We are all concerned about the spectre of an impending diabetes epidemic all over the world, more so in India; but it is much less appreciated that this will be accompanied by an epidemic of chronic kidney disease (CKD), which brings with it a huge burden of cardiovascular disease (CVD) and end-stage renal disease (ESRD), leading to premature death. Approximately one-fourth to one-third of diabetics go on to develop diabetic nephropathy (DN), making it one of the leading causes of CKD and ESRD requiring renal replacement therapy. It is estimated that the number of people with diabetes will rise from 171 million in 2000 to 366 million in 2030, resulting in millions of new cases of CKD, most of them being in the developing world. Since treating ESRD is simply unaffordable for most developing countries, the emphasis has to be on prevention, early detection and slowing of progression from early stages of CKD to ESRD.

The pathogenesis of DN is clearly multifactorial and several genes, proteins and environmental factors are likely to contribute to the onset of the disease. Several metabolic, hemodynamic and intracellular causes have been proposed to play a role in the pathogenesis of DN, though these have not yet been fully elucidated. In this chapter, we look into the various genetic factors potentially associated with the development of nephropathy in patients with diabetes.

PREDICTORS OF DIABETIC NEPHROPATHY

There are various factors which play a role in the development of nephropathy in diabetes. The most well-known amongst them include poor glycemic control, family history of diabetes or hypertension, increased sodium-lithium counter-transport activity in RBCs etc. Previously, it was believed that once albuminuria had become persistent, glycemic control lost its beneficial effect on kidney, but several recent studies documented the importance of glycemic control on progression of nephropathy in patients with Type 1 diabetes. On the other hand, most recent studies have failed to demonstrate any significant impact of glycemic control on progression of nephropathy in Type 2 diabetes. Among the most important putative promoters of progression in kidney disease, blood pressure has been documented to have a close relation with rate of decline of glomerular filtration rate in both Type 1 and Type 2 diabetes. Serum cholesterol concentration has been shown to be another predictor of progression of nephropathy in both types of diabetes. Dietary protein restriction retards the progression of renal disease in both types of diabetes while smoking has been suggested as a determinant for progression of nephropathy in either types of diabetes. The fact that a fairly large number of diabetics goes on to develop nephropathy even in the absence of the above-mentioned factors, led scientists to postulate and investigate about genetic factors leading to this dreadful complication.

EPIDEMIOLOGICAL EVIDENCES

For over two decades it has been suggested that genetic factors are important in the pathogenesis of diabetic nephropathy. This hypothesis was initially based on epidemiological observations, showing that diabetic nephropathy appears to affect only a minority of patients with Type 1 diabetes. The largest prospective epidemiological study to date examined over 300 Scandinavian patients with Type 1 diabetes over a period of 40 years. It was noted that the cumulative incidence of diabetic nephropathy rose to a peak at around 20 years from diagnosis of diabetes, to approximately 30%, and then plateaued, so that the initiation of diabetic nephropathy after 20 years of Type 1 diabetes was uncommon. Similar findings were also noted in a later study from Boston of 292 subjects with Type 1 diabetes over a period of 40 years. These patterns of incidence were all the more remarkable because they contrasted markedly with those seen for diabetic retinopathy, where there is an increase in prevalence of retinopathy with longer duration of diabetes. It is also pertinent to note that no major difference in glycemic control was seen in the patients with nephropathy compared with those without, and a large number of patients remained free of nephropathy despite prolonged severe hyperglycemia. Thus, it was suggested that a subset of patients with Type 1 diabetes are at a particularly high risk of developing nephropathy due to non-metabolic (possibly hereditary) factors.

ETHNIC VARIATION

Present day knowledge states that specific genetic backgrounds might influence development of DN. Indeed, only 30% of patients with Type 1 diabetes and 40% of Type 2 diabetes develop DN irrespective of treatment for diabetes, and DN often shows a familial clustering in siblings with diabetes. It has been noted that...
the prevalence rates of diabetic nephropathy in subjects with Type 2 diabetes show a marked ethnic variation. Higher rates of diabetic renal disease are seen in Indo-Asians in the U.K., in African-Americans¹ and Mexican-Americans in the U.S.A.;¹ in Nauruans¹⁰ and Pima Indians¹¹. The reason for this inter-racial difference in incidence of diabetic nephropathy is unclear; but ethnic variation in genetic susceptibility to nephropathy is a possibility. It is noteworthy that these ethnic groups not only have a very high incidence of Type 2 diabetes, but also a high incidence of hypertension. This suggests that differences in genetic predisposition to hypertension may contribute to the higher prevalence of nephropathy in certain racial groups; although an alternative explanation may be that the presence of hypertension may accelerate an already present renal disease and lead to the condition becoming clinically manifest more quickly.

**STRATEGIES FOR IDENTIFYING SUSCEPTIBILITY GENES**

In view of the complexity involved, it is not surprising that despite investment of significant resources, there has been limited success in identifying genetic variants that modify the risk of developing diabetic nephropathy. Two strategies have been commonly used to identify DN susceptibility loci: the linkage analysis (i.e. family-based studies) or the association analysis (i.e. case-control studies). Both have led to the discovery of many chromosomal and gene regions that may confer susceptibility to DN. The observation of familial clustering of the disease strongly suggests that genetic factors are involved in the development of DN, whereas segregation analyses point to the existence of susceptibility genes¹² and have established that the onset and progression of DN are influenced genetically¹³,¹⁴.

**i. Linkage analysis:**

Genome-wide scans are valuable tools for the identification of loci that could include major genetic components of a disease. Genome-wide scans have identified several DN susceptibility loci. Two complete genome scans for DN have been performed in sibling pairs with Type 2 diabetes. In Pima Indians, four loci with multipoint logarithm of odds (LOD) >1.0 on chromosomes 3, 7, 9 and 20 were identified¹⁵ and in African Americans, evidence for linkage was reported for chromosomes 3, 7 and 18¹⁶. A partial genome-wide scan in Turkish families with nephropathy due to Type 2 diabetes reported linkage on chromosome 18², a finding that was weakly replicated in Pima Indians (LOD = 0.3). Another genome-wide scan amongst patients with nephropathy due to Type 1 diabetes in Finnish population revealed a linkage to a single locus on chromosome 3q with a maximum LOD of 2.67¹⁷. This locus has also been reported by others in Type 1 diabetes patients¹⁸.²⁰. By convention, LOD score greater than 3.0 is considered evidence for linkage. (A score of 3.0 means that likelihood of observing the given pedigree if the two loci are not linked is less than 1 in 1000). On the other hand, LOD score less than -2.0 is considered evidence to exclude linkage.

**ii. Association analysis:**

Association studies are commonly used to test for association between disease and highly correlated single nucleotide polymorphisms (SNPs). These SNPs lie within a candidate gene or region and are selected from the literature or from the HapMap database (www.hapmap.org). Several candidate genes have been identified for association with DN using case-control studies. These were selected considering their positional and/or functional characteristics and the contribution of the corresponding proteins in the pathophysiological axes.

**Limitations of Genome-Wide Association Scans**

The recent Genome-Wide Association Scans (GWAS) have identified only a small proportion of all of the genetic variants that predispose to disease. This limits the utility of such loci to predict an individual’s risk of disease²¹,²², but it also offers hope that many more susceptibility variants await discovery. There are several reasons why the recent GWAS have had limited success in identifying disease susceptibility variants. Firstly, these scans require high statistical genome-wide significance threshold, therefore, there is a high probability of prematurely dismissing true positives (type 2 errors). Hence, in order to reduce the risk of prematurely dismissing true positives, a large number of markers showing the strongest association in stage 1 of a GWAS should be carried forward to stage 2²³. Secondly, the number of cases and controls required to provide adequate power to detect association with disease increases exponentially with decreasing minor allele frequency which prohibitively increases the sample size required for these kinds of scans²⁴. GWAS therefore tend to favours detection of common susceptibility variants which have relatively high minor allele frequencies and are unable to identify rare variants, even if they confer modest risk of disease. The ability of GWAS to detect the majority of the variations that confer susceptibility to complex diseases is therefore dependent on whether the genetic predisposition is largely accounted for by common, low-impact SNPs or by rare variants which confer a much greater individual risk of disease.

Additional features are specifically relevant to GWAS in patients having Type 1 diabetes with nephropathy. Firstly, many susceptibility variants for complex disease have only been detected following meta-analyses employing large numbers of patients²⁵. Given the low prevalence of nephropathy in Type 1 diabetes as compared to Type 2 diabetes, cohorts of this magnitude are very difficult to assemble and replication studies may need to include patients with nephropathy due to Type 2 diabetes. Secondly, the extent of heritability of diabetic nephropathy is less in Type 1 diabetes than that observed in Type 2 diabetes. This implies that it is likely to be much harder to detect nephropathy susceptibility variants as they may be fewer in number, confer on average a lower risk of disease or their signal may be masked by strong environmental influences.
GENETIC BASIS OF DIABETIC NEPHROPATHY

RATIONALE FOR GENE DISCOVERY IN DIABETIC KIDNEY DISEASE

There is enough evidence supporting the concept of genetic susceptibility to nephropathy in patients with diabetes. Discovery of genetic variants that underpin susceptibility to nephropathy could yield important insights into this condition. Firstly, it would permit identification of patients at risk of nephropathy shortly after diagnosis of diabetes rather than much later when persistent microalbuminuria develops, by which time there is already histological evidence of renal injury. This would facilitate targeted therapeutic interventions aimed at primary prevention rather than secondary treatment of established nephropathy. Secondly, and perhaps more importantly, if the susceptibility variants are located in genes that have not previously been implicated in diabetic nephropathy, this may lead to improved understanding of its pathophysiology and development of novel therapies. Table 1 gives a list of various genes studied in relation to diabetic nephropathy.

Amongst the seemingly endless list of genes associated with diabetic nephropathy the most important ones are elaborated below.

RENIN-ANGIOTENSIN ALDOSTERONE SYSTEM

Among the candidate genes suggested for nephropathy, genes of the renin-angiotensin system have attracted the most interest. There is considerable evidence to suggest disturbance of the renin-angiotensin system in diabetic nephropathy. Prorenin, renin, angiotensin-converting enzyme (ACE) and angiotensin II levels are all noted to be elevated in diabetic nephropathy. Furthermore, genes of the renin-angiotensin system have been suggested as being genetic determinants for both hypertension and cardiovascular disease, both of which are common in patients with diabetic nephropathy. Several genes along with their polymorphic forms have been studied in relation to diabetic nephropathy. The most widely studied amongst them are:

i. Angiotensin I-converting enzyme gene

The insertion (I)/deletion (D) polymorphism of the ACE gene is responsible for a large proportion of the genetic variation in serum ACE levels. The II genotype is associated with low levels of serum ACE, whereas the DD genotype is associated with high levels and ID genotypes have intermediate levels of ACE. This gene polymorphism has been investigated for a role in the susceptibility to coronary artery disease, and DD genotype carriers have been noted to be at higher risk of coronary artery disease compared with other genotypes. In diabetic nephropathy two small studies have suggested an association between the D allele of the ACE gene and nephropathy. However, other subsequent large studies examining over 1000 patients with and without nephropathy have shown no association between nephropathy and the D allele. Overall, the cumulative results from a large number of studies suggest that if the ACE gene has any effect, it is likely to be small, and it is not useful as a screening marker for nephropathy.

The ACE D allele has been suggested as a risk factor for...
coronary artery disease in patients with nephropathy. The ACE D allele may have a role in the progression of, rather than susceptibility to, diabetic nephropathy. This is supported by the observation that the presence of the DD genotype confers a reduced responsiveness to ACE inhibition and an increased rate of decline of glomerular filtration rate. However, only long-term prospective studies can truly ascertain whether the ACE gene modulates disease progression.

Recently, the DCCT/EDIC study reported a relative protective effect of the II genotype over the D allele on the development of microalbuminuria and progression to overt proteinuria in 1300 Type 1 diabetic patients, followed for 17 years. The II genotype is also associated with a better response to ACE inhibitors. Interestingly, microalbuminuric Type 2 diabetes patients with the DD genotype present more severe glomerular lesions than patients with the I allele. A large meta-analysis including 47 studies showed that subjects with the II genotype had a 22% lower risk of DN than carriers of the D allele. Summarily, it appears that polymorphisms in the ACE gene may have a role in the progression of DN, rather than in the susceptibility to it.

ii. Angiotensinogen gene

Linkage of the M235T polymorphism of the angiotensinogen gene has been demonstrated in essential hypertension. This polymorphism has also been examined in diabetic nephropathy, but with conflicting results. In a small study of 95 patients with nephropathy, the presence of the TT genotype was associated with nephropathy. However, later studies found no such association although one study has suggested an association of the TT genotype with elevated blood pressure in patients with nephropathy.

iii. Angiotensin II type I receptor gene

The A1166C polymorphism of the angiotensin II type I receptor gene has also been linked to essential hypertension and thus has been examined in diabetic nephropathy. Two large studies suggested no role for this polymorphism in diabetic nephropathy, although one smaller study suggests that the presence of the C allele in combination with poor glycemic control is associated with nephropathy.

ALDOSE REDUCTASE

Aldose reductase (AKR1B1) is a cytosolic enzyme that, in the presence of NADPH, catalyzes the rate-limiting step of the polyol pathway converting glucose into sorbitol. Several mechanisms have been proposed to explain how AKR1B1 activity leads to hyperglycemia-induced lesions in different tissues. Ko et al. were the first to identify seven alleles at the locus of the (AC) dinucleotide repeat sequence upstream of AKR1B1. The commonest allele contains 24 AC repeats and was named Z. Studies have demonstrated a correlation between the Z-2 allele (23 repeats) and increased susceptibility to development of nephropathy in both Type 1 and Type 2 diabetes. Interestingly, diabetic patients, who are homozygous for the XbaI (−) allele of the glucose transporter 1 gene and carry the Z-2 allele, present a 9-fold increased risk of DN, suggesting that this is a particularly high-risk genetic combination. A second AKR1B1 polymorphism has been observed at position -106 of its promoter region. This C106T polymorphism has been identified in both Caucasian and Asian subjects with Type 1 or Type 2 diabetes, and association with DN has been observed. All these results suggest that AKR1B1 polymorphisms play a role in the development of DN.

PEROXISOME PROLIFERATOR-ACTIVATED RECEPTOR GAMMA 2

PPARG2 is the predominant adipose isoform of this receptor and is expressed selectively in the adipose tissue. It modulates the expression of target genes implicated in adipocyte differentiation and glucose homeostasis. Thus, PPARG2 is considered, a major candidate gene for Type 2 diabetes and/or obesity and, recently, for development of nephropathy in Type 2 diabetes. Insulin resistance may be a risk factor for development of nephropathy in Type 2 diabetes and improved insulin sensitivity could be the link between the Pro12Ala polymorphism of PPARG2 and the decreased risk of DN. Several studies have evaluated the association of this genetic polymorphism with nephropathy in Type 2 diabetes. Herrmann et al. showed that the Pro12Ala polymorphism was associated with lower albumin excretion rates among Ala12 carriers, which may indicate a protective effect of this allele and these findings were confirmed by a later study. More recently, Pollex et al. showed that the Ala12 allele carriers have 1.5-fold reduction of albumin/creatinine ratio in urine. All these results indicate a protective correlation between the Ala12 polymorphism and the albumin excretion rate. The mechanism underlying the protective effect of the Ala12 allele is yet unknown.

GLUCOSE TRANSPORTER 1

The glucose transporter SLC2A1 is the major representative of the family of facilitative glucose transporters that are expressed in glomerular, mesangial, endothelial cells and podocytes. SLC2A1 is considered to be pivotal in raising intracellular glucose levels by activating pathogenic pathways. Several workers have tried to determine whether SLC2A1 might be a candidate gene conferring susceptibility to development of DN. These investigations have focused on testing whether the SLC2A1 XbaI SNP is associated with DN using a case-control study design. Although this XbaI SNP was found not to be associated with DN, heterozygosity for the XbaI (−) allele has been linked with a 1.9-fold higher susceptibility to nephropathy in Chinese Type 2 diabetic patients. However, in other studies homozygosity for XbaI (−) allele was seen to be associated with an increased risk for development of DN. Thus, so far, reports regarding the possible role of SLC2A1 XbaI SNP in susceptibility of nephropathy in diabetics are inconsistent. However, a recent meta-analysis concluded that there is, indeed, a significant association between the SLC2A1 XbaI polymorphic
site and DN, but larger studies are needed\textsuperscript{44}. In conclusion, the SLC2A1 gene seems to play an important role in the development of DN.

**GLUTATHIONE S-TRANSFERASE**

The glutathione S-transferases (GSTs) are a family of ubiquitous and multifunctional enzymes which work as one of the antioxidants through their ability to catalyse the conjugation of reduced glutathione with electrophilic compounds\textsuperscript{64}. Over-expression of GSTs has been documented in erythrocytes of CKD patients\textsuperscript{65}. The family of GST enzymes are expressed as various isoforms coded by a variety of genes distributed widely on different chromosomes\textsuperscript{66,67}. Moreover, most of these genetic loci are known to have polymorphic forms of the genes\textsuperscript{68,69}. A few studies have shown that polymorphisms of GST genes lead to change in the expression of the enzyme, either qualitatively or quantitatively\textsuperscript{70} and hence can render individuals susceptible to various diseases including CKD. Amongst all the genetic polymorphisms described in this class of enzymes, the GSTM1 and GSTT1 are most remarkable because these genes are reported to be deleted resulting in absence of the respective isoforms of the enzyme. Apart from this the prevalence of GSTM1 and GSTT1 null variants have been shown to be remarkably high in different population groups from all over the globe. Studies on Indians, although few, show a high prevalence of these genetic variants in normal population\textsuperscript{71,72,73}.

Various studies have been carried out worldwide to study the association of GSTM1 and GSTT1 null variants with different diseases. Although most initial studies concentrated on their role in cancer, some authors have shown the association of these genetic polymorphisms in multifactorial and metabolic diseases like coronary artery disease, chronic renal failure, various neurological disorders, diabetes mellitus and its complications etc. Results from different Asian populations vary regarding the association of GSTM1 and GSTT1 deletions with nephropathy\textsuperscript{74,75}. A recent study on Indian population evaluating the relationship between GST gene polymorphism and susceptibility to end stage renal disease found significant association of both GSTM1 null and GSTT1 null genotypes with ESRD\textsuperscript{76}.

Studies in the area of diabetic nephropathy are not only few but also inconsistent in their results. While Fujita et al found no association of GSTM1 deletion with diabetic nephropathy in Japanese Type 2 diabetes patients\textsuperscript{77}, Yang et al showed that GSTT1 null genotype was a risk factor for development of diabetic nephropathy in the Chinese\textsuperscript{78}. In 2005, Kim et al found that GSTM1 null genotype is associated with development of nephropathy in Type 2 diabetes in the Korean population\textsuperscript{79}. We have also studied the polymorphic GST gene with respect to diabetic nephropathy and found that GSTM1 and GSTT1 double positive genotype seems to have a protective role on the susceptibility to development of nephropathy in patients with Type 2 diabetes. GSTM1 and GSTT1 double deletion appears to be a risk factor for development of nephropathy in Type 2 diabetes\textsuperscript{80}. This novel approach to study the role of xenobiotic metabolizing enzymes in the development and progression of diabetic complications like nephropathy is still in its infancy and needs further validation in large prospective studies.

**CONCLUSION**

The role of genetic factors in the development and progression of diabetic nephropathy, though apparent, has not been conclusively elucidated, as yet. Candidate gene analysis allows us to study both major and minor gene effects, but generally yields conflicting results. The observed discrepancies could be partly explained by differences in the studied populations, particularly dissimilar ethnic backgrounds and genetic heterogeneity, and also by their relatively small sample sizes. The use of single nucleotide polymorphisms in association studies of complex phenotypes has been used most often. At the moment, no single gene with a large effect has been identified and only small effects of a variety of polymorphisms in a number of genes have been reported. Neither linkage analyses nor association studies performed until now support the view of major gene polymorphisms involved in the onset or progression of diabetic nephropathy. Further studies are needed to pin-point genetic predictors to identify population susceptible for development of diabetic nephropathy. This may help clinicians initiate early intervention strategies in susceptible population to prevent or postpone the development of this dreadful complication.

**REFERENCES**


