

Emerging Patterns of Possible Potential Candidate Gene Polymorphisms Associated with Diabetic Retinopathy-a Review



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Abstract : Diabetic retinopathy (DR) is one of the most frequent complications of diabetes and the leading cause of acquired blindness in developed countries. More than 60% of type 2 diabetic patients are prone to the development of retinopathy. Although hyperglycemia is the prime factor associated with the pathogenesis of diabetic retinopathy, the exact mechanism by which retinal damage is inflicted upon is unknown. Studies suggest that many factors like type of diabetes, duration, onset, glycaemic control, environmental, biochemical, growth factors and high blood pressure are involved in the development and progression of diabetic retinopathy. However these factors alone do not explain the occurrence of retinopathy. Subsequently many candidate genes associated with the development and progression of diabetic retinopathy have been identified. This review highlights the molecular genetic aspects of diabetic retinopathy and the results emerging from molecular studies of the potentially involved genes.

Key words : Diabetic Retinopathy, Polymorphism, Candidate gene.

Introduction :

In most developing countries, diabetic retinopathy remains a major cause of visual morbidity. One of the most devastating micro vascular complications of diabetes mellitus is diabetic retinopathy (Balasubramanyam *et al.*, 2002). Diabetic retinopathy is a progressive disease affecting the structure and cellular composition of the microvasculature (Kubawara and Cogan 1962; Sims 1986; Antonelli-Orlidge *et al.*, 1989). Blindness is twenty-five times more common in diabetic conditions than in non-diabetic conditions. The pathogenesis of diabetic retinopathy is multifactorial and not completely understood. Multiple biochemical pathways have been proposed to explain the pathogenesis of diabetic retinopathy. These mainly include promotion of the polyol pathway, increased advanced glycation end products (AGE) formation, activation of protein kinase C (PKC) and increased hexosamine pathway flux (Balasubramanyam *et al.*, 2002). Treatment and control of diabetic retinopathy in the present scenario is efficiently done with

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laser photocoagulation. Unfortunately this is essentially a destructive procedure. Therefore, focusing on and exploiting the genetically identified factors could be a better alternative. The main aim of the current genetic revolution is to introduce highly specific therapeutic measures that carry out, as yet, un-attained molecular precision. Attempts are under way to exploit therapeutically what is known or yet to be known about the genetic aspects of diabetic retinopathy. This raises the question; do we know enough about diabetic retinopathy genetics for such goals to be achieved?

Diabetic retinopathy is most commonly manifested by decreased visual acuity, Hemorrhagic complications ensue from ischemia and the resultant neovascularization. Linkage analysis relates loci on chromosome 3 and 9 (Imperatore *et al.*, 1998) with diabetic retinopathy in addition to other candidate genes. The manner by which mutations in candidate genes result in diabetic retinopathy formation is yet to be established.

Candidate genes and Diabetic Retinopathy : The candidate genes contributing to the development of diabetic retinopathy are the following: Aldose Reductase (ALR2) gene, endothelial Nitric Oxide synthase (eNOS) gene, Vascular Endothelial Growth Factor (VEGF) gene, Hemochromatosis HFE gene, Receptor For Advanced Glycation End products (RAGE) gene, Glucose transporter (GLUT1) gene, Paraxonase 1(PON1) gene, Angiotensin Converting enzyme (ACE) gene, Plasminogen activator inhibitor1 (PAI) gene and β -Adrenoreceptor (β -AR) gene. (Table1)

Aldose Reductase (ALR2) gene : Aldose reductase (ALR2), the first enzyme of the polyol pathway converts glucose to sorbitol in an NADPH-dependent reaction. It may play an important role in the pathogenesis of diabetic microvascular complications and is found in a variety of tissues, including the retinal capillary pericytes, retinal pigment epithelial cells and endothelial cells (Radha *et al.*, 2002). 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 doesn't 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 (Mugregor and Matschinsky, 1986) leading to accumulation of fluid in the cells. Since all of these have an important function in the cells, their alterations may lead to the death of retinal pericytes and hence damage the endothelial cells, an early event in the development of diabetic retinopathy. Several studies have pointed out that a high level of aldose

Table 1 : Gene polymorphisms associated with diabetic retinopathy.

Gene	Region	Nucleotide change	High risk	Low risk	Ethnicity	Reference
Aldose reductase (ALR2)	5' UTR and Promoter Region	(A-C)n dinucleotide repeat sequence	Z-2 allele Z-4 allele Z-2 allele	- - -	Chinese population Japanese population Asian Indian population	Ko <i>et al.</i> 1995. Ikegishi <i>et al.</i> 1999. Kumaramanickavel <i>et al.</i> 2003.
	Intron 8 Promoter	A-C at nucleotide 95	BB homozygote	-	-	Kao <i>et al.</i> 1999.
	Promoter	C (-106) T G (-72) C	CC homozygote	-	Indian population	Kao <i>et al.</i> 1999. Suganthalakshmi <i>et al.</i> 2005.
VEGF	5'UTR,	C (-634) G, T (-1498) C, G (-1190) A, C (-7) T	- 634 Callele	GC, CC genotype	Japanese population	Awata <i>et al.</i> 2002.
RAGE	exon3	Gly 82 Ser	-	Ser82 allele	Asian Indian	Kumaramanickavel <i>et al.</i> 2002.
TGF-β1	Promoter region and Signal peptide sequence	C (-988) A, G (-800) A, C (-509) T, T (869) C-L 10P, G (915) C-R 25P	R 25P	-	Caucasian population	Beranek <i>et al.</i> 2002.

Contd.

Gene	Region	Nucleotide change	High risk	Low risk	Ethnicity	Reference
TNF	Upstream	Allele 4, 8	Allele4	Allele8	Indian population	Kumaramanickavel <i>et al.</i> 2001.
iNOS	Upstream	Penta Nucleotide repeat	210 bp	205bp	Asian Indian population Northern irish population	Kumaramanickavel <i>et al.</i> 2002. Warpeha <i>et al.</i> 1999.
eNOS	Intron 4 and exon 7	Five repeats, four repeats of 27 bp allele	b/b allele five repeats	–	Caucasian population	Taverna <i>et al.</i> 2002.
β 3-adreno receptor	–	Trp 64 Arg	Arg/Arg, Arg/Trp	–	Pima Indians	Sakane <i>et al.</i> 1997.
PAI-1	Promoter	Deletion of 4G allele	4G allele	–	Pima Indians	Nagi <i>et al.</i> 1997.
HFE	–	C 282 Y	C282Y	–	Caucasian population	Peterlin <i>et al.</i> 2003.

reductase in the erythrocyte of both Type 1 and Type 2 diabetic patients is associated with the presence of retinopathy. (Hamada *et al.*, 1991; Hamada *et al.*, 1993; Nishimura *et al.*, 1994)

Human ALR2 gene, encoding aldose reductase has been localized to chromosome 7q35 and consists of 10 exons extending over 18 kilobases of DNA (Graham *et al.*, 1991). There is a growing evidence to implicate ALR2 in the pathogenesis of diabetic microvascular disease. Patients with an early onset of diabetic retinopathy exhibit an abnormal allele (Z-2), one of the seven varying alleles, identified in the (A-C)_n dinucleotide repeat polymorphic marker at the 5' end of the aldose reductase gene from type 2 diabetic patients (Ko *et al.*, 1995). This study highlights that ALR2 or another gene near this locus may contribute to early onset of this complication.

A novel G (-72) C promoter polymorphism in ALR gene of diabetic retinopathy has been reported in International symposium on genes evolution and complex diseases, NCBS, Bangalore by Suganthalakshmi *et al.* 2005

Endothelial Nitric oxide synthase (eNOS) : Nitric oxide synthase regulates endothelium-dependent vasodilatation and blood pressure. Reduced production has been implicated in hypertension, atherosclerosis and diabetes (Tikkanen and Fyhrquist, 1995; Williams *et al.*, 1996). Endothelial nitric oxide synthase mediates the oxidation of L-arginine to produce NO and determines basal vascular wall NO production (Cooke and Dzau, 1997). The vascular endothelial cell is central to the maintenance of normal blood flow, local rheology and the preservation of metabolic homeostasis.

Endothelial cells communicate directly with pericytes and smooth muscle cells via gap junctions and actively regulate capillary and arteriolar tone and calibre by elaborating vasodilators (nitric oxide, adenosine, prostanoids) and vasoconstrictors (endothelin 1, angiotensin II) in response to local metabolic needs. Some recent studies suggest that NOS may have a vasoregulatory role in the retina (Roufail *et al.*, 1995). It is now recognized that aberrations in retinal blood flow in early diabetes are also linked to vascular endothelial dysfunction, thus, endothelial dysfunction in diabetes is likely to have a major effect on the circulation within the retina. (Feener and King, 1997; Stehouwer *et al.*, 1997). In the normal retina, NOS2A gene, one of the three members of the nitric oxide synthase family, is not expressed in the retinal vasculature. High ambient glucose may influence NO release through increased NOS2A expression and reduced constitutive

NOS3 expression in cultured retinal vascular endothelial cells (Chakravarthy *et al.*, 1998)

The gene encoding eNOS is located on chromosome 7q35-36. Cai *et al.* (1998) identified a 27.8% allele frequency of the Glu 288 Asp mutation at exon 7 of the eNOS gene in type 2 diabetic patients, but they found this mutation was not associated with vascular complications or with any of the traditional atherogenic risk factors.

Vascular Endothelial Growth Factor (VEGF) gene : Vascular endothelial growth factor (VEGF), a major mediator of vascular permeability and angiogenesis, may play a pivotal role in mediating the development and progression of diabetic retinopathy. Diabetic retinopathy is characterized by vascular permeability, increased tissue ischemia, angiogenesis and increase oxidative stress. Vascular endothelial growth factor (VEGF), a 45-KDa-homodimeric glycoprotein (Ferrara and Davis-Smyth, 1997), has initially drawn much attention as an important mediator of retinal ischemia-associated intraocular neovascularization (Duh and Aiello, 1999). VEGF is produced from many cell types within the eye, and past studies have shown that VEGF levels are markedly elevated in vitreous and aqueous fluids in the eyes of individuals with proliferative diabetic retinopathy (PDR). Studies indicate that VEGF, appears to present an attractive candidate susceptibility gene for diabetic retinopathy.

The human VEGF gene is organized in eight exons separated by seven introns (Lutty *et al.*, 1996) 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 VEGFR1 and VEGFR2. 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. VEGFR2 promoter is sufficient to induce enhancement in the expression of foreign genes in endothelial cells. Research results evidence polymorphism in 5'UTR and promoter regions of VEGF gene in Japanese diabetic retinopathy patients group (Awata *et al.*, 2002).

Hemochromatosis HFE gene : Proliferative diabetic retinopathy is characterized by active angiogenesis and the formation of fibrovascular tissue at the vitreoretinal interface. This process requires a local production of cell-derived angiogenic factors and synthesis of extracellular matrix components necessary for the anchorage of migrating endothelium. Iron metabolism has been recently

associated with angiogenesis and extracellular matrix remodeling and fibrosis (Simonart *et al.*, 2001). Moreover, emerging scientific evidence has disclosed bi-directional influences between iron metabolism and type 2 diabetes (Fernandez-Real *et al.*, 2002) and in the pathogenesis of diabetic retinopathy. Peterlin *et al.* identified a C282Y HFE gene mutation in the development of proliferative diabetic retinopathy in Caucasians with type 2 diabetes (Peterlin *et al.*, 2003).

Receptor for Advanced Glycation End products (RAGE) gene :

The advanced glycation end products (AGE) are a heterogeneous group of compounds that cause a plethora of adverse effects, including reduction of enzymatic activity, damage to nucleic acids, impaired degradation of proteins, cross-linking of proteins, and induction of cytotoxic pathways.

Most (AGE) effects are mediated through the RAGE receptor and are thought to be involved in the development of diabetic complications (Brownlee, 1994) including retinopathy. RAGE is a 35-kDa polypeptide of the immunoglobulin superfamily of receptors located on chromosome 6 in the HLA region (Neeper *et al.*, 1992), 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 (Liu and Xiang, 1999). 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 al.*, (1998) screened for polymorphisms in the coding regions of RAGE gene and recorded four functional amino acid changes. Associations between the AGE receptor polymorphisms in the promoter region and severity of retinopathy have been described. Polymorphisms in the AGE-R1 gene have also been reported to be associated with the presence and severity of retinopathy (Vlassara, 2001).

Glucose transporter (GLUT1) gene : Elevation of intracellular glucose within retinal vascular cells is believed to be an important causal factor in the development of diabetic retinopathy. The intracellular glucose concentration is regulated by both the rate of glucose metabolism and glucose transport. In the retina, glucose may gain entry into the endothelial cells of the inner blood retinal barrier (BRB) only via transport mediated by the sodium-independent glucose transporter GLUT1 (Takata *et al.*, 1997). Changes in GLUT1 expression may have profound consequences on glucose delivery to the retina and major

implications in the development of diabetic retinopathy. In the human eye, GLUT1 is expressed in the retinal capillary endothelial cells, the retinal pigment epithelium, the non-pigmented epithelium of the ciliary body, the endothelium of Schlem's canal, and the capillaries and posterior pigmented epithelium of the iris (Harik *et al.*, 1990; Mantych *et al.*, 1993).

GLUT 1 represents a unique portal of entry of glucose into the endothelial cells of the inner BRB, changes in retinal endothelial cell GLUT 1 expression and glucose transport may have a major impact in providing substrate to the various pathogenic processes thought to underlie the development of diabetic retinopathy. The gene encoding for GLUT1 is located on chromosome 1p35-p31.3.

Kumagi *et al.* (1994) demonstrated localized upregulation of GLUT1 in retinal endothelial cells of post mortem retina specimens from three individuals with long-standing diabetes, but without clinical evidence of diabetic retinopathy. Aiello *et al.*, (1998) have studied the regulation of the glucose transport system by hypoxia in cultured bovine retinal endothelial cells (BRECs). Such studies suggest that upregulation of retinal GLUT1 occurs in the early stages of diabetic retinopathy, and that the resultant increase in glucose transport plays a role in the progression of diabetic retinopathy.

Paraxonase 1 (PON 1) gene : Serum paraxonase is a glycoprotein that binds to high-density lipoprotein (HDL) and may prevent oxidation of LDL by hydrolyzing lipid peroxides. The gene encoding for PON 1 is located on chromosome 7q21.3. PON genes are members of a multigene family. Recently two other members of the PON family have been found, designated PON 2 and PON 3. The family of paraxonase genes have potential significance for understanding diabetes and its complications. Yon Li Kao *et al.* demonstrated that the allelic frequency of leucine 54(L) was significantly higher in the group with retinopathy than in that without retinopathy (Kao *et al.*, 1998), a variant of PON1 gene (genotype L/L) was strongly associated with the development of diabetic retinopathy suggesting that leucine 54 is a risk factor for 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 angiotensin II (Erelas, 1990). The angiotensin I converting enzyme is encoded by the human ACE gene and has been cloned and sequenced. The ACE gene is located on chromosome 17p23 and spans approximately 21kb

of DNA. A deletion in the polymorphic region of intron 16 of the ACE gene is associated with the prevalence of proliferative retinopathy in type 1 diabetes and suggests that the DD (deletion/deletion) genotype confers susceptibility to proliferative retinopathy independent of diabetic nephropathy (Rabensteiner *et al.*, 1999). However different studies have reported that ACE levels are particularly high in patients with proliferative retinopathy, which suggests that elevated serum ACE levels may be a potential cause of retinal vascular damage in diabetes (Feman *et al.*, 1993).

Plasminogen activator inhibitor 1 (PAI-1) gene : Plasminogen activator inhibitor 1 (PAI-1) is a potent inhibitor of fibrinolysis. The fibrinolytic activity is largely determined by circulating levels of PAI-1. The PAI-1 activity is found to be elevated in subjects with type 2 diabetes and is associated with macrovascular disease in non-diabetic and diabetic subjects (Juhan-Vague *et al.*, 1989; Nagi *et al.*, 1996). The gene encoding for PAI-1 is located on chromosome 7q21.3-q22. Altered PAI-1 expression, through its effect on plasmin production and tissue proteolysis, may alter the endothelial extracellular matrix composition and accumulation seen in diabetic microvascular disease (Cagliero *et al.*, 1991). Nagi *et al.* (1997) reported that in Pima Indians with type 2 diabetes the presence of the deletion 4G allele in the promoter region of the PAI-1 gene was associated with a higher risk of diabetic retinopathy (Nagi *et al.*, 1997).

â3-Adrenoreceptor (â3-AR) gene : Pima Indians have a high frequency of missense mutation, Trp64Arg of the â3-Adrenoreceptor (â3-AR) and those with the mutation have an early onset of diabetes type 2 and a tendency to have a low metabolic rate (Walston *et al.*, 1995). Examination of the relationship between this mutations and proliferative diabetic retinopathy in NIDDM patients has shown that the Arg/Arg or Arg/Trp genotype are more significantly associated with proliferative diabetic retinopathy than the Trp/Trp genotype with an odds ratio of 2.55. These findings suggest that â3-AR gene polymorphism is an important risk factor for proliferative diabetic retinopathy (Sakane *et al.*, 1997).

Conclusion :

Multifactorial influences attributing to the complexity in the pathogenic mechanisms of diabetic retinopathy, may explain the difficulty in associating a single candidate gene abnormality with the condition. Therapeutic intervention in the progression of the diseased condition could easily become possible with the

identification of such a potentially involved, single candidate gene mutation. However, linkage analysis, association studies, differential display and genomewide screening is essential to shed light on the pathogenic picture and the genes of importance.

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Polymorphism in diabetic retinopathy

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Sundaresan P. et al. (2006) *Asian J. Exp. Sci.*, 20 (Supplement), 15-28

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Polymorphism in diabetic retinopathy

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