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Genetics of Diabetic Retinopathy

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ABSTRACT Diabetic retinopathy (DR), a microvascular complication of diabetes in the retina, is one of the leading causes of adult blindness worldwide. Complex interplay of environmental and genetic factors has been known to contribute to its pathology. Variations in several genes have been found to be associated with risk for developing DR in different populations worldwide, which is discussed in this article. Identification of genetic variations underlining the disease would immensely lead to recognition of presymptomatic diabetic individuals susceptible to develop DR and help in planning appropriate clinical management and genetic counseling of patients.

INTRODUCTION

Diabetic retinopathy (DR) is a diabetes related microvascular complication of the retina. Among the leading causes of adult blindness worldwide, DR comes next to cataract, glaucoma and age related macular degeneration (AMD). It is also the second leading cause of blindness due to retinal degeneration in the working age group, contributing to an overall 4.8% blindness across the globe (Resnikoff et al. 2004). India, being the diabetic capital of the world, is feared to end up with an alarming 11.4 million type 2 diabetes mellitus (T2DM) individuals developing this sight threatening disease by 2025, if the present trend of 20% of T2DM population developing DR were to continue (Rema et al. 2005).

DR is the most frightful of all the diabetes related ocular complications, as it could result in irreversible blindness if left untreated. Hyperglycemia and longer duration of diabetes have been known to contribute to pathological proceedings in DR (Frank 2004). Chronic hyperglycemia results in oxidative stress and capillary damage in the retina. In an effort to compensate for the loss, a cascade of events is initiated to form new blood vessels. The thus formed vessels are fragile and leaky, causing edema all over the retina resulting in visual impairment aided by other features such as basement membrane thickening, blood-retina barrier breakdown, loss of pericytes, etc. (Hykin 1996).

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GENETIC CONNECTION

DR is a multifactorial disease with a complex interplay of environmental and genetic factors contributing to its pathology. The role of genetics is well explained by the fact that some diabetic individuals do not develop retinal complications even after a longer duration of uncontrolled diabetes, while certain individuals despite tight metabolic control develop DR within a short span. Identifying the genes that contribute to the disease pathogenesis has been a challenging task for geneticists worldwide due to the innate complexity of the disease in terms of the number of possible genes involved in the disease mechanism, unlike single gene disorders. However a number of studies, both family and population based, have been performed across different populations to identify genes and their variations which are associated with conferring susceptibility to develop DR.

Linkage Analysis

Gene mapping studies using linkage analysis are very difficult to perform in complex diseases like DR due to inherent barriers such as late-onset,

non-availability of parents of the affected individuals, etc. However other family based approaches like sib-pair analysis have been performed to map the genes responsible for DR in a few studies. Imperatore et al. (1998) conducted a study on Pima Indians with T2DM, by performing linkage analysis on 103 sib-pairs. They found suggestive linkage on chromosomes 3 and 9 (LOD=1.36, 1.46 respectively). Recently a genome wide linkage analysis on 211 sibships has revealed an evidence of linkage on chromosome 1p yielding LOD scores of 3.1 and 2.58 by single and multi-point analyses respectively (Looker et al. 2007). A similar study on Mexican Americans with 282 sib-pairs showed linkage to chromosomes 3 (LOD=2.41) and 12 (LOD=2.47) (Hallman et al. 2007). However these studies have only suggested critical genomic regions on certain chromosomes and the possible susceptibility genes are yet to be identified in these loci.

Genetic Association Studies

Substantial amount of research has been done in identifying genetic markers associated with risk for developing DR using indirect approach like case-control association studies in different ethnic populations. Most of the genes that have been screened for variations are implicated in the pathogenic pathways of DR such as polyol pathway, formation of advanced glycation end products (AGE), activation of protein kinase C (PKC) and hypoxia induced angiogenesis. The aldose reductase gene has been widely studied. The enzyme aldose reductase which it codes for is involved in the polyol pathway. It harbors a Z-2 microsatellite repeat upstream to its promoter, which is consistently associated with risk for developing DR across different ethnic populations (Demaine et al. 2000). Moreover, the impact that the Z-2 repeat has on increasing the gene expression has been clearly shown in functional studies (Shah et al. 1998). Two other promoter polymorphisms present in the regulatory region of this gene namely C(-12)G, C(-106)T have been associated with risk for DR in Chinese and Caucasian populations (Li Q et al. 2002).

Another important candidate for DR is vascular endothelial growth factor (VEGF), a potent angiogenic factor implicated in several diseases including diabetic microvascular complications and cancer. Elevated serum and vitreous levels of VEGF have been strongly associated with proliferative diabetic retinopathy (Sydarova and Lee 2005). Increased VEGF expression has been shown to initiate neo-vascularization through a number of pathways (Poulaki et al. 2004). The promoter region of this gene harbors a few polymorphisms of which C(-634)G and T(-1154)C have been strongly associated with DR in Japanese and Caucasian populations respectively (Awata et al. 2002 and Ray et al. 2004). C(-7)T and T(-1498) C polymorphisms were also found to be significantly associated with DR in the Indian population. These polymorphisms have also been shown to have functional significance (Ray et al. 2004).

Hypoxia-induced angiogenesis is an important pathology in DR. A number of growth factors get expressed in hypoxic conditions and have been shown to trigger the hypoxia response elements of VEGF gene bringing about over expression of VEGF leading to neovascularization (Poulaki et al. 2004). Endothelial nitric oxide synthase (eNOS) is one such important partaker of hypoxic events. Knockout animal models of eNOS have shown poor angiogenesis suggesting the contribution of eNOS to DR pathology (Albrecht et al, 2003). Two polymorphisms, T-786C, C774T and a 27bp VNTR in intron 4 of eNOS gene have been associated with risk for developing DR in different populations (Li C et al. 2001; Frost et al. 2003 and Taverna et al. 2002a). An array of other genes having a role in DR disease mechanism have also been investigated and significant associations include peroxisome proliferator-activated receptor (PPAR)-γ gene (Gly482Ser), methylene tetrahydrofolate reductase (MTHFR) gene (C677T), TGF-β gene (R25P), Vit D Receptor (Fok I and Taq I), (Petrovic et al. 2005; Maeda et al. 2003; Beranek et al. 2002 and Taverna et al. 2002b) to name a few. Apart from single nucleotide polymorphisms (SNP), haplotype and linkage disequilibrium analyses have been done in most of these studies to understand the genetic involvement more clearly since an associated SNP need not be the true disease variant and may be in tight linkage with the original disease mutation or may synergistically bring about the disease change along with other variations.

Genetics of DR in India

Molecular genetic participation of DR in India has been in investigation only since the last few

years and very few association studies have been performed for SNPs and microsatellites present in certain candidate genes when compared to the global reports. Rema et al. (2002) studied 322 families with at least two diabetic sibs for the presence of DR in siblings of probands with and without DR. They found that familial clustering was nearly 3 times higher in sibs of those with DR when compared to those without DR (Rema et al. 2002). This suggested that there is an inherited genetic susceptibility among the diabetic individuals in the Indian sub continent for DR. Based on previous reports of association of tumor necrosis factor (TNF) with DR, we investigated a promoter microsatellite $(GT)_n$ for association with DR in a self reported diabetic cohort comprising of 100 patients with DR and 107 individuals without DR. We found a low risk allele ($(GT)_9$) and a high risk allele ($(GT)_{13}$) for DR in this gene (Kumaramanickavel et al. 2001). Later we worked on a pentanucleotide repeat poly-morphism $(CCTTT)_n$ in inducible NOS (iNOS) gene. We found moderate association of two low risk alleles ($(CCTTT)_{13\&17}$) and a high risk allele ($(CCTTT)_{13}$) for DR (Kumaramanickavel et al. 2002a).

The receptor for AGE (RAGE) gains its importance in the disease mechanism of DR through its interaction with AGE and subsequent initiation of a series of intracellular changes. While analysis of polymorphisms in RAGE gene yielded no association with DR in other populations, we reported a protective association of Gly82Ser for development of DR (Kumaramanickavel et al. 2002b). Later we also found that the Z-2 allele of aldose reductase gene was associated with risk for DR in our population, comparable to worldwide results (Kumaramanickavel et al. 2003).

Recently, we found that the 27bp intron4 VNTR of eNOS gene was not associated with DR in a population based south Indian cohort, much to the contradiction of worldwide reports (Uthra et al. 2007). This report was consistent with results of the study by Suganthalakshmi et al. (2006). They also reported significant association of C(-7)T and T(-1498)C promoter polymorphisms of VEGF gene with DR (Suganthalakshmi et al. 2006). Similarly the RAGE gene was analyzed for the association of -429T/C, -374T/A and 63 bp deletion polymorphisms with DR. Broadly the polymorphisms and the possible haplotypes were not associated with DR but the -374T/A was modestly associated with non-proliferative DR (Ramprasad et al. 2007).

Challenges

Although substantial amount of research has been done, analyses of few SNPs or haplotypes are not sufficient to arrive at the gene(s) causing the disease because of the multigene involvement in the pathology. Moreover most of the studies mentioned above have inherent drawbacks such as small sample size, population admixture, interpretation of the statistical results, etc. Therefore analysis of a large sample size with matched cases and controls and more importantly analysis of a large number of genetic variations like genome wide SNP scan would collectively aid in narrowing down the genes and their variations that bring about the DR disease pathology. Moreover in a diversified population as ours, gene mapping studies have to be carried out in multiple centers to identify the genes involved in the disease mechanism. To our knowledge, prevalence studies pertaining to DR in India are very few; the Chennai Urban Rural Epidemiology Study (CURES) showed that the prevalence of DR was 17.6% in south Indian urban diabetic population (Rema et al. 2005). But surprisingly we found in our population based study on rural Tamil Nadu that the prevalence is similar (Unpublished data). However prevalence of DR in rural Tamil Nadu as reported by the Aravind comprehensive eye study is much lower (10.5%) (Nirmalan et al. 2004). Given this non-conformity in the prevalence of DR across different groups from south India, it needs to be explored whether predisposition to develop DR is uniform throughout India or ethnic specific, With our caste-class-region-culture-based Indian population having different lifestyle (activity, smoking, alcohol consumption, etc) and dietary habits that could influence intracellular gene expression (epigenomic) which could lead to varying phenotypes of diabetes complications. Therefore initiating population specific DR prevalence studies combined with genetic and environmental studies by various study groups in India would yield better insight into etiopathology of this disease that would eventually lead to better clinical management of the illness.

APPLICATION OF GENETIC STUDIES IN DR

Identification of genetic variations contri-

buting to the DR disease pathogenesis would have tremendous implication in terms of clinical intervention and management of the disease. A number of inhibitors to key molecules that are involved in detrimental pathways of DR such as ranibizumab, VEGF-Trap (anti-VEGF), LY333531 (anti-PKC), pegabtanib, octreotide (anti-insulin like growth factor-1), are under research (Bainbridge et al. 2003). Analyzing the molecular aspects that govern the development of a disease or predisposition to a disease would achieve desirable clinical outcomes by helping physicians to decide specific management of the disease depending upon the patients' genetic and environmental profile rather than a generalized treatment as laser photocoagulation. Moreover, recognizing an underlying genetic susceptibility would help in counseling presymptomatic individuals to adopt preventive and control measures to delay the onset of disease.

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