Genomics and Proteomics of Type 2 Diabetes in Indians

V Mohan, M Balasubramanyam, V Radha

In its search for the causes of Type 2 diabetes, medicine has now advanced to the molecular level. Genomics and proteomics are opening the way to a new and deeper understanding of pathogenesis of diabetes aiming at the development of precise and targeted interventions. One of the most useful strategies in the search for genes underlying complex diseases such as diabetes is to look at candidate genes. These candidate genes are selected on the basis of their known or presumed functions and they are thought to have some plausible role in pathogenesis of the disease. Some of the candidate genes reported to be associated with Type 2 diabetes include: PPARγ (Peroxisome Proliferator Activated Receptorγ), IRS-1 (insulin receptor substrate 1), KCJN11 (potassium inward rectifier 6.2), SLC2A1 (glucose transporter 1), PPARGC-1 (PPARγ -coactivator-1) and CAPN10 (calpain 10).

At the Madras Diabetes Research Foundation, we have recently studied the plasma cell glycoprotein (PC-1) gene polymorphism K 121 Q, and showed its potential role in genetic susceptibility to type 2 diabetes in both South Asians and Europeans.[1] This study was done in collaboration with Dr. Abate and colleagues at the South Western University of Texas at Dallas and involved south Indians living in Chennai, south Asians living in Dallas (USA) and white Caucasians living in Dallas. The consistent association of this variant allele with type 2 diabetes in the three different ethnic populations characterized by different genetic background and environmental factors suggests that this gene may play an important role in Type 2 diabetes susceptibility. Yet another study by our group showed that the A allele of Thr 394 Thr(G → A) polymorphism of the PGC-1 gene is associated with type 2 diabetes in Asian Indian subjects and the XA genotype was shown to confer a higher risk for type 2 diabetes compared to the GG genotype in our population.[2] The same Thr 394 Thr polymorphism is also associated with increased visceral fat in our population.[3] All these studies point out to the various susceptible genes in conferring a greater risk for developing diabetes in our population which is considered as the world's diabetic capital.

Dr. Mohans' MV Diabetes Specialities Centre and Madras Diabetes Research Foundation, Gopalapuram, Chennai – 600 086.

In this context, the two seminal articles that appear in this issue of JAPI are very relevant to expose further developments occurring in India in this field.

Gene expression profiling in Type 2 diabetes

Microarray technology is expected to initiate a new era in genetic linkage analysis that will facilitate the study of phenotypes of patients and their relatives, providing new impetus and strategies for mapping disease traits. Functional genomics is much more powerful than other techniques in determining mRNA levels—an array can include tens of thousands of probes and can therefore measure the expression of an equivalent number of genes in a single experiment. In this issue of JAPI (page 521-526), Rao *et al*[4] report rare and / or previously unknown phenotypes linked to known genes with significant differential expression in Type 2 diabetes subjects. Such studies are essential in a population where the development of disease not only shows exponential growth but also unprecedented prevalence rates. The study, although of great interest also has certain limitations. Minimum biological replicates (n=3) were used in this study, and much variability can be expected in microarray measurements particularly if few samples are used. The heterogeneity in leukocyte sample preparation is another problem. However, given these limitations, it is of great interest that many clinical disorders (previously not associated with clinical diabetes) were associated with genes that are significantly up-regulated in peripheral blood cells (PBCs) of Type 2 diabetes patients and Rao et al are to be congratulated for their excellent contribution to the literature. While our own study[5] has shown the association of polymorphisms of FABP2 (fatty acid binding protein 2) and Apolipoprotein C-III (APOC III) genes with metabolic syndrome and dyslipidemia in Indians, the work by Rao *et al*[4] further substantiate the differential gene expressions of lipoprotein lipase (LPL) gene and APOC III in Type 2 diabetes patients with dyslipidemia. It is expected that further testing of heterogeneity in diabetes phenotype syndromes may reveal common pathogenic mechanisms and potential candidate genes in the development and progression of Type 2 diabetes.

To some extent, gene expression changes in peripheral blood cells distinguish variable diabetes states[6] and they

could be accessible cell types to study and understand the biochemical perturbations related to diabetes and its complications.[7] For example, the expression profiling in leukocytes of diabetic patients may reflect changes in gene expression related to systemic inflammation and/or oxidative stress. However, gene expression in most instances of pathological situations is also tissue-specific and regulated spatially and temporally. Alterations in gene expressions may occur as a result of specific genetic alterations that underlie diabetes, due to a lack of insulin signaling as a result of insulin deficiency or insulin resistance, or be secondary to the hyperglycemia and altered metabolic state that occurs in diabetes. Therefore, gene expression studies in Indians should be extended to classical insulin-responsive tissues (muscle, fat, pancreas and liver). Any global gene expression changes observed in peripheral blood cells thus need to be interpreted with caution.

Clinical proteomics

Since the result of gene expression is the production of proteins that constitute the targets for therapeutic approaches, the field of proteomics is complementary to that of genomics. Among various applications, clinical proteomics is a rapidly developing area of biomedical research with promise of translation of basic knowledge into clinical applications. Very recently, urinary proteomics has received much attention as a means to better understand renal physiology, to explore the complexity of glomerular disease mechanisms, and to identify novel biomarkers and new therapeutic agents. Clinicians would be interested to know how these newer developments along with the conventional microalbuminuria (MAU) measurements can aid in earlier diagnosis and better understanding of the pathophysiology of diabetic nephropathy,

Microalbuminuria (MAU) is an early sign of incipient renal disease and a marker of its progression. Furthermore, it is a key indicator of the need for intensified treatment in diabetes and hypertension,[8] the most powerful predictor of cardiovascular and atherosclerotic risk[9] and a prognostic marker in the development of end-stage renal disease (ESRD) and mortality.[10] Early screening for MAU can help identify patients who would benefit from renoprotective therapy and antihypertensive treatment. MAU is also indicative of generalized endothelial dysfunction and some suggest that it should also be included as part of the metabolic syndrome.[11] Alhough MAU is the best available noninvasive predictor for risk of diabetic nephropathy, it occurs after several years of diabetes. Some patients with MAU have quite advanced renal pathological changes, for which therapies are less effective than earlier stages of the disease.[12] Additionally, the MAU assay measures just the immunoreactive fraction of albumin.[13] Whereas, 'immunochemically nonreactive albumin' has been claimed to be an earlier predictor of renal damage.[14] Moreover, MAU test gives false-positive results in a number of conditions: uncontrolled hyperglycemia and hypertension, urinary tract infection, stress, febrile conditions, heavy exercise, cardiovascular decompensation, contamination with seminal or menstrual fluid etc. Therefore, along with MAU, more sensitive markers and alternative approaches are essential for diagnosis of early stages of diabetic nephropathy, for assessing the renal state, for optimizing the diabetes care and for monitoring the success of therapy.

Markers other than albumin as predictors of diabetic kidney disease

In this issue of JAPI (page 513-520), using a combined proteomic approach of 2-dimensional gel electrophoresis (2-DGE) and mass spectrometry, Jain et al[15] elegantly demonstrate the presence of 4 additional urinary markers other than albumin, in diabetic patients who tested positive for microalbuminuria. These urinary markers were identified by mass spectrometry as zinc alpha-2 glycoprotein, alpha-1 acid glycoprotein, alpha-1 microglobulin and immunoglobulin. Although the results are preliminary, the appearance of the first three marker proteins preceding the time-appearance of albumin in the samples implies that these additional urinary markers may be early predictors of diabetic nephropathy. The identification of these additional urinary markers is a significant advancement in that they are more specific for tubular and renal abnormalities. Studies such as these, when performed along with creatinine clearance and glomeruar filtration rate measurements may lead not only to better understanding of the pathophysiology, but also to newer targets of therapy of diabetic nephropathy.

Peptides present in the glomerular filtrate may contribute to the progression of renal disease and dysfunction associated with proteinuria. Mass spectrometry allows *de novo* identification of polypeptides. When combined with separation techniques, such as liquid chromatography or two-dimensional gel electrophoresis, mass spectrometry is well suited to the analytical profiling of complex biological mixtures. Recently, Thongboonkert and colleagues[16] created a 2-D proteome map for normal human urinary proteins and using MALDI-TOF mass spectrometry, they have identified a total of 67 proteins representing various cellular components. In a rat model, they have also identified alterations in urinary protein excretion during acute sodium loading[17] and some proteins identified by them such as diphor-1 growth-associated protein (which is regulated by transforming growth factor β) and 1-myc (an oncogene product), had not previously been identified in the urine by other techniques. Findings such as these may lead to identification of new prognostic markers and/or indicators that can be used to monitor therapeutic

outcomes for glomerular diseases. Although proteomics technology may appear expensive and applications to this field are at an early phase, one cannot underestimate the possible identification of clinically useful biomarkers that are more sensitive and specific than MAU for early diagnosis of diabetic renal disease. This is all the more important in the Indian context, because India already has the largest number of diabetic patients in the world and hence significant morbidity from diabetic renal disease can be expected.[18-20]

The allure of the new genomic and proteomic technologies lies in the vast amounts of information that these technologies are expected to generate. We believe that the combination of these approaches will expose new drug targets in the pathogenesis of Type 2 diabetes and hence lead to the design of novel therapeutic treatments. This is perhaps an optimistic vision of what may be expected in the future. From the skeptic's point of view, the cost of applying these technologies and the need for sophisticated instrumentation could limit their use to a few advanced centres. Nevertheless, investment in this type of research will certainly pay dividends in appropriate health care, longer life expectancy and improved quality of life of the diabetic patient.

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