Application Note

Using Gene Logic's BioExpress® System to Reveal Disease Mechanism

Gene Expression Reveals a Distinct Mechanism of Atherosclerosis in Human Diabetes

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Definition and Background

Atherosclerosis is a common cardiovascular disease of large and medium-sized arteries. It is characterized by endothelial dysfunction, vascular inflammation, and the deposition of lipids, cholesterol, calcium, and cellular debris within the intima of the vessel wall. The earliest detectable lesions, fatty streaks, contain lipid-laden macrophages ("foam cells") that are derived from recruited monocytes. More advanced lesions, the fibro-fatty plaques, are the result of continued monocyte recruitment, smooth muscle cell migration and proliferation, and vascular remodeling. Eventually, the plaques thicken the wall of the artery and compromise blood flow. Plaques can also rupture, resulting in superimposed thrombosis and total occlusion of the arterial lumen. This is one of the most common causes of myocardial infarction (heart attack) and cerebrovascular disease including stroke (for reviews see, Libby, 2001; Lucas & Greaves, 2001).

It is difficult to accurately determine the true frequency of atherosclerosis because it is a predominantly asymptomatic condition. However, in the United States alone, approximately 1.5 million myocardial infarctions occur annually, directly resulting in more than 500,000 deaths and more than $100 billion in medical costs. In total, more than 11 million Americans have symptomatic coronary artery disease (CAD), including angina pectoris, congestive heart failure, and other clinical states with or without pre-existing myocardial infarction. Among individuals older than 50 years, more than 30% have some evidence of CAD. Similarly, cerebrovascular disease is responsible for over 200,000 deaths per year in this population.

While several risk factors, such as age, gender, smoking, and hyperlipidemia, among others, are known to be associated with atherosclerosis, hyperglycemia in particular appears to be associated with accelerated progression of atherosclerosis in patients with diabetes mellitus (DM) (Suzuki et al, 2001; Beckman et al, 2002; Hurst & Lee, 2003; Goldberg, 2004). Several epidemiological studies confirm an association between DM and increased prevalence of peripheral vascular disease (PVD), a disease affecting the extremities with atherosclerosis being the main causative agent (reviewed, Dieter et al, 2002). However, the molecular mechanisms underlying the pathogenesis of atherosclerosis, and the way in which diabetes accelerates it, are not fully understood. This Application Note is aimed at elucidating the molecular aspects of the contribution of diabetes to atherosclerosis by using microarray technology to generate global gene expression profiles from human arterial samples in the setting of diabetes.

Materials and Methods

The BioExpress® System is a gene expression database containing more than 18,000 samples

representing normal tissues and diseases such as cancer, inflammation and autoimmune disorders, metabolism, cardiovascular and central nervous system disorders. The present study used two subsets of the BioExpress® System, the Metabolism and Cardiovascular Suites, which contain human samples representing a number of tissues from patients with metabolic and cardiovascular diseases.

In this case study, two groups of peripheral artery samples containing atherosclerosis were examined: a) arteries from patients with PVD but without DM and b) arteries from patients with PVD and DM. These samples were obtained from surgical procedures and followed strict IRB approved protocols, including, informed consent and Gene Logic's standard operating procedures for sample accrual.

The samples were prepared at Gene Logic in accordance with the recommendations of the Affymetrix Expression Analysis Technical Manual. Gene Logic's scientists prepared and optimized detailed sample preparation methods, when needed, to ensure that high quality data was generated from each sample, regardless of the starting material. Only data that passed Gene Logic's stringent quality control metrics were added to the BioExpress® System database for storage and analysis using the Gene Enterprise System® Software.

The Genesis Enterprise System® Software enables the analysis and visualization of the gene expression data in the BioExpress® System, as well as the clinical data associated with the samples used to generate such data, such as patient history, diagnostic tests, medication etc. The Genesis Enterprise System® Software provides tools to allow researchers to perform common research tasks such as building complex queries to identify related samples, visualization to identity outliers within associated samples, principal component analysis to segregate samples into related groups based on gene expression measurements, and the ability to identify genes which discriminate a pattern of regulation. Importantly, the Genesis Enterprise System® Software enables researchers to rapidly reduce complex data sets to biological conclusions by projecting expression data onto over 400 biological pathways, coupling visualizations of clinical data, expression data and reference data. This enables researchers to quickly correlate gene expression changes to clinical parameters.

Results and Discussion

Analysis of gene expression data using Genesis Enterprise System® Software

To determine the transcriptional similarity of the samples within each of the two groups, a Correlation Map employing Pearson's correlation coefficient was plotted. The gene set used to generate the correlation map was the result of the fold change analysis described below. As shown in Fig. 1, the samples within each group showed a high degree of correlation with each other. No outliers were detected in either group of samples. Fold Change (FC) analysis (Fig. 2)

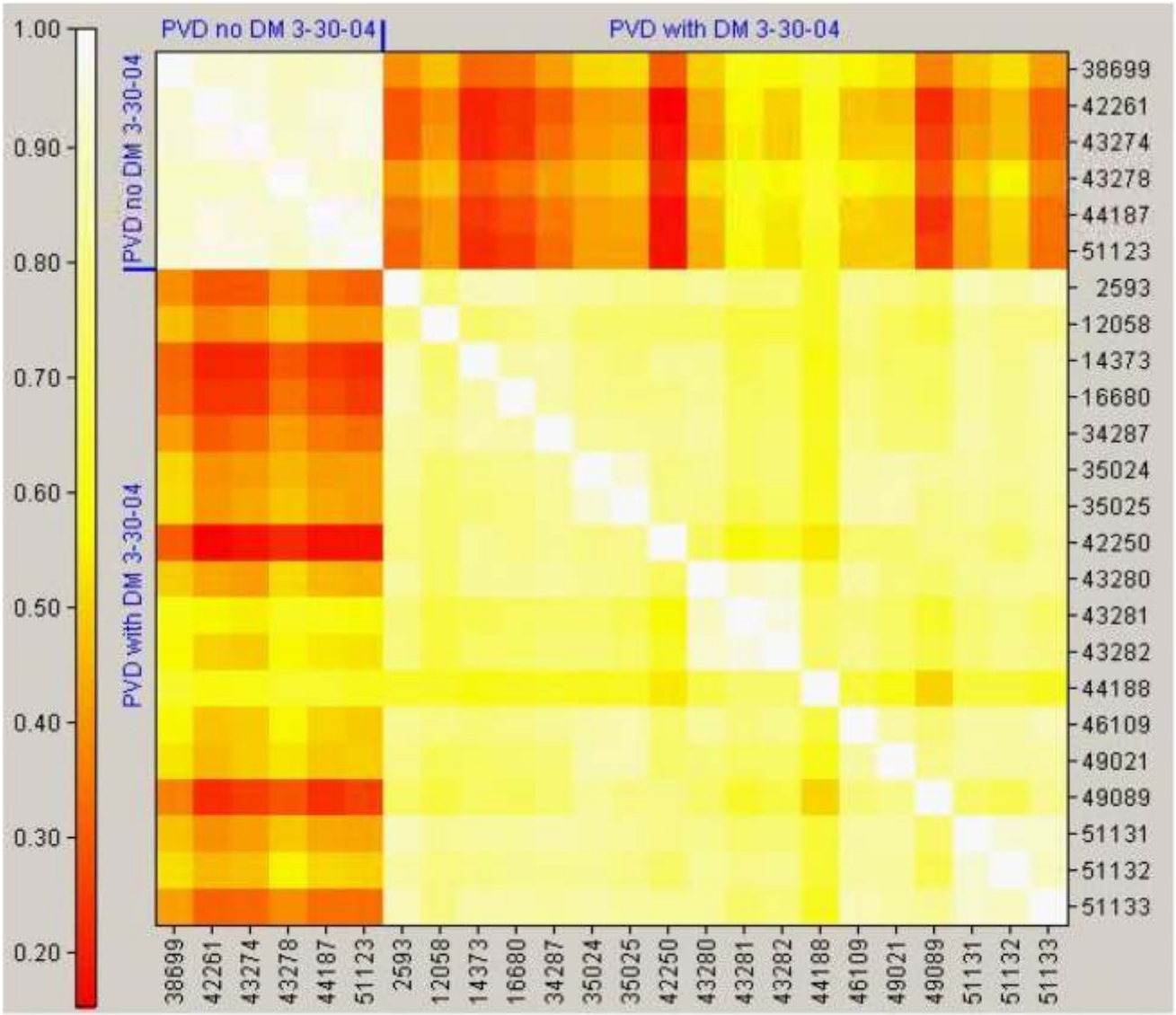

Figure 1: Pearson Correlation Map. The gene set used to generate the correlation map was the result of the fold change (FC) analysis. As illustrated above, the samples within each group showed a high degree of correlation with each other. No outliers are detected in either group of samples. The degree of correlation is indicated by color according to the correlation coefficient scale on the left. ('PVD no DM' indicate Peripheral Vascular Disease samples form patients without Diabetes).

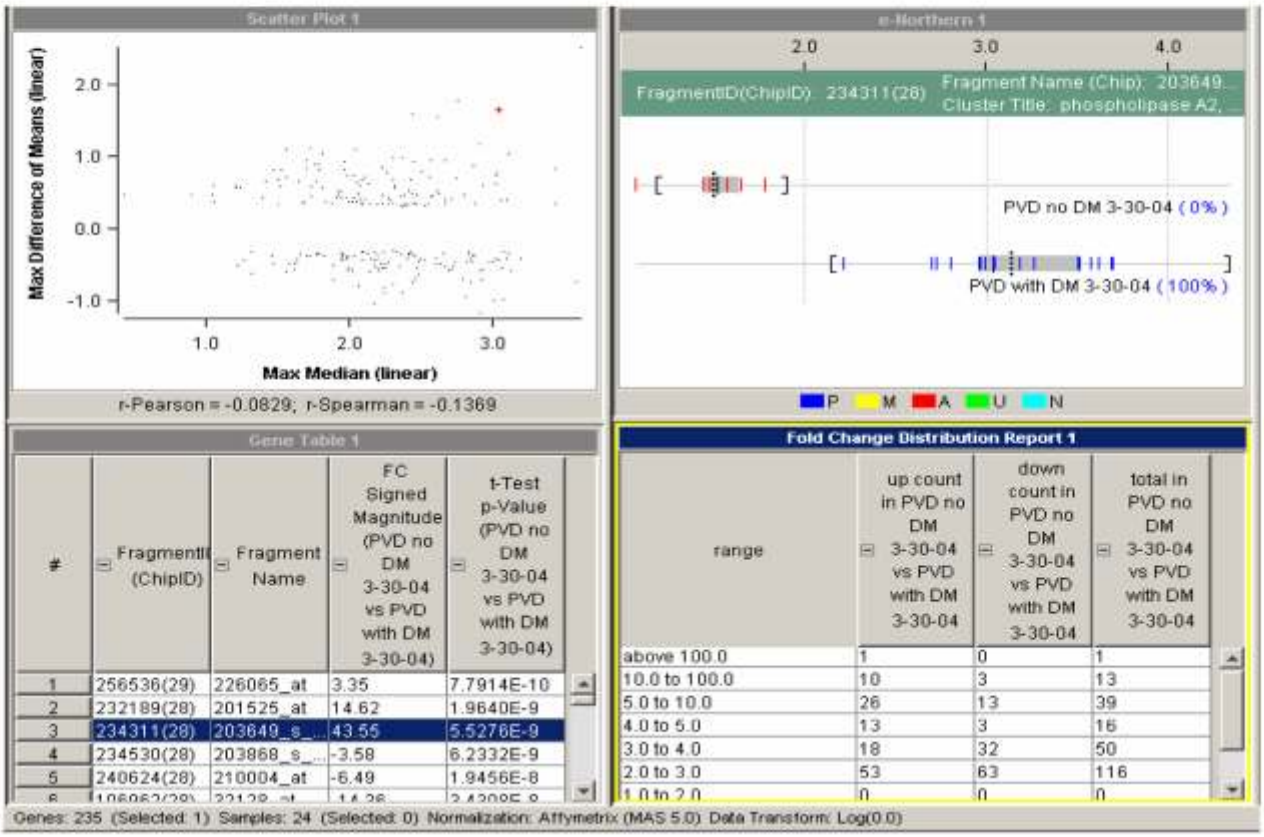

Figure 2: The figure shows a screen-shot of the results obtained using the PCA tool in Gene Logic's Genesis Enterprise System® Software using 235 dysregulated gene fragments.

yielded a set of 235 genes/fragments that were significantly differentially expressed between the two groups of samples, with a threshold setting of FC >2 and p <0.001.

To determine whether the 235 fragments set can discriminate the two groups of samples, Principal Component Analysis (PCA) was performed using Genesis Enterprise System® Software. As can been seen from Fig. 3, this set of fragments clearly separated the samples into two distinct groups.

To further examine the nature of these gene fragments, pathway and other analysis tools within Genesis Enterprise System® Software were used (Table 1). This analysis indicated that the gene fragments that were up-regulated included a collection of growth factors, growth factor receptors, and proteins associated with the extra cellular matrix. The down-regulated cluster consisted primarily of genes correlating with an inflammatory response and cell adhesion molecules.

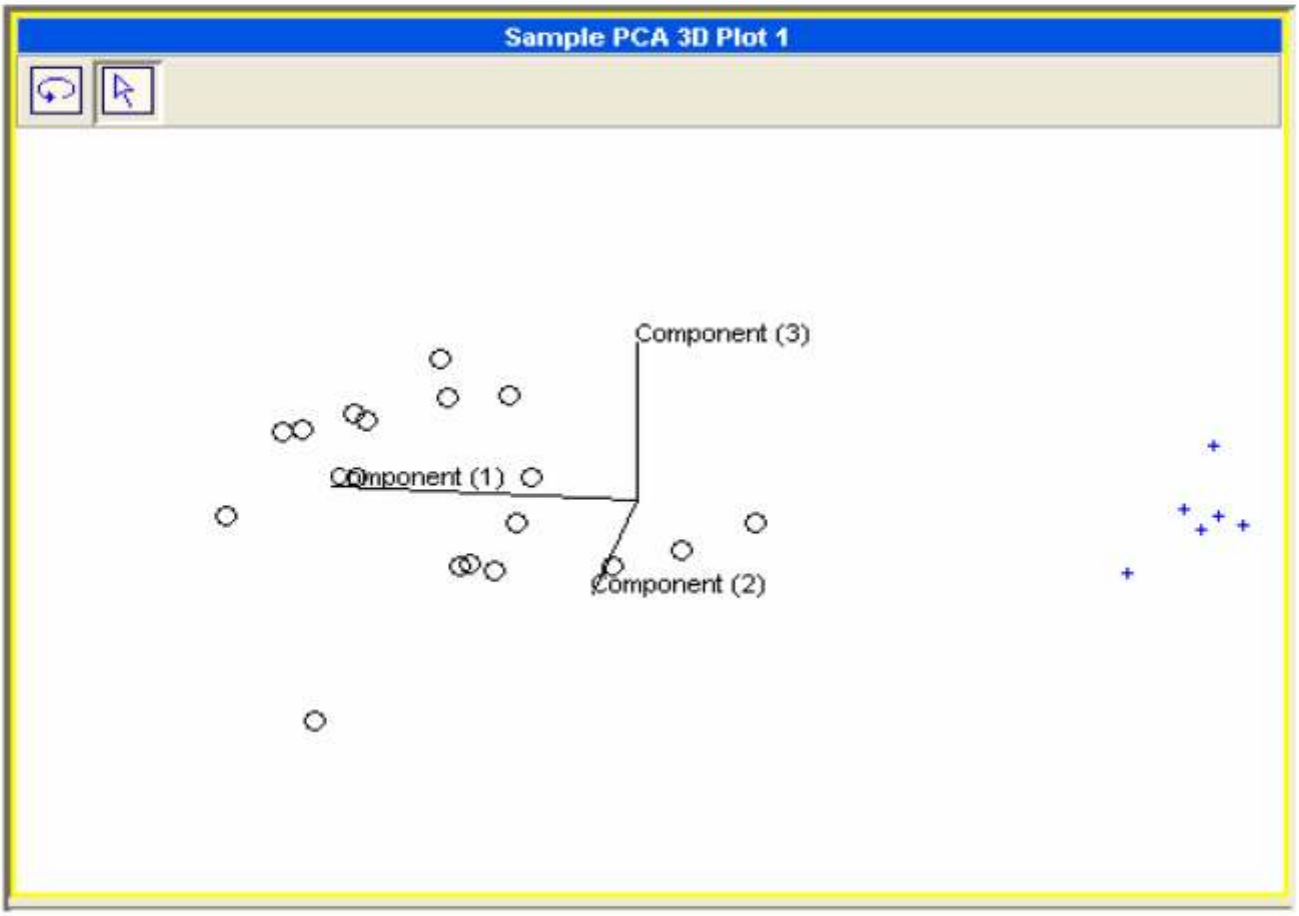

Figure 3: The figure shows a screen-shot of the results obtained using the PCA tool in Gene Logic's Genesis Enterprise System® Software using 235 dysregulated gene fragments.

Examples of up-regulated genes
Vascular endothelial growth factor D
PDGF receptor β
FGF7 and FGFR2
TGF-β receptor II
Insulin-like GF (somatomedin C)
Chemokine C-X-C ligand 14
Spondin 2
Disintegrin & metalloproteinase domain 33
Laminin a2
Dynamin 1
Ankyrin 2

Examples of down-regulated genes
TNF-α superfamily member 11b
Activated leukocyte adhesion molecule
Apolipoprotein C-I, C-II & E
VEGF
MMP 1,7,9 & 19
Chemokine ligand 4, 5, 16 & 18
VCAM 1
IL-18

Table 1: List of up- and down-regulated genes in PVD with Diabetes

Role of CXCL14 in monocyte chemotaxis and angiogenesis

Cellular chemokines, through their involvement in inflammatory recruitment of monocytes/macrophages, fibroproliferative response, and vascular remodeling, are believed to play a central role in atherogenesis (see Reape & Groot, 1999; Ross, 1999). The process appears to be initiated by the accumulation in arterial intima of low-density lipoproteins (LDL) undergoing oxidation and glycation. This provides stimuli for the release of monocyte-attracting chemokines, and for increased production within endothelial cells of molecules promoting adhesion of those monocytes. These conditions favor monocyte transmigration into the arterial intima, where the monocytes internalize the accumulated, modified LDL via endocytosis. This process employs scavenger receptors on the macrophage surface. Differentiating monocytes, in concert with T lymphocytes, exert a modulating effect on lipoprotein metabolism within the intima. This leads to a series of reactions resulting in the generation of lipid peroxides and expression of chemokines, adhesion molecules, cytokines, and growth factors, thereby sustaining an ongoing inflammatory process leading ultimately to lesion formation (Nesto, 2003).

In light of the emerging consensus on this pathogenetic mechanism, and the observation that DM accelerates PVD, one might expect up-regulation of genes expressed in (intimal) monocyte/macrophages correlating with an inflammatory response (apolipoproteins, chemokine ligands), matrix metalloproteases, growth factors (IGF-1, VEGF) and cell adhesion molecules (VCAM, activated leukocyte adhesion molecule). However, we found that, at least, some of these genes were, in fact, down-regulated.

Interestingly, we noted that one inflammatory molecule in particular, chemokine C-X-C ligand 14 (CXCL14/BRAK), is dramatically upregulated in PVD with DM but not in non-diabetics (Figure 4). Kurth et al (2001) have provided evidence that CXCL14/BRAK is selectively chemotactic for monocytes following activation by prostaglandin E2 (PGE2). In the presence of PGE2 found in atherosclerotic arteries, monocytes become exquisitely more sensitive to CXCL14 while losing their chemotactic responsiveness to the more traditional chemokines such as MCP-1, RANTES, stromal cell-derived factor-1, etc. (Beloqui et al., 2004 and Subbiah MT, 1978). It has also been proposed that a fraction of monocytes present in peripheral blood are intrinsically responsive to BRAK in the absence of exogenous activation (Muller, 2004). Kurth et al. had also proposed that fibroblasts are the primary source of BRAK and that BRAK is involved in the homeostasis of monocyte-derived macrophages rather than in inflammation. New evidence indicates that CXCL14 binds with high affinity to immature monocyte-derived dendritic cells and also acts as a chemotactic factor for these cells. Importantly, the same authors showed this chemokine to be a potent inhibitor of angiogenesis, a process closely associated with the pathogenesis of PVD (Shellenberger et al., 2004). Furthermore, another inhibitor of angiogenesis, Angiopoietin-3 (Ang-3), is highly expressed in diabetic patients with PVD emphasizing the role of anti-angiogenic events in the pathogenesis of PVD (Valenzuela et al, 1999).

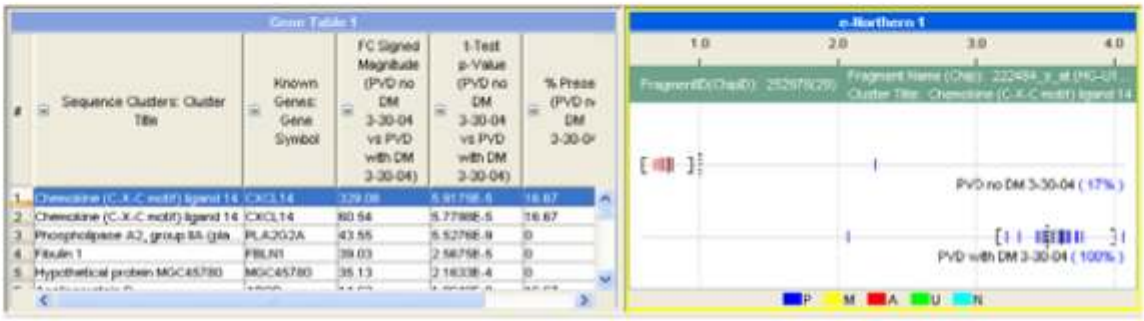

Figure 4: The figure shows results obtained using the Comparative Analysis tool in Gene Logic's Genesis Enterprise System® Software. A. Gene table showing fold change and p-value sorted by highest fold change: One of the CXCL14 fragments is highlighted. B. e-Northern® Plot showing expression of CXCL14 in individual samples.

Conclusions

Taken together these data suggest that DM may affect the development of PVD via mechanisms distinct from those that are observed in non-DM related atherogenesis. A number of novel events of therapeutic importance were observed within this dataset such as dysregulation of Prostaglandin F receptor, MAP kinase pathways, etc. Importantly, we identified a novel monocyte-mediated event in PVD associated with diabetes and described the role of CXCL14 in orchestrating this event. We also suggest preliminary evidence that CXCL14, together with Ang-3 could play a potent anti-angiogenic role in diabetic patients with PVD. This Application Note illustrating molecular events associated with PVD with diabetes demonstrates the power and utility of Gene Logic's BioExpress® System (Metabolism and Cardiovascular Suites) and the Genesis Enterprise System® Software.

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