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The Role of Genetics in Susceptibility to Diabetic Retinopathy

Gerald Liew, MD, MPH[1,2], **Ronald Klein, MD, MPH**[3], and **Tien Y. Wong, MD, PhD**[2,4]

[1] Centre for Vision Research, University of Sydney, NSW, Australia

[2] Centre for Eye Research Australia, University of Melbourne, VIC, Australia

[3] Department of Ophthalmology and Visual Sciences, University of Wisconsin School of Medicine and Public Health, Madison, WI

[4] Singapore Eye Research Institute, Yong Loo Lin School of Medicine, National University of Singapore, Singapore

Introduction

Diabetic retinopathy remains the leading cause of blindness in working-aged adults.(1) Over 4 million adults 40 years and older in the United States are estimated to have diabetic retinopathy, of whom 1 out of every 12 has advanced vision-threatening retinopathy (2). With the projected increase in the world-wide prevalence of diabetes to 380 million people by 2025, (3;4) of whom 40% are expected to have some form of diabetic retinopathy,(2) there is a clearly a need to develop strategies to identify persons at risk of diabetic retinopathy, allowing prevention and early intervention.

Risk Factors for Diabetic Retinopathy

There is already strong evidence that longer duration of diabetes, poorer control of blood glucose and elevated blood pressure are the major factors responsible for the onset and progression of diabetic retinopathy. The Wisconsin Epidemiologic Study of Diabetic Retinopathy (WESDR), a population-based cohort study of diabetes in which participants were first examined in 1980-82, showed that in persons with type 1 diabetes, the prevalence of diabetic retinopathy ranged from 17% in those with diabetes for less than 5 years to almost 100% in those with diabetes for over 15 years.(5) The corresponding figures in persons with type 2 diabetes were 29% and 78%.(6) The importance of good glycemic control for delaying the development and progression of diabetic retinopathy was confirmed in two landmark clinical trials, the Diabetes Control and Complications Trial (DCCT) in persons with type 1 diabetes,(7) and the UK Prospective Diabetes Study (UKPDS) in persons with type 2 diabetes. (8) The UKPDS has further shown the value of tight blood pressure control in delaying the development of diabetic retinopathy complications and well as other microvascular endpoints. (9;10) More recently, the Fenofibrate Intervention and Event Lowering in Diabetes Study (11) indicated that lipid lowering therapy might reduce retinopathy requiring laser treatment.

Nonetheless, diabetic retinopathy occurs even with optimal glucose and blood pressure control. The newly completed ADVANCE trial recruited 11,140 patients with type 2 diabetes and found intensive glucose control to reduce glycosylated hemoglobin to 6.5% or lower had no effect on the 5-year incidence of retinopathy rates. ADVANCE also reported that lowering of blood pressure to near normal levels (approximately 140/80) did not achieve further reduction in

Correspondence to: Tien Yin Wong, MD, PhD, Centre for Eye Research Australia, University of Melbourne, 32 Gisborne Street, East Melbourne 3002, Australia, Tel: +613 9929 8352, Fax: +613 9662 3859, twong@unimelb.edu.au.

progression of diabetic retinopathy.(12) In addition, it is clinically apparent that some patients with poor control of glycemia or blood pressure do not develop diabetic retinopathy even over prolonged periods of time, while others may develop diabetic retinopathy in relatively short periods of time despite good risk factor control. This was prominently illustrated in the Joslin Medalist study which found that almost 50% of older diabetic participants in their study had no evidence of retinopathy despite surviving over 50 years with type 1 diabetes.(13) Finally, a recent observational study of 11,423 participants from three diverse populations,(14) reported that retinopathy signs, mainly retinal microaneurysms, characteristic of diabetes were detectable in 7.4-13.4% of nondiabetic participants, and were present even in individuals with glycosylated hemoglobin levels <5.0%. These results suggest that processes other than hyperglycemia and elevated blood pressure contribute to the development and progression of diabetic retinopathy.

Racial/Ethnic Differences and Familial Concordance in Diabetic Retinopathy

Racial/ethnic differences in the prevalence of diabetic retinopathy may provide insights into relative importance of genetic or environmental risk factors. The Multi-Ethnic Study of Atherosclerosis (MESA) study reported moderate differences in diabetic retinopathy prevalence among different races: 36.7% in African-Americans, 37.4% in Hispanics, 24.8% in whites, and 25.7% in Chinese-Americans.(15) Differences in risk factors such as diabetes duration, glycemic control and hypertension appear to explain the higher prevalence of diabetic retinopathy in African-Americans, but did not explain the higher prevalence observed in Hispanics compared to whites, suggesting that genetic or cultural factors may play a role in the pathogenesis of diabetic retinopathy.(16-19)

Another sign of genetic influence is the increased risk of severe diabetic retinopathy among family members with diabetes,(20) in siblings of affected individuals (approximately 3-fold increased risk) (21) and the moderate heritability of diabetic retinopathy risk (0.52).(22;23) These observations of differential response to risk factors and treatments, racial differences and familial clustering strongly suggest a role for genetic factors in determining susceptibility to diabetic retinopathy.

Identifying Diabetic Retinopathy Genes

Identifying the gene or genes that contribute to the pathogenesis of diabetic retinopathy has been challenging despite being a major focus of research over the past few decades. A large number of putative genes and genetic variants have been reported in the literature but few of these have been consistently replicated. As a result no genes have achieved widespread acceptance as conferring high risk of diabetic retinopathy, in contrast to the situation with *CFH* polymorphisms and risk of age-related macular degeneration. (24) In part, this may be because identifying genes for diabetic retinopathy is more challenging due to the greater complexity of the disease which may have more multifactoral, polygenic and environmental influences than age-related macular degeneration.

Another important factor limiting progress in the search for diabetic retinopathy genes is the lack of uniform assessment and documentation of retinopathy across studies. Some studies have classified retinopathy using a clinical classification into 4 or 5 categories e.g. none, mild, moderate, severe nonproliferative and proliferative(25) while others have used variants of the Early Treatment Diabetic Retinopathy Study (ETDRS) severity scale.(26) A recent systemic review(27) suggested that classification into the 5 step clinical severity scale is not sufficient for genetic analyses and may partly explain the disappointing findings of many studies to date. The more detailed ETDRS severity scale or a similar variant may thus be the preferred means of documenting diabetic retinopathy in genetic studies. However, studies using the full ETDRS scale may be limited in terms of statistical power because of smaller number of persons with

specific detailed levels of retinopathy. One way to overcome this is to pool studies to increase study power. (28)

A further issue with regards to documenting the presence of diabetic retinopathy is the potential for overlap with "nondiabetic" retinopathy, or retinopathy from causes other than hyperglycemia, such as hypertension. Results from many large population-based studies (29; 30) show that on average, between 5-15% of older persons without diabetes have retinopathy, and 1.2-1.8% develop retinopathy lesions per year. Such nondiabetic lesions appear to be transient, with 72% regressing over 5 years.(30) Their pathogenesis is as yet unclear, although hypertension, aging and elevated plasma glucose (below the diabetic threshold) are believed to play a role.(31;32) These nondiabetic retinopathy lesions, namely a retinal microaneurysm or two or a solitary retinal blot hemorrhage are indistinguishable from similar lesions found in early diabetic retinopathy which is used to define early steps of diabetic retinopathy in the ETDRS severity scale and have probably confounded the results and analyses of some genetic studies, especially those examining minimal or mild nonproliferative retinopathy. How to control for the effects of this confounder is not clear at this point, although sensitivity analyses using more severe levels of retinopathy (e.g. proliferative diabetic retinopathy) would presumably exclude many of these confounding lesions though at a cost of reduced power. However, if different genetic factors are associated with minimal or mild retinopathy and proliferative retinopathy, they would be missed by using this approach.

Despite these limitations studies have identified a number of potential susceptibility genes for diabetic retinopathy. Two basic approaches have been used – linkage studies in families, and genetic associations studies which may examine specific candidate genes, or more recently, genome wide association scans.

Linkage Studies

Linkage studies are based on the assumption that regions of the genome with more than the expected number of shared alleles among family members with diabetic retinopathy may contain genetic susceptibility loci. For several decades, this was the dominant study design for examining the genetic basis of complex traits such as diabetic retinopathy. It should be pointed out that gene mapping using linkage analyses are difficult to perform because of the nature of the disease; lower life expectancy in patients with diabetes, and the late onset of diabetic retinopathy often means the parents of diabetic retinopathy affected individuals are no longer alive and available for study. However, modifications in study design such as sib-pair analyses have been conducted which have suggested various chromosomal regions of interest.(Table 1). Two sibship studies have implicated loci on chromosome 3, although the loci do not appear to overlap. Other regions implicated are on chromosomes 1, 9 and 12. It should be noted that none of the regions identified in the three linkage studies in Table 1 have reached levels of accepted genome wide statistical significance.

Candidate Gene Studies

Most research to date has focused on identifying genetic susceptibility to diabetic retinopathy through the candidate gene approach. This study design typically selects participants with and without the disease of interest (i.e. cases and controls) and compares the frequency of genetic variants between the two groups. Most, but not all, studies also attempt to adjust for some confounding factors such as age, diabetes duration and glycosylated hemoglobin levels. Although relatively simple to perform, such studies have the important drawback of lacking study power. As a result, many associations may be spurious and replication in other populations becomes of paramount importance. An important guard against spurious associations is the statement of a robust *a priori* hypothesis implicating the gene of interest in known or suspected diabetic retinopathy pathophysiological pathways. The majority of

candidate gene studies have therefore examined genetic variants implicated in diabetes development or metabolic pathways such as the polyol pathway, formation of advanced glycation end products (AGE) and hypoxia induced angiogenesis through vascular endothelial growth factor (VEGF). The most promising results from these studies are discussed below and summarized in Table 2. A full discussion of the various candidate genes that have been examined is available elsewhere.(27)

Aldose Reductase Gene (ALR2)

The polyol pathway is a major metabolic pathway linking hyperglycemia to diabetes specific tissue complications, and aldose reductase (ALR2) is the first and rate limiting enzyme of this pathway. ALR2 converts glucose to sorbitol in an NADPH-dependent reaction. In the presence of hyperglycemia, sorbitol accumulates intracellularly leading to osmotic stress.(33) In animal models, this results in microaneurysm formation, basement membrane thickening and pericyte loss.(34)

The ALR2 gene has an (A-C) repeat polymorphism at the 5′ end that has been found to be associated with diabetic retinopathy in Hong Kong Chinese,(35;36), Mainland Chinese,(37) Japanese,(38-40) Indians(41), Chileans(42) and Brazilians.(43) (Table 2) In contrast, several other studies have failed to find association of ALR2 gene variants with diabetic retinopathy in Koreans(44) and Euro-Brazilians.(45) While the weight of evidence to date appears to favor ALR2 this should be balanced by the negative findings of clinical trials which have failed to find efficacy of aldose reductase inhibitors in preventing incidence or progression of diabetic retinopathy.(46) A significant limitation of these trials is they did not stratify participants by their ALR2 polymorphism status, so the efficacy of aldose reductase inhibitors is still uncertain at this stage.

Vascular Endothelial Growth Factor Gene (VEGF)

VEGF plays an important role in the neovascularization process in proliferative retinopathy and in breakdown of the blood-retina barrier in the development of diabetic macular edema. Markedly elevated serum and vitreous levels of VEGF have also been reported in eyes of patients with proliferative diabetic retinopathy.(47) The promoter region of VEGF has several polymorphisms, some of which have been associated with diabetic retinopathy.(Table 2) In Japanese(48) and Indian(49;50) populations, the C(-634)G polymorphism is associated, whereas in Caucasian populations,(51-53) the -460C polymorphism may also be associated. The C-634G polymorphism is further reported to increase risk of macular edema in Japanese patients.(54) Studies have also implicated other VEGF single-nucleotide polymorphisms (SNPs) and haplotypes in diabetic retinopathy.(55;56) The majority of published studies appear to replicate associations of VEGF polymorphisms with diabetic retinopathy, highlighting the promise that this gene may hold. Several clinical trials are currently investigating the efficacy of anti-VEGF agents in treatment of diabetic retinopathy.

Receptor for Advanced Glycation End Products Gene (RAGE)

Advanced glycation end products (AGE) result from prolonged exposure of proteins and lipids to hyperglycemia which leads to nonezymatic glycation of these macromolecules. Accumulation of AGEs are believed to contribute to diabetic complications through direct tissue damage(57) as well as through activating specific receptors for AGE (RAGE). RAGE is a member of the immunoglobulin superfamily and the gene maps to chromosome 6p21.3. Activation of this receptor by high circulating levels of AGE leads to secretion of cytokines that hasten the progression of diabetic complications, partly by increasing endothelial permeability.(58) The –374 T/A polymorphism in the RAGE gene has been associated with diabetic retinopathy in a large Scandinavian study of 3,539 Caucasians, with a suggestion the effect may be dependent on glycosylated hemoglobin levels.(59) This polymorphism has also

been associated with diabetic retinopathy in Asian Indians.(60) The Gly82Ser and -429T/C polymorphisms may also increase risk of diabetic retinopathy in Asian Indian(61) and Caucasian(62) populations, respectively, though this was not confirmed in Chinese(63;64) or another Caucasian(65) study.

Candidate Genes with Little or No Independent Replication

A moderate number of other candidate genes have been reported to be associated with diabetic retinopathy in one or more studies,(27) but with little independent replication.(Table 1) These include ACE(66), MTHFR(67), GLUT1(68), PAI-1(69), α2β1 Integrin,(70) and APOE.(71) Many of the studies reporting positive findings have reported borderline or weak associations, had small sample sizes or methodological limitations. When investigated in other populations, most have failed replication.(27)

Genome-Wide Association Studies (GWAS)

These are the latest and largest genetic association studies and often require participation of several thousand individuals. Advances in genotyping technologies and rapid cost reductions have made assaying hundreds of thousands of SNPs feasible, and computational algorithms can relate them to clinical disease and measurable traits. Several GWAS searching for diabetic retinopathy susceptibility loci are ongoing but no findings have been published to date. With the recent successes of GWAS in identifying genetic loci for complex nonmendellian traits such as coronary heart disease, type 1 and 2 diabetes,(72-75) this approach may be the most promising and the results of GWAS in diabetic retinopathy are keenly awaited.

Nonetheless, GWAS have important limitations which may restrict their ability to identify novel diabetic retinopathy related gene loci. Due to the large numbers of SNPs evaluated (approximately 500,000 in most studies), an extremely stringent level of statistical significance (10^{-7} or less) is often required to exclude false positives. However, the cost of guarding against false positives is that SNPs associated with modest relative risks (e.g. 1.3 for heterozygotes and 1.6 for homozygotes) are likely to be missed using such criteria. Another limitation is the use of manufactured SNP chips that provide poor coverage of certain parts of the genome, and do not capture information on non-SNP gene variants such as insertions, deletions and variations in gene copy numbers.(76) These limitations mean that the requirement for replication in independent samples is as great or greater for GWAS as for other genetic study designs.

Genes for Diabetes

Recent genome-wide association studies have reported a number of genetic variants that are consistently associated with risk of type 2 diabetes. These include loci at or near the genes *IGF2BP2, CDKAL1, CDKN2A, CDKN2B, TCF7L2, SLC30A8, HHEX, FTO, PPARG,* and *KCNJ11*.(73-75) These genes are believed to play roles in pancreatic function and control of insulin secretion. Of these loci, the best known is the *TCF7L2*, which codes for a transcription factor involved in lipid metabolism and glucose homeostasis.(77) A weak association of polymorphisms in *TCF7L2* with diabetic retinopathy was reported in one candidate gene study, (78) though this was not confirmed in another.(79) The Pro12Ala polymorphism in *PPARG* has been associated with reduced risk of diabetic retinopathy in one study, (80) with no associations reported in a number of others.(81;82) It is not known if the presence of the other genetic variants increasing susceptibility to type 2 diabetes may also increase susceptibility to diabetic retinopathy, either independently or through their effects on hyperglycemia. A genome linkage study(83) has suggested that the genes for susceptibility to diabetic retinopathy may be distinct from those for diabetes itself, highlighting the complexity of multifactorial interactions in the pathogenesis of diabetes and the development of its major complications. This clearly represents an area of important future research.

Future Research Directions

Although the studies to date have not provided consistent evidence of the genetic variants underlying susceptibility to diabetic retinopathy, they have indicated methodological aspects that are important for future studies to take into account. Standardization of phenotypes is an important aim, either by using the ETDRS severity scale or a modification, and performing sensitivity analyses with increasing severity of retinopathy. Standardization of phenotypes and genotyping protocols would also facilitate pooling of individual patient level data in meta-analyses to increase power. Such pooled studies are required to explore the complex interplay of gene-environment interactions in expression of severity of diabetic retinopathy as well as response to treatment modalities. Population-based cohort studies may play a unique role in addressing these limitations as they often utilize standardized methods of retinopathy assessment from retinal photographs, collect data on multiple environmental risk factors, and can be successfully pooled. (28) They offer the added advantage that the most common and thus important genes in the community associated with diabetic retinopathy may be identified and the population-attributable risk calculated based on the true community prevalence of the genetic variant.(84)

Conclusion

Diabetic retinopathy remains an important cause of blindness and treatment options have limitations. Several lines of evidence point to a considerable genetic influence in susceptibility to diabetic retinopathy and there has been intensive research to uncover the genes responsible. Linkage studies and candidate gene approaches have suggested many potential genetic variants which may underlie the disease but replication of these results has often been inconsistent. These inconsistencies may reflect a lack of standardized documentation of retinopathy and overlap of diabetic retinopathy with ‘nondiabetic’ retinopathy which has been found to occur in 5-15% of participants without clinical diabetes. Despite these limitations, a few fairly consistent associations involving variants in the ALR2, VEGF and RAGE genes have been demonstrated. However, these associations have not been replicated in linkage analyses. No GWAS investigating diabetic retinopathy has been published to date, but given past successes with this approach, it is expected that this type of analysis, particularly if conducted within a population-based cohort setting, will provide novel insights into genetic susceptibility to diabetic retinopathy.

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Table 1

Linkage Studies of Diabetic Retinopathy

Author	Year	Sample size	Population	Diabetes Type	Results	Significance	Genes in region	Comments
Imperatore et.al(85)	1998	103 sibpairs	Pima Indians	2	Linkages on chromosome 3 and 9	LOD 1.36, 1.46 respectively	Chromosome 3: Angiotensin II (type 1) receptor	Weak LOD score
Looker et.al(86)	2007	211 sibships	Pima Indians	2	Linkage on chromosome 1	LOD 2.58	CASP-9, PADI 4, and CLCN-Kb	Region not previously identified
Hallman et.al(83)	2007	282 sibpairs	Mexican Americans	2	Linkage on chromosomes 3 and 12.	LOD 2.41, 2.47 respectively	Chromosome 3: ROBO 2, PROS1, ARL6, IMPG2 Chromosome 12: WNT5B, TULP3, GNB3 WNK1 SCNN1A ING4 OLR1	Regions are not associated with risk of diabetes itself, only with risk of retinopathy

Int Ophthalmol Clin. Author manuscript; available in PMC 2010 January 1.

Table 2

Candidate Gene Studies with Multiple Independent Replication

Gene	Polymorphism	Author	Year	Sample size	Population	Diabetes Type	Adjustment/ Matching for confounders	Significance	Comments
ALR2	(CA)n	Lee at.al (35)	2001	384	Hong Kong Chinese	2	Yes	P<0.05	Implicated, but unlikely to play major role
	(CA)n	Ko et.al (36)	1995	44	Hong Kong Chinese	2	Yes	P=0.007	
	(CA)n	Fujisawa et.al (38)	1999	170	Japanese	2	Yes	P=0.029	
	(CA)n	Ichikawa et.al (39)	1999	117	Japanese	2	No	P=0.039	
	(CA)n	Ikegishi et.al(40)	1999	61	Japanese	2	Yes	P=0.004	Small sample size
	(CA)n	Wang at.al (37)	2003	738	Mainland Chinese	2	Yes	P=0.02	
	(CA)n	Kumaramanickavel et.al (41)	2003	214	Indians	2	Yes	P=0.03	
	(CA)n	Olmos et.al(42)	2000	271	Chileans	2	Yes	P=0.04	ETDRS scale used
	(CA)n	Richeti et.al(43)	2007	64	Brazillian	1	No	P=0.014	NPDR vs PDR
	T allele	Wang at.al (37)	2003	738	Mainland Chinese	2	Yes	P=0.02	
VEGF	C-634G	Awata et.al(48)	2002	268	Japanese	2	Yes	P<0.0018	VEGF serum levels were significantly higher in healthy subjects with the CC genotype
	C-634G	Awata et.al(54)	2005	378	Japanese	2	Yes	P=0.047 for macular edema	Results for macular edema independent of those for diabetic retinopathy
	C-634G	Uthra et.al(49)	2008	213	Indian	2	No	P=0.02	Only in patients with microalbuminuria
	C-634G	Suganthalakshmi et.al(50)	2006	210	Indian	2	Yes	P=0.02	C(-7)T, T(-1498)C polymorphisms also associated.
	C-634G	Szaflik et.al(53)	2008	215	Caucasian	2	No	P<0.05	-460C not associated
	-460C	Ray et.al(51)	2004	267	Caucasian	1 & 2	Yes	P=0.027	Polymorphism increases basal VEGF promoter activity by 71%
	-460C	Churchill et.al(52)	2008	106	Caucasian	1 & 2	Yes	14-SNP haplotype P=0.006	Haplotype analyses showed strong associations
	Multiple SNPs in VEGF-A	Al Kateb et.al(55)	2007	1,369	Caucasian from DCCT	1	Yes	P<0.05	Follow-up of DCCT population (Incident severe retinopathy)

Gene	Polymorphism	Author	Year	Sample size	Population	Diabetes Type	Adjustment/ Matching for confounders	Significance	Comments
	I/D	Buraczynska et.al(56)	2007	919	Caucasian	2	Yes	P<0.001	
RAGE	Gly82Ser	Kumaramanickavel et.al(61)	2002	200	Indian	2	Only age	P=0.03	
	-429T/C	Hudson et.al(62)	2001	215	Caucasian	2	No	P=0.012	Functional studies show differences in polymorphic receptor activity
	-374T/A	Ramprasad et.al(60)	2007	339	Indian	2	No	P<0.05	No association with -429T/C
	-374 T/A	Lindholm et.al(59)	2006	3,539	Caucasian	1	Yes	P=0.03	May be interaction with glycosylated hemoglobin

Table 3

Candidate Gene Studies with Little or No Independent Replication

Gene	Polymorphism	Author	Year	Sample size	Population	Diabetes	Adjustment/ Matching for confounders	Significance	Comments
ACE	I/D	Matsumoto et.al(66)	2000	210	Japanese	2	Yes	P=0.036	Multiple (>10) negative studies.(27;87-89)
MTHFR	C677T	Maeda et.al(67)	2003	156	Japanese	2	Yes	P=0.03	Several negative studies.(45;90)
GLUT1	Xbal	Liu et.al.(68)	1999	255	Chinese	2	No	Weak	Associated with nephropathy.(68;91) Several negative studies.(92)
PAI-1	4G/5G	Nagi at.al(69)	1997	167	Pima Indians	2	Yes	P=0.04	Several negative studies.(93)
α2β1 Integrin	BgIII	Matsubara et.al(70)	2000	227	Japanese	2	Yes	P=0.0036	A few positive studies.(27;94)
APOE	ε4	Santos(71)	2002	58	Mexican	2	No	P<0.05	Weak association with macular edema in this study. Many negative studies.(95)
PPARG	Pro12Ala	Malecki(80)	2008	359	Caucasian	2	Yes	P=0.014	Protective association with diabetic retinopathy. Several negative studies.(81;82)