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

Ayo P. Doumateya, Huichun Xu, Hanxia Huang, Niraj S. Trivedi, Lin Lei, Abdel Elkhawloun, Adebowale Adeyemo, Charles N. Rotimi

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/m²) 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 (HRNRP41, TNKS2), D-myo-inositol (1,4,5)-trisphosphate biosynthesis (PPIP5K1A, PIP4K2A), and regulation of actin-based motility by Rho (ARPC3) were the most significant canonical pathways and overlay with T2D signaling pathway. 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-
Table 1. Characteristics of the Study Participants

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Obese non diabetic (control: n = 6)</th>
<th>Obese diabetic (cases: n = 14)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>41.0 ± 4.6 (36 - 47)</td>
<td>41.5 ± 9.1 (27 - 61)</td>
<td>0.87</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>53.9 ± 7.1 (45.6 - 65)</td>
<td>54.9 ± 7.8 (44.1 - 73.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Waist (inches)</td>
<td>N/A</td>
<td>55.8 ± 3.3 (51 - 60)</td>
<td>-</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>N/A</td>
<td>128.1 ± 25.5 (81 - 172)</td>
<td>-</td>
</tr>
</tbody>
</table>

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.

Materials and Methods

Subjects

A total of 20 morbidly obese African Americans (BMI ≥ 40 kg/m²) 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 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 universal tissue kit on the EZ1 workstation according to the manufacturer’s instructions. RNA quantity and quality were assessed using the Agilent 2100 Bioanalyzer and Nanodrop (Thermo Fisher, Wilmington, DE). 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. 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 homogenous and centrifuged. After centrifugation, the sample separates into three phases. 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.

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#AM1791). 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 (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 (MAGO, 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 (HNRNPA1, HNRNPA1, HNRNPA1, TBRG1, COL4A2, PDIA3).
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Table 2. List of the most highly differentiated genes in omental adipose tissue of morbidly obese and diabetic African Americans

<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Gene name</th>
<th>FC</th>
<th>P-value</th>
<th>FDR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Upregulated transcripts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MYO10</td>
<td>Myosin X</td>
<td>2.3</td>
<td>1.1 × 10⁻⁴</td>
<td>3.8 × 10⁻²</td>
</tr>
<tr>
<td>TBRG1</td>
<td>Transforming growth factor beta regulator 1</td>
<td>2.0</td>
<td>1.8 × 10⁻⁴</td>
<td>4.9 × 10⁻²</td>
</tr>
<tr>
<td></td>
<td>Downregulated transcripts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MALAT1</td>
<td>Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)</td>
<td>-9.8</td>
<td>1.4 × 10⁻⁷</td>
<td>4.9 × 10⁻⁴</td>
</tr>
<tr>
<td>MAGOH</td>
<td>Proliferation-associated (Drosophila)</td>
<td>-6.8</td>
<td>3.6 × 10⁻⁵</td>
<td>1.9 × 10⁻²</td>
</tr>
<tr>
<td>NR1D2</td>
<td>Nuclear receptor subfamily 1, group D, member 2</td>
<td>-6.5</td>
<td>1.1 × 10⁻⁵</td>
<td>9.3 × 10⁻³</td>
</tr>
<tr>
<td>HIRA</td>
<td>Histone cell cycle regulator</td>
<td>-6.5</td>
<td>3.0 × 10⁻⁸</td>
<td>1.8 × 10⁻⁴</td>
</tr>
<tr>
<td>PDIA3</td>
<td>Protein disulfide isomerase family A, member 3</td>
<td>-5.8</td>
<td>1.5 × 10⁻⁷</td>
<td>4.9 × 10⁻⁴</td>
</tr>
<tr>
<td>ARPC3</td>
<td>Actin related protein 2/3 complex, subunit 3, 21 kDa</td>
<td>-5.0</td>
<td>1.0 × 10⁻⁸</td>
<td>1.6 × 10⁻⁴</td>
</tr>
<tr>
<td>HNRNPA1</td>
<td>Heterogeneous nuclear ribonucleoprotein A1</td>
<td>-4.5</td>
<td>1.1 × 10⁻⁴</td>
<td>3.8 × 10⁻²</td>
</tr>
<tr>
<td>HIVEP2</td>
<td>Human immunodeficiency virus type 1 enhancer binding protein 2</td>
<td>-4.4</td>
<td>3.7 × 10⁻⁵</td>
<td>1.9 × 10⁻²</td>
</tr>
<tr>
<td>NBPF10</td>
<td>Neuroblastoma breakpoint family member 21-like</td>
<td>-4.4</td>
<td>2.4 × 10⁻⁶</td>
<td>3.4 × 10⁻³</td>
</tr>
<tr>
<td>NUCKS1</td>
<td>Nuclear casein kinase and cyclin-dependent kinase substrate 1</td>
<td>-4.1</td>
<td>5.7 × 10⁻⁵</td>
<td>2.6 × 10⁻²</td>
</tr>
</tbody>
</table>

FC: fold change; FDR: false discovery rate.

BAX, PIK5K1A, PI4K2A); 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 ≤ -2 for downregulated genes, FC of ≥ 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 ΔΔCt 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...
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 ≥ 40 kg/m² and no diabetes); the remaining 14 individuals were cases (i.e. BMI ≥ 40 kg/m² and diabetes). Fifteen of the 20 subjects were women, and mean BMI and age for the entire sample were 54.6 kg/m² 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 ≤ - 2, FC ≥ 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). 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...
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.
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 (HNRNP41, TNKS2, P = 1.35 × 10^{-3}), D-myo-inositol (1,4,5)-trisphosphate biosynthesis (PIP5K1A, PIP4K2A, P = 3.36 × 10^{-3}), and regulation of actin-based motility by Rho (PIP5K1A, PIP4K2A, ARPC3, P = 3.65 × 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 NFkB, 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, NFkB 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 × 10^{-6}; miR-381-3p, P = 2.40 × 10^{-5}; miR-3679-3p, P = 9.83 × 10^{-5}; miR-494-3p, P = 9.98 × 10^{-5}; miR-141-3p, P = 1.76 × 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 upregulated (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 3. List of the Most Significant Canonical Pathways Among the 68 DEGs (Based on Core Analysis in Ingenuity Pathway Analysis (IPA))

<table>
<thead>
<tr>
<th>Description</th>
<th>P-value</th>
<th>Molecules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telomere extension by telomerase</td>
<td>1.35 × 10^{-3}</td>
<td>HNRNP41, TNKS2</td>
</tr>
<tr>
<td>D-myo-inositol (1,4,5)-trisphosphate biosynthesis</td>
<td>3.36 × 10^{-3}</td>
<td>PIP5K1A, PIP4K2A</td>
</tr>
<tr>
<td>Regulation of actin-based motility by rho</td>
<td>3.65 × 10^{-3}</td>
<td>PIP5K1A, ARPC3, PIP4K2A</td>
</tr>
<tr>
<td>Rac signaling</td>
<td>3.73 × 10^{-3}</td>
<td>PIP5K1A, ARPC3, PIP4K2A</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Top functions</th>
<th>Score</th>
<th>Focus molecules</th>
<th>Network scheme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infectious disease, neurological disease, cancer</td>
<td>49</td>
<td>22</td>
<td>Figure 3A</td>
</tr>
<tr>
<td>Dermatological diseases and conditions, developmental disorder, hereditary disorder</td>
<td>32</td>
<td>16</td>
<td>Figure 3B</td>
</tr>
<tr>
<td>RNA post-transcriptional modification, metabolic disease, cellular development</td>
<td>27</td>
<td>14</td>
<td>Figure 3C</td>
</tr>
<tr>
<td>Cell-to-cell signaling and interaction, cellular assembly and organization, tissue development</td>
<td>20</td>
<td>11</td>
<td>-</td>
</tr>
</tbody>
</table>
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 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; (○) 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.
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].

HNRPNPA1 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
adipocytes impairs glucose uptake [37]. It has also been postu-
lated that the association between T2D and impaired telom-
erase activity and regulation could also explain the occurrence of
certain cancers in diabetics [38]. We observed an overlap be-
tween 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 NFκB, 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 IP3, an important cellular second mes-
senger, may be impaired in T2D. Two of the enzymes (PIP-
5K1A, PIP4K2A) responsible for IP3 synthesis are down-
regulated in our dataset. IP3 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 demon-
strated 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 path-
way found in this study, namely 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 me-
tabolism [43, 44].

Similar to previous reports [9, 19], we observed differen-
tial expression in PIP5K1A, ENG3, PDIA3, transcripts in-
volved in carbohydrates, lipids and small molecules metabo-
lisms. 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 un-
derstand the underline mechanisms of regulation contributing
to the gene expression change seen in this study. Hence us-
ging 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 ho-
meostasis 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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