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Published in:
Ophthalmic Genetics

DOI:
10.1080/13816810.2019.1633549

Publication date:
2019

Document Version
Peer reviewed version

Link to publication in Discovery Research Portal

Citation for published version (APA):
Meng, W., Chan, B., Ezeonwumelu, C., Hebert, H., Campbell, A., Soler, V., & Palmer, C. (2019). A genome-wide association study implicates that the TTC39C gene is associated with diabetic maculopathy with decreased visual acuity. *Ophthalmic Genetics*, *40*(3), 252-258. https://doi.org/10.1080/13816810.2019.1633549

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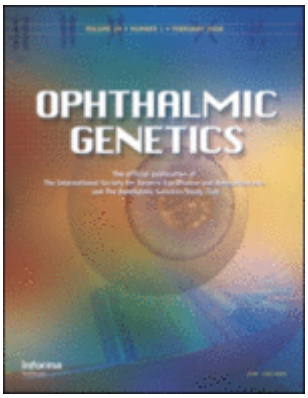

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Journal:	*Ophthalmic Genetics*
Manuscript ID	NOPG-2019-0055.R2
Manuscript Type:	Research Report
Date Submitted by the Author:	02-Jun-2019
Complete List of Authors:	Meng, Weihua; University of Dundee Medical Research Institute, Division of Population Health and Genomics Chan, Brian; University of Dundee Medical Research Institute, Division of Population Health and Genomics Ezeonwumelu, Chinenyenwa; University of Dundee Medical Research Institute, Division of Population Health and Genomics Hébert, Harry; University of Dundee Medical Research Institute, Division of Population Health and Genomics Campbell, Amy; University of Dundee Medical Research Institute, Division of Population Health and Genomics Soler, Vincent; Universite de Toulouse, Unité "Différenciation Epithéliale et Autoimmunité Rhumatoïde" Palmer, Colin; University of Dundee Medical Research Institute, Division of Population Health and Genomics
Keywords:	Diabetic Maculopathy, Visual Acuity, Genome-wide Association Study, TTC39C, Genetics

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A genome-wide association study implicates that the *TTC39C* gene is associated with diabetic maculopathy with decreased visual acuity

Ophthalmic Genetics

Weihua Meng[1] (ORCiD: 0000-0001-5388-8494), Brian W Chan[1](ORCiD: 0000-0002-4614-3483), Chinenyenwa Ezeonwumelu[1], Harry L Hébert[1], Amy Campbell[1], Vencent Soler[2], Colin NA Palmer[1]

[1] Division of Population Health and Genomics, Medical Research Institute, Ninewells Hospital and School of Medicine, University of Dundee, Dundee, UK, DD2 4BF;

[2] Retina unit, Ophthalmology department, Hôpital Pierre Paul Riquet, CHU Toulouse, 31059 Toulouse Cedex 9; Unité "Différenciation Epithéliale et Autoimmunité Rhumatoïde", UMR 1056 Inserm - Université de Toulouse;

Corresponding Author: Weihua Meng w.meng@dundee.ac.uk

Address: Division of Population Health and Genomics, School of Medicine, University of Dundee, Dundee, UK, DD2 4BF. Tel.: +44 1382383419; Fax: +44 1382383802

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ABSTRACT

Background: Diabetic maculopathy is a form of diabetic retinopathy. The visual acuity of one third of patients with diabetic maculopathy will be affected. The purpose of this study was to identify genetic contributors of diabetic maculopathy with decreased visual acuity based on a genome-wide association approach using a well-defined Scottish diabetic cohort.

Methods: We used linked e-health records of diabetic patients to define our cases and controls. The cases in this study were defined as type 2 diabetic patients who had ever been recorded in the linked e-health records as having maculopathy (observable or referable) in at least one eye and whose visual acuity of the eye was recorded to have decreased between the first and the last visual acuity record of that eye in the longitudinal e-health records. The controls were defined as a type 2 diabetic individual who had never been diagnosed with maculopathy or retinopathy in the linked e-health records. Anyone who had laser photocoagulation treatment was also excluded from the controls. A standard genome-wide association approach was applied.

Results: Overall, we identified 469 cases and 1,374 controls within the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) dataset. We found that the *P* value of rs9966620 in the *TTC39C* gene was 4.13×10^{-8}, which reached genome-wide significance.

Conclusions: We suggest that the *TTC39C* gene is associated with diabetic maculopathy with decreased visual acuity. This needs to be confirmed by further replication studies and functional studies.

KEYWORDS

Diabetic maculopathy; Visual acuity; Genome-wide association study; TTC39C; Genetics;

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INTRODUCTION

Maculopathy is defined as any pathological condition affecting the macula, a highly sensitive region located centrally on the retina which is responsible for sharp, clear, and accurate colour vision (1). It is commonly associated with old age, eye surgery/trauma and diabetes mellitus (1). Maculopathy commonly causes impaired vison but in severe cases can lead to blindness (2). According to a report by the World Health Organization in 2014, maculopathy was responsible for 3.1% of all visual impairment and 6.6% of all blindness in 2010 (2,3). Between 1990 – 2010, there was an 81% increase in the number of visually impaired people and a 36% increase in numbers of blind people due to maculopathy(2). Maculopathy and its subsequent visual disturbances are linked to many adverse health issues. One such example is falls, which (especially in geriatric patients) can result in fractures, subsequent reduction in quality of life, and an increased number of years spent with disability (4). The economic impact of maculopathy is enormous, with an estimated cost of over £100M in 2010 in England alone, incorporating aspects such as screening, management and home care (5).

Diabetic maculopathy (DM) is a type of diabetic retinopathy and it is a major eye complication and visual impairment amongst people with diabetes (2). According to the latest diabetic retinopathy guidelines by The Royal College of Ophthalmologists in the UK, DM can be classified as either focal oedema, diffuse oedema, ischemic, or mixed (6). An epidemiological study of DM in Germany showed a prevalence rate of 15% in type 1 and 23% in type 2 diabetic patients (7). Environmental risk factors of DM include longer duration of diabetes, high glycated haemoglobin (HbA1C) levels, prior high-risk proliferative diabetic retinopathy, presence of

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diabetic neuropathy, hypertension, anaemia, elevated serum lipid (triglycerides and cholesterol) levels and raised creatinine levels (7–9).

The genetic mechanism of DM is poorly understood and only limited numbers of genetic studies have been performed, particularly on diabetic macular oedema, a subtype of DM. One of these studies suggested that three polymorphisms in the nitric oxide synthase 3 (*NOS3*) gene were associated with an increased risk of developing diabetic macular oedema (10). The C-634G polymorphism in the vascular endothelial growth factor A (*VEGFA*) gene has also been demonstrated to be associated with development of diabetic macular oedema and diabetic retinopathy, in addition to correlating with macular retina thickness in type 2 diabetics (11). More recently, genetic variations in MicroRNA-146a and the vascular endothelial growth factor C (VEGFC) gene were found to be significantly associated with diabetic macular oedema in patients with type 2 diabetes (12,13). Whilst there have been a few genome-wide association studies (GWAS) performed on diabetic retinopathy across various ethnic groups, no GWAS have yet been performed specifically on DM (14–17). Graham PS et al. has performed a GWAS on diabetic macular oedema although no GWAS significance was generated. (18)

Given the fact that people are living longer with diabetes and the increasing prevalence of diabetic eye complications including maculopathy, it is necessary to understand the genetic mechanisms of DM as we seek new ways to relieve the burden of morbidity that DM creates worldwide. Therefore, this study seeks to identify genetic variants for DM using a GWAS approach in a well-defined diabetes cohort within Scotland.

MATERIALS AND METHODS

Participants

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To identify genetic risk factors for diabetes and its complications, the Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) project was established in 2005. All participants (including diabetic and non-diabetic individuals, mainly Scottish) completed a lifestyle questionnaire, a baseline clinical examination, and provided their biological samples (blood and/or urine). The participants also gave broad consent to allow their health information and biological samples to be used for future scientific research purposes. In addition, the participants gave permission to have their personal information linked to the National Health Service (NHS) medical records anonymously. This information included their personal health status, their general practice clinic visits, outpatient appointments, prescribing history and hospital admissions. Furthermore, their personal information was also anonymously linked with the Scottish Care Information-Diabetes Collaboration (SCI-DC) database, which is another electronic health record system designed to track local diabetic patients and help health professionals to provide better health care in Scotland. Further information about the GoDARTS project is available in the public domain at https://godarts.org. This study has followed the principles of the Declaration of Helsinki. Ethics approval has been granted by Tayside Committee on Medical Research Ethics (REC reference 053/04).

At the time of this study, the GoDARTS project had recruited 9,439 diabetic patients, 6,927 of which were already genotyped by DNA chips. All GoDARTS participants' health information was anonymously linked with their NHS and SCI-DC medical records from June 1996 until June 2011.

Definition of cases and controls

The cases in this study were defined as type 2 diabetic patients who had ever been recorded in the linked e-health records as having maculopathy (observable or referable) in at least one eye

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and whose visual acuity of the eye was recorded to have decreased between the first and the last visual acuity record of that eye in the longitudinal e-health records. The controls were defined as a type 2 diabetic individual who had never been diagnosed with maculopathy or retinopathy in the linked e-health records. Anyone who had laser photocoagulation treatment was also excluded from the controls. A standard genome-wide association approach was applied. The definition of visual acuity is summarized in Supplementary Table S1. The diagnosis of DM and assessment of visual acuity was performed by ophthalmologists within the annual national retinal screening service offered to diabetic patients. The diagnostic criteria for diabetic retinopathy or DM are summarized in the Supplementary Table S2.

Genotyping and quality control

The GoDARTS project used two types of DNA chips to genotype its participants with diabetes. The SNP6.0 (Affymetrix, Santa Clara, CA, USA) chips were used on 3,673 subjects, and the OmniExpress (Illumina, Inc., San Diego, CA, USA) chips were used on 3,254 subjects. The standard genotyping quality-control protocols were established for the above studies (WTCCC2 and SUMMIT) (19,20).

Statistical analysis

The imputation of non-directly genotyped single nucleotide polymorphisms (SNPs) was carried out using SHAPEIT and IMPUTE2 software, with reference files from the 1000 genome phase I datasets (21,22). The cut-off value ($r^2 < 0.3$) recommended by IMPUTE2 was applied to remove poorly imputed SNPs.

Standard quality control steps were applied during data analysis, such as removal of individuals with more than 5% genotype data missing, SNPs with missing genotype of more than 5%, or SNPs with less than 1% minor allele frequency and SNPs that failed Hardy–Weinberg tests ($P <$

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0.000001). PLINK was used as the primary software for data analysis (https://www.cog-genomics.org/plink2) (23). SNPs on sex chromosomes and mitochondria were also excluded. Detection of population stratification and removal of population outliers were performed using the multidimensional scaling (MDS) analysis integrated in PLINK. To indicate the level of stratification, a lambda value was generated by MDS. Samples with pi-hat > 0.125 were removed due to relatedness. *P* values were calculated by the logistic regression tests integrated in PLINK with covariates of age, sex, body mass index (BMI), cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and HbA1c (using the latest available health records). A *P* value of less than 5×10^{-8} was considered to be statistically significant.

This study also used many GWAS related software such as FUMA for generating Manhattan plots, LocusZoom for regional visualization, and FUMA for generating a corresponding Q-Q plot to evaluate differences between the cases and controls caused by potential confounders (e.g. different genotyping laboratories, different DNA extraction methods, etc.) (24,25). SPSS 22 software (IBM Corp., Armonk, NY, USA) was used to compare means of all covariates (except sex) in cases and controls through the independent sample t-test. Sex difference was compared by chi-square test. The whole workflow is shown in Supplementary Figure S1.

RESULTS

After excluding type 1 diabetic samples, population outliers, and related samples from 6,927 diabetic patients with genetic information from Affymetrix and Illumina chips, we were left with a study population of 4,852 unrelated type 2 diabetes individuals for further analysis. Among these patients, we identified 1,240 samples with diabetic maculopathy. However, only 469

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participants had a decreased visual acuity in the corresponding eye and were suitable for inclusion as cases. We also identified 1,374 patients as controls, after excluding patients who had diabetic retinopathy without DM, patients who had previous laser treatment, and patients who were already cases. Therefore, there were 469 cases (males=192, females=277) and 1,374 controls (males=630, females=744) for the further association analysis. The prevalence of diabetic maculopathy with decreased visual acuity in our cohort was 25.4% [469/(469+1,374)]. The means of sex, age, BMI, cholesterol, triglycerides, HDL, LDL, HbA1c were compared between cases and controls. There were statistically significant differences in age, triglycerides, HDL and HbA1c between the cases and controls, but no statistically significant difference in sex, BMI, cholesterol and LDL (Table 1).

Affymetrix SNP6.0 chips contained 704,847 directly genotyped and quality-controlled SNPs and Illumina OmniExpress chips contained 601,394 directly genotyped and quality-controlled SNPs. Overall, 6,717,712 genotyped and imputed SNPs were available for association analysis after standard quality control steps of genotyping and imputation. There was no need to further adjust for population stratification since the lambda value was 1.00, indicating a homogeneous population. We then performed logistic regression tests integrated in PLINK adjusting for sex, age, BMI, cholesterol, triglycerides, HDL, LDL, HbA1c. We found that the SNP rs9966620 in the *TTC39C* gene, reached genome-wide significance (Table 2) with a *P* value of 4.13×10^{-8} and odds ratio of 1.95 (Confidence interval: 1.53-2.47, Figure 1). This finding was supported by the nearby SNPs (rs7243626 and rs7240470) which also showed encouraging *P* values ($P= 5.64 \times 10^{-8}$ and 8.05×10^{-7}, respectively). We calculated the LD among these SNPs using our dataset and these SNPs are highly correlated (R-square>0.8) (Supplementary Table S3). The regional plot around the top SNPs is shown in the Figure 2. The Q-Q plot of the association

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results is shown in the Supplementary Figure S2.

We have moderate power for this GWAS study. Calculated by CaTS, we have 80% power based on 469 cases and 1,374 controls, assuming a minor disease allele frequency of 0.25, a genotypic relative risk of 1.49, a prevalence of diabetic maculopathy with decreased visual acuity in the diabetic population of 25%, and significance level of 5×10^{-8} (26).

We also ran a GWAS on the phenotype of DM (regardless of visual acuity status, 1,240 cases) against the controls used in this study. The result did not show GWAS significance (Supplementary Table S4).

DISCUSSION

In Scotland, patients with diabetes are invited to attend a free annual retinal screening service. This screening service aims to identify diabetic eye complications at an early stage to prevent or delay subsequent visual loss. During the screening, if an eye falls within the diagnosis criteria of diabetic retinopathy or DM (Supplementary Table S2), then the relevant information will be recorded in the e-health linked records. The status of macula is then categorised and recorded as either: no maculopathy, observable maculopathy, or referable maculopathy. Diabetic retinopathy is further classified and recorded based on the Scottish diabetic retinopathy grading scheme (27). Because of the lack of more detailed classification info, it was not possible to phenotype the subtypes of DM (as outlined by The Royal College of Ophthalmologists guidelines) when using population e-health linked records such as GoDARTS (6), Decreased visual acuity is a well-recognised symptom associated with maculopathy. 22% of diabetic patients with decreased visual acuity have maculopathy, compared to 1% in diabetic patients with normal visual acuity (28). It is also estimated that 6 months after laser treatment, the visual

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acuity of 34% of DM patients became worse, 22% became better, and 44% remained the same (29). These statistics were similar for DM patients who did not undergo laser treatment. In this study, 37.8% of patients (469/1240) who had DM also had a decreased visual acuity, which is only slightly higher than the above post-laser treatment statistics, however this could be attributed by the fact that we had a longer follow-up period. A national UK audit also found that a poor baseline visual acuity was associated with a poorer visual prognosis in DM patients (30). In this study, we defined the cases as DM with decreased visual acuity based on the hypothesis that these patients might share common genetic components. We tried to identify the genetic factors which contribute to decreased visual acuity among DM patients. Though this narrow definition will reduce case numbers and the study power, it allows us to generate a more homogeneous case population. A similar approach has been applied when we were defining diabetic neuropathic pain (the cases should not only have evidence of pain provided by prescription records, but also positive evidence of neuropathy provided by a medical test) (31). We also acknowledge that 'decreased visual acuity' in the cases was defined as any visual loss, including both major and minor visual loss, which may be an additional confounding factor. Because there is no literature which indicates if diabetic retinopathy and DM share the same genetic components, we also excluded individuals with diabetic retinopathy with and without DM from the controls to maintain a homogeneous population as we wanted to exclude genetic influence from diabetic retinopathy. The duration of diabetes is an important risk factor for many diabetic complications. However, the GoDARTS dataset does not include a high-quality record of this information. Hence, duration of diabetes therefore could not be adjusted for in our analysis. We also noticed that there were statistical differences of some covariates between cases and controls. For example, the mean HbA1c value was higher in cases than controls,

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which suggest that the controls may have had better controlled diabetes and/or a longer duration of diabetes (32).

We have identified the SNP rs9966620, which has achieved GWAS significance (P=4.13 x 10^{-8}, Odds ratio=1.95). This was supported by 2 nearby SNPs which showed encouraging *P* values. The 3 SNPs are located in the intronic areas with no clear functional roles. The SNP cluster was in the tetratricopeptide repeat domain 39C gene (*TTC39C*), which is a protein-coding gene located on the long arm of chromosome 18. The *TTC39C* gene is expressed in the eyes, and encodes a protein named as TTC39C (33). However, the physiological functions of both the gene and the protein are not known. The protein contains a relatively well-characterized structural motif called the tetratricopeptide repeat (TPR). The TPR is known to be involved in cell cycle regulation, mediating protein-protein interactions, assisting in protein folding and translocation, assembly of multi-protein complexes. The TPR structure also shows flexibility in the mediation of biological activities (34,35). In particular, it has been proposed that this structure plays a role in anaphase: the stage of mitosis when replicated chromosomes are split and the daughter chromatids are moved to opposite poles of the cell (36). Anaphase has been suggested to play an essential role in regulating cell fate of the vertebrate retina (37). Some studies have suggested that central macular thickness, a highly heritable trait with no specific gene identified, is associated with visual acuity (38,39). However, this parameter was not recorded our e-health linked records and so it is still unknown if the *TTC39C* gene is linked with central macular thickness. It has been suggested that the TTC39C protein interacts with the protein from the heat shock protein family B (small) member 1 (*HSPB1*) gene (40). HSPB1 is reported to be upregulated in the rat retina upon optic nerve injury (41). The TTC39C protein has 2 paralogs: TTC39A and TTC39B, although their functions are not clear. Next to *TTC39C*

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are the laminin subunit alpha 3 gene (*LAMA3*) and the calcium binding tyrosine phosphorylation regulated gene (*CABYR*). Of note, the protein of *LAMA3* is laminin subunit alpha-3 and laminins are essential for formation and function of the basement membrane and have additional functions in regulating cell migration and mechanical signal transduction, which suggests it is a possible candidate gene for diabetic maculopathy (42).

We also investigated the top SNPs suggested by other candidate gene studies for diabetic macular oedema. However, none reached statistically significant *P*-values: rs2070744 (*P*=0.286, *NOS3*), rs429358 (*P*=0.59, *APOE*), rs7412 (*P*= 0.35, *APOE*), rs2010963 (*P*=0.30, *VEGFA*), rs17697515 (*P*=0.47, *VEGFC*). Rs2910164 (MicroRNA-146a) was not present in our datasets.

There are limitations in using population level e-health records, especially in differentiating age-related maculopathy from diabetic maculopathy as this information (as well as lens opacity) is not always included. However, the prevalence of age-related maculopathy in a diabetic population is typically low, indicating that this limitation may have had a lessened effect on the study (43). As visual deterioration is associated with foveal oedema, further investigation in a dataset with Optical Coherence Tomography (OCT) data characterising foveal oedema would be valuable to further investigate the findings. A better phenotyping approach of DM for the purposes of research is also required.

In conclusion, we propose that the *TTC39C* gene is associated with DM with decreased visual acuity in a Scottish diabetic cohort using a GWAS approach. Replication studies and functional studies will help to confirm its role in DM.

ACKNOWLEDGEMENTS

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

DISCLOSURE

All authors declare no financial interests or benefit.

FUNDING

1. This study was supported by the Tenovus Scotland under Grant 2015-T15/40.
2. The Affymetrix SNP6.0 chips were funded by the 'Wellcome Trust Case Control Consortium 2' (WTCCC2) project.
3. The Illumina OmniExpress chips were funded by the 'Surrogate markers for Micro- and Macro-vascular hard endpoints for Innovative diabetes Tools' (SUMMIT) project.
4. The GoDARTS project is jointly funded by DIABETES UK and The Wellcome Trust.

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TABLES

FIGURES

FIGURE CAPTIONS

- Figure 1. Manhattan plot of the GWAS on diabetic maculopathy with decreased visual acuity (469 cases and 1,374 controls).
- Figure 2. Regional plot around top SNPs of the TTC39C gene area.
- Supplementary Figure S1. Workflow of the GWAS on diabetic maculopathy with decreased visual acuity in GoDARTS.
- Supplementary Figure S2. Q-Q plot compared expected and observed –Log10(P) values.

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Table 1. Covariates information between cases and controls.

	Cases (mean+SD)	Controls (mean+SD)	*P*
Age , y	67.20±9.50	65.27±10.39	0.0003
Sex , n	192/277 (m/f)	630/744 (m/f)	0.068
BMI , kg/m^2	31.25±4.99	31.77±5.84	0.082
Cholesterol, mmol/L	4.36±0.85	4.40±0.88	0.372
Triglycerides, mmol/L	2.07±1.10	2.29±1.30	0.002
HDL, mmol/L	1.37±0.36	1.33±0.34	0.018
LDL, mmol/L	2.09±0.67	2.10±0.72	0.870
HbA1c, %	8.00±1.46	7.27±1.20	<0.0001

Cases= 469

Controls= 1,374

SD: standard deviation

BMI: body mass index

HDL: high-density lipoprotein

LDL: low-density lipoprotein

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Table 2. SNPs of the GWAS on diabetic maculopathy with decreased visual acuity ($p<1.0x10^{-6}$).

SNPID	Chromosome position (GRCh37)	Gene	Minor Allele	MAF in cases: controls	*P* value (no adjustment)	*P* value	OR±SE
rs9966620	18:21680735	*TTC39C*	A	16.33%:9.64%	$7.23x10^{-8}$	$4.13x10^{-8}$	1.95±0.12
rs7243626	18:21679950	*TTC39C*	T	16.17%:9.51%	$8.84x10^{-8}$	$5.64x10^{-8}$	1.95±0.12
rs7240470	18:21681516	*TTC39C*	T	16.86%:10.33%	$3.20x10^{-7}$	$8.05x10^{-7}$	1.80±0.12
rs11706588	3:126448513	*CHCHD6*	C	20.98%:13.05%	$6.99x10^{-7}$	$4.43x10^{-7}$	1.89±0.13
rs11718070	3:126448560	*CHCHD6*	T	20.98%:13.09%	$8.01x10^{-7}$	$5.13x10^{-7}$	1.88±0.13

SNP: single nucleotide polymorphism

MAF: minor allele frequency

OR: odds ratio

SE: standard error

TTC39C: tetratricopeptide repeat domain 39C

CHCHD6: coiled-coil-helix-coiled-coil-helix domain containing 6

rs11706588 and rs11718080 are included in the table for readers' interest although the *P* values of these 2 SNPs did not reach GWAS significance.

Listed covariates in the Table 1 were used for adjustments.

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Table S1. The definition of visual acuity in GoDARTS.

Visual acuity coding	Description
1	6/4
2	6/5
3	6/6
4	6/9
5	6/12
6	6/18
7	6/24
8	6/36
9	6/60
10	3/60
11	Counting fingers
12	Hand movements
13	Perception of Light
14	No perception of Light

Visual acuity of 6/12 means a testing eye can see at six metres while an eye with normal vision can see at 12 metres away.

Individuals with coding 11 to 14 were removed from study.

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Table S2. Maculopathy and retinopathy grades for the diabetic retinal screen.

MACULOPATHY GRADING		
Maculopathy	**Description**	**Outcome**
M0 (No maculopathy)	No features ≤ 2 disc diameters from the centre of the fovea, enough to classify it as M1 or M2 from definitions below	Rescreen in 12 months
M1 (Observable)	Any hard exudates within a radius of > 1 but ≤ 2 disc diameters of the fovea centre	Rescreen in 6 months or refer to ophthalmology if not feasible
M2 (Referable)	Any hard exudates or blot haemorrhages within a distance of ≤ 1 disc diameter of the fovea centre.	Refer to ophthalmology (these patients will not definitely receive immediate laser treatment and may be kept under surveillance
RETINOPATHY GRADING		
Retinopathy	**Description**	**Outcome**
R0 (No visible retinopathy)	No diabetic retinopathy anywhere	Rescreen in 12 month
R1 (Mild)	Background Diabetic Retinopathy (BDR) – Mild Presence of any one of these: Dot haemorrhages, micro-aneurysms, hard exudates, cotton-wool spots, blot haemorrhages, flames shaped haemorrhages	Rescreen in 12 month
R2 (Observable Background)	Background Diabetic Retinopathy (BDR) – Observable Four(4) or more blot haemorrhages in one hemi-field*	Rescreen in 6 month
R3 (Referable Background)	Background Diabetic Retinopathy (BDR) - Referable Presence of any of the following: • Four(4) or more blot haemorrhages in both superior and inferior hemifields • Venous beading • IrMA	Refer to ophthalmology;
R4 (Proliferative)	Proliferative Diabetic Retinopathy (PDR) Presence of active new vessels or Vitreous haemorrhage	Refer to ophthalmology; Patients are likely to receive laser treatment or other intervention
R6 (Inadequate)	Not adequately visualized; Retina not sufficiently visible for assessment	Technical failure; Patients for alternative screening examination.
IrMA, Intraretinal Microvascular Abnormality. * The hemifields, Superior and Inferior, are demarcated by a line through the fovea centre and optic disc.		

Source: The Scottish Diabetic Retinopathy Grading Scheme 2007 v1.0.[16]

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Table S3. Linkage disequilibrium among 3 SNPs.

SNPID	rs7243626	rs9966620	rs7240470
rs7243626	1	1	0.84
rs9966620	1	1	0.85
rs7240470	0.84	0.85	1

SNP: single nucleotide polymorphism

The values in the table indicate R-square values.

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Table S4. The top SNPs for the GWAS on the phenotypes of DM (regardless of visual acuity, 1,240 cases) against the controls (1,374 samples).

Chromosome	SNP	Positions	Test	NMISS	OR	SE	L95	U95	*P*
1	rs3818329	192627760	ADD	2324	1.865	0.1202	1.474	2.361	2.12×10^{-7}
3	rs12629668	147209179	ADD	2061	1.397	0.06611	1.228	1.591	4.15×10^{-7}
6	rs117482282	165511471	ADD	2361	3.079	0.2224	1.991	4.762	4.27×10^{-7}
13	rs1149833	50750876	ADD	2557	0.747	0.05813	0.6666	0.8372	5.23×10^{-7}
2	rs1406230	29583321	ADD	1943	0.6923	0.07334	0.5996	0.7994	5.35×10^{-7}
1	rs2296022	192627124	ADD	2324	1.799	0.1183	1.427	2.269	6.92×10^{-7}
16	rs35498131	9120809	ADD	1493	0.5596	0.117	0.445	0.7038	6.94×10^{-7}
7	rs140306040	62321151	ADD	2171	0.509	0.1365	0.3895	0.6651	7.47×10^{-7}
7	rs73121760	62313911	ADD	2155	0.5131	0.135	0.3938	0.6685	7.71×10^{-7}
16	rs34300094	9120719	ADD	1487	0.5589	0.1184	0.4431	0.7049	8.98×10^{-7}
2	rs34954281	152225877	ADD	2021	0.6739	0.08045	0.5756	0.789	9.34×10^{-7}

SNP: single nucleotide polymorphism

NMISS: number of individuals for the logistic regression analysis of a specific SNP

OR: odds ratio

SE: standard error

ADD: additive model

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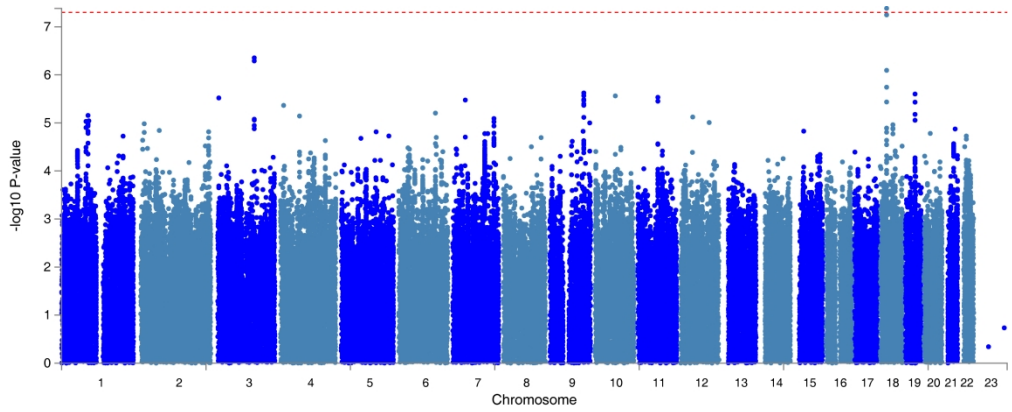

Figure 1. Manhattan plot of the GWAS on diabetic maculopathy with decreased visual acuity (469 cases and 1,374 controls).

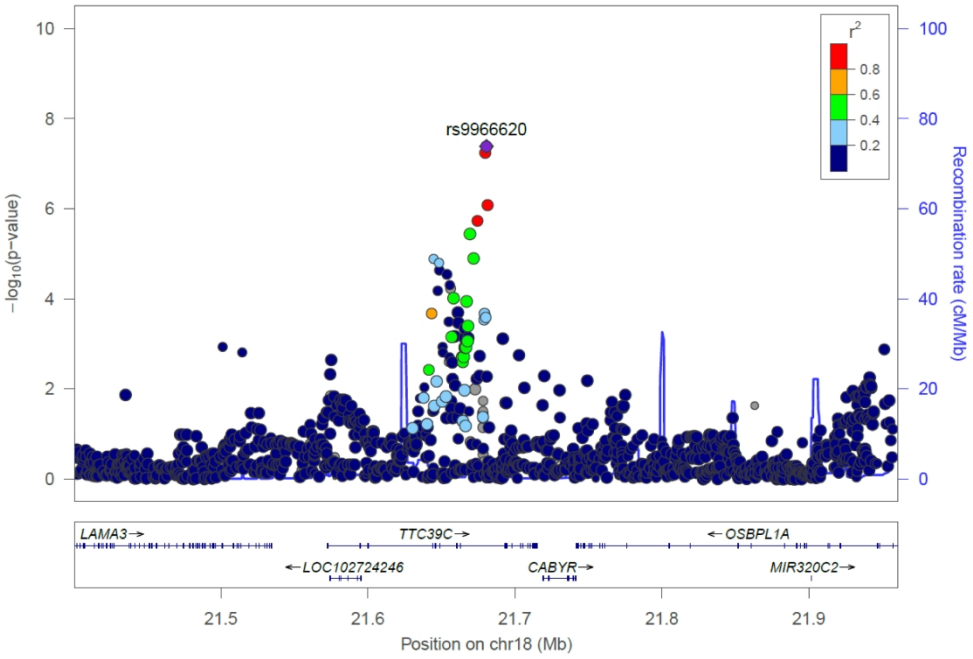

Figure 2. Regional plot around top SNPs of the TTC39C gene area.

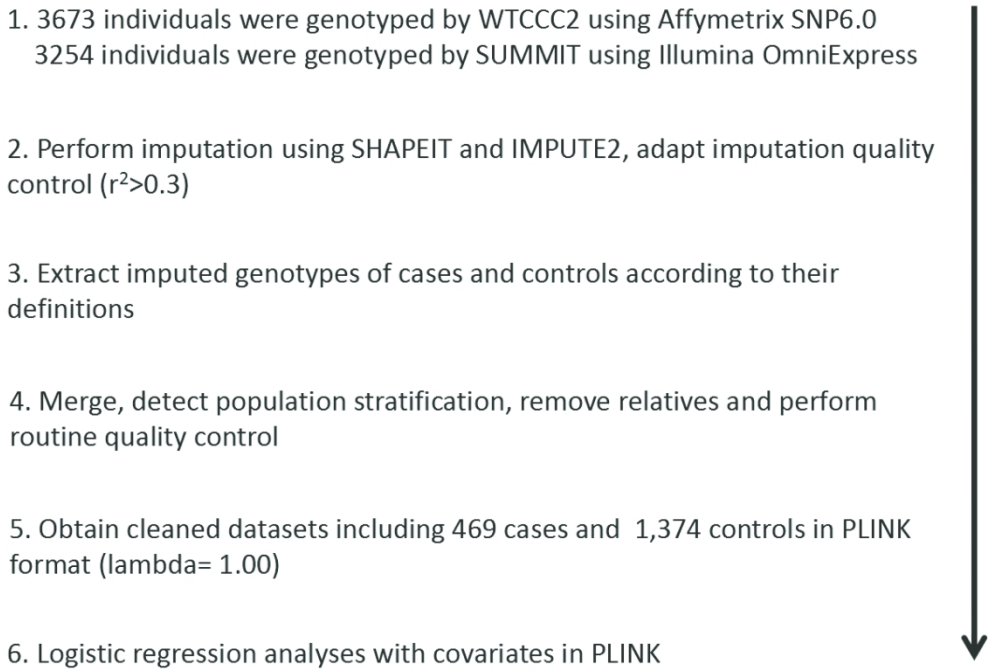

Figure S1. Workflow of the GWAS on diabetic maculopathy with decreased visual acuity in GoDARTS.

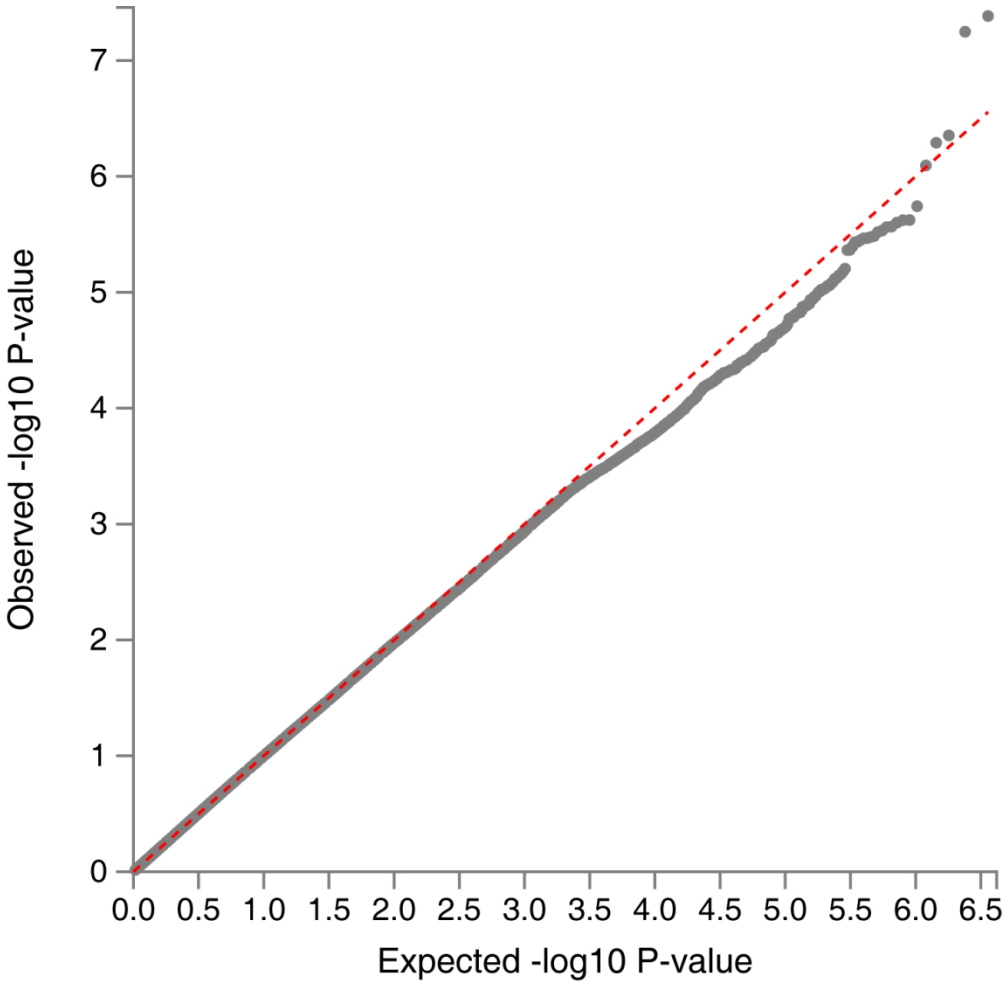

Figure S2. Q-Q plot compared expected and observed –Log10(P) values.