

# Global Gene Expression Profiling in Omental Adipose Tissue of Morbidly Obese Diabetic African Americans

Ayo P. Doumateya<sup>a, c</sup>, Huichun Xu<sup>a, d</sup>, Hanxia Huang<sup>a</sup>, Niraj S. Trivedi<sup>b</sup>, Lin Lei<sup>a</sup>, Abdel Elkahouloun<sup>c</sup>, Adebowale Adeyemo<sup>a</sup>, Charles N. Rotimi<sup>a, c</sup>

## Abstract

**Background:** Adipose tissues play an important role in the pathophysiology of obesity-related diseases including type 2 diabetes (T2D). To describe gene expression patterns and functional pathways in obesity-related T2D, we performed global transcript profiling of omental adipose tissue (OAT) in morbidly obese individuals with or without T2D.

**Methods:** Twenty morbidly obese (mean BMI: about 54 kg/m<sup>2</sup>) subjects were studied, including 14 morbidly obese individuals with T2D (cases) and six morbidly obese individuals without T2D (reference group). Gene expression profiling was performed using the Affymetrix U133 Plus 2.0 human genome expression array. Analysis of covariance was performed to identify differentially expressed genes (DEGs). Bioinformatics tools including PANTHER and Ingenuity Pathway Analysis (IPA) were applied to the DEGs to determine biological functions, networks and canonical pathways that were over-represented in these individuals.

**Results:** At an absolute fold change threshold of 2 and false discovery rate (FDR) of < 0.05, 68 DEGs were identified in cases compared to the reference group. Myosin X (MYO10) and transforming growth factor beta regulator 1 (TBRG1) were upregulated. MYO10 encodes for an actin-based motor protein that has been associated with T2D. Telomere extension by telomerase (*HNRNP1*, *TNKS2*), D-myo-inositol (1,4,5)-trisphosphate biosynthesis (PIP5K1A, PIP4K2A), and regulation of actin-based motility by Rho (ARPC3) were the most significant canonical pathways and overlay with T2D signaling path-

way. Upstream regulator analysis predicted five miRNAs (miR-320b, miR-381-3p, miR-3679-3p, miR-494-3p, and miR-141-3p), as regulators of the expression changes identified.

**Conclusion:** This study identified a number of transcripts and miRNAs in OAT as candidate novel players in the pathophysiology of T2D in African Americans.

**Keywords:** Obesity; Global gene expression; Type 2 diabetes; African Americans

## Introduction

The prevalence of type 2 diabetes (T2D) has risen concomitantly with that of obesity in virtually every country in the world [1, 2]. The International Obesity Task Force estimates that up to 1.7 billion people of the world's population are at a heightened risk of weight-related, non-communicable diseases such as T2D. The International Diabetes Federation predicts that the number of people with diabetes will rise from 194 million today to more than 333 million by 2025. In the US, about 35.7% of adults are obese and more than 11% of adults aged 20 years and older have diabetes, a prevalence expected to increase to approximately 21% by 2050 [3-5]. Ethnic minority groups, including African Americans, have a higher prevalence of obesity and are therefore at higher risk of developing obesity-related co-morbidities than other ethnic groups [6-8]. The molecular mechanisms that underline the development of T2D in obesity are still not well understood [9]. A number of studies have investigated these mechanisms in animal models or in tissues and organs that are directly involved in insulin metabolism such as pancreas, liver, and smooth muscle [9-16].

White adipose tissue (WAT) [17] is now well recognized as an important endocrine organ that produces large number of bio-molecules involved in glucose and lipids metabolism and inflammation [18]. These observations have led to intense investigation of the role of WAT in the pathophysiology of T2D. Gene expression arrays have been used to investigate the expression patterns of visceral adipose tissue (VAT) from morbidly obese and lean individuals [19, 20]. Also, the expression profiles of VAT and subcutaneous adipose tissue (SAT) in non-diabetic obese men have been investigated [21]. Other studies have conducted gene expression profiling in obese in-

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<sup>a</sup>Center for Research on Genomics and Global Health, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

<sup>b</sup>Computational and Statistical Genomics Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

<sup>c</sup>Core Laboratory-Cancer Genetics, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD, USA

<sup>d</sup>University of Maryland, School of Medicine, Baltimore, MD, USA

<sup>e</sup>Corresponding Author: Ayo P. Doumateya, Center for Research on Genomics and Global Health, NHGRI/NIH, 12 South Dr. Bldg 12A-4047 Bethesda, MD 20852, USA. Email: doumateya@mail.nih.gov; Charles Rotimi, Center for Research on Genomics and Global Health, NHGRI/NIH, 12 South Dr. Bldg 12A-4047 Bethesda, MD 20852, USA. Email: rotimic@mail.nih.gov

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**Table 1.** Characteristics of the Study Participants

Parameters	Obese non diabetic (control: n = 6)	Obese diabetic (cases: n = 14)	P-value
Age (years)	41.0 ± 4.6 (36 - 47)	41.5 ± 9.1 (27 - 61)	0.87
BMI (kg/m <sup>2</sup> )	53.9 ± 7.1 (45.6 - 65)	54.9 ± 7.8 (44.1 - 73.5)	0.79
Waist (inches)	N/A	55.8 ± 3.3 (51 - 60)	-
FBG (mg/dL)	N/A	128.1 ± 25.5 (81 - 172)	-

Values presented are mean ± standard deviation; values in parenthesis are ranges. Mean between groups were compared by Student's *t*-test. FBG: fasting blood glucose.

dividuals with insulin resistance, a physiological state often occurring before the development of T2D [22]. A number of pathways and candidate genes have been reported to be associated with obesity including those related to glucose and lipids metabolism, membrane transport, cell cycle promotion and immunity-complements C2, C3, C4 [9]. While these studies have contributed to our knowledge of the pathophysiology of obesity or obesity-related insulin resistance, identifying putative pathways that are more directly involved in obesity-related T2D remains crucially important. For example, it is well documented that insulin secretion and action are impaired in T2D but the exact mechanisms underlying this observation are not well understood especially in insulin-targeted tissue such as adipose tissues (ATs) [23-25]. *In vitro* experiments show that insulin receptor substrate-1 (IRS1), phosphatidylinositol 3-kinase (PI3-kinase) and small Rho family such as TC10 and cell division cycle 42 (*cdc42*) pathways are involved in insulin-induced glucose transport. However, these findings were observed in non-diseased state. In the present study, we utilize a global expression profiling approach to provide insight into gene expression patterns and pathways that may be affected in persons with T2D in the context of obesity.

## Materials and Methods

### Subjects

A total of 20 morbidly obese African Americans (BMI ≥ 40 kg/m<sup>2</sup>) were included in this study. Clinical and demographic data as well as OAT samples were obtained from Zen-Bio, Inc. (Research Triangle, NC). The participants in this study were undergoing elective surgical procedures and agreed to donate their de-identified discarded tissues for research. All participants underwent pre-operative clinical assessments by their physicians and diabetes status was determined at the time by the treating physicians using standard procedures. The characteristics of the 20 obese subjects (14 morbidly obese diabetics - "cases" and six morbidly obese non-diabetics - "reference") are summarized in Table 1. One sample was determined as a microarray hybridization outlier after quality control (QC) and was excluded from subsequent analyses.

### RNA extraction and quantification

Total RNA was extracted from OAT using EZ1 RNA univer-

sal kit and the EZ1 workstation (Qiagen Inc., Valencia, CA). Briefly 100 mg of frozen OAT was disrupted and homogenized in 750 µL of QIAzol lysis reagent using a tissuelyser (Qiagen Inc., Valencia, CA). Then 150 µL of chloroform was added to the homogenate and centrifuged. After centrifugation, the sample separates into three phases. The upper aqueous phase containing the RNA was transferred to a new tube and processed with EZ1 RNA universal tissue kit on the EZ1 workstation following the manufacturer's instructions. RNA quantity and quality were assessed using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) and Nanodrop (Thermo Fisher, Wilmington, DE).

### RNA amplification, labeling, and microarray hybridization

Per RNA labeling, 500 µg of total RNA was used in conjunction with the Illumina® TotalPrep RNA Amplification Protocol (Ambion/Applied Biosystems, Cat#AMIL1791). The hybridization cocktail containing the fragmented and labeled cRNAs was hybridized to the Affymetrix Human Genome U133 2.0 Gene Chip. The chips were washed and stained by the Affymetrix Fluidics Station using the standard format and protocols as described by Affymetrix. The probe arrays were stained with streptavidin phycoerythrin solution (Molecular Probes, Carlsbad, CA) and enhanced by using an antibody solution containing 0.5 mg/mL of biotinylated anti-streptavidin (Vector Laboratories, Burlingame, CA). An Affymetrix Gene Chip Scanner 3000 was used to scan the probe arrays. Probe cell intensity data (Affymetrix CEL files) were generated using Affymetrix GeneChip Command Console (AGCC) software. The microarray platform and data have been submitted to the Gene Expression Omnibus public database at NCBI following MIAME guidelines [26].

### Validation of gene expression results from microarray by quantitative RT-PCR (qRT-PCR)

To validate the microarray results, genes were selected using the following criteria: 1) upregulated gene (*TBRG1*); 2) downregulated genes (*MAGOH*, *PDIA3*, *ARPC3*); 3) genes previously associated with diabetes in humans and animal models or associated with T2D or insulin secretion in genome wide scan studies (*TBRG1*, *COL4A2*, *PDIA3*); 4) genes associated with significant canonical pathways among DEGs (*HNRNP1A1*,

**Table 2.** List of the most highly differentiated genes in omental adipose tissue of morbidly obese and diabetic African Americans

Gene symbol	Gene name	FC	P-value	FDR
Upregulated transcripts				
MYO10	Myosin X	2.3	$1.1 \times 10^{-4}$	$3.8 \times 10^{-2}$
TBRG1	Transforming growth factor beta regulator 1	2.0	$1.8 \times 10^{-4}$	$4.9 \times 10^{-2}$
Downregulated transcripts				
MALAT1	Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)	-9.8	$1.4 \times 10^{-7}$	$4.9 \times 10^{-4}$
MAGOH	Proliferation-associated (Drosophila)	-6.8	$3.6 \times 10^{-5}$	$1.9 \times 10^{-2}$
NR1D2	Nuclear receptor subfamily 1, group D, member 2	-6.5	$1.1 \times 10^{-5}$	$9.3 \times 10^{-3}$
HIRA	Histone cell cycle regulator	-6.5	$3.0 \times 10^{-8}$	$1.8 \times 10^{-4}$
PDIA3	Protein disulfide isomerase family A, member 3	-5.8	$1.5 \times 10^{-7}$	$4.9 \times 10^{-4}$
ARPC3	Actin related protein 2/3 complex, subunit 3, 21 kDa	-5.0	$1.0 \times 10^{-8}$	$1.6 \times 10^{-4}$
HNRNPA1	Heterogeneous nuclear ribonucleoprotein A1	-4.5	$1.1 \times 10^{-4}$	$3.8 \times 10^{-2}$
HIVP2	Human immunodeficiency virus type I enhancer binding protein 2	-4.4	$3.7 \times 10^{-5}$	$1.9 \times 10^{-2}$
NBPF10	Neuroblastoma breakpoint family member 21-like	-4.4	$2.4 \times 10^{-6}$	$3.4 \times 10^{-3}$
NUCKS1	Nuclear casein kinase and cyclin-dependent kinase substrate 1	-4.1	$5.7 \times 10^{-5}$	$2.6 \times 10^{-2}$

FC: fold change; FDR: false discovery rate.

*BAX*, *PIP5K1A*, *PIP4K2A*); and 5) transcripts with expression fold change (FC) lower than 2 were also included in this step (BLCAP, COL4A2).

RNA samples were reverse transcribed using iScript™ cDNA synthesis kit with random primers for the qRT-PCR following the manufacturer's instructions (BIO-RAD, Hercules, CA). The qRT-PCR assay was then carried out on MyIQ system (BIO-RAD, Hercules, CA) using previously synthesized cDNA and TaqMan gene expression assays which include two unlabeled PCR primers and one FAM® dye-labeled TaqMan® MGB probe (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA). A sample volume of 25 µL was used for all assays and contained 12.5 µL of 2× TaqMan universal PCR mix, 1.25 µL of 20× Taqman gene expression assay mix and 11.25 µL of cDNA diluted in RNase-free water. All runs included duplicates of the samples and triplicates of negative control without the target DNA. The thermal cycling conditions were: 25 °C for 5 min, 42 °C for 30 min and 85 °C for 5 min and 4 °C forever.

### Data analysis

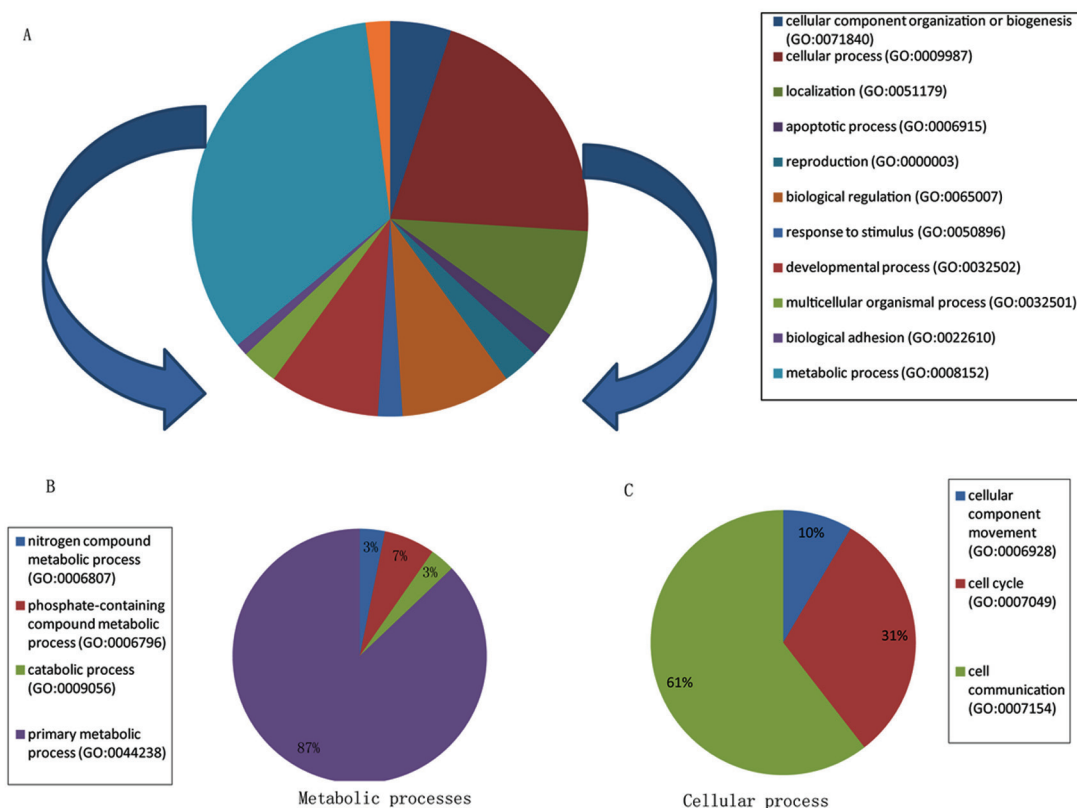
To identify genes or biological pathways that may be associated with T2D in obesity, we determined the gene expression profiles of OAT from morbidly obese diabetics (cases) and morbidly obese non-diabetics (reference). The generated global gene expression data were assessed by two different methods: differential expression analysis and functional classification of differentially expressed genes (DEGs).

Affymetrix microarray CEL files with probe cell information were further processed in Partek Genomics Suite 6.0 (St Louis, MO) and were summarized into probe-set level data with robust multi-array average (RMA) algorithm [27]. QC of

the data and analysis of covariance to identify DEGs among cases and reference were performed subsequently. The threshold for significance in expression change was set at FC of  $\leq -2$  for downregulated genes, FC of  $\geq 2$  for upregulated and false discovery rate (FDR) using Benjamini-Hochberg procedure of  $< 0.05$ . Classification of DEG into biological processes was done using Protein ANalysis Through Evolutionary Relationship (PANTHER, www.pantherdb.org) [28]. Pathways and interaction networks analyses were performed using Ingenuity Pathway Analysis (IPA®) by uploading the 68 DEG's Affymetrix probe identification. The significance of canonical pathways and interaction networks was tested using Fisher's exact test.

To understand the gene expression changes observed in this dataset, we used IPA® upstream regulator analytic tool which is based upon prior knowledge of expected effects between transcriptional regulators and their targeted genes stored in the Ingenuity® Knowledge Base. This tool not only allows identifying potential regulators, which may or may not be differentially expressed, that are involved in the expression change seen but predicts their activation/inhibition. Two statistical measures determined the significance of the upstream regulators identified, the overlap P-value calculated using Fisher's exact test (significance cutoff is  $P < 0.01$ ) and activation Z-score which is used to infer the activation state of the regulators [29].

The qRT-PCR data were analyzed using Relative Expression Software Tool (REST 2009), a stand-alone software developed by Pfaffl and Qiagen (<http://www.REST.de.com>) and uses the  $\Delta\Delta C_t$  method. The expression values were normalized to a reference gene, GAPDH (Applied Biosystems, Life Technologies Corporation, Carlsbad, CA). Two criteria were used to confirm the microarray results: a quantitative measure namely Spearman's coefficient correlation (Rho) between



**Figure 1.** Classification of DEGs among morbidly obese diabetes into biological functions. (A) Pie chart represents all biological functions represented by the 68 DEGs; metabolic and cellular processes denote the two biological classes the most represented. (B) Pie chart represents a breakdown of the metabolic processes shown in A. (C) Pie chart represents a breakdown of the cellular processes shown in A.

qRT-PCR and microarray results and a qualitative measure which is the consistency in the direction of the change in expression between RT-PCR and microarray. The coefficient of correlation and statistical significance were determined using IBM SPSS Statistics v.19; the input data for this analysis were the calculated FCs from the array and the average relative FC from the RT-PCR experiment.

## Results

### Characteristics of study participants

The characteristics of the 20 AA individuals that provided omental adipose tissue (OAT) samples are summarized in Table 1. Six of the 20 individuals were in the reference group (i.e. BMI  $\geq 40$  kg/m<sup>2</sup> and no diabetes); the remaining 14 individuals were cases (i.e. BMI  $\geq 40$  kg/m<sup>2</sup> and diabetes). Fifteen of the 20 subjects were women, and mean BMI and age for the entire sample were 54.6 kg/m<sup>2</sup> and 41.1 years, respectively. Cases and individuals in the reference group were not statistically different with respect to BMI and age. The mean plasma fasting glucose for cases was 128.1 mg/dL.

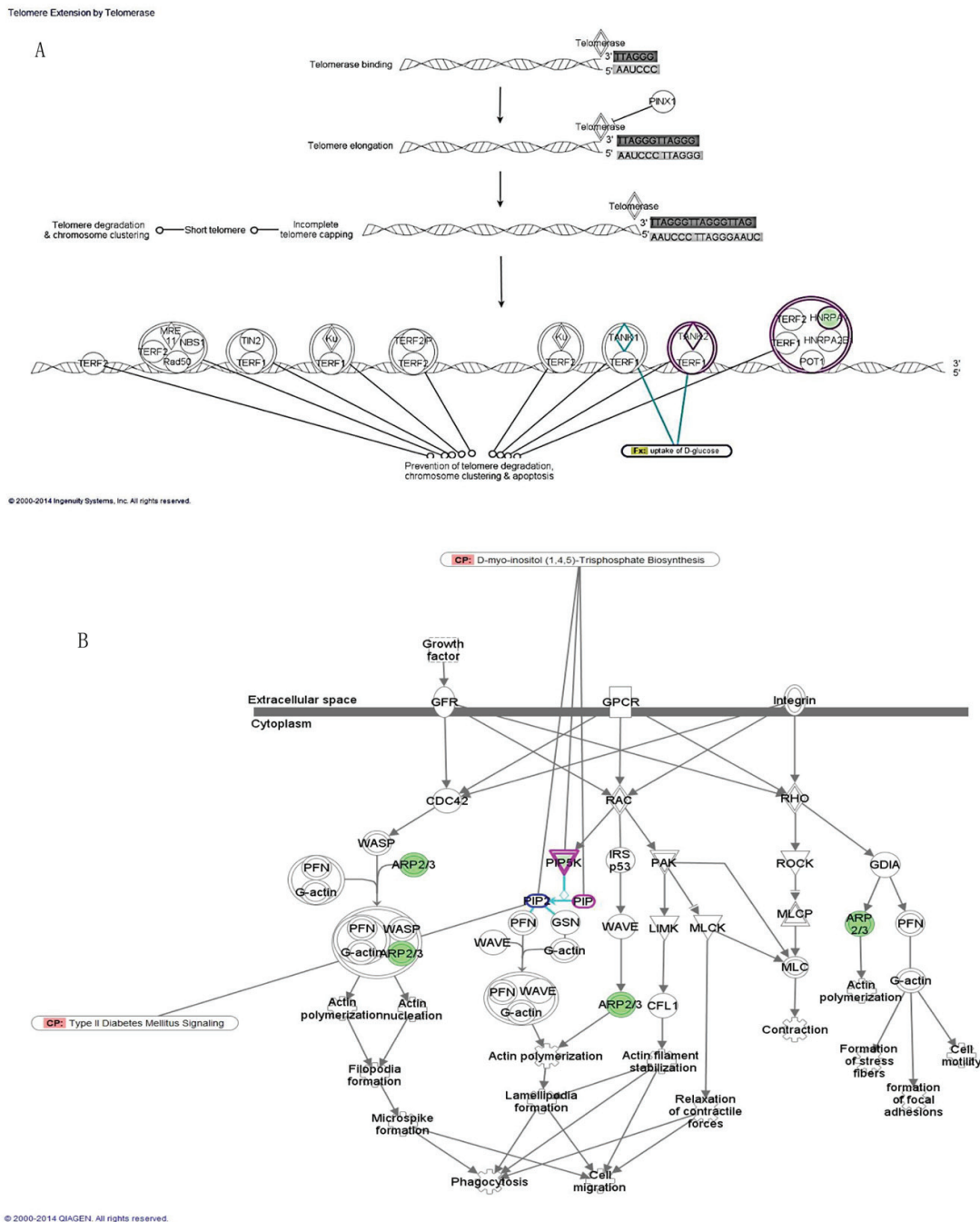
### Global gene expression profiling in OAT

Using an absolute expression FC of 2 (FC  $\leq -2$ , FC  $\geq 2$ ) and FDR of  $< 0.05$ , we identified 68 differentially expressed transcripts in cases relative to the reference group (Supplementary Table 1, [www.jofem.org](http://www.jofem.org)). The most highly DEGs between the two groups are presented in Table 2. About 3% of the DEGs including myosin X (MYO10, FC = 2.3) and transforming growth factor beta regulator 1 (TBRG1, FC = 2.0) were upregulated in the cases while the remaining including metastasis-associated lung adenocarcinoma transcript 1 (MALAT1, FC = -9.8), Mago-Nashi homolog, proliferation-associated (MAGOH, FC = -6.8), actin-related protein 2/3 complex, subunit 3 (ARPC3, FC = -5.0) and PDIA3 (FC = -5.8) were downregulated.

### Functional classification of DEGs in morbidly obese diabetic

To determine the biological functions represented by the DEGs in OAT of these individuals, we evaluated the gene set of 68 DEGs using PANTHER and identified two major biological





**Figure 2.** Top canonical pathways enriched among DEGs of morbidly obese diabetics. (A) Telomere extension by telomerase with overlay of glucose uptake function through TNSK2. (B) Regulation of actin-based motility by rho with intersection of D-myo-inositol (1,4,5)-triphosphate biosynthesis through PIP5K and interaction with type 2 diabetes signaling pathway.

functions mainly metabolic processes and cellular processes (Fig. 1A). In fact, metabolic and cellular processes represent about 55% of all biological processes (Supplementary Table 2, www.jofem.org). A detailed breakdown of these two major biological functions is depicted in Figure 1B and C. Interestingly, 27 of the 34 DEGs classified in the metabolic process

were associated with lipids, carbohydrates, proteins, and nucleobase-containing compound metabolisms. Notably, nucleotide metabolism is the most represented function among the primary metabolic processes (19/27 DEGs, i.e. about 70%, Supplementary Table 3, www.jofem.org). Few of the DEGs were associated with more than one biological functions, e.g.

**Table 3.** List of the Most Significant Canonical Pathways Among the 68 DEGs (Based on Core Analysis in Ingenuity Pathway Analysis (IPA))

Description	P-value	Molecules
Telomere extension by telomerase	$1.35 \times 10^{-3}$	<i>HNRNPA1, TNKS2</i>
D-myo-inositol (1,4,5)-trisphosphate biosynthesis	$3.36 \times 10^{-3}$	<i>PIP5K1A, PIP4K2A</i>
Regulation of actin-based motility by rho	$3.59 \times 10^{-3}$	<i>PIP5K1A, ARPC3, PIP4K2A</i>
Rac signaling	$6.79 \times 10^{-3}$	<i>PIP5K1A, ARPC3, PIP4K2A</i>
RhoA signaling	$7.38 \times 10^{-3}$	<i>PIP5K1A, ARPC3, PIP4K2A</i>

*ING3* which plays a role both in carbohydrate and nucleotide metabolism. Other specialized biological functions were also present including immune system, localization (transport and RNA localization), and response to stimulus (Fig. 1A, Supplementary Table 2, www.jofem.org).

### IPA canonical pathways and interaction networks associated with DEGs in OAT of morbidly obese diabetic

In addition to the biological functions, pathway analysis was carried out using the core analysis feature of IPA to determine the most enriched pathways among the DEGs in obese diabetics. The most enriched canonical pathways were telomere extension by telomerase implicating two DEGs that are involved in nucleotide metabolism specifically in DNA replication and RNA processing (*HNRNPA1, TNKS2*,  $P = 1.4 \times 10^{-3}$ ), D-myo-inositol (1,4,5)-trisphosphate biosynthesis (*PIP5K1A, PIP4K2A*,  $P = 3.4 \times 10^{-3}$ ), and regulation of actin-based motility by Rho (*PIP5K1A, PIP4K2A, ARPC3*,  $P = 3.6 \times 10^{-3}$ ) (Fig. 2, Table 3). All the genes involved in these canonical pathways are downregulated in the obese diabetics compared to the reference group and few play a role in glucose uptake (*TNSK2*) or in T2D signaling (*PIP5K1A*) (Fig. 2A, B).

We also investigated interactions among the DEGs in morbidly obese diabetics and identified four eligible gene networks (Table 4). The three gene networks significantly over-represented are depicted in Figure 3; the top functions of these genes are associated with infectious disease, neurological disease and cancer (network 1, Fig. 3A), dermatological diseases and conditions, developmental disorders, hereditary disorder (network 2, Fig. 3B) and RNA-post-transcriptional modification, metabolic disease, cellular development (network 3, Fig. 3C). These networks identified NFκB, Akt and UBC as hub molecules. Although these hub molecules were not differentially expressed in this dataset, they interact directly or indirectly with number of the identified DEGs. Additionally,

NFκB and Akt are critical in insulin-induced glucose homeostasis and may play key roles in the interactions observed among the DEGs in this dataset as revealed by the overlay of T2D canonical pathway on network 1 (Fig. 3A).

### Upstream regulator analysis

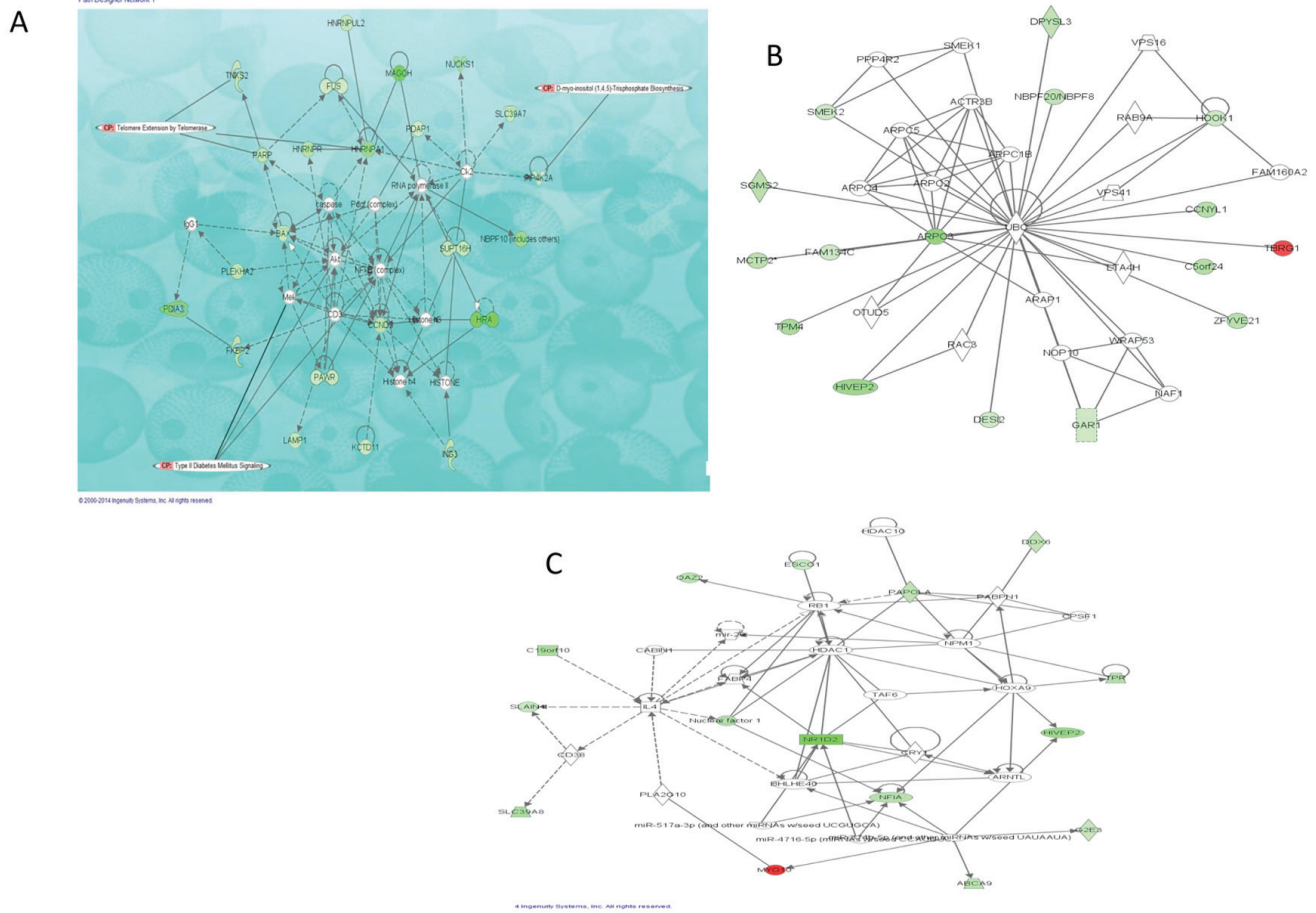
To understand the underline regulation of the expression change seen in this dataset, we used IPA upstream regulator analysis. The five most significant upstream regulators identified that would explain the gene expression changes seen were all microRNAs (miR-320b,  $P = 1.20 \times 10^{-6}$ ; miR-381-3p,  $P = 2.40 \times 10^{-5}$ ; miR-3679-3p,  $P = 9.83 \times 10^{-5}$ ; miR-494-3p,  $P = 9.98 \times 10^{-5}$ ; miR-141-3p,  $P = 1.76 \times 10^{-4}$ ) and were all predicted to be activated based on the activation Z-score. Network association between the top transcriptional upstream regulators and their target genes showed that activation of the regulators resulted in downregulation of all targeted genes except for MYO10 which is up-regulated (Fig. 4). Interestingly, all but two of the identified miRNAs notably miR-320b, miR-141-3p, miR-494-3p were previously implicated in T2D pathophysiology.

### QRT-PCR validation

To validate our microarray data, we evaluated the expression of 11 genes selected from the microarray data using qRT-PCR. The specifics of the gene expression assays are summarized in Supplementary Table 4 (www.jofem.org). Overall, the direction and magnitude of the normalized expression ratios (FC) obtained from qRT-PCR were comparable to those obtained by microarray (Supplementary Figure 1, www.jofem.org). A significant correlation of 0.82 was found among the validation dataset (Spearman's Rho,  $P < 0.002$ ,  $n = 11$ ). Additionally a scatter plot between  $FC_{(RT-PCR)}$  and  $FC_{(microarray)}$  showed a lin-

**Table 4.** Biological Functions and Diseases Associated With the Top Ranked Networks

Top functions	Score	Focus molecules	Network scheme
Infectious disease, neurological disease, cancer	49	22	Figure 3A
Dermatological diseases and conditions, developmental disorder, hereditary disorder	32	16	Figure 3B
RNA post-transcriptional modification, metabolic disease, cellular development	27	14	Figure 3C
Cell-to-cell signaling and interaction, cellular assembly and organization, tissue development	20	11	-



**Figure 3.** Top three networks showing interactions between selected differentially expressed genes in morbidly obese diabetics. Red: transcripts up-regulated in morbidly obese/diabetic AA; green: transcripts down-regulated in morbidly obese/diabetic AA; white: transcripts not differentially expressed in our study but important in the network. The color gradient in the network indicates the strength of expression denoted by FC. → indicates direct interaction between transcript products; ---> indicates indirect interaction between transcript products; (o) indicates autoregulation. (A) Network 1 with overlay of type 2 diabetic signaling and two of the most significant canonical pathways observed among a number of DEGs. (B) Network 2 representing 16 of the DEGs associated with dermatological diseases and conditions as well as developmental and hereditary diseases. (C) Network 3 representing 14 of the DEGs associated with RNA post-transcriptional modifications, metabolic disease, and cellular development.

ear relation between the results from the two methods with all data points falling within the 95% confidence interval (Fig. 5).

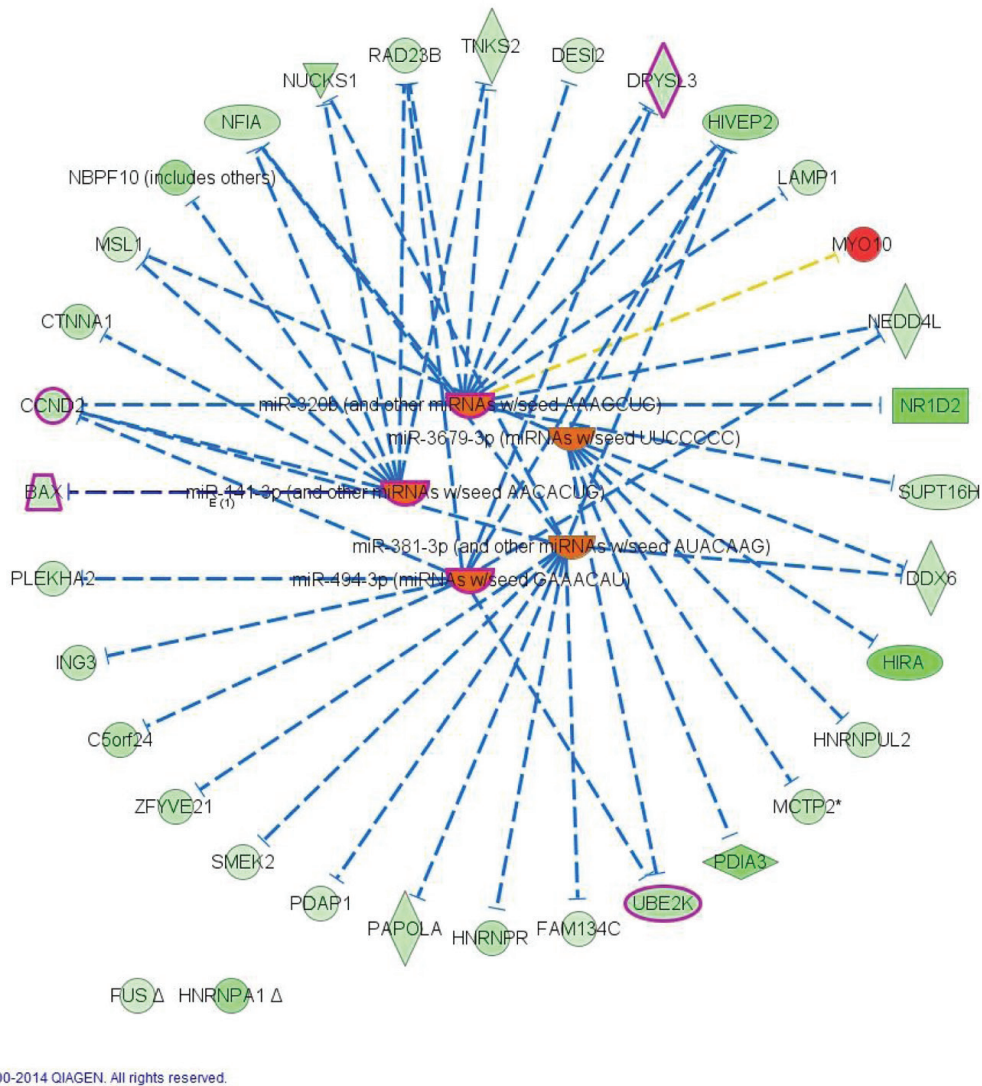
**Discussion**

We evaluated the global gene expression profile of OAT, a tissue that is recognized to play an important role in the pathophysiology of insulin resistance and T2D. We identified 68 genes, two upregulated and 66 downregulated, that were differentially expressed in morbidly obese African Americans with T2D compared to those that are equally obese but without T2D. *MYO10* or myosin X, one of the two most upregulated gene in this study, encodes for an atypical myosin which functions as an actin-based molecular motor and plays a role in integration of F-actin and microtubule cytoskeletons during

meiosis. A genome-wide association study in four European populations reported association between *MYO10* variants and T2D [30]. More importantly, Zhan et al have not only reported an association between *MYO10* variants and metabolic syndrome phenotypes within an adiponectin QTL at 5p14 but also demonstrated that one of the variants had a cis-effect on *MYO10* expression in peripheral white blood cells [30]. Even though the mechanisms involved remain unclear, these results suggest a role for *MYO10* in the pathophysiology of T2D.

The 68 DEGs were grouped in diverse biological functions and canonical pathways. In contrast to other studies [9], genes involved in primary metabolic processes were overwhelmingly represented among the DEGs especially those involved in RNA processing and DNA replication and repair including *FUS*, *HNRNPA1*, *HNRNPR*, *TNKS2*, and *PAPOLA*. RNA processing is a complicated cascade of functions that includes





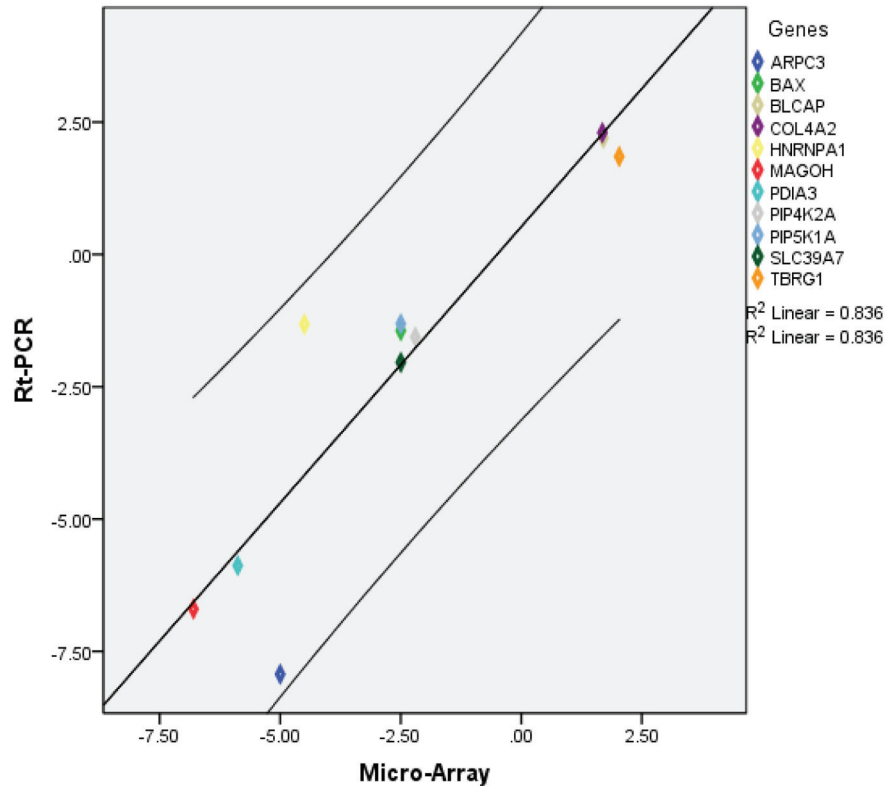
**Figure 4.** Upstream analysis: network interactions between the five top transcriptional regulators and their target DEGs in morbidly obese diabetics. Orange identifies activated upstream regulators. Green identifies down-regulated target DEGs whereas red identifies up-regulated DEGs. Blunt arrowhead indicates inhibition of the target DEG. Purple indicates genes or regulator that overlay with T2D (4 DEGs + 3 upstream regulators).

alternative splicing, polyadenylation, and nuclear export of mature RNA. Decreased expression of RNA processing genes in liver and skeletal muscle has been reported to contribute to metabolic phenotypes associated with obesity [31]. While downregulation of RNA processing genes (e.g. heterogeneous nuclear ribonucleoproteins (HNRNPs) which encode for RNA binding proteins) has been reported to be involved in the pathobiology of obesity, to our knowledge, this study is the first to find a link between downregulation of genes involved in RNA processing and T2D in OAT. Importantly, HNRNPs are key player in the formation of spliceosomes that control alternative splicing of a number of human genes. In fact, disruption of alternative splicing has been shown in a number of disease states [31]. Several genes associated with obesity and insulin

resistance, a key feature of T2D, are regulated by alternative splicing [31, 32]. Moreover, spliceosomes appear also to regulate telomeres length by modulating telomerase activity [33].

HNRNPNA1 and TNKS2, both downregulated in this study, affect telomere maintenance [34] and also belong to the telomere extension by telomerase pathway, the most significantly enriched pathway in this study. In fact, evidence implicating telomere shortening in metabolic diseases including T2D is growing [35]. Though, telomerase activity and shortening of telomeres were the most demonstrated in beta-cells and leukocytes of diabetics [36], this study reveals that such phenomenon may not just be limited to these cells types. Furthermore, TNKS are not only involved in telomere regulation but also in glucose uptake by GLUT4. TNSKS knockdown in





**Figure 5.** Scatter plot showing relationship between microarray and RT-PCR data for selected transcripts. Diagonal line represents the regression line.

adipocytes impairs glucose uptake [37]. It has also been postulated that the association between T2D and impaired telomerase activity and regulation could also explain the occurrence of certain cancers in diabetics [38]. We observed an overlap between T2D signaling and a number of DEGs that are involved in cancer, infections and neurological diseases (Fig. 3A). These interactions are mediated through factors such as NF $\kappa$ B, Akt, and caspase known for their involvement in multiple diseases.

We also found that the biosynthesis of D-myo-inositol (1,4,5)-triphosphate or IP<sub>3</sub>, an important cellular second messenger, may be impaired in T2D. Two of the enzymes (PIP5K1A, PIP4K2A) responsible for IP<sub>3</sub> synthesis are down-regulated in our dataset. IP<sub>3</sub> is involved in the release of intracellular calcium, a mechanism important for number of signaling pathways; therefore any disruption to its synthesis can potentially affect the downstream biological events such as GLUT4 vesicles trafficking that depend on intracellular calcium to maintain glucose homeostasis. Sears et al demonstrated that in the absence of intracellular calcium both glucose transporter translocation and glucose uptake are inhibited *in vitro* [39]. Interestingly, D-myo-inositol (1,4,5)-triphosphate biosynthesis interacts with another significant canonical pathway found in this study, namely the regulation of actin-based motility by Rho through PIP<sub>2</sub>, a precursor of IP<sub>3</sub>. Both PIP<sub>2</sub> and Arp2/3, an actin-related protein that interacts with neural Wiskott-Alrich syndrome protein (N-WASP) in the regulation

of actin-based motility by Rho, control actin polymerization [40]. Arp2/3, which is downregulated in this study, participates in cortical actin regulation and translocation of GLUT4 [41, 42]. Actin remodeling has been shown to be important in the dynamics of insulin-dependent uptake of glucose into target cells by translocation of glucose transporter-4 (GLUT4) and failure in this process may result in an impaired glucose metabolism [43, 44].

Similar to previous reports [9, 19], we observed differential expression in *PIP5K1A*, *ING3*, *PDIA3*, transcripts involved in carbohydrates, lipids and small molecules metabolisms. Though there is a knowledge-based evidence of the potential mechanisms by which the DEGs may be involved in the pathophysiology of T2D, it is equally important to understand the underline mechanisms of regulation contributing to the gene expression change seen in this study. Hence using bioinformatics prediction, we found that most of the DEGs identified are regulated by microRNAs which are all predicted to be in activated state and to inhibit the expression of their respective targeted transcripts. MiR-320b, miR-141-3p, and miR-494-3p have been previously linked to insulin resistance and T2D [45]. However, miR-320b's regulation of glucose homeostasis varies with tissue type. In adipocytes, its expression is increased in insulin resistance state whereas its expression is decreased in plasma of T2D patients [46, 47]. MiR-320 was shown to regulate insulin resistance in adipocytes by target-

ing Akt/PI3K pathways via phosphorylation of Akt and by increasing insulin-stimulated glucose uptake through increased protein expression of the glucose transporter GLUT4 [46]. To the best of our knowledge, miR-381-3p and miR-3679-3p were not previously linked to insulin resistance or T2D and would constitute novel miRNAs detected in OAT of morbidly obese diabetics and implicated in T2D [45].

The design and methods used in this study are not without limitations. One major limitation is the use of commercially acquired samples with limited phenotype data or unavailability of other biological specimens (e.g., serum and plasma) that would have allowed us assessing the association between transcript levels, protein levels and relevant phenotypes or to carry out functional assays to determine the causality of our findings. Cognizant of these limitations, we validated identified transcripts using RT-PCR. Also, this study is cross-sectional in nature and cannot infer causality. It remains imperative to replicate our findings and to determine the generalizability of these findings to other tissues (e.g., muscles) important in the pathophysiology of T2D.

In summary, this study provided key insights into gene expression patterns and pathways that distinguish between subjects with morbid obesity and diabetes versus those that are only morbidly obese. Thus, the expectation is that most of these differences are attributable to the diabetic state. Importantly the data point to the roles of: 1) RNA binding proteins (HNRNPs and TNSKs) in telomere maintenance, 2) enzymes (PIP5K1A, PIP4K2A) that regulate the biosynthesis of second messenger (IP3) important in glucose homeostasis, and 3) actin remodeling (Arp3) and d) microRNA in T2D. The study also provides a resource for future investigations.

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## Conflict of Interest

The authors declare that there is no conflict of interest associated with this manuscript.

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