Genome-Wide Association Study to Identify Genetic Variants Associated With Diabetic Maculopathy

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Purpose. Diabetic maculopathy (including diabetic macular edema [DME]) is the leading cause of vision loss in people with diabetes. We aimed to identify the genetic determinants of diabetic maculopathy.

Methods. We conducted a genome-wide association study (GWAS) in two cohorts with a meta-analysis. The Australian cohort comprised 551 cases of DME and 599 controls recruited from the states of South Australia and Tasmania. The Scottish cohort comprised 1951 cases of diabetic maculopathy and 6541 controls from the Genetics of Diabetes Audit and Research in Tayside Scotland study (GoDARTS). Genotyping, imputation, and association analysis using logistic regression were conducted in each cohort, before combining summary statistics in a meta-analysis using the GWAMA package.

Results. A locus on chromosome 7 reached genome-wide significance in GoDARTS but showed the opposite direction of effect in the Australian cohort. The meta-analysis identified two suggestive associations ($P < 5 \times 10^{-6}$) for diabetic maculopathy risk with similar effect direction; one at chromosome 1 close to the *RNU5E-1* gene and one at chromosome 13 upstream of the *ERICH6B* gene. The two loci were evaluated in silico for potential functional links to diabetic maculopathy. Both are located in regulatory regions and have annotations indicating regulatory functions. They are also expression quantitative trait locus (eQTLs) for genes plausibly involved in diabetic maculopathy pathogenesis, with links to folate metabolism and the regulation of VEGF.

Conclusions. The study suggests several promising SNPs and genes related to diabetic maculopathy risk. Despite being the largest genetic study of diabetic maculopathy to date, larger, homogeneous cohorts will be required to identify robust genetic risk loci for the disease.

Keywords: diabetic macular edema (DME), genetics, genome-wide association study (GWAS), meta-analysis, Mendelian randomization

Diabetic maculopathy is damage to the macula caused by diabetes. Most diabetic maculopathy is classed as diabetic macular edema (DME). DME is the leading cause of blindness in working-age adults in developed countries, affecting 1 in 14 people with diabetes mellitus.[1] It can occur at any stage of the progression from non-proliferative to proliferative diabetic retinopathy (DR)[2] with or without other features of DR. Several clinical and ocular risk factors play an important role in the pathogenesis of DME but these clinical risk factors, even when considered collectively, do not explain DME in its entirety.[3–5] Further, some patients develop DME despite good glycemic control and others with poor glycemic control do not, suggesting a genetic contribution not explained by the conventional risk factors.[6] The genetic basis of DR has been studied extensively over the last decade[7]; however, well-powered studies focusing specifically on DME or maculopathy are still lacking.[8] Most DME-related studies have used a candidate gene approach, have small sample sizes, and have not yielded significant findings. Several genome-wide association studies (GWAS) have investigated DME or the closely related phenotype diabetic maculopathy.[9–11] The largest and only GWAS with significant findings is that of Meng et al.[10] This study identified an SNP in the *TTC39C* gene, rs9966620, as a potential risk SNP for

diabetic maculopathy ($P = 4.13 \times 10^{-8}$), but this has not yet been replicated in an independent cohort. A smaller study by Graham et al.[9] performed a sub-analysis for DME as part of their GWAS investigating DR risk but found no genome-wide significant associations. Recently, Stockwell et al.[11] have performed a multi-ancestry GWAS analysis which suggested that variants in *APOI* are a high-risk locus for DME. Overall, there has been very little progress in understanding the genetics of DME or maculopathy, and GWAS consisting of larger cohorts and/or meta-analyses are required. Here, we expand the two previously reported cohorts[9,12] and present a meta-analysis of the two studies for the largest reported GWAS investigating DME or maculopathy risk to date.

Materials and Methods

Australian Cohort

The GWAS of Australian cohort[9] consisted of Caucasian participants from the Tasmanian Ophthalmic Biobank (TOB; Ethics approval number H0012902) and the Genetic Risk Factors in Complications of Diabetes study (GRFCD; Ethics approval number 86-067). Ethics approval was obtained from all the collaborating institutions, and each participant gave written informed consent in accordance with the Declaration of Helsinki. A GWAS for DME was performed using participants with type 1 (T1) or type 2 (T2) diabetes. Participants included all individuals that were previously included in the GWAS by Graham et al.[9] Cases were defined as participants with diabetes over 18 years of age with clinically diagnosed DME on fundoscopic examination as per the International Clinical Disease Severity Scale (ICDSS)[13] irrespective of the DR status. Controls included patients with T1 and T2 diabetes without any features of DR or DME on the ICDSS scale. The clinical and demographic data were collected retrospectively from a review of medical records, as described previously.[14,15] DR severity was clinically graded as per the ICDSS guidelines.[13]

Baseline characteristics of cases and controls were compared using independent *t*-tests for continuous variables and chi-square tests for discrete traits in R software (version 4.0.2).

Genetics of Diabetes Audit and Research in Tayside Scotland Cohort

The Genetics of Diabetes Audit and Research in Tayside Scotland (GoDARTS) database (https://godarts.org) has been described in detail previously.[10,16] The analysis presented here expanded on a previous GWAS for diabetic maculopathy in the GoDARTS cohort[10] with the inclusion of additional participants with T1 or T2 diabetes. Ethics for this cohort was approved by Tayside Committee on Medical Research Ethics (REC reference 053/04).

The cases were defined as patients with diabetes (T1 or T2) noted as being diagnosed with maculopathy in the e-health records. The controls were defined as diabetics without maculopathy or other types of DR. Retinopathy grading was as per the Scottish Diabetic Retinopathy Grading Scheme 2007 version 1.0,[17] and the status of the macula was recorded as "with or without diabetic maculopathy."

Genotyping, Quality Control, and Statistical Analysis: Australian Cohort

Genomic DNA, obtained from whole blood using standard methods, was genotyped on one of two Illumina arrays (GSA or OmniExpress). The initial processing was done in GenomeStudio and then imported separately for each array type into PLINK (version 1.9/2.0),[18] where further quality control (QC) was performed on each dataset. Briefly, individuals with discordant sex information, a missing genotype rate ≥ 0.03, and heterozygosity ± 6 SDs from the mean were excluded. Genetically related individuals were detected by calculating pairwise identity by descent (IBD)/KING cutoff, and the individual with the lower genotyping rate in any pair with IBD ≥ 0.185 was removed. SNPs were excluded if they had a missing genotype rate ≥ 0.03 and had minor allele frequency (MAF) ≤ 0.01. After thorough QC for each array design (Illumina Omniexpress = 627,516 autosomal SNPs, 1060 individuals, and Illumina GSA = 281,952 autosomal SNPs, 221 individuals), imputation of allele dosage was performed with reference to the HRC panel, version r1.1 2016, using the Michigan imputation server (Minimac4 [1.5.7]). Post imputation, the two array sets were merged and filtered for imputation quality score ($r^2 < 0.8$), MAF ≤ 0.01, missing genotype rate ≤ 0.03, and Hardy–Weinberg equilibrium (HWE) deviation $P \leq 1 \times 10^{-6}$. Ancestral outliers were then identified using principal component analysis (PCA) in PLINK version 2.0 (Supplementary Fig. S1). PCA was performed on pruned sets of autosomal SNPs, selected by a linkage disequilibrium (LD) r^2 threshold of 0.2 in windows of 50 SNPs and a moving step of five SNPs to detect and correct for any population stratification.

For the primary association between SNP genotypes and DME risk, a logistic regression model implemented in PLINK version 2.0 was used under an additive model with allele dosage scores. Covariates shown to alter the risk of DME (duration of diabetes, HbA1c, sex, hypertension, nephropathy status, and diabetes type) were included in the regression model, along with genotyping batch and the first two principal components (PCs), based on the scree plot (Supplementary Fig. S2), to adjust for population stratification. The genome-wide significance threshold was set at $P = 5 \times 10^{-8}$, and the suggestive significance at $P = 5 \times 10^{-6}$. Genomic inflation factors (λ) were calculated to evaluate any residual population stratification in PLINK version 2.0.

Genotyping, Quality Control, and Statistical Analysis: GoDARTS Cohort

Genotyping was done using the Genome-Wide Human SNP Array 6.0 (Affymetrix, USA) or the OmniExpress Array (Illumina, USA) across three batches. Standard genotyping QC protocols were applied as described by Meng et al.[19] and the Pharmacogenetics Study Group et al.[20] Briefly, samples were removed based on individual and SNP call rate > 95%, gender discrepancy, IBD PiHAT > 0.08, heterozygosity ± 3 standard deviation, and HWE deviation $P \leq 1 \times 10^{-6}$. For imputation, the genotype was phased using SHAPEIT and imputed using the 1000 Genomes Reference Panel3 via IMPUTE2 and imputation quality score ($r^2 > 0.4$).

The association between SNP genotypes and maculopathy risk was assessed using a logistic regression additive

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model in PLINK version 1.9. Separate analyses for each of the three batches were adjusted for age, sex, duration of diabetes, HbA1c, diabetes type, hypertension, nephropathy, and the first two PCs. Following these analyses, a meta-analysis was conducted to synthesize the GoDARTS results. Identical genome-wide and suggestive significance levels were used for the Australian cohort.

Meta-Analysis

To increase the association analysis' power, we performed a meta-analysis using the summary statistics from the two datasets in GWAMA.[21] We considered DME and diabetic maculopathy to be equivalent phenotypes and, from here on, refer only to DME, accepting a small number of cases may have other types of maculopathies. A fixed-effects meta-analysis with inverse weighting was performed using odds ratio (OR) and 95% confidence interval (CI). We did not apply genomic control to the meta-analysis results, as limited inflation was detected ($\lambda = 1.004$). All SNPs were analyzed relative to the forward strand of the reference genome, and statistics are presented regarding the alternative allele. SNPs considered significant had a meta-P value $\leq 5 \times 10^{-8}$ and agreement in effect direction across all cohorts. The I^2 statistic was used to quantify statistical heterogeneity between the individual markers between studies.

Data Visualization and Functional Annotation

Q−Q plots and Manhattan plots were created in R with the GWASTools[22] and CMplot package,[23] respectively. Regional association plots were constructed with FUMA version 1.3.7 online tool.[24] Functional annotations for each SNP with suggestive or significant association were generated using the (1) Combined Annotation-Dependent Depletion (CADD)[25] scores, (2) RegulomeDB rank,[26] (3) expression quantitative trait locus (eQTL) mapping using Genotype-Tissue Expression database (GTEx database version 8) and the EyeGEx database[27] at the false discovery rate (FDR) < 0.05.

Phenome-Wide Association Study and Mendelian Randomization

To investigate potential associations between the top SNPs from meta-analysis results and related traits, we conducted a Phenome-Wide Association Study (PheWAS) using a dataset of 4756 GWAS summary statistics from the GWASATLAS platform (https://atlas.ctglab.nl/xtreme). For each locus, those phenotypes that were significantly ($P < 0.05$) associated with all SNPs within that locus were only included. We analyzed SNPs with P values $\leq 5 \times 10^{-6}$ and generated 3 heatmaps for 3 loci, adjusting for multiple comparisons using the Bonferroni method. Phenotypes that reached Bonferroni significance were selected for Mendelian Randomization (MR) studies to determine if these significant findings from PheWAS are causally related to DME. This analysis was conducted using the "TwoSampleMR" package, with data sourced from the IEU OpenGWAS database (https://gwas.mrcieu.ac.uk/). Data preparation steps included clumping and harmonization, with DME results used as outcome data. Our MR methods included inverse variance weighted (IVW), MR Egger, weighted median, simple median, and weighted mode.

Results

Cohort Characteristics

Following all QC and data cleaning, the Australian cohort consisted of 551 cases and 599 controls with genotyped and imputed data for 4,351,713 autosomal SNPs. The GoDARTS cohort comprised 1951 cases and 6541 controls, with data for 4,351,713 autosomal SNPs. Demographic and clinical information for both cohorts is presented in Table 1. In the Australian cohort, the DME cases were significantly older than the controls, with a longer duration of diabetes, poor glycemic control, more likely to be male patients, and significantly higher proportions of comorbid conditions, including hypertension and nephropathy. The GoDARTS cohort showed similar patterns, except the controls were older than the cases and the duration of diabetes in cases was less than seen in the Australian cases.

Association Analysis for DME in Individual Cohorts

No variants reached genome-wide significance in the Australian cohort; however, two loci reached suggestive significance ($P < 5 \times 10^{-6}$; (Fig. 1A; Table 2). The locus on chromosome 6 (top SNP, rs2817108, $P = 1.55 \times 10^{-6}$) is located near *ELOVL5*. This top hit is supported by nine other nearby SNPs in LD (Supplementary Table S1). For the locus on chromosome 4, there were 2 SNPs of suggestive significance, rs76040981 ($P = 4.89 \times 10^{-6}$) and rs75624598 ($P = 4.92 \times 10^{-6}$), both located in the intronic regions of the Protocadherin 7 (*PCDH7*) gene (see Table 2, Supplementary Table S1). The regional plots of the top hits (rs2817108 and rs76040981) of the two suggestive loci for the Australian cohort are given in Supplementary Figure S3.

The most statistically significant locus in the GoDARTS cohort was on chromosome 7, top SNP rs1549395, $P = 5.39 \times 10^{-8}$ (Fig. 1B; see Table 2) in a gene-poor region down-

Table 1. Baseline and Clinical Characteristics of Diabetic Maculopathy Cases Compared to Controls in the Australian and GoDARTS Cohort

Australian Cohort	Cases ($N = 551$)	Controls ($N = 599$)	P Value
Age, y	64.36 (12.8)	60.53 (17.6)	**<0.001**
Diabetes duration, y	20.87 (10.0)	12.87 (8.1)	**<0.001**
HbA1c %	8.63 (1.6)	7.58 (1.4)	**<0.001**
Sex: M	331 (60.0%)	318 (53.0%)	**0.017**
Hypertension: Yes	461 (83.6%)	396 (66.1%)	**<0.001**
Nephropathy: Yes	196 (35.5%)	64 (10.6%)	**<0.001**
Diabetes type: T2	459 (83.3%)	469 (78.2%)	**0.032**
GoDARTS cohort	**Cases ($N = 1951$)**	**Controls ($N = 6541$)**	**P value**
Age, y	64.60 (11.7)	73.34 (10.6)	**<0.001**
Diabetes duration, y	12.8 (10.5)	14.1 (10.9)	**<0.001**
HbA1c %	8.2 (1.2)	7.3 (1.0)	**<0.001**
Sex: M	1389 (71.2%)	3950 (60.4%)	**0.02**
Hypertension: Yes	1122 (57.5%)	4467 (68.3%)	**<0.001**
Nephropathy: Yes	630 (32.3%)	1066 (16.3%)	**<0.01**

Data are presented as means (SD) for continuous variables and numbers, and percentage (%) for categorical variables. Independent t-test was conducted for continuous variables and Chi-square test for categorical variables.

Significant P values are in bold.

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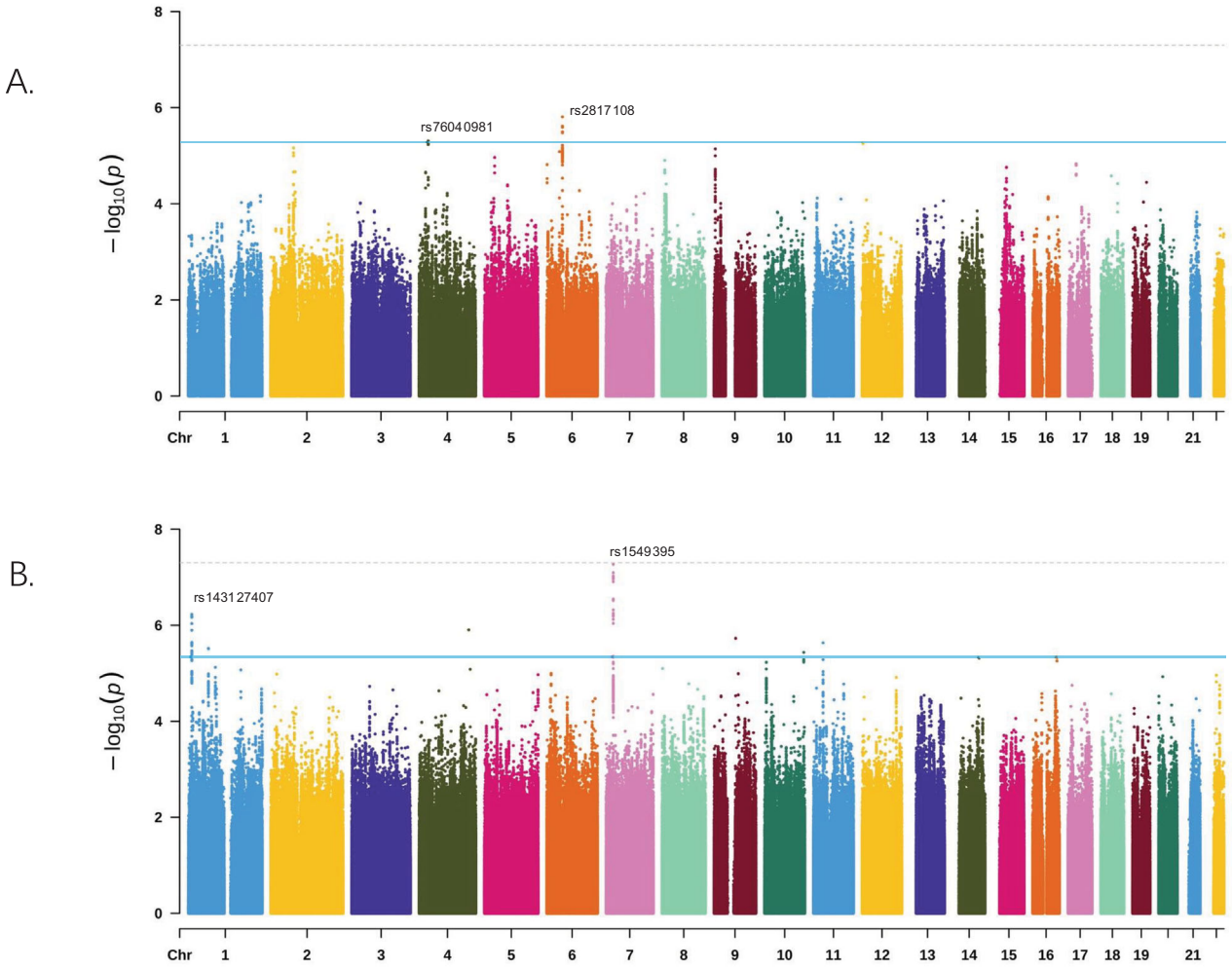

Figure 1. Manhattan plot for association analysis of diabetic maculopathy. (**A**) Australian cohort (diabetic macular edema). (**B**) GoDARTS cohort (diabetic maculopathy). The x-axis represents chromosomal position, and the y-axis represents the −log10 (*P* values) of association for each SNP tested. The *blue horizontal line* corresponds to the threshold for genome-wide suggestive association ($P \leq 5 \times 10^{-6}$).

stream of *STK31*, with 17 nearby SNPs in LD supporting this SNP. Another suggestive locus was identified on chromosome 1 close to the small nuclear RNA, U5E Small Nuclear 1 (*RNU5E-1*) gene, with top SNP rs143127407, $P = 5.92 \times 10^{-7}$, and multiple other SNPs in LD. Several other individual SNPs also reached suggestive significance (Supplementary Table S2). The regional plots for the leading two suggestive loci (chr7 rs1549395 and chr1 rs143127407) are presented in Supplementary Figure S4.

Q−Q plots (Supplementary Fig. S5) show adequate control of the underlying population structure in both cohorts. Neither cohort showed replication of the top-ranked SNPs in the other cohort (see Table 2).

Meta-Analysis for DME

The Q−Q plot (see Supplementary Fig. S5) shows low genomic inflation ($\lambda = 1.004$), suggesting good control of the population structure in the meta-analysis. No loci reached genome-wide significance. The 3 top-ranked loci included the 2 highlighted in the GoDARTS GWAS (chromosomes 7 and 1), and one novel locus on chromosome 13 (lead SNP = rs9534181 and upstream of *ERICH6B*) reached suggestive significance (Fig. 2; Table 3). The complete list of suggestive SNPs from the meta-analysis is provided in Supplementary Table S3. The lead SNP on chromosome 7 in the meta-analysis (rs7776503, $P = 1.12 \times 10^{-7}$) was absent from the Australian cohort. Other SNPs in LD with rs7776503 showed suggestive *P* values and were present in both datasets; however, they demonstrated an opposite effect direction and a high degree of statistical heterogeneity ($I^2 > 80\%$; see Supplementary Table S3). The lead SNP on chromosomes 1 (rs143127407, $P = 3.90 \times 10^{-7}$) and 13 (rs9534181, $P = 3.29 \times 10^{-6}$) were supported by several surrounding SNPs in LD showing similar *P* values, with a consistent direction of effect between the 2 studies and low to medium heterogeneity. None of the suggestive loci from the Australian cohort reached suggestive significance in the meta-analysis and there was a high degree of statistical heterogeneity ($I^2 > 90\%$) at these SNPs (see Table 2, Supplementary Table S1).

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Table 2. Lead SNPs for Suggestive ($P < 5 \times 10^{-6}$) and Significant ($P < 5 \times 10^{-8}$) Associations With Diabetic Maculopathy Risk in the Australian and GoDARTS Cohort, With Replication and Meta-Analysis Statistics for Each SNP

			Australian Cohort					Replication in GoDARTS		Meta-Analysis			
Locus*	**Position†**	**rsID**	**Alt**	**Case Freq**	**Control Freq**	**OR**	***P* Value**	**OR**	***P* Value**	**OR**	***P* Value**	**Effect**	**I^2**
ELOV5	6:53107202	rs2817108	C	0.36	0.29	1.80	1.55×10^{-6}	1.05	0.182	1.11	0.005	++	0.94
PCDH7	4:31129897	rs76040981	C	0.05	0.08	0.31	4.89×10^{-6}	0.93	0.339	0.85	0.028	++	0.94
			GoDARTS Cohort					Replication in Australian		Meta-Analysis			
Locus*	**Position†**	**rsID**	**Alt**	**Case Freq**	**Control Freq**	**OR**	***P* Value**	**OR**	***P* Value**	**OR**	***P* Value**	**Effect**	**I^2**
STK31	7:24005226	rs1549395	C	0.36	0.40	1.24	5.39×10^{-8}	0.89	0.311	1.20	1.44×10^{-6}	+−	0.87
RNU5E-1	1:11962135	rs143127407	A	0.05	0.07	0.65	5.92×10^{-7}	0.77	0.306	0.67	3.90×10^{-7}	−−	0.00

Alt, alternate allele; Freq, frequency of the Alt allele; I^2, statistical heterogeneity; OR, odds ratio.
* Locus assigned to gene within or nearest to the association signal.
† Genomic positions are based on hg19.
+Indicates increased risk.
–Indicates decreased risk.
All statistics adjusted for sex, HbA1c, diabetes duration, nephropathy status, hypertension, diabetes type, batch, and the first two principal components.
Genome-wide significant *P* values are in bold font.

Functional Annotation

Functional annotations of the top SNPs with a consistent direction of effect in the meta-analysis (chr 1 and chr13) are presented in Supplementary Table S4. The locus on chromosome 1 (lead SNP rs143127407, nearest gene *RNU5E-1*), extends over a 73 kbp region from rs72640280 (1:11943792, hg19) to rs4130774 (1:23110641 2, hg19) and contains a small nuclear RNA gene (*RNU5E-1*) and the protein-coding genes, *KIAA2013* and *PLOD1*. None of the SNPs assessed in the meta-analysis in this region have a CADD score > 15. RegulomeDB annotations indicate this region contains transcription factor binding sites and DNase peaks (ranks 1–5) as well as eQTLs (ranks 1a-1f), collectively suggesting that it may have a strong regulatory function. Specifically, rs2336381 has a RegulomeDB rank of 1f, indicating this SNP is an eQTL and located in transcription factor binding and DNase peak regions. The GTEx database reveals eQTLs for several nearby genes (*MTHFR, PLOD1, KIAA201,* and *NPPA*) in multiple tissues, including the brain and thyroid. The locus on chromosome 13 (lead SNP rs9534181, nearest gene *ERICH6B*) covers a 47 kbp region with no annotated genes. The CADD and RegulomeDB annotations do not highlight the region as a major regulatory locus, although SNP rs9534182 received a RegulomeDB rank of 2c, indicating it is located in a transcription factor binding site and DNase peak. All the suggestively associated SNPs at this locus were marked as eQTLs in GTEx for gene *LINC01055* in subcutaneous adipose tissue. None of the SNPs at either locus were associated with significant eQTL expression in eye tissues according to data from the EyeGEx database.

PheWAS and MR Results

PheWAS analyses were performed at 3 top loci in chromosomes 1, 7, and 13, respectively, each adjusted for multiple testing using Bonferroni-corrected *P* values (Supplementary Fig. S6). Within each locus, phenotypes significantly associated with multiple SNPs were highlighted by black boxes and a red asterisk. Overall, 19 phenotypes on chromosome 1, 4 on chromosome 7, and 2 on chromosome 13 showed these potentially significant associations (Supplementary Table S5).

MR analyses investigated the associations between DME and each significantly identified phenotype from the PheWAS. Notably, the exposure extreme body mass index (EBMI; IEU GWAS ID: "ieu-a-85") potentially influences DR, evidenced by a significant IVW *P* value of 0.002. "EBMI" was defined by categorizing individuals in the top 5% of the body mass index (BMI) distribution as cases and those in the bottom 5% as controls.[28] In the heterogeneity analysis, both the IVW and MR Egger *P* values are greater than 0.05 (Supplementary Table S6), indicating no significant heterogeneity between the genetic tools used. Figure 3 and Supplementary Figure S7 plot SNP effects on EBMI against their effects on DME. In addition to EBMI, arm fat percentage (right side) and monocyte cell count also showed significant effects in the IVW analysis. All MR results are available in the supplementary zip file.

Discussion

This meta-analysis reports two suggestive risk loci for DME or diabetic maculopathy with a similar direction of effect in the 2 study cohorts; one at chromosome 1, close to the

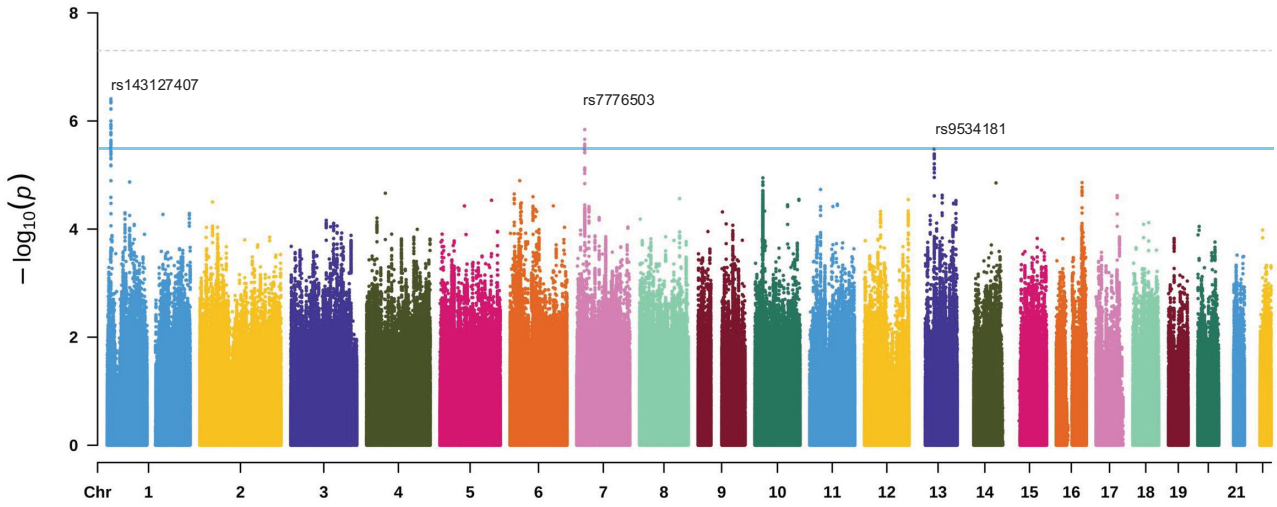

Figure 2. Manhattan plot for meta-analysis for diabetic macular edema/maculopathy. The x-axis represents chromosomal position, and the y-axis represents the −log10 (*P* values) of association for each SNP tested. The *blue horizontal line* corresponds to the threshold for genome-wide suggestive association ($P \leq 5 \times 10^{-6}$).

Table 3. Lead SNPs for Top Three Loci in the Meta-Analysis for Diabetic Maculopathy Risk

Locus*	Position†	rsID	EA	EAF	OR	Meta-Analysis *P* Value	Effect	I^2
AC009508.1	7:23972603	rs7776503	C	0.39	1.24	1.14×10^{-7}	+?#	NA
RNU5E-1	1:11962135	rs143127407	A	0.06	0.67	3.90×10^{-7}	−−	0.00
ERICH6B	13:46194814	rs9534181	A	0.13	1.28	3.29×10^{-6}	++	0.33

EA, effect allele; EAF, effect allele frequency; *ERICH6B*, glutamate-rich protein 6B; OR, odds ratio; *RNU5E-1*, RNA, U5E small nuclear 1; rsID, SNP identification number; SNP, single nucleotide polymorphism.

I^2 represents the statistical heterogeneity between the individual markers between studies.

* Locus assigned to gene within or nearest to the association signal.

† Genomic positions are based on hg19.

SNP is absent in the Australian cohort.

RNU5E-1 gene, and one at chromosome 13, upstream of the *ERICH6B* gene. The *RNU5E-1* locus was also a top-ranked region in the GoDARTS GWAS, but the *ERICH6B* locus was only observed in meta-analysis. A locus on chromosome 7 reached genome-wide significance in GoDARTS but showed the opposite direction of effect in the Australian cohort, indicating this locus is not likely to influence DME risk; however, this needs validation in future studies. The Australian cohort identified two additional suggestive loci (chromosome 6 upstream of *ELOVL5* and chromosome 4 within *PCDH7*), but neither locus was replicated in the GoDARTS cohort and both showed substantial heterogeneity. Given the two meta-analysis cohorts are of European ancestry, we would expect a true risk locus to have a similar effect direction in each cohort. The absence of this expected uniformity suggests that these loci are less likely to influence DME risk, although it could also reflect insufficient statistical power.

The SNPs highlighted on the chromosome 1 locus are associated with a decreased risk of developing DME/maculopathy, indicating the minor or alternate alleles are protective for the diabetic complication. The locus lies close to *RNU5E-1*, a small nuclear RNA (snRNA) gene predicted to be involved in pre-mRNA splicing.[29] Evidence of splicing abnormalities involving snRNA has been reported in previous studies in relation to diabetes and diabetes-related complications.[30,31] This suggestive locus was also associated with significant allele-specific expression of *MTHFR*, *PLOD1*, *KIAA2013*, and *NPPA*, two of which are strong candidates for a role in DME pathogenesis. The MTHFR enzyme is fundamental to folate metabolism, with impaired activity resulting in hyper-homocysteinemia.[32] Evidence suggests that homocysteine activates vascular inflammation through inflammatory cytokines, including VEGF,[33] which is an important molecule in DME pathogenesis. *MTHFR* has been proposed to be linked with T2 diabetes susceptibility[34] and diabetes-related complications, including DR.[35–38] Furthermore, many individual studies and meta-analyses reported significant associations between the *MTHFRC677T* polymorphism and T2 diabetes.[39,40] The same polymorphism has been linked to the progression of DR, particularly in Asians.[41] The *NPPA* gene belongs to the natriuretic peptide family. Studies report elevated levels of natriuretic peptides in patients with DR and suggest these peptides have a regulatory role on VEGF.[42,43] Natriuretic peptides also play an essential role in regulating plasma volume, blood pressure, and renal excretion, which can contribute to fluid retention and may increase DME risk.[44]

The chromosome 13 locus corresponding to *ERICH6B* is associated with an increased risk of development of DME. *ERICH6B* is a protein coding gene and although there is no prior evidence suggesting a role in diabetes or DME, it has been previously associated with macular dystrophy syndrome.[45]

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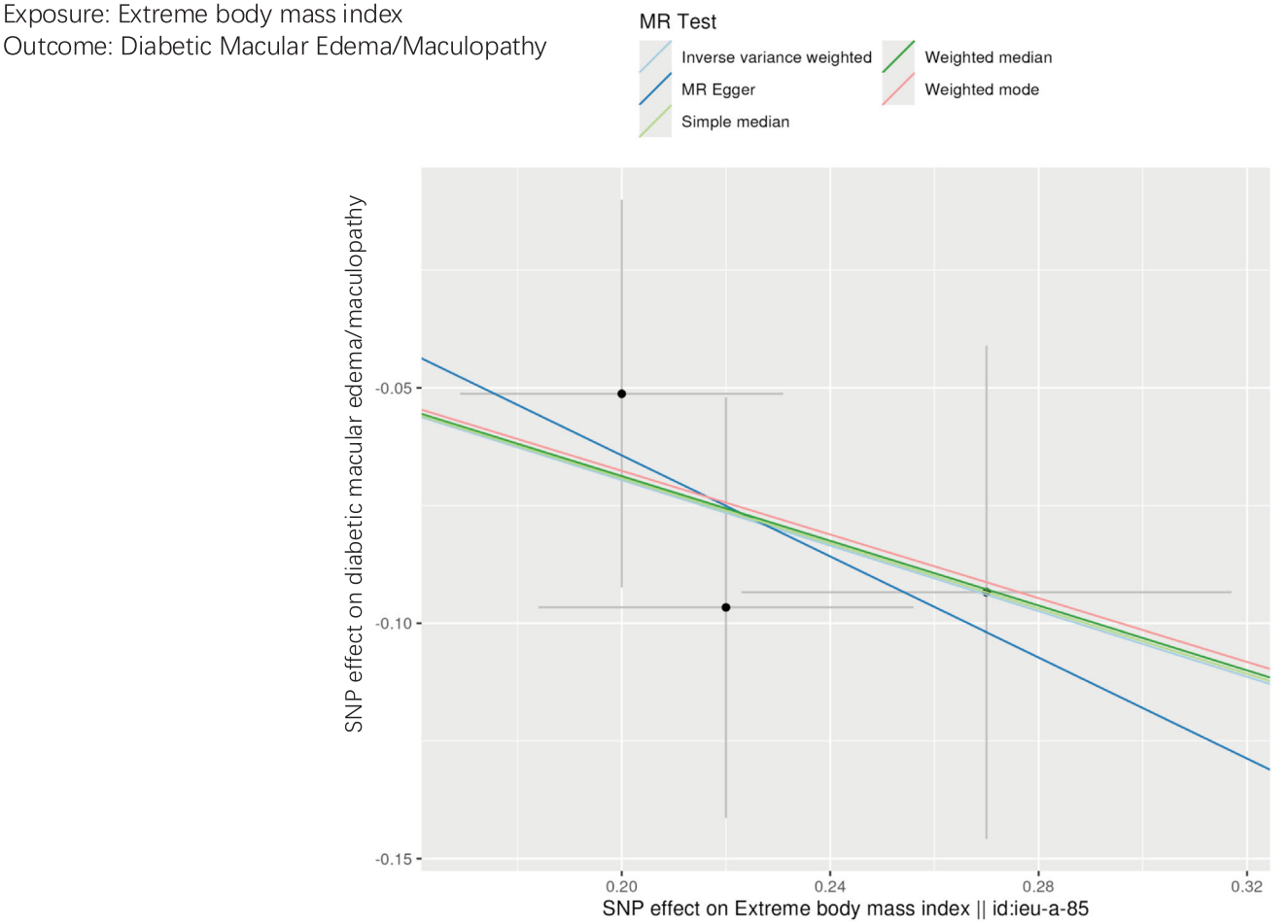

Figure 3. Plot of Mendelian Randomization results between extreme body mass index (EBMI) and diabetic macular edema/maculopathy. *Five different color lines* mean different statistical methods used within the MR framework. The IVW method shows a significant negative association, whereas other methods suggest consistency in effect direction. The significant results found in the IVW, weighted median, and simple median methods demonstrate a consistent negative effect of EBMI on diabetic macular edema/maculopathy. These results highlight the robustness of the negative association, although the methods differ in sensitivity and statistical power due to underlying assumptions and treatment of instrumental variable validity.

The meta-analysis combined two previously published but now expanded cohorts.[9,10] Of note, the previously reported loci identified in the original analyses of each cohort were not replicated in the expanded datasets (i.e. *MLRP19* in the Australian cohort and *TTC39C* in GoDARTS). This is consistent with a general lack of replication of GWAS loci, as discussed previously,[46,47] and further highlights the need to interpret results from small studies cautiously. For further analysis, we have included results for the top SNPs reported in Stockwell et al.'s study[11] in Supplementary Table S7; none of these SNPs reached statistical significance in our meta-analysis.

As mentioned above, for many of the putative loci (chromosome 7, chromosome 6, and chromosome 1), substantial heterogeneity was apparent. Heterogeneity from a variety of sources is an important consideration in any GWAS, especially for meta-analyses.[48,49] The GoDARTS case definition for diabetic maculopathy was broader than the specific DME definition used in the Australian cohort. Diabetic maculopathy can include both DME and diabetic ischemic maculopathy. Therefore, the chromosome 7 locus identified in GoDARTS may be associated with general maculopathy, not specifically with DME. In addition to differing phenotype definitions from different studies, heterogeneity may be caused by underlying population differences[50,51] or differences in genotyping platforms, imputation accuracy, and genotyping errors.[52] Although meta-analysis offers advantages in terms of sample size and power, it cannot replace an adequately powered primary association study. Pooled analysis of primary genotypic data from independent studies, using consistent and standardized protocols, maximizes information on covariates contributing to heterogeneity.[53]

The PheWAS results provide exploratory insights, revealing that loci on chromosome 1 are predominantly associated with body composition traits (e.g. hip circumference, arm fat, leg fat, trunk fat, and BMI). Notably, osteoarthritis of the knee and plateletcrit have emerged as novel areas of interest associated with DME, as reported in recent studies.[54,55] On chromosome 7, certain SNPs have been linked to anxiety, with approximately three-quarters of patients with DME experiencing anxiety during treatment.[56] SNPs on chromosome 13 have been found to be associated with monocyte count, which is reported to influence DME.[57]

Further MR analysis indicates that only extreme BMI, and not general BMI indices, significantly negatively impacts DME, supporting its role as a significant negative predictor for clinically significant DME ($P < 0.001$).[58] This observation underscores the unique pathophysiological relevance of extreme BMI in the genetic mechanisms underlying DME.

One important consideration is that both participating cohorts adjusted for heritable covariates (including HbA1c and hypertension) in the logistic regression model. It has been suggested that using these covariates in GWAS could elevate spurious loci to statistical significance.[59] Analysis without adjusting for such covariates could be an approach taken in future studies. It is also worth noting that the covariates used in this study showed significant imbalances between cases and controls. Whereas the adjustment can reduce biases, it does not completely eliminate them due to residual confounding, unmeasured confounding, and overadjustment. Both DME and DR are complications of diabetes that share some pathophysiological features, including retinal vascular damage, suggesting potential shared genetic risk factors. At the same time, not all people with DR develop DME, suggesting that there might also be unique genetic factors that contribute to the progression from DR to DME.[60]

In conclusion, this meta-analysis, the largest GWAS of diabetic maculopathy risk to date (combined cases = 2506 and combined controls = 7140), suggests several promising SNPs related to disease risk.

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Data Sharing: The summary statistics of the meta-analysis results of this study will be shared upon request.

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