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The Genetic Study of Diabetic Retinopathy: Recruitment methodology and analysis of baseline characteristics.

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ABSTRACT

BACKGROUND: Diabetic retinopathy (DR) is a blinding disease of increasing prevalence, caused by a complex interplay of genetic and environmental factors. Here we describe the patient recruitment methodology, case and control definitions, and clinical characteristics of a study sample to be used for genome-wide association (GWAS) analysis to detect genetic risk variants of DR.

METHODS: 1669 participants with either type 1 (T1) or type 2 (T2) diabetes mellitus (DM) aged 18 to 95 years were recruited in Australian hospital clinics. Individuals with T2DM had disease duration of at least 5 years, and were taking oral hypoglycemic medication, and/or insulin therapy. Participants underwent ophthalmic examination. Medical history and biochemistry results were collected. Venous blood was obtained for genetic analysis.

RESULTS: 683 diabetic cases (178 T1DM and 505 T2DM participants) with sight-threatening DR, defined as severe non-proliferative DR (NPDR), proliferative DR (PDR) or diabetic macular edema (DME) were included in this analysis. 812 individuals with DM but no DR or minimal NPDR were recruited as controls (191 with T1DM and 621 with T2DM). The presence of sight-threatening DR was significantly correlated with DM duration, hypertension, nephropathy, neuropathy, HbA1C and BMI. DME was associated with T2DM ($p<0.001$), whereas PDR was associated with T1DM ($p<0.001$).

CONCLUSIONS: Adoption of a case-control study design involving extremes of the DR phenotype makes this a suitable cohort, for a well-powered GWAS to detect genetic risk variants for DR.

Keywords: diabetic retinopathy, diabetic macular edema, genome wide association study.

INTRODUCTION

Diabetic retinopathy (DR) is the leading cause of blindness in working age adults[1]. The Australian National Diabetes Information Audit and Benchmark exercise (ANDIAB) from 2009 reports that diabetic Australians with access to large diabetes centres for screening and treatment have a prevalence of DR of 29%, and a rate of blindness approaching 1%[2]. The diabetic complication rates of those living in remote Australia with limited access to healthcare are much greater, with a rate of vision threatening retinopathy between 7 and 11% [3-5].

Landmark trials over the last 30 years have been crucial in substantiating an association between the development and progression of DR and risk factors including diabetes mellitus (DM) duration, glycemic control, hypertension and hyperlipidemia[6-8]. The focus of recent research on DR susceptibility has moved to the contribution of genetic susceptibility to DR development and severity. The Diabetes Control and Complications Trial (DCCT) was the first large-scale trial to investigate and determine a significant correlation between patients and their first-degree relatives in relation to the presence and severity of DR, independent of glycemic control [9]. Significant familial aggregation of DR severity across a range of ethnic groups was subsequently demonstrated, with the degree of heritability dependent on ethnicity[10]. The heritable component of sight-threatening DR has been reported to be between 25% and 50%[10,11].

Genome-wide association studies (GWAS) have been conducted to investigate the genetic contribution to DR susceptibility, but to date, results have not been reproducible across studies and studies have lacked appropriate statistical power to detect variants of even modest effect size[12,13]. A number of functional candidate genes for DR susceptibility have

been studied, including aldose reductase (*ALR*), angiotensin converting enzyme (*ACE*), vascular endothelial growth factor (*VEGF)* and nitric oxide synthase (*NOS*)[14]. The efficacy of inhibiting the VEGF pathway in the treatment of diabetic macula edema (DME) demonstrates the clinical relevance of research aimed at elucidating the genetic risk factors, and the associated molecular pathways involved in the pathogenesis of sight-threatening DR. Such improved understanding is required to facilitate the development of personalized approaches, and novel therapeutic strategies to reduce the burden of diabetes associated visual morbidity.

The sample presented in this paper is a current representation of Australian type 1 (T1) and type 2 (T2) diabetes patients from an era in which the importance of glycemic control and systemic vascular risk factors have been well understood. The target population includes individuals with DM affected by potentially sight-threatening complications of DM, as well as individuals who have DM without retinopathy. The eventual outcome of this study is to investigate the influence of genetics on the development and severity of DR, specifically using GWAS methodology to identify candidate single-nucleotide polymorphisms (SNPs). An understanding of the molecular pathogenesis of DR would have significant implications for screening strategies and the development of novel therapies. The present report describes the recruitment methodology, case control definitions, and the clinical characteristics of the sample to be studied.

METHODS

Ethics approval:

In South Australia, ethics approval was obtained from the Southern Adelaide Health Service Flinders University Clinical Research Ethics Committee (Flinders Medical Centre

and Repatriation General Hospital), and the Human Research Ethics Committees (HREC) of the Royal Adelaide Hospital and Queen Elizabeth Hospital. In New South Wales and Victoria, the HREC of Southeastern Sydney and Illawara Northern Hospital Network (Sydney Eye Hospital), and the HREC of Melbourne Health (Royal Melbourne Hospital) approved this study. The ACT Health HREC approved this study in the Australian Capital Territory. The study adhered to the tenets of the Declaration of Helsinki.

Participant recruitment and consent:

In 2007 recruitment of participants began from three tertiary hospitals in metropolitan Adelaide, South Australia: The Flinders Medical Centre, the Royal Adelaide Hospital and the Queen Elizabeth Hospital. The project was expanded to the Royal Melbourne Hospital (Melbourne, Victoria) in 2009, the Sydney Eye Hospital (Sydney, New South Wales) in 2010, and the Repatriation General Hospital (Adelaide, South Australia), and The Canberra Hospital (Canberra, Australian Capital Territory) in 2011. Moorfields Eye Hospital (National Institute for Health Research (NIHR) Biomedical Research Centre at Moorfields Eye Hospital NHS Foundation Trust and UCL Institute of Ophthalmology, London, United Kingdom) has become the first international recruitment centre for this study. Recruitment from this site, along with participants from ongoing recruitment throughout Australia will be used for replication analysis following GWAS.

Ophthalmology, endocrinology and renal clinics of these hospitals were used to identify and recruit participants with diabetes meeting eligibility criteria. A combination of sequential recruitment, and opportunistic recruitment with a bias towards patients with more severe diabetic complications occurred. Written, informed consent was obtained from all participants following explanation of the nature and possible consequences of the study.

Case and control definitions

Eligibility criteria for this study required that participants were at least 18 years of age and had either T1DM of any duration, or T2DM of at least 5 years duration. All participants must be taking oral hypoglycemic medication, or be on insulin therapy, or both. Individuals with diet-controlled DM were excluded.

Cases included those with sight threatening DR, meeting at least 1 of the following criteria:

1. Worst ever retinopathy grading of severe non-proliferative diabetic retinopathy (NPDR) or proliferative diabetic retinopathy (PDR) in at least 1 eye;
2. The presence or history of DME in at least 1 eye.

Controls were defined as those whose retinopathy grading had never been worse than minimal NPDR, and who had no current or previous history of DME in either eye. Those with mild or moderate NPDR were recruited for the longer term study of issues such as progression, but excluded from specific analyses comparing cases and controls for the purpose of the planned GWAS.

Clinical data collection:

Full ophthalmic examination was performed according to existing site specific clinical practice. Acuity was assessed with either Snellen or logMAR charts, and recorded in the database as Snellen equivalent. Examination of the anterior segment included specific assessment of any lens opacity, and also the presence or absence of rubeosis. Intraocular pressure was measured with either a Goldmann tonometer, or an iCare tonometer (iCare Finland Oy, Revenio Group Corporation, Helsinki, Finland). Retinal examination was performed with slit lamp biomicroscopy. Retinopathy status was clinically

graded according to the Early Treatment Diabetic Retinopathy Study criteria with the following stages: no DR, minimal NPDR, mild NPDR, moderate NPDR, severe NPDR, PDR, or DME[15]. Worst ever retinopathy status for the worse eye was used in the analyses. Participants were classified as having DME irrespective of their DR grading if they were found to have DME in either one or both eyes. Those who had received laser treatment or intravitreal injections for sight-threatening DR were graded according to their retinopathy status prior to treatment (ie their worst ever grading). Participants with active disease were documented with color photography, fundus fluorescein angiography (FFA), and optical coherence tomography (OCT) of the macula as clinically indicated at the discretion of the treating clinician, but the clinical grading as determined at the time of slit lamp biomicroscopy was used for classification. Ophthalmologists were contacted to obtain ophthalmic examination data of participants recruited who attended private ophthalmology clinics.

For individuals in whom FFA was clinically indicated, a digital mydriatic retinal camera was used to obtain retinal photographs. Prior to photography, pupils were further dilated with phenylephrine hydrochloride 2.5%. Macular OCT, including high definition macular scans, was performed for all patients with DME using a Cirrus HD-OCT (Carl Zeiss Meditec, California, USA). Patients who had a history of previous laser treatment but with currently inactive disease were not subjected to photography or OCT.

A detailed questionnaire was administered to collect relevant information regarding social, demographic and medical history. Information collected included sex, age, ethnicity and lifestyle factors (cigarette smoking and alcohol history). Medical history data included DM type and duration, family history of DM, co-existing risk factors (systemic hypertension, hypercholesterolemia), history of vascular disease (ischemic heart disease (IHD),

cerebrovascular accident (CVA) or transient ischemic attack (TIA), peripheral vascular disease (PVD)), and history of diabetic complications (peripheral neuropathy, diabetic nephropathy and details of renal transplant or dialysis). Ophthalmic history was completed by the treating ophthalmologist and included details of past cataract surgery, age related macular degeneration (ARMD), glaucoma, retinal detachment, vitreous hemorrhage, rubeosis/iris neovascularisation and past treatment for DR (focal laser, macular grid laser, pan retinal photocoagulation (PRP), intravitreal injections, and vitrectomy). The year of development of sight-threatening DR was also documented.

Height, weight and blood pressure measurements were taken at the time of recruitment. Individuals were classified as having hypertension if they were on pharmacologic treatment for hypertension, or they had a systolic or diastolic blood pressure reading greater than or equal to 140mmHg or 90 mm Hg respectively.

Results of the participants' previous blood tests were accessed from the medical record where available. The most recent serum lipid profile results (total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides) were recorded. Hypercholesterolemia was defined as a total cholesterol greater than or equal to 5.5mM, or current use of lipid-lowering medication. Renal function tests (serum creatinine, urine albumin, and albumin-creatinine ratio) were also obtained. The most recent renal function results available were used for analyses except for those who were on dialysis or who had received a renal transplant, in which case results immediately prior to the start of dialysis or transplantation were used. Nephropathy was defined as the presence of microalbuminuria (30 – 300 mg/d) or macroalbuminuria (> 300 mg/d). The mean of three HbA1c levels was used for each participant. The 3 most recent values immediately prior to recruitment were used, except in cases of sight-threatening DR where 3 HbA1c values in the year of onset of

sight-threatening DR development were taken.

Collection of DNA and serum:

A venous blood sample was obtained from each participant by a trained venipuncturist. Two 9ml Vacuette EDTA tubes of venous blood were collected and stored at 4 degrees Celsius. DNA was extracted from the blood samples using the QiaAmp Blood Maxi Kit (Qiagen, Valencia, California). An 8ml Vacuette serum tube was also collected from a subset of approximately half the participants when the proximity of the recruitment site facilitated the timely processing of the sample. These serum tubes were centrifuged at 2700g for 10 mins within 2 hours of collection. The serum was transferred to 1ml microfuge tubes, labeled and stored at -80 degrees Celsius for future analysis.

Data analysis:

All data recorded onto the data collection form were entered into a password protected Microsoft Office Access 2003 version 5.1 for Windows XP professional (Microsoft corporation, USA) electronic database. Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 20.0 for Mac OS X (IBM SPSS Statistics 20.0, SPSS Inc., USA). Case and control groups were compared for differences in age and sex with a Mann-Whitney U test and a chi-squared test respectively. Data was characterized using basic descriptive statistics, and correlations between variables and phenotypes were assessed. Variables were analysed for differences between DM type using the Mann-Whitney U test and a chi-squared test. Analyses were considered for sight-threatening DR, PDR and DME subgroups, for T1DM and T2DM combined, and also stratified for type of DM. P values <0.05 were considered statistically significant. Odds ratios (OR) are presented with corresponding 95% confidence intervals. Power calculations were made with the online program Genetic Power Calculator[16].

RESULTS

Cases and controls

A total of 1669 Caucasian individuals with DM were recruited for inclusion in GWAS analysis. Included were 683 cases with sight threatening DR and 812 diabetic controls as defined in methods. Of the cases, 215 patients with DME also had co-existing PDR or severe NPDR. A further 174 (10%) participants had DR that met neither sight threatening case or control criteria (ie. mild or moderate NPDR) for the purpose of the genome-wide association study. The sample comprised 407 (24%) T1DM and 1262 (76%) T2DM patients. Retinopathy gradings by type of DM are presented in table 1.

Baseline characteristics of case and control groups were compared (Table 2). A Chi-square test for independence (with Yates Continuity Correction) indicated significantly more male cases compared with controls for T2DM alone (χ^2=6.9, p=0.008), and all DM irrespective of type (χ^2=9.97, p=0.002). Age distribution was found to violate the assumption of normality for T1DM and T2DM patients alone and combined. A Mann-Whitney U Test revealed no significant difference in age between case and control groups when pooling all diabetics (p=0.196). Analysis by DM type found that T1DM controls were significantly younger than cases (U=11957.5, p<0.001), but that T2DM controls were significantly older than cases (U=137741, p=0.001). The baseline differences found between case and control groups will be accounted for in multivariate models for future analyses.

Clinical variables

Duration of DM ranged from 0-70 years with a mean duration of 18 years. The prevalence of co-morbidities was high, with 76% of participants having hypertension, 66% of participants having hyperlipidemia, and a mean body mass index (BMI) of 30.8 (range

14.3-70.5; SD 6.6). Suboptimal diabetic control as determined by HbA1C was also common, with the sample having a mean HbA1C of 8.1% (range 2.2-15.3%; SD 1.6). Duration of diabetes, total cholesterol, systolic blood pressure, HbA1C and BMI were all unimodally distributed.

Complications of DM (DR, nephropathy, neuropathy, IHD or stroke) were noted in 80% (1331 patients) of the sample. Of 741 patients who had urine albumin measurements available, 38% had nephropathy (26% with microalbuminuria, and 13% with macroalbuminuria), with 17 of these patients on dialysis and 5 having received renal transplants. 29% of patients had neuropathy, 24% had ischemic heart disease and 9% had a history of stroke. Clinical characteristics stratified by DM type are presented in table 3, with p-values comparing these variables between T1DM to T2DM participants.

The correlation of clinical variables with type of DR was also examined (Table 4). Sight threatening DR, PDR and DME were assessed in separate models. Statistically significant ($P < 0.05$) correlations with one or more subgroups of DR were found for duration of diabetes, diabetes type, hypertension, nephropathy, neuropathy, HbA1C and BMI. Correlations were strongest for HbA1c, duration of diabetes, and nephropathy. Interestingly, PDR was correlated with T1DM ($p<0.001$), whereas DME was correlated with T2DM ($p<0.001$).

Power

Power for the planned GWAS was calculated using a specific type 1 error rate of $5x10^{-8}$ to allow for multiple hypothesis testing in a genome-wide association scan. Assuming a disease prevalence of any retinopathy of 0.3, this study has 94% power, to detect associated SNPs with an odds ratio of 1.3, if the minor allele frequency is at least 0.2.

DISCUSSION

This manuscript describes the 683 cases and 812 controls for which clinical information and DNA have been collected. A GWAS to identify genes relating to the pathogenesis of DR in this study sample is currently underway.

A number of candidate gene studies have investigated SNPs associated with DR. A meta-analysis of previous candidate gene studies up to the year 2008 found that the biggest limitations in this field was varying case-control definitions and suboptimal power[14]. The largest candidate gene analysis of genes previously associated with DR, DN, T2DM and atherosclerotic cardiovascular disease has since been performed for 2691 participants with T2DM from the CARe consortium, and did not find substantial evidence for a DR gene[17]. The most compelling results thus far have come from investigation of the Aldose reductase (*AKR1B1)* and *VEGFA* genes.

Aldose reductase, involved in the polyol pathway, consumes NADPH while reducing glucose to sorbitol, and in turn contributes to microvascular damage through the exacerbation of intracellular oxidative stress[18]. The *AKR1B1* gene has been investigated in a number of ethnic groups and DM types with varying findings. The z-2 allele of a short tandem repeat microsatellite marker has been associated with increased risk of DR in both T1DM and T2DM, whereas the z+2 allele has been shown to be protective against retinopathy in T1DM[14]. However, more recent multivariate analysis which accounted for confounding risk factors (including duration of DM and glycemic control), did not confirm these associations between the *AKR1B1* gene and DR,[19] indicating confounding factors contributing to the reported associations. No studies to date have specifically investigated the contribution of this gene to the development of DME.

VEGF has been shown to contribute to the development of diabetic microvascular complications including DR and DME through its role in angiogenesis, and the associated increase in vascular permeability[20]. A number of GWAS and candidate gene studies have reported associations of various *VEGFA* SNPs, though results have not been consistent between studies[14]. Two particular SNPs that combined represent nearly 50% of the VEGF variance have been assessed in a large case control study including 6920 T2DM participants, but neither appeared to be directly associated with the development of DR, DME or diabetic nephropathy (DN)[21]. The clinical benefit of intravitreal anti VEGF therapy for PDR and DME is now well substantiated[22,23], however the complex genetic link between VEGF and diabetic microvascular complications may be indirect and requires further detailed investigation.

Suboptimal power also remains problematic in recent GWAS, and as a result, genome wide significance has not been reached in any published study, nor has there been any consistency in genes identified between studies[11,24-26]. Studies so far have typically included samples from the entire range of DR severity, with case-control definitions comparing no DR with any DR[24], or any DR with severe DR[11,25]. Given that the DR variants under investigation are likely to have small to moderate effect sizes, large sample sizes are required in order to reach statistical significance when using population sampling. However, there is good recent evidence to suggest that focusing on recruitment and analysis of phenotypic extremes results in systematically larger effect sizes than the overall population effect size[27]. This strategy has specifically been used in the discovery of rare variants, and is postulated to reduce the required sample size by as much as fourfold compared with sampling from the general population[27]. The case-control definitions adopted for the current study differ from GWAS performed to date by involving

analysis of phenotypic extremes. We have successfully employed this strategy using advanced glaucoma cases to detect two genome wide significant loci in a study of 650 cases[28]. We expect results of the current study to be consistent with a sufficiently powered GWAS and the potential for detection of susceptibility loci for DR.

Data from long-term prospective population based studies indicate that those with T2DM may be more susceptible to DME than those with T1DM, and that the difference in the incidence of DME between these groups becomes more pronounced as duration of DM increases[29,30]. Preliminary descriptive statistics of the present study sample are consistent with this finding, with double the prevalence of DME in T2DM compared with T1DM participants. The implication of this association during an era of rapidly increasing incidence of T2DM, on vision related quality of life and health care costs is particularly noteworthy. DME is the major vision threatening complication of DR and thus determining independent genetic risk factors for DME is imperative in achieving improved treatment strategies and outcomes.

The complex integration between genetic susceptibility and environmental risk factors in the development of diabetic microvascular complications requires further investigation with large sample sizes and clear *a priori* case and control definitions in order to obtain meaningful results. Specific analysis of DME is warranted given the lack of current understanding of the genetic risk factors involved, and the associated visual morbidity of this type of DR. The size of the current sample has the capacity to detect genome wide significance. The potential outcome of this study is the identification of candidate genes involved in the development and progression of PDR and DME, to assist in the development of novel management strategies targeting this blinding disease of increasing prevalence.

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REFERENCES

1. National Diabetes Data Group (U.S.), National Institute of Diabetes and Digestive and Kidney Diseases (U.S.), National Institutes of Health (U.S.). Diabetes in America. 1995.

2. National Association of Diabetes Centres. Australian National Diabetes Information Audit and Benchmarking: Final report 2009. 2010;:1–470.

3. Landers J, Henderson T, Abhary S, Craig J. Prevalence and associations of diabetic retinopathy in indigenous Australians within central Australia: the Central Australian Ocular Health Study. Clin. Experiment. Ophthalmol. 2010;38:393–7.

4. Taylor HR, Xie J, Fox S, Dunn RA, Arnold A-L, Keeffe JE. The prevalence and causes of vision loss in Indigenous Australians: the National Indigenous Eye Health Survey. Med. J. Aust. 2010;192:312–8.

5. Jaross N, Ryan P, Newland H. Incidence and progression of diabetic retinopathy in an Aboriginal Australian population: results from the Katherine Region Diabetic Retinopathy Study (KRDRS). Report no. 2. Clin. Experiment. Ophthalmol. 2005;33:26–33.

6. Kobrin Klein BE. Overview of Epidemiologic Studies of Diabetic Retinopathy. Ophthalmic Epidemiol 2007;14:179–83.

7. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. N. Engl. J. Med. 1993;329:977–86.

8. Mitchell P, Foran S, Foran J. Clinical Practice Guidelines for the Management of Diabetic Retinopathy. Canberra: National Health and Medical Research Council, 2008.

9. Diabetes Control and Complications Trial/Epidemiology of Diabetes Interventions and Complications (DCCT/EDIC) Study Research Group. Clustering of long-term complications in families with diabetes in the diabetes control and complications trial. The Diabetes Control and Complications Trial Research Group. Diabetes 1997;46:1829–39.

10. Arar NH, Freedman BI, Adler SG, Iyengar SK, Chew EY, Davis MD, et al. Heritability of the Severity of Diabetic Retinopathy: The FIND-Eye Study. Investigative Ophthalmology & Visual Science 2008;49:3839–45.

11. Grassi MA, Tikhomirov A, Ramalingam S, Below JE, Cox NJ, Nicolae DL. Genome-wide meta-analysis for severe diabetic retinopathy. Human Molecular Genetics 2011;20:2472–81.

12. Looker HC, Nelson RG, Chew E, Klein R, Klein BEK, Knowler WC, et al. Genome-Wide Linkage Analyses to Identify Loci for Diabetic Retinopathy. Diabetes 2007;56:1160–6.

13. Hallman DMD, Boerwinkle EE, Gonzalez VHV, Klein BEKB, Klein RR, Hanis CLC. A

genome-wide linkage scan for diabetic retinopathy susceptibility genes in Mexican Americans with type 2 diabetes from Starr County, Texas. Diabetes 2007;56:1167–73.

14. Abhary S, Hewitt AW, Burdon KP, Craig JE. A systematic meta-analysis of genetic association studies for diabetic retinopathy. Diabetes 2009;58:2137–47.

15. Group ER. Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991;98:786–806.

16. Purcell SS, Cherny SSS, Sham PCP. Genetic Power Calculator: design of linkage and association genetic mapping studies of complex traits. Bioinformatics 2002;19:149–50.

17. Sobrin L, Green T, Sim X, Jensen RA, Tai ES, Tay WT, et al. Candidate gene association study for diabetic retinopathy in persons with type 2 diabetes: the Candidate gene Association Resource (CARe). Investigative Ophthalmology & Visual Science 2011;52:7593–602.

18. Brownlee M. Biochemistry and molecular cell biology of diabetic complications. Nature 2001;414:813–20.

19. Abhary S, Burdon KP, Laurie KJ, Thorpe S, Landers J, Goold L, et al. Aldose Reductase Gene Polymorphisms and Diabetic Retinopathy Susceptibility. Diabetes Care 2010;33:1834–6.

20. Bates DOD, Harper SJS. Regulation of vascular permeability by vascular endothelial growth factors. Vascul Pharmacol 2002;39:225–37.

21. Bonnefond A, Saulnier P-J, Stathopoulou MG, Grarup N, Ndiaye NC, Roussel R, et al. What is the contribution of two genetic variants regulating VEGF levels to type 2 diabetes risk and to microvascular complications? PLoS ONE 2012;8:e55921–1.

22. Mitchell P, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, et al. The RESTORE Study. Ophthalmology 2011;118:11–1.

23. Michaelides MM, Kaines AA, Hamilton RDR, Fraser-Bell SS, Rajendram RR, Quhill FF, et al. A Prospective Randomized Trial of Intravitreal Bevacizumab or Laser Therapy in the Management of Diabetic Macular Edema (BOLT Study). Ophthalmology 2010;117:1078–87.

24. Huang Y-C, Lin J-M, Lin H-J, Chen C-C, Chen S-Y, Tsai C-H, et al. Genome-wide Association Study of Diabetic Retinopathy in a Taiwanese Population. Ophthalmology 2011;118:7–7.

25. Fu Y-P, Hallman DM, Gonzalez VH, Klein BEK, Klein R, Hayes MG, et al. Identification of Diabetic Retinopathy Genes through a Genome-Wide Association Study among Mexican-Americans from Starr County, Texas. J Ophthalmol doi:10.1155/2010/861291.

26. Sheu WH-H, Kuo JZ, Lee I-T, Hung Y-J, Lee W-J, Tsai H-Y, et al. Genome-wide association study in a Chinese population with diabetic retinopathy. Human

Molecular Genetics [Internet] 2013 doi:10.1093/hmg/ddt161.

27. Guey LT, Kravic J, Melander O, Burtt NP, Laramie JM, Lyssenko V, et al. Power in the phenotypic extremes: a simulation study of power in discovery and replication of rare variants. Genet Epidemiol 2011;35:236-46.

28. Burdon KP, Macgregor S, Hewitt AW, Sharma S, Chidlow G, Mills RA, et al. Genome-wide association study identifies susceptibility loci for open angle glaucoma at TMCO1 and CDKN2B-AS1. Nat. Genet. 2011;43:574–8.

29. Klein R, Klein BE, Moss SE, Cruickshanks KJ. The Wisconsin Epidemiologic Study of Diabetic Retinopathy. XV. The long-term incidence of macular edema. Ophthalmology 1995;102:7–16.

30. Mitchell PP, Moffitt PP. Update and implications from the Newcastle diabetic retinopathy study. Aust N Z J Ophthalmol 1990;18:13–7.

TABLES:

Table 1: Retinopathy gradings by diabetes type. Cases are defined as those with a worst ever retinopathy grading of sight threatening DR (severe NPDR, PDR and/or DME) in at least 1 eye. Controls are defined as those whose retinopathy grading has never been worse than minimal NPDR, and have had no history of DME.

	T1DM	**T2DM**	**Combined**
	N (%)	N (%)	N (%)
N	407	1262	1669
Cases	178 (43.7)	505 (40.0)	683 (40.9)
Controls	191 (46.9)	621 (49.2)	812 (48.7)
No DR	139 (34.2)	539 (42.7)	678 (40.6)
Mild/Mod NPDR	60 (14.7)	265 (21.0)	325[†] (19.4)
PDR	144 (35.4)	258 (20.4)	402 (24.1)
DME	59 (14.5)	354 (28.1)	413 (24.7)
Any DR	268 (65.8)	723 (57.3)	991 (59.4)

T1DM indicates type 1 diabetes; T2DM, type 2 diabetes; DR, diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; DME, diabetic macular edema.

[†]Co-existent DME was found in 151 of the 325 participants with mild or moderate NPDR.

Table 2: Features of cases and controls for all participants, and by diabetes type. Cases are defined as those with a worst ever retinopathy grading of sight threatening DR (severe NPDR, PDR and/or DME) in at least 1 eye. Controls are defined as those whose retinopathy grading has never been worse than minimal NPDR, and have had no history of DME.

	Cases (Sight threatening DR)	**Controls** (No DR + minimal NPDR)	
Combined			
N	683	812	
Males (%)	401 (58.9)	410 (50.5)	$\chi^2(1, n=1494)=9.97$ $p=0.002$
Mean age ± SD	60±15	60±17	*Mann-Whitney U=266539*
Age range	21-91	18-95	*z=-1.29*
Median Age	61	63	*p=0.196*
T1DM			
N	178	191	
Males (%)	104 (58.4)	94 (49.2)	$\chi^2(1, n=369)=2.8$ $p=0.095$
Mean age ± SD	47±16	39±15	*Mann-Whitney U=11957.5*
Age range	21-79	18-85	*z=-4.93*
Median age	46	37	*p<0.001*
T2DM			
N	505	621	
Males (%)	297 (58.9)	316 (50.9)	$\chi^2(1, n=1125)=6.9$ $p=0.008$
Mean age ± SD	64±11	67±12	*Mann-Whitney U=137741*
Age range	33-91	23-95	*z=-3.51*
Median age	65	67	*p=0.001*

DR indicates diabetic retinopathy; NPDR, non-proliferative diabetic retinopathy. Sight-threatening DR is defined as severe NPDR or PDR and/or DME.

Table 3: Clinical characteristics of the cohort. The number of patients contributing data to each variable is given (N). Data is presented as mean +/- standard deviation or N (%). Where relevant, the range is also given. P-values are derived from Mann-Whitney U test for continuous variables and chi-squared test for categorical variables comparing TIDM to T2DM participants.

Variable	T1DM		T2DM		
	N	Mean ± SD (Range)	N	Mean ± SD (Range)	P-value
Total	407		1262		
Age at recruitment (years)	407	44 ± 16 (18-85)	1262	66 ± 12 (23-95)	<0.001
Duration of diabetes (years)	406	23 ± 13 (0-70)	1258	16 ± 9 (0-67)	<0.001
Serum creatinine (umol/L)	356	95 ± 88 (24-921)	1005	114 ± 116 (32-1238)	<0.001
Total cholesterol (mmol/L)	351	4.7 ± 1.1 (1.1-10.4)	994	4.3 ± 1.1 (1.3-10.9)	<0.001
Systolic Blood Pressure (mmHg)	361	131 ± 18 (90-197)	1057	137 ± 19 (90-240)	<0.001
HbA1C (%)	394	8.4 ± 1.6 (4.6-14.4)	1113	8.0 ± 1.6 (2.2-15.3)	<0.001
Body mass index	381	27.8 ± 5.3 (18.4-63)	1148	31.8 ± 6.7 (14.3-70.5)	<0.001
	N	N (%)	N	N (%)	P-value
Sex (male)	407	217 (53.3)	1262	695 (55.1)	0.749
Family history of diabetes	362	200 (55.2)	1243	806 (64.8)	<0.001
Hypertension	406	201 (49.5)	1262	1072 (84.9)	<0.001
Hyperlipidaemia	407	193 (47.4)	1261	911 (72.2)	<0.001
Nephropathy	406	117 (28.8)	1259	426 (33.8)	0.041
Dialysis	334	2 (0.6)	1083	15 (1.4)	0.269
Renal transplant	334	1 (0.3)	1083	4 (0.4)	1.000
Neuropathy	362	67 (18.5)	1258	412 (32.8)	<0.001
Peripheral vascular disease	362	39 (10.8)	1258	179 (14.2)	0.103
Ischemic heart disease	362	41 (11.3)	1259	364 (28.9)	<0.001
Cerebrovascular accident	362	16 (4.4)	1258	131 (10.4)	<0.001
Smoking (current or ex-smoker)	407	204 (50.1)	1262	664 (52.6)	0.320

T1DM indicates type 1 diabetes; T2DM, type 2 diabetes.

Table 4: Correlation of clinical variables with retinopathy grading. Pearson correlations are presented with corresponding p-values. Cases are defined as those with a worst ever retinopathy grading of sight threatening DR (severe NPDR, PDR and/or DME) in at least 1 eye.

Variable	**Cases** (Sight Threatening DR)	**PDR**	**DME**
Duration of DM	**0.37 (<0.001)**	**0.42 (<0.001)**	**0.30 (<0.001)**
T2DM	-0.03 (0.26)	**-0.13 (<0.001)**	**0.11 (<0.001)**
Hypertension	**0.12 (<0.001)**	**0.11 (<0.001)**	**0.14 (<0.001)**
Hyperlipidemia	0.02 (0.39)	0.02 (0.59)	0.05 (0.09)
Nephropathy	**0.23 (<0.001)**	**0.28 (<0.001)**	**0.19 (<0.001)**
Neuropathy	**0.17 (<0.001)**	**0.19 (<0.001)**	**0.14 (<0.001)**
HbA1C	**0.34 (<0.001)**	**0.36 (<0.001)**	**0.34 (<0.001)**
BMI	0.04 (0.17)	0.03 (0.37)	**0.07 (0.02)**

DM indicates diabetes mellitus; T2DM, type 2 diabetes; DR, diabetic retinopathy; PDR, proliferative diabetic retinopathy; DME, diabetic macular edema. Sight-threatening DR is defined as severe NPDR or PDR and/or DME.