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·Commentary·

Susceptibility genes for diabetic retinopathy

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Abstract

• Diabetic retinopathy (DR) is a sight-threatening chronic complication of diabetes mellitus and is the leading cause of acquired blindness in adults. Long-term exposure to the hyperglycemia of diabetes patients leads to the development of DR. Several studies have provided evidence that good diabetes control is important to prevent DR. However, emerging evidence suggests that genes are a significant contributor to an individual's risk of retinopathy. This evidence is from evaluations of familial aggregation and different incidence of DR in racial and ethnic groups. Some groups of patients develop DR despite good control and some escape retinopathy despite poor control. This suggests that the genes are involved in the susceptibility to DR. Genes suggested as having a role include those encoding aldose reductase, nitric oxide synthase, receptor for advanced glycation end products, angiotensin converting enzyme, vascular endothelial growth factors and pigment epithelium-derived factor. An understanding of the role of susceptibility genes will ultimately allow the development of novel therapeutic strategies. This article reviews the role of genetic factors in the etiology and progression of DR.

• KEYWORDS: diabetic retinopathy; susceptibility gene

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INTRODUCTION

Diabetes mellitus is a worldwide medical problem and is a significant cause of mortality. Micro- and macrovascular complications are highly prevalent among the diabetes patients[1]. Diabetic retinopathy (DR), one of the most important complications in both Type 1 and Type 2 diabetes, has become the leading cause of vision loss and blindness in working-age adults in both developed and developing countries. Visual loss results mainly from central macular edema, and less frequently from proliferative diabetic retinopathy (PDR). The development of these pathological changes is strongly related to hyperglycemia [2]. Although the Diabetes Control and Complications Trial showed that a tight control of hyperglycemia can reduce the incidence of retinopathy, it is clear that hyperglycemia alone does not explain the development of this complication. It may be absent in some patients with poor glycemia control even over a long period time, while others may develop retinopathy in a relatively short period despite good glycemia control, possibly as a result of hereditary factors. The strongest evidence for a genetic predisposition towards DR derives from twin, family and transracial studies. Early reports from identical twins study showed that the stage of retinopathy has been found to be similar, demonstrating the importance of inherited factors in the etiology of diabetes retinopathy[3].

Over the past several years, some studies were under way evaluating genetic links to diabetic retinopathy. The genes that influence these conditions may be the suitable candidate genes. The search for candidate genes that predict risk of DR is important for a number of reasons. First, it will define a group of diabetic subjects who are predisposed to retinopathy at the time of diabetes diagnosis. This group could then be offered careful follow-up and possible early therapeutic intervention. Second, insights into the pathogenesis of the condition may be developed by establishing the identity and function of the candidate genes, ultimately facilitating new therapeutic approaches [4]. In order to identify these genes, there is a range of approaches from limited evaluations of single genetic polymorphism in small case-control studies to systematic evaluations of the human genome, using genome scans and linkage analysis in a large collection of families. Broadly speaking, there are two molecular strategies, candidate gene analysis and whole genome scans, and two analytical approaches, association studies and linkage studies. Association studies compare the frequency of specific alleles of a genetic marker between different populations, conventionally case-control populations. Linkage studies evaluate the inheritance of a genetic locus in families. Molecular genetic analysis combining association and linkage is the most powerful approach for assessing genetic contributions to diabetes complications[5].

The following genetic loci have been the focus of investigation regarding a possible role in the development of DR. This article reviewed the current status of these genes and their association with DR.

ALDOSE REDUCTASE GENE

Although prolonged exposure to hyperglycemia is the primary factor associated with the development of most diabetes

complications, it is also evident that genetic factors play an important role in determining the risk for the microvascular complications. The polyol pathway as one of the physiological mechanisms linking hyperglycemia has been considered important in the development of diabetes retinopathy[6].

Aldose reductase is the first and rate-limiting enzyme in the polyol pathway[7]. It converts glucose to sorbitol, and sorbitol dehydrogenase converts sorbitol to fructose[8,9]. Aldose reductase is widely expressed in tissues, including retinal capillaries and pericytes. An osmoregulatory role for this enzyme is supported by the rapid and specific increases in its activity and gene expression, which occur upon exposure to hypertonic media. Increased glucose flux through this enzymatic pathway with intracellular sorbitol accumulation leads to several abnormalities in cellular metabolism, which may contribute to the death of retinal pericytes and hence damage to endothelial cells, an early event in the development of diabetic retinopathy [10,11]. 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[12,13].

Human aldose reductase gene, the gene encoding aldose reductase has been localized at the chromosome 7q35 and consists of 10 exons extending over 18 kilobases of DNA. There is growing evidence supporting that aldose reductase gene has been strongly associated with diabetic microvascular disease. Variants in the aldose reductase gene may cause increased level or activity of the enzyme, and thus contribute to diabetic retinopathy [14,15]. Polymorphism of this gene have been suggested to exert an effect on the natural history of DR. This finding was supported by an early study that showed an (A-C)n dinucleotide repeating polymorphic marker at 5' end of the aldose reductase gene was first described and Z-2 was associated with early onset of DR in Type 1 diabetes[16], which was similar to several current studies suggesting that in type 2 diabetics having similar glycemic control, the (AC) 23 allele is related to a progression rate of retinopathy 8.9 times higher than in diabetics who lack it [17] and the Z-2 and C-106 alleles are associated with the microvascular complications while the Z+2 and T-106 may be protective factors[18-20]. Kao *et al*[21] have identified a single substitution of A for C at 95th nucleotide of intron 8 in 164 adolescents with type 1 diabetes in whom DR was assessed and have reported that the BB genotype was significantly more common in adolescents with early DR than those without retinopathy. The study suggested that polymorphism of the aldose reductase gene was associated with DR. Chromosome 7q35 was considered a candidate genetic locus for susceptibility to DR[21]. Two more studies performed on Asian, European and African populations had shown that the frequency of the C (-106)T polymorphism of the aldose reductase gene had a significant association with retinopathy and that type 2 diabetics with the CC genotype were more susceptible to developing retinopathy than those with the CT or TT genotype, giving additional evidence for the CC genotype as genetic marker of retinopathy [22-25]. Thus, genotyping of the 106C>T polymorphism in the aldose reductase gene could be useful as a tool in the identification of diabetic patients who are more prone to develop DR, and thereby require a more intensive treatment in order to prevent the progression of DR.

NITRIC OXIDE SYNTHASE GENE

Nitric oxide (NO) plays a pivotal role in the regulation of vascular homeostasis and is produced in endothelial cells by endothelial nitric oxide synthase (eNOS). The intraluminal release of nitric oxide mediates local vasodilatation, antagonizes platelet aggregation and inhibits vascular smooth muscle proliferation [26]. In response to stimuli such as hypoxia and stress, the vascular endothelial cells synthesize nitric oxide from L-arginine by a constitutive, calcium/calmodulin-dependent enzyme known as eNOS [27]. Abnormality in nitric oxide availability plays an important role in the pathophysiology of diabetic vascular disease, which involves impaired endothelium-dependent relaxation. A growing amount of clinical and experimental evidence suggests that the pathogenisis of diabetic retinopathy is associated with a heterogeneous and complex constellation of retinal disorders in the nitric oxide pathway including increased ocular NO levels, aberrant retinal NO utilization, impaired NO-mediated vasodilation, oxidative and nitrative stress, dysregulation of NO synthase isoforms, and endothelial NO synthase uncoupling [28-30]. Therefore, human eNOS gene, as well as other NOS genes, represents a plausible candidate gene responsible for DR.

The locus for the eNOS gene is on the long arm of chromosome 7. The gene contains 26 exons spanning approximately 21 kilobases of genomic DNA, encodes a messenger RNA of 4052 nucleotides, and is present as a single copy in the haploid human genome [31]. Three polymorphisms in the eNOS gene have been widely studied: a single nucleotide polymorphism (SNP) in the promoter region(T-786C), a SNP in exon 7 (Glu298Asp), and a variable number of tandem repeats (VNTR) in intron 4[32,33].

Several studies have shown significant associations between eNOS polymorphism and the development or severity of diabetic retinopathy in patients with type 1 diabetes mellitus (T1DM)[34-36], while a few studies have reported no association between eNOS polymorphism and DR in T2DM patients[37-39]. A group from Paris reported a strong association between the eNOS4b/a endothelial nitric oxide synthase polymorphism and severe DR [40] and their another finding suggested that T-789C and C774T eNOS polymorphism affected the onset pattern of severe DR [41]. Therefore, whether eNOS gene should be considered as a candidate gene in DR or not await a large-scale prospective study.

GENE OF RECEPTOR FOR ADVANCED GLYCATION END PRODUCTS

Diabetes mellitus is associated with oxidative and carbonyl stress, microinflammation and eventually autoimmune reaction. Advanced glycation end products are represented by a heterogeneous group of compounds, which are formed from Schiff bases and amadori products when reducing sugars such as glucose react nonenzymatically with amino groups in proteins, lipids, and nucleic acids through a series of reactions. The ability to form cross-links to and between proteins, and their interactions with a class of binding sites on endothelial cells and monocytes, as well as other cell types lead to tissue damage in diabetic complications [42]. Among several etiopathological mechanisms proposed in diabetic retinopathy, advanced glycation end products (AGEs) formed due to nonenzymatic glycation of proteins is one of the key components causing microvascular complications[43].

AGEs can exert biological activity via specific receptors, among them the best known is receptor for advanced glycation end products (RAGE). RAGE, a 35kDa protein, has been isolated and cloned form the bovine lung and has been classified as a member of the immunoglobulin superfamily, which is expressed on endothelial cells, mononuclear phagocytes and vascular smooth muscle cells [44]. The AGE RAGE interaction are thought to be involved in the development of diabetic complications, including retinopathy. The RAGE gene is located on chromosome 6p21.3 in the major histocompatibility coplex locus in the class Ⅲ region [45]. Genetic polymorphism in the RAGE could influence AGEs processing in tissues or reactions following the AGE binding to RAGE, and thereby accelerates the development and severity of glucose-mediated tissue damage. Several RAGE gene polymorphisms have shown association with the pathological states of diabetic complications[46]. Hudson *et al* [47] screened for polymorphisms in the coding regions of RAGE gene and found seven polymorphisms in exons and two in introns. Meanwhile, four functional amino acid changes were detected: Gly82Ser(exon3), Thr187Pro(exon 6), Gly329Arg(exon 8), and Arg389Gln (exon 10)[47]. Gly82Ser polymorphism is particularly interesting because of relatively high prevalence and the polymorphism results in the creation of an Alu I restriction site (AG/CT). Later study from Asian populations investigated the frequency of Gly82Ser polymorphism in exon 3 of the RAGE gene and its association with DR. Their study suggested that Ser82 allele in the receptor for AGE gene is a low-risk allele for developing DR [48]. Therefore, polymorphisms resulting in functional amino acid changes in the RAGE gene may influence development of DR by altering the AGE-RAGE interaction.

ANGIOTENSIN CONVERTING ENZYME GENE

Angiotensin converting enzyme(ACE) is a zinc metallopeptidase widely distributed on the surface of endothelial and epithelial cells. It has been known that ACE contributes to the regulation of systemic hemodynamics by converting angiotensin I to angiotensin Ⅱ, a potent vasoconstrictor that increases intraglomerular pressure and glomerular filtration. Therefore, it has been suggested that an elevation of ACE plasma concentration may be associated with the microvascular complication of diabetes[49]. The gene encoding ACE is located on the long arm of chromosome 17 (17q23), which is 21 kilo bases (kb) long and comprises 26 exons and 25 introns [50]. In 1990, Rigat et al first found a polymorphism involving the presence (insertion, I) or absence (deletion, D) of a 287-bp sequence of DNA in intron 16 of this gene [51]. Later studies showed that the involvement of the I/D polymorphism was not limited to ACE levels in plasma, and was also detected in tissue ACE levels[52,53].

Because of the central role of ACE in the renin-angiotensin system, numerous studies have addressed the role of the I/D polymorphism in microvascular disorders, particularly in diabetes [54,55]. Preliminary studies suggested that ACE levels are elevated in type II diabetes, chiefly in patients with retinopathy[56]. Matsumoto *et al*[57] reported a significant relationship between the presence of the D allele polymorphism in the ACE gene and advanced diabetic retinopathy in Japanese subjects with type 2 diabetes. However, several studies examined the ACE insertion/deletion (I/D) polymorphism and found no association with DR [58-65]. The reason for these conflicting results is unclear. There are some possible reasons: one is the racial difference of the subject populations; another is the difference in the method of selecting diabetics for the studies. So additional studies are required to confirm the association between ACE gene and retinopathy in different ethnic populations.

VASCULAR ENDOTHELIAL GROWTH FACTOR GENE

Vascular endothelial growth factor (VEGF) is an endothelial cell-specific mitogen *in vitro* and an angiogenic inducer in a variety of models *in vivo*. It has been implicated in the pathogenesis of diabetic retinopathy and suggested that the up-regulation of the VEGE gene is mainly due to hypoxia caus ed by the obstruction of small arteries in the retina [66]. Ele-vations of VEGF levels in the aqueous and vitreous humor of human eyes with proliferative retinopathy secondary to diabetes and other conditions have been described. These studies demonstrated a temporal correlation between VEGF elevations and active proliferative retinopathy[67].

The human VEGF gene is organized in eight exons separated by seven introns and is localized to chromosome 6p21.3. The coding region spans approximately 14kb [68]. Previous studies have shown that VEGF expression is increased in patients with diabetic microvascular complications and found that polymorphisms in the promoter region of VEGF are associated with susceptibility to diabetic microvascular complicat-

ions [69]. Awata *et al* [70] found that C-634G polymorphism in the 5'-untranslated region of the vascular endothelial growth factor gene was significantly associated with DR. Later they confirmed the important role of C-634G in susceptibility to retinopathy and analyzed the C-2578A and G-1154A polymorphisms of VEGF, but neither was associated with the development of DR[71].

PIGMENT EPITHELIUM-DERIVED FACTOR GENE

Pigment epithelium-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors. It was first purified from conditioned medium of human retinal pigment epithelial cells as a factor with potent neuronal differentiating activity[72]. Recently, PEDF has been shown to be a highly effective inhibitor of angiogenesis in cell culture and animal models. PEDF inhibits the growth and migration of cultured endothelial cells, and it potently suppresses ischemia-induced retinal neovascularization [73-75]. PEDF levels in aqueous or vitreous humour decreased in patients with diabetes, especially those with proliferative diabetic retinopathy (PDR)[76-78]. Furthermore, PEDF knockout mice showed several retinal abnormalities, such as morphological alterations and increased microvessel density [79]. These observations suggest that the loss of PEDF activity in the eye may contribute to the pathogenesis of PDR. A recent study identified four polymorphisms in the PEDF SNPs and found rs12150053 and rs12948385 were significantly associated with diabetic retinopathy. The findings examined that the GA or AA genotype of rs12948385 was a risk factor for DR. Therefore, PEDF is an attractive candidate gene for DR[80,81].

THE FUTURE

It is clear that the dissection of the genetics of diabetic retinopathy is far from easy. The methodology used so far has had a number of flaws, whereas some clues to the role of genes in retinopathy may have been gleaned and no clinically significant genetic marker has been found. In the future, a combined approach will certainly be required. This involves the use of association studies in large populations followed by analysis within families. The latter will usually involve a transmission disequilibrium test analysis of the frequency of transmission of designated alleles from heterozygous parents to affected offspring. This method has the advantage of requiring fewer pedigrees than affecting sib pair analysis and requires DNA only from both parents and the affected proband.

Futhermore, with the development of the human genome project, the genomic sequence data will supply a wealth of information for the identification of mutations in various disease complications such as diabetic retinopathy and offer the greatest hope for identifying the principal genetic components of diabetes complications. There is a growing need for large-scale studies on the susceptibility genes for treatment and prevention of diabetic retinopathy.

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