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ADVANCED GLYCATION END PRODUCTS IN THE PATHOGENESIS OF DIABETIC RETINOPATHY: A REVIEW

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ABSTRACT

Advanced glycation end products are a group of biochemical species formed by the chronic exposure of vital biomolecules to hyperglycemia. All events leading to diabetic retinopathy such as, adhesion of leukocytes and monocytes to capillary endothelial cells, apoptosis of endothelial cells and pericytes, dismantling of blood retinal barrier, increased expression of pro-angiogenic factors and decreased the expression of anti-angiogenic factors in the retinal endothelial cell are linked to an increased formation of advanced glycation end products. Expression of receptors for the advanced glycation end products and its gene polymorphism are found to have a major implication in the development and progression of diabetic retinopathy. Soluble extracellular ligand binding domains of these receptors called as sRAGEs that circulate freely in plasma are shown to scavenge advanced glycation end products and prevent damages. So there lies a large scope for research with respect to the role of sRAGE in the treatment and management of diabetic retinopathy.

Key words: Diabetic retinopathy, advanced glycation end products, receptors for advanced glycation end products, angiogenesis.

INTRODUCTION

Diabetic retinopathy (DR) is considered to be the most serious complication and one of the major causes of blindness in diabetic patients [1,2]. It is a disorder affecting the microvasculature of the retina. Accumulation of leukocytes on the capillary wall, capillary basement membrane thickening, pericyte dropout, blood retinal barrier breakdown, capillary hyper perfusion and subsequent angiogenesis are the key mechanisms in the pathogenesis of retinopathy. These are linked with inflammation, oxidative stress and growth factor expression in the retina [3-6]. Hyperglycemia can trigger most of these events through the formation of advanced glycated end products (AGE). AGEs bind to specific receptors on the target tissues called as receptors for advanced glycation end products (RAGEs) and bring about tissue damage.

AGEs are found to be involved in the inflammation and production of reactive oxygen species (ROS) which can further damage both intracellular and extracellular proteins which maylead to microvascular abnormalities and endothelial dysfunction. Glycosylation can modify the structure, stability, signaling property and receptor affinity of a protein. Many *in vitro* as well as *in vivo* studies have proposed that besides proteins even lipids and nucleotides can get glycated and have additive effects on AGE induced damages [7-12].

Membrane bound forms of RAGEs have been implicated in most of the pathogenic effects of AGEs. Soluble extracellular ligand binding domains of RAGEs called as sRAGEs which circulate freely in plasma, are formed by alternative splicing of RAGE mRNA and have shown to scavenge AGEs and prevent damages caused by them (Figure 1) [8-14]. Several studies have shown that blocking AGE binding to RAGEs or prevention of AGE production itself can significantly minimize microangiopathy and other related effects of hyperglycemia. In this regard, several studies have been undertaken to study RAGE gene polymorphism in diabetic retinopathy [15,16]. In this review, we illustrate some of the mechanisms of AGEs proved to be involved in the development of microangiopathy particularly diabetic retinopathy.

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Formation of AGEs

Chronic hyperglycaemia can lead to non-enzymatic glycation of lysine and arginine residues in proteins such as collagen, albumin which have longer half-life. Unlike enzyme mediated reactions, these nonenzymatic glycations are comparatively sluggish, and do not get saturated even at high glucose levels [17].

Glucose and its reactive derivatives non-enzymatically react with free amino groups of proteins to form Schiff's bases which further undergo several molecular rearrangements to form highly reactive ketoamines known as Amadori products. These can cross-link irreversibly to form a network of structurally as well as functionally altered proteins known as advanced glycation end products [18-21]. This reaction is called as Milliard reaction [Figure 2]. Usually AGEs are detected and destroyed by macrophages and excreted through the kidneys [18]. But, excessive generation and low eliminations of AGEs can lead to their accumulation in the tissues and extracellular matrices predisposing them to damage.

Apart from glycation of proteins, peroxidation of lipids can also contribute to the generation of AGEs. Glycated proteins produce carboxymethyl-lysine, pentosidine, carbonyls etc. while lipid peroxidation generates highly reactive aldehydes such as, methylglyoxal, glyoxal, and 3-deoxyglucosane [22]. All of these intermediates further help in stabilization of advanced glycation end products (Figure 3).

Receptors for advanced glycation end products (RAGE): The AGE signal transducers

Advanced glycation end products act through their receptors ie. RAGEs. These are expressed by almost all the tissues. The cell types which express these receptors include, vascular endothelial cells, pericytes, macrophages, lymphocytes, glomerular epithelial cells etc. It is found that, in normal physiological conditions expression of RAGE is very low but can be drastically induced by the availability of AGEs [8,14]. Studies have shown that all different types of AGEs with no structural similarity can bind to a single type of receptor on the target cells So RAGEs are considered as 'pattern recognition receptors' [14].

The membrane bound RAGEs based on their degree of glycosylation, can have molecular weights ranging from 45 to 50 kDa. It has an extracellular ligand binding domain, a transmembrane domain and an intracellular domain with 339, 22 and 42 amino acids respectively. The ligand binding domain shares the structure of one half of the immunoglobulin molecule and contains, one V-type immunoglobulin domain which is responsible for binding to most of its ligands and two C-type immunoglobulin (C1and C2)domains (Figure 4) [23-25]. It has been proposed that RAGEs can dimerize or multimerize upon binding of ligands [26].

Advanced glycation end products and retinal endothelial cell apoptosis

Diabetic patient's blood has high levels of cell adhesion molecules that attract leukocytes over vascular endothelium, leading to endothelial cell damage and dysfunction. In vivo studies in animals and in vitro studies with cultured human endothelial cells, demonstrated an increased expression of cell adhesion molecules such as Intracellular Adhesion Molecule-1 (ICAM-1), Vascular Cell Adhesion Molecule-1 (VCAM-1), Monocyte Chemoattractant Protein-1 (MCP) etc in hyperglycemic conditions (Figure 5) [27-29]. Similar effects were observed when human endothelial cells were incubated with advanced glycated bovine serum albumin (AGE-BSA) for 6 hours [30]. Pei Tian *et al*. observed more apoptosis of retinal endothelial cells (REC) derived from diabetic individuals when they co-cultured them with leukocytes as compared to that of non-diabetic subjects. The same study showed reversal of these effects after berberine therapy [31].

Advanced glycation end products can activate NFκB pathway

NFκB is shown to be involved in the pathogenesis of several diseases associated with inflammation and angiogenesis. It belongs to the category of "rapid-acting" primary transcription factors, i.e., transcription factors that are present in cells in an inactive state and do not require new protein synthesis in order to become activated. NF-κB is widely used by eukaryotic cells as a regulator of genes that control cell proliferation and cell survival. It was found to be involved in the regulation of gene transcription of certain inflammatory genes like, VCAM-1, MCP-1, COX2 that are involved in the retinal epithelial cell death, endothelial cell proliferation, angiogenesis and retinopathy [32-35]. Further, LR90 a newly found inhibitor of advanced glycation end product was shown to reduce the transcription of pro-inflammatory genes, including monocyte chemoattractant protein-1 and cyclooxygenase-2 in a dose-dependent manner [36].

Renu A. Kowluru et al. have shown an increased expression of NF-kB,caspase-3,and oxidative stress markers, which lead to increase in apoptosis of the REC and pericytes incubated with AGE-Bovine serum albumin (AGE-BSA) and diabetic sera as compared to those incubated with normal bovine serum albumin [37]. This shows that AGE can act through multiple pathways and bring about endothelial dysfunction and retinopathy.

Protein kinase-C are a group of molecules that are involved in the pathology of microangiopathy through, a decreased eNOS (vasodilator), increased endothelin-1 (potent vasoconstrictor), TGF-β, NFκB which causes, vascular occlusion, increased vascular permeability, basement membrane thickening etc. [38-43].

Renu A. Kowluru et al. had also demonstrated an inhibition of the AGE-BSA induced effects on REC and pericytes when an antibody specific for PKCα and PKCε were used before their exposure to glucose [37]. This indicates all the effects shown by AGE-BSA were indirectly related to the activation of NFκB via PKC pathway. There was a remarkable trend towards NFκB activation in the endothelial cells exposed either to hyperglycaemia or AGE-albumin. This shows that hyperglycaemia itself independently can initiate the pathogenesis of vascular abnormality in the initial stage through an increase in the NFκB activation. But for progression of the same may require AGEs and several other factors (Figure 6).

Advanced glycation end products can dismantle anti-oxidant defense system

Hyperglycaemia induced oxidative stress in diabetes is a well-known fact. The reactive oxygen species (ROS) such as superoxide radical, H_2O_2 etc. attack membrane lipids, proteins and even DNA and cause irreversible damage. They are thus involved in the tissue damage and pathogenesis of several diseases [44, 45]. Our body has a well-equipped defense system against ROS, which include non-enzymatic antioxidants such as Vitamin A, C, E, glutathione; enzymatic antioxidants like, superoxide dismutase, catalase, and glutathione peroxidase (Figure 7) [46].

Both *in vivo* and in vitro studies have shown that AGEs are found to be involved in the destruction of anti-oxidant defense system in the diabetic tissues An *in vitro* study has shown decreased Glutathione and Vit.C and increased expression of NF-κB, other inflammatory genes controlled by NF-κB like, tissue factor and endothelin-1 when cultured bovine aortic endothelial cells were incubated with AGE-albumin [47].

Increased formation of malondialdehyde (a lipid peroxidation end product) along with an upsurge in NFκB expression were seen in capillary endothelial cells exposed to advanced glycation end products. Infusion of AGE-albumin into healthy animals found to increase lipid peroxidation and NFκB mRNA expression on the capillary walls and tissues. All these effects were reversed by treatment with AGE specific antibodies and anti-oxidants [48].

Ulriche Denis et al. have found an increase in the apoptosis of pericytes when treated with AGE-BSA which was then shown to be completely subdued by treatment with antioxidants [49]. Subhadip Choudhuri et al. have found a drastic increase in the levels of N^{ε}-carboxy methyle lysine (an advanced glycation end products) and ROS levels in the serum of proliferative diabetic retinopathy patients when compared to normal controls [50].

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Advanced Glycation End Product can Induce Blood-Retinal Barrier Break Down

Blood retinal barrier is a tightly packed mass of cells that regulated the exchange of molecules between the blood and retinal tissue. It is formed by retinal vascular endothelial cells lining the capillary and the retinal pigment epithelium from retinal side. These cells are tightly packed and contain tight junctions made up of tight junction proteins that help in cell adhesion [53-55]. Disruption of blood retinal barrier can lead to the development of diabetic retinopathy [56]. When vascular endothelial cells were co-cultured with AGE-albumin there was a notable decrease in the tight junction molecules such as, VE-cadherin, β-catenin and γ-catenin and this loss was positively correlated with the exacerbated vascular permeability and endothelial cell migration (Figure 9) [57].

Intraperitoneal injection of AGE-albumin increased ICAM-1 expression, retinal leukostasis, blood–retinal barrier breakdown and VEGF expression in diabetic C57/BJ6 and RAGE-transgenic mice (figure 9). All these effects were reversed by systemic administration of sRAGE in both diabetic C57/BJ6 and RAGE-transgenic mice [58].

Pericytes are the cells lining the capillary endothelial cells, which gives strength to the capillary and also prevent endothelial cell migration and it anchors the capillary structure of the surrounding matrix [59]. Pericytes are sensitive to advanced glycation end products. Pericyte apoptosis is an important event in breakdown of the blood retinal barrier and pathogenesis of retinopathy. Treatment with anti-sense oligonucleotide molecules complementary to the RAGE gene mRNA have led to restoration of defects caused by AGEs and prevented the death of pericyte in culture. A threefold increase in parasite apoptosis was observed when treated with AGE-

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albumin. This was linked to induced oxidative stress in pericytes [60].

Abnormal angiogenesis in the retina and link with AGEs

Development of the new set of blood vessels from already existing blood vessels is angiogenesis. There is always a balance between pro-angiogenic and anti angiogenic factors in normal subjects. But retinal hypoxia due to capillary endothelial damage can induce the expression of angiogenic factors with simultaneous suppression of anti-angiogenic factors in the retinal tissue [61-64]. Further, loss of pericytes as explained above, also aid in the proliferation of capillary endothelial cells. The new blood vessels formed will be fragile and have a tendency to bleed inside the vitreous cavity and which may cause loss of visual acuity in the diabetic individual [65, 66]. Loss of visual acuity can also occur when there is a contraction of the vitreous and subsequent retinal detachment [67].

The DNA synthesis and endothelial cell division was found to be enhanced by AGE-albumin *in vitro*. This effect is found to be mediated by AGE induced production of VEGF a potent pro-angiogenic factor. This was further confirmed by using monoclonal antibodies against human VEGF [68]. An increased release of VEGF, TNFα, IL-8 and tissue factor are observed in Monocyte cell lines incubated with extensively glycated human serum albumin [69]. Ming Lu *et al*., showed dose and time dependent induction of VEGF mRNA in the ganglion, inner nuclear, and retinal pigment epithelial cell layers of the retina in diabetic rats [70]. VEGF expression, PKC and oxidative stress pathways are shown to be activated by increased AGEs in bovine retinal endothelial cells [71]. , Pigment Epithelial Derived Factor (PEDF), an anti-angiogenic factor secreted by the retinal pigmented epithelium is found to be decreased with exposure to advanced glycated end products. VEGF induced microvascular permeability also decreased by successive treatment with PEDF (Figure 10) [72].

Increased glycation and crosslinking of vitreous collagen was demonstrated in an *in vitro* study by incubating it with concentrated glucose solution [73]. Glycation and metal-mediated glycoxidation of vitreous collagen was demonstrated by Sulochana KN et al. in 2003 which was found to be inhibited by the addition of lysine and inositol respectively [74]. Increased accumulation of fructosamine (an advanced glycation end product) is seen in vitreous of diabetic animals within 6 months of induction of diabetes [75]. Thus, accumulation of AGEs induced cross linkage of vitreous collagen molecules may have a role in vitreous contraction and retinal detachment seen in severe proliferative diabetic retinopathy (Figure 10).

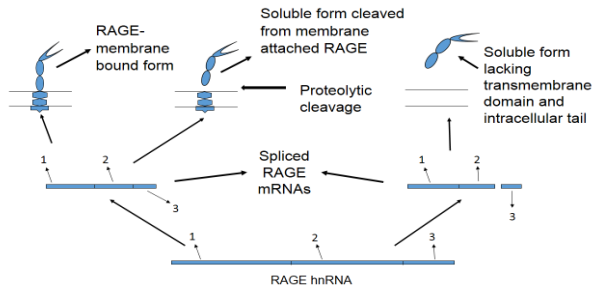
RAGE gene polymorphism and retinopathy

Polymorphism in a gene is a change in a single nucleotide or a sequence of nucleotide bases causing alteration in its expression. Balasubbu et al. and also two meta-analyses had demonstrated a significant association of SNP rs2070600 (G82S), polymorphism G1704T, –374T/A and Gly82Ser in the RAGE gene with diabetic retinopathy [76, 77, 78].

In 2013, Yuichiro Higashimoto *et al*., in an *in vitro* study have demonstrated a potential therapeutic effects of phosphorothioate aptamers (oligonucleotides containing phosphate analogue) where they found to repress AGE induced effects such as, DNA synthesis, expression of RAGE, VEGF and PAI -1 mRNA in HUVEC cell lines [79]. Li Yang *et al*., found that G82S polymorphism of RAGE is associated with circulating sRAGE levels in Chinese Population. DR subjects with the S/S genotype have a significantly higher serum sRAGE levels than controls and the subjects with G/S genotype [80].

Zhi Xiang Ng et al. demonstrated a significantly elevated NF-κB, p65, plasma MCP-1, AOPP, pentosidine levels and a lower sRAGE levels in DR patients with 2245GA genotype as to those with wild-type 2245GG. This study confirms the association of the RAGE gene with pro-inflammatory, oxidative-glycation markers and circulating sRAGE in diabetic patients with retinopathy [81].

Fig 1. Forms of Receptors for advanced glycation end products



1. *Sequences for extracellular ligand binding domain*, **2.** *Sequences for transmembrane domain*, **3.** *Sequences for Intracellular domain*

Fig 2. Formation and fate of AGEs

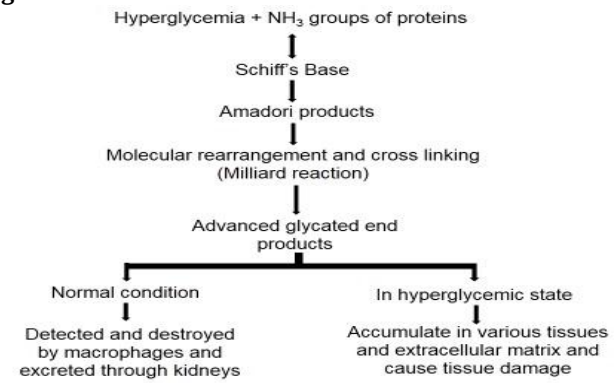


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Fig 3. Contribution of protein glycosylation and lipid peroxidation in formation and stabilization of AGEs

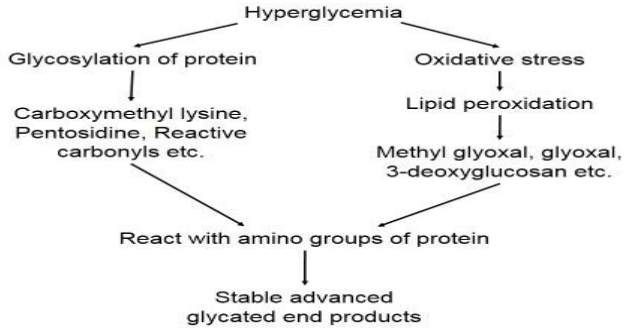


Fig 4. Structure of RAGE

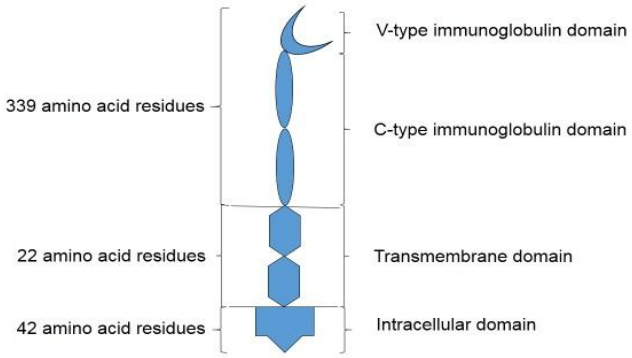


Fig 5. AGE induced leukostasis leading to retinopathy

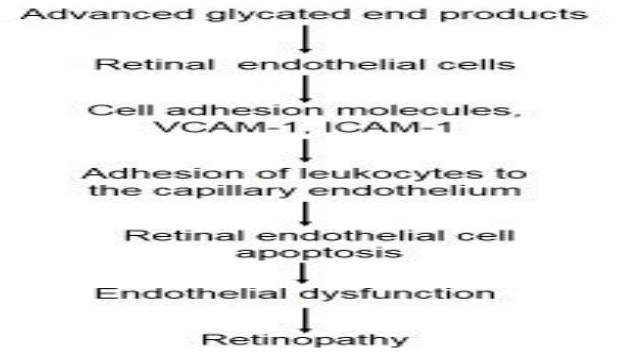


Fig 6. Protein kinase-C and NFκB in the pathogenesis

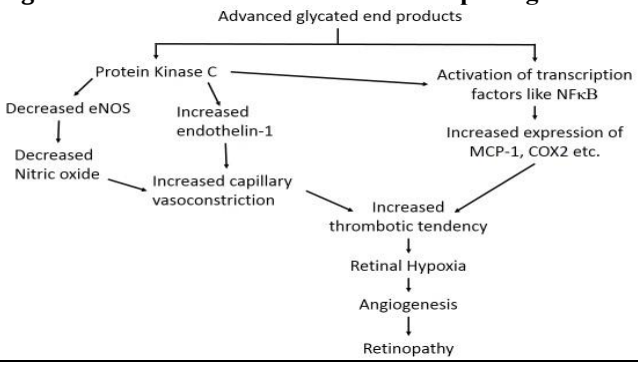


Fig 7. Antioxidant defense system

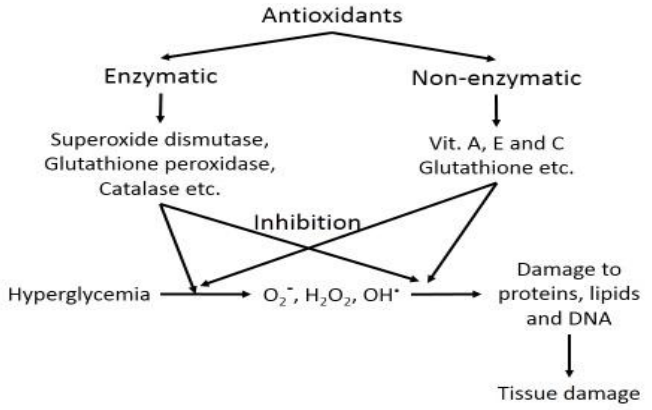


Fig 8. AGE induced oxidative stress leading to retinopathy

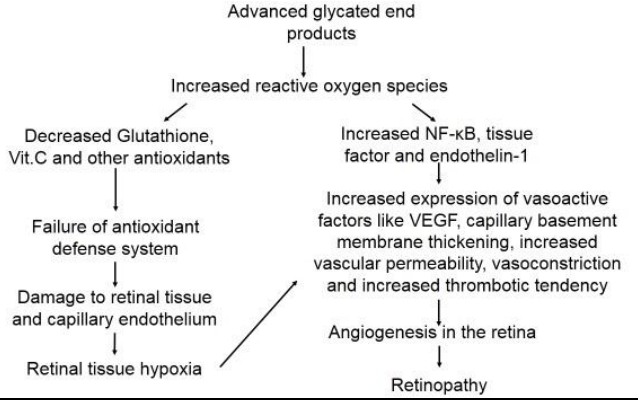


Fig 9. Advanced glycation end product can induce blood-retinal barrier breakdown and retinopathy

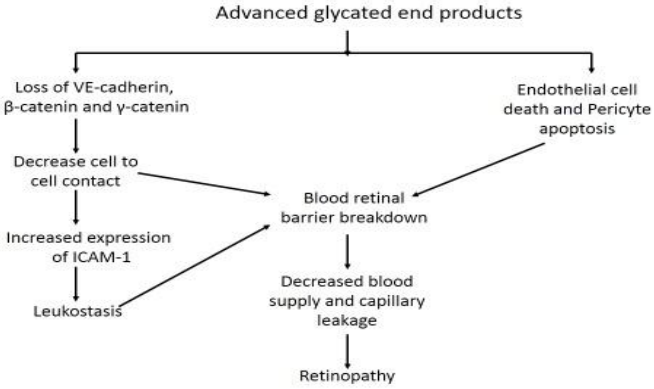
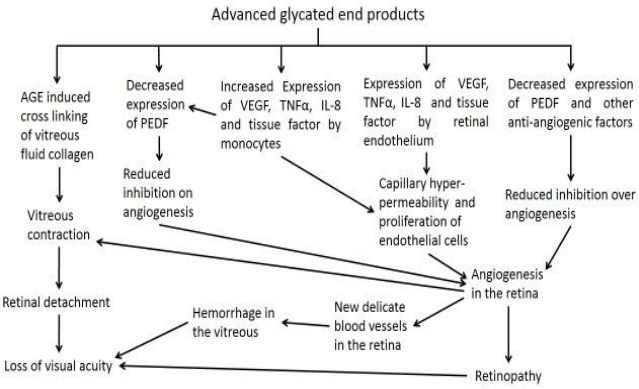


Fig 10. AGE induced angiogenesis and Pathogenesis of retinopathy



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Questions yet to be answered in AGE research

Effects of glycation of the tight junctional proteins and its effects on cell to cell signaling and blood retinal barrier destruction is yet to be studied. A study should be done to know if there is any interaction between advanced glycated proteins of capillary endothelial cells and the immune cells in accumulation of immune cells on the capillary wall. Actual mechanism and reason behind the modification of membrane bound RAGE to free sRAGE is still an area of active research. The ratio of these two forms and their impact on the development of diabetic complications is yet to be established. As most studies done focused only on single nucleotide polymorphism, studies on epigenetics, copy number variations and other aspects of the RAGE gene may throw more light on to the role of the RAGE gene in retinopathy.

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