

## Genetic Epidemiology of Adult Onset Type 2 Diabetes in Asian Indian Population: Past, Present and Future

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**ABSTRACT** Incidence and prevalence of Type 2 Diabetes mellitus (T2DM) continue to rise in Indian populations. Despite known roles for obesity, sedentary lifestyles and diet, genetic predisposition accounts for significant risk. The identification of susceptibility loci for both monogenic and typical (oligogenic) diabetes have introduced novel genes, pathways and mechanisms of diabetes pathogenesis. Very little data is available on T2DM susceptibility loci in Asian Indian population. An extensive consortium based approach is required to identify the susceptibility locus and genes responsible for common form of familial diabetes in India. By defining the genetic susceptibility loci, such studies will eventually facilitate a direct, systematic exploration of the interactions of environmental factors, obesity, insulin resistance, and genetic predisposition in the pathogenesis of T2DM and prediabetic traits and also will open new pathways of exploration and therapy. This article is a systematic review of genetic epidemiology of adult onset Type 2 Diabetes in Asian Indian Population and related research initiatives in India and abroad.

“Arise, Awake, and stop not till the goal is achieved”

-Swami Vivekananda

### INTRODUCTION

Type 2 diabetes mellitus (T2DM; MIM125853) encompasses a diverse set of diseases marked by elevated levels of plasma glucose. Extensive studies in humans and animal models have led to a model for the pathogenesis of type 2 diabetes (T2DM) in which early and persistent insulin resistance in insulin target tissues in combination with the progressive decline in glucose-stimulated insulin secretion from the pancreatic  $\beta$ -cells results in postprandial hyperglycemia. Subsequently, overt fasting hyperglycemia surfaces with hepatic insulin resistance and elevated hepatic glucose production. Details of this general schema remain uncertain; however, both animal models and monogenic causes of T2DM suggest complex pathways in which insulin action and insulin secretion are intertwined. Thus, Type 2 diabetes is likely to be a heterogeneous disorder that may result from defects in one or more diverse molecular pathways. Rare monogenic forms of T2DM (mostly MODY) account for only 5% of diabetes. Most monogenic forms of diabetes are caused by defects in insulin secretion as compared to syndromic or common multifactorial T2DM in which insulin resistance and obesity play the major role.

Three key defects mark the onset of hyperglycemia in T2DM: increased hepatic glucose production, diminished insulin secretion, and impaired insulin action. Unfortunately, at the time of hyperglycemia, glucose and possibly lipid toxicity obscure the primary defects. Prospective and cross-sectional analyses of euglycemic individuals at risk (relatives of T2DM individuals) circumvent this dilemma, and suggest a key early and predictive role of reduced insulin sensitivity in T2DM pathogenesis. Nonetheless, few individuals with genetic insulin resistance develop diabetes, and 25% of nondiabetic individuals may have insulin sensitivity as low as that seen in T2DM. The increase in hepatic glucose production is a late event, occurring only at the onset of hyperglycemia and glucose intolerance. Consequently, the importance and timing of a  $\beta$ -cell defect have been hotly debated. Obese individuals, those with a family history of diabetes, and individuals with impaired glucose tolerance (IGT) are all characterized by absolute hyperinsulinemia until the development of overt hyperglycemia. In contrast, when  $\beta$ -cell function is viewed in the context of reduced insulin sensitivity, considerable data support the early failure of insulin secretion in T2DM pathogenesis. Animal models also support this concept: both reduced insulin secretion and

reduced  $\beta$ -cell mass precede diabetes in the GK rat model, yet an experimental mouse with isolated, pro-found muscle and adipose tissue insulin resistance develops only IGT without overt diabetes. Despite much study, the signals that cause normal  $\beta$ -cell compensation and hyperinsulinemia, the mechanisms of this compensation, the point in the pathogenesis of T2DM where this compensatory mechanism fails, and the etiology of this failure all remain subjects of speculation. Insulin sensitivity and insulin secretion deteriorate in parallel in most human T2DM. The remarkable difficulty in uncoupling these two defects may suggest a common mechanism.

Despite the diverse phenotypic nature of T2DM, monozygotic and dizygotic twin studies, family studies, and marked differences in disease prevalence across populations all provide convincing evidence for an important role of genetic susceptibility loci in T2DM pathogenesis. Based on epidemiological data, the total sibling relative risk ( $\lambda_s$ ) has been estimated at 3-4, although the number of loci that contribute to this risk is unclear (Barroso 2005; Elbein 2002; Kahn et al. 1996; McCarthy 2004; O'Rahilly et al. 2005; Permutt et al. 2005). Identification of the genetic components of type 2 diabetes is the most important area of diabetes research because elucidation of the diabetes genes (alleles) will influence all efforts toward a mechanistic understanding of the disease, its complications, and its treatment, cure, and prevention (Olefsky 2002).

### EPIDEMIOLOGY OF T2DM IN INDIA

The worldwide prevalence of diabetes for all age-groups was estimated to be 2.8% in 2000 and is predicted to be 4.4% in 2030 by the World Health Organization (WHO) (Wild et al. 2004). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030. WHO has predicted that India would experience the largest increase (48% increase in total population and 168% increase in population with >65 years of age) in type 2 diabetes and would have the greatest number of diabetic individuals in the world by the year 2030 (31.7 million in 2000 to 79.4 million in 2030).

Data on the prevalence of type 2 DM in sub-continental Indians is limited, considering the socio-economic and rural-urban disparity, and

the great cultural, geographical and racial diversity of our country. The WHO 2030 prediction on India was extrapolated from the National Urban Diabetes Survey (NUDS) published by Diabetes Epidemiology Study Group in India (DESI) (Ramachandran et al. 2001). This Study was conducted on 11,216 subjects 20 years or older, from six metropolitan cities (Chennai, Bangalore, Hyderabad, Mumbai, Calcutta and New Delhi). Age standardized prevalence of diabetes and impaired glucose tolerance were found to be 12.1% and 14.0 % respectively in NUDS study with no gender difference. Subjects under 40 years of age had a higher prevalence of IGT than diabetes (12.8% vs. 4.6%,  $p < 0.0001$ ). Diabetes showed positive and independent association with Body Mass Index (BMI), Waist to Hip Ratio (WHR), family history, sedentary physical activity etc. The DECODE -DECODA study group on behalf of the European Diabetes Epidemiology Group and the International Diabetes Epidemiology Group found that DM prevalence in Indians starts increasing at a BMI of 15-20 kg/m<sup>2</sup> compared with greater than 25 kg/m<sup>2</sup> in Chinese, Japanese and Europeans (DECODE-DECODA Study Group 2003).

Lack of proper representation from different socioeconomic and geographic regions (e.g. overrepresentation from higher economic group population from metro cities) is probably a major factor for over or under estimation of DM prevalence in India by different published epidemiological studies. Recently a random multistage cross-sectional population survey was undertaken to determine the prevalence of type 2 diabetes mellitus (DM) in subjects aged 25 years and above in India by the Diabetes India group, Mumbai called Prevalence of Diabetes in India study (PODIS) (Sadikot et al. 2004). The study was carried out in 77 centers (42 urban and 35 rural) to reflect the size and heterogeneity of the Indian population. 18 363 (9008 male and 9355 female) subjects were studied of which 10 617 (5379 males and 5238 females) were from urban areas and 7746 (3629 males and 4117 females) from rural areas. Blood samples were taken after 10-12 hr. of fasting and 2 hr. after 75 g of oral glucose intake. Subjects were categorized as having impaired fasting glycemia (IFG) or DM using the 1997 American Diabetes Association (ADA) or having impaired glucose tolerance (IGT) or DM using the 1999 WHO criteria. The

age and gender-standardized prevalence rate for DM using the ADA criteria was 3.6% whilst that using the WHO criteria was 4.3% for total Indian cohort. This study observed a significant difference in DM prevalence between rural and urban population. The respective standardized prevalence of DM, using the two criteria was 4.7 and 5.6%, respectively in the urban Indian population and 2.0 and 2.7% in the rural Indian population. The standardized prevalence rate for IGT in the total Indian, urban and rural populations was 5.2, 6.3 and 3.7%, respectively under WHO criteria. This study showed that use of the ADA criteria would underestimate the prevalence of DM by not diagnosing subjects showing a poor response to a glucose challenge. Other than differences in DM prevalence between urban and rural region, this study also observed significant difference of DM prevalence between different geographical regions of India. West (4.02% and 4.34%) and South (3.83% and 4.42%) showed significantly higher prevalence of DM and IFG compared to North (3.12% and 3.67%), East (2.79% and 2.76%) and Central (2.58% and 2.39%) region. This difference in DM prevalence is more striking in urban regions as compared to rural regions. There was no significant difference in IFG prevalence in rural regions of different geographic zones (personal communication Diabetes India).

### GENETICS OF T2DM

Although physiologic and epidemiologic studies provide clues to the pathophysiology underlying T2DM, the molecular mechanisms for the strong genetic predisposition cannot be explained. Many laboratories have chosen a genetic approach to define early T2DM pathophysiology. This approach is supported by:

1) The observation of a wide spectrum of diabetes prevalence in different ethnic group's world wide. Part of this observed ethnic variability can be attributed to non-genetic environmental and cultural factors; however, the observation that the disease prevalence varies substantially among ethnic groups that share similar environment, supports the idea of genetic factors contributing to disease predisposition.

2) Apart from genes, families share environments, culture and habits, yet familial aggregation of the disease is another source of

evidence for a genetic contribution to the disease. Different observations like the nearly 4-fold increased risk for T2DM in siblings of a diabetic proband compared with the general population ( $\lambda_s$  of 3.5 to 4), the increase in risk of T2DM in the offspring of one affected parent to an odds ratio (OR) of 3.4–3.5 and to 6.1 if both parents are affected etc. substantiate the role of genetic factors in T2DM etiology.

3) Twin studies suggesting that T2DM susceptibility can be explained by only a few genetic loci (oligogenic inheritance). Several studies of twin concordance rates have been undertaken in T2DM, and estimates for concordance rates ranged from 0.20 to 0.91 in Monozygotic (MZ), while in Dizygotic (DZ) the range was 0.10–0.43. In spite of several caveats in twin studies, the evidence is compelling that T2DM has a substantial genetic component (Barroso 2005).

4) Data from different laboratories supporting a genetic basis for insulin sensitivity and insulin secretion.

Single gene causes of T2DM, account for fewer than 5% of all cases. Interestingly, most single gene disorders impair insulin secretion or  $\beta$ -cell mass rather than insulin action. Researchers world wide have undertaken genome-wide linkage mapping studies in multiple populations to identify loci for common T2DM susceptibility genes or related quantitative traits including insulin sensitivity, insulin secretion, and obesity. Loci for T2DM have been reported on chromosomes 1q21, 2q, 3, 5, 11q, 12q, and 20q, among others (Stern 2002). *NIDDM1* was mapped to 2q in Hispanic sib pairs (Hanis 1996) and subsequently localized the gene to calpain10 (CAPN10) using linkage disequilibrium (Horikawa 2000). Among the best replicated regions is chromosome 1q21-q24, which was mapped independently in Utah Caucasians and in Pima Indians and confirmed in English, French, Amish, Chinese and several other studies. To date, more than 50 linkage studies (genome wide and fine mapping on smaller intervals) have been conducted in a variety of populations with a number of chromosome regions demonstrating at least suggestive evidence for linkage (LOD > 2), but only a few regions have shown significant evidence for linkage (LOD > 3.6). Fewer regions still have been replicated in multiple studies. Linkage studies in T2DM are facing similar problems as encountered in other complex

diseases i.e., a lack of replication of peaks of linkage and difficulty in identifying the underlying genes.

Progress in gene identification for more common, multifactorial forms of type 2 diabetes has been slower, but there is now compelling evidence that common variants in the PPARG, KCNJ11, CAPN10 and HNF4 $\alpha$  genes influence T2DM susceptibility. Three common intronic variants of CAPN10 that showed linkage to T2DM in Hispanics, were associated with T2DM in Hispanics and Finns, altered gene transcription, and reduced muscle mRNA levels. However, its role in other populations is unclear. Non-coding variants of the sulfonylurea receptor gene (ABCC8) were associated with altered insulin secretion and T2DM in multiple populations. The Pro12Ala variant of the PPAR $\gamma$ 2 gene has been associated with alterations in BMI, insulin sensitivity, and most recently diabetes risk. Several studies suggest a role for the glycogen targeting subunit of type 1 protein phosphatase (PPP1R3) in insulin sensitivity and T2DM. Evidence is less clear for the insulin VNTR and a common variant of insulin receptor substrate 1 (IRS1, Gly972Arg) that may impair insulin secretion and action. Recent independent publication from Finnish and Ashkenazi Jews population showed polymorphisms near P2 promoter of HNF4 $\alpha$  that is associated with Type 2 Diabetes. In genetic association database (<http://geneticassociation.db.nih.gov/>) 562 entries (as of August 2005) can be obtained after a search using Type 2 diabetes as broad phenotype category keyword and 124 entries after filtering for positive association [Association Y/N? = Y]. Associations of many of those variants have not been replicated in other population or another cohort and experimental evidence towards elucidating the role of most of those variants in common multifactorial form of T2DM pathogenesis is lacking.

Asian Indians (people from India, Pakistan, Bangladesh, Sri Lanka etc.) have surprisingly high prevalence of type 2 diabetes compared to Caucasians. The high racial predisposition is evident from the studies in native Indians as well as migrant populations in any part of the world. However, the incidence of obesity, an important risk factor in the development of type 2 diabetes, is significantly lower in Asian Indians compared to Caucasians. Though westernization of lifestyle with dietary changes and lack of exercise may

play a role in increased prevalence of type 2 diabetes in migrant Asian Indians, various epidemiological studies have shown that these factors alone are not sufficient to explain this finding. Excessive insulin resistance in Asian Indians compared to Caucasians may be one of the contributing factor. This difference in the degree of insulin resistance may be explained by either an environmental or a genetic factor or by combination of both (Abate and Chandalia 2001; Dhawan 1994; Gopalan 2001; Mohan 2004; Radha et al. 2003; Ramachandran et al. 2004; Ramachandran 2005; Snehalatha 2003).

### **Search for T2DM gene(s) in Asian Indian Population**

To date, only one published genome wide linkage analysis towards finding the T2DM loci in Asian Indian population is available. A 10cM density genome wide scan with 403 microsatellite markers was performed in a set of 99 complex families (including 535 individuals) of North-Eastern Indian origin (Hindu or Muslims, whose ancestors had migrated from the port of Calcutta) ascertained from Mauritius and in a second cohort of 35 complex Tamil Indian families ascertained through at least two T2DM affected individuals from Pondichery, India (Francke et al. 2001). Model free two point and multipoint linkage analysis was performed using the Mapmaker-sibs (MLS) and maximum-likelihood binominal (MLB) for autosomal markers. A suggestive linkage with T2DM was observed for 3q22 region (MLS LOD= 2.06) at marker D3S1292 (164.85cM, 1 LOD interval= 156.68-173.88 cM), 1q44 region (MLS LOD=2.14) at marker D1S2836 (318.92cM) and 8q23 region (MLS LOD=1.73) at marker D8S1784 (131.88cM) in Mauritian pedigrees. Dense marker analysis on chromosome 8q23 improved the linkage for T2DM at 121.98 cM (D8S1779, MLS LOD= 2.55, 1 LOD interval= 109.67-143.09cM). Nominal replication of T2DM linkage was observed only for 3q22 region (MLS LOD=1.36) at marker D3S1292 and a weak evidence for a novel linkage region at D16S407 (MLS LOD= 1.14, 16p13-pter) in Pondicherian pedigrees. Ordered-subset analysis based on family body mass index ranking showed a suggestive evidence for linkage with T2DM at position 273cM on chromosome 2q37 (D2S125, 273cM, MLS=3.03) including 24 Mauritian T2DM families with the lowest BMI. Other interesting

regions that emerged through the same analysis are 8q23 (D8S514, 129cM, MLS=2.26), 16q12 (D16S415, 67cM, MLS=1.96) and 19p13.3 (D19S414, 15cM, MLS=2.20). Recently a study has been undertaken by University of Pittsburgh, USA and Guru Nanak Dev University, India in Khatri Sikh population (North Indian) which promises a genome wide search to map Asian Indian diabetes (T2DM) susceptibility gene by family based linkage study (Sanghera et al. 2005)

Some genetic studies have been performed on Indian Type 2 diabetic subjects in the southern part of India, but most of those studies are under powered, ill designed, and used monogenic disease approach to solve complex disease genetics. Many of these studies looked at one or two exonic mutation in case-control association study approach, but they are too rare to account for common familial form of T2DM in Indian population, although may explain certain rare monogenic forms of T2DM. Except a few reports (Das and Maji 1999; Dixit et al. 2005; Gill et al. 1990, 1991; Hitman et al. 1987; Inamdar 2000) most of the genetic association studies are on Asian Indians of South Indian (Dravidian) origin. Considering the high genetic/ ethnic heterogeneity in this geographic region those studies are extremely inadequate.

Study of PuVII RFLP site in Islet amyloid polypeptide (IAPP) or amylin gene failed to find any significant association with T2DM in a well-characterized population of 62 unrelated Dravidian subjects with non-insulin-dependent diabetes mellitus and 56 normal Dravidian controls (McCarthy 1992) from South India. McCarthy (1993) also studied the role of glucokinase (GCK) gene in a cohort of 168 South Indian Type 2 diabetic subjects and 70 racially-matched control subjects. Out of two (CA)<sub>n</sub> marker studied GCK(3') marker showed a significant difference between the Type 2 diabetic subjects and control subjects ( $p = 0.009$ ) with an increase of the z allele (78.0% vs. 66.4%) and a decrease of the z + 2 allele (13.7% vs. 25.0%) amongst the diabetic subjects. Linkage analysis was negative for this region. Hitman et al. (1995) examined the role of a missense mutation in the insulin receptor substrate-1 gene (IRS-1) at codons 972 (glycine to arginine) with restriction enzymes BstNI in a population from South India and failed to find any significant association with T2DM. Baker et al. (1994) observed a significant difference in genotype distribution of apolipoprotein D (apo

D) genotypes between diabetic subjects ( $n = 110$ ) and controls ( $n = 88$ ;  $p = 0.004$ ) of South Indian ethnicity studied through Taq 1 polymorphism analysis. Pontiroli et al. (1996) evaluated the allele and genotype frequencies of GLUT1 and GLUT4 restriction fragment length polymorphism (RFLP), revealed by digestion with XbaI for GLUT1 and KpnI for GLUT4, in Asian Indian population. Positive results were found for the XbaI RFLP and showed an association of allele 1 with type 2 diabetes. Cassell et al. (1999) investigated the exon 8 insertion-deletion variant of UCP2 gene as a marker for glucose and body weight homeostasis in 453 South Indian subjects and found an association in women between the UCP2 exon variant and body mass index ( $p = 0.018$ ). These findings were replicated in a separate group of South Indian subjects ( $n = 143$ ,  $p < 0.001$ ) irrespective of sex. No association was found between UCP2 and Type II (non-insulin-dependent) diabetes.

Leprêtre et al. (1998) reported a study of 10 candidate genes presumably involved in diabetes or insulin resistance or obesity among Pondichurian Tamil Indians. Forty-nine families with at least two affected patients in the sibship (567 individuals) were selected and tested by PCR-RFLP techniques for reported mutations in 10 diabetes or obesity candidate genes namely: glucagon receptor (GCG-R), insulin receptor substrate 1 (IRS-1), insulin receptor (INSR), human b3 adrenergic receptor (Hb3AR), fatty acid binding protein 2 (FABP2), mitochondrial tRNA<sup>Leu</sup>(UUR), sulphonylurea receptor (SUR22), human uncoupling protein (UCP1) and the glycogen associated regulatory subunit of protein phosphatase-1 (PPP1R3). No mutations were found in glucokinase, glucagon receptor and mitochondrial genes in any of the 49 probands. No evidence of association between any of these gene variants and non-insulin-dependent diabetes mellitus (NIDDM) or quantitative traits related to NIDDM (including body mass index, waist/hip ratio, insulinaemia, glycaemia, triglycerides and total cholesterol) was found in this cohort. However, this study cannot exclude that these genes may contribute to the polygenic background of the metabolic syndrome in Pondichery, India.

Using Starch gel electrophoresis Das and Maji (1999) studied the Phosphoglucosyltransferase (PGM1) and Glyoxalase I (GLO) enzyme isoforms in an East Indian (Bengali speaking) cohort of

195 NIDDM and 195 control individuals, but failed to find any genotypic association.

Cassell et al. (2002) evaluated whether 4 SNP (UCSNP 44, 43, 19 and 63), haplotype or haplotype combination of Calpain 10 (CAPN10) gene contribute to increased susceptibility to impaired fasting glucose (IFG)/impaired glucose tolerance (IGT) and type 2 diabetes in a South Indian population. Two study groups were used: 95 families ascertained through a proband with type 2 diabetes and 468 subjects recruited as part of an urban survey (69.1% with normal glucose tolerance, 12.8% with IFG/IGT, and 18.2% with T2DM). Family-based association studies did not reveal any excess transmission for any of the four individual SNP alleles to probands with type 2 diabetes, also found no evidence of excess transmission of any haplotype to type 2 diabetic offspring. In the urban survey, there was no difference between the genotype frequencies of normoglycemic control subjects, IFG/IGT, and T2DM for the UCSNP44, 43 and 19 variants. However, the presence of the uncommon allele 2 (T) of UCSNP63 was significantly increased in IFG/IGT subjects, with a frequency of 17% compared with control subjects (4%,  $P=0.001$ ). Although there was only a slight nonsignificant increase in frequency of allele 2 in the unrelated type 2 diabetic subjects (5.9%), there was a significant increase (11%) in the type 2 diabetic probands ( $P=0.02$ ). Analysis of the five common haplotypes found the frequency of the 1112 haplotype also significantly increased in both the urban IFG/IGT subjects (global  $P = 0.001$ ) and the probands (global  $P = 0.004$ ). Interestingly in this study UCSNP19 was not ( $P= 0.009$ ) in Hardy-Weinberg equilibrium. Further analysis found that the 1112/1121 heterozygous haplotype combination was associated with an increased risk of IFG/IGT in urban subjects (OR =10.74,  $P =0.001$ ) and urban type 2 diabetic subjects (OR=6.52,  $P=0.015$ ) and probands (OR=5.78,  $P = 0.025$ ). Due to relative infrequency (frequency= 0.05-0.09) of the "at-risk" combinations in the South Indian population this study suggests that calpain 10 is not a common determinant of susceptibility to type 2 diabetes. Analysis of the same population genotype data set by North et al. (2003) using a neural network based method revealed arguably stronger evidence of association for UCSNP63 of CAPN10.

Venkatesan et al. (2003) examined the relation of Pro12Ala variant in PPAR- $\gamma$ 2 in Type 2

diabetes, insulin resistance and obesity. This variant was examined using PCR-RFLP technique on 1145 randomized subjects from the population based ongoing Chennai Urban Rural Epidemiological Study (CURES). 1015 subjects (88.6%) had the PPAR- $\gamma$ 2 Pro12Pro genotype. 125 subjects (10.9%) had Pro12Ala genotype and 5 subjects (0.4%) had Ala12Ala genotype. In males, the prevalence of Pro12Ala was more in controls (14.4%) compared to Insulin resistant (IR) (9.3%,  $p= 0.50$ ), Impaired Glucose Tolerant (IGT) (8.9%,  $p=0.61$ ) and Diabetic (6.8%,  $p=0.076$ ) subjects, suggesting a protective role of this polymorphism. However, no such trend was seen in females. Further, the Pro12Ala did not show any association with BMI. Authors also investigated the interaction between the Pro12Ala of PPAR- $\gamma$ 2 and Gly482Ser of PGC-1 (PPAR-gamma coactivator-1) gene. Gly482Ser polymorphism was genotyped also by using PCR-RFLP in a subset of these samples ( $n=163$ ). In this preliminary study no indication for the additive effects of the two polymorphisms on the diabetes status was observed. Further in a cohort of 87 type 2 diabetic subjects and 81 subjects with Normal Glucose Tolerance (NGT) of CURES study Mohan et al. (2005) analyzed Thr394Thr (G $\rightarrow$ A), Gly482Ser and +A2962G polymorphism of PGC-1 $\alpha$  gene and found that Thr394Thr polymorphism in the PGC-1 $\alpha$  gene is associated ( $p=0.001$ ) with higher visceral and central abdominal fat and with type 2 diabetes in urban Asian Indians. There was no association between Gly482Ser or +A2962G polymorphisms and body fat distribution.

Abate et al. (2003) studied if plasma cell membrane glycoprotein (PC)-1 K121Q and insulin receptor substrate-1 (IRS-1) G972A polymorphisms contribute significantly to susceptibility to insulin resistance in Asian Indians. This study recruited 638 individuals originated from the Asian Indian subcontinent (India, Pakistan, and Bangladesh) by public advertisement and offering free screening for cardiovascular risk factors at University of Texas Southwestern Lipid and Heart Disease Risk Management Clinic (Dallas, TX). A significantly higher insulin area under the curve during oral glucose tolerance testing ( $P < 0.0001$ ) and lower insulin sensitivity during hyperinsulinemic-euglycemic clamps ( $P = 0.04$ ) were found in Asian Indians with PC-1 121Q variant compared with Asian Indians with wild-type PC-1. IRS-1 G972A was not associated

with any change in insulin sensitivity. This study concluded that the PC-1 K121Q polymorphism associates with primary insulin resistance in migrant Asian Indians and relatively high frequency of this polymorphism thus may be one factor contributing to insulin resistance susceptibility in Asian Indians. Chiu et al. (2000) investigated the role of the G→A polymorphism in the hepatic glucokinase (GCK) promoter on insulin sensitivity and beta cell function in 63 normotensive Asian Indians with normal glucose tolerance recruited from local Indian temples of metropolitan Los Angeles area. Compared to the GG group, the GA/AA group had a lower hepatic insulin sensitivity  $ISI_H$  ( $p=0.002$ ), a lower total body insulin sensitivity  $ISI_M$  ( $p=0.009$ ), a higher Beta cell function %B ( $p=0.014$ ) measured under Homeostasis model assessment, and a higher insulogenic index  $dI/dG$  ( $p=0.030$ ). Multivariate analysis revealed that this polymorphism is an independent determinant for  $ISI_H$  ( $p=0.019$ ) and along with age, waist-hip ratio, gender, and diastolic blood pressure accounted for 51.5% of the variation of  $ISI_H$ . However, this polymorphism was a weak, but independent determinant for  $ISI_M$  ( $p=0.089$ ) and %B ( $p=0.083$ ). Furthermore, it had no independent effect on  $dI/dG$  ( $p=0.135$ ). These data suggest that the G→A polymorphism in the hepatic GCK promoter is associated with hepatic insulin resistance in Asian Indians.

Jackson et al. (2004) investigated the role of three polymorphisms in three genes namely: Ala45Thr polymorphism of neurogenic differentiation-1 [*NEUROD1*], Ser199Phe polymorphism of Neurogenin-3 [*NEUROG3*] and Ala98Val polymorphism of Hepatic nuclear factor-1 $\alpha$  [*TCF1* or *HNF-1 $\alpha$* ] in T2DM. They have also studied the effect of interactions between these variants using PCR/RFLP assays in 454 subjects recruited as part of a population survey in South India. Additionally, 97 South Indian parent-offspring trios were studied. Only TCF1 polymorphism showed genotypic ( $p=0.037$ ) as well as allelic ( $p=0.02$ ) association with T2DM in case-control study. The families were not studied because there was insufficient power for analysis due to the low variant allele frequency of TCF1 polymorphism. NEUROD-1 and NEUROG3 markers were not significant in both case-control and family based study. Polymorphisms of all three genes were associated with either fasting blood glucose (FBG) and/or 2-h blood glucose (BG) in either the total data set or when restricted

to a normoglycemic population. A monotonically increasing effect, dependent on the total number of risk-associated alleles carried, was observed across the whole population ( $P < 0.0001$  for FBG and 2-h BG) which indicates the possible role of these polymorphisms in overall glucose intolerance in South Indian population.

Abate et al. (2005) evaluated the role of Ectonucleotide pyrophosphate phosphodiesterase or ENPP1 (also called Plasma cell glycoprotein-1, PC-1) genes' exon4 K121Q polymorphism in prediction of type2 diabetes in two Asian Indian populations that differ in susceptibility to diabetes and environmental exposure. Two cohorts included 679 nonimmigrant South Asians participants of the Chennai Urban Rural Epidemiological Study (CURES) living in Chennai, India (223 with type 2 diabetes) and 1,083 migrant (new immigrants or first generation from India, Pakistan, or Bangladesh) South Asians living in Dallas, Texas (121 with type 2 diabetes) recruited by public advertisement and by offering free screening for cardiovascular risk factors at the University of Texas Southwestern Lipid and Heart Disease Risk Management Clinic. Patients with type 2 diabetes were included in these cohorts if they had diabetes onset before the age of 60 years. The prevalence of subjects carrying the polymorphic ENPP1 121Q allele was 25% in the nondiabetic group and 34% in the diabetic group of South Asians living in Chennai ( $P=0.01$ ). The prevalence in the nondiabetic and diabetic groups were 33 and 45% ( $P=0.01$ ) for the South Asians living in Dallas. This study supports the hypothesis that ENPP1 121Q predicts genetic susceptibility to type 2 diabetes in South Asians.

In most of these published Type 2 diabetes genetic association studies investigators selected these variations in putative functional candidate genes, which might affect insulin signaling cascade directly or indirectly. Due to lack of high throughput genotyping techniques and data analysis algorithm none of these studies have implemented unbiased genome wide association analysis or extensive linkage disequilibrium (LD) based approach. Recent findings have revived interest in the role played by the brain in both glucose homeostasis and the mechanism linking obesity to type2 diabetes. Also there is mounting evidence of changes in  $\beta$ -cell mass (genes involved in  $\beta$ -cell growth and survival) and concomitant change of insulin

secretion in the pathogenesis of type 2 diabetes. A whole genome association study followed by an advanced pathway analysis can only lead to an unbiased conclusion about genes involved and mode of their interactions in T2DM pathogenesis in Asian Indians.

### **Search for Genetic Factors Associated with Diabetic Complications in Asian Indians**

Microvascular and macrovascular complications in relation to diabetes mellitus are responsible for major morbidity and mortality. Prevention of these complications should be the aim while managing diabetes. Retinopathy, nephropathy and neuropathy are microvascular complications and macrovascular complication affects the heart, brain and foot (Maji 2004). Ethnic differences in prevalence of diabetic complications indicate the involvement of genetic factors in these phenotypes. Several studies suggest that genetic factors could be promoting the severity and rapidity of onset of micro and macrovascular complications in diabetic patients (Rema M et al. 2002).

Hawrami (1991) by southern blot hybridization technique tried to evaluate the role of several HLA region markers for predisposition to diabetic retinopathy in a cohort of South Indian Type 2 (non-insulin-dependent) diabetic patients. Patients were subdivided into those with exudative maculopathy (n = 53), proliferative retinopathy (n = 40) and patients free from diabetic retinopathy with a minimum disease duration of 15 years (n = 45). This study suggests that there is a genetic predisposition to proliferative retinopathy in Type 2 (non-insulin-dependent) diabetes of South Indian origin and that this is determined by polymorphism of the heavy chain immunoglobulin genes [using restriction enzyme Pvu II and probe for the switch region of the immunoglobulin IgM heavy chain gene (S mu)] located on chromosome 14.

Kumaramanickavel et al. (2001) studied a dinucleotide microsatellite repeat length polymorphism [(GT)<sub>n</sub>] upstream to the promoter region of tumor necrosis factor (TNF) gene in an Indian cohort (mixed south and north Indian ethnicity) of 100 patient with diabetic retinopathy, 107 patients without retinopathy and 50 control individuals without diabetes or retinopathy to evaluate the role of this gene in susceptibility for the development of retinopathy. In this study

population authors observed 18 alleles ranging from 97 to 131 base pairs (bp). Allele 4 (103 bp) had a higher prevalence (9.81%) in the Diabetic Non Retinopathy (DNR) group compared to that in the Diabetic Retinopathy (DR) group (2.5%; P=0.002) and control group (2%; P=0.039). When the allele 4 data were separated into those of north and south Indian patients, results were still significant, the P value for north Indians being 0.003 and for the south Indians being 0.008. Patients with retinopathy and allele 8 (111 bp) had a tendency to develop proliferative diabetic retinopathy (PDR). In this study of Indian subjects, it is suggested that allele 4 is a low risk allele for developing retinopathy and allele 8 (111 bp) shows an association with PDR. Considering the cohort size and number of observed alleles of the TNF microsatellite this study is extremely underpowered and also not in agreement with a previously published study of Hawrami et al. (1996) on a Dravidian (South Indian) cohort. Fifteen (15) alleles were observed for the same marker in a cohort of unselected NIDDM (n = 76), unselected IDDM (n = 99), non-diabetic controls (n = 96), NIDDM subjects with maculopathy (MAC), n = 55, NIDDM subjects with proliferative retinopathy (PR), n = 53, and Long term diabetic without retinopathy (LTD), n = 46, studied by Hawrami et al. (1996). After correction for the number of alleles studied, only the allele 9 (B9) had a significantly different distribution between patients with proliferative retinopathy and those without retinopathy (p = 0.04).

Kumaramanickavel (2002) also studied a pentanucleotide microsatellite repeat located 2.5 kb upstream of the transcription start site of the inducible nitric oxide synthase (iNOS) gene by polymerase chain reaction (PCR) in a cohort of 199 unrelated Asian Indian patients with 15 or more years of type 2 diabetes (divided into two groups: diabetic retinopathy and diabetic nonretinopathy). This study identified eleven alleles (ranging from 175 to 225 bp) of which allele 210 bp was significantly associated with retinopathy (p = 0.044, OR = 2.03; 95% CI 0.96-4.35). Alleles 200 and 220 bp were also significantly associated with no retinopathy and no serious retinopathy complications, respectively. From this study authors concluded that in Asian Indian population, allele 210 bp of the iNOS gene is a high-risk allele for developing retinopathy and alleles 200 and 220 bp protect an



individual from developing retinopathy or its complications.

In a similar study design polymorphisms in (CA)<sub>n</sub> di-nucleotide repeat present in upstream of the promoter of the aldose reductase (ALR2) gene was studied by Kumaramanickavel et al. (2003) to evaluate its association with diabetes retinopathy in the Asian Indian population. 105 diabetic patients with retinopathy and 109 diabetic patients without retinopathy were screened and 13 alleles of this marker were identified. The Z-2 allele (136 bp) showed an association with the DR group (13.81%) with a significant p value ( $p = 0.029$ ) when compared with the DNR group (7.34%). The Z-2 allele also showed a significant association with the DR patients who had proliferative retinopathy and maculopathy ( $p = 0.004$ ). This study infers Z-2 allele as a high-risk allele for diabetic retinopathy in the Asian Indian patients.

Gly82Ser polymorphism in exon 3 of the receptor for advanced glycation end products (RAGE) gene was studied by Kumaramanickavel et al. (2002) in a cohort of 100 diabetic patients with retinopathy (DR), 100 diabetic patients without retinopathy (DNR) and 50 control individual of Asian Indian origin (heterogeneous ethnicity) by PCR-RFLP method. The frequency of the Ser82 allele was significantly higher, 18% in the DNR group compared to 7% in the DR group ( $P=0.03$ ). The same genotype was 2% in the control group. This result suggests that Ser82 allele in the RAGE gene is a low-risk allele for developing DR in Asian Indian patients who have type II diabetes. This report has not been replicated in any other Indian cohort and due to ethnic heterogeneity this observed association may be a type I statistical error due to population stratification.

An ongoing population-based study called “Sankara Nethralaya-Diabetic Retinopathy Epidemiology and Molecular Genetic Study (SN-DREAMS 1)” promises to estimate the prevalence of diabetes and diabetic retinopathy in urban Chennai, Tamil Nadu, South India, and also to elucidate the clinical, anthropometric, biochemical and genetic risk factors associated with diabetic retinopathy in an anticipated cohort of total of 5830 participants (Agarwal et al. 2005)

South Asian type 2 diabetic patients have been shown to have a higher prevalence of nephropathy when compared to Europeans. Viswanathan et al. (2001) studied the association

of Angiotensin-converting enzyme (ACE) gene polymorphism and diabetic nephropathy in a cohort of 109 South Indian type 2 diabetic patients. The patients were subdivided into two groups: nephropathic ( $n=86$ ) and normoalbuminuric patients ( $n=23$ ). ACE gene insertion (I)-Deletion (D) polymorphism was analyzed by PCR amplification. The D allele was present in 80.2% of the nephropathic patients and 56.5% of the normoalbuminuric patients ( $P=0.039$ ; odds ratio 3.12). This study showed a positive association between the D allele (ID and DD genotype) of the ACE polymorphism and diabetic proteinuria in South Indian type 2 diabetic patients.

Dixit et al. (2005) studied the association of Cholesteryl ester transfer protein (CETP) TaqI B, D442G, and Apolipoprotein E (APOE) Hha I polymorphisms with T2DM and its complications in a North Indian cohort of 136 patients and 264 healthy controls ascertained mostly from SGPPI, Lucknow, Uttar Pradesh. Polymorphisms were detected using PCR-RFLP. CETP TaqI B polymorphism was not associated with the T2DM but B1B2 genotype was significantly ( $p=0.028$ ) associated with high risk of hypertension in diabetic patients ( $OR=3.068$ , 95% CI 1.183-7.958). In North Indians D442G variation in CETP gene was found to be absent. Frequency of APOE HhaI polymorphism was also not different between patients and controls. In diabetic patients having neuropathy and retinopathy significantly different levels of total-cholesterol [ $p=0.001$ ] and [ $p=0.029$ ] respectively] and LDL-cholesterol [ $p=0.001$ ] and [ $p=0.001$ ] respectively] were observed when compared to patients with T2DM only. However, lipid levels did not show any correlation with the CETP TaqI B and APOE Hha I genetic polymorphisms. This study suggests that CETP TaqI B and APOE HhaI polymorphism may not be associated with type II diabetes mellitus in North Indian population; however CETP TaqI B polymorphism may be associated with hypertension along with T2DM.

Guettier et al. (2005) evaluated the association between Ala54Thr polymorphism in the fatty acid-binding protein 2 (FABP2) gene as well as the T-455C and C-482T polymorphisms in the apolipoprotein C-III (APOC3) gene promoter with metabolic syndrome (MS) and dyslipidemia, defined according to National Cholesterol Education Program Adult Treatment Panel III in Asian-Indians (70 controls and 110 patients with

diabetes from the Chennai Urban Population Study). This study showed that controls carrying FABP2 Thr54 were more likely to have MS than noncarriers ( $P=0.031$ ; odds ratio=6.9 with a 95% confidence interval of 1.1, 43.9). Those carrying at least one polymorphic allele in both genes had a higher likelihood of having MS than wild type ( $P=0.003$ ; odds ratio=12.1 with a 95% confidence interval of 1.88, 77.6). Dyslipidemia was associated with the polymorphism as well. The polymorphisms were not associated with MS in patients with diabetes. Association of the polymorphisms with MS and dyslipidemia indicate their contribution to the high cardiovascular disease prevalence in this population.

#### **Indian Diabetes Research- working with “Ostrich attitude”**

Diabetes mellitus is described as ‘Madhumeha’ in ancient Indian Sanskrit literature dealing with health care systems, and is duly acknowledged in modern medical texts. However, detailed descriptions of the disease process and therapeutics prescribed in these classics could not get proper recognition (Tiwari 2005).

Arunachalam and Gunasekaran (2002) have mapped and evaluated diabetes research in India based on papers published during 1990–1999 and indexed in PubMed, Science Citation Index (SCI) and Biochemistry and Biophysics Citation Index (BBICI) and citations to each one of these papers up to 2000. They have also assessed the extent of international collaboration in diabetes research, based on papers indexed in SCI and BBICI. There is an enormous mismatch between the disease burden and the share of research performed in India. 837 unique papers from India consisting of 667 articles, 111 meeting abstracts, 31 letters, 25 notes and three editorials. Nearly 59.6% of Indian papers are covered by SCI. The 531 papers from India indexed in PubMed amount to 1.11% of the 47,877 papers from all over the world. India’s share of diabetes papers in SCI is 0.98% and in BBICI is 1.61%. In 1995, over 14% of the world’s diabetes patients were in India and yet India accounted for about 1% of research in diabetes. In all, 371 Indian diabetes papers have been cited 1657 times. More than 55% of Indian papers were not cited at all. India has published a very large percentage of their papers in low-impact journals: 578 (69%) Indian papers in journals of impact factor (IF) less than 1.0. Two

private research institutions (attached to hospitals) located in Chennai are among the leading producers of diabetes research papers in India. Diabetes Research Centre, Chennai, founded in 1972, has published 74 papers, and of these 45 was cited 289 times. Madras Diabetes Research Foundation (the parent hospital was founded in 1991) has published 27 papers, of which 16 were cited 53 times. More than 16% of the 534 Indian papers in diabetes indexed in SCI and BBICI (86 papers from 37 institutions) had resulted from collaboration with foreign authors. In diabetes research, India had collaborated with the UK in 40 papers and USA in 22 papers.

Similar situation prevails in the area of genetic epidemiology of T2DM research in India. Most of the research in this area was carried out on South Indian samples and with a few exceptions most of those research works are spearheaded by UK, US or French laboratories, as evidenced from the location of corresponding author of those publications and no acknowledgement to Indian Government funding agencies like Indian Council of Medical Research (ICMR), Department of Biotechnology (DBT), Department of Science and Technology (DST) or Council for Scientific and Industrial Research (CSIR).

Under the mission mode programs of Department of Biotechnology, Government of India, a major proposal on molecular genetic studies of type2 diabetes and diabetic retinopathy has been developed at Madras Diabetes Research Foundation, Chennai (<http://dbtindia.nic.in/r&d/humangenome.html>) with objectives to identify and characterize various genes that predispose to Type2 diabetes and its intermediary phenotypes such as Insulin Resistance, impaired glucose tolerance, perform gene expression and protein profiling of components of the signaling molecules, analyze drug responses in different single nucleotide polymorphisms (SNPs) of PPAR- $\gamma$  gene and elucidate genetic factors predisposing to diabetic retinopathy in India and to attempt clinico-genetic correlations of DR phenotype. The recent annual report of DBT indicate the progress of this project (DBT 2004 – 2005 Annual Report 2005) but no research publication appeared till date in any Pubmed Indexed journal acknowledging this mission mode project of DBT, India.

A public-private knowledge alliance between Nicholas Piramal India Limited, Mumbai and Institute of Genomics and Integrative Biology

(a Council of Scientific and Industrial Research Laboratory) was initiated on November 2001 as “GENOMED” project with a major research objective (Flag Ship Project) of developing biomarkers (Genetic markers) for Type 2 Diabetes. Till the final closing of this three years project on January 2005, this project failed to publish any research article or patent which can be evidenced as achievement of this project. Lessons can be learned from failures of such high investment projects, which may be useful for future successful project formulation and execution.

Incidence and prevalence of T2DM continue to rise in Indian populations. Despite known roles for obesity, sedentary lifestyles, and diet, genetic predisposition accounts for significant risk. The identification of susceptibility loci for both monogenic and typical (oligogenic) diabetes have introduced novel genes, pathways and mechanisms of diabetes pathogenesis. An extensive consortium based approach is required to identify the susceptibility locus and genes responsible for common form of familial diabetes in India. By defining the genetic susceptibility loci, such studies will eventually facilitate a direct, systematic exploration of the interactions of environmental factors, obesity, insulin resistance, and genetic predisposition in the pathogenesis of T2DM and prediabetic traits and also will open new pathways of exploration and therapy. It can be argued that India needs to strengthen research capabilities by increasing investment in these areas of research considerably. Substantive facilitation of international collaboration in research and support of cross-disciplinary research between basic life sciences researchers and medical researchers will facilitate this process. It is not the time to work with an “*Ostrich-attitude*” to avoid problems, but to face the problem and solve it to achieve the yet elusive goal. Time has come for Indian researchers and research policy makers to switch gear and give new direction towards taking diabetes research in India to global standard.

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