Hepatic Gene Expression in Morbidly Obese Women: Implications for Disease Susceptibility

Marshall B. Elam[1,2], George S. Cowan Jr[3], Robert J. Rooney[4], M. Lloyd Hiler[3], Chandrahasa R. Yellaturu[2], Xiong Deng[2], George E. Howell[2], Edwards A. Park[2], Ivan C. Gerling[1], Divyan Patel[4], J. Christopher Corton[5], Lauren M. Cagen[2,*], Henry G. Wilcox[2], Malay Gandhi[6], Micheal H. Bahr[3], Micheal C. Allan[6], Linus A. Wodi[6], George A. Cook[2], Thomas A. Hughes[6] and Rajendra Raghow[1,2]

The objective of this study was to determine the molecular bases of disordered hepatic function and disease susceptibility in obesity. We compared global gene expression in liver biopsies from morbidly obese (MO) women undergoing gastric bypass (GBP) surgery with that of women undergoing ventral hernia repair who had experienced massive weight loss (MWL) following prior GBP. Metabolic and hormonal profiles were examined in MO vs. MWL groups. Additionally, we analyzed individual profiles of hepatic gene expression in liver biopsy specimens obtained from MO and MWL subjects. All patients underwent preoperative metabolic profiling. RNAs were extracted from wedge biopsies of livers from MO and MWL subjects, and analysis of mRNA expression was carried out using Affymetrix HG-U133A microarray gene chips. Genes exhibiting greater than twofold differential expression between MO and MWL subjects were organized according to gene ontology and hierarchical clustering, and expression of key genes exhibiting differential regulation was quantified by real-time–polymerase chain reaction (RT-PCR). We discovered 154 genes to be differentially expressed in livers of MWL and MO subjects. A total of 28 candidate disease susceptibility genes were identified that encoded proteins regulating lipid and energy homeostasis (*PLIN, ENO3, ELOVL2, APOF, LEPR, IGFBP1, DDIT4*), signal transduction (*MAP2K6, SOCS-2*), postinflammatory tissue repair (*HLA-DQB1, SPP1, P4HA1, LUM*), bile acid transport (*SULT2A, ABCB11*), and metabolism of xenobiotics (*GSTT2, CYP1A1*). Using gene expression profiling, we have identified novel candidate disease susceptibility genes whose expression is altered in livers of MO subjects. The significance of altered expression of these genes to obesity-related disease is discussed.

Obesity (2009) **17,** 1563–1573. doi:10.1038/oby.2009.49

INTRODUCTION

Morbid obesity is associated with increased risk of a many metabolic, proliferative, and inflammatory diseases, including dyslipidemia, hypertension, hyperglycemia, and thrombosis (1). Obese individuals also elicit a chronic low-level inflammatory state due to adipose-derived pro-inflammatory cytokines, including transforming growth factor-β2, tumor necrosis factor-α, and interleukin-6 (2). As a result of both enhanced lipid synthesis and the actions of adipokines, morbidly obese (MO) individuals have a high prevalence of hepatic steatosis and are at increased risk to develop nonalcoholic steatohepatitis, hepatic fibrosis (3,4), and gallstones (5,6). MO individuals also experience increased incidence of postoperative infections and impaired wound healing (7,8), as well as increased risk of certain cancers (9–13). The presence of morbid obesity is also predictive of poor response to interferon therapy in hepatitis C (14,15).

Although the altered hormonal and nutritional milieu that accompanies obesity has been invoked as an etiologic factor in many diseases, the cellular and molecular mechanisms underlying increased risk of a wide range of diseases in MO remain largely undefined. Application of genomics offers great promise in this regard by identifying genes that are differentially expressed in target tissues of obese individuals (16). Analysis of mRNA isolated from tissue samples with gene microarray chips enables simultaneous assessment of expression of thousands of mRNAs. This approach has been successfully used to define patterns of gene expression in adipose tissue of obese humans (17,18) and livers of obese animals (19). However, profiling of hepatic gene expression has, until recently, not

[1]Department of Medicine and Research Service, Veterans Affairs Medical Center, Memphis, Tennessee, USA; [2]Department of Pharmacology, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA; [3]Department of Surgery, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA; [4]Genome Explorations Inc. Memphis, Tennessee, USA; [5]US-EPA, Research Triangle Park, Durham, North Carolina, USA; [6]Department of Medicine, University of Tennessee Health Sciences Center, Memphis, Tennessee, USA; *Deceased. Correspondence: Marshall B. Elam (melam@utmem.edu)

Received 8 May 2008; accepted 8 October 2008; published online 5 March 2009. doi:10.1038/oby.2009.49

been widely reported from MO humans due to the limited availability of liver tissue.

Individuals with morbid obesity who undergo bariatric surgery for weight loss represent a unique opportunity to examine the effects of obesity and weight loss on hepatic gene expression. In individuals who undergo bariatric surgery, the volume of food the stomach will accommodate is reduced by gastric banding, gastric bypass, or other bypass procedures, with resulting weight loss of between 63 to 102 pounds, depending upon the procedure (20). Some patients return 6–12 months after gastric bypass (GBP) for ventral hernia repair following massive weight loss (MWL). These two unique populations of patients may be ideal to assess potential changes in hepatic gene expression in MO subjects before and after weight loss since a wedge biopsy can be obtained from the liver under direct visualization during laparotomy for either bariatric surgery or ventral hernia repair. Since MWL following bariatric surgery is accompanied by reversal of many of the metabolic complications of obesity, including dyslipidemia, insulin resistance, and hyperglycemia (20), an examination of changes in gene expression before and after MWL may provide insight into genome-wide consequences of morbid obesity on the liver as it responds to reversion to the "pre-obese" state.

We report a study of 31 female subjects who underwent either GBP (N = 22) or ventral hernia repair 1 year following GBP (N = 9). We assessed metabolic profiles of all patients prior to surgery and compared hepatic gene expression profiles in a subset of 13 MO and 5 MWL subjects for whom biopsy specimens were available. We observed that numerous genes involved in the metabolism of nutrients and xenobiotics, signaling in response to hormones/cytokines, and cell growth and apoptosis were differentially expressed in livers of MO and MWL individuals. Among the aberrantly expressed genes, we have identified a number of candidate genes that may enhance susceptibility of MO individuals to a wide range of illnesses.

METHODS AND PROCEDURES

Subjects and sample collection

Study participants were screened at the Obesity Wellness Center in the Department of Surgery, University of Tennessee Health Science Center. A total of 31 women participated in this study; 22 MO subjects underwent GBP procedures for weight loss, and 9 underwent ventral hernia repair after experiencing MWL following GBP. MWL subjects underwent abdominoplasty on average, 1 year after their initial GBP surgery, following attainment of stable weight. There was no overlap between GBP and MWL subjects, that is, none of the MWL subjects had been studied at the time of their prior GBP surgery. Subjects with a history of triglyceride-related pancreatitis or with hypertriglyceridemia (>1,000 mg/dl), chronic renal disease, liver disease (transaminase elevation >2 times upper limit of normal), current malignancy, or hypoalbuminemia were excluded from the study. Patients who had been hospitalized within the past 3 months for intercurrent illness were also excluded. Patients treated with corticosteroids, androgens, and lipid-lowering agents within 4 weeks of study were also excluded, as were patients with diabetes requiring insulin or oral hypoglycemic therapy. Patients scheduled for GBP or ventral hernia repair underwent preoperative metabolic profiling in the University of Tennessee General Clinical Research Center. Body weight was determined using a calibrated digital electronic scale. Fasting levels of plasma lipoproteins, glucose, insulin, and thyroid hormones were measured. After preoperative studies were completed, participants were transferred to the surgical wards where they received standard preoperative care and remained fasting until completion of surgery the next morning.

Surgical procedures and liver biopsy

An extended Roux-en-Y gastric bypass coupled with a horizontal gastric pouch was accomplished. The gastric pouch of ~30 ml capacity was fashioned by dividing the proximal stomach horizontally between two 9 cm linear staplers (4.8 mm staple length). The retro-colic, retrogastric Roux-en-Y alimentary limb was 90 cm in length. The common (distal) small intestinal limb from the enteroenterostomy to the cecum was 180–240 cm in length. The biliopancreatic (afferent) limb consists of the remainder of the measured small intestine. Abdominoplasty was performed to revise redundant abdominal wall and repair associated ventral (incisional) hernias and diastasis recti in postbypass patients who had lost >80 pounds. During surgery, a wedge biopsy specimen of liver was obtained under direct visualization from a standardized site, the edge of the anterior–superior portion of the left lobe of the liver. A portion of the liver biopsy sample was immediately placed in RNAStat-60 (Tel-Test, Friendswoop, TX) and the remaining sample was snap-frozen in liquid N_2 and stored at −70 °C.

Laboratory tests

Blood samples were analyzed in the Clinical Research Center core laboratory. Serum chemistry was determined by standard autoanalyzer techniques in a commercial laboratory.

Microarray gene expression analysis

RNA samples, from 13 MO and 5 MWL subjects, were processed using standard protocols for short oligonucleotide arrays (Affymetrix HG-133A, Santa Clara, CA) as described (21) and outlined in detail in **Supplementary Appendix 2** online. A total of 154 probe sets exhibiting differential expression between the MO and MWL groups were identified using the following criteria: (i) a twofold or greater change in group mean signal values, (ii) a mean GCOS-generated detection P value ≤ 0.065 (M) for at least one group, and (iii) a Welch t test P value ≤ 0.05 for significance of differences between MO and MWL samples. Gene annotation, gene ontology (GO) information, and biochemical pathway information were obtained from the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov), NetAffx (http://www.affymetrix.com), Gene Ontology Consortium (http://amigo.geneontology.org), Kyoto Encyclopedia of Genes and Genomes (http://www.genome.jp/kegg), and WebGestalt (http://bioinfo.vanderbilt.edu/webgestalt). Heat maps were generated and clustered using GeneMaths XT (Applied Maths, Austin, TX). Hierarchical clustering of samples was performed by the Combined Linkage Method based on Pearson correlation distance. Hierarchical clustering of probe sets was performed by the Unweighted Pair-Group Method using Arithmetic Averages based on Euclidean distance.

Quantitative assessment of gene expression by real-time polymerase chain reaction (RT-PCR)

Findings of altered expression of key genes in each of the major Gene Ontogeny (GO) groups as judged by microarray analysis were corroborated by RT-PCR performed with a LightCycler 480 System using SYBR Green 1 dye (LightCycler 480 SYBR Green 1 Master Mix, Roche Diagnostics, Indianapolis, IN) intercalation to detect DNA amplification. Expression of both the target gene and control gene (cyclophilin D) within each sample were quantified based on their respective threshold cycle values. Target gene threshold cycle values were normalized to threshold cycle values of cyclophilin D and then expressed as the ratio of MO target gene expression to MWL target gene expression. Sources and specific primer sequences used for RT-PCR are provided in the table in **Supplementary Appendix 1** online.

Statistical methods

Significance of differences between plasma analytes and anthropometric variables between MO and MWL subjects was determined by Student's *t* test (continuous variables) or χ^2 (Fisher's exact test for noncontinuous variables) using a microcomputer statistical package (SAS Institute, Cary, NC). Statistical consultation was provided by the Biostatistics Department of the University of Tennessee Health Sciences Center.

Statement of ethics

All regulations concerning the ethical use of human volunteers in research were followed in the conduct of this study. All participants gave written informed consent prior to conduct of any study-related procedures. The Institutional Review Boards of the University of Tennessee Health Sciences Center, Memphis, and Baptist Memorial Hospital, Memphis, approved these studies.

RESULTS

Clinical and demographic characteristics of the study population

As shown in Table 1, the average age of participants was 36 and 39 years for MO and MWL patients, respectively; African-Americans and whites were represented in both groups. MWL individuals were on average 102 pounds lighter than MO. Similarly, the BMI was significantly lower in MWL subjects. Plasma cholesterol and triglyceride levels were higher than expected for age in the MO participants and were significantly lower in MWL subjects (Table 1). Conversely, high-density lipoprotein cholesterol (HDL-C) was lower than expected for age and sex in MO and was significantly higher in MWL subjects (Table 1). Fasting insulin levels were higher in MO subjects, reflecting the presence of insulin resistance (Table 1). Similarly, mean plasma glucose was marginally higher in MO compared to MWL subjects (Table 1). Although free T4 was similar in MO and MWL subjects, thyroid-stimulating hormone (TSH) levels were significantly higher in MO (Table 1). This is consistent with previous reports of increased TSH in MO humans (22). With the exception of bilirubin, which was slightly higher in MWL subjects, serum chemistries were similar in the MO and MWL groups (Table 1). Serum transaminase levels did not differ between MO and MWL groups (Table 1). Thus, MWL following GBP was accompanied by reversal of many of the metabolic abnormalities of MO, including dyslipidemia, hyperinsulinemia, and hyperglycemia.

Profiles of hepatic gene expression

Microarray analysis revealed that 154 unique genes met our criterion for twofold differential expression between MO and MWL subjects. The relative expression of genes of individual patients is shown as a heat map (Figure 1), and as ratios of group means of MO vs. MWL participants (Table 2) revealed, the vast majority of differentially expressed genes were down-regulated (143 genes decreased vs. 11 increased) in livers of MO subjects. Examination of the gene expression patterns by hierarchical clustering (Figure 1) reveals heterogeneity in gene expression among MO subjects, in part, related to race (obese white females vs. obese black females, Figure 1) suggesting ethnic differences in hepatic gene expression in response to obesity (Figure 1). Despite this heterogeneity, we were able to detect significant differences in hepatic gene expression of a large number of genes between MO and MWL subjects (**Supplementary Appendix 3** online).

Based on GO functional characteristics, the differentially expressed genes were initially organized into nine groups (**Supplementary Appendix 3** online). The majority of differentially expressed genes belonged to the GO functional categories of Inflammation, Protein Metabolism, Lipid Metabolism, and Xenobiotic Metabolism (Figure 2). Smaller numbers of genes were classified as being related to signal transduction, cell cycle, cell proliferation, and adhesion (Figure 2). Each of the differentially expressed genes was examined for their potential role in diseases associated with obesity using information derived from the Unigene and National Center for Biotechnology Information Protein databases and from PubMed search combining the gene and/or protein product name with the keywords "obesity" and "liver". From the original list of 154 differentially expressed genes, a total of 28 unique genes in four functional groups were identified as having a potential role in the susceptibility of MO individuals to specific diseases (Table 2). The unabridged list of 154 differentially expressed genes organized by GO functional categories is shown in **Supplementary Appendix 3** online.

Table 1 Clinical and demographic data for MO and MWL study subjects

Variable/group	MO-GBP (*N* = 22)	MWL-post-GBP (*N* = 9)	*P*[a]
Age (years)	36.2 ± 1.4	39.2 ± 2.5	0.27
African American, *N* (%)	5 (22.7%)	4 (44.0%)	0.38
Hypertension (%)	22.7%	11.0%	0.64
Weight (pounds)	301 ± 17	199 ± 13	0.002
BMI	54.5 ± 3.9	32.0 ± 1.8	0.001
Waist–hip ratio	0.86 ± 0.02	0.81 ± 0.02	0.14
Cholesterol (mg/dl)	171 ± 7	114 ± 9	0.0002
LDL-C (mg/dl)	101 ± 6	65 ± 12	0.008
Triglyceride (mg/dl)	157 ± 26	63 ± 9	0.04
HDL-C (mg/dl)	40 ± 2	50 ± 2	0.002
Fasting glucose (mg/dl)	105 ± 5	84 ± 4	0.03
Fasting insulin (μU/ml)	20.2 ± 2.2	4.7 ± 0.9	0.0002
Albumin (mg/dl)	3.6 ± 0.1	3.7 ± 0.1	0.66
Creatinine (mg/dl)	0.8 ± 0.0	0.7 ± 0.1	0.26
Bilirubin (mg/dl)	0.4 ± 0.0	0.6 ± 0.1	0.03
AST (U/l)	23.0 ± 3.5	26.2 ± 3.0	0.57
ALT (Ul)	35.2 ± 5.0	32.4 ± 4.6	0.73
TSH (μU/ml)	2.1 ± 0.2	1.4 ± 0.3	0.049
Free T4 (ng/dl)	1.1 ± 0.0	1.1 ± 0.1	0.96

Data are mean ± s.e.m. for anthropometric and laboratory parameters. ALT, alanine aminotransferase; AST, aspartate aminotransferase; GBP, gastric bypass surgery; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MO, morbidly obese; MWL, massive weight loss, post GBP; TSH, thyroid-stimulating hormone.
[a]Significance of differences between obese and lean participants determined by Student's *t* test for continuous variables and χ^2 (Fisher's exact test) for noncontinuous variables.

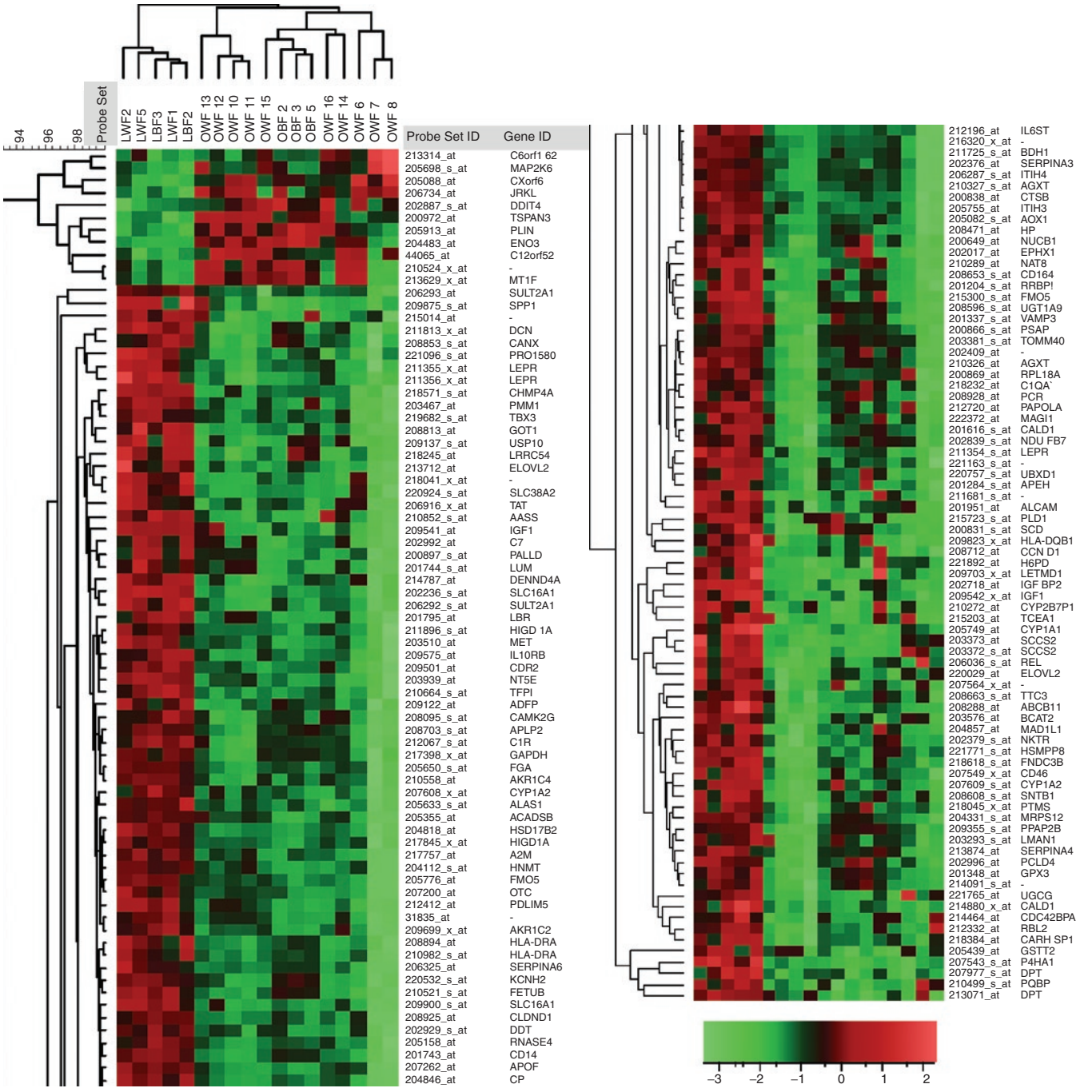

Figure 1 Heat map of differentially expressed genes showing gene expression level in each individual compared to the average expression of that gene in all subjects (**a** and **b**). Red indicates higher expression and green lower expression in liver biopsy samples from 5 massive weight loss and 12 morbidly obese patients. B = Black; F = female; L, massive weight loss; O, morbidly obese; W = white.

Findings of the 28 differentially expressed genes identified as candidate genes for disease susceptibility in MO are discussed by GO category in the Results and Discussion sections.

Differential expression of genes related to lipid metabolism

Expression of mRNA encoding two genes, Perilipin *(PLIN)* and *Enolase 3 (ENO3)*, was significantly higher in livers of MO compared to MWL participants (**Table 2**). Expression of other lipid-related genes, including the *monocarboxylate transporter (SLC16A1)* and *elongation of very long chain fatty acids-like 2 (ELOVL2)*, was markedly lower in MO, as was the gene for *Apolipoprotein F.*

Differential expression of genes related to inflammation, cell proliferation, and tissue repair

The gene for the inflammatory modulator *osteopontin (SPP1)* was expressed at lower levels in livers of MO compared to MWL patients (**Table 2**). Expression of genes encoding the

Table 2 Selected candidate disease susceptibility genes arranged by GO functional category

Affymetrix probe set ID #	Gene title	Gene ID	Unigene cluster	Expression MO/MWL	GO category (functional)
Genes related to lipid metabolism					
205913_at	**Perilipin**	**PLIN**	**Hs.103253**	**7.13**	**Lipid storage/lipolysis**
204483_at	**Enolase 3 (β, muscle)**	**ENO3**	**Hs.224171**	**4.36**	**Glycolysis**
209122_at	Adipose differentiation-related protein	*ADFP*	Hs.3416	0.41	Intracellular lipid transport
207262_at	**Apolipoprotein F**	**APOF**	**Hs.534302**	**0.32**	**Cholesterol transport**
220029_at	Elongation of very long chain fatty acids-like 2	*ELOVL2*	Hs.408557	0.29	Fatty acid biosynthesis
202236_s_at	Solute carrier family 16 member 1	*SLC16A1*	Hs.75231	0.28	Mevalonate transport
Genes related to inflammation, cell proliferation, and tissue repair					
200972_at	Tetraspanin 3	*TSPAN3*	Hs.5062	2.48	Cell proliferation
210524_x_at	DEAD box polypeptide 42	*DDX42*	Hs.591164	2.39	DNA replication
209875_s_at	**Secreted phosphoprotein 1 (osteopontin)**	**SPP1**	**Hs.313**	**0.47**	**Immune response (Interferon)**
213071_at	Dermatopontin	*DPT*	Hs.80552	0.45	Extracellular matrix, connective tissue
201744_s_at	Lumican	*LUM*	Hs.406475	0.40	Collagen fibril organization, tissue repair
209823_x_at	Major histocompatibility complex, class II, DQ β 1	*HLA-DQB1*	Hs.409934	0.39	Immune response
208894_at	Major histocompatibility complex, class II, DR-α	*HLA-DRA*	Hs.520048	0.37	Immune response
207977_s_at	Dermatopontin	*DPT*	Hs.80552	0.36	Extracellular matrix, connective tissue
210982_s_at	Major histocompatibility complex, class II, DR-α	*HLA-DRA*	Hs.520048	0.35	Immune response
207543_s_at	**Prolyl-4-hydroxylase, α-polypeptide I**	**P4HA1**	**Hs.500047**	**0.29**	**Collagen synthesis**
Genes related to signal transduction					
205698_s_at	**Mitogen-activated protein kinase, kinase 6**	**MAP2K6**	**Hs.463978**	**2.43**	**Signal transduction**
211354_s_at	Leptin receptor	*LEPR*	Hs.23581	0.38	Energy reserve metabolism
203372_s_at	Suppressor of cytokine signaling 2	*SOCS2*	Hs.485572	0.33	IGF-1 signaling
202718_at	**Insulin-like growth factor binding protein 2, 36 kDa**	**IGFBP2**	**Hs.438102**	**0.05**	**Regulation of cell growth**
Genes related to metabolism of xenobiotics, hormones, and bile acid					
202887_s_at	**DNA-damage-inducible transcript 4**	**DDIT4**	**Hs.523012**	**4.11**	**Response to energy depletion, hypoxia**
206292_s_at	**Sulfotransferase family, cytosolic, 2A, member 1**	**SULT2A1**	**Hs.515835**	**0.35**	**Bile acid/steroid hormone metabolism**
204818_at	Hydroxysteroid (17-β) dehydrogenase 2	*HSD17B2*	Hs.162795	0.29	Estrogen biosynthesis
215300_s_at	Flavin containing monooxygenase 5	*FMO5*	Hs.642706	0.28	Xenobiotic metabolism
210272_at	Cytochrome P450, family 2, subfamily B, polypeptide 7 pseudogene 1	*CYP2B7P1*	Hs.529117	0.28	Xenobiotic metabolism
205439_at	Glutathione S-transferase θ 2	*GSTT2*	Hs.1581	0.26	Glutathione transferase activity
207609_s_at	Cytochrome P450, family 1, subfamily A, polypeptide 2	*CYP1A2*	Hs.1361	0.25	Xenobiotic metabolism
208288_at	**ATP-binding cassette, subfamily B, member 11**	**ABCB11**	**Hs.158316**	**0.24**	**Bile acid transport**
205749_at	**Cytochrome P450, family 1, subfamily A, polypeptide 1**	**CYP1A1**	**Hs.72912**	**0.23**	**Xenobiotic metabolism**

Genes identified as having a high likelihood of being related to disease susceptibility were selected from the list of 154 genes that met criteria for differential expression in livers of morbidly obese ($N = 13$) vs. MWL ($N = 5$) patients. Genes detected by two different probe sets and those with more than one dominant GO function are listed more than once. Genes in bold print have been confirmed using real-time polymerase chain reaction. The full list of all differentially expressed genes is presented in a series of tables in **Supplementary Appendix 3** online.
MO, morbidly obese; MWL, massive weight loss, GO, gene ontology.

major histocompatibility (MHC) Class II cell surface glycoprotein *HLA-DRA* and *HLA-DQB1* were also lower in livers of MO subjects. Expression of a number of genes related to connective tissue synthesis and repair including *prolyl-4-hydroxylase, α-polypeptide 1 (P4HA1)*, *Lumican (LUM)*, and *Dermatopontin (DPT)* were also lower in livers of MO individuals (**Table 2**). On the other hand, expression of the cell surface glycoprotein *tetraspanin 3 (TSPAN3)* was higher in livers of MO patients, as was expression of the RNA helicase *Dead Box Polypeptide 42 (DDX42)*.

Differential expression of genes related to signal transduction

Expression of several genes related to signal transduction was lower in livers of MO patients, including *insulin-like growth factor binding protein-1 (IGFBP-1)*, *suppressor of cytokine signaling 2 (SOCS-2)*, and the *leptin receptor (LEPR)* (**Table 2**). Conversely, expression of the gene encoding *mitogen-activated protein kinase kinase 6 (MAP2K6)* was higher in livers of MO subjects (**Table 2**).

Expression of genes related to metabolism of xenobiotics, bile acids, and steroid hormones

A panel of genes involved in the metabolism and detoxification of xenobiotics were differentially expressed in livers of MO compared to MWL subjects. Phase I genes whose expression was lower in MO subjects include three members of the *cytochrome P450 family (CYP1A1, CYP1A2, CYP2B7P)* (**Table 2**). Phase II genes that were also expressed in lower levels in MO included *flavin-containing monooxygenase (FMO5), glutathione S-transferase T2 (GSTT2), and sulfotransferase 2A1 (SULT2A1)* (**Table 2**). Expression of the gene *hydroxysteroid (17-β) dehydrogenase 2 (HSD17B2)* was lower in livers of MO subjects, as was the expression of *ATP-binding cassette sub-family B (ABCB11)*. In contrast, hepatic expression of the *DNA-damage inducible transcript-4 (DDIT4)* was significantly higher in livers of MO subjects.

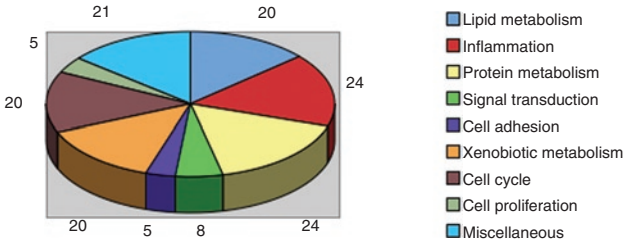

Figure 2 Distribution of differentially expressed genes by GO functional category (some genes are listed twice, i.e., duplicate genes identified by multiple probe sets and genes with overlap between major functional categories). GO, gene ontology.

Confirmation of microarray mRNA expression findings by RT-PCR

We selected a subset of genes from each of the four (GO) functional categories that were judged from microarray analysis to be differentially expressed and extended these results by quantitative RT-PCR. Higher expression of *PLIN* and *ENO3* in livers of MO subjects was confirmed by RT-PCR (20.9- and 8.6-fold, respectively), as was lower expression of *ApoF* (0.6-fold) (**Figure 3a**). Reduced expression of *SPP1*, *insulin-like growth factor binding protein 2 (IGFBP-2)*, and *P4HA1* in MO was corroborated by RT-PCR (0.7-, 0.2-, and 0.2-fold, respectively), as was higher expression of *MAP2K6* (8.2-fold). Similarly, RT-PCR analysis confirmed higher expression of the stress-response gene *DDIT4* (3.5-fold) and markedly lower expression of the xenobiotic metabolizing gene *CYP1A1* (0.1-fold) in MO subjects, as well as lower expression of genes related to bile acid metabolism, SULT2A1, and *ABCB11* (0.6-fold for both) in MO.

Ethnic differences in hepatic gene expression in obesity

The apparent heterogeneity of gene expression in individual black vs. white MO individuals notwithstanding we were able to detect racial differences in expression of some of the genes of interest analyzed by RT-PCR (**Figure 3b**). For example, expression of *PLIN* and *ENO3* that was higher in MO as a

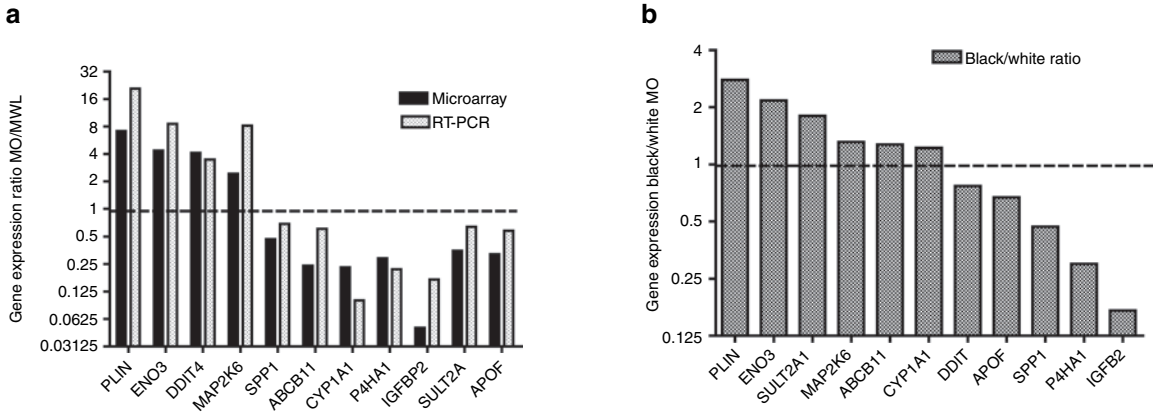

Figure 3 Corroboration of microarray gene expression by RT-PCR. (**a**) Comparative analysis of hepatic gene expression by microarray and their corroboration by RT-PCR. Data are the ratio of gene expression in livers of morbidly obese (MO) vs. massive weight loss (MWL) subjects assessed by both microarray analysis and RT-PCR. *N* = 12 (MO) and 5 (MWL). (**b**) Difference in expression of key genes by race (RT-PCR). Ratio of gene expression in white (*N* = 9) vs. black (*N* = 3) MO females.

Table 3 Summary of candidate genes for disease susceptibility in morbid obesity identified by microarray and RT-PCR

Disease/process	Gene (ID)	Gene function
Hepatic steatosis	Perilipin (*PLIN*)	Lipid storage, lipolysis
	Enolase 3 (*ENO3*)	Cholesteryl ester synthesis
Dyslipidemia (low HDL)	Apolipoprotein F (*ApoF*)	CETP inhibitor
Hepatitis, liver injury, interferon response	Osteopontin (*SPP1*)	Immune-response
	HLA-DRA	Antigen presentation
	HLA-DQB1	Antigen presentation
Impaired wound healing	Dermatopontin (*DPT*)	Collagen fibril formation
	Prolyl-4-hydroxylase (*P4HA1*)	Collagen synthesis
	Lumican (*LUM*)	Collagen fibril formation
Dysregulation of satiety and energy metabolism	Leptin receptor (*LEPR*)	Satiety, energy metabolism
	Insulin-like-growth factor binding protein-2 (*IGFBP-2*)	Adipocyte development (IGF-1)
Insulin resistance	Mitogen activated protein kinase-kinase-2 (*MAP2K6*)	Cytokine signaling (TNF-α)
Cancer	Cytochrome *P450* (*CYP1A1, CYP1A2*)	Xenobiotic metabolism
	Glutathione-S-transferase (*GSTT2*)	Xenobiotic metabolism
Impaired drug metabolism	Cytochrome *P450* (*CYP2B7B1*)	Xenobiotic metabolism
Gallstones, cholestasis	Sulfotransferase (*SULT2A1*)	Sulfonation of bile acids
	ATP-binding casssette (*ABCB11*)	Bile acid transport

CETP, cholesteryl ester transfer protein; HDL, high-density lipoprotein; RT-PCR, real-time polymerase chain reaction; TNF-α, tumor necrosis factor-α.

group was even greater (2.8- and 2.2-fold greater) in obese black women compared to obese white. Expression of the bile acid metabolizing enzyme *SULT2A1*, which was lower in the obese subjects as a whole, was 1.8-fold higher in obese black subjects, indicating that decreased expression was manifested primarily in white subjects. Expression of the cholesteryl ester transfer protein (CETP) inhibitor *ApoF*, which was lower in the obese subjects was correspondingly lower in MO blacks (0.67-fold), as was the inflammatory response gene *SPP1* (0.5-fold) and the collagen synthesis gene *P4HA1* (0.3-fold) (**Figure 3b**). Similarly, the *IGFBP2*, whose expression was markedly lower in all obese subjects, was even lower (0.2-fold) in MO blacks compared to MO whites.

DISCUSSION

We report here that altered gene expression profiles in livers of MO humans after MWL reflect key molecular consequences of morbid obesity and its close association with dyslipidemia, hepatic steatosis, steatohepatitis, gallstones, cancer, and impaired wound healing (Summarized in **Table 3**).

Functional significance of altered expression of genes related to lipid metabolism

Altered expression of genes related to lipid metabolism was not entirely unexpected, given the increased circulating levels of fatty acid and glucose in the MO. In humans with morbid obesity, hepatic steatosis or nonalcoholic fatty liver is a frequent finding (23). Thus, higher expression of *Perilipin (PLIN),* a key lipid storage protein, likely reflects a compensatory response to increased hepatic lipid content. Conversely, reduced hepatic Perilipin expression in MWL subjects likely reflects resolution of hepatic steatosis following GBP surgery (23). Higher expression of *Perilipin* in livers of MO may also contribute to the development of hepatic steatosis since Perilipin acts as a fatty acid trap and reduces lipolysis in response to PPARγ activators (24). In addition to its role in lipid storage, Perilipin may also play an important role in the pathogenesis of obesity itself. Perilipin-null mice are resistant to the development of obesity and exhibit upregulation of genes involved in β-oxidation and electron transport chain with concomitant reduction in expression of genes involved in lipid biosynthesis (25). Conversely, polymorphisms at the *PLIN* locus are associated with increased risk of obesity in women (26). Higher expression of *Enolase 3 (ENO3)*, an enzyme mediating cholesterol ester synthesis, in livers of MO may also represent a compensatory response to increased lipid delivery to the liver and may be an important factor in accumulation of hepatic cholesteryl ester in obesity (27). Hepatic expression of the fatty acid elongase *ELOVL2* was lower in livers of obese subjects. *ELOVL2* is a member of a family of substrate specific enzymes that carry out elongation of polyunsaturated fatty acids (28). Although the significance of lower expression of *ELOVL2* in MO is unknown, the linkage of another member of this family, *Elov16,* with insulin resistance underscores the potential role of altered fatty acid metabolism in the pathogenesis of the metabolic syndrome (29).

Expression of the gene encoding the apoprotein *ApoF* was significantly lower in livers of MO subjects. As an inhibitor of CETP-mediated remodeling of HDL3 and HDL2 particles (30), lower *APOF* expression would be expected to result in decreased cholesterol content of HDL via increased CETP activity. Therefore, *APOF* is a candidate gene for lower plasma levels of HDL-C in obesity.

Functional significance of altered expression of genes related to inflammatory response and extracellular matrix

It has been postulated that a low-grade inflammatory state seen in many MO patients is mediated by increased secretion of adipokines (31). Therefore, it is noteworthy that expression of *osteopontin (SPP1)*, a gene whose product is involved in the regulation of immune inflammatory reactions, was expressed at lower levels in livers of MO patients compared to those who had undergone MWL. Similarly, expression of genes encoding *HLA-DRA* and *HLA-DQB1* glycoprotein was also lower in livers of MO. These immune-response proteins serve an important role in antigen presentation to T-lymphocytes (32). One potential explanation for these findings is that chronic elevation in adipokines in MO may result in compensatory

downregulation of signals that regulate the inflammatory response. In addition, the high prevalence of steatohepatitis and hepatic fibrosis in MO (4) may be correlated with our observation of altered expression of *SPP1*, *HLA-DQB1,* and *HLA-DRA*. *SPP1* plays an important role in the pathogenesis of inflammatory and fibrotic diseases (33,34). Upregulation of *SPP1* occurs early in the development of steatohepatitis, and *SPP1* appears to be an important factor in the progression of nonalcoholic steatohepatitis to cause liver injury and fibrosis (33). In this regard, downregulation of *SPP1* might be considered a protective compensatory response to cytokines. *HLA-DRA* and *HLA-DQB1* are members of the MHC Class II cell surface glycoproteins that regulate presentation of antigens (32). Expression of the major histocompatibility Class II genes is induced by cytokines, in particular IFN-γ (35). The *HLA-DQB1* gene locus is an important determinant of susceptibility to autoimmune hepatitis (36), whereas the *HLA-DQB1* locus plays a role in drug-induced liver injury (37). Reduced response to antiviral treatment in obese individuals with Hepatitis C has been postulated to result from impaired interferon signaling (38). Therefore, lower expression of *SPP1* and *HLA-DQB1* may play a role in both the impaired response of MO individuals to interferon therapy and increased risk for hepatic fibrosis in chronic hepatitis (39).

MO individuals are also at increased risk of postoperative infection and delayed wound healing (7,8,40,41). Impaired wound healing in obesity may be related to altered extracellular matrix proteins due to lower expression of *P4HA1, DPT,* and *LUM*. Dermatopontin is an extracellular matrix protein that associates with collagen and plays a role in collagen fibril formation (42). Similarly, both prolyl-4-hydroxylase and Lumican play important roles in synthesis and posttranslational modification of collagen fibrils.

Functional significance of altered expression of genes related to IGF and leptin signaling

Expression of the gene encoding IGFBP-2 was markedly lower in livers of MO subjects. This is consistent with a previous report of decreased plasma levels of IGFBP-2 in obese humans (43). IGFBP-2 is thought to modulate the effects of IGF-I on adipocyte development in obesity, and overexpression of IGFPB-2 protects against diet-induced obesity in mice (44). Our finding of lower hepatic expression of the leptin receptor (*LEPR)* may also have important implications in the pathogenesis of obesity. Downregulation of leptin signaling in the liver has been previously described in obese animal models (45). In addition, lower hypothalamic *LEPR* expression and/or signaling has been postulated to play an important role in dysregulation of satiety in MO humans (46). Insofar as it may reflect a generalized downregulation of *LEPR* in response to high circulating leptin levels, the observed downregulation of hepatic *LEPR* in MO is a significant finding. Leptin signaling also affects peripheral tissues by promoting fatty acid oxidation and glucose transport in muscle and adipocyte, respectively (47); however, the significance of attenuated leptin signaling in the liver remains unknown.

Hepatic expression of the dual specificity protein kinase, *MAP2K6*, was higher in livers of MO individuals. *MAP2K6* is a key component of the p38 MAP Kinase signaling pathway. The *MAP2K6* gene product, MAP Kinase Kinase 6 (MKK6), which activates p38, has been implicated in the induction of insulin resistance by tumor necrosis factor-α (48). Thus, the finding of upregulation of this key component of the p38 MAP kinase signaling pathway in livers of MO humans is particularly intriguing.

Functional significance of altered expression of genes related to metabolism of xenobiotic compounds, bile acids, and steroids

Hepatic expression of many genes that regulate the metabolism of chemicals, carcinogens, and free radicals was altered in livers of MO subjects. We observed decreased expression of a number of cytochrome P450 (*CYP)* genes including *CYP1A1, CYP1A2,* and *CYP2B7B1* in livers of MO subjects. CYP1A1 metabolizes xenobiotics, such as aflatoxin B1, caffeine and acetaminophen, and polycyclic aromatic hydrocarbons. Polymorphisms of *CYP1A1* are associated with increased risk of colon cancer (49). CYP1A2 is primarily thought to play a role in metabolism of steroids, fatty acids, and xenobiotics (50). The *CYP2B7P1* gene product is a component of the CYP2B6 enzyme that metabolizes many drugs including benzodiazepines and buproprion (51). Thus, metabolism of certain xenobiotics may be compromised in individuals with morbid obesity. Hepatic expression of *glutathione S-transferase (GST) θ 2* was also lower in MO. Gene polymorphisms of GSTs are known to affect individual susceptibility to toxicity from xenobiotics and carcinogens (52), and polymorphisms of *GSTT2* specifically are associated with increased risk of colon cancer (53). Obesity is associated with increased risk of endometrial, breast, colon, esophageal, and liver cancers (9–13). This has been attributed both to environmental factors and to the changes in hormone and cytokine levels that accompany obesity. Our findings raise the interesting possibility that obesity-related alterations in xenobiotic metabolizing enzymes may also contribute to the predisposition of MO subjects to neoplastic disease.

Hepatic expression of the *DNA-damage inducible transcript-4 (DDIT4*) was significantly higher in livers of MO. *DDIT4* encodes a protein (TP801 or REDD1) that inhibits mTOR function to control cell growth in response to energy stress and hypoxia (54). The *DDIT4* product REDD1 also plays a role in the generation of reactive oxygen species and the p53-dependent DNA damage response (55). Thus, enhanced expression of *DDIT4* may reflect an adaptive response to increased energy stress accompanying over-nutrition in morbid obesity.

Expression of *HSD17B2* was lower in MO. *HSD17B2* plays an important role in the conversion of estradiol, testosterone, and 5α-dihydrotestosterone to estrone and androstenedione (56). *HSD17B2* may therefore be a candidate gene for higher plasma levels of testosterone and estradiol observed in MO. Through its role in regulating tissue levels of active estrogen and androgen, *HSD17B2* may play an important role in cellular proliferation in hyperplastic and neoplastic disorders (56).

Expression of *SULT2A1* and *ABCB11* was lower in MO. *SULT2A1* plays an important role in the detoxification and clearance of bile acids by catalyzing their sulfonation (57). *ABCB11* is a major transport protein that facilitates the enterohepatic circulation of bile salts (58). Decreased expression of *ABCB11* has been implicated in cholestatic liver disease (58). MO patients have a known predisposition to develop gallbladder disease (6). Based on our data, we speculate that altered expression of these genes involved in bile acid, steroid, and xenobiotic metabolism reflects a common theme of regulation by receptors, including the aryl hydrocarbon receptor, constitutive androstane receptor, and the pregnane X receptor (59).

Difference in hepatic gene expression in obese subjects by race

Hierarchical analysis indicated the presence of racial heterogeneity in hepatic gene expression among MO subjects. Although the number of subjects in general and African-American subjects in particular were too few to allow a systematic evaluation of racial differences in gene expression, we compared expression of selected key differentially expressed genes in white and African-American subjects by RT-PCR. Expression of *Perilipin (PLIN)* and *Enolase 3 (ENO3)* genes were disproportionately higher, and expression of the *SPP1* and *P4HA1* was disproportionately lower in MO African-Americans. Ethnic differences in expression of these and other genes may reflect differential racial sensitivity to liver disease in MO. Indeed, the frequency of hepatic steatosis and nonalcoholic fatty liver disease–related cirrhosis is lower in African-Americans vs. whites and Hispanics (60). In light of the observed heterogeneity in hepatic gene expression, study of racial differences in hepatic gene expression in a larger population of African-American, white, and other ethnic groups is warranted.

We successfully corroborated the microarray findings of altered gene expression for 11 key disease susceptibility genes by RT-PCR. On the other hand, it is important to note that RT-PCR analysis failed to corroborate differential expression of several other genes, including *Haptoglobin, Interleukin 10 receptor β, Interleukin 6 Signal Transducer (IL6ST), and α-2 Macroglobulin (A2M).* This may be related to the inherent difficulty of detecting small (less than twofold) changes in gene expression using RT-PCR and/or the use of primers and probe sets that detect different isoforms of mRNAs by the two techniques.

Limitations of the study and future Prospects

It is important to interpret the findings of this (and any human) study in light of certain limitations. First, the obese subjects we studied represent an extreme form of obesity leading to surgical intervention for weight loss. Thus, these MO subjects may not be representative of individuals with lesser degrees of obesity. Second, as the number of men seeking GBP was too few to obtain meaningful data, only women were studied. Therefore, until more information on sex-specific hepatic gene expression is obtained, these data should be interpreted to reflect the effect of obesity on hepatic gene expression specifically in women. Third, we focused only on one level of gene regulation, mRNA expression, which does not always predict tissue-specific expression of the encoded protein. Therefore, full assessment of the significance of altered expression of the mRNA species observed in the current study would require more detailed investigation of gene expression. Fourth, it is important to remember that although the MWL subjects experienced significant weight loss, their "post-obese" BMI is still greater than that of normal lean individuals. In addition, changes in the gene expression profile may reflect specific effects of weight loss and caloric restriction following GBP. Thus, these individuals should not be considered "normal" but rather reflect the effect of MWL on expression of obesity-related genes. Insofar as altered gene expression in MWL reflects the ability of weight loss to reverse the hormonal and metabolic milieu of morbid obesity, the MWL group provides insights into patterns of gene expression associated with obesity. In addition, as pointed out in the Discussion, there appears to be significant ethnic variation in hepatic gene expression in MO women. As the microarray analyses included both white and African-American women, this variability may have affected our ability to detect significant differences in hepatic gene expression in either ethnic group. Despite this variability, we were in fact able to detect a number of differentially expressed genes. We should also point out that although we have focused our discussion on a selected group of 28 genes that can be directly linked to specific disorders associated with obesity, our analysis identified a larger number (154) of genes. Many of the additional genes identified by microarray analysis may also have significance for diseases associated with obesity that is not readily apparent. The reader is referred to the complete list of differentially expressed genes in the **Supplementary Appendix 3** online.

These caveats notwithstanding, we have identified key molecular signatures of hepatic gene expression in MO patients who have undergone MWL after bariatric surgery. Through the use of transcriptome analysis, we have identified a number of novel gene targets that may play a role in the pathophysiology of obesity and in the increased susceptibility of MO individuals to a wide range of disorders.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

ACKNOWLEDGMENTS

This material is based on work supported in part by the Medical Research Service, Office of Research and Development, Department of Veterans Affairs (M.B.E., R.R.); the University of Tennessee General Clinical Research Center, Grant #MO1-RR0021, NIDDK RO1-DK75504-01 (M.B.E., R.R., C.Y.); and by a grant from the Vascular Biology Center of Excellence, University of Tennessee Health Sciences Center–Memphis. We would like to thank Dr Grant Somes of the UT Biostatistics Division for providing statistical consultation and Dr William L. Taylor of the Molecular Resource Center of Excellence for providing facilities and assistance in RT-PCR assays. We would also like to thank Poonam Kumar for technical assistance. We would also like to thank the staff of the UT Obesity Wellness Center and the Operating room staff of UT-Bowld Hospital and Baptist Memorial Hospital Memphis for their assistance. R.R. is a Senior Research Career Scientist of the

Department of Veterans Affairs. This work was reviewed by the EPA but does not necessarily reflect official Agency Policy. Mention of trade names or commercial products does not constitute endorsement or recommendation by EPA.

DISCLOSURE

The authors declared no conflict of interest.

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