Introduction

Diabetes encompasses a heterogeneous group of the diseases, growing all over the globe at an alarming rate, where type 2 diabetes (T2D) accounts for >95% of all diabetes. In 2015, India was the second leading country with 69.2 million affected individuals which is estimated to be increased by 2040 to ~123 million (IDF, 2015). The progression in diabetic condition is known to cause secondary micro- and macrovascular complications. These complications are encountered at relatively early age now a day in India; and are found to be the major cause of morbidity and mortality, affecting severely to the life of an individual. Moreover, the disease exhibits the utmost health burden to an economy of the country, which greatly hampers growth of the country. All the present treatments and/or preventive measures are not much helping in lowering epidemics of diabetes.

The rationale of the rise in diabetes is thought to be chiefly due to environmental factors such as life style change (urbanization), dietary habits (junk food, oily food), increasing prevalence of obesity, physical inactivity, smoking; which are modifiable risk factors. Beside this, one cannot avoid non modifiable risk factors like age and genetic predisposition. Thus far, the best explanation for such a hike in diabetes incidences is that environmental factors stated above act on a genetically predisposed individuals; where contribution of genetic factors have been substantially proven by showing heritability of the diabetes (Florez, Hirschhorn, & Altshuler, 2003). The present review explains the role of genetic predisposition in T2D and diabetic retinopathy (DR).

Genetics of Type 2 Diabetes

The substantial progress has been made in defining the genetic factors that increase the risk for specific subtypes of diabetes that are mostly monogenic (e.g., MODY - maturity-onset diabetes of the young, T1D - type1 diabetes). However, the involvement of genetic risk factors of T2D susceptibility remains to be vague till date due to its complex polygenic and multifaceted pathophysiology. The insulin resistance together with other causative factors like aging, obesity, and reduced exercise is responsible for the T2D development (Saltiel and Kahn, 2002). Besides, ability of the pancreatic β cells to produce insulin is gradually reduced, due to which
imbalanced is caused in carbohydrate and lipid homeostasis (Bell and Polonsky, 2001). Furthermore, genetic contribution to the T2D seems to be less potential than that for T1D, with overall genetic risk ratio ranging from 2 to 4 (Weijnen et al., 2002). Earlier studies on migrant Indians across the globe have reported that Asian Indians seize higher risk for developing T2D and related metabolic abnormalities compared to other ethnic groups (Mohan et al., 2007).

**Approaches of Studying Genetics**

Genetic architecture of disease based on the hypothesis, the “common-disease, common-variant” will not justify the inheritance of diseases including T2D. Several scientists have explained evidently that the important fraction of common and rare disease phenotypes could be explained by several rare variants (Pritchard, 2001). Ultimately, empiric studies will characterize genetic architecture for each phenotype of interest. In elucidating genetics of T2D and its secondary complications, apart from considering common allelic variants, geneticists are also working for the identification of rare genetic risk alleles.

Initially, the **family-based linkage studies and twin studies** were undertaken for studying the inheritance of specific loci that may influence the disease development. Such studies were successful in identifying specific gene/loci responsible for developing chiefly monogenic form of the disease but not the complex polygenic form like T2D.

The **candidate gene approach** involves examination of numerous genes encoding proteins strongly related to T2D pathophysiology, generally in case-control studies. This type of study is generally executed on small size of population, and they often yield conflicting outcomes. To overcome this problem, meta-analyses have been carried out to pinpoint the few genes that might have shown cumulative evidence for a relationship with T2D and its related complications.

The most recent approach is **GWAS (Genome wide association studies)** – evolved from the concept of candidate gene studies only, but allows the screening of large numbers of genetic variations (around 30,000 at a time) in very large sample size (approximately in 1000-5000 and more). This is possible due to advancement in the genotyping technology and sequencing of DNA.
Genes in Type 2 Diabetes

Although, T2D have relatively low genetic attributes than T1D; results of several familial linkage studies have reported a number of chromosomal regions that may confer significant risk of developing T2D. The heritability estimates for T2D greatly vary in different studies. The earlier approach were based on familial linkage studies where, the most promising and validated chromosomal regions have been found on chromosomes 1q21-q24, 2q37, 12q24 and 20 in association with T2D (Demenais et al., 2003). Apart from these the regions on chromosomes 1q25.3, 2q37.3, 3p24.1, 3q28, 10q26.13, 12q24.31, and 18p11.22 were showing strong evidences for linkage with T2D in more than one study (Florez, Hirschhorn, & Altshuler, 2003). Among these, many loci were validated in the different populations like Finnish Caucasian families, U.K. Caucasians, U.S. Caucasians, and American Mexican where the outcomes of the linkage studies were discrepant. Since the approach has a poor resolution, it identifies the regions that could include millions of base pairs and hundreds of genes. Such studies were successful in revealing calpain 10 (CAPN10) and transcription factor 7-like 2 (T-cell specific, HMG-box) (TCF7L2) genes that were considerably found associated with T2D.

Calpain 10

The finding of linkage analysis with T2D in Mexican Americans to chromosome 2q37 (named NIDDM) (Hanis et al., 1996), led to the identification of susceptibility variants within the Calpain-10 (CAPN10) gene (Horikawa et al., 2000). CAPN10 is the first gene to be detected for the diabetes susceptibility through a genome scan and it was replicated in Botnia region of Finland and the German population of Saxony. A reassessment of the linkage data in similar population has shown non-significant evidence of linkage towards T2D in the initial scan (Cox et al., 1999; Hanis et al., 1996). All the different approaches were failed to show its linkage consistency (Florez, Hirschhorn, & Altshuler, 2003). Then Horikawa et al., (2000) had done the positional cloning of NIDDM1 and had undertaken a relatively are investigation of common variants, with 20 markers typed over 7 cM region at the locus. They identified a haplotype of three-marker polymorphisms apparently linked with T2D predisposition, where further study recognized association of A/G polymorphism in intron 3 of the CAPN10 gene with T2D. The authors emphasized that only
heterozygotes (A/G) were at higher risk; while homozygotes (G/G) showed no linkage with T2D. Additional analysis identified a combination of two different haplotypes of the three markers conferring the highest risk to T2D. All these SNPs, were found to be located in non-coding regions of CAPN 10 gene, commonly known as SNP-43, -19, and -63; establishing risk to T2D by threefold. Since common variants of the CAPN10 gene are correlated with its transcript levels in skeletal muscle and adipose tissue (Sreenan et al., 2001), they seem to be affecting the risk of T2D by disturbing transcriptional regulation of CAPN 10 (Horikawa et al., 2000).

CAPN10 is calcium activated proteases and ubiquitously expressed in most of the tissues (Ma et al., 2001) and it seems to influence both insulin secretion and resistance. As calcium plays a vital role in insulin release in response to secretagogues; CAPN10 has been implicated in the T2D pathogenesis. CAPN10 appears to facilitate the actin reorganization, which is required for glucose-induced release of insulin and hence inhibition of calpains prevents the insulin secretion (Parnaud et al., 2005). CAPN10 was found in human pancreatic islet cells where positive correlation of CAPN10 levels with insulin release has been evidently reported. Role of CAPN10 is implicated in the first phase of insulin exocytosis and its binding to the plasma membrane impairs proteolysis of SNAP-25 (Marshall et al., 2005). CAPN10 has been thought to induce lipotoxic apoptosis, an apoptosis in response to fatty acids, which is found in T2D pathophysiology. During abnormal glucose homeostasis, occurring in diabetes, the CAPN10 expression is distorted in the pancreas, muscle, and fat cells. Low level of Calpain-10 expression in skeletal muscle was also found to be associated with insulin resistance (Baier et al., 2000). Calpain levels in the diabetic vasculature are elevated and inhibition of Calpain reduces the vascular dysfunction found in chronic diabetes, which suggests a role of calpain in the pathophysiology of diabetic vascular disease (Stalker et al., 2005). Further, the expression of the CAPN10 gene was detected in retina, due to which we hypothesize that the SNPs influencing the CAPN10 level may damage the retinal cells (Ma et al., 2001).

Subsequent to the report of CAPN 10, many researchers have carried out large studies to examine the correlation of genetic variations in CAPN10 with T2D development. Horikawa et al. (2000) has proposed the specific model however, it have not been
widely validated even among the investigations carried out in closely related populations (Baier et al., 2000; Daimon et al., 2002; Fingerlin et al., 2002; Hegele et al., 2001; Rasmussen et al., 2002; Tsai et al., 2001). In several other studies, no association was observed between SNPs of CAPN10 and metabolic traits (Fingerlin et al., 2002; Rasmussen et al., 2002). On the other hand few studies indicated that other sequence variants (Evans et al., 2001) or haplotype combinations (Malecki et al., 2002) might be related with the disease. SNP-43 of CAPN10 has been found to increase levels of total cholesterol that may cause T2D (Zaharna, Abed, & Sharif, 2007), while SNP-19 and haplotype 111 have attributed the risk of T2D in Tunisian population (Ezzidi et al., 1987). Although, haplotype of SNPs-44,-43, -19 and -63 polymorphisms may be associated with T2D; none of them appears to be associated with T2D independently (Bodhini et al., 2011), though SNP-44 and SNP-43 may directly change susceptibility to T2D by regulating the transcription. Several reports for the ethnic differences have been also reported in patient with T2D in Caucasian and Asian subjects. Due to inconsistent association have been reported between genetic variations of CAPN10 and T2D related phenotype; a complete picture of genotype-phenotype correlation of CAPN10 variants remain yet to emerge. This could be owing to a number of causes that includes population-specific environment, gene-gene interactions, or ethnicity dependent patterns of Linkage disequilibrium (LD) (Gabriel et al., 2002). At last, it is challenging to define the appropriate statistical thresholds for confirming association (Dahlman et al., 2002).

In summary, low concentration of CAPN10 leads to increased protein kinase C activity, reduced insulin signaling that in turn causes insulin resistance. Some experimental studies have shown the possibility of calpains as potential markers for prognosis of T2D (Hsieh et al., 2008). Moreover, researchers have used calpain inhibitors that suggested the participation of CAPN10 and other calpains in secretion as well as action of insulin and susceptibility of T2D. Therefore, CAPN10 is possibly considered as promising molecular target for the prevention and treatment of T2D.

**TCF7L2**

The discovery, that genetic variants of TCF7L2 gene was the first to indicate the success of the ‘hypothesis-free’ association approach which was the large-scale GWAS study conducted by Sladek et al., (2007). However, TCF7L2 was initially
identified by a linkage study that had showed a strong linkage mapping to chromosome 10q in Mexican-American subjects (Ehm et al., 2000). This region was later fine-mapped in other ethnicities that identified risk locus in introns of the TCF7L2 gene (Busfield et al., 2002; Grant et al., 2006; Helgason et al., 2007). TCF7L2, a transcription factor, plays a role in the Wnt – signalling. Further studies to elucidate the role of TCF7L2 in T2D development, has shown alterations in TCF7L2 expression or function impairing pancreatic islet function might be through disturbing the regulation of proglucagon gene expression, which leads the reduction in insulin secretion (Lyssenko et al., 2007).

**Important genes associated with T2D**

Till date, many candidate gene studies are carried out in association with T2D, where Pro12Ala variant in the peroxisome proliferator-activated receptor gamma-2 (PPARG2, encodes the target for thiazolidinedione drugs used as the T2D treatment) gene and Glu23Lys polymorphism in potassium inwardly rectifying channel, subfamily J, member 11, (KCNJ11, encodes for the part of the target for sulphonylureas class of diabetes drug) are the most common polymorphisms reported in multiple studies for influencing the risk of T2D (Prokopenko et al., 2008). Their sample sizes of the studies were fairly large that found each copy of the susceptibility allele increasing 15–20% risk of developing T2D. Across a swathe of replication studies, it has become clear that TCF7L2 variants confer substantial risk to the T2D development, than those in PPARG and KCNJ11 (Prokopenko et al., 2008). Moreover, the candidate gene approach detected genetic variations found to be correlating other genes like insulin receptor substrate 1 (IRS1) and IRS-2, Wolfram syndrome 1 (wolframin) (WFS1), HNF1 homeobox A (HNF1A), HNF1 homeobox B (HNF1B) and HNF4A found to be correlated with T2D (Ali, 2013).

The latest approach to study genetics of such complex diseases is GWAS where as much as thirty genes were discovered that imparted risk to T2D in various studies (Ali, 2013). However, except TCF7L2 and KCNQ1 that showed nearly 1.4 odds ratio, most of the genes indicated odds ratio ranging from 1.04 - 1.3 for attributing the risk of T2D development (Ali, 2013). Further, the discovery of various risk genes has opened new path in understanding the regulation of glucose homeostasis and the pathogenesis of T2D. For instance, prior to such genetic association studies, no one
could have expected the role of TCF7L2 in conferring T2D risk through regulation of glucose metabolism. In summary, there is ample evidence that T2D, in part, genetically determined. Similarly, the genetic risk for diabetic retinopathy may be defined by yet another set of genes than those that modify the risk for diabetes alone.

**Diabetic retinopathy**

Diabetic retinopathy (DR) — including its the most severe form, proliferative diabetic retinopathy (PDR) remains to be a principal cause of vision loss in working-age people. By the 2025, it is estimated that 40% of the total diabetic patients would have some form of DR (King et al., 1998). Currently, a total of approximately 150 million people worldwide are affected with DR and expected to raise almost two fold by the year 2025 (Patel et al., 2008). DR, a well recognized consequence of long-standing and poorly controlled diabetes mellitus (DM), is known to cause significant vision loss. Present development in treatment and therapies intended to slower the disease progression, is expected to be overpowered by the rapidly rising incidences of T2D and thus of DR across the world in the near future. The increase in DR prevalence demands for an identification of persons at risk of DR. This allows applying early intervention, in turn aiding help in delayed onset or decreased severity of the disease. To achieve this goal, one should be meticulous with the exact molecular mechanisms and pathophysiology of the disease which is hitherto poorly understood in the case of DR. The basic key mechanisms through which DR leads to vision loss are 1) vascular leakage and exudation of proteins occur in macula that results in a macular edema, and 2) closing of retinal capillaries that leads to retinal ischemia, followed by secondary neovascular proliferation with altered permeability which is accompanied with vitreous hemorrhage and ultimately cause tractional retinal detachment (Simó-Servat et al., 2013). Both of these conditions are sight threatening and can end up with blindness if not intervened in between.

**Genetics of DR**

Although lack in glycemic control, long standing diabetes, high blood pressure are well established contributors of diabetic retinopathy, some people harboring these factors remain free from DR; whereas, others with strong glycemic control and normal blood pressure end up with diabetic retinopathy even at short duration of
diabetes (Simó-Servat et al., 2013). Various clinical studies have also revealed that such conventional risk factors do not entirely explain the onset and severity of DR (Patel et al., 2007). Presently, preventive measures for the disease are strict control of blood glucose levels and hypertension, and regular eye check up according to current screening guidelines. However, ADVANCE Trial study has reported that control of these known risk factors did not show any effect on lowering the 5-year incidence of DR or DR progression (Harris et al., 1998). The other significant risk factor appears to be is ethnicity because of racial and ethnic variations in DR prevalence and severity. One of the reasons behind this could be prevalence of the known risk factors for DR amongst the population. The other major cause could be high occurrence of diabetes in particular ethnicity/ race imparting obvious increase in DR incidences. It has been evidently reported that risk of developing DR was twice in Hispanic diabetic patients compared to non-Hispanic white diabetic patients (Leslie and Pyke, 1982). The observation was confirmed even after controlling the results for known risk factors, suggesting apparent role of ethnicity in the disease susceptibility (Leslie and Pyke, 1982). Moreover, there are enough evidences that heritability of the PDR is estimated to be on an average ranging from 25% to 50% (Patel et al., 2007); where for twins concordance for DR was the highest with 68% and 95% in type 1 and type 2 diabetes, respectively (Warpeha et al., 2003). All these findings, urge for the possible role of genetic predisposition in DR development and progression which is now evidently reported (Leslie and Pyke, 1982; Richeti et al., 2007).

Genetic factors divergently affect the onset or the severity of DR and the genetic association studies would aid the identification of genetic variants which are associated with an increased risk of DR, revealing the better understanding toward the disease prevention and treatment. The location and function of the found genome variants may further lead to the identification of gene/s and molecular pathways involved in the etiology of DR. Such genetic approach may facilitate to generate algorithm for the early identification of diabetic individuals who are prone to the complication and may get benefit from extensive prevention programs.

**Potential Genes Associated with DR**

To find out genes responsible for the disease, the main strategies applied are; linkage studies in families, candidate genes studies and Genome-Wide Association Studies
Review of Literature

(GWAS) – a subset of the candidate gene approach at very large scale. Most of the studies have examined genetic variants in the candidate genes of metabolic pathways related to DR pathology such as the polyol pathway, the advanced glycation end products (AGE) formation and oxidative stress leading to hypoxia inducing angiogenesis through vascular endothelial growth factor (VEGF). We have discussed few important genes conferring risk to DR development and progression in the further sections.

**Aldose Reductase Gene**

Aldose reductase (ALR2) is the first and an important regulatory enzyme of the polyol pathway that is responsible for diabetic complications. ALR2 converts glucose to sorbitol and found to be upregulated in states of hyperglycemia and hypoxia (Robison et al., 1989). Hence, diabetic condition leads to intracellular sorbitol accumulation, creating osmotic stress (Engerman and Kern, 1984), which plays a crucial role in the development of DR by altering cellular metabolism in eye (Simó-Servat et al., 2013; Engerman and Kern, 1984). Experiments on animal models had showed that Intracellular accumulation of sorbitol causes microaneurysm, thickening of the basement membrane and pericyte loss in the retina (Vinores et al., 1993). ALR2 is expressed in retinal pericytes and is also found in the vascular endothelium (Abhary et al., 2010). The ALR2 gene is located on chromosome 7q35. Many studies carried out in various ethnicities that have found associations of three ALR2 SNPs with DR: the promoter SNP rs759853, SNP rs9640883 and one microsatellite located at 5’ of the ALR gene. Although, the risk allele was found to be different among various ethnic groups (Simó-Servat et al., 2013), a meta-analysis study carried out in 2010 has reported the association of ALR2 gene with DR (Liew, Klein, & Wong, 2009). The (CA)n microsatellite polymorphism has been found to be associated with DR in Indians, Hong Kong and Mainland Chinese, Japanese, and Brazilians (Park et al., 2000) and not in Koreans (Santos et al., 2003) and Euro-Brazilians (Stitt, 2003). On the other hand, T allele of the promoter SNP rs759853 was conferring the protection against DR. Finally, SNP rs9640883 was observed in association with DR. However, the association might be due to the relationship between ALR2 gene and the age at onset of diabetes, therefore, the gene has effect on diabetes duration, instead of a direct relation with increased DR risk (Liew, Klein, & Wong, 2009). Most of the
studies related to polymorphisms of the ALR2 gene and DR predisposition have been performed in Asian populations, but currently a significant relationship of a particular haplotype has been observed with the development of severe DR in Caucasian subjects, too (Richeti et al., 2007). Thus, ALR2 remains to be associated with DR more often, making it a strong candidate for the DR predisposition.

**Receptor for Advanced Glycation End Products Gene (RAGE)**

Prolonged hyperglycemia compel non-enzymatic glycation of proteins and lipids that forms Advanced glycation end products (AGEs) and their accumulation leads to various diabetic complications by causing direct tissue damage (Goldin et al., 2006), as well as, by activating the specific AGE receptors known as RAGE (Hudson et al., 2002). RAGE is a 35-kD transmembrane receptor that gets activated by high circulating levels of AGE consequently stimulates secretion of cytokines. These cytokines along with AGEs cause a proinflammatory cascade and increase endothelial permeability that accelerates the progression of diabetic complications (Simó-Servat et al., 2013; Hudson et al., 2002). In this regard, RAGE appears to be a good candidate for studying its polymorphisms influencing DR development. Thus far, minimum 20 different polymorphisms have been identified within the RAGE gene for diabetes (Ramprasad et al., 2007); where associations between the SNPs and DR were inconsistent among various investigations (Lindholm et al., 2006). The -374A T/A SNP located within a transcription factor-binding site of RAGE gene and may influence the transcription of RAGE. Large Scandinavian study among the Caucasians, suggested that –374 T/A SNP has been associated with DR and the effect may be dependent on glycosylated hemoglobin levels (Kang, Tian and Jia, 2012). Similarly, in Asian Indians, this polymorphism has been correlated with DR (Lindholm et al., 2006). Other few studies have also identified an association of the -374 T/A polymorphism with sight-threatening DR, which was contradictory to the other studies (Patel et al., 2008).

Gly82Ser and -429 T/C polymorphisms also seem to raise the risk of DR among Asian Indians and Caucasians, respectively; although the association did not show significance in many populations, like Chinese and Caucasian (Patel et al., 2008). Two meta-analysis studies have not shown the significant association of -374A, Gly82Ser, and -429T/C SNPs with DR in Caucasian and Asian populations
(Niu et al., 2012; Ng et al., 2012). Interestingly, the meta-analysis conducted in East-Asian predominating population has found 1704 T allele to increase the risk of DR (Ng et al., 2012). Further studies are required to validate these findings.

**Endothelial Nitric Oxide Synthase (eNOS)**

eNOS catalyses the formation of the potent vasodilator nitric oxide (NO) from, and it has been implicated in the pathology of diabetic vascular complications. The eNOS knockout diabetic mice have shown to exhibit advanced retinal vascular complications (Fukumura et al., 2001; Abhary et al., 2009). NO is also known to increase oxidative stress by free radical generation, which also plays a role in pathogenesis of retinal neovascularization. The polymorphisms of eNOS (NOS) gene have been related to an elevated risk of DR development (Cilenˇsek et al., 2012). In two large studies, an association, between either AA genotype of the a/b polymorphism in intron 4 (4a/b) or haplotype A val Ins and PDR has been reported in Caucasians (Crispim et al., 2010; Mamoulakis et al., 2009). In West African cohort study, the 4a/b polymorphism has been found to confer increased risk of DR (Bazzaz et al., 2010). Conversely, recent studies, including a meta-analysis, have not observed a significant relationship of this polymorphism with the early onset of microangiopathy (Santos et al., 2012; Zhao et al., 2012). Moreover, another meta-analysis study (Chen et al., 2007) has suggested significant association between the A allele of the 4a/b polymorphism and a reduced risk of DR. Similarly, the C allele of T-786C SNP has been associated with DR (Khalfaoui et al., 2009), which has not been incorrigible in other studies (Cilenˇsek et al., 2012; Zhao et al., 2012; Bazzaz et al., 2010). The C allele of T-786C SNP seems to be a protective factor for PDR, while G894T SNP did not show association with DR. (Patel et al., 2007). The conflicting research results for an association between SNPs of eNOS gene and DR might be due to ethnicity’s effect on the disease susceptibility (Bazzaz et al., 2010). Convincingly, eNOS having a significant role in disease evolution and hence further studies should be carried out.

**Vascular endothelial growth factor (VEGF)**

The substantial over expression of VEGF was demonstrated in fibrovascular membranes and vitreous samples of patients with proliferative diabetic retinopathy,
suggesting its possible contribution to the development of PDR (Aiello et al., 1994; Petrović, 2013). It has been observed that single-nucleotide polymorphisms (SNPs) in growth factors, such as VEGF, basic fibroblast growth factor (BFGF), and insulin-like growth factor (IGF), influence expression of their genes. Several studies have so far demonstrated the importance of gene polymorphisms VEGF, BFGF, and IGF in the pathogenesis of PDR (Simo et al., 2008).

VEGF is an endothelial cell-specific mitogen, secreted primarily from retinal pigmented epithelial cells, pericytes, astrocytes, muller cells, glial cells and endothelial cells. It has attributed a major contribution to the development of DR and diabetic macular edema (DME) (Patel et al., 2008; Aiello, 2005). Both hyperglycemia induced hypoxia or ischemia were proved to stimulate the VEGF production, through activation of DNA binding protein called hypoxia-inducible factor 1, in turn elevating expression of VEGF and its receptor in diabetic retinas (Patel et al., 2007). Thus, VEGF is a mediator of neovascularization that induced by ischemia and is responsible for altering retinal capillary permeability in diabetic retina by increasing phosphorylation of proteins involved in tight junctions (Petrović, 2013; Antonetti et al., 1999; Lip et al., 2004).

VEGF has high-affinity binding to tyrosine kinase receptors that includes VEGFR-1 and VEGFR-2, where binding of VEGF with VEGFR-2 modifies endothelial cell morphology, actin organization, chemotaxis, and cellular proliferation. Endothelial receptor tyrosine kinase, Tie-2, is differentially regulated by two ligands, Ang I and Ang II. Ang I induced activation of Tie-2, regulates capillary sprouting, endothelial cell survival, and pericyte recruitment to vessels, whereas, Ang II inhibits the effect of Ang I by inhibiting Tie-2 phosphorylation (Hammes et al., 2011). Chronic hyperglycemia and tissue hypoxia also induce angiotensin (Ang) II levels found to be upregulated in PDR patients (Watanabe et al., 2005; Enge et al., 2002) where it induces sprouting of blood vessels in the presence of VEGF (Figure 2.1). Opposite to this, absence of AngII induces vessel regression (Han et al., 2014). Moreover, VEGF expression is upregulated by protein kinase C, activated by hyperglycemia induced high levels of diacylglycerol. Further, hypoxia and capillary nonperfusion induced VEGF production plays a chief role in the retinal neovascularisation. However, the genes and pathways leading to capillary nonperfusion are yet not understood entirely.
**Figure 2.1:** Regulation of the Ang-Tie system affecting diabetic vasoregression and angiogenesis. [(A) Pericyte-derived AngI dominates AngII stimulates Tie-2 phosphorylation in endothelial cells. The activated Tie-2 regulates endothelial cell division and induces intercellular contacts and junctions, and hence maintaining retinal vasculature and promoting the formation of the blood-retinal barrier (B) Diabetes-induced vasoregression is a result of Ang-2 upregulation in the absence of hypoxia. Retinal endothelial cells and glial cells (Müller cells) express AngII that blocks Tie-2 phosphorylation. The increased AngII induces vascular cell reduction and progressive capillary occlusion (C). The rising areas of nonperfusion stimulate the upregulation of hypoxia-induced factors such as VEGF and AngII. In pericytes, under hypoxic conditions Notch3 gets activated that induces AngII production. High VEGF levels without a succinct gradient and accumulated AngII destabilize vessels, results in endothelial cell proliferation and pericyte activation. P, phosphate.] (Hammes et al., 2011).
For developing comprehensive anti-neovascularization therapies will need identification of components that regulate normal angiogenesis. For example, current treatments that target VEGF, VEGFR, protein kinase C and integrins has shown promising efficacy besides the laser treatments such as panretinal photocoagulation, which causes severe peripheral damage and night vision loss, and cannot stop retinal neovascularization in all cases. Further research is going in the field to find new drug targets by elucidating exact molecular mechanism of DR (Ladomery, Harper and Bates, 2007).

The VEGF gene is located in chromosome 6 (6p21.3) consisting of eight exons, and its alternate splicing refers to the two VEGF protein families, namely, pro-angiogenic and anti-angiogenic (Bleda et al., 2012). Certain SNPs of VEGF are thought to play a role in contributing risk or protection against DR (Simó-Servat et al., 2013). Till date, mostly SNPs in the promoter and 5’ region of the VEGF gene have found to be associated with DR. amongst these SNPs, Bleda et al., (2012) has found a relation between the C/A genotype of the promoter polymorphism -2578 C/A, and a genetic susceptibility for DR in a Caucasian population. Contradictorily, the relationship has not been confirmed in many other populations (Cilenˇsek et al., 2012; Ladomery, Harper and Bates, 2007). Moreover, sample size of the earlier study was too low, challenging their findings, which were ultimately found to be not much significant in association with DR (Ladomery, Harper and Bates, 2007) The -634 C/G polymorphism at 5’ UTR of VEGF is the most studied and in Japanese and Indian populations, it has shown association with DR, which has not been found in Caucasian populations (Park et al., 2002). The -634 C/G polymorphism has been further reported in Japanese diabetic patients for increasing risk of macular edema (Awata et al., 2005) The other polymorphism in this region is -460 T/C and it was found to be non-significant in imparting risk to DR in Asian populations, whereas, it may be associated with DR among Caucasians (Park et al., 2002).

The +405 C/G appears to be interesting SNP, which was found to be genotyped frequently with regard to DR development and it is also implicated in a number of diseases, predominantly those that involve angiogenic basis, like DR. Simó-Servat et al, (2013) have reviewed several studies conducted on this SNP, in which Japan have demonstrated an correlation of the CC genotype or the C allele of +405 C/G SNP with
the occurrence of DR (Patel et al., 2008). However, Caucasian population did not show this association (Patel et al., 2008). Interestingly, it has been indicated that the C allele is responsible for the higher serum levels of VEGF (Vailati et al., 2012), as well as, the elevated gene expression of VEGFA in the human retina (Awata et al., 2002).

Additionally, the SNP has shown increased risk of developing diabetic macular edema (Fegghi et al., 2011). In contrast, studies in Caucasian population have found GG genotype of this polymorphism (compared with the CC genotype) as an independent predictor of PDR (Vicenti et al., 1996; Suganthalakshmi et al., 2006). However, the CG genotype has been identified significantly associated with PDR in Indian ethnicity (Uthra et al., 2008; Kim et al., 2009). Nevertheless, a meta-analysis has not found any statistically significant association between the G allele of this polymorphism and risk of developing DR (Cilensˇek et al., 2012). A meta-analysis by Han et al., (2014) has found significant relationship of +936 C/T (rs3025039) polymorphism with genetic predisposition of DR in Asian populations; while Kim et al. found the same in Korean population (Carter et al., 2011). However, Japanese population demonstrated non-significant association of this polymorphism with the progression of DR (Fegghi et al., 2011). The association between PDR and the 2994 C polymorphism of SRp55 in a Caucasian population was observed where SRp55, a splicing factor, controls the alternative splicing of the exon 8 of VEGF pre-RNA (Al-Kateb et al., 2007). This splicing event plays a chief role in balancing pro- and anti-angiogenic isoforms of VEGF. Numerous studies have indicated other VEGF polymorphisms and haplotypes in DR (Buraczynska et al., 2007; Chen et al., 2008) majority of which appears to be corroborated for revealing their assured association.

Thus far, -634 C/G polymorphism in 5’ UTR of the VEGF was the most often tested polymorphism; nonetheless, meta-analysis showed lack of association of -634 C/G polymorphism with PDR (Ladomery, Harper and Bates, 2007). However, studies regarding other polymorphisms are less conclusive, as a disease-associated SNP in one population may not confer a risk in another population. The main reason for this discrepancy might be racial differences in the studied populations. Other reasons might be due to variations in the inclusion criteria of cases, sampling bias, sample sizes, and so forth.
Other Important genes associated with DR

A number of other candidate genes have been also implemented to be associated with diabetic retinopathy in one or more studies, are found to be less interested due to little independent replication or lack of association. The erythropoietin (EPO) gene encodes for the angiogenic factor expressed in the retina and the kidney and linked to angiogenesis, vasculogenesis, and neuroprotection (Watanabe et al., 2005) due to which it was considered as a candidate gene for DR. The increased EPO mRNA and protein concentrations in the vitreous of was found in PDR patients (Watanabe et al., 2005), which was associated with allele (T) of promoter polymorphism rs1617640 (Tong et al., 2008). The study included 19 SNPs from 11 candidate genes that has indicated an association of functional EPO promoter polymorphism (rs1617640) with both PDR and nephropathy in a European-American population (Williams et al., 2012). However, no association was observed with PDR when it was analyzed independently from the renal failure (Uhlmann et al., 2006). It should not be circumvented that EPO might play a neuroprotective role in the early development of DR and protects the retinal pigment epithelium against the increased permeability caused by chronic diabetes (Patel et al., 2008). On the other hand, EPO gets activated synergistically with VEGF in developing advanced DR, thus stimulating PDR formation (Tong et al., 2008). Therefore, additional studies to explain this contradictory role of EPO in the pathogenesis of DR are required.

Certain factors like angiopoietin-1, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), erythropoietin (EPO), and their corresponding receptors contribute to the pericyte recruitment and vessel maintenance (Simó-Servat et al., 2013; Antonetti et al., 1999; Tong et al., 2008) due to which they are thought to be involved in DR progression. Moreover, impaired expression of integrins and proteins that mediate cell communication and extracellular matrix are also thought to be responsible for the occurrence of PDR (Han et al., 2014). Hence, the investigation of candidate genes incorporated in cellular communication and the extracellular matrix have also been carried out for finding their association with DR and/or PDR. They includes, namely, APOE, b-3 adrenergic receptor gene (b-3AR), plasminogen-activating factor (PAI-1), Paraoxonase 1 (PON1), a2b1 integrin, collagen IV, tumour necrosis factor alpha (TNF-a), G-protein b-3 subunit and neuropeptide Y (Richeti
et al., 2007). However, in some cases, consistent associations of these genes with frequency or severity of DR was not observed, whereas, some others cases have shown significant associations but not replicated in additional populations, rendering their unclear role in genetic predisposition to DR. The other genes include ACE, MTHFR and GLUT1; which may have reported borderline or poor associations, had small sample sizes or some limitations in the methodology of study (Park et al., 2002).

The discrepancy in the outcomes of the studies may reflect the lack of an accurate definition of DR (i.e. use of different diagnosis criteria), the small sample sizes, underlying genetic differences among the study populations due to ethnic/racial variations, diverse pattern of linkage disequilibrium among the various populations, and differences in study design in terms of methods of genotyping or screening and statistics used in the study. In addition, the duration of diabetes, hyper tension and poor glycemic control are key factors which should be counted in the analysis of the results. Finally, the overlap of DR with ‘non-diabetic’ retinopathy that occurs in subjects without clinical diabetes, could also be a confounding factor.

**Conclusion**

Extensive efforts continue to identify genetic factors in T2D and DR. Considerable evidence exists that T2D is a multifactorial disease, which involves multigenic determinants. Hitherto, only less than 10% of the overall genetic variance responsible for the T2D has been studied; large part remains to be unrevealed. However, GWAS conducted till now have revealed many genetic variations in genes like TCF7L2, CAPN 10, KCNQ1, CDKN2B, FTO, PPARG, KCNJ11 imparting significant risk to T2D; which was not possible earlier using conventional strategies. Although, the consistent replications and/or associations of these variations are lacking; till date TCF7L2 remains to be the most significantly associated gene with T2D. Other genetic variations not encompass as severe association alone but their concordances with each other and environmental factors may prove determinant for T2D.

Despite the understanding of initial events in DR pathogenesis, factors leading to extracellular matrix modifications, pericyte loss, capillary non-perfusions, endothelial cell proliferation, and overall deregulation of these events are less understood. More
recently GWAS approach is undertaken to recognize specific genetic variants and combinations of susceptibility loci associated with DR to gain knowledge about the molecular mechanisms governing its pathology. Thus, more frequent examinations of high-risk individuals with T2D may provide more efficient management of DR and delaying its onset. A large number of putative genes have been screened and/or genotyped for its variants and some of which exhibit consistent associations with DR such as VEGF, ALR2, and RAGE genes. However, these results have not been replicated in multiple populations. Further advanced studies need to be conducted in various ethnicities to achieve the widespread acceptance of genes highly affecting the DR development.

It is expected that GWA studies will provide novel insights to genetic susceptibility of DR in the near future, for which there is a need to conduct larger crosssectional studies and well-powered meta-analyses. The identification of genetic factors related to the disease and its subsequent study to analyze their role in the disease development may provide us an insight to an unrevealed etiology of the type 2 diabetes and diabetic retinopathy. It has been substantially proved that screening of genetic predisposition can find the people at high risk of diabetes and its related traits. Ultimately, the proposed genetic approach would permit us to design more target based therapeutic strategies to circumvent the devastating complication of diabetes.

**Future Guidelines**

It is well evident that T2D and DR are polymorphic and multigenic in nature, which makes the study complicated to find the genetic predisposing factors underlying both the diseases. To overcome the rationale behind the inconsistency of the results; standardization of phenotypes is an important aim in DR, either by using the ETDRS severity scale or a modification, and performing sensitivity analyses with increasing severity of retinopathy. This approach with standardized genotyping protocols would also facilitate meta-analyses to increase power. Ultimately, the studies would allow the exploration of the complex interplay of gene-environment interactions in causing severity of DR and responding to treatment modalities. Population-based cohort studies may play a unique role against these limitations as they often utilize standardized methods of retinopathy assessment, collect data on multiple environmental risk factors, and thus can be successfully pooled. Such cohort studies
have been never carried out in India, demands strongly for conducting a large study in Indian populations keeping the aspects in consideration discussed above.

Moreover, large number of relatively common allelic variants imposes risk to DR and PDR, possibly interlinked and interacting with environmental influences, each individually harboring modest increase in relative risk. Identification of these variants in an individual with DR may help in the appropriate medical management, promising its application in the prevention and treatment of DR in term of personalized medicine. Further, the studies would also aid a direction to understand the basis of the DR pathogenesis.