Application Note

Translational Approaches to Understanding Metabolic Syndrome

Use of clinical and animal model data identifies Stearoyl-CoA-Desaturase (SCD) as a potential target

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Introduction

In the United States and other developed countries, obesity has risen to an epidemic level. As a result, metabolic diseases are currently receiving a great deal of attention from the medical community. This Application Note will demonstrate how scientists at Gene Logic conducted a study to determine the gene expression changes occurring in overweight individuals suffering from metabolic syndrome and how this led to the identification of a putative pharmacological target for the disease. In addition, we will show how an animal model was used to validate these findings. The BioExpress® System, a database of gene expression measurements from human samples, curated by board-certified pathologists, and the Genesis Enterprise System® Software, an extensive collection of warehousing and analysis tools, were used to perform the analyses. This study highlights how researchers can rapidly perform translational research to develop novel hypotheses that can be then validated in the laboratory.

Definition and Background

With recent trends in sedentary lifestyles and ever increasing serving sizes of high-caloric foods in diets, metabolic disorders such as obesity, diabetes, and metabolic syndrome are on a sharp increase in the general population, especially in the developed world. Metabolic syndrome (also known as insulin resistance syndrome or syndrome X) is a metabolic disorder that has recently been recognized by the World Health Organization (WHO) and the National Cholesterol Education Program (NCEP). It is a cluster of risk factors that includes dyslipidemia (hypertriglyceridemia and low levels of high-density lipoprotein [HDL] cholesterol), elevated blood pressure, impaired glucose tolerance, and central obesity that is closely associated with insulin resistance. And according to the criteria defined by the National Cholesterol Education Program-Adult Treatment Panel (NCEP-ATP III, 2001), having any three of these five risk factors is diagnostic of metabolic syndrome.

Currently, it is estimated that more than forty-seven million American adults meet the NCEP criteria for metabolic syndrome (Mokdad et al, 2003). Numerous epidemiologic studies have shown that individuals with metabolic syndrome and insulin resistance have a threefold increase in cardiovascular disease and a significant risk of cardiovascular mortality (Baillie et al, 1998; Laaksonen et al, 2004). Thus, identifying these high-risk individuals is critical to providing appropriate therapy.

The NCEP-ATP III has also identified metabolic syndrome as an indication for vigorous

lifestyle intervention. Effective interventions include diet, exercise, and judicious use of pharmacologic agents to address specific risk factors. In a large number of randomized, controlled studies, substantial improvement in all aspects of metabolic syndrome and severe obesity was observed with only a moderate degree of weight loss (for a review, see Busetto, 2001). Specific dietary changes appropriate to address different aspects of metabolic syndrome include reducing saturated fat intake to lower insulin resistance, reducing sodium intake to lower blood pressure, and reducing high-glycemic-index carbohydrate intake to lower triglyceride levels. At present, however, the mechanism by which caloric intake regulates various aspects of metabolic syndrome and insulin resistance is far from clear.

New research has shown that adipose tissue is a complex endocrine organ capable of producing many important hormones referred to as adipokines. Several inflammatory mediators such as TNF-a, IL-1ß, IL-6, IL-8, IL-10, TGF-ß, nerve growth factor, and acute-phase response mediators such as plasminogen activator inhibitor-1, haptoglobin and serum amyloid A (SAA) that are secreted by the adipose tissue, appear to play a significant role in the pathogenesis of metabolic syndrome, insulin resistance and obesity (reviewed, Trayhurn & Wood, 2004). Furthermore, a large body of experimental evidence has also established an association between visceral obesity, insulin resistance, and the metabolic syndrome in particular (see Vega, 2004; Freedland, 2004 for recent reviews).

The purpose of this Technical Application Note is to discern the molecular changes in the adipose tissue of metabolic syndrome patients, before and after weight loss intervention. These molecular changes were identified using Affymetrix GeneChip® arrays, in order to find specific genes whose expression patterns change in response to weight loss. This knowledge could be used to develop novel therapeutics or diagnostics.

Materials and Methods

Details of the Human Clinical Study

Ten adults with a Body Mass Index (BMI) of >30 (obese) who met NCEP-ATP III criteria for metabolic syndrome were recruited for this study. Patients with eating disorders, cancer, steroid therapy, liver diseases, myocardial infarction, or pregnancy were excluded. Weight loss intervention averaged 8 weeks (range, 7.5 to 10 weeks). The program involved induction with a protein-sparing, very low calorie (cal) diet of approximately 600-800 cal daily, supervised by a team of physicians, registered dieticians, and behaviorists. It consisted of meal replacement products (Nutrimed-Plus; Robard Corp., Mount Laurel, NJ); each serving containing 200 cal, 6 g of fat, 26 g of protein, and 10 g of carbohydrate. These products were used alone or in combination with lean beef, fish, or poultry. Daily prescribed protein intake was 1.5 g/kg of a predetermined goal weight; daily fluid intake was a minimum of 2 liters.

Subcutaneous adipose tissue was harvested from the umbilical region under local

anesthesia. The accrual procedure followed strict IRB approval and Standard Operating Procedures provided by Gene Logic. The samples were prepared at Gene Logic and followed the recommendations of the Affymetrix Expression Analysis Technical Manual. Gene Logic's scientists prepared optimized detailed methods, when needed, to ensure that high quality data was generated from each sample. Only those samples that passed Gene Logic's stringent quality control metrics were added to the BioExpress® System database for storage and analysis using the Genesis Enterprise System® Software.

The BioExpress® System is a gene expression database containing more than 18,000 samples representing diseases such as cancer, inflammation and autoimmune disorders, oncology, metabolism, cardiovascular, and central nervous system disorders. The present study used a portion of the database, the Metabolic Disorders Data Suite, that contains human samples representing diabetes and obesity, including the samples from the ten adults described above. The Metabolic Disorders Data Suite also contains animal samples obtained as a result of carefully controlled studies with a focus on metabolic disorders.

The Genesis Enterprise System® Software enables the storage of the gene expression data as well as the clinical data associated with the samples such as patient history, diagnostic tests, medication, etc. The Genesis Enterprise System® Software provides standard tools to allow researchers to perform common research tasks, such as building complex queries to identify related samples, visualizations to identify 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, and providing the ability to quickly correlate gene expression changes to clinical parameters, including currently used markers.

Details of the Animal Model Study

A separate animal study was conducted using Sprague-Dawley rats to (1) investigate changes in expression of genes in peripheral tissue that may be involved in the development of the body weight set point, and (2) determine if post-natal overfeeding and underfeeding causes permanent changes in the expression pattern of these genes, leading to abnormal body weight set points during adulthood.

Animals in three groups (Normal Nutrition, Chronic Postnatal Overfeeding (CPO), and Chronic Postnatal Underfeeding (CPU)) were sacrificed at 11 days, 16 days, 21 days, and 60 days of age. Brain regions and peripheral organs were collected, processed, and run over microarrays.

Results and Discussion

In this study, subcutaneous adipose tissue samples were identified and assembled into two groups. The first, 'pre-diet' group, consisted of samples (n = 11) from patients with metabolic syndrome before the (average) 8 week weight loss intervention described above. The second, 'post-diet' group included samples (n = 7) from the same patients after the weight loss intervention. On average, a 10% reduction in body weight was achieved by each patient.

A screen shot from the Genesis Enterprise System® Software is shown in Fig. 1, detailing some of the clinical information collected on each patient sample contained in the BioExpress® System. Analyses can be run using any of the hundreds of clinical attributes present.

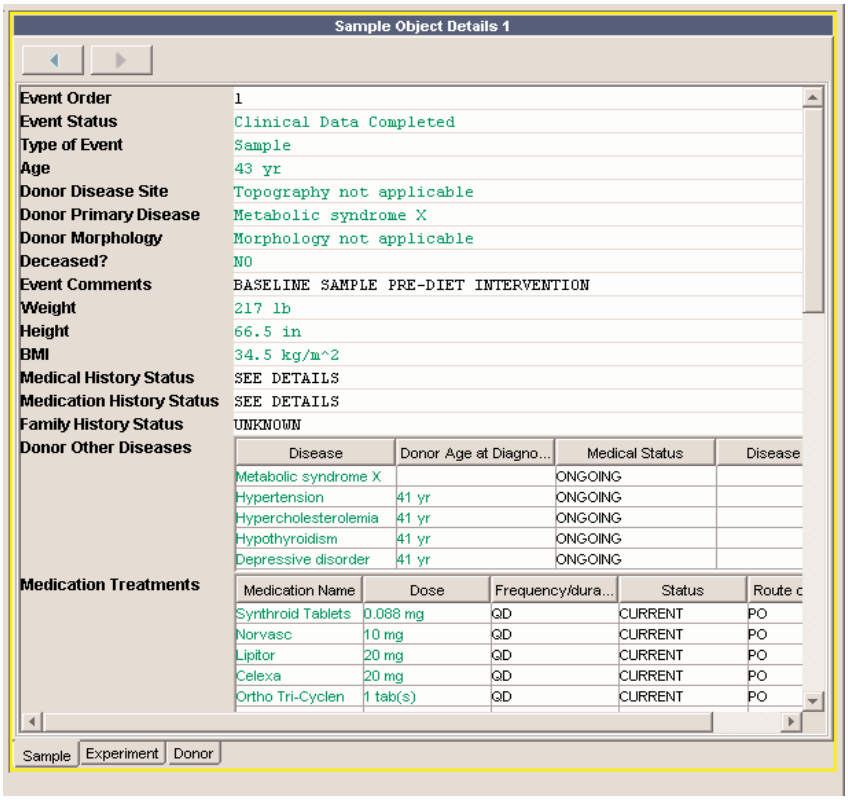

Figure 1. The figure shows a screen-shot of the results obtained by querying on the patients clinical data using Gene Logic's Genesis Enterprise System® Software.

Analysis of Gene Expression Data from the Clinical Study

In order to discern the effects of weight loss on gene expression in adipose tissue, a fold-change (FC) analysis of the two groups of samples (pre- and post-diet) was performed. The FC analysis yielded a set of 702 genes/fragments that were significantly differentially expressed between the two groups with a FC magnitude of >1.5 and a p value of <0.05.

As expected, the expression of several genes involved in lipid metabolism such as glycerol-3-phosphate acyltransferase, stearoyl-CoA desaturase (SCD), fatty acid synthase, fatty acid desaturase, diacylglycerol O-acyltransferase, and stress proteins such as heat shock protein (HSP70) and serum amyloid A (SAA) were all down-regulated upon weight loss (Fig. 2). SCD in particular was down-regulated by at least four-fold after 8 weeks of weight loss intervention (Fig. 3).

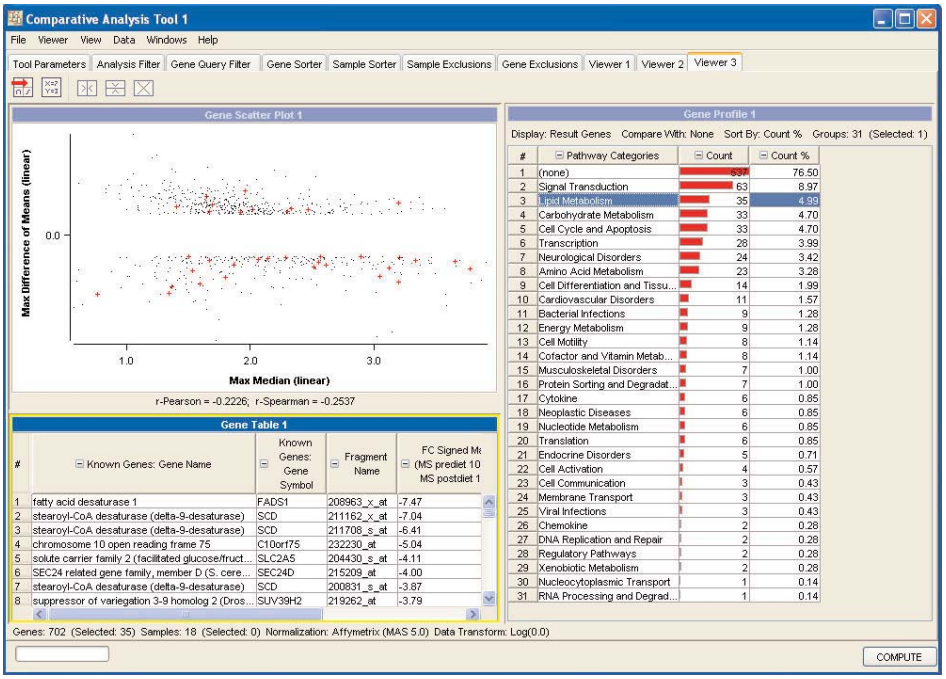

Figure 2: The figure is divided into three interlinked-panels. The top left panel shows a Gene Scatter plot indicating the up- and down-regulated genes in pre-diet sample set compared to the post-diet sample set. The Gene Profile panel to the right was created to show the "Pathway Categories." Here the count is the total number of Affymetrix fragments within the "Pathway Category" that has undergone dysregulation (up or down). The Lipid Metabolism Pathway Category selected highlights all the fragments that have undergone dysregulation in the Gene Scatter plot as indicated by the red cross marks. The lower panel shows the dysregulated list of genes sorted by the Fold Change Magnitude. Clearly SCD is one of the most down-regulated genes.

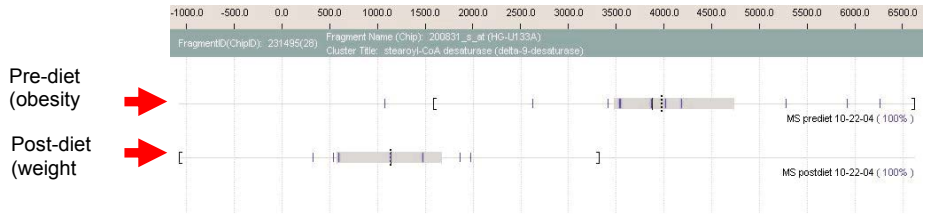

Figure 3. An e-Northern® report from the Genesis Enterprise System® Software showing down-regulation of SCD in patients with metabolic syndrome upon weight-loss.

In contrast, certain molecules associated with inflammation, such as the macrophage scavenger receptor 1, chemokine C-C ligand 18, apolipoprotein CI, and molecules associated with signaling such as IGF binding proteins, were up-regulated upon weight loss (Data not shown).

Although the mechanisms by which SCD deficiency leads to these metabolic changes are unknown, Dobrzyn et al (2004) observed that the phosphorylation and activity of AMP-activated protein kinase (AMPK), a metabolic sensor that regulates lipid metabolism during increased energy expenditure, is significantly increased in the livers of SCD knockout mice. In parallel with the activation of AMPK, the authors also observed that acetyl-CoA carboxylase activity was decreased, resulting in decreased intracellular levels of malonyl-CoA. Lower malonyl-CoA concentrations are known to derepress carnitine palmitoyltransferase 1 (CPT1). Indeed, in SCD-deficient mice, both CPT1 and CPT2 activities were significantly increased, suggesting increased mitochondrial oxidation of fatty acids (Dobrzyn P et al, 2004).

The adipose tissue from patients with metabolic syndrome demonstrated decreased expression of acetyl-CoA carboxylase upon weight loss, while levels of carnitine palmitoyltransferase 1 expression increased as obtained using the BioExpress® System (not shown). These results not only corroborate the results reported in the literature with animal models, but also underscore the power, ease and speed with which the BioExpress® System can be employed to test new hypotheses and confirm existing ones. Stearoyl-CoA desaturase (SCD)

SCD is a central lipogenic enzyme which catalyzes the rate-limiting step in the biosynthesis of monounsaturated fatty acids. Increased SCD activity and alterations in monounsaturated fatty acids have been implicated in various diseases including cancer, diabetes, atherosclerosis, and obesity (Natmbi, 1999). Recently, compelling biochemical and genetic evidence for its role in obesity and insulin resistance has been reported (Ntambi et al, 2002; Rahaman et al, 2003). Thus, diet-induced obesity in mice fed a high-fat 'cafeteria' diet, resulted in a significant rise in SCD expression together with similar changes in several other molecules involved in lipid metabolism (Lopez et al, 2003). In a corollary experiment, mice with a targeted disruption of SCD have reduced body adiposity,

increased insulin sensitivity, and are resistant to diet-induced obesity (Ntambi et al, 2002).

Analysis of Gene Expression Data from the Rodent Study

Although the human and the rodent physiology are different, there are number of advantages of using animal models, such as ease of measuring calorie intake, controlled diet environment, etc. The use of this CPO/CPU model could help identify patterns of gene expression in normal development and identify those that specifically change in response to the nutritional challenges. It is important to use this model to be able to focus on a limited number of genes that are important, specifically for development of the body weight set point, as well as in identifying those genes that may be responsible for the development of an obese phenotype.

From the human clinical study above, the SCD expression is clearly down regulated post-diet. It follows that overweight/obesity due to excess caloric intake should cause an increase in SCD expression and weight loss should lead to its decline. Therefore, we investigated the gene expression changes in this rodent study. Indeed, wild-type rat pups allowed excessive postnatal milk supply (obese due to CPO) show a four-fold increase in SCD expression in adipose tissue, while severely malnourished pups (underweight due to CPU) have decreased expression, when compared to pups on a 'normal' postnatal milk supply (Fig. 4). Taken together, the present data illustrate how SCD may be a potential drug target for metabolic syndrome. Thus, small molecules that inhibit SCD could be developed as novel therapeutics for this complex metabolic disorder.

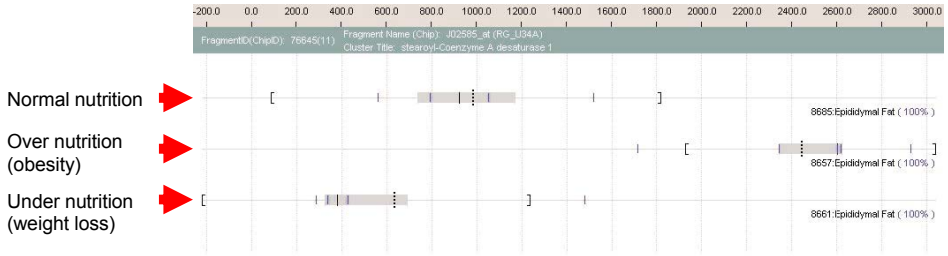

Figure 4. An e-Northern® report showing expression of the SCD gene in a rat model of juvenile obesity. The experiment was set-up as follows: in a control group, a mother rat was allowed to feed 8 new-born pups. In the CPO group, a mother rat was allowed to feed only 3 new-born pups thus ensuring an abundant milk supply. In contrast, the third group, the CPU group, a mother rat was allowed to support as many as 14 new-born pups, thereby severely limiting the milk supply. As can be seen, SCD mRNA levels were dramatically up-regulated in the CPO group, when compared to the control group of animals, much like that observed in the human obesity study. In contrast, severe weight loss due to chronic under-nutrition (CPU group) dramatically reduced expression of SCD.

Conclusions

This Application Note demonstrates the power and utility of Gene Logic's BioExpress® System and Genesis Enterprise System® Software. Using metabolic syndrome as an example of a disease whose etiology is poorly understood, this paper clearly illustrates how gene targets and biomarkers can be identified and validated in silico. More importantly, this study illustrates the application of a reference database such as the BioExpress® System for translational approaches to understanding disease.

Postscript: As this article was getting ready to be published, we encountered another publication from Merck which suggests the use of SCD as a putative pharmacological target (Jiang et al, 2005)

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