Peripheral Genotype-Phenotype Correlations in Asian Indians with Type 2 Diabetes Mellitus

PV Rao*, X Lu++, P Pattee+++, M Turner++++, Suguna Nandgaonkar**, Bhanu T Paturi***, CT Roberts Jr.+++++, SR Nagalla+

Abstract

Objective: A genome-wide scan of gene expression in leucocytes in Asian Indians with type 2 diabetes was performed and correlated with their known phenotype.

Methods: Microarray gene profiling of 13,474 sequence-verified, non-redundant human cDNAs was done to study leukocyte gene expression in Asian Indians with type 2 diabetes (DM: n=3) and matched controls (n=3).

Results: Significant differential expression (fold change <0.3 or >3) was noted for 897 genes in DM vs. controls. The 147 known genes in this category belonged to following broad functional groups (%): enzyme (32), nucleic acid binding (22), ligand binding or carrier (10), signal transducer (9), transporter (7), structural protein (6), cell adhesion (3), tumor suppressor (3), transcription factor binding (2), enzyme inhibitor (2), chaperone (2), cell cycle regulator (1), and defense/immunity protein (1). The 20 genes with at least a 3-fold change, annotated with known phenotypic associations in the current gene databank (phenotype association, fold change) were aspartoacylase (Canavan disease, 9.96), growth hormone receptor (Laron dwarfism, idiopathic short stature, 8.25), lipoprotein lipase (familial chylomicronemia syndrome, lipoprotein lipase deficiency, 8.00), vitamin D (1,25-dihydroxyvitamin D3) receptor (involutional osteoporosis, vitamin D resistant rickets, 7.94), intercellular adhesion molecule 1 human rhinovirus receptor (cerebral malaria susceptibility, 7.16), peroxisomal membrane protein 3 35-kDa (Refsum disease, infantile form, Zellweger syndrome, 3.60), Bardet-Biedl syndrome 2 (Bardet-Biedl syndrome, 5.87), ribosomal protein S19 (Diamond Blackfan anemia, 5.85), apolipoprotein C-III (hypertriglyceridemia, 5.44), argininosuccinate lyase (argininosuccinicaciduria, 5.22), myosin VA (Griscelli syndrome-type pigmentary dilution with mental retardation, 4.92), lysozyme (renal amyloidosis, 4.17), SAM domain, SH3 domain and nuclear localisation signals 1 (Cherubism, 4.12), von Hippel-Lindau syndrome (hemangioblastoma, cerebellar, somatic, von Hippel-Lindau syndrome, 3.94), early-onset breast cancer 1 (BRCA1, papillary serous carcinoma of the peritoneum, 3.73), UDP-N-acetylglucosamine-2-epimerase/N-acetylmannosamine kinase (inclusion body myopathy, autosomal recessive, sialuria, 3.53), apolipoprotein A-I (amyloidosis, 3 or more types, hypoalphalipoproteinemia, 3.29), midline 1 Ophit/BBB syndrome (Opitz G syndrome, type I, 3.28), ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide (familial hemiplegic migraine, 3.05), Canavan disease, Zellweger syndrome, infantile Refsum disease, Griscelli syndrome, cherubism, breast cancer, peritoneal papillary serous carcinoma, Opitz G/BBB syndrome, and familial hemiplegic migraine (FHM) are phenotypes not previously reported in association with type 2 DM, but whose underlying genes were up-regulated in this peripheral genome scan of Asian Indians.

Conclusion: Rare and/or previously unknown phenotypes linked to known genes with significant differential expression in type 2 DM are reported. Further testing of heterogeneity in diabetes phenotype syndromes may reveal common pathogenic mechanisms and potential candidate genes responsible for type 2 DM. ©

INTRODUCTION

Microarray-based gene profiling and improved methods for analyzing large datasets from a single experiment now provide the opportunity to simultaneously analyze the expression of thousands of genes in multifactorial diseases such as type-2 diabetes mellitus (DM). In human pancreas, muscle, fat, and liver tissues, mRNA levels of about 800 genes are modulated in DM. Many of these are targets of the insulin/insulin...
receptor signaling and belong to functional classes that can account for most of the biological and metabolic effects of diabetes. Furthermore, gene expression changes in peripheral blood cells (PBCs) also distinguish variable diabetic states. Identification of candidate gene expression in PBCs raises the possibility of using easily accessible biomarkers to identify and monitor diabetes.

We have employed a translational research approach comprised of unbiased global profiling of leukocyte gene expression signatures in a defined Asian Indian population to discover potential novel biomarkers for type-2 DM development, progression, and response to therapy. These data and annotated phenotype-genotype correlations are reported in this study. New DM-associated disease entities are described and new inheritance patterns identified for some syndromes in association with DM in Asian Indians.

**MATERIALS AND METHODS**

Microarray gene profiling of 13,474 sequence-verified, non-redundant human cDNAs was done to study leukocyte gene expression in Asian Indians with type 2 DM (DM: n=3) and controls (C: n=3) matched for age and gender. Informed consent was obtained from the subjects following the institution (Nizam’s Institute of Medical Sciences, Hyderabad) review board guidelines for human subjects.

Each of two pooled (DM, C) leukocyte tissue samples was extracted by TriReagent™ (Molecular Research Corp.) to isolate RNA according to the manufacturer’s protocol. Total RNA (10 µg) was reverse-transcribed with Superscript II (Invitrogen) using poly-T primer and labelled with Cy5 by an amino-allyl labeling protocol. Each sample was hybridized separately to 2 human cDNA arrays. Detailed microarray protocols are available on our supplemental website at: http://www.medir.ohsu.edu/~geneview/.

Mean signal intensity was adjusted for local background by subtracting the median background intensity. For normalization, data for each array was exported to Arraystat™ statistical software (Imaging Research, version 1.0, Revision 2.0). The Arraystat normalization parameters used were ‘Proportional model with offsets, no outlier exclusion’ which log-transforms the data (base 10) and globally centers the transformed data within study and control samples.

Modified ANOVAs (Arraystat’s F* test) and significance of differences between means (z-test) were determined using a pooled error model. Centered expression values and test results were exported to Excel. Normalized means and differences between means were converted from log10 to log2 for ease of comparison with the literature.

Datasets were merged and adjustment for multiple testing was done on the p values of the statistical tests in merged data set using the False-Discovery Rate (FDR) correction with the level of acceptable false positives set at 0.05 for each statistical test. Significant regulation was defined as a fold change between study and control subjects greater than 3 or less than -3 (using a common error model and a modified z test, all 3-fold changes were statistically significant at p<0.05 after FDR correction for multiple comparisons).

Peripheral genome data (DM vs.C) annotated from local database that was downloaded from GenBank (http://www.ncbi.nih.gov/Genbank) and MGI/OMIM (http://www.informatics.jax.org/) on October 20, 2004 is presented here. This data included accession number, gene name, gene symbol, molecular function, broad function, biological process, broad process, cellular component, broad cell component, phenotype, chromosome, cytogenetic position, summary function, other annotations, and tissues.

**RESULTS**

Significant differential expression (fold change <0.3 or >3) was seen for 897 genes in DM vs. controls. Of these, 147 known genes fell into the following broad function categories (%): enzyme (32), nucleic acid binding (22), ligand binding or carrier (10), signal transducer (9), transporter (7), structural protein (6), cell adhesion (3), tumor suppressor (3), transcription factor binding (2), enzyme inhibitor (2), chaperone (2), cell cycle regulator (1), and defense/immunity protein (1).

Of all known genes with a 3-fold change, 20 were annotated with phenotypic associations according to current gene database database as shown in Table 1.

**DISCUSSION**

These data identify significant changes in the expression levels of 897 genes in human leucocytes isolated from Asian Indians with DM. Approximately half were expressed sequence tags (ESTs) with unknown functions. The remainder could be classified into broad functional categories such as enzyme, nucleic acid binding, ligand binding or carrier, etc. Certain of them can support most of the biological and metabolic effects in DM and are potential novel candidates in diabetes pathologies. Further elaboration on these functional categories is not within the scope of the present study pending more data from this ongoing study.

This article focuses on the clinical disorders associated with genes that are significantly up-regulated in PBCs of type 2 DM patients. Of these genotype-phenotype associations, previously known clinical associations with diabetes as available in medical literature are first listed here, followed by rarer phenotypes.

Laron syndrome (LS) is due to insulin-like growth factor-I (IGF-I) deficiency causing dwarfism, acromicria, organomicrocira, marked obesity, insulin resistance, retardation of skeletal maturation and osteoporosis, as...
Table 1: Phenotypes linked to known genes with significant expression in Asian Indians with type 2 DM

<table>
<thead>
<tr>
<th>Accession number</th>
<th>Gene Name</th>
<th>Gene Symbol</th>
<th>Cytogenetic Position</th>
<th>Phenotype</th>
<th>Fold change</th>
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<tr>
<td>N71653</td>
<td>Aspartoacylase (aminoacylase 2, Canavan disease)</td>
<td>ASPA</td>
<td>17pter-p13</td>
<td>Canavan disease</td>
<td>9.96</td>
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<tr>
<td>W05000</td>
<td>Growth hormone receptor</td>
<td>GHR</td>
<td>5p13-p12</td>
<td>Laron dwarfism, Short stature, idiopathic</td>
<td>8.25</td>
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<tr>
<td>AA633835</td>
<td>Lipoprotein lipase</td>
<td>LPL</td>
<td>8p22</td>
<td>Chylomicronemia syndrome, familial Lipoprotein lipase deficiency</td>
<td>8.00</td>
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<tr>
<td>AA485226</td>
<td>Vitamin D (1,25- dihydroxyvitamin D3) receptor</td>
<td>VDR</td>
<td>12q12-q14</td>
<td>Osteoporosis, involutional, Rickets, vitamin D-resistant, type IIB</td>
<td>7.94</td>
</tr>
<tr>
<td>R77293</td>
<td>Intercellular adhesion molecule 1 (CD54), human rhinovirus receptor</td>
<td>ICAM1</td>
<td>19p13.3-p13.2</td>
<td>Malaria, cerebral, susceptibility to</td>
<td>7.16</td>
</tr>
<tr>
<td>R88992</td>
<td>Peroxisomal membrane protein 3, 35kDa (Zellweger syndrome)</td>
<td>PXMP3</td>
<td>8q21.1</td>
<td>Refsum disease, infantile form, Zellweger syndrome-3</td>
<td>6.00</td>
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<tr>
<td>N93740</td>
<td>Bardet-Biedl syndrome 2</td>
<td>BBS2</td>
<td>16q21</td>
<td>Bardet-Biedl syndrome 2</td>
<td>5.87</td>
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<tr>
<td>H41165</td>
<td>Ribosomal protein S19</td>
<td>RPS19</td>
<td>19q13.2</td>
<td>Anemia, Diamond-Blackfan</td>
<td>5.85</td>
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<tr>
<td>N53169</td>
<td>Apolipoprotein C-III precursor (Apo-CIII)</td>
<td>ICAM1</td>
<td>22p13</td>
<td>Hypertriglyceridemia</td>
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<tr>
<td>AA486741</td>
<td>Argininosuccinate lyase</td>
<td>ASL</td>
<td>7cen-q11.2</td>
<td>Argininosuccinic aciduria</td>
<td>5.22</td>
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<tr>
<td>N67810</td>
<td>Myosin VA (heavy polypeptide 12, myoxin)</td>
<td>MYO5A</td>
<td>15q21</td>
<td>Griscelli syndrome, type 1</td>
<td>4.92</td>
</tr>
<tr>
<td>N63943</td>
<td>Lysozyme (renal amyloidosis)</td>
<td>LYZ</td>
<td>12q15</td>
<td>Amyloidosis, renal</td>
<td>4.17</td>
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<tr>
<td>H77697</td>
<td>SAM domain, SH3 domain and nuclear localisation signals, 1</td>
<td>SAMSN1</td>
<td>21q11</td>
<td>Cherubism</td>
<td>4.12</td>
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<td>H90415</td>
<td>Breast cancer 1, early onset</td>
<td>BRCA1</td>
<td>17q21</td>
<td>Breast cancer-1, Papillary serous carcinoma of the peritoneum</td>
<td>3.73</td>
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<td>T68440</td>
<td>Glucosamine (UDP-N-acetyl)-2-epimerase/N-acetylmannosamine kinase</td>
<td>GNE</td>
<td>9p13.2</td>
<td>Inclusion body myopathy, autosomal recessive, Sialuria</td>
<td>3.53</td>
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<tr>
<td>AA453673</td>
<td>Arginase, liver</td>
<td>ARG1</td>
<td>6q23</td>
<td>Arginemia</td>
<td>3.33</td>
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<tr>
<td>R97710</td>
<td>Apolipoprotein A-I</td>
<td>APOA1</td>
<td>11q23-q24</td>
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<td>3.29</td>
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<tr>
<td>AA598640</td>
<td>Midline 1 (Opitz/BBB syndrome)</td>
<td>MID1</td>
<td>Xp22</td>
<td>Opitz G syndrome, type 1</td>
<td>3.28</td>
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<tr>
<td>R73570</td>
<td>ATPase, Na+/K+ transporting, alpha 2 (+) polypeptide</td>
<td>ATP1A2</td>
<td>1q21-q23</td>
<td>Alternating hemiplegia of childhood, Migraine, familial hemiplegic, 2</td>
<td>3.05</td>
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</table>

As well as muscular and central nervous tissue underdevelopment, and a series of biochemical changes, including hypercholesterolemia. Growth hormone (GH) receptor gene expressed significant association in the present study, and it is well known that tissue resistance to GH action occurs as an inborn (Laron dwarf) or acquired (fasting, diabetes mellitus, chronic renal failure) defect.3

The lipoprotein lipase (LPL) gene demonstrated significant regulation in this study. LPL deficiency and subsequent chylomicronemia increase free fatty acids and insulin resistance, and promote gluconeogenesis and hyperglycemia, a vicious cycle leading to type 2 DM.4 Severe hypertriglyceridemia is associated with the presence of the apoC-III S2 allele5 and expression of this genotype was significant in the study. The alleles of the apoA-I gene that were differentially expressed in the present study are known to be associated with hypoalphalipoproteinemia in diabetes and coronary artery disease (CAD).6

Vitamin D (1,25- dihydroxyvitamin D3) receptor gene expression was significant in the present study. Idiopathic phosphate diabetes, diagnosed on the basis of a maximal rate for tubular reabsorption of phosphate (TmPO4/GFR) of 0.77 or less and associated with osteoporosis and vitamin D resistance, is a well known clinical entity.7

Interestingly, the intercellular adhesion molecule 1 (CD54) gene linked to susceptibility to cerebral malaria was expressed in subjects with type 2 DM. Polymorphisms in the interleukin-12 (IL-12) B gene influence the susceptibility to type 1 diabetes, and homozygosity for a polymorphism in the IL12B promoter was associated with increased mortality in Tanzanian children with cerebral malaria.8

Bardet-Biedl syndrome (BBS) is a genetic disorder with the primary features of obesity, pigmentary retinopathy, polydactyly, renal malformations, mental retardation, and hypogonitalism. Patients with BBS are also at increased risk for DM, hypertension, and congenital heart disease.9 Congenital pure red cell aplasia (Diamond-Blackfan anemia) is treated with high doses of prednisone, along with erythrocyte transfusions, and is often associated with transient diabetes, which may,
in part, be a true genetic predisposition to diabetes. In von Hippel-Lindau disease, although the main endocrine manifestations are pheochromocytoma and paraganglioma, the presence of pancreatic disease has also been variably reported with diabetes as the commonest clinical association. Multiple cerebellar hemangioblastomas accompanied with congenital deafness, juvenile diabetes mellitus, and retinal angioma was also reported in an adult. Genes linked to these syndromes were significantly up-regulated in the study subjects with type 2 DM.

Dysfunction of the argininosuccinate lyase enzyme results in decreased arginine and impaired production of NO (nitric oxide), which inhibit endothelial cell growth and promotes atherogenesis during the early stages of DM. Significant expression of the argininosuccinate lyase gene, as seen in this study, might relate to the increased atherogenesis characteristic of DM.

The lysozyme and apoA-I genes that are linked to one or more types of amyloidosis were significantly expressed in the present study subjects. Several lysozyme gene mutations have been reported as causative for (pre-dialysis, renal) amyloidosis, but no association with diabetes has been described. However, there is growing evidence from experimental and clinical studies that oxidative stress may be implicated in the pathogenesis of DM and in dialysis-related amyloidosis, one of the complications of end-stage renal disease (ESRD).

Inclusion body myositis (IBM) is an inflammatory myopathy in which one of the significant associations was DM in about 20% of the cases. Other phenotypic associations of the gene linked to IBM are mutations in glycoconjugate biosynthesis resulting in phenotypes related to the inborn metabolic defect sialuria, but no clinical association with DM was reported.

In addition to the aforementioned renal (pre-dialysis) amyloidosis and sialuria, the following are the other rare phenotypes that might also be associated with type 2 DM as reported in this study.

Canavan disease, an inherited leukodystrophy due to aspartoacylase deficiency, typically starts in the first months of life with megalencephaly, muscular hypotonia and developmental standstill, and has not yet been associated with DM. In the present study, aspartoacylase gene expression was significant in subjects with type 2 DM (9.96) as compared to controls.

A group of genetic diseases are caused by peroxisomal biogenesis, i.e., Zellweger syndrome and infantile Refsum disease. A generalized defect of peroxisomal function is due to a deficiency in 2-methylacyl-CoA racemase, which fails to initiate peroxisomal beta-oxidation to primary bile acids in neonatal cholestasis. None of these peroxisomal diseases has yet been reported to be associated with diabetes, but the related genes were expressed significantly in the present study subjects.

Griscelli syndrome is a rare disorder with poor prognosis, and is characterized by silver-grey hair in childhood and variable cellular immunodeficiency. Recurrent episodes of fever and lymphohistiocytic infiltration of organs lead to hepatosplenomegaly, lymphadenopathy, pancytopenia, and progressive neurological impairment. No clinical association of DM with this disorder has been reported.

Cherubism is a non-neoplastic bone disease characterized by clinically evident bilateral, painless enlargements of the jaws, which is said to give the patient a cherubic appearance, and has not yet been reported in association with DM.

In the present study, BRCA1 gene expressions for breast cancer and peritoneal papillary serous carcinoma (PPSC) were significant. Though dysfunction of insulin and insulin-like growth factor-I (IGF-I) action are implicated in breast cancer, diabetics were not at increased risk. PPSC, a rare tumor involving the surface of the peritoneum, with prevalence in female patients and which originates from a single or multicentric focus, was not yet reported in diabetics.

Deficiency of liver arginase (AI) causing hyperargininemia, a disorder characterized by progressive mental impairment, growth retardation, and spasticity and punctuated by sometimes fatal episodes of hyperammonemia, has not been described with diabetes.

Opitz G/BBB syndrome was originally described as two distinct entities, the BBB syndrome with cleft lip/palate and mental retardation, and the G-syndrome characterized by gastrointestinal anomalies. Subsequently, both syndromes were merged and reclassified as Opitz BBB/G syndrome. Opitz BBB/G syndrome is a monogenic disorder characterized by malformations of the ventral midline, and is not yet clinically linked to diabetes.

Familial hemiplegic migraine (FHM) is a rare autosomal dominant subtype of migraine with aura. Cerebellar ataxia, confusion without hemiparesis, and progressive cognitive dysfunction are other features. In a case of FHM suffering from prolonged right-sided hemiparesis and aphasia, positron emission tomography revealed glucose hypometabolism in left hemisphere, but no association with clinical diabetes was ever reported.

The hypothesis that heterozygous carriers of the above-mentioned genes for certain genetic syndromes may be predisposed to type 2 DM need to be further tested by comparing diabetes incidence in blood relatives to that in spouse controls or among all adult family members of patients with one of these syndromes. This provides further evidence for the phenotypic and genotypic heterogeneity in diabetes and may provide clues to the search for candidate genes responsible for diabetes phenotypes.
Previous gene-profiling studies of DM-related genes concentrated on pancreas, muscle, and fat. It is more logical, however, to explore the genes in peripheral tissues, as all metabolic functions in diabetes are exclusively measured in blood or blood cells, and genes expressed in PBCs may also mimic those in classical insulin-responsive tissues. We previously reported the differential expression of the IGF-I receptor gene in PBCs in patients with decreased serum IGF-I levels due to Laron Syndrome. Of particular relevance to the present study is the recent report of decreased insulin resistance in transgenic animals with altered NF-kappaB signaling in myeloid cells. Additionally, PBCs represent attractive targets for monitoring of disease presence, progression, or response to therapy. The data presented here represent the first ongoing peripheral genome study in diabetes.

Microarray gene profiling in diabetes has largely been confined to laboratories and to animal studies. Although these arrays were not specifically geared to represent tissues and pathways affected by diabetes, they have been used in both type 1 and type 2 DM research. The identification of candidate genes in diabetes by genomewide scan on the basis of quantification of their expression level and the subsequent application of this knowledge to transgenic technology is a logical step toward realization of the new genes-to-mechanisms paradigm.

Acknowledgements

The authors wish to thank colleagues at Nizam’s Institute of Medical Sciences and the Oregon Health and Science University for technical and informatics support, and Dr. Rakesh Mittal, Senior Deputy Director General, Indian Council of Medical Research, for assistance in gaining approvals from the Ethics Committee, Nizam’s Institute of Medical Sciences; Ministry of Health and Family Welfare, Government of India; and the Ministry of Science and Technology, Government of India.

REFERENCES


### Announcement

**RSSDI – 2005**

33rd Annual Conference

23rd, 24th and 25th September 2005, Bangalore

The highlights of the Conference include the following:

- The Conference Venue is in the midst of IT icons surrounded by greenery, go-karting etc.
- The Conference building is aesthetically designed to have an excellent acoustics.
- More than 3000 delegates are expected to attend.
- As part of the Conference, CME is also organized.
- Nationally and Internationally acclaimed faculties shall address the delegates using the state-of-the-art audio-visuals.
- Parallel scientific sessions.
- Many pharma companies have agreed to display their products and services.
- The following is the delegate fees:

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* Participation in CME needs a separate registration fee.

- Accommodation, transport and sight seeing shall be arranged on request.
- Accompanying children and spouses shall have fun time at the venue itself.

For further details, please contact: Dr. KR Narasimha Setty, Organising Secretary, RSSDI-2005, 132/18, 22nd Cross, III Block, Jayanagar, Bangalore – 560011.

Phone:+91-080-57726555; Fax: +91-080-51307737  Email: krssetty@touchtelindia.net; Web: www.rssdi2005.com

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### Announcement


Registration Fee : Upto 31st August - Rs. 300/-; Upto 30th September- Rs. 500/-

* October onward registration: Rs. 1,000/-

(Please send DD in favour of “Endocrinology Update, SGRH”) Registration form can also be downloaded from www.SGRH.com

Note : Kindly register early. Limited registration.

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