Genomics and proteomics of diabetes*

Today, the medical industry worldwide is building upon the knowledge, resources and technologies emanating from the human genome project to further our understanding of genetic and proteomic contributions to human health. While the West has begun to integrate aspects of genomics and proteomics into bedside applications, this area of specialization is still in its infancy in India. In order to assess the perception and long-term vision of the Government of India, academia and the pharmaceutical industry in terms of scientific developments in the field of genomics and proteomics in general, and of diabetes in particular, a two-day symposium was held recently in Chennai. Over 200 registered participants from all over the country directly benefited by the symposium.

During the inaugural function, the Madras Diabetes Research Foundation (MDRF) honoured Kiran Mazumdar Shaw, Chairman and Managing Director, Biocon Limited, Bangalore, with the 'Second MDRF Award for Innovation in R&D'. Stressing on the importance of research she said, 'a deeper understanding of cellular, molecular factors and genomics was necessary for a newer approach towards drug discovery'. Addressing the gathering, T. S. Rao, Adviser, Department of Biotechnology, Government of India, said centres of excellence were necessary to tackle diabetes at the grass roots level and congratulated MDRF for taking the lead in studying genomics and proteomics of diabetes in India. In his inaugural address, Jan C. Duke, Dean of Research and Education, Karolinska Institute (KI), Sweden, mentioned that if glucose was the only cause for diabetes, then with the advent of insulin it could have been cured. 'There is something deeper than that and to tackle it, research efforts should be

taken up collectively by both basic and clinical scientists utilizing the state-of-the-art technologies', he added.

G. Norstedt (KI) talked about the technological expertise available in KI in the area of genomics and metabolomics. He emphasized the need for computational knowledge to integrate these techniques and to obtain a systematic insight into different biological problems on experimental and clinical diabetes and associated metabolic disorders. Dwaipayan Bharadwaj (Institute of Genomics and Integrative Biology, New Delhi) spoke on 'The Indian genome variation consortium', whose aim is to discover informative repeat and single nucleotide polymorphisms in over 1000 genes of biomedically important metabolic and genetic networks. Through systematic bioinformatics analysis, he claimed that majority of the genes harbouring these variations in type 2 diabetes are clustered in or near the insulin-signalling network and are identified as potential sites for post-translational modifications. He summarized that type 2 diabetes mellitus may result from a large number of single nucleotide polymorphisms that impair modular domain function and post-translational modifications involved in signalling. Purnananda Guptasarma (Institute of Microbial Technology, Chandigarh) delivered a talk focusing on why two-dimensional gel electrophoresis (2-DGE) is still the method of choice for separating and analysing proteins by mass spectrometry, especially for performing protein identification tasks. He explained the evolutionary developments in 2-DGE protein separation techniques and briefed on different forms of mass spectrometry, and on how innovative applications of mass spectrometry aid in tasks ranging from examination of protein modifications to protein sequencing.

While receiving the 'Dr Mohan's DSC Gold Medal Oration Award', C. B. Sanjeevi (KI) explained how genomic studies could be transformed into bedside applications of predicting and preventing type 1 diabetes mellitus (T1DM). He claimed that approximately 89% of the newly diagnosed patients carry the high-risk susceptibility haplotypes (HLA-DQ8 and DQ2) and the remaining 11% develop

T1DM due to an interaction of genes and environmental factors. Several peptides were identified for each autoantigen pre-proinsulin, GAD65 and IA-2 that carried DQ6 motifs. He emphasized that docking studies using these peptides open up future studies of estimation of T-cells reacting to these peptides in newly diagnosed children with T1DM and prediabetic children positive for autoantibody markers and high-risk genetic markers for T1DM.

As the National Health Service in Sweden serves the entire population, it is possible to have biobanks, registers and health records, all based on the personal identity numbers providing a unique resource for the integration of experimental, clinical and epidemiological data for clinical studies. This was referred to by Jan C. Duke (KI) as 'structured clinical information'. In order to exploit this resource and its potential in genomic medicine, KI has developed a new Biobank Information Management System in collaboration with IBM. KI also hosts the largest twin register in the world, containing data on 85,000 pairs of twins, an invaluable tool to separate genetic and environmental factors involved in various diseases. Establishment of a large cohort with structured clinical information will enable us to collect genetic, environmental and lifestyle factors of potential importance for studying the underlying causes of major diseases, including diabetes.

Drawing on the results of the Chennai Urban Population Study (CUPS) and the Chennai Urban Rural Epidemiology Study (CURES), V. Mohan (MDRF, Chennai) revealed a strong association of ethnicity difference with genetic polymorphisms. While some genes like the Pro12Ala polymorphism of PPARg gene are protective against diabetes in Europeans, they do not appear to offer protection among Indians. Unlike in other ethnic groups, the Thr394Thr (G→A) polymorphism of PGC-1 is strongly associated with diabetes as well as body fat in Indians. Similarly, the Gly1057Asp polymorphism of IRS-2 gene predisposes Indians to diabetes, particularly in the presence of obesity. Though genetic factors undoubtedly play a major role in the predisposition of diabetes in Indians, environmental factors

contribute to over 50% of the risk. In CUPS, physical inactivity and family history of diabetes had a cumulative effect on the risk of diabetes. In CURES, analysis on gene–diet interaction revealed that adiponectin gene polymorphism [+10211 T>G] contributes to insulin resistance and diabetes, and also showed a cumulative effect with glycemic load. These studies indicate gene–environment interactions playing a major role in increasing the excess risk for diabetes in Asian Indians.

Progressive renal disease in certain proportion (20–30%) of individuals with type 2 diabetes and familial clustering provides clear evidence for genetic contribution to the development of end-stage renal disease (ESRD). B. K. Thelma (University of Delhi) hypothesized that RAAS (renin–angiotensin–aldosterone) pathway has a crucial role in the development of diabetic kidney disease. To test this hypothesis, a total of 12 SNPs from six genes, namely renin, angiotensinogen, angiotensin converting enzyme I, angiotensin II type 1 receptor and aldosterone synthase gene from the RAAS pathway and a related gene chymase were genotyped. This study revealed that SNPs Met235Thr in AGT, T>C (-344) in aldosterone synthase, and G>A (-1903) in CMA genes are significantly associated with diabetic renal disease. Although microalbuminuria is currently the only diagnostic tool available for early diagnosis of diabetic nephropathy, there are several limitations of the use of microalbuminuria as an index of renal dysfunction. Using the proteomics approaches of 2-DGE and mass spectrometry, Utpal Tatu (Indian Institute of Science, Bangalore) described the possibility of identifying additional protein markers that would augment accurate prediction of diabetic nephropathy. He claimed that using a larger cohort of diabetes patients, his team is currently developing a mass spectrometry-based high throughput diagnostic approach to detect protein markers in the urine sample that would predict the development of diabetic kidney disorders.

In his talk on 'Functional genomics approaches in modern biology', M. R. S. Rao (Jawaharlal Nehru Centre for Advanced Scientific Research, Bangalore) disclosed how during the course of the human genome project, several new technologies have been developed that are now available for scientists to address various fundamental questions pertaining to life processes, development and disease pro-

cesses. While appraising the disease gene-mapping studies, he emphasized the need to understand the biological relevance of each polymorphism and its effect on gene functions. Quoting the advent of genome-scanning technologies that have now uncovered an unexpectedly large extent of structural variation in the human genome such as deletions, duplications and large-scale copy-number variants (collectively termed as copy-number polymorphisms), Rao explained how these molecular changes could explain and contribute to human diversity and disease susceptibility.

Saurabh Ghosh (Indian Statistical Institute (ISI), Kolkata) delivered a talk on the advantages of multivariate phenotypes over binary end points in the genetic dissection of complex traits. Mapping a multivariate phenotype traditionally uses some function of quantitative values of sib-pairs or other sets of relatives as a response variable and marker IBD (identity-by-descent) scores as explanatory variables and is thus susceptible to violations in distributional assumptions. Ghosh introduced a new statistical model of linear regression formulation in which the response and explanatory variables are interchanged and the methodology can simultaneously incorporate qualitative and quantitative traits and can use data on n siblings as $(n - 1)$ independent observations. An application of the method was illustrated using data on alcoholism-related phenotypes from the COGA (Collaborative Study on the Genetics of Alcoholism) study.

C. G. Ostenson's (KI) talk dealt with the mechanism of impaired insulin release from the pancreatic islet b-cells, which is one of the crucial components in the pathogenesis of type 2 diabetes. Corroborating their previous studies in diabetic Goto-Kakizaki (GK) rat, marked decreases in exocytotic SNARE (soluble N-ethylmaleimide attachment protein receptor) complex proteins (VAMP-2, syntaxin-1A, SNAP-25, nSec1 and Munc13-1) were demonstrated in isolated islets of type 2 diabetic patients. Gene expression (Affymetrix microarray chips and qPCR) studies demonstrated several islet metabolic perturbations that may lead to reduced production of energy-rich ATP and thereby impair b-cell stimulus-secretion coupling for glucose.

In an expository talk, Partha Majumder (ISI, Kolkata) provided the necessary background and compulsions for adopting study designs to map genes for common

diseases in which data are not collected on families but on pairs of individuals. He described the statistical framework for analysis of such data and highlighted some essential precautions to be taken in such study designs with illustrations from type 2 diabetes studies.

While explaining how diabetic retinopathy (DR) is set to impose a major health concern in the country, M. Rema (MDRF) questioned why some diabetic subjects with good glycemic control develop DR, whereas others even with poor control of diabetes do not develop DR. She gave an overview of evidences of genetic factors in DR that come from twin studies, familial aggregation in sibships, linkage studies and case-control association studies. Referring to her familial clustering study on type 2 diabetic subjects with an increased risk of DR of about 3.5 times among sibships, Rema outlined the origin of on-going studies that aim at unravelling the genetic basis of DR. The AGE–RAGE pathway has been considered as a key-signalling event that is altered in diabetic complications, including retinopathy. Increased RAGE signalling triggers upregulation of transcription factors such as NFkB which through turning on several genes, mediate capillary occlusion and cell proliferation. G. Kumaramanickavel (Sankara Nethralaya, Chennai) explained how Gly82 polymorphism of the *RAGE* gene increases the risk for diabetic retinopathy in the presence of abnormal microalbuminuria and in insulin users. Studying the –374 T/A, –429 T/C and 63 bp deletion polymorphisms of *RAGE* gene for their association with diabetic retinopathy, S. Ramprasad (MDRF) showed an association of –374 polymorphism with non-proliferative diabetic retinopathy. Bhanuprakash Reddy (National Institute of Nutrition (NIN), Hyderabad) cautioned about the alarming increase in the prevalence of obesity/diabetes, which further exacerbates concern about retinal degenerations. He disclosed the efforts of the NIN in developing a obese mutant rat (WNIN-Ob rat) that mimics an animal model with retinal degeneration arising as a consequence of obesity. He also described the microarray analysis data on obese rat retina with photoreceptor degeneration.

Type 1 diabetes (T1D) is a multifactorial autoimmune disease, characterized by T-cell-mediated destruction of the insulin-secreting pancreatic islet b-cells. Although 18 genetic loci referred to as 'IDDM loci' have been shown to contribute to

susceptibility and disease pathogenesis, there exists ethnicity-related variations. N. K. Mehra (All India Institute of Medical Sciences, New Delhi) gave a brief overview on the unique genetic factors contributing susceptibility to T1D in Indians. The classical ancestral haplotype AH8.1 (HLA-A1 B8 DR3) which has a strong association with T1D in Caucasians, is rather rare in the healthy as well as T1D patients in India and has been replaced by a variant AH8.1v that differs from the Caucasian AH8.1 at several gene loci. Similarly, AH8.2 (HLA-A26 B8 DR3) is the main diabetes susceptibility DR3 haplotype in the Indian population that resembles the Indian AH8.1v rather than classical Caucasian AH8.1. Additionally, HLA-DR3 haplotypes HLA-A24B8DR3 (AH8.3), A3B8DR3 (AH8.4), A31B8DR3 (AH8.5) and A33B58DR3 occur in the Indian population with relatively higher frequency. Showing the association of T1D with INS-VNTR, CTLA4 and other allele-specific genes, Mehra said that more such studies were warranted in India to better understand the disease pathogenesis and to design newer therapies.

Tropical calcific pancreatitis (TCP) is a type of idiopathic pancreatitis unique to tropical countries with a variable clinical picture compared to the commonly occurring pancreatitis due to alcohol consumption. Although the disease classically presents as recurrent abdominal pain in childhood, exocrine pancreatic insufficiency and large pancreatic calculi; many patients may initially present with diabetes. Diabetes secondary to TCP is known as Fibrocalculous Pancreatic Diabetes (FCPD). Contrary to the previous mutations identified in cationic trypsinogen gene (PRSS1), G. R. Chandak (Centre for Cellular and Molecular Biology, Hyderabad) reported a new line of genetic basis in that mutation N34S in *SPINK1* gene coding for Pancreatic Secretory Trypsin Inhibitor (PSTI) protein, was equally prevalent in both TCP and FCPD. A strong association of mutations in the signal peptide region of Cathepsin B (CTSB) with TCP, independent of N34S *SPINK1* mutation was suggested to be responsible for premature intra-pancreatic activation of trypsinogen in these patients.

Alok Kanungo (Cuttack Diabetes Research Foundation) gave an overview of the genetics of malnutrition-modulated diabetes mellitus (MMDM). MMDM patients are typically young at onset, with low body mass index, require insulin treatment

for glycemic control, exhibit insulin resistance, and do not develop ketosis on withdrawal of insulin. Genetic analysis revealed that both T1D and MMDM in eastern Indians are associated with DR3-DQ2, but not DR4-DQ8. However, in autoantibody-negative MMDM patients, an association with DR7-DQ2 is identified suggesting that there is a different immunogenetic background to MMDM than to T1D. Additional experiments showed that polymorphism in *MICA* gene but not SUMO4 M55V was associated with MMDM again indicating the preposition that MMDM is immunogenetically different from T1D.

A number of adipocytokines are secreted from adipose tissue, many of which play a role in insulin resistance and vascular endothelial function. Resistin, a newly identified adipocytokine could serve as a link between diabetes, obesity and inflammation. Though well studied in animals, the role of resistin in humans remains debatable. In an attempt to analyse the differences between human and mouse resistin gene, Nasreen Ehtesham (NIN) showed data related to transcriptional regulation of resistin. Mouse resistin showed TZD (thioazolidinedione) responsiveness by virtue of a PPAR*g* response element (PPRE) present in the intron of the gene. *In silico* analyses of the upstream sequences of human resistin revealed the presence of several transcription factor-binding sites, which are likely to play a role in its regulation. Interestingly, human resistin potentiates the inflammatory response in macrophages involving NF*k*B pathway. Radha Venkatesan (MDRF) reported on investigations that contribute to the common polymorphisms in *LPL* gene with dyslipidemic traits, obesity and type 2 diabetes. There was a significant association of the H+ allele of *Hin*dIII with low HDL-cholesterol and elevated triglyceride levels. The Ser allele of Ser 447 Ter was also strongly associated with low HDL cholesterol levels. With respect to the promoter -T93G polymorphism, subjects with XG genotype (TG+GG) had higher body mass index, waist circumference, total abdominal fat and subcutaneous fat compared to those with TT genotype. With respect to –G53C, subjects with the XC (GC+CC) genotype had 0.527 and 0.531 times lower risk for developing type 2 diabetes and obesity. Based on these observations, a thorough investigation on *LPL* gene in dyslipidemia is warranted.

Delivery of insulin safely and non-invasively has been a long-sought-after goal to allow early initiation of insulin therapy and ensure compliance. Shrikumar Suryanarayan (Biocon Ltd) elegantly highlighted how oral route for insulin delivery has several advantages over the other delivery systems. This route of insulin delivery mimics the natural route of insulin secretion, i.e. by the pancreas into the portal vein, targetting the liver, which plays a major role in maintaining glucose homeostasis. Because of a portal–peripheral gradient, it is possible to maintain high concentration of insulin at the liver, while still maintaining normal insulin levels in the periphery. This has potentially several advantages in terms of being able to switch-off hepatic glucose output without causing peripheral hyperinsulinemia, and consequent dangers of hypoglycaemia, especially in cases where hepatic insulin resistance is high and greater levels of insulin would be required. Explaining the previous experience with an insulin analogue called HIM2 that is resistant to enzymatic degradation with better bioavailability in humans, Suryanarayan said that Biocon is currently researching and developing an improved version of this molecule called IN105. The molecule has already been tested in animals and early human trials are currently underway.

Much of the signalling events in healthy and diseased human cells can only be studied at the protein level, and there is increasing evidence to link minor changes in the expression of some of these proteins and their post-translational modifications with type 2 diabetes. As a prerequisite to study the target-tissue specific proteomics of type 2 diabetes, M. Balasubramanyam (MDRF) showed data on culturing and characterizing of human skeletal muscle cells and assessing insulin signalling readouts by glycogen synthesis and glucose uptake assays. Using peripheral blood cells from patients with type 2 diabetes and its microangiopathy, he claimed a central role for increased oxidative stress in post-translational modifications (Hb glutathionylation, poly (ADP)-ribosylation, telomere shortening, etc). Highlighting the importance of clinical proteomics in bedside applications, Balasubramanyam showed protein spots with region-specific differences in plasma samples from control and prediabetes subjects and patients with type 2 diabetes. Identification of differentially expressed proteins (biomarkers)

from these spots by mass spectrometry is underway.

P. Venkatesan (Tuberculosis Research Centre, Chennai) briefed how computational tools could be used to classify biological sequences, detect weak similarities, separate protein-coding regions from non-coding regions in DNA sequence, predict molecular structure and function, and reconstruct the underlying evolution history as a means to our understanding of life and evolution as well as to the discovery of new drugs and therapies. His talk on 'computational molecular biology' majorly dwelt around machine learning approaches such as artificial neural networks, hidden Markov models, Markov chain Monte Carlo and belief networks that are ideally suited for analysing biological data.

Type 2 diabetes is associated with increased frequency of vascular restenosis due to accelerated vascular smooth muscle cell motility from the media to neointima. Nitric oxide (NO) is an established inhibitor of growth factor-induced vascular smooth muscle cell (VSMC) motility. Contrary to this, Madhulika Dixit (Germany) showed data that NO stimulates the motility of VSMCs cultured for several days in the presence of insulin. Additionally, chronic insulin exposure of these cells also abrogated the ability of NO to inhibit PDGF-induced motility. Showing a role for both tyrosine phosphatase and kinases in NO-mediated motility of VSMCs, Dixit related how these molecular mechanisms underlie the pathogenesis of vascular disease in hyperinsulinemic diabetes.

The symposium also included a presentation of research papers both oral and poster by Ph D students and junior faculty of several prestigious Indian university and research institutions and also from Sweden. Through this symposium it was made possible to bring together researchers working on different aspects of genomics and proteomics of diabetes and its complications on a single scientific platform to focus on current trends and needs and emphasize on what should be the future directions for translational research application in this field.

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MEETING REPORT

Art of petrography: Eyes of a petrographer and mind of a petrologist*

The DST-sponsored contact programme on 'Art of Petrography' was held recently at the Banaras Hindu University (BHU), Varanasi. The course was aimed at reviving the mastery on careful petrographic studies to extract and interpret invaluable petrological information frozen in rock textures at meso-, micro- and even at nano domains of igneous, sedimentary and metamorphic rocks. There were 40 participants from all over India. Teachers (4) and research scholars (27) from different colleges/universities, junior-level scientists/researchers from GSI (3), NGRI (4), and State Directorate of Geology (2) constituted the list of participants. Twenty-two distinguished speakers were on the faculty. The lecture gallery with modern audio-visual system made the setting perfect for teaching and learning.

Anand Mohan (Department of Geology, BHU) while welcoming the participants, briefly highlighted the enormous poten-

tial and scope of petrographic studies of rocks. The inaugural lecture, 'A journey to the centre of the earth', was delivered by A. K. Gupta (Allahabad) on recent understanding of deeper parts of the earth through experimental, mineralogical and phase petrological studies. M. Joshi (BHU), reported on the methods of optical mineralogy and polarizing microscope, which are essential for petrographic studies. B. K. Chatterjee (BHU) discussed petrography of carbonate sediments and their diagenesis emphasizing that carbonate sediments are born, not made, unlike siliciclastics. I. B. Singh (Lucknow) discussed at length the petrographic aspects of sandstone including its changes with depth of burial. He also demonstrated the staining techniques used to differentiate primary and secondary matrix in clastic rocks. The nature of metamorphic reactions and technique of graphical representations in triangular diagrams of mineral assemblages related to different metamorphic reactions was taken up by Anand Mohan. S. Dasgupta (Jadavpur) concentrated on how to recognize key mineral assemblages in 'appropriate rocks' to unravel ultrahigh temperature metamorphic imprint, draw-

ing examples from the Eastern Ghats belt. Reaction modelling and reconstruction of metamorphic P–T path from metamorphic reaction textures, especially coronas and reaction rims, was discussed by S. K. Bhowmik (IIT, Kharagpur). K. Das (ISM) dealt with recognition of extreme conditions of metamorphism from inclusions and intergrowths in porphyroblasts. The time relationship between deformation, crystallization and metamorphism was outlined by R. K. Lal (Varanasi). The techniques and essence of identification of shear zone fabric both in field and under microscope was explained by T. R. K. Chetty (NGRI, Hyderabad). Textures and structures in ductile shear zone, including shear sense determination were also discussed by A. K. Jain (IIT, Roorkee) with the help of numerous photomicrographs. R. S. Sharma (Jaipur) discussed the origin and interpretation of igneous textures from common phase diagrams. Overview of igneous textures and their variability was given by J. P. Shrivastava (Delhi). Y. J. Bhaskar Rao (NGRI) provided insights into new trends and applications on zircon *in situ* U–Th–Pb geochronology, Hf and Nd isotopic systematics and trace element geo-