GENE REGULATORY NETWORKS IN METABOLIC SYNDROME

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Metabolic syndrome (MetS) is a complex worldwide epidemic disorder defined by a cluster of concomitantly occurring conditions, including dyslipidemia, hypertension, hyperglycemia, or abdominal obesity. MetS is chronic in nature, negatively affecting the life quality of patients, who also have an increased risk of cardiovascular disease and type 2 diabetes. Efforts to prevent MetS, and to better diagnose and treat patients, rely on a better understanding of this syndrome.

In an effort to demonstrate the applicability of compendium-wide analyses in bringing high-quality insights into the pathophysiology of MetS, we used the GENEVESTIGATOR® toolset and the underlying rich compendium of curated data from the areas of obesity, nutrition, type 2 diabetes, and other metabolic disorders. Our analysis highlights the gene regulatory networks involved in the inflammation-immune responses in MetS and other related conditions. Using two different transcriptomic approaches, we identified gene signatures of subcutaneous adipose tissue (SAT) specific for certain metabolic disorders. Using the first approach, we confirmed the effectiveness of current treatment interventions which resulted in signatures very different from our identified signature. We could also confirm the translatability of our finding across platforms. Using the second approach, we identified a metabolic signature for MetS, obese, overweight, and control subjects, in which genes previously reported to be involved in these conditions were identified. One specific cluster of interest was identified, containing genes mainly associated with the inflammation-immune response of SAT. We could show that these genes are also highly expressed in immune cells, confirming the pro-inflammatory state of SAT in these conditions. Lastly, we identified co-regulated genes in obese/insulin resistant patients, all of which have been previously associated with MetS, and thereby providing a list of genes that can be used for further computational or laboratory validation.

INTRODUCTION

Metabolic syndrome (MetS) is a complex disorder which lacks uniform definition criteria (Kassi *et al.*, 2011). One accepted definition of MetS is that a patient fulfills three out of five risk factors: elevated triglycerides combined with high low-density lipoprotein and/or low high-density lipoprotein in their serum (jointly referred to as dyslipidemia), elevated arterial blood pressure (hypertension), dysregulated glucose homeostasis, or abdominal (visceral) obesity (Paley & Johnson, 2018). MetS is estimated to affect over 20% of the global adult population (Paley & Johnson, 2018), and is correlated with an increased risk of cardiovascular disease and type 2 diabetes (Kassi *et al.*, 2011). Treatment of MetS and the related conditions is rather complex. It includes changes in lifestyle, with emphasis on weight loss through diet and regular exercise, as well as pharmacotherapy of the associated pathological conditions.

The current treatment of MetS targeting weight loss is supportive of the hypothesis that abdominal obesity is the predominant risk factor for MetS (Paley & Johnson, 2018). As far back as 1991, factors promoting insulin

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resistance (IR), which are active in abdominal obesity, were described. The close association between abdominal obesity and IR as well as MetS were highlighted (Björntorp, 1991). More recent research suggests that obese subjects with a similar body mass index can be classified into two groups which reflect a different metabolic health status (Das *et al.*, 2015). This classification is based on a substantial heterogeneity in the gluco- and cardio-metabolic health phenotypes of the subjects. Dozens of studies performed up to now have demonstrated significant dysregulation in the adipose tissue transcriptome in obesity, highlighting adipose tissue as a key tissue in obesity. However, identification of genes underlying the observed heterogeneity and determination of gene signatures that would distinguish metabolically healthy from unhealthy phenotypes, and hence the associated risk of MetS, remains challenging.

One way to approach this challenging task of gene signature identification is to use transcriptomic data. GENEVESTIGATOR® contains more than 220 human studies from the area of endocrinology, nutrition, and metabolism, comprising more than 10 000 manually curated samples. When combined and analyzed in concert, the results provide a more comprehensive picture than single experiments alone would. In this study, we performed a comparative gene expression analysis of subcutaneous adipose tissue samples derived from normal, obese, IR, and MetS patients. The aim was to demonstrate that compendium-wide analyses can bring high-quality insights into the pathophysiology of MetS and lead to a better understanding of this complex disorder.

RESULTS AND DISCUSSION

We employed two transcriptomic approaches to investigate gene expression in our conditions of interest:

I. GENE SIGNATURE OF SUBCUTANEOUS ADIPOSE TISSUE OF METS/IR/OBESE PATIENTS

In the first transcriptomic approach, we utilized the perturbations tool from the GENE SEARCH toolset to identify differences in gene expression of subcutaneous adipose tissue (SAT) between obese subjects with IR (IR/obese) and healthy individuals (HS-01078, GSE26637). For identifying a signature, the Affymetrix Human Genome U133 Plus 2.0 Array was chosen as a data selection, whereby the comparison of HS-01078 insulin resistance study 2/normal subcutaneous adipose tissue was used to identify differentially regulated genes. 25 up-regulated and 25 down-regulated genes were selected for further analysis in the Signature tool of the SIMILARITY SEARCH toolset to identify other correlated studies.

A similar gene expression pattern was found mainly in comparisons of IR/obese patients with controls (Figure 1A), as anticipated. Among the most different perturbations identified (Figure 1B), we observed treatment interventions reducing MetS symptoms, such as obese patients after successful weight reduction, and IR/obese subjects with good response to thiazolidinedione therapy (a group of oral anti-diabetic drugs designed to treat patients with type 2 diabetes). Moreover, using data from the collection of studies profiled with the Illumina HumanHT-12 V3.0 expression bead chip platform, we were able to identify conditions reducing MetS such as diet/training, confirming the translatability of our findings across different transcriptomic technology platforms (Figure 1C).

II. GENES DIFFERENTIALLY EXPRESSED IN METABOLIC SYNDROME AND RELATED PATHOLOGICAL CONDITIONS

The second transcriptomic approach was based on differential expression (DE) analysis of SAT from MetS, obese and overweight patients, and healthy controls (HS-02321, GSE24883) using the Differential Expression tool of GENEVESTIGATOR®. We performed all possible comparisons and selected the top 20 up-regulated and the top

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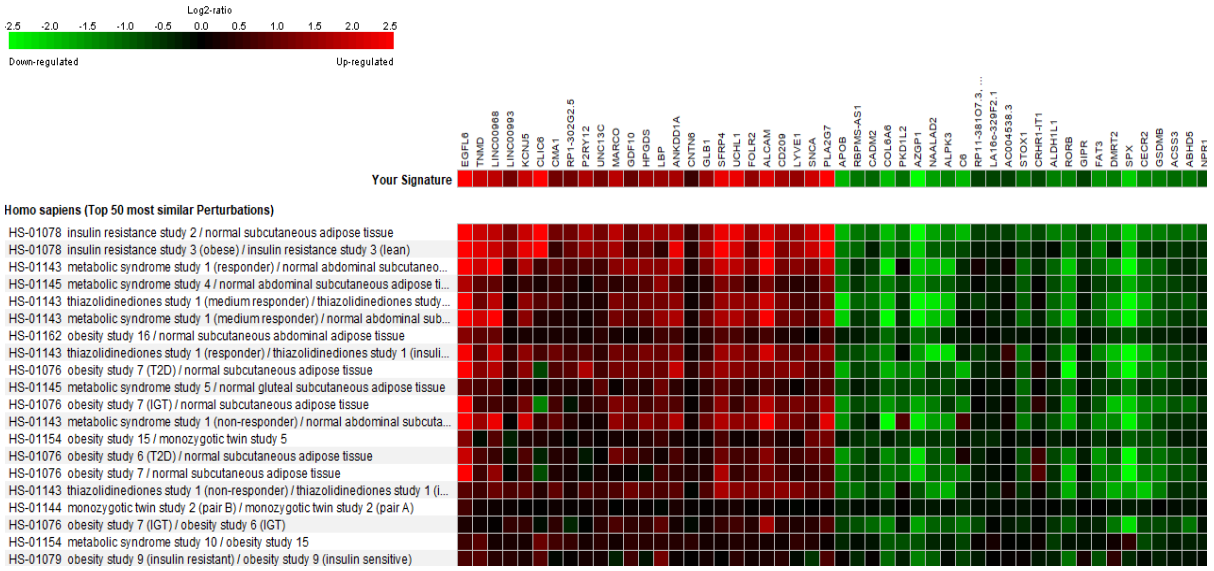
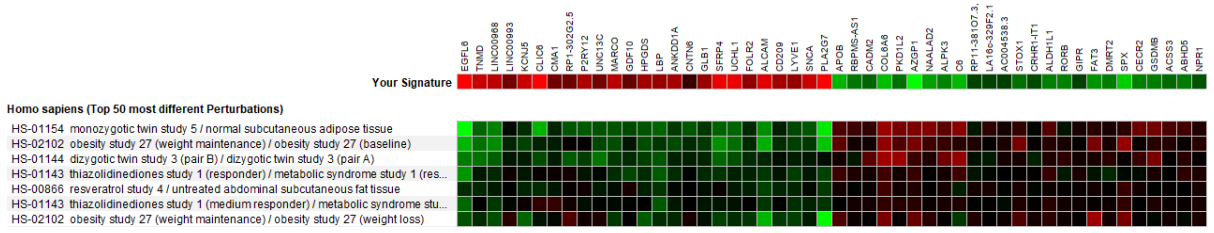
20 down-regulated genes from each analysis to create a metabolic signature (Exceptions: DE analysis of overweight SAT vs. normal SAT resulted in only 16 down-regulated genes, and no DE genes were found in obese SAT vs. overweight SAT under FDR = 0.05). The specificity of the final set of 148 DE genes (= metabolic signature) was further confirmed using the Hierarchical Clustering tool of GENEVESTIGATOR®, separating patients from the HS-02321 study into MetS, obese, overweight (with respect to the inter-stage phase) and controls clusters, which are marked in colored rectangles (Figure 2).

(A)



(B)



(C)

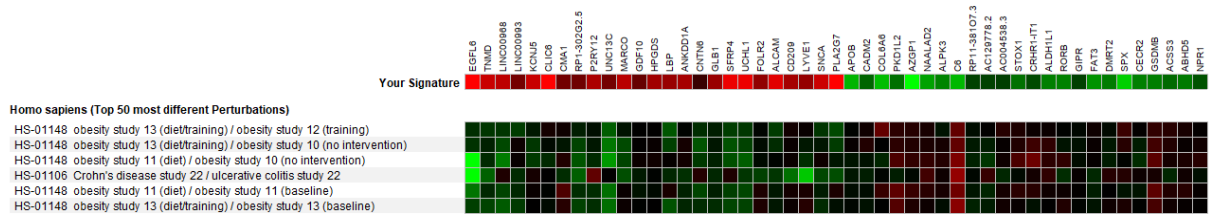


Figure 1. *Perturbations identified using the Signature tool from the SIMILARITY SEARCH panel compared with the signature of IR/obese SAT vs. normal SAT. In (A) and (B), analysis was performed on data from the Affymetrix Human Genome U133 Plus 2.0 Array with a gene expression fold-change > 0.5 and a p-value < 0.05. (A) Perturbations showing the gene expression patterns most similar to the signature. (B) Perturbations showing the gene expression patterns most different from the signature. (C) The most different perturbations generated using the Illumina Human HT-12 V3.0 expression bead chip platform.*

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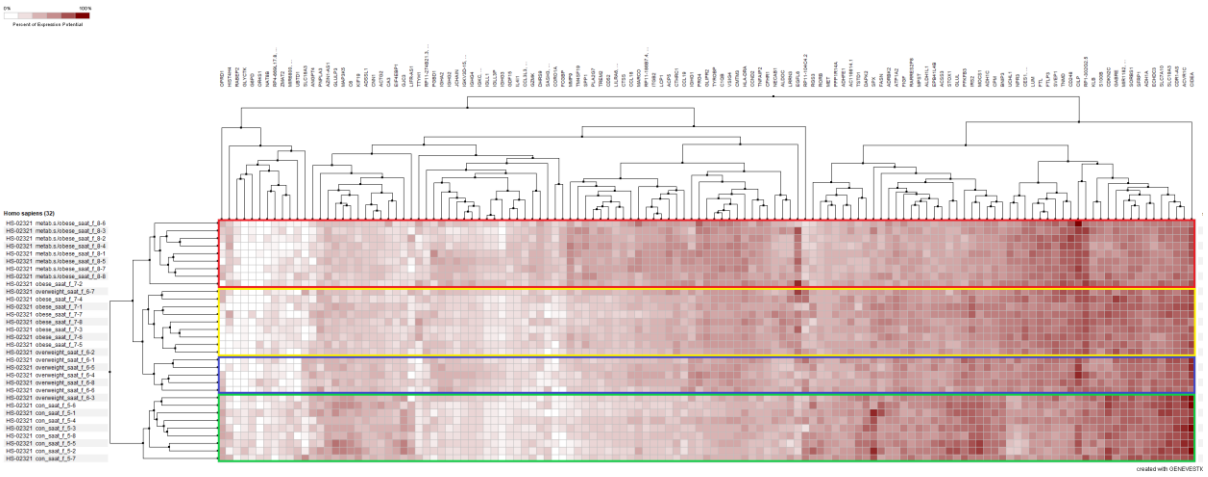

Figure 2. *Hierarchical clustering of the metabolic signature genes across MetS and related metabolic conditions (HS-02321, Euclidean Distance, Optimal-leaf ordering). The colored rectangles are representative of different groups: MetS patients are marked in red, obese in yellow, overweight in blue, and controls in green.*

The identified metabolic signature contains genes previously described to be associated with MetS pathology, e.g. *TNMD*, *EGFL6*, or *MMP9*. Genetic variation in *TNMD* (tenomodulin) is associated with a risk of type 2 diabetes and central obesity (Ruiz-Ojeda *et al.*, 2019). Increased expression of *EGFL6* (epidermal growth factor-like domain multiple-6) is related to obesity (Oberauer *et al.*, 2010), while *MMP9* (matrix metalloproteinase 9) is connected with common health problems of diabetic patients, like diabetic retinopathy (Mohammad *et al.*, 2012) or wound healing (Nguyen *et al.*, 2018).

Next, we focused on gene clusters that emerged from the hierarchical clustering (Figure 2). A preliminary analysis revealed two gene clusters (1,2) connected with metabolism and immune response in MetS and related conditions (Figure 3).

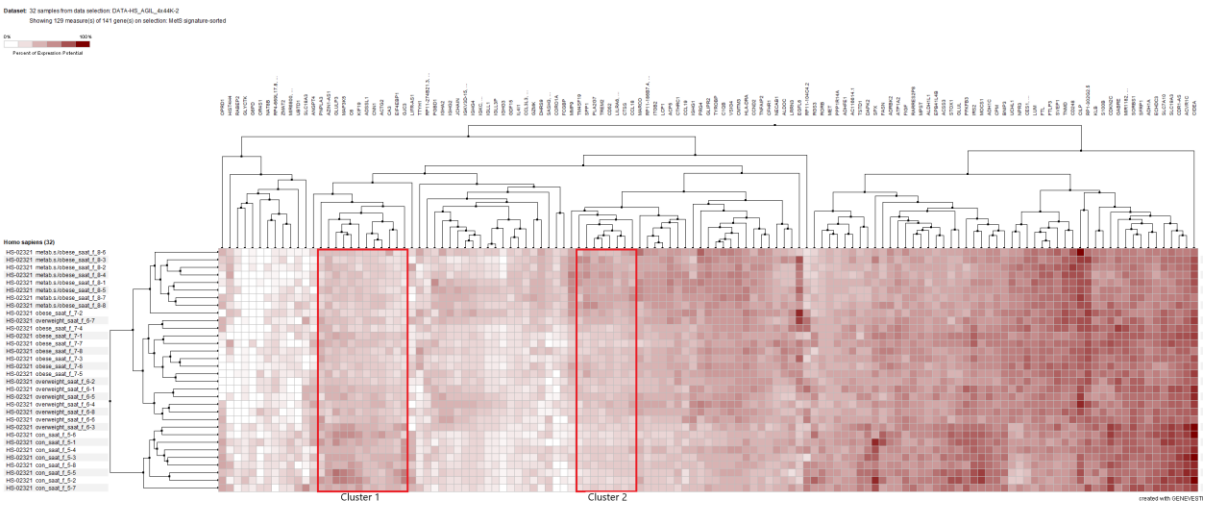

Figure 3. *Gene clusters associated with metabolism and immune response in MetS and related conditions (HS-02321). Two identified clusters are outlined with red rectangles.*

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Cluster 1 contains, among others, genes involved in lipid metabolism in adipocytes. Genes in cluster 2 are mainly associated with the inflammation-immune response of the SAT in MetS and obesity (Figure 3).

Chronic low-grade inflammatory conditions have been implicated as a major factor in MetS initiation (Ferranti and Mozaffarian, 2008), therefore we further subjected genes from Cluster 2 to a detailed analysis. To identify cell types with specific expression of Cluster 2 genes, we utilized the Hierarchical Clustering tool again. This time, we selected the option to sort genes according to Anatomy (tissues and cell types). This analysis verified that genes from Cluster 2 are highly expressed in immune cells like macrophages, leukocytes, and dendritic cells (Figure 4), confirming the pro-inflammatory state of SAT.

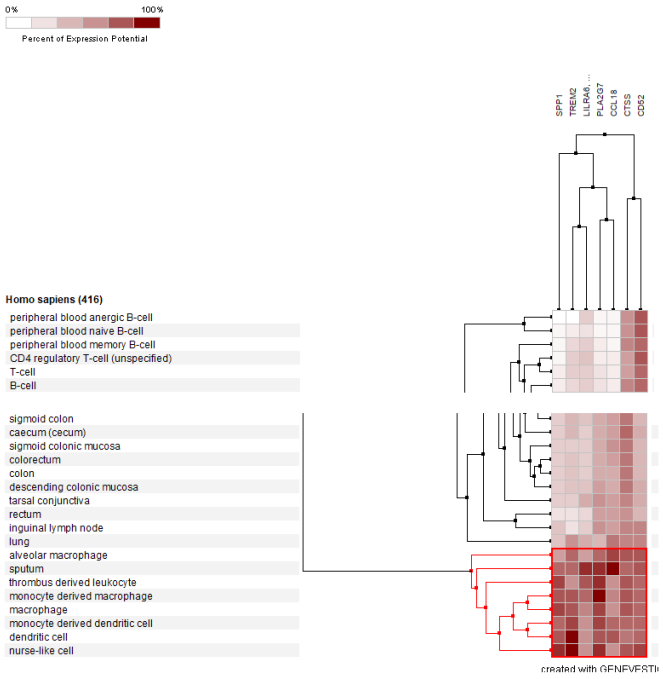

Figure 4. *Cell-type specific expression of Cluster 2 genes. The Affymetrix Human Genome U133 Plus 2.0 Array platform was selected for the analysis across all Anatomies. High expression levels of genes from Cluster 2 were detected in immune cells.*

To explore the gene regulatory network around Cluster 2, co-expression analysis for a chosen target gene was performed. We chose *PLA2G7* (phospholipase A2 group VII), encoding circulatory macrophage-derived factor, which is associated with obesity and type 2 diabetes (Jackisch *et al.*, 2018), and searched for genes which are similarly regulated. To obtain biologically relevant results, we selected IR/obesity perturbations on the Affymetrix Human Genome U133 Plus 2.0 Array platform in which we had previously detected *PLA2G7* to be significantly regulated. This analysis revealed several positively correlated clusters, of which one of the most dominant is marked by a red circle in Figure 5. The marked cluster contains 12 genes, all of which have known associations with MetS, especially obesity and diabetes, such as *MSR1*, or *LPXN*. The cluster can be used as a starting point for further laboratory or computer validations.

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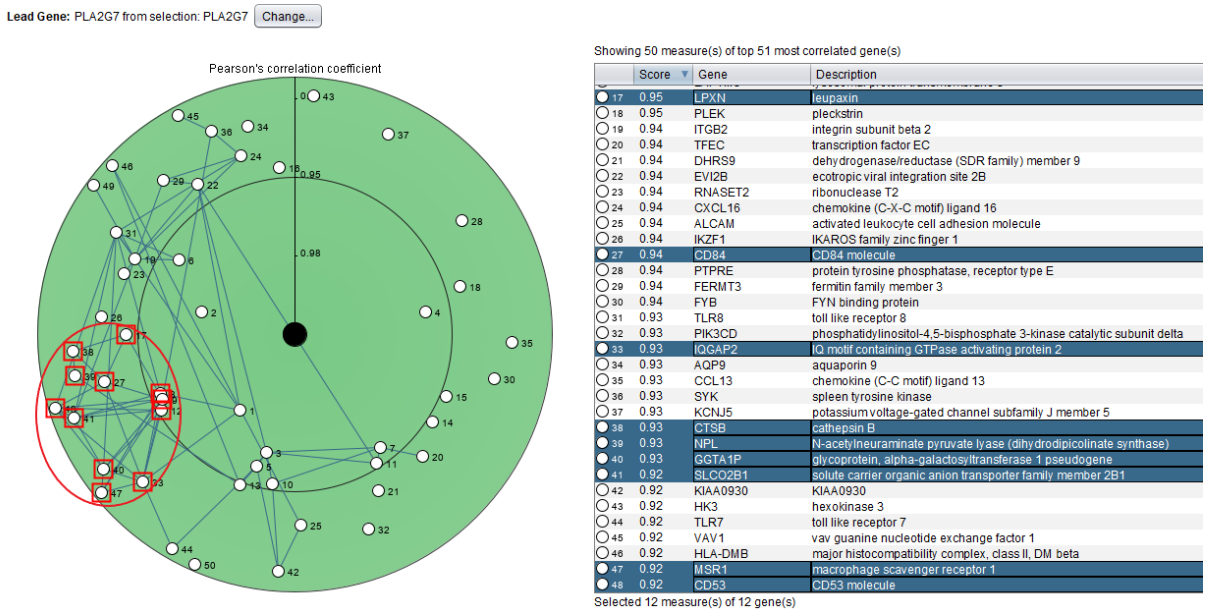

Figure 5. *Genes co-regulated with PLA2G7 in response to IR/obesity perturbations as defined using the Co-Expression tool. The Affymetrix Human Genome U133 Plus 2.0 Array platform was selected and filtered for studies containing comparisons of IR/obese SAT vs. normal SAT (HS-01076 (GSE27949), HS-01078 (GSE26637), HS-01079 (GSE15773/GSE20950), HS-01143 (GSE13070)).*

CONCLUSION

Microarray and RNA-seq high-throughput technologies facilitate gene expression analyses of a broad spectrum of conditions, including diseases, effects of environmental factors, or drug treatments. GENEVESTIGATOR® is an effective analytical tool that contains high-quality curated expression data and enables compendium-wide analysis for disease investigations and drug discovery. In this example study, we demonstrated how GENEVESTIGATOR® can be utilized to identify genes specifically regulated in the complex multi-factorial disorder, MetS. Additionally, our analysis gave insights into the gene regulatory networks involved in the inflammation-immune response in MetS, type 2 diabetes, and obesity.

SELECTED DATA AND SETTINGS FOR GENEVESTIGATOR®

Gene signature of subcutaneous adipose tissue of MetS/IR/obese patients
Gene Search Perturbations tool: Data selection: HS-01087 vs. HS_AFFY_U133PLUS_2 (full platform)
Signature tool: Data selection: HS_AFFY_U133PLUS_2 (full platform), HS_ILLU_HT12_V3 (full platform)

Genes differentially expressed in metabolic syndrome and related pathological conditions
Differential expression: Data selection: HS-02321
Hierarchical clustering: Data selection: HS-02321, HS_AFFY_U133PLUS_2 (full platform)
Co-expression: Data selection: HS-01076, HS-01078, HS-01079, HS-01143. Gene selection: PLA2G7

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