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Genes and Diabetic Retinopathy

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Several recent studies have provided evidence that good diabetes control is important to prevent diabetic retinopathy. However, some groups of patients develop diabetic retinopathy despite good control and others escape retinopathy despite poor control. This suggests the role of genetic factors in susceptibility to retinopathy. This article reviews the role of genetic factors in determining diabetic retinopathy.

Key Words: Diabetic retinopathy, genetics, candidate genes, India

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Diabetes mellitus is one of the most common causes of blindness in the developed world and is rapidly becoming an important cause in developing countries as well.¹ Blindness due to diabetes mellitus could have a variety of reasons including an increased prevalence of cataract, glaucoma and retinopathy. Two significant forms of sight-threatening retinopathy are proliferative diabetic retinopathy and diabetic maculopathy. Diabetic retinopathy is one of the most important complications in both Type 1 and Type 2 diabetes. The duration of diabetes and glycemic control are the two most important factors in the development of retinopathy.^{2,3} However, these factors alone do not explain the occurrence of retinopathy. It may be absent in some patients with poor glycaemic control even over a long period of time, while others may develop retinopathy in a relatively short period despite good glycaemic control. This raises the possibility of a genetic predisposition to retinopathy. Supportive evidence for a genetic role for retinopathy derives from twin, family and transracial studies demonstrating the importance of inherited factors in the aetiology of diabetes and its complications.⁴

Assessment of familial clustering with respect to complications of diabetes is difficult for several reasons. None of the complications is a simple trait that is consistently present or absent in each individual. Rather, there is a steady increase in the prevalence of detectable

complications with long-standing diabetes and a subsequent further temporal increase in their severity, once they are established.⁷ Hence it is important to account for the effects of both duration of disease and its severity. Moreover, the importance of metabolic control in the prevention of diabetic complications cannot be underestimated. This has been clearly shown by the Diabetes Control and Complications Study (DCCT)⁸ and the United Kingdom Prospective Diabetes Study (UKPDS).⁹ However, in both these studies there were subgroups of patients who developed retinopathy despite fairly good control of diabetes and others seemed to be protected from retinopathy despite poor control of diabetes.^s

Genes and Diabetic Retinopathy

Over the past several years, progress has been made in identifying some of the genes associated with diabetic retinopathy. Our knowledge of the physiology and pathophysiology of diabetic retinopathy leads us to hypothesize on the possible genetic influences on the development of this complication; the genes that influence these conditions may be the suitable candidate genes. *Family linkage* studies, using multiplex families in which two or more patients are affected by the condition, are a useful way of examining the influence of such genes. A second approach is to use *case control (association)* studies. This involves collecting samples from patients with only retinopathy and a control group without retinopathy and then comparing the allele frequencies of the candidate gene. Candidate genes have been identified in diabetic retinopathy by these two approaches.

The possible candidate genes contributing to the development of diabetic retinopathy are genes for Aldose Reductase (ALR), Nitric Oxide Synthase (NOS) genes, genes for Receptor for Advanced Glycation End

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products (RAGE), genes coding for Angiotensin Converting Enzyme (ACE gene), Human Leucocyte Antigen (HLA) genes and genes for Vascular Endothelial Growth Factors (VEGF) (Table 1). This article reviews the current status of these genes and their association with diabetic retinopathy.

Aldose Reductase Gene

Although prolonged exposure to hyperglycaemia is the primary factor associated with the development of most microvascular complications, additional risk factors also play a role. One of the physiological mechanisms linking hyperglycaemia to diabetes-specific tissue damage is the polyol pathway. This is summarised in Figure 1.

Aldose reductase (ALR2) is the rate-limiting enzyme of this pathway and converts glucose to sorbitol in an NADPH-dependent reaction. It is found in a variety of tissues including the endothelial and retinal pigment epithelial cells. Its affinity to glucose is low and therefore enough sorbitol is not produced under euglycaemic conditions. In the presence of hyperglycaemia, however, sorbitol accumulates because it does not readily diffuse out of the cell and this causes osmotic stress. Concomitant with the accumulation of sorbitol is the decrease in Na⁺/K⁺ dependent ATPase leading to accumulation of fluid in the cells. Since all of these have important functions in the cells, their alterations may lead to the death of retinal pericytes and hence damage to endothelial cells, an early event in the development of diabetic retinopathy.

Several studies have pointed out that a high level of aldose reductase in the erythrocyte of both Type 1 and Type 2 diabetic patients is associated with the presence of retinopathy.¹⁴⁻¹⁶

Human ALR2 gene, the gene encoding aldose reductase has been localized to chromosome 7q35 and consists of 10 exons extending over 18 kilobases of DNA.¹⁷ There is growing evidence to implicate ALR 2 in the pathogenesis of diabetic microvascular disease. The abnormal expression and activity of this enzyme seem to play an important role in diabetic complications. Ko et al⁸ have identified an (A-C)_n dinucleotide repeat polymorphic marker at the 5' end of the aldose

Table 1. Possible candidate genes associated with diabetic retinopathy

Aldose Reductase (ALR) Gene
Nitric Oxide Synthase (NOS) Gene
Receptor for Advanced Glycation End Products (RAGE) Gene
Angiotensin Converting Enzyme (ACE) Gene
Human Leucocyte Antigen (HLA) Gene
Vascular Endothelial Growth Factor (VEGF) Gene
Tumor Necrosis Factor- α (TNF- α) Gene

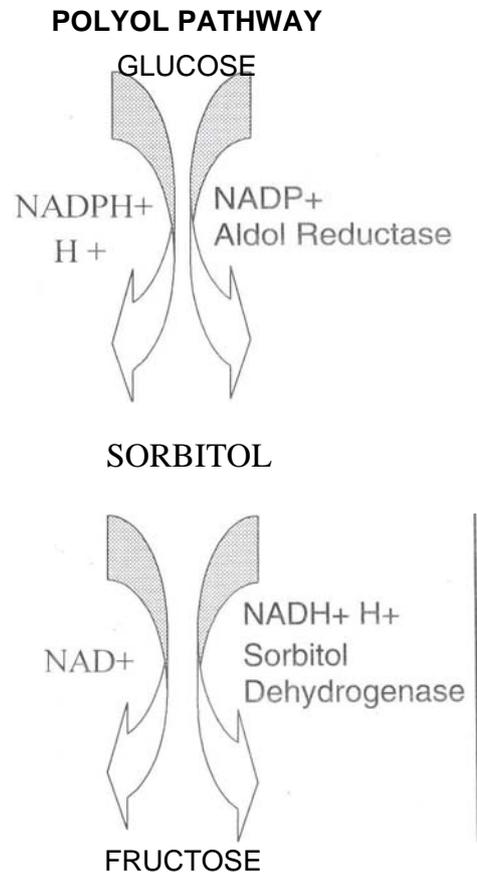


Figure 1. Polyol pathway of glucose metabolism: Glucose is converted to sorbitol in the presence of Aldol Reductase and then to fructose in the presence of enzyme, Sorbitol Dehydrogenase.

reductase gene, in Chinese type 2 diabetic patients and have reported the presence of 7 alleles at this locus and a strong association of one of the alleles (2-2) in patients with early onset of diabetic retinopathy. The study suggests that ALR2 or another gene near this locus may contribute to early onset of this complication, but this may not be the sole contributing factor. Previous studies using restriction fragment length polymorphism (RFLP) analysis have shown that aldose reductase, as well as the adjacent T-cell antigen receptor constant beta chain locus, confer susceptibility to diabetic microvascular disease.¹⁹

Nitric Oxide Synthase gene

The earliest detectable abnormality in the retinal circulation is an increase in blood flow. Pathological changes soon follow which eventually are incompatible with normal vision. Evidence suggests that endothelium-mediated vasodilation is defective and

reduced in diabetes along with an increase in vasoconstrictor activity. It is now recognised that aberrations in retinal blood flow in early diabetes are also linked to vascular endothelial dysfunction.²⁰ The retinal circulation which is devoid of extrinsic innervation, is dependent entirely on endothelial mediated autoregulation and hence, endothelial dysfunction in diabetes is likely to have a major effect on circulation within the retina.

Three members of the nitric oxide synthase gene family have been identified: neuronal (NOS1), inducible (NOS2A), and endothelial (NOS3), all of which could play a role. In the diabetic retina, NOS2A is not expressed in the retinal vasculature under normal conditions. High ambient glucose may influence NO release through increased NOS2A expression and reduced constitutive NOS3 expression in cultured retinal vascular endothelial cells.²¹ Studies have reported that (CCTTT)_n repeat in the promoter region of NOS2A gene was significantly associated with the absence of diabetic retinopathy.²² Therefore, NOS2A is considered a candidate gene in diabetic retinopathy.

Gene for Receptor for Advanced Glycation End products (RAGE)

Advanced glycation end products of proteins (AGEs) result from the prolonged exposure of proteins to aldoses, such as glucose, and have been shown to be present in the plasma and to accumulate in tissues at an accelerated rate in diabetes.²³ The ability to form crosslinks to and between proteins, and their interactions with a class of binding sites on endothelial cells and monocytes, as well as other cell types leads to tissue damage in diabetic complications, as shown in Figure 2.

Three AGE-binding proteins now referred to as AGE-RI, AGE-R2 and AGE-R3 have been described. Among the cell-associated receptors interacting with AGE are Receptor for AGE (RAGE) and ScR II.

Most AGE effects are mediated through the RAGE receptor and are thought to be involved in the development of diabetic complications²⁴ including retinopathy. RAGE is a member of the immunoglobulin superfamily, which is expressed on endothelial cells, mononuclear phagocytes and vascular smooth muscle cells. Pilot studies have demonstrated that RAGE contributes to the enhanced adherence of diabetic red cells to the endothelium. In addition, AGEs can stimulate endothelial proliferation.²⁵ Uu et al²⁶ reported a negative association of Gly 82 Ser polymorphism in the RAGE gene with microvascular complications in Type 2 diabetes. However, it has been proposed that intracellular AGE formation occurs in retinal pericytes and this may affect DNA function and result in capillary damage in the retina.

Sequence variants within the RAGE gene may influence the development of complications by the AGE-RAGE interactions. Hudson et al²⁷ screened for polymorphisms in the coding regions of RAGE gene and recorded four functional amino acid changes. The RAGE gene is located in the HLA region on chromosome 6. Associations between the AGE receptor polymorphisms in the promoter region and severity of retinopathy have been described. Polymorphisms in the AGE-RI gene have also been reported to be associated with the presence and severity of retinopathy.²⁸

Human Leucocyte Antigen (HLA) genes

The search for markers of genetic predisposition to proliferative retinopathy has focussed primarily on antigens encoded by the HLA region. These studies have had inconsistent results and hence the association of HLA region to diabetic complications has come under close scrutiny both in Type 1 and Type 2 subjects.

Agardh et al²⁹ have reported that Type 1 patients who are positive for DR3 -DQ2/DR4 - DQ8 stand greater risk of developing severe retinopathy at a young age. Investigations have been carried out to assess if inherited polymorphism of complement C4 is associated with genetic susceptibility to microvascular complications in

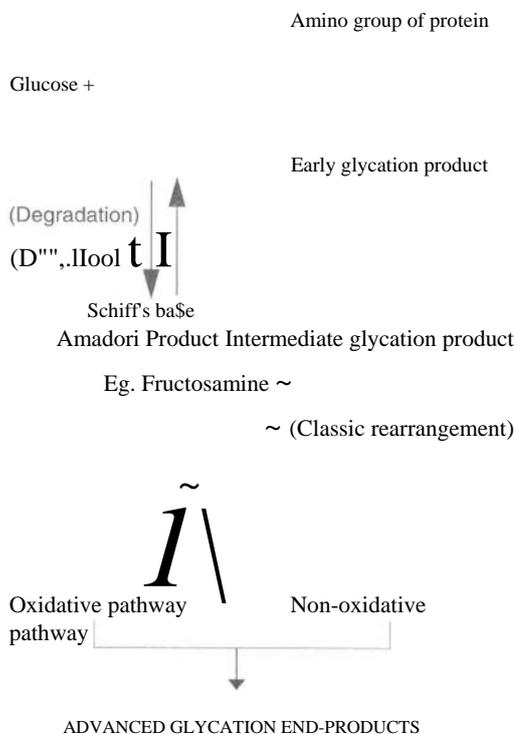


Figure 2. Formation of AGEs from glucose - Reversible early products give rise to irreversible advanced products through generation of highly reactive carbonyl compounds.

Type 2 diabetic patients. The same investigations have shown an association between chain complement C4 allotypes to microvascular complications in Type 1 diabetes.³⁰ Some published reports also suggest the lack of influence of major histocompatibility complex genes on diabetic retinopathy^Y

Studies on South Indian Type 2 Diabetic Patients with Retinopathy

During the last 10 years our group in collaboration with the Royal London Hospital, UK has been investigating the genetic factors in the development of diabetic retinopathy in South Indian Type 2 diabetic patients. Our studies indicated the possible role of genetic factors on predisposition to proliferative diabetic retinopathy in Type 2 diabetes.

In one of our early studies (1991),³² we looked for association of MHC region and immunoglobulin heavy chain gene (Gm types) with diabetic retinopathy. Type 2 diabetic patients were divided into those with either predominantly exudative or proliferative retinopathy and another group of patients free from diabetic retinopathy but with a minimum duration of diabetes of at least 15 years. The genetic analyses included regions such as HLA-DQBI, HLA-DQIA, HLA-DRA, insulin gene hypervariable region and the switch region of the immunoglobulin heavy chain gene (Sm). Differences in genotype distributions between the study groups were detected only with the Sm probe that detected the polymorphism in the switch region of IgA. The results of the study suggested that there was a genetic predisposition of proliferative diabetic retinopathy in Type 2 diabetes in south India and this seems to be determined by polymorphism of the heavy chain immunoglobulin genes located on chromosome 14.

The second study looked for association of proliferative retinopathy and tumour necrosis factor- α (TNF- α) within the MHC class III region in Type 2 diabetes.³³ It has been suggested that TNF- α secretion determined by TNF- α genotype possibly leads to an alteration in the pericyte / endothelial cells ratio, thus leading to the development of retinal microvascular changes. TNF- α also acts in the pathogenesis of retinopathy by inducing a hypercoagulatory state through the effect on platelet activating factors, thromboxine and protein kinase C. Our study demonstrated a strong association of Type 2 diabetic patients with retinopathy with the TNF- α allele suggesting the possibility that TNF- α plays an important role in the pathogenesis of proliferative diabetic retinopathy.

Angiotensin Converting Enzyme (ACE) Gene

Angiotensin Converting Enzyme (ACE), an endothelial ectoenzyme secreted in plasma plays a key role in regulating systemic and renal circulations by activating angiotensin I into the vasoconstrictor peptide

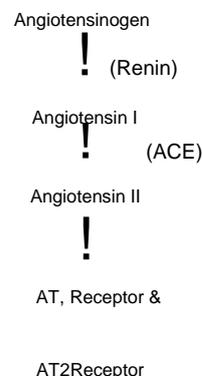


Figure 3. Angiotensin converting enzyme (ACE): The Angiotensin converting enzyme activates the Angiotensin I and Angiotensin II. It regulates the systemic and renal circulation.

angiotensin II³⁴ (Figure 3). The angiotensin I converting enzyme is encoded by the human ACE gene which has been cloned and sequenced. The ACE gene is located on chromosome 17p23 and spans approximately 21kb of DNA.³⁵

The ACE gene polymorphism, first described by Rigat et al.^{36,37} conventionally refers to the insertion (I) or deletion (D) of a 287 bp sequence in intron 16 of the gene. Subjects with DD genotypes have the highest level of plasma ACE, while those with II phenotypes have lowest levels and those with ID phenotypes exhibiting intermediate levels of plasma ACE.

The development of a single-step method for detection of the ACE gene (*I/D*) polymorphism by use of polymerase chain reaction (PCR),³⁸ which amplifies the DNA, facilitated the large-scale studies that followed the first report by Rigat et al.³⁶ Interest in gene polymorphism research has exploded over the last few years allowing DNA genotyping of patients with retinal, renal and cardiovascular complications. However, the data obtained so far have produced conflicting results which may be due, among other reasons, to a high genetic heterogeneity. Lack of any relationship between an insertion / deletion polymorphism in the Angiotensin I Converting Enzyme gene was reported by different groups in both Type 1 and Type 2 diabetes. These studies were carried out on diabetic patients in different populations including Caucasians, French and Japanese.³⁹⁻⁴¹

Fujisawas and coworkers⁴² recently carried out a meta-analysis of data from literature on ACE *I/D* polymorphism and diabetic retinopathy. The authors analysed various studies on a total of 2010 diabetic

patients with and without retinopathy in both Type 1 and Type 2 diabetes and found no association of the 1/ D polymorphism with diabetic retinopathy. In a metaanalysis, although it is possible to analyse a large population, there are a number of limitations including the small size of single studies and the effect of publication bias. However, there is a need to plan a large-scale prospective study.

Recent reports have provided evidence for the association of the ACE gene with proliferative retinopathy.⁴³ ACE levels are high in patients with proliferative retinopathy,⁴⁴⁻⁴⁶ which suggests that elevated serum ACE levels may be a potential cause of retinal vascular damage in diabetes. The DD allele phenotype has been strongly linked to highest plasma concentrations of ACE. It has also been proposed that the potential value of identifying a patient with a DD genotype could be the early therapeutic intervention which reduces the further progression of diabetic retinopathy.

Vascular Endothelial Growth Factor (VEGF) gene

In human beings, the vasculature is quiescent except during the physiological cycles of reproduction. During the formation of new blood vessels or angiogenesis, in response to unknown stimulus which stimulates various growth factors, endothelial cells break down and the integrity of the basement membrane is lost. Vascular Endothelial Growth Factor (VEGF) is an endothelial cell specific angiogenic and permeability-inducing factor that has been implicated in the pathogenesis of diabetic retinopathy.⁴⁷

Neoretinal vascularisation is associated with retinal ischaemia and hypoxia which induces VEGF production. Various clinical studies have evaluated the correlation between diabetic retinopathy and intraocular VEGF concentrations. VEGF concentrations were markedly elevated in both the vitreous and aqueous fluid of patients with proliferative diabetic retinopathy compared with samples from patients without diabetes and with non-proliferative diabetic retinopathy. The observation of retinal VEGF expression early in diabetic retinopathy suggests that VEGF may also play a role as a permeability-inducing factor leading to progression of the early stages of retinopathy.⁴⁸

VEGF is a dimeric heparin-binding protein with a molecular weight of approximately 46 KD. The human VEGF gene is organized in eight exons separated by seven introns⁴⁹ and is found on chromosome 6. Four molecular species of the human VEGF family have been generated and the target specificity of this growth factor seems to be restricted to vascular endothelial cells. Two VEGF tyrosine kinase receptors have been identified,

namely VEGF R1 and VEGF R2. The genes for these two receptors have been studied and two of their promoters have been shown to contain a 5' flanking sequence essential for endothelial specific expression. VEGF R2 promoter is sufficient to induce enhancement in the expression of foreign genes in endothelial cells. Research is continuing on the regulation of gene expression of VEGF and its two receptors. To summa rise, VEGF is always expressed in neovascular membranes and that VEGF receptor binding activity is found in greater quantities in vitreous aspirates of diabetic patients with proliferative retinopathy. This constant expression of VEGF in fibrous and vitreous membrane samples taken from diabetic patients suggests that this angiogenic factor may play a role in the extension of neovascular fronts and could therefore be a major candidate in the auto-amplification of the neovascular process.⁵⁰

The enhanced expression of these genes in certain tissues suggests both alternative splicing of mRNA and the role of the excess glucose molecule in regulating the gene expression of diabetic patients.

The Future

With the current advances in genomics and the outcome of the human genome project, major progress is being made in cataloging and mapping genes expressed in the retina. The process of sequencing the mRNAs expressed in various tissues is also underway. These genes, known as expressed sequence tags (ESTs), now allow recognition of the genes expressed in various tissues and also of the transcriptional units among genomic sequences.

The genomic sequence data will soon supply a wealth of information for the identification of mutations in various disease complications such as diabetic retinopathy and for developing strategies for treatment and prevention of these complications. There is a growing need for large-scale studies on well characterised Type 2 diabetic patients with and without diabetic retinopathy as this could help to finally resolve the issue of whether or not diabetic retinopathy has a major genetic basis.

Postscript

Subsequent to the acceptance of this manuscript, a publication by Govindaswamy Kumaramanikavel et al in Diabetes Research and Clinical Practice (2001; 54:8994) has shown the association between TNF ~ polymorphism and diabetic retinopathy. They have identified allele 4 (103 base pair) with (GT)⁹ repeat as a low risk allele for developing diabetic retinopathy and allele 8 (111 base pair) as high risk for developing proliferative diabetic retinopathy in Type 2 diabetic patients of south Indian origin.

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