

## Genetic predisposition to type 2 diabetes among Asian Indians

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**Genes play an important role in the development of diabetes mellitus. Type 2 diabetes is a polygenic disorder with multiple genes located on different chromosomes contributing to its susceptibility. Analysis of the genetic factors is further complicated by the fact that numerous environmental factors interact with genes to produce the disorder. Only a minority of cases of type 2 diabetes are caused by single gene defects and one example is maturity onset diabetes of the young (MODY). Till date knowledge of the genetics of type 2 diabetes is limited. Consistent with the complex web of physiologic defects in type 2 diabetes, the genetics of the disorder involves a large number of susceptibility genes, each with a relatively small effect. In this article, the studies on genetics of diabetes in Asian Indians are reviewed. As Asian Indians have an increased susceptibility to diabetes and have increased insulin resistance, they are a unique population for carrying out genetic studies. There appears to be certain genes which predispose Indians to diabetes while other genes (for example Pro 12 Ala polymorphism of PPAR gamma gene) which afford protection against diabetes and insulin resistance to Caucasians, do not appear to protect Indians. Further studies are needed to unravel the genetics of diabetes in South Asians .**

**Key words** Asian Indians - candidate genes - linkage studies - MODY - polymorphisms - South Asians - type 2 diabetes

Type 2 diabetes (T2D) is a complex heterogeneous group of conditions characterized by elevated levels of plasma glucose, caused by impairment in both insulin secretion and action. The recent global epidemic of T2D is indicative of the importance of environmental triggers such as rapid changes in lifestyle related to changing patterns and increasing physical inactivity. However, there is strong evidence from twin, family, and epidemiological

studies for genetic factors contributing to the aetiology of type 2 diabetes.

Multiple lines of evidence support the view that genetic components play an important role in the pathogenesis of type 2 diabetes:

(i) The prevalence of type 2 diabetes varies widely among populations. Part of the observed

ethnic variability can be attributed to non genetic environmental factors; however, the observation that the disease prevalence varies substantially among ethnic groups that share a similar environment supports the idea that genetic factors also contribute to predisposition to the disease<sup>1</sup>.

(ii) Familial aggregation of the disease is another source of evidence for a genetic contribution to the disease although, admittedly, families also share common environmental traits. The odds ratio for offspring of a single affected parent is 3.5 compared to those with no parental diabetes history and this increases to 6.1 if both parents are affected<sup>2</sup>.

(iii) The high concordance in monozygotic twins (over 80%) and the 50 per cent decline in dizygotic twins provides compelling evidence for a genetic component in the aetiology of type 2 diabetes<sup>3,4</sup>.

(iv) Data from various studies are in support of a genetic basis for measures of both insulin sensitivity and insulin secretion<sup>5,6</sup>.

### **Evidence for increased genetic predisposition to diabetes in Indians**

According to the recent projections of World Health Organization (WHO), India already leads the world with the largest number of diabetic subjects (nearly 40 million) and it is predicted that this number would reach almost 80 million by the year 2030<sup>7</sup>. This would represent approximately 20 per cent of the total diabetic population of the world. Recent population based studies reveal a rising prevalence of diabetes in the urban areas of India with figures ranging between 12-16 per cent<sup>8,9</sup>. While environmental factors certainly play a major role in the diabetes epidemic, this usually occurs on a background of genetic susceptibility.

Mohan *et al*<sup>10</sup> first showed that plasma insulin levels are higher in Asian Indians compared to matched groups of Europeans. Later Sharp, Mohan and colleagues<sup>11</sup> showed that Asian Indians are more

insulin resistant. Though body mass index (BMI) an indicator of obesity, is lower among Indians, for any given BMI, the waist to hip ratio was higher among Indians compared to other ethnic groups<sup>12</sup>. Further, at any BMI, Indians also had higher body fat; and even when matched for body fat, Indians had greater insulin resistance compared to other ethnic groups<sup>13-15</sup>. These studies suggest that Indians seem to be genetically more prone to diabetes and insulin resistance.

Various studies on migrant Indians have consistently shown higher prevalence of diabetes among Indians compared to the indigenous population<sup>16</sup>. Various epidemiological studies report that prevalence rates are very high in migrant Indians<sup>17,18</sup>. This clearly indicates that Indians have a predilection to diabetes probably due to genetic predisposition.

One factor contributing to insulin resistance is obesity and among Caucasians, most people with type 2 diabetes are obese. Studies in Asian Indians reveal that even a moderate degree of obesity can produce insulin resistance as fat tends to accumulate more in the abdominal region in people of this ethnic group. Individuals with abnormal fat distribution, characterized by a high waist to hip ratio or a high truncal to peripheral skinfold thickness ratio appear to be particularly predisposed to developing insulin resistance<sup>19</sup>. There are now data to suggest that Asian Indians are more susceptible to developing truncal obesity, which might account for their propensity to insulin resistance<sup>14,16</sup>. This cluster of metabolic abnormalities is referred to as the "Asian Indian Phenotype"<sup>20</sup>.

Factors that determine the distribution of body fat are not known; the possibility that abnormal insulin action at the level of adipose tissue could promote the accumulation of truncal fat cannot be excluded. Studies on low birth weight and insulin resistance in Indian neonates have shown that new born Indian babies have higher insulin levels and greater adiposity compared to Caucasians<sup>21</sup>. These

studies underscore the importance of genetic susceptibility in Indians towards developing diabetes and insulin resistance.

Studies carried out in the 1980's in a group of 135 Asian Indian and 146 European diabetic patients attending a diabetic clinic in the UK showed that 45 per cent of Asian Indians had a first degree relative with diabetes compared to 36 per cent of the Europeans<sup>22</sup>. Of greater interest was the fact that 10 per cent of Asian Indians compared to 1 per cent of Europeans had two diabetic parents. Various studies looked at the prevalence of diabetes among the offspring of two type 2 diabetic parents in India. These studies showed that 55-60 per cent of offspring had diabetes or impaired glucose tolerance<sup>23,24</sup>. This was considerably higher than those reported for prevalence of diabetes in offspring of American or European diabetic parents<sup>25,26</sup>.

In the recent population based study called Chennai Urban Population Study (CUPS) conducted on 1262 individuals in Chennai in southern India, we assessed the influence of familial factors. The prevalence of type 2 diabetes was higher among subjects who had a positive family history of diabetes (18.2%) compared to those without (10.6%,  $P=0.0015$ ). Moreover, 9.3 per cent of subjects with family history of diabetes had impaired glucose tolerance (IGT- a pre-diabetic stage) compared to 5.0 per cent of subjects without a family history. The overall prevalence of glucose intolerance (diabetes + IGT) among subjects with two diabetic parents was significantly higher (55%) than those who had one diabetic parent (22.1%,  $P=0.005$ ) or those with two non-diabetic parents (15.6%,  $P<0.0001$ ). The odds ratios of the risk for diabetes among subjects with one diabetic parent was 2.5 and this increased to 6.62 in subjects who had both parents affected by diabetes<sup>24</sup>. These findings again reinforce the importance of genetic susceptibility in Indians towards developing diabetes and insulin resistance.

The genetics of type 2 diabetes can be considered under two broad groups: genetics of monogenic forms

of diabetes and genetics of polygenic forms of diabetes.

### Genetics of monogenic forms of diabetes

Monogenic forms of diabetes are a consequence of rare mutations in a single gene<sup>27</sup>. These mutations substantially change the structure and subsequently the function of a protein. Monogenic forms are characterized by high phenotypic penetrance, early age of diagnosis, and a distinct clinical picture. Genetic background plays a critical role in their pathogenesis, while the environment only slightly modifies the clinical picture. Monogenic forms constitute a very small proportion of type 2 diabetes (<5%). They have a clearly defined inheritance model and the ascertainment of multigenerational families is relatively easy due to early age of diagnosis. The known forms of monogenic type 2 diabetes are characterized either by severe defect in insulin secretion (gene defects involving the  $\beta$ -cell) or profound decrease in insulin sensitivity but the former is more common. Of these, the most common and important form is the maturity onset diabetes of the young (MODY). MODY is a monogenic subtype of type 2 diabetes, characterized by an autosomal dominant inheritance, and an age of onset at 25 yr or younger and non insulin dependent diabetes. It is estimated that 2-5 per cent of all patients with type 2 diabetes may have MODY. Our earlier studies showed a high prevalence of MODY in south Indians (4.8%) (as defined at that time)<sup>28</sup> besides delineating the insulin responses in them<sup>29</sup> and beta cell response in the offspring of MODY<sup>30</sup>. MODY is genetically heterogeneous and with atleast six different MODY forms, namely MODY 1, MODY 2, MODY 3, MODY 4, MODY 5, MODY 6. The different forms are caused by mutations in the genes encoding hepatocyte nuclear factor-4 $\alpha$  (HNF-4 $\alpha$ ), glucokinase, hepatocyte nuclear factor-1 $\alpha$  (HNF-1 $\alpha$ ), insulin promoter factor-1 (IPF-1), hepatocyte nuclear factor-1 $\beta$  (HNF-1 $\beta$ ) and neuro D, respectively. MODY 1-3 seem to be the most common in the world<sup>31-36</sup>. All of the known MODY genes have been considered as possible candidates for gene defects in late -onset type 2 diabetes mellitus also.

Recently, we studied 73 unrelated young south Indian diabetic subjects (with at least one parent diabetic) for mutations in hepatocyte nuclear factor 1- $\alpha$  (*HNF-1- $\alpha$* ). Only 6-8 per cent of south Indian MODY had any mutation in *HNF-1- $\alpha$* . Four novel missense mutations, one silent mutation, and promoter mutations were identified in the hepatocyte nuclear factor 1- $\alpha$ . These results demonstrate that MODY3 mutations in south Indians may be different from that observed in Western populations<sup>37</sup>.

In Asian Indians, type 2 diabetes occurs earlier and often overlaps with MODY, but the genetics of the latter is unknown. Our group performed a study to estimate the prevalence of a common polymorphism of the *HNF1- $\alpha$*  gene, the Ala 98 Val, in five different types of diabetes in Asian Indians including MODY and a control group of glucose-tolerant subjects to evaluate its role in conferring risk of diabetes in Asian Indians<sup>38</sup>. We compared the age of onset for the disease with the three associated genotypes and found that the mean age of diabetes onset was earliest in the homozygote Val/Val genotype (mean age of 24.8 yr) compared to Ala/ Val (mean age of 29.9 yr), and Ala/Ala (mean age of 35.7 yr). The Val/Val genotype thus appears to trigger the condition almost 11 yr earlier than those with the Ala/Ala genotype. This study clearly shows that in Asian Indians, the Ala98Val polymorphism of *HNF1- $\alpha$*  gene is associated with MODY and with earlier age at onset of type 2 diabetes<sup>38</sup>. This is indeed the first report showing an association of this polymorphism with MODY and younger age at onset of type 2 diabetes.

### Genetics of polygenic forms of type 2 diabetes

The genetics of the common variety of type 2 diabetes, also called polygenic or multifactorial is a result of the interaction between the environment and multiple genes. The susceptibility is associated with frequent polymorphisms that create amino acid variants in exons or influence the expression of genes in the regulatory parts<sup>39</sup>. Alleles of these

polymorphisms are present in both healthy individuals and type 2 diabetes patients, although with different frequencies. These sequence variants are associated with just a limited increase in the risk of developing the disease. They can be considered susceptibility variants, but by themselves are not causative factors that unequivocally determine the disease.

Two broad approaches have been used to define the genetic predisposition of type 2 diabetes. First, the molecular events in diabetes pathogenesis have been examined directly by testing the role of sequence variants of specific candidate genes<sup>39</sup>. This approach called the candidate gene approach, focuses on the search for an association between type 2 diabetes and sequence variants in or near biologically defined candidate genes which have been chosen based on their known physiological function. The importance of these or other nearby variants is tested by comparing the frequency in type 2 diabetes mellitus (T2DM) subjects and in control non diabetic individuals. A second approach is known as a genome-wide linkage scan strategy<sup>40</sup> in which regularly spaced markers are traced in families and sibling pairs for segregation with type 2 diabetes. No prior knowledge of gene or gene effects is necessary, but the genetic locus must have sufficient impact on the disease susceptibility in order to be detectable.

### Type 2 diabetes susceptibility genes discovered by candidate gene approach

Extensive studies have been conducted on genes identified through physiologic pathways. Consistent with the very large numbers of studies, many have shown modest associations in single populations, but these associations have not yet been replicated in other populations. Therefore our focus was on those genes for which reasonable replication has been achieved already. In each case, like those genes identified through linkage, the effect of these variants is relatively small, with an odds ratio <1.4 and in most cases approaching 1.2 or less<sup>39</sup>.

One of the main candidate genes that is implicated in adipogenesis, insulin resistance and type 2 diabetes is the peroxisome proliferator activated receptor- $\gamma$  (*PPAR- $\gamma$* ) gene. This is a transcription factor that is involved in adipogenesis and in regulation of adipocyte gene expression and glucose metabolism. Within a unique domain of *PPAR- $\gamma$*  2 gene that enhances ligand-independent activation, a common Pro12Ala polymorphism has been identified<sup>41</sup>. This polymorphism has been shown to be associated with obesity. Using a family based design to control for population stratification, it was reported that the Ala allele of this polymorphism was associated with a decreased risk of type 2 diabetes<sup>42</sup>.

*PPAR- $\gamma$*  gene: The frequencies of the common Pro12Ala polymorphism of *PPAR- $\gamma$*  in south Indians living in Chennai was found to be 19 per cent while that in South Asians in Dallas was 18 per cent and in Caucasians in Dallas it was 20 per cent. The Caucasian diabetic subjects had significantly lower prevalence of *PPAR $\gamma$ -12Ala* when compared with the Caucasian non diabetic subjects (20 vs. 9%,  $P=0.006$ ). However, there were no significant differences between diabetic and non diabetic subjects with reference to the Pro12Ala polymorphism among the South Asians living in Dallas (20 vs. 23%) and the Indians in Chennai (19 vs. 19.3%) (Fig. 1). Although Caucasians carrying *PPAR $\gamma$ -Pro12Ala* had lower 2 h plasma insulin levels at 2 h of oral glucose tolerance test (OGTT) than the wild-type (Pro/Pro) carriers ( $76 \pm 68$  and  $54 \pm 33$  U/ml, respectively,  $P=0.01$ ), no differences in either fasting or 2 h plasma insulin concentrations were found between South Asians (Indians) carrying the *PPAR $\gamma$ -Pro12Ala* polymorphism and those with the wild-type genotype either at Chennai or at Dallas (Fig. 2). We concluded that despite the frequency of the Ala allele at the *PPAR $\gamma$ -Pro12Ala* locus being the same in individuals of South Asian descent, as in Caucasians, this particular polymorphism does not appear to improve insulin sensitivity or decrease risk for type 2 diabetes in South Asians (Asian Indians), as it does in Caucasians. Our study thus supports the hypothesis that the Pro12Ala polymorphism is

protective against diabetes in Caucasians but not in South Asians<sup>43</sup>. If confirmed by larger studies, the hypothesis generated in this study may help us understand the increased susceptibility to insulin resistance and excessive risk for type 2 diabetes observed in South Asians.

*Plasma cell glycoprotein (PC-1) gene:* We carried out another genetic study in collaboration with the Dallas group in the same populations on plasma cell glycoprotein (*PC-1*) gene polymorphism K121Q (ENPP1 K121Q), to evaluate the role of this polymorphism in prediction of type 2 diabetes. The three study groups included 679 south Indians living in Chennai, India (223 with type 2 diabetes); 1,083 migrant South Asians living in Dallas, Texas (121 with type 2 diabetes); and 858 Caucasians living in Dallas, (141 with type 2 diabetes). Patients with type 2 diabetes were included in these cohorts if they had diabetes onset before the age of 60 yr. The prevalence of subjects carrying the polymorphic ENPP1 121Q allele was 25 per cent in the nondiabetic group and 34 per cent in the diabetic group of South Asians living in Chennai ( $P<0.01$ ). The prevalence in the non diabetic and diabetic groups were 33 and 45 per cent ( $P<0.01$ ) for the South Asians living in Dallas and 26 and 39 per cent ( $P<0.003$ ) for the Caucasians<sup>44</sup>. Our study supports the hypothesis that ENPP1 121Q predicts genetic susceptibility to type 2 diabetes in both South Asians and Caucasians. If our findings are confirmed in a larger sample and in other populations, the ENPP1 121Q variant may provide an important genetic marker to identify people at risk type 2 diabetes.

*PGC-1 alpha gene:* Studies on some of the genetic polymorphisms of peroxisome proliferator activated receptor-co-activator-1 alpha (*PGC-1*) gene by our group showed that this gene could be a strong candidate gene for type 2 diabetes and also for obesity measures such as body fat. We examined the relationship of three polymorphisms, Thr394Thr, Gly482Ser and +A2962G, of the *PGC-1* gene with type 2 diabetes in Asian Indians chosen from the

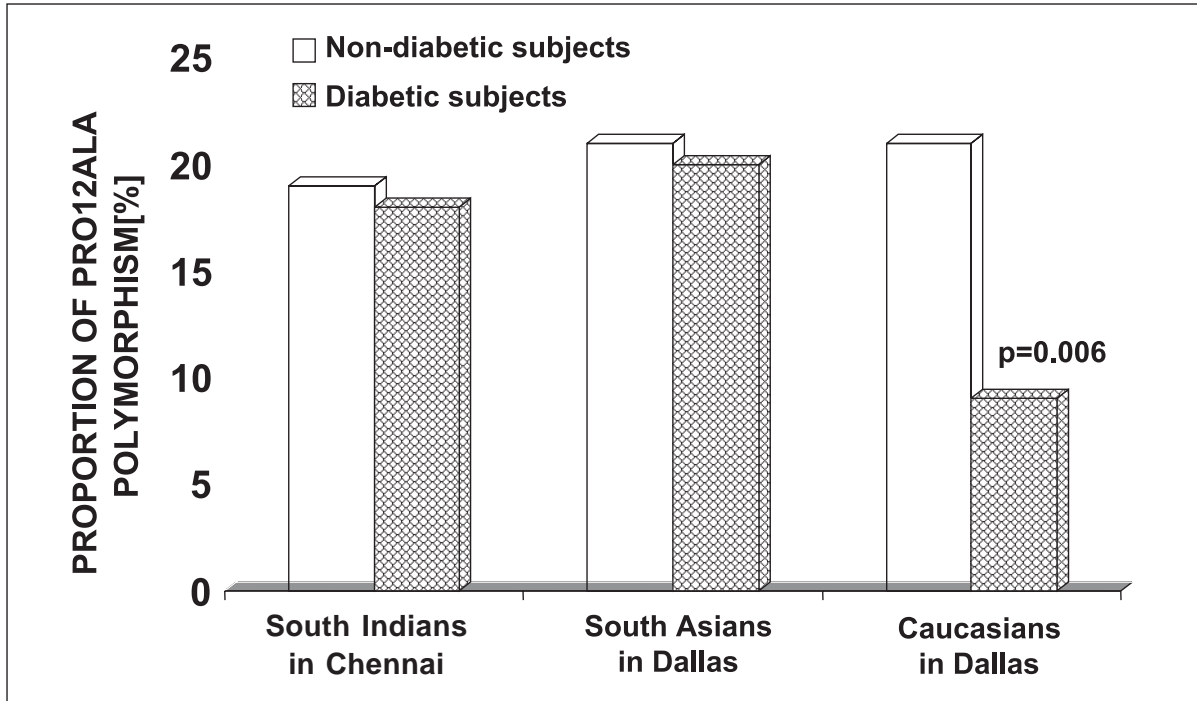


Fig. 1. PPAR- $\gamma$  gene: *Pro12Ala* polymorphisms in Asian Indians and Caucasians (adopted from Ref. 43 with permission from American Diabetes Association).

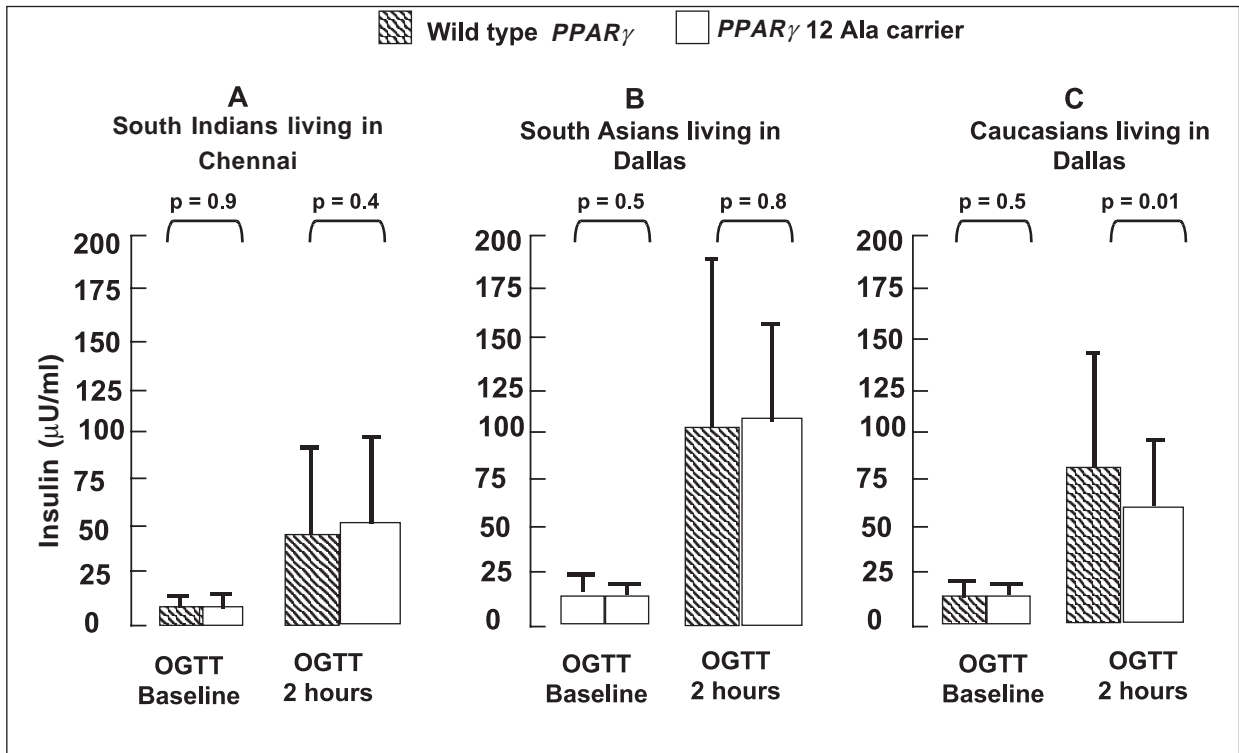


Fig. 2. PPAR- $\gamma$  gene: *Pro12Ala* polymorphisms and insulin resistance<sup>43</sup>.



Chennai Urban Rural Epidemiology Study (CURES) in southern India. With respect to the Thr394Thr polymorphism, 20 per cent of the type 2 diabetic patients had the GA genotype compared with 12% of the normal glucose tolerant subjects ( $P=0.0004$ ). The frequency of the A allele was also higher in type 2 diabetic subjects compared with normal glucose tolerant (NGT) subjects ( $P=0.002$ ). Our study shows that the A allele of Thr394Thr (G -A) polymorphism of the *PGC-1* gene is associated with type 2 diabetes in Asian Indian subjects and the XA genotype confers 1.6 times higher risk for type 2 diabetes compared with the GG genotype in this population<sup>45</sup>. We further extended this study and investigated whether these polymorphisms were related to body fat in Asian Indians<sup>46</sup>. Visceral, subcutaneous and total abdominal fat were measured using computed tomography, whereas dual X-ray absorptiometry was used to measure central abdominal and total body fat. The genotype and allele frequencies of Thr394Thr polymorphism were significantly higher in type 2 diabetic subjects compared to those in NGT subjects and the odds ratio for diabetes for the susceptible genotype, XA of Thr394Thr polymorphism, was 2.53 (95% confidence intervals: 1.30–5.04,  $P=0.009$ ). Visceral and subcutaneous fat were significantly higher in NGT subjects with XA genotype of the Thr394Thr polymorphism compared to those with GG genotype. Abdominal and non abdominal fat were also significantly higher in the NGT subjects with XA genotype compared to those with GG genotype<sup>46</sup>. Our studies clearly show that among Asian Indians, the Thr394Thr (G-A) polymorphism is associated with type 2 diabetes and also with total, visceral and subcutaneous body fat.

*Insulin receptor substrate-2 (IRS-2) gene:* We also studied the association of insulin receptor substrate-2 (*IRS-2*) Gly1057Asp (G1057D) polymorphism with type 2 diabetes and obesity in Asian Indians and this yielded interesting results<sup>47</sup>. The genotype frequency of the *IRS-2* G1057D polymorphism was significantly different between the NGT and type 2 diabetic groups in the total study subjects and among

the obese subjects. The DD genotype showed an increased susceptibility to diabetes with an odds ratio of 2.19 when compared to the GG and GD genotype, among the obese subjects, but not in non obese subjects. We also explored the possible interaction of this polymorphism with obesity, and found that the DD genotype increases susceptibility to type 2 diabetes by interacting with obesity. The major finding of this study is that in Asian Indians, the D1057D genotype of *IRS-2* is susceptible to diabetes by interacting with obesity. Table I summarizes the findings of the recent genetic studies done in type 2 diabetes.

Both physiologic and genetic data are inconsistent for the glycine to arginine (G972R) variant of the insulin receptor substrate 1 (*IRS1*) gene<sup>48</sup>. Despite evidence that this variant alters insulin action on *IRS1*, the association with T2DM has been inconsistently replicated<sup>49</sup>.

The beta cell potassium channel comprises two subunits, the potassium channel encoded by the gene *KCNJ11*, and the regulatory subunit (SUR1 encoded by *ABCC8*) that binds sulphonylureas and ATP. Variants in both subunits have been associated with type 2 diabetes mellitus. Two variants in *ABCC8* were initially associated in smaller studies<sup>50,51</sup>, and some subsequent studies appeared to confirm the association<sup>52</sup>. Other studies however, have not confirmed the association<sup>53</sup>. A coding glutamine to lysine at position 23 in the single exon (E23K) was shown to lower the sensitivity of the potassium channel to ATP and thus reduce insulin secretion *in vitro*<sup>54</sup>. Gloyn *et al*<sup>55</sup> subsequently showed a modest association of the E23K variant with T2DM in nearly 2500 British case-control subjects, and in a meta-analysis confirmed association with an OR of 1.16. Overall, E23K appears to have a role comparable to the *PPARG P12A* polymorphism.

Several genetic studies have been performed earlier on type 2 diabetic subjects in the southern part of India. Initial studies were focused on the insulin gene<sup>56</sup> and islet amyloid polypeptide (IAPP)<sup>57</sup>

and revealed that both these genes are unlikely to represent major susceptibility factors for the development of type 2 diabetes. Earlier studies by other groups have shown a variant in the *IRS* gene to be associated with decreased insulin sensitivity and impairment of insulin stimulated PI 3- kinase activity<sup>58</sup>. A meta-analysis of one of the polymorphisms in *IRS-1* gene (Gly972 Arg) in four populations comprising a small south Indian population, Finnish, French and Danish Caucasian population showed a significant association of this polymorphism with type 2 diabetes<sup>59</sup>.

Other genes studied in south Indian type 2 diabetic subjects include glucokinase and the glucose transporters, GLUT 1 and GLUT 4. The glucose transporter (*GLUT*) gene is an attractive candidate since it acts as a sensor to the  $\beta$ -cell and as a major signaling molecule. These studies have shown that glucokinase acts as a minor gene influencing the development of type 2 diabetes, and that the *GLUT1* polymorphism may contribute to susceptibility to type 2 diabetes<sup>60</sup>.

A study on the haplotype combinations of Calpain 10 gene was found to show increased risk of both impaired fasting glucose (IFG)/impaired glucose tolerance (IGT)/and type 2 diabetes in south Indians<sup>61</sup>. The study reported that the at risk haplotype combination 112/121 and its intrinsic variants (UCSNP 43, -19, -63) were infrequent in south Indian type 2 diabetic subjects.

Genes associated with obesity like the uncoupling proteins the UCP2 and UCP3, were studied in a small group of south Indians<sup>62</sup>. This study showed a lack of association of UCP2 with type 2 diabetes. Another study suggested UCP 3 to be associated with a high waist to hip ratio. A study on 10 candidate genes: the glucagon receptor, insulin receptor substrate 1, insulin receptor, human beta 3 adrenergic receptor, fatty acid binding protein 2, mitochondrial tRNA [Leu (UUR)], sulphonylurea receptor, human uncoupling protein and the glycogen-associated

regulatory subunit of protein phosphatase-1 genes suggested that none of them were associated with type 2 diabetes in south Indians studied at Pondicherry<sup>63</sup>. However, it must be emphasized that most of these studies were carried out on small numbers and thus may be grossly underpowered for such association studies.

### **Type 2 diabetes susceptibility genes identified by linkage studies**

Linkage studies have helped in the identification of many genes. A few important ones have been detailed below:

In the region of linkage on chromosome 2q Horikawa and colleagues<sup>64</sup> identified 3 common intronic variants of a previously unknown gene called calpain 10 (*CAPN10*). Both a single intronic variant (UCSNP-43: G to A) and a specific haplotype combination defined by three polymorphisms (UCSNP-43, -19, and -63) were associated with type 2 diabetes in Mexican Americans, with lesser evidence of an association in a Northern European population (Botnia). Surprisingly, individuals with a combination of two different haplotypes (121/112) were at the highest risk of type 2 diabetes. In further support of *CAPN10* as a susceptibility gene, Baier and colleagues<sup>65</sup> showed altered gene transcription and reduced muscle mRNA levels in muscle biopsies from Pima Indians with type 2 diabetes. An initial meta-analysis also supported an association with type 2 diabetes<sup>66</sup>, but a subsequent meta-analysis of over 5000 cases and 5000 controls suggested an effect size that was clearly less than that suggested in the earlier studies<sup>67</sup> and raised questions about its role in type 2 diabetes.

Although initial studies of the HNF4 $\alpha$  gene, an obvious candidate on chromosome 20, failed to find any association with type 2 diabetes<sup>68</sup>, subsequent studies based on the discovery of an upstream, beta cell specific promoter and first exon prompted a re-evaluation. Two studies found evidence for



association with type 2 diabetes that could partially explain the linkage signal<sup>69,70</sup>.

HNF 4 $\alpha$  has a complex expression pattern, which includes elaborate alternative splicing, and is expressed in many tissues, including the liver and pancreas. Three of the isoforms are transcribed by an alternative P2 promoter, located 6 kb upstream of the P1 promoter and the rest of the coding exons. Transcripts from the P1 and P2 promoters have been detected in pancreatic beta cells, but the P2 promoter is suggested to be the primary transcription start site in these cells. In total, 4 SNPs flanking the P2 promoter were associated with type 2 diabetes in various populations. However, subsequent replication has been inconsistent with some showing positive, and others negative, results. Damcott reported replication in the Old Order Amish<sup>71</sup>, and Muller and colleagues reported replication in Pima Indians with OR of 1.3<sup>72</sup>. Winckler and colleagues, on the other hand, found no replication in 7883 Caucasians from several populations<sup>73</sup>. Although these SNPs appear to have a role in T2DM susceptibility, the effect is probably smaller than originally reported.

The *PTPN1* gene (chromosome 20q13) codes for protein tyrosine phosphatase 1B (PTP1B), which

negatively regulates insulin signaling by dephosphorylating the phosphotyrosine residues of the insulin receptor kinase activation domain. Multiple noncoding SNPs in the *PTPN1* gene (PTP1B) on chromosome 20q13 were recently implicated in T2DM in Caucasian and Mexican-American populations<sup>74,75</sup>. All of the associated SNPs were present in a single 100-kb haplotype block that encompassed the *PTPN1* gene. Haplotype frequencies were significantly different between case and control subjects with a single common haplotype contributing strongly to the evidence for association, with an OR of 1.3. Further, the same haplotypes were associated with glucose homeostasis measured in 811 Hispanic subjects<sup>74</sup>. However, Florez *et al*<sup>76</sup> failed to replicate the findings in a large Caucasian study. Further studies are needed to determine the role of the *PTPN 1* gene.

Adiponectin is a strong candidate for T2DM given the clear role of plasma adiponectin levels in insulin sensitivity and the fall in adiponectin levels with obesity and T2DM. Adiponectin maps to the region of replicated linkage on chromosome 3q. A large number of studies have examined adiponectin variants with metabolic traits, T2DM, and risk of conversion of impaired glucose tolerance to T2DM. Many studies found evidence that promoter variants in the *APM1* gene were

**Table I.** Summary of recent genetic studies in type 2 diabetes in Indians

	Author & references	Association studies
Type 2 diabetes genes		
<i>PPAR <math>\gamma</math></i> (Pro12Ala)	Radha <i>et al</i> <sup>43</sup>	Pro12Ala polymorphism not protective against diabetes or insulin resistance in South Asian unlike in Caucasian
<i>PGC-1<math>\alpha</math></i> (Thr394Thr)	Vimalleswaran <i>et al</i> <sup>45,46</sup>	Associated with type 2 diabetes and with body fat
<i>PC-1 (K121Q)</i>	Abate <i>et al</i> <sup>44</sup>	Associated with type 2 diabetes
<i>IRS-2</i> (Gly1057Asp)	Bodhini <i>et al</i> <sup>47</sup>	D1057D genotype susceptible to diabetes by interacting with obesity
<i>MODY</i> genes	Radha <i>et al</i> <sup>37</sup>	<i>MODY3</i> mutations in south Indians different from that observed in Western populations
	Anuradha <i>et al</i> <sup>38</sup>	Association of Ala98Val of <i>HNF1A</i> with younger age at onset

associated with T2DM in French and Swedish Caucasian and Japanese populations, and some data support a role of the *APMI* locus in controlling adiponectin levels<sup>77-79</sup>. We have recently identified a noncoding variant which is

strongly associated with type 2 diabetes and with lower adiponectin levels (unpublished observation). However, *APMI* variants did not appear to account for the 3q27 linkage in French families. Zacharova and colleagues<sup>78</sup> showed that *APMI* variants at

**Table II.** Association of *TCF7L2* polymorphism (rs12255372) with type 2 diabetes in different populations

Population	<i>P</i> value of minor allele frequency	Sample size	Reference
Iceland	4.8x10 <sup>-8*</sup>	Total: 2116 NGT: 931 T2DM: 1185	Grant <i>et al</i> <sup>80</sup>
Denmark	0.0048*	Total:767 NGT:539 T2DM: 228	
USA	9.4x10 <sup>-9*</sup>	Total:891 NGT: 530 T2DM: 361	
US (DPP)	0.002**	Total: 3509	Florez <i>et al</i> <sup>81</sup>
U.K.	2.2x10 <sup>-8*</sup>	Total: 6736 NGT: 2158 T2DM: 2574	Groves <i>et al</i> <sup>82</sup>
Finnish	0.0002	Total: 2104 NGT: 953 T2DM: 1151	Scott <i>et al</i> <sup>83</sup>
Amish	0.04	Total: 618 NGT: 342 T2DM: 276	Damcott <i>et al</i> <sup>84</sup>
Scandinavia Poland, US	4.07 x10 <sup>-13</sup>	Total: 8310 NGT: 4278 T2DM: 4132	Saxena <i>et al</i> <sup>85</sup>
French	7.6 x10 <sup>-28</sup>	Total: 4866 NGT: 2367 T2DM: 2499	Cauchi <i>et al</i> <sup>86</sup>
U.S.	<0.0001	Total: 3520 NGT: 1947 T2DM: 1573	Zhang <i>et al</i> <sup>87</sup>
Dutch Breda	0.003	Total: 1422 NGT: 920 T2DM: 502	van Vliet-Ostapchouk <i>et al</i> <sup>88</sup>
Asian Indians	0.001	Total: 2069 NGT: 1038 T2DM: 1031	Bodhini <i>et al</i> <sup>89</sup>
Asian Indians	4.0x10 <sup>-5</sup>	Total: 1354 NGT: 399 T2DM: 955	Chandak <i>et al</i> <sup>90</sup>

NGT, normal glucose tolerance; T2DM, type 2 diabetes mellitus

\**P* value for relative risk; \*\**P* value for Hazard ratio for the TT genotype

positions 45 (G allele) in exon 2 and 276 in intron 2 (T allele) were associated with risk of converting from impaired glucose tolerance to T2DM in the STOP-NIDDM trial. On the other hand, no association was observed in Pima Indians<sup>79</sup>. Larger studies are needed to determine the magnitude of risk imparted by *APM1* variation.

### **Transcription factor 7 like 2 (*TCF7L2*) gene and type 2 diabetes**

The most exciting and promising gene associated with type 2 diabetes is the *TCF7L2* gene. Grant and colleagues<sup>80</sup> searched for a T2DM susceptibility gene under the suggestive linkage peak on chromosome 10q in an Icelandic population. They identified a single nucleotide polymorphism in intron 3 of the transcription factor 7 like 2 gene (*TCF7L2*). Strong associations were also found in a Danish population and a United States Caucasian population, with a relative risk of 1.45 for heterozygotes and 2.41 in homozygotes. Two additional noncoding SNPs were closely correlated with this SNP. By far, this gene has shown greatest promise as a strong candidate for type 2 diabetes risk since positive replication has been reported by virtually all the studies conducted so far<sup>81-88</sup>, including our own study in south Indian population<sup>89</sup> and by Chandak *et al*<sup>90</sup> in a western Indian population studied at Pune where the intronic SNP has been shown to be associated with type 2 diabetes (Table II). Although the exact role of *TCF7L2* is unknown, it appears to act through the Wnt pathway.

The search for diabetes susceptibility genes on most other chromosomes is ongoing. Despite initial identification through a linkage signal, which would be expected to select relatively strong effects, each of these variants has only modest individual effect (odds ratio <1.4). Available data suggest that with respect to type 2 diabetes which is a polygenic disease (i) the effects of individual

variants are likely small; (ii) multiple variants probably contribute to replicated linkage signals, and (iii) most variants are noncoding and regulatory rather than altering protein structure.

The eventual genetic landscape of T2DM will integrate a large number of genes, many with multiple variants. As these susceptibility genes are located in different pathways in the complex physiologic network that causes T2DM, we can expect many of the variants to interact with each other. Sorting out these complex gene-gene interactions will require very large sample sizes. Different environmental stresses, population differences in activity, and different diets clearly cause some genes to manifest as a disease phenotype. The effect of a common gene variant between populations that have very different diets and exercise habits might be expected to be totally different, thus explaining some instances of lack of replication. Given the likely extensive role of intronic and intergenic DNA in determining phenotype, a major role of sequence variants in noncoding regions in T2DM pathogenesis should be anticipated. Ignoring this wealth of knowledge will impede our continued quest to understand the role of genes in T2DM susceptibility. Our increased understanding of such phenomena will throw new light on how common variants can alter disease susceptibility, and this is essential to understand the physiologic importance of the genetic associations that are uncovered. The utility of genetic approaches will depend on a holistic understanding of the interactions among the genes, and also between genes and the environment.

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