smoking, laboratory measurements including HbA1c, and lipid profiles. Statistical analyses were performed with SPSS Statistics for Windows, version 24.0 (IBM Corp., Armonk, NY, USA) (Supplementary Methods).

## Results

### Baseline characteristics

The baseline characteristics of the subjects (994 males and 824 females) according to the *TCF7L2* rs7903146

polymorphism are summarized in Supplementary Table S1. The majority of the patients (1679; 92.4% of the total patients analysed) were identified with C/C genotype with the remaining 139 patients having either heterozygous C/T ( $n=137$  or 7.5% of the total patients) or homozygous T/T ( $n=2$  or 0.1% of the total patients) at the *TCF7L2* rs7903146 site. No significant difference was found in anthropometric and biochemical variables between groups.

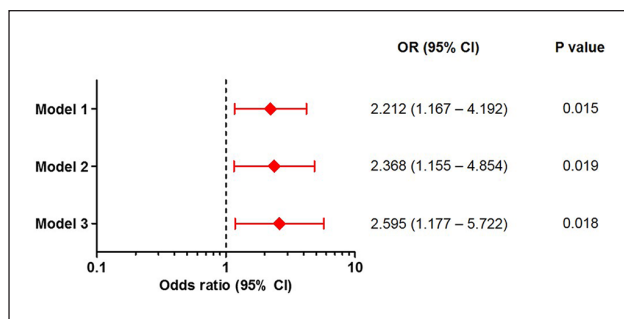
### Clinical risk factors for PAD

The prevalence of PAD was 10.7% ( $n=195$ ) in patients with T2D, and significantly higher ( $p < 0.008$ ) in patients with long ( $\geq 10$  years;  $n=100$  out of 771 patients or 13%) versus short ( $< 10$  years;  $n=95$  out of 1047 patients or 9.1%) durations. Supplementary Table S2 shows odds ratios (ORs) of PAD. Age [OR=1.047, 95% confidence interval (CI)=1.032–1.062], male (OR=1.670, 95% CI=1.223–2.280), presence of familial history of diabetes (OR=1.616, 95% CI=1.180–2.213), current smoking (OR=2.138, 95% CI=1.344–3.401) and comorbid hypertension (OR=2.536, 95% CI=1.801–3.572) were associated with PAD (all  $p < 0.05$ ). Serum total cholesterol ( $\geq 200$  mg/dL), triglyceride ( $\geq 250$  mg/dL) and low-density lipoprotein (LDL) cholesterol ( $\geq 100$  mg/dL) were also associated with PAD, with ORs of 1.866 (95% CI=1.166–2.986), 1.975 (95% CI=1.101–3.541) and 2.272 (95% CI=1.294–3.990), respectively (all  $p < 0.05$ ). However, overweight (BMI  $\geq 25$  kg/m<sup>2</sup>) and hyperglycaemia (HbA1c  $\geq 7.0\%$  or fasting plasma glucose  $\geq 126$  mg/dL) and lower high-density lipoprotein (HDL) cholesterol levels (male  $< 40$  mg/dL, female  $< 50$  mg/dL) showed no significant association with PAD.

### Association between the *TCF7L2* polymorphism rs7903146 and PAD

Overall, the distribution of the genotypes in the patients with PAD was as follows: C/T or T/T ( $n=20$  patients or 14.4%) and C/C ( $n=175$  patients or 10.4%) (OR=1.444, 95% CI=0.877–2.379,  $p=0.149$ ) (Supplementary Table S3). In patients with a long duration of diabetes ( $\geq 10$  years,  $n=771$ ), the PAD in patients with T/T or C/T genotypes was more prevalent at 23.3% ( $n=14/60$  patients) compared to 12.1% patients with C/C genotype ( $n=86/711$  patients) ( $p=0.015$ ). However, patients with a shorter duration of diabetes and exhibiting PAD were found to have a non-significant difference ( $p=0.410$ ) comparing the possessing either T/T or C/T genotypes (7.6% of the population or  $n=6/79$ ) versus the C/C genotype (9.2% or  $n=89/968$ ) (Supplementary Table S3).

Figure 1 shows univariate and multivariate analyses for the association between *TCF7L2* rs7903146 polymorphism and PAD, and the strongest association was observed with PAD in the subgroup with long duration of diabetes (Figure 1). The risk allele (T) *TCF7L2* rs7903146 showed



**Figure 1.** Odds ratio of peripheral arterial disease for *TCF7L2* polymorphism rs7903146 for patients with long duration of diabetes ( $\geq 10$  years) ( $n=771$ ). Data are expressed as ORs and 95% confidence intervals. Multivariate logistic regression models were adjusted for the following covariates: model 1, unadjusted; model 2: age, sex and body mass index; and model 3: adjusted for the same covariates of model 2 plus presence of familial history of diabetes, smoking, glycated haemoglobin, total cholesterol and triglycerides.

a highly significant association with PAD (model 1, unadjusted OR=2.212, 95% CI=1.167–4.192,  $p=0.015$ ), even after adjustment of age, sex, BMI (model 2, adjusted OR=2.368, 95% CI=1.155–4.854,  $p=0.019$ ). After further adjustment for presence of familial history of diabetes, smoking habit, laboratory measurements including HbA1c, and lipid profiles (model 3), the results remained unchanged, showing that *TCF7L2* T-allele of rs7903146 was strongly associated with PAD (adjusted OR=2.595, 95% CI=1.177–5.722,  $p=0.018$ ).

## Discussion

In this study, we identified that the specific allele variant, rs7903146, from the *TCF7L2* gene was significantly associated with PAD in only patients with long-standing diabetes more than 10 years in duration after adjusting well-known cardiometabolic risk factors.

Since a primary clinical feature of PAD shows atherosclerotic change in the mid-to-small arteries, we hypothesized that genetic variation of *TCF7L2* gene rs7903146 could be associated with increased risk of PAD in patients with T2D. The *TCF7L2* gene is located on chromosome 10 and encodes the transcription factor *TCF7L2* protein.<sup>7</sup> This protein can influence the Wnt signalling pathway as a  $\beta$ -catenin transcriptional partner.<sup>5</sup> Several SNPs in the *TCF7L2* gene have shown association with increased risk of T2D and gestational diabetes mellitus.<sup>5</sup> Of particular interest in the specific role of *TCF7L2* appeared to be involved in the remodelling process of blood vessels. This could be explained by the recent possible mechanism of Wnt signalling pathway regulating vascular smooth muscle cell differentiation.<sup>8</sup> Transcriptional activation of *TCF7L2* also activates the nuclear factor- $\kappa$ B signalling pathway, which regulates inflammatory response.<sup>9</sup>

According to numerous large-scale genome-wide association studies (GWAS), *TCF7L2* was associated with reduced beta-cell function and diabetes has been reported.<sup>10</sup> More importantly, *TCF7L2* SNP rs7903146 has been identified as the most significant variant associated with diabetes (40% increased risk of diabetes per allele).<sup>11</sup> In terms of the association between rs7903146 and PAD, the Atherosclerosis Risk in Communities (ARIC) study did not significantly identify an association with the prevalence of PAD.<sup>12</sup> The difference in our findings may likely be due to the heterogeneous population of Black (27.1%) and White (72.9%) patients in the ARIC study compared to a more uniform racial group in this study. In addition to racial differences from our study, *TCF7L2* SNP might be difficult to have a clear impact in groups with a population with normal glucose tolerance.

Several limitations of this study could be considered. Study subjects were primarily Korean T2D patients. Because of the genetic differences among ethnicity, future studies will need to examine the importance of racial/ethnic differences with this specific SNP. Second, PAD does not occur at the early stage of diabetes, so it can be challenging to obtain sufficient number of PAD group in patients with a shorter duration of diabetes.

## Conclusion

This study indicates that the *TCF7L2* SNP, rs7903146, is associated with a higher prevalent PAD in patients with a long duration of T2D. We believe that this additional new information about the genetic association with this specific variant and PAD in patients with long duration of T2D will contribute to a better understanding and development of strategic therapeutic protocols in the treatment of this special population.

## Authors' Note

The part of our data was presented as a poster exhibition at the 55th EASD Annual Meeting of the European Association for the Study of Diabetes. The authors thank the staff and participants of National Biobank of Korea in Chungbuk National University Hospital for their important contributions. The biospecimens for this study were provided by the Chungbuk National University Hospital, a member of the National Biobank of Korea, which is supported by the Ministry of Health, Welfare and Family Affairs. T.K.O. and E.J.K. conceived and designed the study. Y.H.L. and G.W.W. conducted the experiment. T.K.O. conducted all data quality control measures. E.J.K. conducted statistical analyses and drafted the manuscript. T.K.O., E.J.K., H.J.J. and D.-H.L. have contributed discussion of our study findings. All authors read and approved the final version of the manuscript.

## Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

## Funding

The author(s) disclosed receipt of the following financial support for the research, authorship and/or publication of this article: E.J.K. was supported by research grant from the Chungbuk National University Hospital in 2019.

## ORCID iD

Eu Jeong Ku  <https://orcid.org/0000-0001-5533-4989>

## Supplemental material

Supplemental material for this article is available online.

## References

1. Forsythe RO, Brownrigg J and Hinchliffe RJ. Peripheral arterial disease and revascularization of the diabetic foot. *Diabetes Obes Metab* 2015; 17: 435–444.
2. Fowkes FG, Murray GD, Butcher I, et al. Ankle brachial index combined with Framingham Risk Score to predict cardiovascular events and mortality: a meta-analysis. *JAMA* 2008; 300: 197–208.
3. Adler AI, Stevens RJ, Neil A, et al. UKPDS 59: hyperglycemia and other potentially modifiable risk factors for peripheral vascular disease in type 2 diabetes. *Diabetes Care* 2002; 25: 894–899.
4. Fuchsberger C, Flannick J, Teslovich TM, et al. The genetic architecture of type 2 diabetes. *Nature* 2016; 536: 41–47.
5. Jin T and Liu L. The Wnt signaling pathway effector TCF7L2 and type 2 diabetes mellitus. *Mol Endocrinol* 2008; 22: 2383–2392.
6. American Diabetes Association. Classification and diagnosis of diabetes: standards of medical care in diabetes-2018. *Diabetes Care* 2018; 41: S13–S27.
7. Castrop J, van Norren K and Clevers H. A gene family of HMG-box transcription factors with homology to TCF-1. *Nucleic Acids Res* 1992; 20: 611.
8. Srivastava R, Zhang J, Go GW, et al. Impaired LRP6-TCF7L2 activity enhances smooth muscle cell plasticity and causes coronary artery disease. *Cell Rep* 2015; 13: 746–759.
9. Lamberti C, Lin KM, Yamamoto Y, et al. Regulation of beta-catenin function by the IκB kinases. *J Biol Chem* 2001; 276: 42276–42286.
10. Grant SF, Thorleifsson G, Reynisdottir I, et al. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; 38: 320–323.
11. Helgason A, Palsson S, Thorleifsson G, et al. Refining the impact of TCF7L2 gene variants on type 2 diabetes and adaptive evolution. *Nat Genet* 2007; 39: 218–225.
12. Bielinski SJ, Pankow JS, Folsom AR, et al. TCF7L2 single nucleotide polymorphisms, cardiovascular disease and all-cause mortality: the Atherosclerosis Risk in Communities (ARIC) study. *Diabetologia* 2008; 51: 968–970.