INTRODUCTION

Type 2 diabetes (T2D) is a group of metabolic diseases which has increased at an alarming rate all over the globe (International Diabetes Federation, 2011), with India being the most affected region often known as diabetes capital of the world (Mohan et al., 2007) where prevalence is reaching epidemic proportions (Ramachandran et al., 2001; Anjana et al., 2011; Walia et al., 2014). T2D is characterised by hyperglycaemia resulting from insulin resistance and insufficiency (American Diabetes Association, 2016). The prevalence of T2D varies in different ethnic groups from <6% in most populations to over 50% among the Pima Indians (Najaipoor et al., 2004). According to the International Diabetes Federation (2013), of the 382 million people reported to have T2D worldwide, nearly 35 million people are from the Middle East. It has been estimated that the number of T2D will shoot up to 592 million by 2035. Currently, there are 176 million people that are yet undiagnosed (International Diabetes Federation, 2013). In India the prevalence of T2D is 10.4% in rural Tamil Nadu (South India; Raman et al., 2014), 16.1% in Mumbai (West India; Sunita et al., 2014), 11.5% in Kolkata (East India; Kumar et al., 2008); 8.15% in Jammu (North India; Shora et al., 2014) and 16.4% in Chandigarh (North-West India; Walia et al., 2014). With the impact of globalisation and industrialisation, the tendency towards physical inactivity, hence, overweight and obesity are mounting in the populations of Punjab as in other societies of the world. The prevailing lifestyle conditions in the background of susceptible genetic factors are the leading causes of T2D (Arnold et al., 2009; Gutch et al., 2014). Uncontrolled hyperglycaemia is further mounting the burden of secondary complications (Pal et al., 2011). In North Indian population, the increase in diabetes and related complications has been observed due to transition from agriculture to a sedentary lifestyle/physical inactivity associated with consumption of high calorie and fat rich diet (Gutch et al., 2014).

Diabetes is recognised as a global epidemic and its major microvascular complication, diabetic retinopathy (DR), is a leading cause of blindness and is also expected to rise at an alarming rate (Tarr et al., 2013). A meta-analysis of DR-based studies conducted between 1980-2008 revealed a global prevalence of 34.6% across 35 countries (Yau et
al., 2012). In India, DR is presently the 6th leading cause of blindness (Raman et al., 2016) with a prevalence of 10.3% in the Southern India (rural Tamil Nadu; Raman et al., 2014), 14.5% in Western India (Mumbai; Sunita et al., 2014), and an overall estimate of 21.24% across India (Gadkari et al., 2016). The region wise distribution of DR is represented in Figure 1.1.

![Figure 1.1 Region wise prevalence of diabetic retinopathy in India (A) Prevalence of Diabetic Retinopathy in population-based studies. (B) Prevalence of Diabetic Retinopathy in self-reported diabetics (C) Prevalence of Sight-threatening Diabetic Retinopathy in self-reported diabetics. (Source: Adapted from Raman et al., 2016)](image)

Clinically DR can be classified based on the “The International Clinical Disease Severity Scale for DR” (Wilkinson et al., 2003). This scale is based on the findings of the Wisconsin Epidemiological Study of Diabetic Retinopathy (WESDR) and The Early Treatment for Diabetic Retinopathy Study (ETDRS; Wu et al., 2013):

- **Proliferative Diabetic Retinopathy (PDR):** It is characterised by the neovascularisation of the disc, retina, iris, angle and vitreous haemorrhage or tractional retinal detachment.

- **Mild Non-Proliferative Retinopathy (NPDR):** It is characterised by the presence of microaneurysms.
b2) **Moderate NPDR**: It is associated with intraretinal haemorrhages or venous beading in the presence of microaneurysms.

b3) **Severe NPDR**: This results due to the blockage of blood vessels in the retina, which in turn causes ischemia. Patients with severe NPDR usually have 50% higher chances of rapid progression, in the absence of any intervention by laser treatment (Ferris et al., 1996).

**Diabetic Macular Oedema (DME)**: It can occur in both cases of PDR and NPDR and can be similarly classified as Mild, Moderate and Severe depending upon the distance of the exudates and thickening from the centre of the fovea (Wilkinson et al., 2003).

Several biochemical pathways are implicated in the pathophysiology of DR, although the underlying molecular mechanisms are still unclear (Figure 1.2; Klein et al., 1989; Ola and Nawaz, 2012; Tarr et al., 2013). The hyperglycaemia in diabetes causes accumulation of advanced glycation end products (AGEs); inflammation, neuronal dysfunction and increased oxidative stress in the retina (Bhagat et al., 2009). These biochemical changes coupled with stress leads to increased vascular permeability and reduction in the capillary density resulting in neovascularisation (Bandello et al., 2013). The accumulation of AGEs causes retinal apoptosis (Lecomte et al., 2005) and modifications in the basement membrane (Kim et al., 2010). The increased oxidative stress leads to loss of pericytes, formation of acellular capillaries, vascular leakage and thickening of basement membrane (Zheng et al., 2009; Li et al., 2010). Oxidative stress in the retina results in the impairment of other biochemical pathways such as vascular inflammation (Al-Shabrawey et al., 2008), receptor for advanced glycation end products (RAGE) activation (Warboys et al., 2009), activation of protein kinase-C (PKCs; Pricci et al., 2003); and activation of nuclear factor Kappa-B (NF-κB; Zheng et al., 2012). Moreover, the activation of inflammation pathway leads to the upregulation of pro-inflammatory cytokines such as tumour necrosis factor-alpha (TNF-α); interleukin-1beta (IL-1β) and vascular endothelial growth factor (VEGF; Krady et al., 2005; Li et al., 2009; Yang et al., 2013a). The increased levels of VEGF molecule in the retina causes neovascularization and leakage of blood from the blood-retinal-barrier (BRB; Hu et al., 2013), which is the primary event in DR.
Introduction

DR is a multifactorial disease contributed jointly by genetic and environmental factors in the disease development and progression (Tuomi et al., 2014). The role of genetics in DR development can be understood by the fact that some diabetic individuals do not develop retinal changes/problems even after a longer duration of uncontrolled diabetes, however, certain individuals develop DR within a short span despite having a tight metabolic control (Mohan et al., 2005). The most prevalent risk factors associated with DR are hypertension (Rani et al., 2009; Pang et al., 2011; Raman et al., 2014), male gender (Varma et al., 2007; Giloyan et al., 2014; Raman et al., 2014), duration of diabetes (Raman et al. 2010; Chatziralli et al., 2010; Raman et al., 2014) and obesity (Raman et al., 2010; Pang et al., 2011; Sikka et al., 2014). Interestingly, the Madrid Diabetes Study (MADIABETES) cohort reported the use of aspirin as an independent risk factor for DR development [Hazard ratio=1.12 (95% confidence interval=0.84-1.49)] (Salinero-Fort et al., 2013).

In complex disorders, the identification of genes that contribute to disease pathogenesis is a challenging task for the researchers (Uthra et al., 2008). Many genome-wide linkage and candidate gene studies among different populations have reported variations associated with the risk of DR (Uthra et al., 2008). However, linkage analysis in
complex disorders such as DR is difficult to accomplish due to the variable ages at onset limiting the possibility of sampling multiple individuals over generations (Liew et al., 2009).

Earlier studies on candidate gene screening focused on VEGF (Fan et al., 2014; Yang et al., 2014; Yuan et al., 2014), RAGE (Kang et al., 2012; Ng et al., 2012a; Ng et al., 2012b; Yang et al., 2013a), eNOS (Santos et al., 2012; Zhao et al., 2012; Narne et al., 2014; Qian-Qian et al., 2014), TNFα (Meng et al., 2014; Sesti et al., 2015); ALR (Abhary et al., 2010; Deng et al., 2014); MTHFR (Santos et al., 2003; Sun et al., 2003; Errara et al., 2006) and ACE (Lu et al., 2012; Li et al., 2013; Liang et al., 2013). Further, with the advent of Genome-Wide Association Studies (GWAS), some additional variants could be associated with the risk of DR in Mexican-Americans (Fu et al., 2010); Taiwanese (Huang et al., 2011; Sheu et al., 2013); Japanese (Awata et al., 2014) and White Australian & Indian (Burdon et al., 2015) populations. But none of these studies have been conclusive so far.

Some of the potentially associated variants have been screened across multiple DR cohorts across different regions of India with variable results. In a Southern Indian population, VEGF rs2010963 (Uthra et al., 2008); RAGE rs1800625 (Ramprasad et al., 2007; Balasubbu et al., 2010) & rs2070600 (Balasubbu et al., 2010; Uthra et al., 2010); the ALR (AC)ₙ repeat (Balasubbu et al., 2010) did not demonstrate any significant association with DR. In an East Indian (Bengali) population, VEGF rs833061 exhibited risk of DR (Paine et al., 2012a). Similarly, Cheema et al. (2012) reported a protective role of eNOS 4a/b aa genotype with DR in two independent cohorts from Northern India, while the same could not be replicated in South Indian cohorts (Uthra et al., 2007a; Narne et al., 2014). In another North Indian population, Singh et al. (2014) reported a marginal association of TLR-4 rs10759931 with the risk of DR. In another dataset from the North-West Indian population, the RAGE (Gly82Ser) and the MnSOD (Val16Ala) variants exhibited risks of DR under the recessive and dominant models, respectively (Vanita, 2014). In our earlier study conducted on the present cohort, TNF-α rs1800629 indicated a lack of association, while the ADIPOQ rs2241766 exhibited risk of DR (Sikka et al., 2014).
**1.1 Hypothesis and Rationale**

Based on the pathophysiological link between various diseases related to metabolic syndrome, genetic variants from different genes involved in pathways (such as AGE, polyol, inflammation, neuronal dysfunction and increased oxidative stress) related to metabolic disorders might also be implicated with DR across different populations within India and globally. Thus, we hypothesize that the pathogenesis of DR would be implicated by multiple genes with varying magnitudes of effect. Moreover, the understanding of its underlying molecular mechanism is still in its early phase. Also, there is scarcity of genetic data available in the literature from the North Indian population (Cheema *et al*., 2012; Sikka *et al*., 2014; Singh *et al*., 2014; Vanita, 2014) and the major limitations of these studies pertain to the screening of limited number of single nucleotide polymorphisms (SNPs) and on smaller sample sizes.

Therefore, the present study was designed in an attempt to explain the potentially associated 112 SNPs from 57 genes and 14 intergenic SNPs to fill these lacunae towards understanding the genetic factors contributing to the susceptibility of DR. To the best of my knowledge, the present study is perhaps the first comprehensive study to analyse the association of many candidate genes involved in DR development and would help to provide a baseline data for the DR association variants in North Indian population. The understanding of genotype-phenotype correlation will help in identifying the modifiable risk factors for DR, which would greatly help in disease management and in reducing the economic burden.

**1.2 Objectives:**

The proposed objectives of this study are:

- To determine the various clinical and genetic risk factors responsible for DR in a large cohort sampled from a North Indian population in Punjab.

- To undertake a genotype-phenotype correlation to understand the implications of genetic variants on the clinical phenotypes of DR.