IDENTIFICATION OF CANDIDATE ANTI-OBESITY TARGETS

by

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ABSTRACT OF THE THESIS

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Over the past decade, obesity has become a major health issue in the western world, and is very rapidly becoming a concern for eastern countries as well. In the most basic sense, obesity results from an imbalance between energy intake and energy expenditure. However, the complex disease that is obesity arises as a result of the insufficient energy consumption, resulting in adiposity, the increase of triglyceride storage in adipocytes, body weight gain, and an increased risk of obesity co-morbidities. As the prevalence of obesity is expected to increase over the next 20 years, the identification of novel anti-obesity targets has become paramount in the search for an efficacious and compliance-friendly obesity therapy. In an effort to identify and classify potential anti-obesity targets as experimental candidates for obesity therapy, WAT (white adipose tissue) from $Hmga2^{-/-}$, $Lep^{ob}.Lep^{ob}$ double knockout mice, $Hmga2^{+/+}$ mice, $Hmga2^{-/-}$ mice, and Lep^{ob}/Lep^{ob} mice were run on microarray to determine gene expression profiles for each genotype. Exclusion criteria were added, and a list of 138 adipocyte-specific genes remained. The genes were then characterized through bioinformatics according to 6 parameters: 1) chromosomal location in mouse genome; 2)

chromosomal location in human genome; 3) subcellular localization; 4) function; 5) knockout phenotype in mice; 6) obesity-related QTLs in human genome. After characterization, each gene was evaluated in greater detail and filtered to form a list of candidates for experimental analysis to evaluate anti-obesity effectiveness. The list of 138 genes was finally narrowed to 10 genes, 6 of which were present in the top 25 most highly expressed genes in adipocyte-rich WAT. Preliminary experimental analysis reveals diminished weight gain and overall decreased body weight in mice upon peptide inhibition for one gene from the filtered list. While we have only begun evaluating one gene, thus far, the results are encouraging in that the method used to develop and filter such a list of genes is proving to be validated. Further experimental research is required in order to fully validate each potential candidate; however such an approach, to determine solid candidates, appears promising.

Acknowledgement

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LIST OF ABBREVIATIONS

BAT	Brown Adipose Tissue
BMI	Body Mass Index
CB_1	Cannabinoid-1
CCR	Chemokine (C-C) Receptor
CHF	Congestive Heart Failure
DEXA	Dual X-ray Absorptiometry
ECM	Extracellular Matrix
ER	Endoplasmic Reticulum
EST	Expressed Sequence Tag
EST	Expressed Sequence Tag
FDA	Food and Drug Administration
GLP-1	Glucagon-Like Peptide-1
GPCR	G-Protein Coupled Receptor
HMGA2	High Mobility Group AT-hook protein 2
HMGIC	High Mobility Group AT-hook protein 2
IGF	Insulin-Like Growth Factor
JNK	c-Jun N-Terminal Kinase
KO	Knockout
Lep^{ob}	Leptin-Deficient
LOD	Logarithm of Odds
Mbp	Mega Basepair
MGI	Mouse Genome Informatics
MRI	Magnetic Resonance Imaging
NCBI	National Center for Biotechnology Information
NPL	Non-Parametric Linkage
qRT-PCR	Quantitative Real-Time Polymerase Chain Reaction
QTL	Quantitative Trait Locus
RIO	Rimonabant-In-Obesity
STS	Sequence Tagged Site
TAG	Triacylglycerol
UniProt	The Universal Protein Resource
WAT	White Adipose Tissue
WHO	World Health Organization

Chapter 1: Introduction

1.1 The Disease

Over the past decade, obesity has become a major health issue not only in the western world, but is very rapidly becoming a concern for eastern countries as well. In the most basic sense, obesity results from an imbalance between energy intake and energy expenditure. However, the complex disease that is obesity arises as a result of the insufficient energy consumption, resulting in adiposity, the increase of triglyceride storage in adipocytes, body weight gain, and an increased risk of obesity co-morbidities (Schutz, 2004).

The prevalence of obesity is expected to increase over the next 20 years. It is estimated that one-third of the U.S. adult population is currently obese; however, that number is expected to jump to one-half by 2030. Currently, two-thirds of the U.S. adult population is overweight or obese and by 2030, it is estimated that 86 percent of the adult population will be overweight or obese (Wang, 2008). Given these figures, obesity is expected to carry with it a heavy burden on the healthcare system; both monetarily and pathologically, with an estimated $698 to $785 billion in direct healthcare costs (Wang, 2008).

The significance of treating obesity lies not only in its aesthetic issues, but maybe more importantly in its co-morbidities. Obesity has been linked to many cardiovascular and metabolic disorders, as well as cancer; and has been associated with morbidity and mortality (Crowley, 2008; Table 1).

Table 1: Obesity Co-morbidities

Disease Type	***Disease***
Cardiovascular	Hypertension, Cardiovascular disease, CHF
Metabolic	Type 2 Diabetes Mellitus, Insulin resistance, Dyslipidaemia, Hyperuricaemia/Gout, Polycystic ovarian syndrome, Impaired fertility
Other	Cancer, Gallbladder Disease, Reflux oesophagitis, osteoarthritis, Lower back pain, Sleep apnea, Non-alcoholic fatty liver disease, Psychological illness

Co-morbidities linked to obesity include several metabolic and cardiovascular diseases as well as cancer and structural illnesses.

1.2 Diagnosis of Obesity

At present, there are several different ways to diagnose obesity and measure adiposity. The most common method used is the Body Mass Index (BMI), in which an individual's body mass is measured in relation to his or her height:

$$\frac{\text{Body Weight (kg)}}{\text{Body Height } (m^2)}$$

The World Health Organization (WHO) classifies a BMI$\geq$25.00 to be overweight, and a BMI$\geq$30.00 to be obese (WHO, 2004; Table 2). Despite being the most common method of obesity diagnosis, this is a less-than-adequate system for measuring adiposity. Body weight is not a precise indicator of adiposity, indiscriminately including muscle and bone mass, as well as water weight. Moreover, the inability to discern between types of adiposity limits the assessment of risk factors; specifically, visceral adiposity has been strongly linked to certain disorders such as type 2 diabetes mellitus and cardiovascular disease (Crowley, 2008). The cheap cost and easy access to this system of weight monitoring favor its wide use; however, other methods are used which provide greater insight into the type of adiposity and the specific amount of adipose tissue. Anthropometry is a method used to measure skin folds that incorporate subcutaneous fat, thereby determining the percentage of body fat. This system is used often; however, there is potential for error during the measurement and interpretation. Furthermore, this method does not account for visceral and bone marrow fat. Bioelectrical impedance analysis is another method used to measure body fat content. This system measures the opposition of electric flow through the body to determine fat-free body mass, and by subtraction from total body mass, the body fat. Not having been perfected, this method

also yields error, and does not provide a "map" of adiposity. Scanning methods such as magnetic resonance imaging (MRI) or dual x-ray absorptiometry (DEXA) give greater details about the types of adiposity (i.e. subcutaneous, visceral, bone marrow) and a better idea of overall body fat percentage for a particular patient. These methods are typically used when being treated by a specialist (Crowley, 2008).

Overall, the diagnosis of obesity is not a problem in the clinical setting; that is, obesity is not frequently misdiagnosed. Moreover, minute variations in measurement of body fat percentage yield no dissimilar outcomes. The dilemma in modern medicine is the development of an efficient and compliance-friendly way to prevent and/or treat obesity.

Table 2: WHO Classification of Weight Using BMI

Classification	***BMI (kg/m^2)***
Underweight	<18.50
Normal Range	18.50 – 24.99
Overweight	≥25.00
Obese Class I	30.00 – 34.99
Obese Class II	35.00 – 39.99
Obese Class III	≥40.00

The internationally accepted World Health Organization (WHO) BMI classification organizes an individual's weight range as underweight, normal, overweight, or obese.

1.3 Current Pathways in the Targeting of Obesity

Current anti-obesity research focuses primarily on the central and peripheral nervous system and endocrine signaling. Sibutramine©, an FDA approved anti-obesity medication, targets the norepinephrine and serotonin pathways by blocking neuronal uptake; thereby decreasing appetite and increasing satiety. Orlistat©, another FDA approved anti-obesity medication, is a gastric lipase inhibitor, preventing absorption of dietary fat. Although these current medications have shown efficacy as pharmacological alternatives for weight loss, side effects such as increased heart rate and blood pressure, abdominal cramping, liquid stools, oily spotting, and incontinence discourage patient compliance and clinical recommendations (Chaput, 2006).

Current areas of research continue to include the nervous and endocrine system, along with gastrointestinal metabolism. A large area of investigation is the endocannabinoid receptors and their effects on satiety and subsequent weight loss. Studies with cannabinoid-1 (CB_1) receptors agonists resulted in an increased food intake in rats (Williams, 1998). Furthermore, antagonist compounds have shown specificity for the CB_1 receptor. Treatment with CB_1 antagonists was shown to reduce food intake in rodents and humans, and has been shown to result in weight loss in humans as evidenced by the approval of Rimonabant© for anti-obesity treatment in Europe. One major drawback of targeting CB_1 receptors is the correlation to psychiatric problems. Psychiatric illness became the major reason for patients dropping out of the Rimonabant –in-obesity (RIO) clinical trials, with symptoms such as irritability, depressed mood, agitation, insomnia, and headache (Viveros, 2008). Additional areas of concern are possible drug interactions with other medications targeting the endocannabinoid system,

such as acetaminophen, blood pressure effects, and heart rate effects. As promising as it may be to target this system, it is not without shortcomings.

Another major area of research for anti-obesity treatment is endocrine signaling, primarily effecting appetite and satiety. One example of this is the targeting of Ghrelin, a plasma peptide associated with appetite behavior. Intravenous infusion of this peptide in humans has shown to increase appetite and food intake (Wren, 2001). Thus, the antagonism of Ghrelin is a highly researched area for anti-obesity treatment. Another example is the Glucagon-like peptide-1 protein (GLP-1). GLP-1 is produced mainly in the distal ileum and colon, and delays gastric emptying, inhibits glucagon secretion, stimulates glucose-induced insulin secretion, increases insulin sensitivity, improves glucose blood levels in diabetic patients, and increases satiety (Chaput, 2006). GLP-1 agonists have the potential to treat both obesity and diabetes; however, dosing of such a drug may pose a problem in the effective treatment of both illnesses. Moreover, as is the case with many potential drugs, an effective and compliant drug delivery system may be a problem, with oral GLP-1 agonist compounds showing decreased efficacy than injectable analogues (Chaput, 2006).

Current anti-obesity research focuses on an array of systemic systems, most of which target appetite suppression and satiety. These drug targets all have one thing in common: none of them target the adipocyte itself. The targeting of these various systems may address obesity; however, other significant systemic mechanisms may be affected, such as cardiac output, psychiatric wellness, and gastrointestinal integrity, as well as compromise of co-morbidities. Targeting of the adipocyte may address obesity specifically without drastically affecting other mechanisms; however, investigation of

adipocyte-specific genes has been somewhat suspect due to the inability to obtain a large and pure enough pre-adipocyte cell population with which the adipocyte population may be compared.

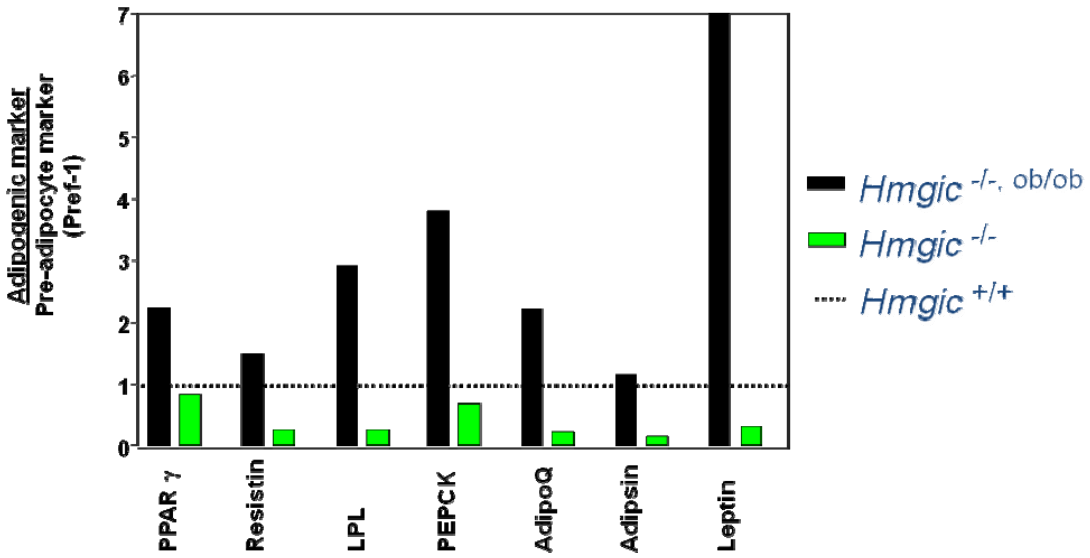
1.4 HMGA2

The high mobility group AT-hook protein 2 (HMGA2; HMGIC) is a member of general class on non-histone nuclear proteins (Berg, 2000). The HMGA family of proteins has many functions including cell-cycle control, chromosomal organization, differentiation, and cellular senescence (Malek, 2008). This protein family is referred to as architectural transcription factors due to their non-histone DNA-binding ability as well as their capacity to recruit and interact with several other transcription factors and form multiprotein enhanceosome complexes.

Previous work in the lab led to the characterization of HMGA2 as a protein involved in the proliferative expansion of undifferentiated pre-adipocytes (Anand, 2000). The protein, which is typically detected during mouse embryogenesis and in tumors, was shown to be detected one week after beginning a high fat diet. Furthermore, the HMGA2 knockout mice (pygmy mice) were 47% that of the weight of wild-type mice on a standard diet by 30 weeks of age. In addition, a double homozygous null of HMGA2 and Lep^{ob} (leptin-deficient) weighed only 27% that of wild-type mice after 30 weeks on a standard diet (Anand, 2000). The hypothesis put forth is that HMGA2 expression is increased as a result of the expanding pre-adipocyte population required in order for adipocyte number to increase during obese conditions, and its absence retards pre-adipocyte expansion resulting in fewer differentiated adipocytes. Further investigation showed a high ratio of known adipocyte-specific marker expression to pre-adipocyte-specific marker expression (Pref-1) in the WAT (white adipose tissue) of $Hmga2^{-/-}$, Lep^{ob}/Lep^{ob} mice; $Hmga2^{-/-}$ mice showed a low ratio (less than one) (unpublished; Figure 1). These results, coupled with the increased expression of pre-adipocyte markers in

WAT of Hmga2$^{-/-}$ mice on standard diet compared to wild-type also on standard diet, suggest that Hmga2$^{-/-}$ WAT is predominantly pre-adipocytes; whereas Hmga2$^{-/-}$, Lep^{ob}/Lep^{ob} WAT is predominantly adipocytes (unpublished). The findings led to the development of a new model for determining adipocyte-specific genes and possible obesity targets.

Figure 1: Ratio of Adipocyte:Pre-adipocyte Expression



qRT-PCR was used to determine expression of adipocyte and pre-adipocyte-specific markers. Whole white adipose tissue (WAT) was acquired from three different samples ($Hmgic^{-/-}$, Lep^{ob}/Lep^{ob}; $Hmgic^{-/-}$; $Hmgic^{+/+}$) and marker expression was detected. Adipocyte-specific marker expression was significantly higher in the $Hmgic^{-/-}$, Lep^{ob}/Lep^{ob} double knockout mice; while, pre-adipocyte-specific marker expression was higher in $Hmgic^{-/-}$ mice.

Chapter 2: Identification and Validation of Adipocyte-specific Genes

2.1 Introduction

In order to identify new adipocyte-specific genes, the above-mentioned model was used to isolate enriched populations of adipocytes and pre-adipocytes. This model allowed for the identification of possible adipocyte-specific genes in a true *in vivo* system, in contrast to the common method of separation of WAT into adipose and stromal vascular fractions. Upon identification of the adipocyte-specific genes, subsequent validation was necessary in order to identify potential anti-obesity targets and continue with target-validation experiments.

Over the past decade, bioinformatics has become commonplace in the research arena. Sparked by the completion of the Human Genome Project, the creation of databases and organized materials has allowed scientists to easily search for and acquire information pertaining to their specific interests. The quality and quantity of the information available can itself be used as criteria for the further filtering of the adipocyte-specific genes into obesity-specific targets.

The characterization of genes according to their chromosomal location, subcellular localization, and function can be effectively accomplished through bioinformatics data mining. Several online databases such as GeneCards®, UniProt (Universal Protein Resource), and NCBI (National Center for Biotechnology Information) provide a plethora of detailed information for genes and proteins in many different species, most importantly mouse and human.

One powerful tool used in the identification of genes as putative targets for disease-state phenotypes is QTL (Quantitative Trait Locus) proximity (Seaton, 2002). QTL mapping is a common method used to identify chromosomal regions associated with a phenotype. In the case of obesity, as with many diseases, several QTLs have been found, due to the polygenic nature of the disease. Discovery of both mouse and human QTLs in close proximity to candidate genes can provide strong genetic evidence for those genes as putative targets of a disease phenotype (Wuschke, 2007).

In order to further classify the adipocyte-specific genes/ESTs as possible obesity-specific genes and subsequent targets, each gene was characterized according to 6 parameters: 1) chromosomal location in mouse genome; 2) chromosomal location in human genome; 3) subcellular localization; 4) function; 5) knockout phenotype in mice; 6) obesity-related QTLs in human genome. These parameters served to provide a comprehensive picture of each gene as it relates to obesity, which then allowed the list of genes to be narrowed, through quantitative and qualitative scrutiny, to a small and concise list of genes for further experimental analysis.

2.2 Materials & Methods

2.2.1 Determination of Adipocyte-specific Genes

In order to identify adipocyte-specific genes, we looked at expressional changes between populations of enriched pre-adipocytes and enriched adipocytes via Hmga2 modulation in mice. In collaboration with Dr. Abu Sayed, C57Bl/6J mice were crossed to produce four different genotypes: $Hmga2^{+/+}$; Lep^{ob}/Lep^{ob}; $Hmga2^{-/-}$; $Hmga2^{-/-}$, Lep^{ob}/Lep^{ob}. WAT was then isolated from each of the four genotypes and their gene expression profiles were detected and compared by gene microarray analysis. Briefly, total RNA was extracted from adipose tissue samples by using RNAeasy kit (Qiagen). Ten micrograms of total RNA was used to prepare cRNA for Affymetrix Genechip Murine Genome U74 microarray as described in the Affymetrix manual. Hybridization, washing, and scanning were performed as described in the same manual. Exclusion criteria were established in order to narrow the list of genes and create a hierarchy of adipocyte-specific gene expression. Genes were filtered using Genespring 5.0 to include only those genes which showed more than 5-fold hybridization intensity in the $Hmga2^{+/+}$ mice compared to the $Hmga2^{-/-}$ mice and the $Hmga2^{-/-}$, Lep^{ob}/Lep^{ob} mice compared with $Hmga2^{-/-}$ mice. Expression levels were assessed for over 36,000 genes and ESTs. From the list obtained by combining the former criteria, genes expressed at low levels (less than 1000 intensity) in all samples were excluded. In addition, immune-responsive genes were also excluded.

Upon filtering the genes into a list meeting all criteria, the genes were validated by qRT-PCR of the "Top 25" genes (genes expressed the highest in the double knockout)

from the WAT of wild-type C57Bl/6J mice ($Hmga2^{+/+}$), distinct from the mice used in the microarray experiments. The WAT tissue was separated into adipose and stromal vascular fractions, and the level of expression was assessed for genes in the top 25. Separation of adipocytes from stromal vascular fraction was carried out from fat pads using collagenase digestion. Adipose tissues were minced and placed on collagenease buffer for 30 minutes at 37°C. After total homogenization, samples were fractionated by spinning at 400 x g for 2 min at 4°C; the upper fractions containing adipocytes were taken out by a Pasteur pipette. The adipocyte fractions and the bottom stromal vascular fractions were centrifuged again at 2800rpm for 7 minutes at 4°C. The pellet of SV cells was washed several times with cold PBS.

Reverse transcription of 1μg total RNA was carried out using strand cDNA synthesis kit for RT-PCR (Invitrogen). The cDNA was brought to 200μl by TE buffer and 2μl of the diluted cDNA was used per 10μl PCR reaction using Sybr Green reagents or Taqman master mix from Applied Biosystems. PCR was performed in an ABI Prism 7900HT sequence detection system.

2.2.2 Update of Gene Identities

In order to begin characterizing the genes and ESTs, the list had to first be updated to ensure the correct genes were being investigated and that all ESTs were up-to-date and had not been matched to genes. To do this, the accession number for each gene and EST was searched in the NCBI database (http://www.ncbi.nlm.nih.gov/sites/gquery). The sequence was obtained, and for further confirmation, was BLAST searched against the mouse genome. Each gene and EST in the list was updated as such. From the

BLAST results screen, mouse genomic contig sequences were checked to ensure that EST locations were not overlapping genes. ESTs were also mapped on the genome by using the "Map Viewer" function on the BLAST results screen (heading for each hit) and run in the MGI (Mouse Genome Informatics) database (http://www.informatics.jax.org/). The chromosomal location for each EST was searched in the MGI database by specifying the chromosome number and searching for genes which overlapped the chromosomal location of the EST. The updating was done intermittently every 2-3 months to ensure all identities were up-to-date.

2.2.3 Mouse Chromosomal Locations

Chromosomal locations of each gene and EST were determined for the mouse genome. Using the MGI database, genes were entered in the search box on the "Genes and Markers Query Form" and searched; chromosomal locations were documented. Locations were double checked by using the "Map Viewer" function in the NCBI database. EST locations were searched similarly to the updating process. The EST sequence was BLAST searched against the mouse genome and the location was documented from the map viewer. All mouse chromosomal locations for genes were documented from the MGI database (which all referenced NCBI); while all EST chromosomal locations were documented from NCBI map viewer.

2.2.4 Human Chromosomal Locations

Determination of human homologs of genes was made through the MGI database. Genes were searched for on the "Genes and Markers Query Form" and the gene name was clicked to open the gene detail page; human homologs were documented from the

"Mammalian Orthology" link. The homologous gene symbol was then run through the GeneCards ® database, specifically the "Gene/Marker" search under GeneLoc (http://genecards.weizmann.ac.il/geneloc-bin/aliases.pl). The human chromosomal location was then documented from the gene page (referencing Entrez/NCBI). All human chromosomal gene locations were documented from GeneCards®.

2.2.5 Function & Subcellular Localization

Function and localization for each gene was determined using the previously mentioned databases, GeneCards® and UniProt. First, human gene symbols were searched through the GeneCards® database using GeneLoc. The gene function and localization was documented from the individual gene card and confirmed through publication. For further confirmation, the UniProt database (http://www.uniprot.org/) was searched for each gene symbol, and function and localization were compared to the gene card. Any additional information was added. All information was verified by referenced publications.

2.2.6 Knockout Phenotype in Mice

Obesity-related knockout phenotypes were investigated for each gene on the list. To do this, GeneCards® were opened for each gene, and the gene symbol link was clicked for the MGI mutant phenotypes. On the MGI gene detail page, "All phenotypic alleles" was clicked, and knockout corresponding to the obesity-related phenotype was chosen. Under the "Phenotype summary" chart, the genotype link was opened which corresponded to the desired phenotype. The corresponding publication was then accessed to verify and the phenotype was documented.

2.2.7 Obesity-related QTLs in Human Genome

Investigation of QTLs proximal to or within the genes was initially accomplished using the comprehensive publication by Rankinen *et al.* QTLs were filtered according to their cytogenetic location and subsequently narrowed by LOD score and molecular location. Once QTLs located on the same band as the gene were identified, the original publications were investigated for the QTL boundaries. Original papers were found at the web address referenced in the publication by Rankinen *et al.*: http://obesitygene.pbrc.edu/. In order to normalize all QTL data, locations and distances from genes were documented according to their molecular localization, with the maximum allowed distance from the gene start site limited to 10Mbp. This was accomplished using the NCBI search engine tool "UniSTS", a database for sequence tagged sites (STSs), as defined by PCR primer pairs. The primer pair(s) defining the QTL boundaries was run through the database and the molecular locations were documented. The QTL distance from the gene start site was calculated and documented.

2.2.8 Elimination of Genes

Once the six parameters were investigated, each gene was thoroughly analyzed for a possible role in obesity. The findings in the investigation of each of the six parameters were scrutinized both quantitatively and qualitatively for each gene. A preliminary decision was then made for each gene in regards to the gene's potential as an anti-obesity target. The remaining genes were then investigated thoroughly for roles in lipid metabolism, adipogenesis, and obesity. Once each remaining gene was investigated, a final round of elimination was conducted, leaving only the most promising

candidate genes for further experimental evaluation as anti-obesity targets.

2.3 Results

2.3.1 Identification of Adipocyte-specific Genes

Upon meeting all criteria, a list of 138 potential adipocyte-specific genes remained, including 16 ESTs (Expressed Sequence Tags) (Table 3). Furthermore, the 25 most highly expressed genes in the double knockout (Top 25) contained 6 known adipocyte markers: AdipoQ, Plasma Membrane S3-12, Resistin, PEP Carboxykinase, Cell Death-inducing DFFA-like Effector C, and Leptin. Next, the list was validated by qRT-PCR of 12 genes in the top 25 from WAT of wild-type mice. Consistent with the model above, the 12 genes tested showed at least 5-fold higher expression in the adipose fraction compared to the stromal vascular fraction (Table 4). These results provided an independent validation of the Hmga2 model used to determine adipocyte-specific genes for the purpose of identifying putative obesity targets.

Table 3: List of 138 Adipocyte-specific Genes

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM1	Transmembrane Protein 45b	HM26	Phospholipase D Family, Member 4
HM2	Sorbin and SH3 Domain Containing-1	HM27	S100 calcium binding protein A9
HM3	Chemokine (C-C motif) ligand 6	HM28	Krupel-like Factor 15
HM4	Est3	HM29	Ribonucleoprotein, PTB-binding 2
HM5	Est5	HM30	Coatomer Protein Complex, Subunit Gamma 2, Antisense 2
HM6	Carbonic Anhydrase III	HM31	Leucine Rich Alpha 2 Glycoprotein
HM7	AdipoQ	HM32	Galectin-12
HM8	Plasma Membrane S3-12	HM33	TBC1 Domain Family, Member 10C
HM9	Fatty Acid Desaturase 3	HM34	Mesoderm Specific Transcript
HM10	Neuronatin	HM35	Best12
HM11	Best25	HM36	Rac/Cdc42 Guanine Nucleotide Exchange Factor (GEF) 6
HM12	Atrial natriuretic peptide C-type receptor	HM37	CD1D Antigen
HM13	Resistin	HM38	Synuclein, Gamma (Breast Cancer-specific Gene 1)
HM14	Best23	HM39	Olfactomedin-like 1
HM15	Matrix Metallopeptidase 19	HM40	Carboxypeptidase M
HM16	PEP Carboxykinase	HM41	Coiled-Coil Domain Containing 80
HM17	Amine Oxidase, Copper-containing	HM42	CD299 Antigen
HM18	cest1	HM43	Est27
HM19	Unknown Carboxylesterase	HM44	Leukotriene C4 Synthase
HM20	Cell Death-inducing DFFA-like Effector C	HM45	Arylacetamide Deacetylase-like 1
HM21	Secreted Frizzled-like Protein 5	HM46	Paternally Expressed 13
HM22	Cytochrome P450, Family 2, Subfamily D, Polypeptide 22	HM47	Liver Glycogen Phosphorylase
HM23	G Protein-coupled Receptor 109A	HM48	CDC42 Effector Protein 2
HM24	Inter-alpha (globulin) Inhibitor H5	HM49	cest6
HM25	Leptin	HM50	UDP-Gal:betaGlcNAc Beta 1,3-galactosyltransferase, Polypeptide 2

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM51	Interferon, Alpha-Inducible Protein 27-like 2A	HM76	Coronin, Actin Binding Protein 1A
HM52	Phosphatase and Actin Regulator 2	HM77	ADAMTS-like 5
HM53	WD Repeat Domain, Phosphoinositide Interacting 1	HM78	cest10 hypothetical
HM54	Best40	HM79	Protocadherin 7
HM55	Decay Accelarating Factor for Complement	HM80	Centromere Autoantigen H
HM56	Retinol Binding Protein 4, Plasma	HM81	Acid Phosphatase 2, Lysosomal
HM57	Lactotransferrin	HM82	Docking Protein 3
HM58	Pleckstrin	HM83	Ankyrin Repeat and Sterile Alpha Motif Domain Containing 3
HM59	ATP-binding Cassette, Sub-family D, Member 2	HM84	Sodium Channel, Voltage-gated, Type VII, Alpha
HM60	Phospholipase C, Beta 1	HM85	Resistin-like Alpha
HM61	Solute Carrier Family 1, Member 3	HM86	Thyroid Hormone Responsive SPOT14 (Rat) Homolog
HM62	RAS p21 Protein Activator 2	HM87	Cyclin M3
HM63	ADP-ribosylation-like 4	HM88	Host Cell Factor C1
HM64	Ras Homolog Gene Family, Member Q	HM89	Best46
HM65	Eukaryotic Translation Initiation Factor 4E Member 3	HM90	Limb Bud and Heart Development Homolog
HM66	ATP-binding Cassette, Sub-family A, Member 9	HM91	Chemokine (C-C Motif) Ligand 5
HM67	Best28	HM92	Best48
HM68	Unknown	HM93	Solute Carrier Family 16 (Monocarboxylic Acid Transporters), Member 12
HM69	Best43	HM94	Best50
HM70	Rho GTPase Activating Protein 25	HM95	Matrix Metalloproteinase 9
HM71	Best35	HM96	Kelch-like 22 (Drosophila)
HM72	Myeloperoxidase	HM97	Best52
HM73	Transmembrane Protein 38a	HM98	Triggering Receptor Expressed on Myeloid Cells-like Protein 2
HM74	Histocompatibility (Minor) HA-1	HM99	TSPY-like 3
HM75	Copine II	HM100	Chemokine Binding Protein 2

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM101	HRAS-like Suppressor 3	HM120	Eosinophil-associated Ribonuclease 1
HM102	Protein Tyrosine Phosphatase, Receptor Type, C	HM121	Sprouty Protein with EVH-1 Domain 1
HM103	Cest14	HM122	EGF-like Module Containing, Mucin-like, Hormone Receptor-like Sequence 4
HM104	Cest15	HM123	Alcohol Dehydrogenase, Iron Containing, 1
HM105	Insulin-like Growth Factor 2 Receptor	HM124	Transmembrane Protein 26
HM106	Napsin A Aspartic Peptidase	HM125	Niban Protein
HM107	Kelch-like 2, Mayven (Drosophila)	HM126	Guanine Nucleotide Binding Protein, Alpha Inhibiting 1
HM108	G Protein-Coupled Receptor 18	HM127	Beta Parvin
HM109	Cyclin-dependent Kinase-like 4	HM128	Cd209 Antigen
HM110	cest17	HM129	Adrenergic Receptor, Beta 3
HM111	Best57	HM130	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 3
HM112	Glycophorin C	HM131	Integrin Alpha 1
HM113	Best60	HM132	G-protein signalling modulator 3 (AGS3-like, C. elegans)
HM114	ATP-binding Cassette, Sub-family D, Member 2	HM133	Best68
HM115	Aest4	HM134	Nicotinamide N-methyltransferase
HM116	RAB3 GTPase Activating Protein Subunit 2	HM135	Extra Cellular Link Domain-containing1
HM117	GTPase, IMAP Family Member 5	HM136	Proteasome (Prosome, Macropain) Subunit, Beta type 9 (Large Multifunctional Peptidase 2)
HM118	DENN/MADD Domain Containing 2D	HM137	Guanylate Nucleotide Binding Protein 2
HM119	Centrosomal Protein 68	HM138	Acyl-CoA Synthetase Long-chain Family Member 6

Gene microarray technology was used to determine the expression profiles in WAT of the four mouse models ($Hmga2^{+/+}$; Lep^{ob}/Lep^{ob}; $Hmga2^{-/-}$; $Hmga2^{-/-}$, Lep^{ob}/Lep^{ob}) for over 36,000 genes and ESTs. After all exclusion criteria were met, a list of 138 genes remained. The list was ordered based on level of expression in the $Hmga2^{-/-}$, Lep^{ob}/Lep^{ob} WAT from greatest to least.

Table 4: Validation of Hmga2 Model by qRT-PCR

Gene #	***Gene***	***Adipose Fraction / Stromal Vascular Fraction***
HM3	Chemokine (C-C motif) Ligand 6	5
HM7	AdipoQ	147
HM8	Plasma Membrane S3-12	37
HM10	Neuronatin	49
HM12	Atrial Natriuretic PeptideC-type Receptor	45
HM13	Resistin	104
HM16	PEP Carboxykinase	103
HM17	Amine Oxidase, Copper-containing	26
HM19	Carboxylesterase	39
HM20	Cell Death-inducing DFFA-like Effector C	64
HM21	Secreted Frizzled-like Protein 5	21
HM25	Leptin	60

Validation of Hmga2 model by qRT-PCR of 12 genes in top 25 from WAT of wild-type mice. Column 3 indicates the expressional difference between adipose fraction and stromal vascular fraction. Example: AdipoQ showed 147-fold greater expression in adipose fraction.

2.3.2 Gene Identities

The gene identities were updated to ensure that the latest data was being used in the model; in all, 16 new genes were identified as adipocyte-specific (Table 5). Three new genes were identified in the Top 25. Previously designated as an EST, HM4 (Est3) was identified as 1-acylglycerol-3-phosphate O-acyltransferase 9 (AGPAT9), an acyltransferase involved in the synthesis of triacylglycerol. Apolipoprotein L6, one member of the apolipoprotein L family of genes was also identified as HM5 (Est5). In addition, HM11 was found to be Activin A receptor, type IC, a ser/thr protein kinase. HM35 (Best12) was also identified as Microtubule Associated Monoxygenase, Calponin and LIM; and HM43 was determined to be Early B-cell factor 1, a transcriptional activator. The 11 remaining newly identified genes were not in the top 50 genes expressed in the double homozygous knockout.

As listed in table 4, both HM68 and HM69 were identified as Proteasome (Prosome, Macropain) Subunit, Beta Type 5 and Transmembrane protein 97 respectively. In addition, HM78, HM89, HM92, HM94, HM97, HM110, HM111, HM113, and HM133 were all ESTs which were subsequently identified as genes.

Table 5: Updated Gene Identities

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM1	Transmembrane Protein 45b	HM26	Phospholipase D Family, Member 4
HM2	Sorbin and SH3 Domain Containing-1	HM27	S100 calcium binding protein A9
HM3	Chemokine (C-C motif) ligand 6	HM28	Krupel-like Factor 15
HM4	1-acylglycerol-3-phosphate O-acyltransferase 9	HM29	Ribonucleoprotein, PTB-binding 2
HM5	Apolipoprotein L6	HM30	Coatomer Protein Complex, Subunit Gamma 2, Antisense 2
HM6	Carbonic Anhydrase III	HM31	Leucine Rich Alpha 2 Glycoprotein
HM7	AdipoQ	HM32	Galectin-12
HM8	Plasma Membrane S3-12	HM33	TBC1 Domain Family, Member 10C
HM9	Fatty Acid Desaturase 3	HM34	Mesoderm Specific Transcript
HM10	Neuronatin	HM35	Microtubule Associated Monoxygenase, Calponin and LIM
HM11	Activin A Receptor, Type IC	HM36	Rac/Cdc42 Guanine Nucleotide Exchange Factor (GEF) 6
HM12	Atrial natriuretic peptide C-type Receptor	HM37	CD1D Antigen
HM13	Resistin	HM38	Synuclein, Gamma (Breast Cancer-specific Gene 1)
HM14	Best23	HM39	Olfactomedin-like 1
HM15	Matrix Metallopeptidase 19	HM40	Carboxypeptidase M
HM16	PEP Carboxykinase	HM41	Coiled-Coil Domain Containing 80
HM17	Amine Oxidase, Copper-containing 3	HM42	CD299 Antigen
HM18	cest1	HM43	Early B-Cell Factor 1
HM19	Unknown Carboxylesterase	HM44	Leukotriene C4 Synthase
HM20	Cell Death-inducing DFFA-like Effector C	HM45	Arylacetamide Deacetylase-like 1
HM21	Secreted Frizzled-like Protein 5	HM46	Paternally Expressed 13
HM22	Cytochrome P450, Family 2, Subfamily D, Polypeptide 22	HM47	Liver Glycogen Phosphorylase
HM23	G Protein-coupled Receptor 109A	HM48	CDC42 Effector Protein 2
HM24	Inter-alpha (globulin) Inhibitor H5	HM49	cest6
HM25	Leptin	HM50	UDP-Gal:betaGlcNAc Beta 1,3-galactosyltransferase, Polypeptide 2

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM51	Interferon, Alpha-Inducible Protein 27-like 2A	HM76	Coronin, Actin Binding Protein 1A
HM52	Phosphatase and Actin Regulator 2	HM77	ADAMTS-like 5
HM53	WD Repeat Domain, Phosphoinositide Interacting 1	HM78	RNA Binding Motif Protein 25
HM54	Best40	HM79	Protocadherin 7
HM55	Decay Accelarating Factor for Complement	HM80	Centromere Autoantigen H
HM56	Retinol Binding Protein 4, Plasma	HM81	Acid Phosphatase 2, Lysosomal
HM57	Lactotransferrin	HM82	Docking Protein 3
HM58	Pleckstrin	HM83	Ankyrin Repeat and Sterile Alpha Motif Domain Containing 3
HM59	ATP-binding Cassette, Sub-family D, Member 2	HM84	Sodium Channel, Voltage-gated, Type VII, Alpha
HM60	Phospholipase C, Beta 1	HM85	Resistin-like Alpha
HM61	Solute Carrier Family 1, Member 3	HM86	Thyroid Hormone Responsive SPOT14 (Rat) Homolog
HM62	RAS p21 Protein Activator 2	HM87	Cyclin M3
HM63	ADP-ribosylation-like 4	HM88	Host Cell Factor C1
HM64	Ras Homolog Gene Family, Member Q	HM89	Hypothetical Protein LOC55196
HM65	Eukaryotic Translation Initiation Factor 4E Member 3	HM90	Limb Bud and Heart Development Homolog
HM66	ATP-binding Cassette, Sub-family A, Member 9	HM91	Chemokine (C-C Motif) Ligand 5
HM67	Best28	HM92	5830443L24Rik
HM68	Proteasome (Prosome, Macropain) Subunit, Beta Type 5	HM93	Solute Carrier Family 16 (Monocarboxylic Acid Transporters), Member 12
HM69	Transmembrane Protein 97	HM94	C130050O18Rik
HM70	Rho GTPase Activating Protein 25	HM95	Matrix Metalloproteinase 9
HM71	Best35	HM96	Kelch-like 22 (Drosophila)
HM72	Myeloperoxidase	HM97	Endoplasmic Reticulum-golgi Intermediate Compartment 1
HM73	Transmembrane Protein 38a	HM98	Triggering Receptor Expressed on Myeloid Cells-like Protein 2
HM74	Histocompatibility (Minor) HA-1	HM99	TSPY-like 3
HM75	Copine II	HM100	Chemokine Binding Protein 2

Gene #	*Gene Name*	*Gene #*	*Gene Name*
HM101	HRAS-like Suppressor 3	HM120	Eosinophil-associated Ribonuclease 1
HM102	Protein Tyrosine Phosphatase, Receptor Type, C	HM121	Sprouty Protein with EVH-1 Domain 1
HM103	Cest14	HM122	EGF-like Module Containing, Mucin-like, Hormone Receptor-like Sequence 4
HM104	Cest15	HM123	Alcohol Dehydrogenase, Iron Containing, 1
HM105	Insulin-like Growth Factor 2 Receptor	HM124	Transmembrane Protein 26
HM106	Napsin A Aspartic Peptidase	HM125	Niban Protein
HM107	Kelch-like 2, Mayven (Drosophila)	HM126	Guanine Nucleotide Binding Protein, Alpha Inhibiting 1
HM108	G Protein-Coupled Receptor 18	HM127	Beta Parvin
HM109	Cyclin-dependent Kinase-like 4	HM128	Cd209 Antigen
HM110	V-set and Immunoglobin Domain Containing 8	HM129	Adrenergic Receptor, Beta 3
HM111	Proline Rich 16	HM130	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 3
HM112	Glycophorin C	HM131	Integrin Alpha 1
HM113	Expressed Sequence AI480653	HM132	G-protein signalling modulator 3 (AGS3-like, C. elegans)
HM114	ATP-binding Cassette, Sub-family D, Member 2	HM133	Hypothetical Protein LOC69066
HM115	RIKEN cDNA A430104N18 gene	HM134	Nicotinamide N-methyltransferase
HM116	RAB3 GTPase Activating Protein Subunit 2	HM135	Extra Cellular Link Domain-containing1
HM117	GTPase, IMAP Family Member 5	HM136	Proteasome (Prosome, Macropain) Subunit, Beta type 9 (Large Multifunctional Peptidase 2)
HM118	DENN/MADD Domain Containing 2D	HM137	Guanylate Nucleotide Binding Protein 2
HM119	Centrosomal Protein 68	HM138	Acyl-CoA Synthetase Long-chain Family Member 6

Gene identities were updated as described above. The NCBI database was used to search sequences and obtain the latest sequence data. Sixteen new genes were identified as adipocyte-specific. The list was ordered based on level of expression in the $Hmga^{-/-}$, Lep^{ob}/Lep^{ob} WAT.

2.3.3 Human Homologs & Chromosomal Location

In order to determine gene candidacy as a potential target for anti-obesity treatment, human homologs of the mouse genes had to be identified. In all, 119 of the mouse genes had known human homologs (Table 6). All but 3 of the Top 25 and 4 of the Top 50 mouse genes had human homologs. The identification of homologous genes in humans was a primary requirement for the further classification of genes as possible anti-obesity targets.

Table 6: Mouse & Human Genomic Localizations

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM1	Transmembrane Protein 45b	chr9: 31175760 -- 31176503	chr11: 129190951 -- 129235108
HM2	Sorbin and SH3 Domain Containing-1	chr19: 40345351 -- 40567123	chr10: 97061518 -- 97311161
HM3	Chemokine (C-C motif) ligand 6	chr11: 83401384 -- 83406589	n/a
HM4	1-acylglycerol-3-phosphate O-acyltransferase 9	chr5: 101327329 -- 101327613	chr4: 84676248 -- 84746052
HM5	Apolipoprotein L6	chr15: 76883233 -- 76884362	chr22: 34374370 -- 34394402
HM6	Carbonic Anhydrase III	chr3: 14841124 -- 14841843	chr8: 86537710 -- 86548526
HM7	AdipoQ	chr16: 23061907 -- 23073362	chr3: 188043157 -- 188058946
HM8	Plasma Membrane S3-12	chr17: 55693886 -- 55703096	chr19: 4453192 -- 4468716
HM9	Fatty Acid Desaturase 3	chr19: 10126986 -- 10127157	chr11: 61325947 -- 61415582
HM10	Neuronatin	chr2: 157251570 -- 157253963	chr20: 35583021 -- 35585509
HM11	Activin A Receptor, Type IC	chr2: 58119864 -- 58120248	chr2: 158097150 - 158193645
HM12	Atrial natriuretic peptide C-type Receptor	chr15: 11789107 -- 11789973	chr5: 32747297 -- 32823013
HM13	Resistin	chr8: 3655776 -- 3657388	chr19: 7639972 -- 7641340
HM14	Best23	chr9: 85117153 -- 85117992	n/a
HM15	Matrix Metallopeptidase 19	chr10: 128202918 -- 128203836	chr12: 54515481 -- 54523002
HM16	PEP Carboxykinase	chr2: 172796012 -- 172802210	chr20: 55569543 -- 55574922
HM17	Amine Oxidase, Copper-containing 3	chr11: 101146694 -- 101155516	chr17: 38256727 -- 38263667
HM18	cest1	chr12: 113704525 -- 113704654	n/a
HM19	Unknown Carboxylesterase	chr8: 96145353 -- 96168854	chr16: 65552638 -- 65566552
HM20	Cell Death-inducing DFFA-like Effector C	chr6: 113390410 -- 113400291	chr3: 9883398 -- 9895740
HM21	Secreted Frizzled-like Protein 5	chr19: 42251285 -- 42251938	chr10: 99516369 -- 99521760
HM22	Cytochrome P450, Family 2, Subfamily D, Polypeptide 22	chr15: 82198612 -- 82198803	chr22: 40852445 -- 40856827
HM23	G Protein-coupled Receptor 109A	chr5: 124124193 -- 124126114	chr12: 121751793 -- 121753857
HM24	Inter-alpha (globulin) Inhibitor H5	chr2: 10071424 -- 10174382	chr10: 7641238 -- 7748940

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM25	Leptin	chr6: 29010221 -- 29023876	chr7: 127668567 -- 127684917
HM26	Phospholipase D Family, Member 4	chr12: 113216654 -- 113216797	chr14: 104462232 -- 104470618
HM27	S100 calcium binding protein A9	chr3: 90778560 -- 90781173	chr1: 151596954 -- 151600127
HM28	Krupel-like Factor 15	chr6: 90439740 -- 90440725	chr3: 127544168 -- 127558929
HM29	Ribonucleoprotein, PTB-binding 2	chr4: 100649750 -- 100650300	chr1: 64983366 -- 65071500
HM30	Coatomer Protein Complex, Subunit Gamma 2, Antisense 2	chr6: 30738266 -- 30738876	chr7: 129933318 -- 130004108
HM31	Leucine Rich Alpha 2 Glycoprotein	chr17: 55712930 -- 55715251	chr19: 4479439 -- 4491079
HM32	Galectin-12	chr19: 7663701 -- 7664134	chr11: 63030132 -- 63040815
HM33	TBC1 Domain Family, Member 10C	chr19: 4184357 -- 4184762	chr11: 66927988 -- 66934136
HM34	Mesoderm Specific Transcript	chr6: 30688164 -- 30698476	chr7: 129913282 -- 129933369
HM35	Microtubule Associated Monoxygenase, Calponin and LIM	chr6: 120962963 -- 121047209	chr22: 16650412 -- 16705531
HM36	Rac/Cdc42 Guanine Nucleotide Exchange Factor (GEF) 6	chrX: 53578293 -- 53578877	chrX: 135575372 -- 135691913
HM37	CD1D Antigen	chr3: 87081753 -- 87085368	chr1: 156416361 -- 156421310
HM38	Synuclein, Gamma (Breast Cancer-specific Gene 1)	chr14: 33199366 -- 33203881	chr10: 88708365 -- 88712997
HM39	Olfactomedin-like 1	chr7: 107381769 -- 107382534	chr11: 7463289 -- 7489178
HM40	Carboxypeptidase M	chr10: 117086663 -- 117087647	chr12: 67531222 -- 67643287
HM41	Coiled-Coil Domain Containing 80	chr16: 45015990 -- 45047220	chr3: 113806097 -- 113842667
HM42	CD299 Antigen	chr8: 4102776 -- 4103709	chr19: 7734081 -- 7740491
HM43	Early B-Cell Factor 1	chr11: 44821187 -- 44821592	chr5: 158058006-158459347
HM44	Leukotriene C4 Synthase	chr11: 50079894 -- 50081955	chr5: 179092457 -- 179156119
HM45	Arylacetamide Deacetylase-like 1	chr3: 27434001 -- 27434117	chr3: 173831129 -- 173911702
HM46	Paternally Expressed 13	chr15: 72633032 -- 72633194	n/a
HM47	Liver Glycogen Phosphorylase	chr12: 71109380 -- 71116151	chr14: 50441687 -- 50480984
HM48	CDC42 Effector Protein 2	chr19: 5917543 -- 5918527	chr11: 64838907 -- 64846476
HM49	cest6	n/a	n/a

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM50	UDP-Gal:betaGlcNAc Beta 1,3-galactosyltransferase, Polypeptide 2	chr1: 145408345 -- 145409938	chr1: 191414798 -- 191422407
HM51	Interferon, Alpha-Inducible Protein 27-like 2A	chr12: 103843219 -- 103856950	n/a
HM52	Phosphatase and Actin Regulator 2	chr10: 12903666 -- 12904043	chr6: 143971093 -- 144188885
HM53	WD Repeat Domain, Phosphoinositide Interacting 1	chr11: 109392501 -- 109392878	chr17: 63929017 -- 63965235
HM54	Best40	chr19: 33048411 -- 33048890	n/a
HM55	Decay Accelarating Factor for Complement	chr1: 132266572 -- 132267347	chr1: 205561476 -- 205600934
HM56	Retinol Binding Protein 4, Plasma	chr19: 38181771 -- 38189952	chr10: 95341434 -- 95351491
HM57	Lactotransferrin	chr9: 110864124 -- 110887140	chr3: 46452500 -- 46481657
HM58	Pleckstrin	chr11: 16873745 -- 16874044	chr2: 68445920 -- 68478088
HM59	ATP-binding Cassette, Sub-family D, Member 2	chr15: 90976593 -- 91019566	chr12: 38232813 -- 38300237
HM60	Phospholipase C, Beta 1	chr2: 135166090 -- 135166699	chr20: 8060908 -- 8813547
HM61	Solute Carrier Family 1, Member 3	chr15: 8581338 -- 8581902	chr5: 36642446 -- 36724193
HM62	RAS p21 Protein Activator 2	chr9: 96351339 -- 96354211	chr3: 142688616 -- 142813887
HM63	ADP-ribosylation-like 4	chr12: 40543703 -- 40544452	chr7: 12693006 -- 12697084
HM64	Ras Homolog Gene Family, Member Q	chr17: 86871627 -- 86903426	chr2: 46623379 -- 46662924
HM65	Eukaryotic Translation Initiation Factor 4E Member 3	chr6: 99592256 -- 99604687	chr3: 71814568 -- 71885465
HM66	ATP-binding Cassette, Sub-family A, Member 9	chr11: 109916838 -- 109917487	chr17: 64482368 -- 64568731
HM67	Best28	chr9: 21349334 -- 21349775	n/a
HM68	Proteasome (Prosome, Macropain) Subunit, Beta Type 5	chr14: 53568325 -- 53572219	chr14: 22564900 -- 22573949
HM69	Transmembrane Protein 97	chr11: 78358012 -- 78366972	chr17: 23670353 -- 23679378
HM70	Rho GTPase Activating Protein 25	chr6: 87428571 -- 87429288	chr2: 68815472 -- 68907469
HM71	Best35	n/a	n/a
HM72	Myeloperoxidase	chr11: 87609775 -- 87620605	chr17: 53702201 -- 53713295
HM73	Transmembrane Protein 38a	chr8: 75516075 -- 75516266	chr19: 16632938 -- 16660830
HM74	Histocompatibility (Minor) HA-1	chr10: 79433890 -- 79434599	chr19: 1018174 -- 1037627

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM75	Copine II	chr8: 97459122 -- 97459658	chr16: 55684007 -- 55739377
HM76	Coronin, Actin Binding Protein 1A	chr7: 126490921 -- 126493181	chr16: 30102427 -- 30107898
HM77	ADAMTS-like 5	chr10: 79743844 -- 79751518	chr19: 1456023 -- 1464019
HM78	RNA Binding Motif Protein 25	chr12: 84992380 -- 85024073	n/a
HM79	Protocadherin 7	chr5: 58421014 -- 58421464	chr4: 30331135 -- 30753851
HM80	Centromere Autoantigen H	chr13: 101859943 -- 101860229	chr5: 68521131 -- 68541940
HM81	Acid Phosphatase 2, Lysosomal	chr2: 91013336 -- 91013911	chr11: 47217429 -- 47226939
HM82	Docking Protein 3	chr13: 55532857 -- 55533513	chr5: 176861539 -- 176869992
HM83	Ankyrin Repeat and Sterile Alpha Motif Domain Containing 3	chr16: 4863152 -- 4863885	chr16: 4686514 -- 4724201
HM84	Sodium Channel, Voltage-gated, Type VII, Alpha	chr2: 66474908 -- 66475221	chr2: 166970135 -- 167051727
HM85	Resistin-like Alpha	chr16: 48762176 -- 48764089	n/a
HM86	Thyroid Hormone Responsive SPOT14 (Rat) Homolog	chr7: 97288127 -- 97292768	chr11: 77452555 -- 77457045
HM87	Cyclin M3	chr1: 36472164 -- 36472270	chr2: 96845718 -- 96864848
HM88	Host Cell Factor C1	chr7: 127595050 -- 127595527	chrX: 152866198 -- 152890452
HM89	Hypothetical Protein LOC55196	chr6: 149284770 -- 149285741	chr12: 32003620 -- 32037308
HM90	Limb Bud and Heart Development Homolog	chr17: 72826186 -- 72846946	chr2: 30307899 -- 30336393
HM91	Chemokine (C-C Motif) Ligand 5	chr11: 83341975 -- 83346718	chr17: 31222608 -- 31231490
HM92	5830443L24Rik	chr5: 105254455 -- 105255310	n/a
HM93	Solute Carrier Family 16 (Monocarboxylic Acid Transporters), Member 12	chr19: 34734332 -- 34735188	chr10: 91180340 -- 91285293
HM94	C130050O18Rik	chr5: 139890223 -- 139892046	n/a
HM95	Matrix Metalloproteinase 9	chr2: 164639436 -- 164647040	chr20: 44070954 -- 44078607
HM96	Kelch-like 22 (Drosophila)	chr16: 17706182 -- 17706945	chr22: 19125806 -- 19180122
HM97	Endoplasmic Reticulum-golgi Intermediate Compartment 1	chr17: 26698471 -- 26792295	chr5: 172193947 -- 172312292
HM98	Triggering Receptor Expressed on Myeloid Cells-like Protein 2	chr17: 47777597 -- 47778105	chr6: 41265993 -- 41276902
HM99	TSPY-like 3	chr2: 152913813 -- 152914296	chr20: 30240610 -- 30241824

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM100	Chemokine Binding Protein 2	chr9: 121807582 -- 121820182	chr3: 42825980 -- 42883779
HM101	HRAS-like Suppressor 3	chr19: 7642003 -- 7652827	chr11: 63098825 -- 63138471
HM102	Protein Tyrosine Phosphatase, Receptor Type, C	chr1: 139879826 -- 139991716	chr1: 196874424 -- 196993035
HM103	Cest14	chr6: 117834302 -- 117834613	n/a
HM104	Cest15	chr2: 94212264 -- 94212515	n/a
HM105	Insulin-like Growth Factor 2 Receptor	chr17: 12596840 -- 12597072	chr6: 160310121 -- 160447573
HM106	Napsin A Aspartic Peptidase	chr7: 44440482 -- 44454908	chr19: 55553546 -- 55560743
HM107	Kelch-like 2, Mayven (Drosophila)	chr8: 67632022 -- 67633113	chr4: 166348238 -- 166463749
HM108	G Protein-Coupled Receptor 18	chr14: 121046614 -- 121047024	chr13: 98704972 -- 98711999
HM109	Cyclin-dependent Kinase-like 4	chr17: 80432482 -- 80472160	chr2: 39259192 -- 39310177
HM110	V-set and Immunoglobin Domain Containing 8	chr1: 174400090 -- 174400390	n/a
HM111	Proline Rich 16	chr18: 51428907 -- 51429761	chr5: 119827918 -- 120050864
HM112	Glycophorin C	chr18: 32671334 -- 32672789	chr2: 127130154 -- 127170716
HM113	Expressed Sequence AI480653	chr16: 30875246 -- 30877222	chr3:196270302 -- 196473166
HM114	ATP-binding Cassette, Sub-family D, Member 2	chr15: 90973638 -- 90974180	chr12: 38232813 -- 38300237
HM115	RIKEN cDNA A430104N18 gene	chr11: 87570433 -- 87570769	n/a
HM116	RAB3 GTPase Activating Protein Subunit 2	chr1: 186984957 -- 186985539	chr1: 218388258 -- 218512419
HM117	GTPase, IMAP Family Member 5	chr6: 48682787 -- 48683184	chr7: 150065384 -- 150071669
HM118	DENN/MADD Domain Containing 2D	chr3: 106630570 -- 106631033	chr1: 111531319 -- 111548554
HM119	Centrosomal Protein 68	chr11: 20138389 -- 20139347	chr2: 65136999 -- 65167618
HM120	Eosinophil-associated Ribonuclease 1	chr14: 42982528 -- 42983224	n/a
HM121	Sprouty Protein with EVH-1 Domain 1	chr2: 116871468 -- 116872031	chr15: 36331808 -- 36433533
HM122	EGF-like Module Containing, Mucin-like, Hormone Receptor-like Sequence 4	chr17: 55446206 -- 55446777	chr19: 6908004 -- 6946250
HM123	Alcohol Dehydrogenase, Iron Containing, 1	chr1: 9561886 -- 9562142	chr8: 67507287 -- 67543596
HM124	Transmembrane Protein 26	chr10: 68175729 -- 68175971	chr10: 62836414 -- 62883214

Gene #	*Gene Name*	*Mouse Chr. Pos.*	*Human Chr. Pos.*
HM125	Niban Protein	chr1: 153483568 -- 153484155	chr1: 183026787 -- 183210305
HM126	Guanine Nucleotide Binding Protein, Alpha Inhibiting 1	chr5: 17778984 -- 17803445	chr7: 79602076 -- 79686661
HM127	Beta Parvin	chr15: 84142092 -- 84143443	chr22: 42726506 -- 42896439
HM128	Cd209 Antigen	chr8: 3847972 -- 3849240	n/a
HM129	Adrenergic Receptor, Beta 3	chr8: 28691725 -- 28694537	chr8: 37939673 -- 37943341
HM130	myeloid/lymphoid or mixed-lineage leukemia (trithorax (Drosophila) homolog); translocated to, 3	chr4: 87241380 -- 87241670	chr9: 20331663 -- 20612542
HM131	Integrin Alpha 1	chr13: 116077186 -- 116077602	chr5: 52119531 -- 52285242
HM132	G-protein signalling modulator 3 (AGS3-like, C. elegans)	chr17: 34198174 -- 34199810	chr6: 32266521 -- 32299822
HM133	Hypothetical Protein LOC69066	chr11: 106890897 -- 106891761	chr17: 63417679 -- 63420164
HM134	Nicotinamide N-methyltransferase	chr9: 48343843 -- 48357120	chr11: 113633763 -- 113688448
HM135	Extra Cellular Link Domain-containing1	chr7: 110641780 -- 110642567	chr11: 10535990 -- 10546855
HM136	Proteasome (Prosome, Macropain) Subunit, Beta type 9 (Large Multifunctional Peptidase 2)	chr17: 33792272 -- 33797624	chr6: 32929916 -- 32935606
HM137	Guanylate Nucleotide Binding Protein 2	chr3: 142558034 -- 142575394	chr1: 89290590 -- 89303631
HM138	Acyl-CoA Synthetase Long-chain Family Member 6	chr11: 54207647 -- 54208175	chr5: 131170735 -- 131375678

2.3.4 Subcellular Localization & Function

The function and subcellular localization for each protein was next investigated. The analysis resulted in the characterization of 38 enzymes and 18 membrane receptor proteins (Table 7). Within the Top 25, we found 8 enzymes and 2 membrane receptor proteins; and within the Top 50 were 16 enzymes and 4 membrane receptor proteins. Given the presence of enzymes and membrane receptors, the potential for a "druggable" target is good, with enzymes and membrane receptor proteins constituting 40% of the proteins in both the Top 25 and Top 50.

Enzymes such as AGPAT9 (HM4) and FADS3 (HM9) are particularly interesting due to their known role in fatty acid metabolism. Although targets are not limited to roles in fatty acid metabolism and lipid droplet modulation, and despite the fact that they are both localized to the ER membrane (possibly making them difficult to target), these two enzymes are interesting candidates due to their function alone.

In addition, 16 total secreted proteins were identified, with 4 of them also characterized as enzymes (HM15, HM57, HM77, and HM95). These enzymes are particularly desirable due to their localization and subsequent accessibility as a target. These peptides are also frontline candidates as potential anti-obesity targets, and so require further bioinformatics and subsequent experimental research.

Two particular membrane receptors, HM23 and HM129, show promise from a purely localization and functional aspect. HM23, GPR109a, is a G-protein coupled receptor known to have anti-lipolytic activity upon receptor activation (Richman, 2007). HM129, ADRB3, is a well-studied G-protein coupled receptor which has been targeted in

the past as a possible anti-obesity therapy. Although attempts to find an efficient and desirable agonist for the receptor have come up dry, citing poor side effect profiles, ADRB3 remains a possible candidate given its localization and function in lipolysis. Given their function in lipolysis and their localization on the cell surface, these two receptors may prove to be efficiently "druggable" and so, provide a possible treatment for obesity.

Table 7: Subcellular Localization & Function

Gene #	*Subcellular Localization*	*Function*
HM1	Membrane	Membrane Receptor
HM2	Cytoplasm (cell-cell jcns, cytoskeleton, plasma membrane)	Required for insulin stimulated glucose transport - recruits CBL to insulin receptor
HM3	Secreted	Chemokine activity; inflammatory response; cellular calcium ion homeostasis; heparin binding; binds CCR1, CCR2, CCR3; induces release of gelatinase B
HM4	ER membrane	Enzyme; Catalyzes first step in de novo synthesis of triacylglycerol
HM5	Cytoplasm	Lipid transporter activity; may effect movement of lipids in cytoplasm or binding of lipids to organelles
HM6	Cytoplasm	Enzyme; Cellular ion transport and pH homeostasis; reversible hydration of carbon dioxide
HM7	Secreted	Insulin sensitizing - antiatherosclerotic properties
HM8	Plasma Membrane	PAT family member known for fat storage within cells; coats lipid droplets
HM9	ER membrane (multipass)	Enzyme; Unsaturation of fatty acids through addition of double bonds
HM10	Unknown	NNAT is a member of the proteolipid family of amphipathic polypeptides and is believed to be involved in ion channel transport or channel modulation
HM11	Plasma Membrane (transmembrane)	Enzyme; Ser/Thr kinase forms receptor complex; involved in activation of SMAD through phosphorylation steps
HM12	Membrane (Single-pass)	The ligand natriuretic peptides regulate blood volume, blood pressure, ventricular hypertrophy, pulmonary hypertension, fat metabolism, and long bone growth
HM13	Secreted	Horomone which may suppress Insulin's ability to stimulate glucose uptake into adipocytes
HM14	n/a	n/a
HM15	ECM (Secreted)	Enzyme; Endopeptidase that degrades components of ECM
HM16	Cytoplasm (2 isozymes: mitochondrial and cytoplasmic)	Enzyme; Gluconeogenesis
HM17	Membrane (Single-pass)	Enzyme; Inflammatory response; cell adhesion; amine metabolism
HM18	n/a	n/a
HM19	n/a	n/a
HM20	Cytoplasm	Apoptotic pathway
HM21	Secreted	Wnt signaling pathway
HM22	ER membrane (Peripheral)	Enzyme; Metabolism of drugs and chemicals by oxidation; electron transport
HM23	Membrane (Multipass)	Membrane Receptor; Niacin receptor (Anti-lipolytic); GPCR
HM24	Unknown	Hyaluronan Metabolism; serine-type endopeptidase inhibitor activity

Gene #	*Subcellular Localization*	*Function*
HM25	Secreted	Regulation of food intake and/or energy expenditure
HM26	Membrane (Single-pass)	Enzyme; Hydrolase activity; A phosphatidylcholine + H(2)O = choline + a phosphatidate
HM27	Unknown	Inflammatory respone; cell-cell signaling; regulation of integrin biosynthesis; actin cytoskeleton re-organization; leukocyte chemotaxis
HM28	Nucleus	KLF15 plays an essential role in adipogenesis in 3T3-L1 cells through its regulation of PPAR gamma expression
HM29	Nucleus; Cytoplasms	RNA binding; nucleotide binding; protein binding
HM30	Cytoplasm, Golgi membrane (cytoplasmic face)	ER to Golgi vesicle-mediated transport
HM31	Secreted	Protein binding
HM32	Nucleus	Lactose binding; Galectin-12 is required for adipogenic signaling and adipocyte differentiation; adipocyte apoptosis
HM33	Unknown	GTPase activator activity; Ras pathway
HM34	Membrane (Single-pass)	Enzyme; Proteolysis; amino peptidase activity
HM35	Cytoplasm	Enzyme; Monooxygenase activity; electron transport; aromatic compound metabolism
HM36	Intracellular	Guanine Nucleotide Exchange Factor Activity; GTPase activator
HM37	Membrane (Single-pass)	Membrane Receptor; The function of CD1d is to present lipid based antigens to natural killer (NK) T cells; positive regulation of T cell mediated cytotoxicity
HM38	Cytoplasm; Perinuclear; Centrosome	A novel marker for breast cancer prognosis; may be involved in exocytosis of synaptic vesicles; putatively involved in the pathogenesis of neurodegenerative diseases; overexpressed in breast tumors; neurofilament network integrity
HM39	Secreted	n/a
HM40	Plasma Membrane (lipid anchor, GPI anchor)	Enzyme; Proteolysis; aromatic compound metabolism; removes C-terminal residues (Arg and Lys); anatomical structure morphogenesis
HM41	ECM (Secreted)	Down-regulated in cancer and after osteoblastic differentiation. Up-regulated by dihydrotestosterone (DHT)
HM42	Membrane (Single-pass); Secreted	Membrane Receptor; Pathogen recognition receptor for immune system in liver
HM43	Nucleus	Transcriptional activator
HM44	Membrane (Multipass)	Enzyme; Catalyzes the conjugation of leukotriene A4 with reduced glutathione to form leukotriene C4; Leukotriene C(4) = leukotriene A(4) + glutathione
HM45	Membrane (Single-pass)	Enzyme; Hydrolysis of 2-acetyl MAGEs
HM46	n/a	n/a
HM47	Unknown	Enzyme; Glycogen metabolism
HM48	Intracytoplasmic membrane (peripheral)	Organization of actin cytoskeleton; pseudopodia; regulation of cell shape
HM49	n/a	n/a

Gene #	*Subcellular Localization*	*Function*
HM50	Golgi Membrane (Single-pass)	Enzyme; Protein amino acid glycosylation
HM51	n/a	n/a
HM52	Unknown	Actin binding; protein phosphatase inhibitor activity
HM53	Golgi; endosomes; cytoplasmic vesicles	Autophagy; protein trafficking for M6P receptor recycling
HM54	n/a	n/a
HM55	Cell Membrane	Cell surface protein. The expression of CD55 is down-regulated in hyperlipidemia, which might be influenced by obesity, abdominal distribution of adipose tissue and inflammatory status of hyperlipidemia, but not by blood lipids. The expression of CD55 is related with complement activation
HM56	Secreted	Delivers retinol stores from liver to peripheral tissues; RBP4 is an adipokine that induced insulin resistance in mice
HM57	Secreted	Enzyme; Serine-type endopeptidase activity; humoral immune response; defense response to bacteria
HM58	Intracellular	Major protein kinase C substrate of platelets
HM59	Peroxisomal Membrane (multi-pass)	Enzyme; Transporter; ATPase activity; fatty acid metabolic process
HM60	Nucleus & Cytoplasm	Enzyme; Lipid metabolism; intracellular signal cascade; hydrolase activity
HM61	Membrane (Multipass)	Glutamate and Aspartate transporter (rapidly removes glutamate from synapse after release); symporter (co-transports sodium and glutamate)
HM62	Cytoplasm; perinuclear	Inhibitory regulator of RAS-cAMP pathway; intracellular signal cascade; RAS-GTPase activator protein
HM63	Nucleus	Enzyme; GTPase activity; nucleotide binding; rRNA processing
HM64	Intracellular; cytoplasmic; cell membrane	Enzyme; GTPase activity; small GTPase mediated signal transduction; causes formation of filopodia; cell response regulation
HM65	Cytoplasm	RNA binding; translational initiation; binds G-cap of mRNA
HM66	Membrane (Multipass)	Enzyme; May play role in monocyte differentiation and lipid homeostasis; ATPase activity; transport
HM67	n/a	n/a
HM68	Cytoplasm; nucleus	Enzyme; Chymotrypsin activity; threonine endopeptidase activity; response to oxidative stress; ubiquitin-dependent protein degradation
HM69	Membrane (Multipass)	Membrane Receptor; Regulation of cell growth; molecular function
HM70	Intracellular	GTPase activator activity for Rho-type GTPases
HM71	n/a	n/a
HM72	Lysosome	Enzyme; Response to oxidative stress; defense response; anti-apoptpsis; oxidoreductase activity;oxidant-generating enzyme which promotes microbicidal activity
HM73	Membrane (Multipass)	Membrane Receptor
HM74	Intracellular	Intracellular signaling cascade

Gene #	***Subcellular Localization***	***Function***
HM75	Unknown	Family of ubiquitous Ca^{2+}-dependent, phospholipid-binding proteins; may be involved in cell division and growth
HM76	Cytoplasm; cytoskeleton; nucleus;	Mitosis; cell motility; transport; actin skeleton regulator
HM77	Secreted	Enzyme; DNA binding; metalloendopeptidase activity
HM78	n/a	n/a
HM79	Cell membrane (single-pass)	Homophilic cell adhesion; calcium ion binding
HM80	Nucleus	Chromosome segregation; mitosis; kinetochore assembly
HM81	Lysosomal membrane	Enzyme; Hydrolase; phosphatase; skeletal development, lysosomal organization
HM82	Cytoplasm	Negative regulator of JNK signaling in B cells; may modulate Abl; insulin receptor binding; Ras protein signal transduction
HM83	Unknown	Regulation of transcription
HM84	Membrane (Multipass)	Voltage gated sodium channel complex; ion transport; muscle contraction
HM85	n/a	n/a
HM86	Nucleus	May play role in lipogenesis; lipid metabolic process; regulation of transcription from RNA polymerase II promoter; mRNA is rapidly upregulated by lipogenic stimuli
HM87	Cell Membrane (Multi-pass)	Probable metal transporter; protein binding
HM88	Nucleus & Cytoplasm	Cell cycle regulation; transcription from RNA polymerase II promoter; reactivation of latent virus; regulation of protein complex assembly; regulation of transcription
HM89	n/a	n/a
HM90	Nucleus	Regulation of transcription from RNA polymerase II promoter
HM91	Secreted	Chemotaxis; cellular calcium ion homeostasis; exocytosis; cell motility; inflammatory response; chemoattractant
HM92	n/a	n/a
HM93	Cell Membrane (Multi-pass)	Transporter activity; symporter; transports monocarboxylates
HM94	n/a	n/a
HM95	Secreted	Enzyme; Peptidoglycan metabolic process; proteolysis; skeletal development; ECM organization; macrophage differentiation; metalloendopeptidase activity; positive regulation of apoptosis
HM96	Unknown	Protein binding
HM97	ER membrane (multipass); ER-Golgi intermediate compartment membrane (multi-pass)	Possible role in transport between ER & Golgi
HM98	Cell membrane (single-pass)	Membrane Receptor; Cell surface receptor which may play role in adaptive and innate immune response
HM99	Nucleus	Nucleosome assembly

Gene #	*Subcellular Localization*	*Function*
HM100	Plasma Membrane (multi-pass)	Membrane Receptor; GPCR; immune response; chemotaxis; multicellular organismal development; rhodopsin-like receptor activity; CC & CXC receptor activity
HM101	Membrane	Negative regulation of progression through cell cycle; tumor suppressor may be involved in interferon-dependent cell death
HM102	Plasma Membrane (single-pass)	Enzyme; Negative regulation of T-cell mediated cytotoxicity; negative regulation of cytokine & chemokine signaling pathway; protein tyrosine phosphatase activity; cell surface receptor linked signal transduction; hydrolase activity
HM103	n/a	n/a
HM104	n/a	n/a
HM105	Lysosomal membrane; endosome; plasma membrane; nuclear envelope lumen	Membrane Receptor; Receptor-mediated endocytosis; transport; insulin-like growth factor receptor activity; binds IGF2 and internalizes it for degradation in lysosome; binding promotes glucose transport
HM106	Unknown	Enzyme; Proteolysis; pepsin A activity; peptidase activity
HM107	Actin cytoskeleton	Intracellular protein transport; actin binding; may play role in organizing actin cytoskeleton
HM108	Cell Membrane (Multi-pass)	Membrane Receptor; GPCR; Receptor for N-arachidonyl glycine; may contribute to regulation of the immune system
HM109	Cytoplasm	Enzyme; Protein serine/threonine kinase activity; ATP binding
HM110	Unknown	Receptor activity
HM111	Unknown	n/a
HM112	Plasma Membrane (single-pass)	Membrane Receptor; Stability of red blood cells; organ morphogenesis; protein amino acid N-linked & O-linked glycosylation; possible role in immune response
HM113	Membrane (Single-pass)	n/a
HM114	Peroxisomal Membrane (multi-pass)	Enzyme; Transporter; ATPase activity; fatty acid metabolic process
HM115	n/a	n/a
HM116	Cytoplasm	Regulation of GTPase activity; intracellular protein transport; GTPase activator activity; heterodimerization activity; involved in regulated exocytosis of neurotransmitters and horomones
HM117	Outer mitochondrial membrane	Enzyme; Required for mitochondrial integrity & T-cell survival; GTP binding
HM118	Unknown	n/a
HM119	Centrosome	n/a
HM120	n/a	n/a
HM121	Membrane (peripheral)	Membrane Receptor; Stem cell factor receptor binding; inactivation of MAPK activity; multicellular organismal development
HM122	Cell Membrane (Multi-pass)	Membrane Receptor; Neuropeptide signaling pathway; GPCR activity; EGF receptor signaling
HM123	Unknown	Enzyme; Oxidoreducatase activity; methanol dehydrogenase
HM124	Membrane (Multipass)	Membrane Receptor

Gene #	***Subcellular Localization***	***Function***
HM125	Cellular	Molecular function
HM126	Plasma membrane (heterotrimeric G-protein complex)	Enzyme; GPCR signaling pathway; GTPase activity; hormonal regulation of adenylyl cyclase
HM127	Cell junction, focal adhesion (cytoplasmic side)	Cell adhesion; actin binding; parvins are a family of proteins involved in linking integrins & associated proteins with intracellular pathways that regulate actin cytoskeletal dynamics and cell survival
HM128	Cell membrane (single-pass); secreted	Membrane Receptor; Endocytosis; immune response; cell adhesion; intracellular signaling cascade; receptor activity; sugar binding
HM129	Cell Membrane (Multi-pass)	Membrane Receptor: GPCR; negative regulation of diet induced thermogenesis & body size; carbohydrate metabolic process; regulation of lipolysis
HM130	Nucleus	DNA-dependent regulation of transcription
HM131	Membrane (Single-pass)	Membrane Receptor; Translation; integrin-mediated signaling pathway; cell adhesion; cell-matrix adhesion; neutrophil chemotaxis
HM132	Cytoplasm	Immune response; transcription; patterning of blood vessels; cell fate determination; GTPase activator activity
HM133	Unknown	n/a
HM134	Cytoplasm	Enzyme; Nicotinamide N-methyltransferase activity
HM135	Cell membrane (Single-pass)	Membrane Receptor; Glycosaminoglycan catabolic process; transport; cell motility; cell adhesion; cell-matrix adhesion; hyaluronic acid binding
HM136	Cytoplasm; nucleus	Enzyme; Ubiquitin-dependent protein catabolic process; immune response; antigen processing & presentation; proteolysis; threonine endopeptidase activity
HM137	Cell Membrane (lipid anchor, cytoplasmic side)	Enzyme; Immune response; GTPase activity; induced by interferon gamma during macrophage activation
HM138	Outer mitochondrial membrane; peroxisomal membrane; plasma membrane (single-pass); microsomal membrane	Enzyme; Lipid metabolic process; acyl-CoA metabolic process; long-chain-fatty-acid-CoA ligase activity; catalytic activity; adipocytokine signaling pathway

2.3.5 Knockout Phenotype in Mice

In order to gain insight into possible implications of knocking out the genes *in vivo*, we looked up known obesity-related phenotypes for existing knockout mice. In total, 27 genes were found to have obesity-related phenotypes upon being knocked out in mice; 11 of which were in the Top 25 and 15 of which were in the Top 50 (Table 8). A reduction in weight gain compared to wild-type was seen in 14 of the genes, while an increase in weight gain was seen in 5 of the 27 genes (Figure 2).

Two genes in the Top 25, both having a KO phenotype of increased weight gain, have been well-studied and their roles in obesity and adipogenesis well-established. Both AdipoQ (HM7) and Leptin (HM25) are known adipogenic markers which have been investigated as possible anti-obesity treatments, and were previously used to validate our list of possible anti-obesity targets. Therefore, it was not surprising to discover that the knockout mice showed significant differences in body weight and body fat percent; specifically, increased body weight and increased body fat percent.

Despite the fact that only ~19% of the genes in the list of 138 had known obesity-related knockout phenotypes, these results are significant in that ~55% of the obesity-related phenotypes found were in the Top 50 genes. Furthermore, ~55% of the body-weight-related phenotypes found were also in the Top 50 genes. These findings suggest a possible cluster of obesity-related genes, which may prove to be validated targets, located in the Top 50.

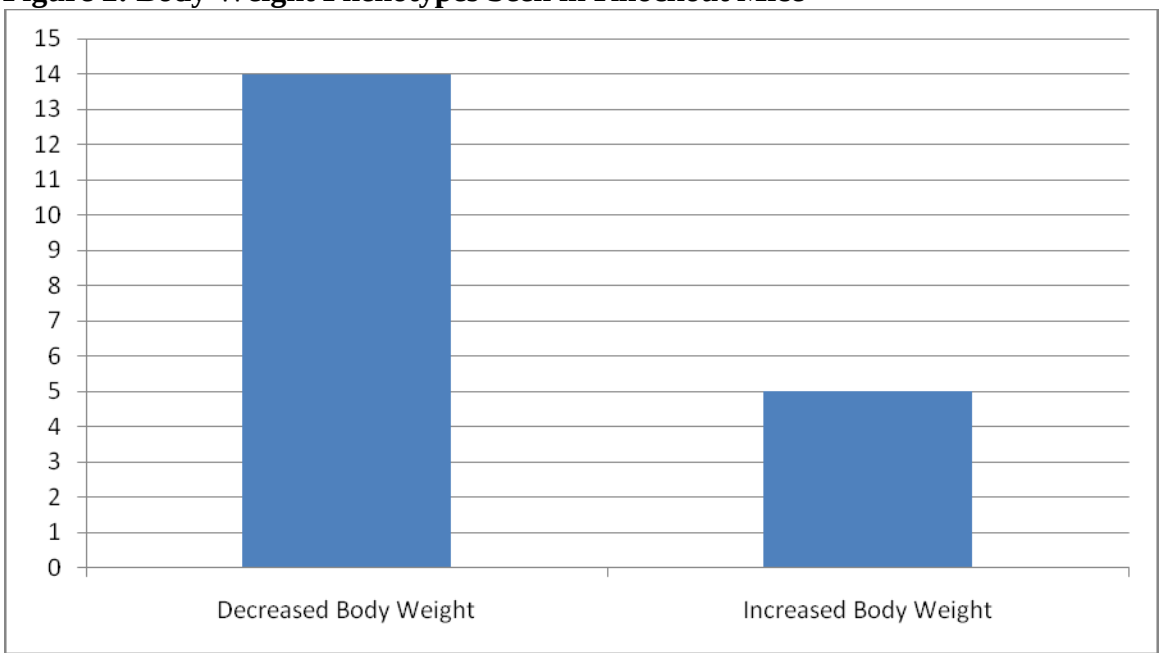
Table 8: Knockout Phenotype

Gene #	*Knockout Phenotype*
HM1	n/a
HM2	KO mice develop adipocyte hypertrophy, but no difference in WAT mass
HM3	n/a
HM4	n/a
HM5	n/a
HM6	n/a
HM7	Increased percent body fat, increased adipose tissue amount, increased body weight
HM8	n/a
HM9	n/a
HM10	n/a
HM11	Reduction in weight gain on high fat diet compared to wild-type
HM12	KOs lack normal body fat deposit; increased body length; decreased body weight
HM13	Decreased triglyceride levels
HM14	n/a
HM15	Mmp19-null mice develop a diet-induced obesity due to adipocyte hypertrophy
HM16	Deacreased fat pads
HM17	KO mice have higher body weight than wild-type; epididymal and inguinal depots are heaveier in KO; increased leptin expression in KO
HM18	n/a
HM19	n/a
HM20	Fat pads smaller in KO; white adipocytes from KO had multiple small lipid droplets vs one large droplet in wild-type; reduced weight gain in KO mice on high fat diet
HM21	n/a
HM22	n/a
HM23	Decreased free fatty acid levels and plasma triglyceride levels
HM24	n/a
HM25	KOs had increased body weight, decreased lean body mass, increased percent body fat
HM26	n/a
HM27	n/a
HM28	n/a
HM29	n/a
HM30	n/a
HM31	n/a
HM32	n/a
HM33	n/a
HM34	Decreased body weight; decreased fetal size
HM35	n/a
HM36	n/a
HM37	50% of 15 week old KO mice are diabetic compared with 0% of wild-type controls
HM38	n/a
HM39	n/a
HM40	n/a
HM41	n/a
HM42	n/a
HM43	Decreased body size compared to wild-type littermates
HM44	n/a
HM45	n/a

Gene #	*Knockout Phenotype*
HM46	n/a
HM47	n/a
HM48	n/a
HM49	n/a
HM50	Decreased body weight in homozygous males
HM51	n/a
HM52	n/a
HM53	n/a
HM54	n/a
HM55	n/a
HM56	n/a
HM57	n/a
HM58	n/a
HM59	Accumulation of very long chain fatty acids in adrenal gland, dorsal root ganglia, spinal cord, sciatic nerve, and serum in KO mice
HM60	Decreased body size, weight, and length in KO
HM61	KO mice gained weight more slowly than wild-type mice
HM62	n/a
HM63	n/a
HM64	n/a
HM65	n/a
HM66	n/a
HM67	n/a
HM68	n/a
HM69	n/a
HM70	n/a
HM71	n/a
HM72	n/a
HM73	n/a
HM74	n/a
HM75	n/a
HM76	n/a
HM77	n/a
HM78	n/a
HM79	n/a
HM80	n/a
HM81	KO has decreased body weight
HM82	n/a
HM83	n/a
HM84	n/a
HM85	n/a
HM86	KO mice have reduction in rate of weight gain due to fat mass on moderate and low fat diets
HM87	n/a
HM88	n/a
HM89	n/a
HM90	n/a

Gene #	***Knockout Phenotype***
HM91	n/a
HM92	n/a
HM93	n/a
HM94	n/a
HM95	KO mice have decreased body size
HM96	n/a
HM97	n/a
HM98	n/a
HM99	n/a
HM100	n/a
HM101	KO showed increased lipolysis; KO prevented obesity induced by high fat and leptin deficiency
HM102	Constitutive phosphatase activity showed some mice were extremely thin and lethargic with undigested food present in their enlarged stomachs
HM103	n/a
HM104	n/a
HM105	n/a
HM106	n/a
HM107	n/a
HM108	n/a
HM109	n/a
HM110	n/a
HM111	n/a
HM112	n/a
HM113	n/a
HM114	Accumulation of very long chain fatty acids in adrenal gland, dorsal root ganglia, spinal cord, sciatic nerve, and serum in KO mice
HM115	n/a
HM116	n/a
HM117	n/a
HM118	n/a
HM119	n/a
HM120	
HM121	Decreased body weight in KO mice
HM122	n/a
HM123	n/a
HM124	n/a
HM125	n/a
HM126	n/a
HM127	n/a
HM128	n/a
HM129	Increased percent body fat and adipose tissue amount in KO mice
HM130	Decreased body size in KO mice
HM131	n/a
HM132	n/a
HM133	n/a
HM134	n/a
HM135	n/a
HM136	n/a
HM137	n/a
HM138	n/a

Figure 2: Body Weight Phenotypes Seen in Knockout Mice



Previously published knockout studies of mice lacking genes in our list were reviewed for obesity-related phenotypes. Knockouts effecting body weight are shown here, with 14 genes in the list resulting in a knockout phenotype of decreased body weight (3 genes in the Top 25 and 6 genes in the Top 50) and 5 genes resulting in a knockout phenotype of increased body weight (4 genes in the Top 25).

2.3.6 Obesity-related QTLs in the Human Genome

The mapping of QTLs is a method used to locate chromosomal regions which segregate with a particular phenotype within a given population. QTLs located within or in close proximity to genes may implicate those genes in the involvement of the manifestation of the phenotype. QTL proximity to a gene can be very informative and, along with other data, may provide enough support to continue investigating the role of a gene in phenotype manifestation, particularly through experimental means. Thus, we set out to find all obesity-related QTLs within 10Mbp of the transcriptional start site for each gene in the list. QTLs within 10Mbp were found for 6 genes in the Top 25, with 4 genes having more than one corresponding QTL (Table 9). Twelve genes within the Top 50 had corresponding obesity-related QTLs, 4 of which only had one associated QTL.

Four genes on the list had an associated obesity-related QTL within 1Mbp, 2 of which were in the Top 25 (HM16 & HM25) and a third in the Top 50 (HM27). The fourth gene, HM52, was found within the QTL bordered by primers D6S403 and D6S1003. The QTL had a strong Lod score (4.2) and was linked to BMI and plasma leptin and fasting state insulin levels in non-diabetic individuals from the SAFADS study (Arya, 2002).

The QTLs listed in the table below provide sufficient evidence for possible linkage of the genes to obesity, suggesting a plausible genetic component in which one or more of these genes may play a strong role in the development of obesity. This data further validates the genes' involvement in obesity, supporting their role as potential targets.

Table 9: Human Obesity-related QTLs

Gene #	*QTL*	*QTL Location*
HM1	**D11S912** - BMI (Lod=3.6)	**D11S4464-D11S912** [123131837-128129307]
HM10	**D20S601** - Respiratory Quotient (Lod=3); **D20S438** - BMI (Lod=3.5); **D20S107** - BMI (p=0.0014)	**D20S477-D20S601** [22485606-34194318] **D20S438** [37485121-37485266] **D20S107** [38315970-38316241]
HM16	**D20S211** - BMI (Lod=3.16); **D20S149** - % Body Fat (NPL=2.57), BMI (Lod=3.2), % Body Fat (Lod=3.2)	**D20S211** [51602082] **D20S149** [54947547]
HM17	**D17S2180** - Abdominal SubQ Fat (Lod=2.2)	**D17S2180-D17S1301** [44028109-70192530]
HM23	**D12S2078** - Abdominal SubQ Fat (Lod=2.9); **D12S2070** - % Fat Intake (p=0.002), BMI (Lod=3.57), Wasit Circumference (Lod=3.05)	**D12S2070** [114567092] **D12S2078** [126527093]
HM25	**D7S2847** - BMI (Lod=2.36); **D7S514** - BMI (p=0.002), Extremity Skinfolds (Lod=3.1)	**D7S2847** [118606748] **D7S514** [126815125]
HM27	**S100A1** - Fat Intake (p=0.001); **D1S394** - Fat Intake (p=0.00081)	**S100A1** [151870751-151871236] **D1S394** [155586041-155586352]
HM30	**D7S514** - BMI (p=0.002), Extremity Skinfolds (Lod=3.1); **D7S504** - BMI (p=0.04, 0.001); **D7S1804** - BMI (Lod=4.9)	**D7S514** [126815125] **D7S504** [127402642] **D7S1804** [131930166]
HM34	**D7S514** - BMI (p=0.002), Extremity Skinfolds (Lod=3.1); **D7S504** - BMI (p=0.04, 0.001); **D7S1804** - BMI (Lod=4.9)	**D7S514** [126815125] **D7S504** [127402642] **D7S1804** [131930166]
HM35	**D22S264** - Abdominal SubQ fat (Lod=2); **D22S1685** (D20S608) - Leptin (Lod=3.44)	**D22S264** [19103232] **D22S1685** [22367551]
HM45	**D3S2427** - BMI (Lod=3.3), Waist Circumference (Lod=2.4), BMI (Lod=3.4)	**D3S2427** [177267495]
HM50	**D1S222** - Fat Intake (p=0.0002)	**D1S222** [186037020]

Gene #	*QTL*	*QTL Location*
HM52	**D6S1009** - BMI (Lod=2.79), Waist Circumference (Lod=3.3); **D6S403-D6S1003** - BMI, Leptin, Insulin (Lod=4.2)	**D6S1009** [137343885] **D6S403-D6S1003** [139754952-144635990]
HM63	**D7S2477-D7S3056** - BMI (Lod=2.53); **D7S2557** - Long-term burden in BMI (Lod=2.9); **D7S3051** - BMI (Lod=2.7)	**D7S2477-D7S3056** [257304-4459565] **D7S2557** [15238861] **D7S3051** [18251053]
HM64	**D2S1356** - BMI (p=0.0004) **D2S1352** - BMI (p=0.0004)	**D2S1356** [43227025] **D2S1352** [50687218]
HM75	**D16S3253** - BMI (Lod=3.21); **D16S2620** - BMI (Lod=2.6)	**D16S3253** [53200000] **D16S2620** [60670959]
HM79	**D4S3350** - BMI (Lod=9.2); **D4S2632** - BMI (Lod=6.1); **D4S2397**- Abdominal SubQ fat (Lod=2.4), BMI (Lod=4.1)	**D4S2397** [26866965] **D4S3350** [33856973] **D4S2632** [35380539]
HM90	**D2S367** - Leptin (Lod=2.7), Adiponectin (Lod=2.7); **D2S1788** - Leptin (Lod=4.9), Fat mass (Lod=2.8), BMI (Lod=3.08), Leptin (Lod=7.5)	**D2S367** [34294652] **D2S1788** [36113810]
HM105	**D6S264** - BMI, Leptin, Insulin (Lod=4.9)	**D6S264** [166629240]
HM106	**D19S418** - BMI $\geq$ 35 (Lod=3.21)	**D19S418** [60237858]
HM108	**D13S779** - BMI (Lod=2.82)	**D13S779** [100301956]
HM109	**D2S1788** - Leptin (Lod=4.9), Fat Mass (Lod=2.8), BMI (Lod=3.08), Leptin (Lod=7.5), BMI (p=0.0006, 0.008); **D2S1356** - BMI (p=0.0004)	**D2S1788** [36113810] **D2S1356** [43227025]
HM110	**D1S194-D1S196** - Waist Circumference (Lod=3.71)	**D1S194-D1S196** [163703895-165870752]
HM112	**D2S347** - BMI (Lod=3.42, 4.44)	**D2S347** [123966318]
HM118	**D1S1631** - Fat intake (p=0.00002)	**D1S1631** [105462175]
HM124	**D10S107** - Obesity (p=0.0005); **D10S1646** - Waist Circumference (Lod=2.5)	**D10S107** [53830795] **D10S1646** [68292844]

Gene #	*QTL*	*QTL Location*
HM129	**D8S1121** - BMI (Lod=3.21)	**D8S1121** [35920931]
HM133	**D17S1290** - Abdominal SubQ Fat (Lod=2.2); **D17S944** - BMI ≥ 35 (Lod=3.16); **D17S1301** - Abdominal SubQ Fat (Lod=2.2)	**D17S1290** [53686448] **D17S944** [58790038] **D17S1301** [70192530]
HM134	**D11S2000** - BMI (Lod=3.35), % Body Fat (Lod-2.8); **D11S2366** - % Body Fat (Lod=2.1, 2.8); **D11S1998** - BMI (Lod=2.7)	**D11S2000** [105063951] **D11S2366** [107022834] **D11S1998** [117202970]
HM135	**D11S419** - BMI (p=0.003)	**D11S419** [15913066]

2.4 Discussion

Traditional anti-obesity target research focuses on the central and peripheral nervous system and endocrine signaling, and gastrointestinal metabolism. However, in an effort to identify novel adipocyte-specific genes and potential anti-obesity targets, we used an *in vivo* model to isolate enriched populations of adipocytes and pre-adipocytes with the intention of discovering novel adipocyte-specific genes expressed in the culprit cell-type of obesity: the adipocyte. After all exclusion criteria were met, we obtained a list of 138 genes, which contained several well-known adipocyte markers in the top 25.

In order to validate the obtained list of genes as obesity-specific for the ultimate purpose of identifying potential anti-obesity targets, the genes had to first be characterized based on six parameters: chromosomal location in mouse genome, chromosomal location in human genome, subcellular localization, function, knockout phenotype in mice, and obesity-related QTLs in human genome. A study published in 2006 by Zheng *et al.* found that 50% of successful drug targets are enzymes, while 23% are receptors. Channels and transporters make up 12% of successful drug targets, and the remaining biochemical classes comprise a total of 15% of successful targets. Furthermore, prediction of protein druggability based sequence homology to known, successful, drug targets revealed a "druggable genome" which includes G-protein coupled receptors, nuclear hormone receptors, ion channels, protein kinases, proteases, metallopeptidases, phosphodiesterases, and several other validated target families (Hajduk, 2005). Given this data, the existing list may be narrowed to one which contains genes with the strongest published evidence for obesity-relatedness; a list which contains potential targets that may be experimentally validated.

Genes in this list should have strong evidence within the given parameters for obesity-relatedness. However, because expressional changes between pre-adipocytes and adipocytes is not the primary parameter for further narrowing the list and ultimately, validation, genes not within the Top 50 may be included when accompanied with strong evidence towards a role in obesity.

2.4.1 Transmembrane Protein 45b, HM1

HM1, transmembrane protein 45b, had the highest expression in the double homozygous ($Hmga2^{-/-}$, Lep^{ob}/Lep^{ob}) by over 2-fold. Furthermore, there was a ~19-fold increase in expression in the double homozygous compared to the $Hmga2^{-/-}$. As shown above, the start site for HM1 is ~1Mbp from a known BMI QTL. In addition, the amino acid sequence of the protein indicates that it may be a potential transmembrane protein, which offers increased accessibility to the peptide. Taken together, the significant increase and relatively high expression of the gene in double homozygous mice, along with the proximity of the gene to a BMI QTL, offers strong evidence for a possible role in obesity and merits further validation through *in vitro* or *in vivo* experimentation.

2.4.2 1-acylglycerol-3-phosphate O-acyltransferase 9, HM4

A transmembrane protein potentially localized to the endoplasmic reticulum, 1-acylglycerol-3-phosphate O-acyltransferase 9 (HM4), also serves as a potential target for obesity treatment. HM4 shows enzymatic transferase activity with a role in triacylglycerol synthesis (Cao, 2006). Known by the acronym AGPAT9, HM4 is highly expressed in omental adipose tissue (Agarwal, 2007) and epididymal fat of mice, and shows ~60-fold increased expression in 3T3-L1 adipocytes versus pre-adipocytes (Cao,

2006). Despite lack of support via knockout and QTL studies, this gene, given its location on the list, enzymatic activity, and role in lipid metabolism, is supported as a potential target for obesity treatment.

2.4.3 Atrial Natriuretic Peptide C-type Receptor, HM12

Atrial natriuretic peptide C-type receptor (NPR3), HM12 is a receptor present on the plasma membrane. One of its ligands, ANP, has been shown *in vivo* and *in vitro* to represent a novel lipolytic pathway in humans (Sengenes, 2000). HM12 has a known function in fat metabolism and ANP clearance. Knockout studies have shown that NPR3-null mice have minimal fat pads and decreased body weight (Jaubert, 1999). Furthermore, humans who are homozygous for an NPR3 promoter variant linked to decreased expression of NPR3 have shown a decreased incidence of overweight and obesity (Dessi-Fulgheri, 2004). In addition, these individuals have reduced abdominal adiposity. Functioning in the clearance of ANP, a known lipolytic stimulant, inhibition of NPR3 may serve to increase lipolysis, as suggested by the knockout studies. NPR3-null mice have also been shown to have increased concentrations on ANP in plasma (Matsukawa, 1999). Given this, and the documented activity assays for NPR3, HM12 represents a strong candidate for further evaluation (Anand-Srivastava, 2000).

2.4.4 Matrix Metallopeptidase 19, HM15

A particular secreted protein, MMP19 (HM15), also shows some promise as a potential target. Matrix metallopeptidase 19 is a secreted enzyme which is an active proponent of the extracellular matrix, degrading various components of the ECM through its endopeptidase activity. Knockout studies show that MMP19-null mice develop diet-

induced obesity through hypertrophy of adipocytes (Pendas, 2004). Given its role in ECM maintenance, MMP19 may act in restricting the excess accumulation of fatty acids in adipocytes. This might explain the phenotype present in MMP19-null mice. Modulation of MMP19 activity through activation of the peptide itself or through an activator may have an obesity-suppressing role, limiting the accumulation of fatty acids. Further analysis is certainly supported for MMP19 as a possible target.

2.4.5 Amine Oxidase, Copper-containing 3, HM17

AOC3 (HM17) is an enzyme (monoamine oxidase) known to be involved in inflammatory response, cell adhesion, and amine metabolism. The enzyme is a transmembrane peptide which is composed of two identical 90kDa dimers (Bono, 1998). The peptide exists in a soluble form, likely cleaved from the extracellular region of the transmembrane peptide, which circulates in serum (Kurkijarvi, 2000). Transgenic mice overexpressing AOC3 on endothelium only appeared "fatter" than normal mice, with reduced caloric intake over 20 weeks. By week 64, transgenic mice had a greater BMI than normal mice. Analysis revealed heavier epididymal and subcutaneous fat pads in transgenic mice along with lower fasting glucose levels (Bour, 2007). This pattern was seen in a separate study when diabetic patients were evaluated for AOC3 levels. Results showed that diabetic patients had increased soluble AOC3 levels in serum, with higher levels in patients on insulin treatment as well as hypoinsulinemic patients (Heniquez, 2003). Furthermore, HM17 expression was seen to increase in a differentiation-dependent manner in 3T3-L1 and 3T3-F442A cells. Semicarbazide-sensitive amine oxidase function, a property of the peptide, was also seen to increase. Studies in mice

also showed increased AOC3 expression in the adipocyte fraction versus the stromal vascular fraction, and in adipocytes versus whole adipose tissue (Bour, 2007).

In addition, AOC3 knockout mice on normal chow gained more weight and had a higher percent body mass than wild-type mice (Bour, 2009). Knockout mice showed heavier epididymal and inguinal fat pads than wild-type mice, in addition to increased leptin expression (Stolen, 2004). QTL data shows the presence of and abdominal subcutaneous fat QTL 5.8Mbp away from the gene starts site. Taken together, these data suggest a role for AOC3 in glucose clearance and metabolism, and insulin regulation and/or sensitivity. Furthermore, a role in adipose regulation is suggested. AOC3 may function in energy storage and regulation, raising or lowering the tendency to store energy as fat, as opposed to another form, such as carbohydrates. Since both knockout and overexpression result in weight gain in mice, there is no direct correlation between AOC3 activity and weight gain. Although it is not clear how this gene functions in obesity, it is apparent that there is a correlation and further research should be done.

2.4.6 Cell Death-inducing DFFA-like Effector C, HM20

CIDEC (HM20) is a cytoplasmic peptide with possible enzymatic activity. Found to be highly expressed in murine WAT and BAT, as well as 3T3-L1 adipocytes, CIDEC shows much potential for further study (Puri, 2007). The peptide contains a CIDE-N domain, or caspase-activated nuclease domain, which may confer enzymatic activity. Studies have shown that 293T and 3T3-L1 cells transfected with CIDEC took on the characteristics of a cell undergoing apoptosis (Liang, 2003 & Keller, 2008). With three known isoforms, the same study showed that both isoforms 1 and 2 induce apoptosis.

Studies have also shown the localization of CIDEC to the lipid droplets in the cytosol of 3T3-L1 cells (Puri, 2007). Furthermore, transfection of 3T3-L1 pre-adipocytes with CIDEC resulted in both increased total neutral lipid and increased lipid droplet size. Subsequent siRNA silencing resulted in increased basal glycerol release (Puri, 2007). This data suggests a possible role for CIDEC in lipid droplet formation along with the peptide's known role in apoptosis.

Knockout studies have shown reduced weight gain and a decrease in overall body weight of CIDEC knockout mice on high fat diet and standard fat diet, respectively (Nishino, 2008). In addition, knockout mice had reduced plasma levels of glucose in fed state on both diets, as well as fasted state on a high fat diet. Knockout mice also had reduced amounts of epididymal and subcutaneous WAT with small multilocular lipid droplets. This data proves consistent with the above findings.

Additional studies have shown that depletion of CIDEC by RNAi in HW adipocytes results in the formation of numerous, small lipid droplets (Nishino, 2008). Three days post RNAi treatment, intracellular TAG content was shown to be significantly reduced with an accompanying increase in lipolytic activity. This increase in lipolysis was also seen in isolated white adipocytes from knockout mice. Although there is no known colorimetric or enzymatic assay for CIDEC, a role for the peptide in adipogenesis is strongly suggested. Collectively, these data provide sufficient evidence for further experimental study.

2.4.7 Mesoderm Specific Transcript, HM34

HM34 (PEG1) presents as a strong candidate for further experimental evaluation. A proteolytic enzyme, PEG1 is a transmembrane protein belonging to the AB hydrolase superfamily. The PEG1 start site has been localized to within 3.1Mbp, 2.5Mbp, and 2.0Mbp of a BMI and extremity skinfolds QTL and two BMI QTLs, respectively. All three QTLs have strong LOD scores and p values.

Knockout studies revealed that mice lacking PEG1 had decreased body weight and decreased fetal size compared to wild-type littermates. Furthermore, an increase in the expression of PEG1 was seen in the adipose tissue of ob/ob mice and diet-induced obese mice (Kamei, 2007 and Takahashi, 2005). The increased level of expression was seen in the adipocyte fraction only and correlated to the increased weight of the WAT (Takahashi, 2005). Transgenic mice overexpressing PEG1 displayed increased adipocyte size and increased adipogenic gene expression. The same study found increased PEG1 expression in db/db mice as well. Concurrent *in vitro* studies showed enhanced differentiation and lipid accumulation in PEG1 transfected 3T3-L1 cells. Collectively, this data provides strong support for the further study of this gene. Genetic QTL and knockout studies show a consistency in results which correlate to results seen in the *in vitro* studies. Compartmental localization of the peptide would be helpful; however, it is not immediately necessary to further analyze PEG1's status as a potential anti-obesity target.

2.4.8 ATP-binding Cassette, Sub-family D, Member 2, HM59 and HM114

The peroxisomal membrane enzyme ABCD2 (HM59 and HM114) is another gene with an abundance of evidence prompting further investigation. An ATPase with transporter activity, ABCD2 has been localized to the peroxisomal membrane and is known to play a role in fatty acid metabolism (Holzinger, 1999; Holzinger, 1997; and Weinhofer, 2002). Knockout studies showed accumulation of very long chain fatty acids (C20 and C22 lipids) in serum, along with adrenal glands, dorsal root ganglia, the spinal cord, and the sciatic nerve in mice lacking ABCD2 (Liu, 2009). In addition, a decrease in ABCD2 levels was seen in the WAT of fasting mice; while re-feeding the mice a high card/low fat diet resulted in re-induction of ABCD2 in the WAT (Weinhofer, 2005). Furthermore, expressional analysis of various tissues showed ABCD2 to be most prominent in adipose tissue, particularly epididymal, inguinal, and retroperitoneal fat (Liu, 2009). The same study showed upregulation of ABCD2 during adipogenesis of NIH3T3-L1 cells. This data suggests a role for ABCD2 in lipid clearance. Although no direct evidence of linkage between ABCD2 and obesity, and weight gain, is reported, the enzyme's potential role in lipid metabolism and/or clearance supports further investigation. Given its role in fatty acid metabolism, future studies may show that modulation of ABCD2 activity, under certain conditions, may result in anti-obesity effects.

2.4.9 Ras Homolog Gene Family, Member Q, HM64

RHOQ (HM64) is a lipid-anchored enzyme localized to the cytoplasmic face of the cell membrane. With roles in cell response regulation, polarization processes, and

potentially trafficking, RHOQ has no apparent role in adipogenic processes or obesity. However, QTL searches revealed two strong BMI QTLs located within 3.4Mbp and 4.1Mbp of the RHOQ start site. Furthermore, *in vitro* studies revealed upregulation of RHOQ upon induction of differentiation of 3T3-L1 fibroblasts (Imagawa, 1999). Studies in rats on high fat diets showed downregulation of RHOQ in the epididymal fat pads compared to normal diet rats (Jun, 2008). In addition, studies linking mouse TC10-alpha (one isoform of mouse RHOQ) to insulin-stimulated glucose uptake have been published, showing a 35% decrease in uptake in TC10-alpha knocked down 3T3-L1 adipocytes (Chang, 2007). Given the GTPase activity of the enzyme, quantitative biochemical assays may be conducted in high volume, allowing for an efficient search method for activity-modulating agents. There is little published evidence relating RHOQ to adipogenesis or obesity; however, the few published studies do suggest a potential role for RHOQ in obesity and obesity-related illness. These studies, along with the genetic linkage analyses, provide enough support for further experimental analysis.

2.4.10 Thyroid Hormone Responsive SPOT14 (Rat) Homolog, HM86

HM86 (THRSP) is a nuclear peptide belonging to the SPOT14 family (Grillasca, 1997). Expressional studies indicate that THRSP is expressed primarily in lipogenic tissues, such as brown and white adipose tissue, liver hepatocytes, and lactating mammary glands (Moreau, 2009). Previous studies indicate that THRSP plays a role in the signal transduction of hormone-related and nutrient-related induction of lipid metabolism (LaFave, 2006 and Freake, 2003). One study revealed an increased rate of *de novo* lipogenesis in transfected HepaRG cells overexpressing THRSP, along with increased accumulation of free fatty acids (Moreau, 2009). Knockout studies also

indicated a role for THRSP in lipid metabolism, with THRSP-null mice exhibiting marked reductions in the rate of weight gain on moderate fat diets compared to wild-type mice (Anderson, 2009). This reduction in weight gain, although not as dramatic, was seen in null mice on a low fat diet as well. This reduction in weight gain was due to the reduced fat mass, as opposed to lean mass, in null mice. Furthermore, THRSP-null mice were observed to have ingested more food than wild-type mice when adjusted for body weight, indicating an increased metabolic rate in the null mice. Another study in humans showed decreased expression of THRSP in the adipose tissue of obese individuals who fast compared with non-obese individuals (Kirchner, 1999). Although no enzymatic or small molecule binding properties have been discovered for THRSP, the above data suggest a strong role for THRSP in lipid metabolism with effects seen on weight gain and lipid accumulation in cells. The data also hints at the possibility of expressional changes and activity modulation being the result of the lipid-rich or lipid-poor state; however, the study done by Moreau *et al.* indicates that THRSP expressional changes alone are sufficient to induce lipogenic-related metabolic changes in hepatocytes. Collectively, these findings all serve to support the further investigation of HM86 as a possible anti-obesity target.

2.4.11 Future Analysis

With the list of 138 genes narrowed to only the most promising candidate genes, experimental analysis to evaluate each gene's potential as a target may begin. Initial experiments should be comprised of knockout studies in mice and *in vitro* expressional modulation studies, with results being compared to those already published. The role of each peptide in obesity (target validation) must be ascertained prior to discovery of

activity-modulating agents. Preliminary studies have shown decreased weight gain in mice on an AOC3 inhibitor when compared to mice not ingesting the inhibitor. Although these studies are preliminary, and are of no statistical significance yet, they are encouraging in that the method used to identify the gene is proving to be validated, as the results are similar to those already published.

Once clarification of the role AOC3 plays in obesity is completed, subsequent studies may then involve the development of assays to monitor peptide activity and for the immediate purpose of identifying small molecule antagonists or agonists. Upon discovery of one or more small molecule activity modulators, *in vivo* and *in vitro* studies may be performed for the purpose of evaluating the efficacy of the modulators and the competence of the individual peptide as an anti-obesity target. The critical step, however, is the confident validation of the target as having a role in obesity, and identifying the mechanism. Once validated, the search for, and utilization of, activity-modulating agents may begin, and trials for an anti-obesity therapy may ensue.

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