A genome-wide association study suggested that the mitogen-activated protein kinase 14 gene (MAPK14) is associated with diabetic foot ulcer

DR WEIHUA MENG (Orcid ID : 0000-0001-5388-8494)

Article type : Original Article

A genome-wide association study suggested that the mitogen-activated protein kinase 14 gene (*MAPK14*) is associated with diabetic foot ulcer

W. Meng,[1] A. Veluchamy,[1] H.L. Hébert,[1] A. Campbell,[1] H.M. Colhoun,[2] C.N.A. Palmer [3]

[1] Division of Population Health Sciences, Ninewells Hospital and School of Medicine, University of Dundee, Dundee, UK, DD2 4BF

[2] Institute of Genetics and Molecular Medicine，Western General Hospital，Crewe Road，University of Edinburgh, Edinburgh, UK，EH4 2XUT

[3] Centre for Pharmacogenetics and Pharmacogenomics, Ninewells Hospital and School of Medicine, University of Dundee, Dundee, UK, DD1 9SY

Correspondence: W.Meng, PhD, E-mail: (w.meng@dundee.ac.uk)

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/bjd.15787

Address: Division of Population Health Sciences, Ninewells hospital and School of Medicine, University of Dundee, Dundee, UK, DD2 4BF. Tel.: +44 1382383419; fax: +44 1382383802

Keywords: genetics, genome-wide association study, diabetes, diabetic foot ulcer, *MAPK14*, MAPK pathway.

Short title: a Genome-wide association study and diabetic foot ulcer

Funding statement: This study is funded by a Tenovus grant (2015-T15/40). The GoDARTS project was jointly funded by DIABETES UK and The Wellcome Trust.

Disclosures: The authors have no conflict of interest to declare.

What's already known about this topic?

The genetics of diabetic foot ulcer is poorly understood though it is considered as a complex disorder that genetics plays a role.

What does this study add?

We have performed a GWAS study which has suggested that a skin-related gene *MAPK14* was associated with diabetic foot ulcer with neuropathy evidence. This is the first GWAS study on diabetic foot ulcer. We also firstly calculated the narrow sense heritability of this disorder.

Summary

Background: Diabetic foot ulcer (DFU) is a devastating complication of diabetes.

Objectives: We aimed to identify genetic contributors of DFU based on a genome-wide association study approach using a Scottish diabetic cohort.

Methods: A genome-wide association approach was applied. A case was defined as a diabetic patient (type 1 or type 2) who had ever been recorded in the linked e-health records as having a foot ulcer (current or previous) in at least one foot as well as a positive result of the monofilament test in the longitudinal e-health records. A control in this study was defined as a diabetic individual (type 1 or type 2) who has never been recorded as having a foot ulcer in either foot in the linked e-health records and the monofilament test results of any foot was once recorded to be positive in the longitudinal e-health records.

Results: We have 699 DFU cases and 2,695 controls in the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) dataset. The lowest *P* value of rs80028505 (Chr6p21.31) in the *MAPK14* gene was 2.45×10^{-8}. The narrow-sense heritability of this phenotype is 0.06.

Conclusions: We suggest that the *MAPK14* gene is associated with DFU.

Diabetic foot ulcer (DFU) is a major and devastating complication of diabetes. According to the National Institute for Health and Care Excellence guideline 2015, DFU is defined as a localized injury to the skin and/or underlying tissue, below the ankle, in a person with diabetes.[1] It has been reported that around 25% of people with diabetes will develop DFU at some stage in their lifetime.[2] Although the majority of DFU cases (60%-80%) will heal without intervention or after treatment, 10%-15% of them will remain active and 5%-24% of all patients with DFU will eventually undergo a lower limb amputation.[3] In fact, DFU accounts for 85% of all lower limb amputations and 50% of bed occupancy in diabetic patients in the UK

are due to diabetic related foot problems.[4,5] The quality of life in patients with DFU, especially those with amputations, is significantly affected. These individuals normally have increased disability, high morbidity and higher mortality.[6] In addition, the cost of treating DFU is huge. It is estimated that £650 million is spent on foot ulcers or amputations each year by the National Health Service (NHS) in the UK.[1] This is equivalent to every 1 in £150 of the whole NHS cost.

Epidemiological studies have suggested multiple risk factors for DFU: diabetic neuropathy, peripheral vascular disease, biomechanical factors, previous foot ulceration, poor glycaemic control, longer duration of diabetes, smoking, ethnicity, retinopathy, nephropathy, insulin use, poor vision, age and male sex.[4] Among these, diabetic neuropathy has been indicated to be the strongest initiating factor for DFU. A study has shown that 63% of diabetic foot ulcers were due to peripheral sensory neuropathy.[7] This is followed by peripheral vascular disease which, while not suggested as a cause of ulceration alone, is usually found in combination with diabetic neuropathy and other factors.[8] Further research on epidemiological risk factors can provide great value in terms of disease prevention and treatment.

At the moment, the genetic mechanism of DFU is not clearly understood. It is assumed that DFU is a common complex disorder determined by both genetic and environmental factors. A previous gene study has suggested that rs699947 in the vascular endothelial growth factor (*VEGF*) gene was associated with DFU.[2] There is increasing evidence that epigenetic changes, referring to molecular modification to genes, can have an impact on the development of DFU through affecting the healing ability of tissues.[9] So far, there are no linkage studies which have reported genetic loci of DFU. In addition, no genome-wide association studies (GWAS) have been published so far on DFU. GWAS is a hypothesis-free genetic association study to identify genes for complex disorders based on phenotype information and genetic information of a population or a cohort.[10]

The purpose of this study was to use a GWAS approach to identify genetic variants for developing a DFU condition upon the presence of peripheral neuropathy, based on phenotype information and genetic information from a Scottish diabetic cohort. To our knowledge, this is the first GWAS in DFU.

Material and methods

Participants

The Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) project was established in 2005 to identify genetic risk factors for diabetes and its complications. Participants including diabetic and non-diabetic individuals are all required to complete a lifestyle questionnaire, a baseline clinical examination and provide their biological samples (blood and urine). All participants provided broad informed consent for their health information from the NHS and biological samples to be anonymously linked to the study for future scientific research. The linked health information includes their personal health status, their general practice clinic visits, outpatient appointments, prescribing history and hospital admissions. In addition, participants' personal information is anonymously linked with the Scottish Care Information-Diabetes Collaboration (SCI-DC) database, which is an electronic health record system specifically designed to provide clinical information, to support diabetic screening services and to provide data for national and local audit programmes. Further information about the GoDARTS project and SCI-DC database can be found in the public domain at (http://diabetesgenetics.dundee.ac.uk/ and http://www.sci-diabetes.scot.nhs.uk/) The research followed the tenets of the declaration of Helsinki. Tayside Committee on Medical Research Ethics (REC reference 053/04) has granted the ethics approval.

So far, 9,439 diabetic patients have been recruited by the GoDARTS project and 7,424 of them have been genotyped by DNA chips. All participants' health information was anonymously linked with their NHS and SCI-DC database records from June 1996 until June 2014.

Definitions of cases and controls

A case of DFU in this study was defined as a diabetic patient (type 1 diabetes (T1D) or type 2 diabetes (T2D)) who had ever been recorded in the linked e-health records as having a foot ulcer (current or previous) in at least one foot as well as a positive monofilament test result recorded in the longitudinal e-health records.

A control in this study was defined as a diabetic individual (T1D or T2D) who has never been recorded as having a foot ulcer in either foot in the linked e-health records while having a positive monofilament test result recorded in either foot in the longitudinal e-health records.

The monofilament test is a neurological test on diabetic patients to check their peripheral sensation.[11] During the test, a monofilament is pressed at 10 sites on both feet (5 sites each) with approximately 10 g of pressure for a short time (2 s). Absence of sensation in at least two out of five sites in one foot is a positive test, suggesting peripheral neuropathy.

Genotyping and quality control

Two sets of DNA chips were applied in the GoDARTS project to genotype its diabetic participants. The Affymetrix SNP6.0 chips (used on 3,884 subjects, Affymetrix, Santa Clara, CA, USA) were sponsored by the Wellcome Trust Case Control Consortium 2 (WTCCC2) project and the Illumina OmniExpress chips (used on 3,540 subjects, Illumina, Inc., San Diego, CA, USA) were funded by the Surrogate markers for Micro- and Macro-vascular hard endpoints for Innovative diabetes Tools (SUMMIT) project.[12,13] Both projects (WTCCC2 and SUMMIT) used standard genotyping quality-control protocols.[12,13]

Statistical analysis

Software SHAPEIT and IMPUTE2 were applied to impute non-directly genotyped single nucleotide polymorphisms (SNPs) using reference files from the 1000 genome phase I datasets.[14,15] Badly imputed SNPs were removed based on a cut-off value ($r^2 < 0.3$) suggested by IMPUTE2.

Standard quality control steps were frequently applied during data manipulation stages using PLINK, such as removal of individuals with more than 5% missing genotype data, SNPs with missing genotype of more than 5%, SNPs with less than 1% minor allele frequency and SNPs that failed Hardy–Weinberg tests ($P < 0.000001$).[16] SNPs on X, Y chromosomes and mitochondria were not routinely included. The multidimensional scaling (MDS) analysis integrated in PLINK was used to detect population stratification in the cohort. A lambda value (indicating the level of stratification and generated by MDS) should be very close to 1, suggesting minimum ancestry mixture. If two samples in the cohort have a pi-hat > 0.125 (indicating relatedness), then one of them was removed randomly from further association analysis. Logistic regression tests integrated in PLINK were used to generate association *P* values, adjusting for covariates including age, sex, body mass index (BMI), cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), HbA1c and duration of diabetes. A *P* value of less than 5×10^{-8} was considered to be genome-wide significant variants. The linkage disequilibrium (LD) among the top SNPs were also calculated by PLINK.

In this study we also used multiple GWAS related software for different purposes such as SNPnexus for SNP functional annotation, HaploView for generating Manhattan plots, LocusZoom for regional visualization and SNPEVG for a corresponding Q-Q plot to evaluate differences between cases and controls caused by potential confounders (e.g different genotyping laboratories or different DNA extraction methods).[17-20] The SPSS 22 software (IBM Corp., Armonk, NY, USA) was used to compare means of all covariates (except gender)

between cases and controls through independent sample t-tests. Gender difference was compared by chi-square test. The whole workflow is shown in Figure. 1.

We also calculated narrow sense heritability (or chip heritability, estimation of the phenotypic variance explained by the SNPs) based on common SNPs in both chips using GCTA software.[21]

Results

We have identified 914 samples with positive foot ulcer records and 6,460 samples without foot ulcer records from 7,424 diabetic patients with genetic information from the GoDARTS project. After applying monofilament test results, only 764 DFU cases and 3,174 controls with positive monofilament test results were suitable for further analysis based on the definitions used in this study. After removing related samples (543 samples) and population outliers (1 sample), we were left with a cleaned study population of 699 DFU cases (males=463, females=236, T2D samples=662, T1D samples=37) and 2,695 diabetic control individuals (males=1,453, females=1,242, T2D samples=2,584, T1D samples=111).

The prevalence of DFU in our case-control population was 20.6% (699/ (699+2,695)). The means of sex, age, BMI, cholesterol, triglycerides, HDL, LDL, HbA1c, duration of diabetes were compared between cases and controls. There were statistically significant differences in sex, LDL, HbA1c and duration of diabetes between cases and controls, while there was no significant difference in age, triglycerides, BMI, cholesterol and HDL (Table 1).

Overall, 6,706,850 genotyped and imputed SNPs were available for association analysis after standard quality control steps of genotyping and imputation. No further adjustment based on population stratification was applied since the lambda value is 1.007, indicating a homogeneous population. Logistic regression tests integrated in PLINK were then performed, adjusting for sex, age, BMI, cholesterol, triglycerides, HDL, LDL, HbA1c and duration of

diabetes. We identified that the SNP rs80028505 in the *MAPK14* gene, reached genome-wide significance with a lowest *P* value of 2.45×10^{-8} and an odds ratio of 1.71 (95% confidence interval: 1.41-2.06, Fig. 2) (Table 2). A cluster of SNPs in the *MAPK14* gene also showed GWAS significant *P* values ($P < 5 \times 10^{-8}$). The regional plot of the *MAPK14* region is shown in Figure. 3. We calculated the LD among these SNPs (top 10 SNPs with lowest *P* values) using our dataset and they are all highly correlated ($r^2 > 0.8$) (Supplementary Fig 1). The Q-Q plot of the association results is shown in the Supplementary Figure. 2. The narrow sense heritability of DFU (with neuropathy evidence) is 0.06, adjusting with all covariates. We also provided the GWAS results of DFU using T2D samples only to remove the influence brought by T1D samples (Supplementary Table 1).

Discussion

In Scotland, patients with diabetes are invited to attend an annual free foot screening and to have their feet checked by podiatrists.[22] The screening aims to identify diabetic foot complications at an early stage to prevent or delay serious consequences such as lower-limb amputation. During the screening, podiatrists not only record the clinical conditions of foot ulcers, if any (including area, size and depth), but also clinical characteristics which might be linked with DFU such as presence or absence of foot pulses, nerve sensation and vibration functions, previous ulceration history, significant structural foot deformity, presence of callus, amputation history and self-care ability. However, in the current version (June, 2014) of e-health records provided by SCI-DC to researchers, the detailed descriptions of ulcers such as area, size and depth are not available. DFU is categorized as current ulcer (left leg and right leg) and previous ulcer (left leg and right leg) in a longitudinal manner based on examination dates. This is the background of the DFU case definition used in this study.

To achieve a more homogeneous case and control definition, we further adapted positive monofilament test results (evidence of neuropathy) into the sample selection. As we know from the results, 150 DFU patients (n=914-764, 16.4% of 914 samples) did not have positive monofilament test results while having a positive foot ulcer record (current or previous). This may suggest that the underlying genetic mechanisms of DFU in these patients might not be the same as other DFU patients (n=764). This stringent definition reduced case numbers and the study power, but generated a more homogeneous case population. A similar approach has been successfully applied when defining diabetic neuropathic pain, which is another complication of diabetes (a case should not only have pain evidence provided by prescription records but also have neuropathy evidence provided by positive monofilament tests).[23] In terms of controls, 4,250 foot ulcer-free diabetic samples (n=7,424-3,174, 57% of 7424 samples) had negative monofilament results while 3,174 samples had positive monofilament results. Despite the control definition used in the study, we also tried to use the group of 4,250 samples as controls but no SNPs achieved GWAS significance in this study design (Supplementary Fig 3). This further illustrated the importance of defining a correct homogeneous control population. After removing related samples and population stratification outliers, our current GWAS answered one question: when cases and controls are likely to have diabetic neuropathy, which SNPs (or genetic components) contribute to foot ulceration in a diabetic population?

The prevalence of DFU (current and/or previous DFU) in our cohort was 20.6% (699/(699+2,695)). This is higher than general reported DFU prevalence of 5-7% in Caucasians.[24] It is mainly due to the fact that we adapted monofilament results into the case and control definitions. Especially, we removed a lot of samples (4,250) from the controls due to lack of neuropathy evidence. This step is necessary for a genetic study, though it is normally not required to estimate disease prevalence in a general epidemiological study. Furthermore, we also used previous foot ulceration history as part of the case definition to increase the case number.

We have identified the SNP rs80028505, which has achieved GWAS significance (P=2.45 x 10^{-8}, odds ratio=1.71). This SNP was supported by a cluster of nearby SNPs which also showed GWAS significant *P* values. The SNP cluster was in the mitogen-activated protein kinase 14 gene (*MAPK14*), which is a protein-coding gene located on chromosome 6. This gene is widely expressed in multiple organs including the skin and soft tissues.[25] The MAPK14 protein, an enzyme also called p38-α, is one of the four p38 MAPKs which play an essential role in the cascade of cellular responses evoked by extracellular stimuli such as pro-inflammatory cytokines or physical stress leading to direct activation of transcription factors.[26] Evidence from a diabetic mouse model has suggested that p38 MAPK was phosphorylated in the wounded skin and using a p38 MAPK inhibitor, the level of phosphorylation was significantly reduced and wound healing was accelerated. This was evidenced by reduced wound width, accelerated re-epithelialization, increased granulation and reduced inflammatory cell infiltration into the wound.[27] However, the effect of the MAPK pathway to wound healing was controversial in some studies. For example, activation of the MAPK pathway has been suggested to promote cell collective migration, a biological process involved in tissue formation and repair.[28] By applying a MAPK inhibitor to a diabetic rat wound model, the rate of wound healing was reported to be reduced by 20%.[29] It was also reported that MAPK inhibitors can reverse cutaneous wound healing effects in a non-diabetic mouse wounding model.[30] In fact, both acute and chronic wound healing abilities are impaired in diabetes and the MAPK pathway has been confirmed to be activated.[31-33] The MAPK pathway is also involved in other types of ulcers, such as venous ulcer,[34] gastric ulcer,[35,36] and corneal ulcer.[37] It is noticed that most SNPs in the *MAPK14* gene affect the *MAPK14* gene expression (P = 10^{-7}) according to the Genotype-Tissue Expression (GTEx) portal, particularly in skin.[38]

There was statistical difference in sex between cases and controls indicating gender is a risk factor for DFU. This is consistent with other studies suggesting that males are more likely to have DFU.[39]

We have moderate power for this GWAS study. Calculated by CaTS, we have 80% power based on 699 cases and 2,695 controls, assuming a minor disease allele frequency of 0.25, a genotypic relative risk of 1.40, a prevalence of DFU in the diabetic population of 20%, and significance level of 5×10^{-8}.[40] We also first reported that the narrow sense heritability of this phenotype was 0.06. This heritability excludes effects of gene-gene interaction, gene-environment interaction, etc.

When defining cases and controls in the study, we only considered neuropathy as evidenced by a positive monofilament test, since it is the strongest risk factor.[7] We did not consider characteristics such as the status of foot pulses which indicates the existence of peripheral vascular disease. This greatly decreased the complexity of defining samples and statistical analysis. For readers' interest, we also included the GWAS results of DFU using T2D samples only. We noticed that the *P* values of the top SNPs increased slightly. This was probably due to a reduced samples size. The reported SNP (rs699947) in the *VEGF* gene was not associated with DFU in our dataset (*P*=0.53).[2]

In conclusion, we propose that the *MAPK14* gene is associated with DFU in a Scottish diabetic cohort using a GWAS approach. Replication studies and functional studies of this gene will help to confirm its role in DFU and to provide insights to facilitate the treatment of DFU.

Acknowledgements

The authors of this manuscript would like to thank all the participants recruited in the GoDARTS study. We are grateful for the support from the Health Informatics Centre in the School of Medicine, University of Dundee, for their help in data linkage.

References:

1. Internal Clinical Guidelines team. *Diabetic Foot Problems: Prevention and Management.*
   London: National Institute for Health and Care Excellence (UK). 2015 Aug.
2. Amoli MM, Hasani-Ranjbar S, Roohipour N, et al. VEGF gene polymorphism association with diabetic foot ulcer. *Diabetes Res Clin Pract* 2011;**93**:215-9.
3. Alexiadou K, Doupis J. Management of diabetic foot ulcers. *Diabetes Ther* 2012;**3**:4.
4. Merza Z, Tesfaye S. The risk factors for diabetic foot ulceration. *The Foot* 2003;**13**:125-9.
5. Adler AI, Boyko EJ, Ahroni JH, et al. Lower-extremity amputation in diabetes. The independent effects of peripheral vascular disease, sensory neuropathy, and foot ulcers. *Diabetes Care* 1999;**22**:1029-35.
6. Sinha R, van den Heuvel WJ, Arokiasamy P. Factors affecting quality of life in lower limb amputees. *Prosthet Orthot Int* 2011;**35**:90-6.
7. Reiber GE, Vileikyte L, Boyko EJ, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. *Diabetes Care* 1999;**22**:157-62.
8. Boulton AJ. The pathogenesis of diabetic foot problems: an overview. *Diabet Med* 1996;**13**:S12-6.
9. Jhamb S, Vangaveti VN, Malabu UH. Genetic and molecular basis of diabetic foot ulcers: Clinical review. *J Tissue Viability* 2016;**25**:229-36.
10. McCarthy MI1, Abecasis GR, Cardon LR, et al. Genome-wide association studies for complex traits: consensus, uncertainty and challenges. *Nat Rev Genet.* 2008;**9**:356-369.
11. Booth J. Assessment of peripheral neuropathy in the diabetic foot. *J Tissue Viability* 2000;**10**:21-5.
12. GoDARTS and UKPDS Diabetes Pharmacogenetics Study Group; Wellcome Trust Case Control Consortium 2, Zhou K, et al. Common variants near ATM are

associated with glycemic response to metformin in type 2 diabetes. *Nat Genet* 2011;**43**:117-20.

13. Fagerholm E, Ahlqvist E, Forsblom C, et al. SNP in the genome-wide association study hotspot on chromosome 9p21 confers susceptibility to diabetic nephropathy in type 1 diabetes. *Diabetologia* 2012;**55**:2386-93.
14. Howie BN, Donnelly P, Marchini J. A flexible and accurate genotype imputation method for the next generation of genome-wide association studies. *PLoS Genet* 2009;5:e1000529.
15. Delaneau O, Marchini J, Zagury JF. A linear complexity phasing method for thousands of genomes. *Nat Methods* 2012;**9**:179-81.
16. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007;**81**:559-75.
17. Dayem Ullah AZ, Lemoine NR, Chelala C. A practical guide for the functional annotation of genetic variations using SNPnexus. *Brief Bioinform* 2013;**14**:437-47.
18. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;**21**:263-65.
19. Pruim RJ, Welch RP, Sanna S, et al. LocusZoom: regional visualization of genome-wide association scan results. *Bioinformatics* 2010;**26**:2336-7.
20. Wang S, Dvorkin D, Da, Y. SNPEVG: a graphical tool for GWAS graphing with mouse clicks. *BMC Bioinformatics* 2012;**13**:319.
21. Stang D. The Scottish Diabetes Foot Action Group: Delivering comprehensive screening services for people with diabetes in Scotland. May 2013. Available at http://patientsafety.health.org.uk/sites/default/files/resources/the_scottis_diabetes_foot_action_group.pdf (last accessed 22 December 2016)
22. Yang J, Benyamin B, McEvoy BP, et al. Common SNPs explain a large proportion of the heritability for human height. *Nat Genet* 2010;**42**:565-9.

23. Meng W, Deshmukh HA, van Zuydam NR, et al. A genome-wide association study suggests an association of Chr8p21.3 (GFRA2) with diabetic neuropathic pain. *Eur J Pain* 2015;**19**:392-9.
24. Walters DP, Gatling W, Mullee MA, et al. The distribution and severity of diabetic foot disease: a community study with comparison to a non-diabetic group. *Diabet Med* 1992;**9**:354-8.
25. https://www.ncbi.nlm.nih.gov/UniGene/ESTProfileViewer.cgi?uglist=Hs.485233
26. Waugh HV, Sherratt JA. Macrophage dynamics in diabetic wound dealing. *Bull Math Biol* 2006;**68**:197-207.
27. Medicherla S, Wadsworth S, Cullen B, et al. p38 MAPK inhibition reduces diabetes-induced impairment of wound healing. *Diabetes Metab Syndr Obes* 2009;**2:**91-100.
28. Nardini, JT, Chapnick, DA, Liu, X, et al. The effects of MAPK activity on cell-cell adhesion during wound healing. 2015; arXiv:1506.07081.
29. Lima MH, Caricilli AM, de Abreu LL, et al. Topical insulin accelerates wound healing in diabetes by enhancing the AKT and ERK pathways: a double-blind placebo-controlled clinical trial. *PLoS One* 2012;**7**:e36974.
30. Escuin-Ordinas H, Li S, Xie MW, et al. Cutaneous wound healing through paradoxical MAPK activation by BRAF inhibitors. *Nat Commun* 2016;**7**:12348.
31. Guo S, Dipietro LA. Factors affecting wound healing. *J Dent Res* 2010;**89**:219-29.
32. Igarashi M, Wakasaki H, Takahara N, et al. Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. *J Clin Invest* 1999;**103**:185-95.
33. Gogg S, Smith U, Jansson PA. Increased MAPK activation and impaired insulin signaling in subcutaneous microvascular endothelial cells in type 2 diabetes: the role of endothelin-1. *Diabetes* 2009;**58**:2238-45.

34. Raffetto JD, Vasquez R, Goodwin DG, et al. Mitogen-activated protein kinase pathway regulates cell proliferation in venous ulcer fibroblasts. *Vasc Endovascular Surg* 2006;**40**:59-66.

35. Chang X, Luo F, Jiang W, et al. Protective activity of salidroside against ethanol-induced gastric ulcer via the MAPK/NF-κB pathway in vivo and in vitro. *Int Immunopharmacol* 2015;**28**:604-15.

36. Jia YT, Wei W, Ma B, et al. Activation of p38 MAPK by reactive oxygen species is essential in a rat model of stress-induced gastric mucosal injury. *J Immunol* 2007;**179**:7808-19.

37. Terai K, Call MK, Liu H, et al. Crosstalk between TGF-beta and MAPK signaling during corneal wound healing. *Invest Ophthalmol Vis Sci* 2011;**52**:8208-15.

38. Carithers LJ, Ardlie K, Barcus M, et al. A Novel Approach to High-Quality Postmortem Tissue Procurement: The GTEx Project. *Biopreserv Biobank* 2015;**38**:311-9.

39. Al-Rubeaan K, Al Derwish M, Ouizi S, et al. Diabetic foot complications and their risk factors from a large retrospective cohort study. *PLoS One* 2015;10:e0124446.

40. Skol AD, Scott LJ, Abecasis GR, et al. Joint analysis is more efficient than replication-based analysis for two-stage genome-wide association studies. *Nat Genet* 2006;**38**:209-13.

Legends

Fig 2. The Manhattan plot of the GWAS on diabetic foot ulcer (699 cases and 2,695 controls)

SNPs with $P > 0.01$ were not included.

Fig 3. The regional plot of the *MAPK14* region in chromosome 6

Table 1 Clinical characteristics of diabetic foot ulcer cases and controls

Covariates	Cases	Controls	*P*
Sex (male:female)	463:236	1,453:1,242	**<0.001**
Age (years)	68.73±9.06	68.48±9.20	0.52
BMI* (kg/m^2)	31.22±5.15	31.35±5.41	0.54
Cholesterol (mmol/L)	4.31±0.82	4.37±0.84	0.06
Triglycerides (mmol/L)	2.29±1.33	2.19±1.26	0.09
HDL* (mmol/L)	1.35±0.33	1.36±0.34	0.37
LDL* (mmol/L)	2.00±0.60	2.07±0.63	**0.01**
HbA1c (mmol/L)	7.88±1.50	7.54±1.26	**<0.001**
Duration of diabetes (years)	21.31±9.00	18.10±8.12	**<0.001**

*BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein
A chi-square test was used to test the difference of gender frequency between cases and controls and an independent t test was used for other covariates.
Covariates were presented as mean ± standard deviation.

There were statistically significant differences in sex, LDL, HbA1c and duration of diabetes between cases and controls.

Table 2 Top 10 SNPs of the GWAS on the diabetic foot ulcer (cases N=699 vs controls N=2,695)

SNPID	Chromosome position (hg19)	Gene	Minor Allele	MAF in cases: controls	*P* value (no adjustment)	*P* value	OR±SE	Imputed or genotyped by
rs80028505	6:35998388	*MAPK14*	T	14.02%:8.99%	2.84×10^{-8}	2.45×10^{-8}	1.71±0.10	Imputed
rs16883819	6:35997768	*MAPK14*	T	13.97%:8.97%	2.92×10^{-8}	2.82×10^{-8}	1.70±0.10	Imputed
rs112201657	6:36019076	*MAPK14*	C	14.25%:9.20%	3.49×10^{-8}	2.91×10^{-8}	1.70±0.10	Imputed
rs3761980	6:35993906	*MAPK14*	C	13.88%:8.94%	4.01×10^{-8}	3.57×10^{-8}	1.69±0.09	Both chips
rs6932598	6:35999080	*MAPK14*	A	13.88%:8.94%	4.23×10^{-8}	3.76×10^{-8}	1.69±0.09	Imputed
rs60481532	6:35994942	*MAPK14*	T	13.90%:8.97%	4.43×10^{-8}	4.01×10^{-8}	1.69±0.09	Imputed
rs58390233	6:36005100	*MAPK14*	A	13.88%:8.96%	4.72×10^{-8}	4.20×10^{-8}	1.69±0.09	Imputed
rs2237096	6:36008002	*MAPK14*	A	13.88%:8.96%	4.72×10^{-8}	4.20×10^{-8}	1.69±0.09	Imputed
rs56715462	6:36011649	*MAPK14*	G	13.88%:8.96%	4.57×10^{-8}	4.43×10^{-8}	1.69±0.09	Imputed
rs61763101	6:35996413	*MAPK14*	T	13.88%:8.97%	4.85×10^{-8}	4.53×10^{-8}	1.69±0.09	Imputed

rs3761980 is also located in the *SLC26A8* gene，which has no evidence relating with skin.

rs3761980 and rs60481532 are located in the 5-upstream region of the *MAPK14* gene while other SNPs are located in the intronic regions of the gene.

SNP: single nucleotide polymorphism

MAF: minor allele frequency

OR: odds ratio

SE: standard error

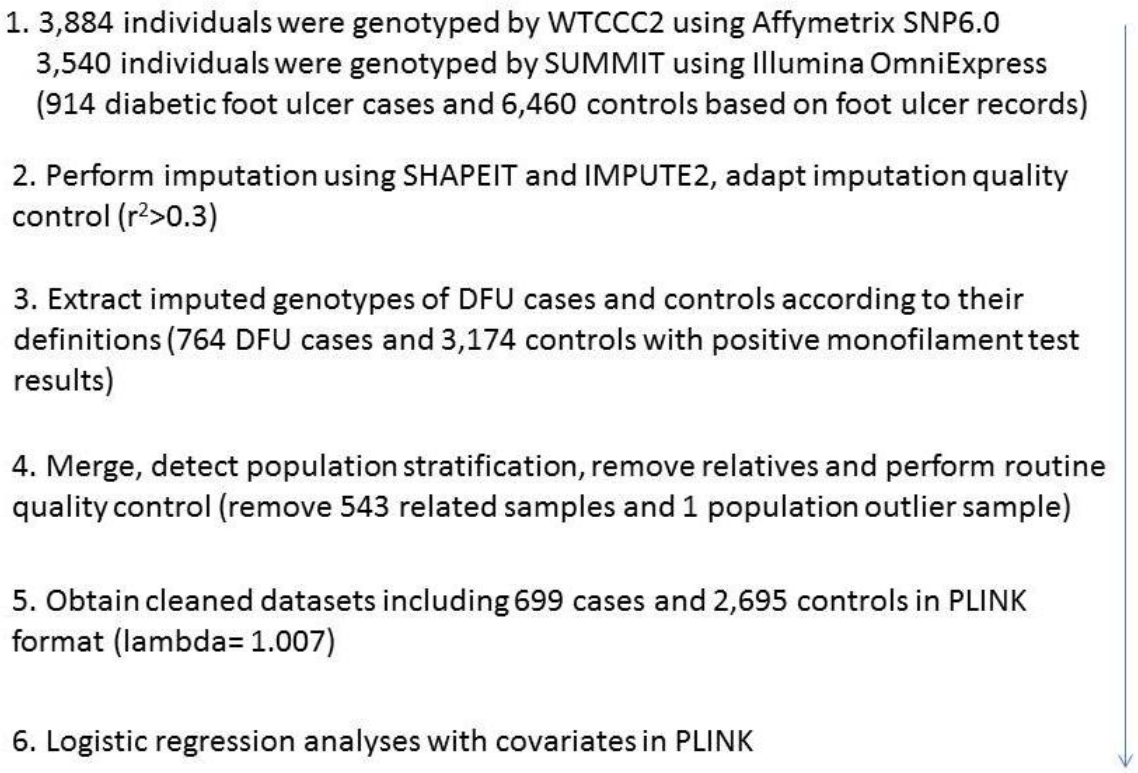

Fig 1. Workflow of the GWAS on diabetic foot ulcer in GoDARTS