RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2012.38.340

ScienceAsia 38 (2012): **340–348**

Association of the *PLEKHO2* and *PLEKHH1* gene polymorphisms with type 2 diabetic retinopathy in a Taiwanese population

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Received 30 Mar 2012
Accepted 6 Sep 2012

ABSTRACT: Diabetic retinopathy (DR) is a chronic retinal disorder, in which the retinal microvasculature is gradually altered, ultimately leading to blindness. Previous observations on clinical variations of the onset and severity of DR in various patients and populations suggest that genetic polymorphisms contribute to DR development. The present study was undertaken in an attempt to uncover new genetic factors contributing to the development of DR in a Taiwanese population. A well-defined Taiwanese population comprising persons with type 2 diabetes mellitus (T2DM) ($n = 749$) was recruited for this study. We conducted a genome-wide association study in an independent set of 174 patients with DR and 575 without DR, using Illumina HumanHap550-Duo BeadChip. Eleven single nucleotide polymorphisms (SNPs) with the most significant test statistics ($p \leqslant 1 \times 10^{-5}$) were selected from one of the models. Of the selected SNPs, rs832882 (G/A) ($p = 2.29 \times 10^{-6}$; odds ratio (OR) = 1.49; 95% confidence interval (CI) = 1.11–2.00) and rs3742872 (G/A) ($P = 1.19 \times 10^{-15}$; OR = 1.95; 95% CI = 1.02–3.72), both identified as having the most significant association with DR, are located in the intronic region of the gene encoding the pleckstrin homology (PH) domain-containing proteins family O member 2 (*PLEKHO2*) and family H member 1 (*PLEKHH1*), respectively. Functional prediction analysis strengthened the likelihood of participation of *PLEKHO2* and *PLEKHH1* in the development of DR. The current findings suggest that the rs832882 and rs3742872 polymorphisms may be harbouring retinopathy susceptibility in a Taiwanese population, and implicate the pathological role of PH domain-containing proteins in DR development.

KEYWORDS: SNPs, T2DM, pleckstrin, hyperglycaemia

INTRODUCTION

Diabetic retinopathy (DR), a frequent and severe complication of diabetes, is a chronic retinal disorder in which the retinal microvasculature is gradually altered, ultimately leading to blindness[1,2]. Many risk factors, including the duration of diabetes, control of blood glucose, and blood pressure affect the development of DR[3–5]. However, these risk factors alone are unable to account for the incidence and progression of DR[6,7]. In clinical observations, DR development is controllable in some cases of type 2 diabetes mellitus (T2DM), whereas it quickly occurs in other T2DM patients despite attempts to control it. Therefore, clinical variations in the onset and severity of DR in different patients could be the result of an interplay between non-genetic risk factors and various genetic polymorphisms.

Variations in the DNA sequences of an individual strongly affect gene expression, gene reaction to the environment, and disease development[8,9]. Previous observations and studies on DM in relation to familial aggregation[10] and racial differences[11,12] revealed that genetic influence is an additional risk factor in DR development, aside from duration of diabetes and glycaemic control. Genetic influences increase the possibility of genetic predisposition to DR. To date, various genetic polymorphisms in the genes encoding aldose reductase (*ALR2*)[13], receptor for advanced glycation end products (*RAGE*)[14], vascular endothelial growth

factor (*VEGF*)[15], 5-10-methyllenetetrahydrofolate reductase (*MTHFR*)[16], and apolipoprotein-E (*APOE*)[17] have been identified as being associated with DR development. Coincidentally, the reactions involving these genes, such as the activation of aldose reductase, non-enzymatic glycation, hypoxia-induced angiogenesis, and diacylglycerol-protein kinase C, are all initiated by hyperglycaemia[18,19].

Identifying DR genetic susceptibilities has remained a challenge for geneticists worldwide because of the innate complexity of DM. Currently, most of the potential DR susceptibility genes identified from different populations were obtained from a small number of DM patients[20–22]. The pleckstrin homology (PH) domain, comprising approximately 100–120 amino acids, is first identified in pleckstrin, a major substrate of protein kinase C (*PKC*) in platelets[23,24]. Many of the proteins containing PH domains are involved in intracellular signal cascades and cytoskeletal remodelling[25–27]. Any genetic defects in the PH domain-containing proteins are possibly associated with various cancers and other serious human diseases, including retinal degenerative diseases[28,29]. The aim of the current study is to determine the presence of a single nucleotide polymorphism (SNP) deposit and uncover new genetic factors contributing to DR development in a well-classified Taiwanese population. We present the results on polymorphism in the genes encoding the PH domain-containing proteins *PLEKHO2* and *PLEKHH1*, which confer susceptibilities to DR. To the best of our knowledge, the current study is the first to address the possible association between SNPs in the gene encoding PH domain-containing proteins and DR in a Taiwanese population.

MATERIALS AND METHODS

Clinical subjects

T2DM patients ($n = 749$) previously admitted at the China Medical University Hospital, Taichung, Taiwan were examined for signs of retinopathy based on international scales for severity of clinical DR proposed by the American Academy of Ophthalmology. Ophthalmologic examinations used for this study for all T2DM patients included visual acuity, fundoscopic examination, and fundus photography. In addition to collecting patient profiles, including gender, age, age at diagnosis of diabetes, and ocular history, other clinical information, such as systolic and diastolic blood pressure, waist and hip circumferences, body mass index (BMI), and haemoglobin A1C (HbA1C) levels, were also determined. Of the 749 T2DM patients, 174 were diagnosed with DR. All subjects, including T2DM patients with and without retinopathy, were of Han Chinese origin, which accounts for 98% of the Taiwanese population. Detailed clinical characteristics, including gender, age, BMI, HbA1C, fasting plasma glucose, and duration of diabetes, are summarized in Table 1.

Genotyping

Genetic data on T2DM patients with and without DR, which were acquired from a genome-wide association study (GWAS) using Illumina HumanHap550-Duo BeadChip and contain more than 550 000 SNP markers, were analysed to identify SNP markers associated with DR. Genomic DNA was extracted using the PUREGENE DNA Isolation Kit (Gentra Systems, Minneapolis, MN) and genotyping was performed using Illumina HumanHap550-Duo BeadChip (Illumina Inc.) following the manufacturer instructions. After hybridization, the resulting fluorescence intensity data were saved as an Illumina BeadStudio project file. Several criteria were followed to ensure that the genotype data are of high quality, as described in a previous study[30]. All SNPs of poor quality were removed from the dataset.

Statistical analysis

To compare the clinical data of T2DM patients with and without DR, a Student's t-test and a Chi-square (χ^2) test were used to determine continuous and categorical variables, respectively. To evaluate the allele frequency and genotype distribution between T2DM patients with and without DR, six single-point methods, namely, genotype, allele, Cochran-Armitage trend test, and dominant, recessive and additive models, were applied to the T2DM association analysis. The most significant test statistic acquired from the six models was considered. SNPs with P values less than 10^{-5} were chosen for a stepwise regression analysis to determine the significant SNPs. Using multiple comparisons corrected by the Bonferroni method, SNPs with P values less than 10^{-5} were considered significant associations with DR. To evaluate the association of SNPs with DR, the odds ratio (OR) values with 95% confidence intervals (CIs) were calculated to estimate the relative risk presented by a particular allele and genotype. The most common genotype in T2DM patients without DR was regarded as the reference. To identify SNPs among the selected candidates in the linkage disequilibrium (LD) region, two LD measures, pairwise Lewontin's disequilibrium coefficient (D') and correlation coefficient (r^2) were calculated in a 250 kb-long flanking region between SNPs and the

Table 1 Clinical characteristics of the T2DM patients.

	DR+ ($n = 174$)	DR− ($n = 575$)	P value* (DR+ versus DR−)
Gender			
male	50.6%	53.9%	0.440†
female	49.4%	46.1%	
Age at study (mean, years)	58.0	61.9	< 0.001
Age at diagnosis T2D (mean ± SD, years)	47.7 ± 9.3	50.2 ± 9.2	0.002
Duration of diabetes (mean ± SD, years)	14.8 ± 8.3	8.3 ± 6.5	< 0.001
HbA1C (mmol/mol)	67 ± 15	61 ± 15	< 0.001
DR severity scales‡			
Non-proliferative DR (NPDR)			
mild NPDR	31.0%		
moderate NPDR	25.9%		
severe NPDR	1.7%		
Proliferative DR	41.4%		
Proportion of macular oedema	25.3%	1.6%	< 0.001†
BMI (kg/m^2)	25.2 ± 4.0	25.1 ± 3.8	0.612
Proportion of BMI categories			
underweight (BMI < 18.5)	4.0%	1.4%	0.065†
normal ($18.5 \leqslant$ BMI < 24)	30.7%	35.1%	
overweight ($24 \leqslant$ BMI < 27)	30.7%	34.4%	
obese (BMI $\geqslant 27$)	34.7%	29.2%	
Systolic blood pressure (mmHg)	135 ± 19	128 ± 16	< 0.001
Diatolic blood pressure (mmHg)	75 ± 11	76 ± 11	0.067
Waist (cm)	90 ± 10	89 ± 10	0.147
Hip (cm)	97.0 ± 8.1	97.1 ± 7.6	0.892
Smoking status			
no	71.6%	64.9%	0.243†
former	14.2%	16.4%	
current	14.2%	18.7%	

Abbreviations: DR+, with diabetic retinopathy; DR−, without diabetic retinopathy; SD, standard deviation; HbA1C, haemoglobin A1C; NPDR, non-proliferative DR; BMI, body mass index.

* Student's t-test.

† Chi-square test.

‡ According to the American Academy of Ophthalmology proposed international scales for severity of clinical diabetic retinopathy.

selected candidate SNP from all the HapMap-SNPs of a Han-Chinese population in Beijing (HCB) and a Japanese population in Tokyo (JPT) using SNP Annotation and Proxy Search (SNAP version 2.1)[31]. The threshold of the haplotype block was set at 0.2 of correlation coefficients (r^2). In this study, two SNPs with $r^2 \geqslant 0.7$ were considered to have good correlation.

Functional prediction analysis

To identify the genes containing candidate SNPs that confer a susceptibility to DR in a more detailed, functional prediction, an online gene-annotation portal, BioGPS (http://biogps.gnf.org), was used to identify the genes based on the similarity of the gene expression patterns with a cut-off value of Pearson's correlation coefficient ($\geqslant 0.9$) via the Gene Atlas expression data[32]. The protein interaction network was predicted using the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING 8.3) and the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database. In STRING 8.3 (http://string-db.org), four major sources, namely, genomic context, high-throughput experiments, co-expression, and previous knowledge, were used to predict the interactions between the proteins via direct and indirect association methods[33]. A predicted association of two genes/proteins was defined by confidence scores (CS) (low confidence, scores < 0.4; medium, 0.4–0.7; and high, > 0.7).

Table 2 Summary of SNPs associated with diabetic retinopathy in type 2 diabetes.

db SNP ID (Nearest gene)	Chr.	Functional class	Risk allele* (non-risk allele)	P value† (best model)	$-\log(P)$	OR (95% CI)‡
rs7546260	1	Intronic	G (T)	2.59×10^{-11}	10.59	0.58 (0.30–1.13)[g]
(*TNN*)						0.67 (0.36–1.26)[a]
rs2238038	12	Intronic	T (C)	3.70×10^{-8}	7.43	1.28 (0.77–2.14)[g]
(*CACNA1C*)						1.44 (0.90–2.32)[a]
rs3794300	12	Intronic	T (G)	5.02×10^{-8}	7.30	1.35 (0.81–3.25)[g]
(*CACNA1C*)						1.47 (0.92–2.37)[a]
rs11062134	12	Intronic	G (A)	3.70×10^{-8}	7.43	1.28 (0.77–2.14)[g]
(*CACNA1C*)						1.44 (0.90–2.32)[a]
rs12579529	12	Intronic	G (A)	7.66×10^{-9}	8.12	1.28 (0.77–2.14)[g]
(*CACNA1C*)						1.49 (0.92–2.40)[a]
rs3742872	14	Intronic	A (G)	1.19×10^{-15}	14.93	1.85 (0.94–3.62)[g]
(*PLEKHH1*)						1.95 (1.02–3.72)[a]
rs832882	15	Intronic	A (G)	2.29×10^{-6}	5.64	1.57 (1.10–2.22)[g]
(PLEKHO2)						1.49 (1.11–2.00)[a]
rs2911275	16	Intronic	C (A)	7.43×10^{-6}	5.13	0.81 (0.58–1.14)[g]
(*CMIP*)						0.99 (0.77–1.29)[a]
rs413223	17	Intergenic	C (T)	6.62×10^{-10}	9.18	0.68 (0.43–1.06)[g]
(*RGS9*/*GNA13*)						0.76 (0.50–1.15)[a]
rs444394	17	Intergenic	C (A)	6.63×10^{-10}	9.18	0.71 (0.46–1.09)[g]
(*RGS9*/*GNA13*)						0.78 (0.52–1.17)[a]
rs2009217	18	Intergenic	G (A)	1.55×10^{-7}	6.81	0.90 (0.55–1.48)[g]
(Unknown)						1.03 (0.65–1.62)[a]

Abbreviations: dbSNP ID, SNP database identification; Chr, chromosome; OR, odds ratio; CI, confidence interval; *TNN*, tenascin N; *CACNA1C*, calcium channel, voltage-dependent, L type, alpha 1C subunit; *PLEKHH1*, pleckstrin homology domain containing proteins family H member 1; *PLEKHO2*, pleckstrin homology domain containing protein family O member 2; *CMIP*, c-Maf-inducing protein; *RGS9*, Regulator of G-protein signalling 9; *GNA13*, Guanine nucleotide-binding protein subunit alpha-13.

* Allele with higher frequency in DR patients as compared to non-DR patients.

† P value (best model): p-value obtained from the model with the most significant statistic. These include genotype, allele, trend, additive, dominant, and recessive models.

‡ ORs with 95% CIs were calculated for the risk allele.

g Dominant model was used for genotypic frequency test.

a Allelic frequency test.

SNP ID with significant results in odds ratio are set in italics.

RESULTS

Association analysis

Eleven SNPs ($P \leqslant 1 \times 10^{-5}$) not previously considered as DR-related markers were selected from among the models having the most significant test statistic; these markers were considered to be significantly associated with DR in a Taiwanese population (Table 2). To further evaluate the selective SNPs associated with DR, OR values with 95% CIs between T2DM patients with and without DR were calculated to estimate the relative risk presented by a particular allele and genotype. As a result, SNP rs832882, which has the most statistical significance, was selected based on the OR in genotypic (OR = 1.57; 95% CI = 1.10–2.22) and allelic frequencies (OR = 1.49; 95% CI = 1.11–2.00) between T2DM patients with and without retinopathy. In contrast, SNP rs3742872 was chosen as the second most significant locus susceptible to DR, based on the OR in the allelic frequency (OR = 1.95; 95% CI = 1.02–3.72). The OR in the genotypic frequency of rs3742872 (OR = 1.85; 95% CI = 0.94–3.62) was not as significant as that in the allelic frequency (Table 2). Interestingly, both rs832882 and rs3742872 genomic positions are located in the intronic region of the gene containing the PH domain.

LD analysis

Candidate SNPs rs832882 and rs3742872 were characterized, as were their corresponding SNPs, as having high LD values for underlying DR-associated variants. Therefore, SNPs around the two candidate

SNPs were further analysed using the LD values r^2 and D'. As a result, four SNPs, namely, rs1107528 ($r^2 = 0.739$ and $D' = 1$), rs6494499 ($r^2 = 0.739$ and $D' = 1$), rs9302235 ($r^2 = 0.739$ and $D' = 1$), and rs3743046 ($r^2 = 0.712$ and $D' = 1$), were identified as the candidate SNP rs832882. Meanwhile, three SNPs, namely, rs749270 ($r^2 = 1.000$ and $D' = 1$), rs3742875 ($r^2 = 0.829$ and $D' = 1$), and rs6573785 ($r^2 = 0.829$ and $D' = 1$), were identified as the candidate SNP rs3742872 (Fig. 1). Of these, rs1107528, rs6494499, and rs9302235, found in the LD region with rs832882, are located in the intronic region of *PLEKHO2*, whereas rs3743046 is located in 5′-UTR of the gene-encoded 5′-to-3′ DNA helicase (*PIF1*) (Fig. 1a). SNPs rs749270 and rs3742875, found in the LD region around rs3742872, are located in the intronic and 3′-UTR regions of *PLEKHH1*, respectively, whereas rs6573785 is located in the intronic region of the gene-encoded phosphatidylinositol N-acetylglucosaminyltransferase subunit H (*PIGH*) (Fig. 1b). Nonetheless, risk-SNPs in close proximity to these genes are generally believed to be pathogenetically important based on their location alone.

Functional prediction analysis

Using STRING 8.3 and KEGG database, a strong association (CS = 0.800) was found between *PLEKHO2* and *PDGFRB*, a tyrosine kinase receptor of the platelet-derived growth factor subunit B (*PDGFB*) in the cytokine-cytokine receptor interaction pathway. Meanwhile, a weak association (CS = 0.508) was predicted between *PLEKHH1* and osteopontin (*OPN*). An indirect or direct crosstalk between *PLEKHO2* and *PLEKHH1* possibly occurs via *PDGFRB*, *PDGFB*, and *SPP1* (*OPN*), as predicted by various evidence (Fig. 2). Moreover, with the assistance of BioGPS, the nerve injury-induced protein 1 (*NINJ1*) was identified based on a similar expression pattern with *PLEKHO2* (correlation coefficient = 0.9289). Several genes, including the nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, epsilon (*NFKBIE*), endothelin converting enzyme 2 (*ECE2*), putative scavenger receptor cysteine-rich domain-containing protein (*LOC619207*), interleukin 21 receptor (*IL21R*), coiled-coil domain-containing protein 28B (*CCDC28B*), zinc finger and BTB domain-containing protein 32 (*ZBTB32*), cation transport regulator-like protein 1 (*CHAC1*), and *KIAA1797*, were also selected for *PIF1* (correlation cutoff $\geqslant$ 0.9) because SNP rs3743046, which was identified in the LD analysis as the candidate SNP rs832882, occurred in the 5′-UTR of *PIF1*.

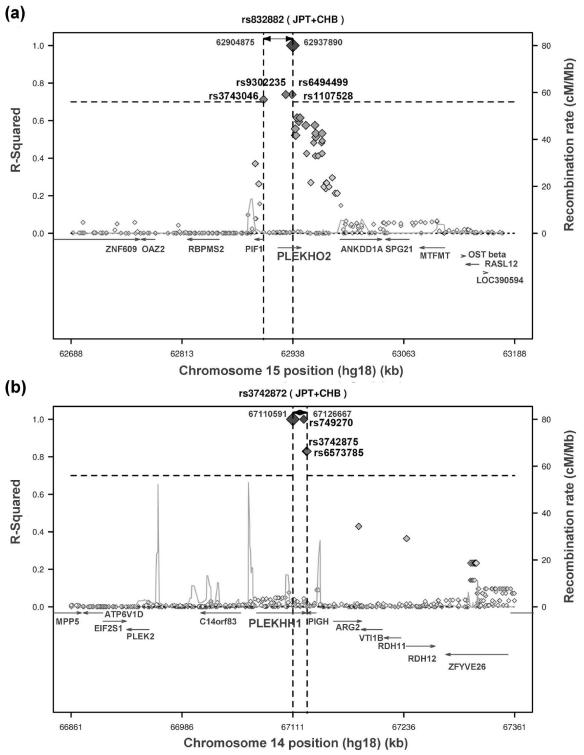

Fig. 1 Regional linkage disequilibrium (LD) plot for SNPs associated with DR in T2DM. Measure of linkage disequilibrium value (r^2) and recombination rate in centimorgans per million bases (cM/Mb) were calculated in 250 kb of the LD region around (a) rs832882 and (b) rs3742872, shown in the left-hand and right-hand y-axis of the plot, respectively. Gene annotations were taken from NCBI, and are shown as horizontal lines below the plot.

DISCUSSION

A GWAS has previously identified several SNPs associated with T2DM in persons of Han Chinese descent residing in Taiwan[30]. In the current study, emphasis was placed on identifying SNPs potentially associated with Type 2 DR in this population. We found that SNP rs832882, which is located in the intronic region of *PLEKHO2*, showed the strongest association with DR. However, the role of the biological function and pathological mechanisms of *PLEKHO2* in DR is unclear, despite its extensive expression in various tissues, including the heart, thyroid, lymph node, bone marrow, and retina. Interestingly, *PDGFB* was identified for potential association with *PLEKHO2* in the cytokine-cytokine receptor interaction pathway. At present, several proteins, including PDGFB, PDGFRB, VEGFA, and the fms-related tyrosine kinase 1 (FLT1), are believed to be responsible for

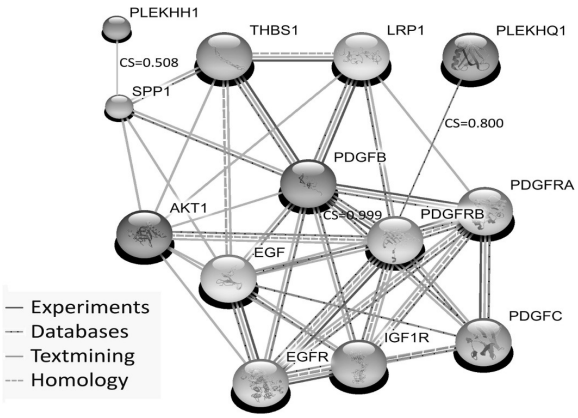

Fig. 2 Potential interaction networks of *PLEKHO2* and *PLEKHH1*. Interaction networks were predicted by STRING 8.3 via databases comprised of experimental data, computational prediction methods, and public text collections, and were comprised of eleven proteins with $>$ 0.95 CS for each interaction. The different line styles indicate the types of evidence for each interaction. Full name of the genes involved in the network is listed as follows: *AKT1*, RAC-α serine/threonine-protein kinase; *EGF*, pro-epidermal growth factor precursor; *EGFR*, epidermal growth factor receptor precursor; *IGF1R*, insulin-like growth factor 1 receptor precursor; *LRP1*, low-density lipoprotein receptor-related protein 1 precursor; *PDGFB*, platelet-derived growth factor B chain precursor; *PDGFC*, platelet-derived growth factor C precursor; *PDGFRA*, alpha platelet-derived growth factor receptor precursor; *PDGFRB*, beta platelet-derived growth factor receptor precursor; *PLEKHH1*, pleckstrin homology domain containing, family H (with MyTH4 domain) member 1; *PLEKHQ1*, pleckstrin homology domain containing, family Q member 1 (*PLEKHO2*); *SPP1* (*OPN*), osteopontin precursor (bone sialoprotein-1) (secreted phosphoprotein 1); *THBS1*, thrombospondin-1 precursor.

recruiting pericytes to newly formed vessels and for maintaining vascular integrity. In diabetic patients, cellular responses such as oxidative stress, DNA damage, and apoptosis caused by high glucose occur in most cell types. Some cell types, such as retinal cells, are specifically vulnerable to hyperglycaemia. In the retina, pericyte loss is mainly caused by hyperglycaemia, and eventually develops into DR[34]. A previous study showed that *PDGFB* or *PDGFRB* gene-knockout mice presented identical phenotypes leading to pericyte loss[35], which developed into early DR[36]. In a recent study on oxygen-induced retinopathy, a decreased PDGFB was found in the retina[37,38]. Nonetheless, the predicted association between the *PDGFB/PDGFRB* system and *PLEKHO2* suggests that the genetic variant rs832882 can be a potential risk that can lead to DR.

Besides, the strongest association with DR is found with SNP, LD is also significant between SNPs rs832882 and rs3743046. SNP rs3743046 is located in the 5′-UTR of *PIF1*, which can negatively regulate telomerase activity. *PIF1* has been implicated in the maintenance of mitochondrial genome stability[39]. In diabetic patients, an accumulation of reactive oxygen species (ROS) by mitochondrial respiration would considerably impair mitochondrial DNA (mtDNA) given mtDNA's higher vulnerability to ROS compared with nuclear DNA. The genetic variation rs3743046, which occurred in the 5′-UTR of *PIF1*, might alter gene expression and subsequently result in mitochondrial genomic instability and DNA damage. Using BioGPS and the Gene Atlas expression data, several genes were selected based on expression patterns similar to *PIF1*. Of these genes, *NFKBIE* and *ECE2* are potentially associated with DR development. *NFKBIE* inhibits the activity of the redox-sensitive transcription factor and the nuclear factor-kappa B (*NF-*κ*B*)/proto-oncogene c-Rel (*REL*) dimeric complexes. *NF-*κ*B* activation was found in the pericytes of diabetic retinal vessels, which resulted from accelerated pericyte loss[40]. Some diabetic patients are unable to express *PIF1* possibly because of the genetic variant rs3743046. A decreased *PIF1* expression can directly or indirectly reduce *NFKBIE* expression in diabetic pericytes. In such pericytes, *NF-*κ*B* would be readily transferred to the cell nucleus in an active form, as a consequence of low expression of *NFKBIE*. On the other hand, *ECE2* can convert the large endothelin-1 into the most potent vasoconstrictor, endothelin-1 (*EDN1*). A recent study showed that the messenger RNA (mRNA) level was markedly more elevated in patients with DR than in those without DR[41]. This result is attributed to *NF-*κ*B* activation[42].

The second strongest association with DR was found in SNP rs3742872, which is located in the intronic region of *PLEKHH1*. Despite the weak association between *PLEKHH1* and *OPN*, an elevated *OPN* has been reported in patients with DR[43]. In addition, rs3742872 is located not only in the 3′-UTR of *PLEKHH1* containing a binding site for microRNA, but also in the 5′-UTR of *PIGH*, suggesting a potential role in the gene regulation of *PLEKHH1* and *PIGH*. Nonetheless, the two SNPs (rs832882 and rs3742872) identified in the current study are located in the intronic region of the gene-encoded PH domain-containing proteins. Therefore, it is difficult to predict

any significant association between polymorphism and gene expression. PH domains comprising approximately 100–120 amino acids can bind to phosphoinositides (PI) with high affinity[44]. It has been shown that the PI plays a significant role in the phosphatidylinositol 3-kinase (*PI3K*)/RAC-alpha serine/threonine-protein kinase (*AKT*) pathway, where the uncoupling of insulin signals at *PI3k-Akt* take place in the retina in response to hyperglycaemia[45]. As with that which occurs in the *PI3K*/*AKT* signalling pathway, it is possible that PLEKHO2 or PLEKHH1 becomes carried by PIs to the membrane, where they are activated for cell survival. Any genetic factor occurring in these genes might result in retinopathy. However, because of the limited number of DR patients recruited for the association study, we used genomic data from all DR patients for the analysis, regardless of their severities. Therefore, we were unable to identify the potential genes specific to the DR subgroups. Nonetheless, the potential DR susceptibility genes (*PLEKHO2* and *PLEKHH1*) identified in the current study may be involved in the early stage of DR development. Further analysis of the DR subgroups (mild and moderate NPDR) is essential to validate the findings of the present study, if the number of DR patients were large enough. On the other hand, the rs832882 and rs3742872 polymorphisms were found to be harbouring retinopathy susceptibility in the Taiwanese population, but further analysis of polymorphism in the genes encoding the PLEKHO2 and PLEKHH1 from different populations is indispensable to clarify the pathological role of the PH domain-containing proteins in DR development.

In conclusion, both association and LD analysis identified several loci harbouring retinopathy susceptibility. A functional prediction analysis strengthened the candidate gene-containing retinopathy susceptibility loci in their pathological roles in the retina of patients with diabetes. Our findings suggest that the rs832882 and rs3742872 polymorphisms may be harbouring retinopathy susceptibility in the Taiwanese population, and implicate the pathological role of the PH domain-containing proteins in DR development. Further studies enrolling larger numbers of patients from different populations are needed to confirm this observation.

Acknowledgements: This study was supported by China Medical University of Taichung grants CMU97-100.

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