World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v18.i11.1141

World J Gastroenterol 2012 March 21; 18(11): 1141-1153
ISSN 1007-9327 (print) ISSN 2219-2840 (online)

EDITORIAL

Genetically modified mouse models for the study of nonalcoholic fatty liver disease

Perumal Nagarajan, M Jerald Mahesh Kumar, Ramasamy Venkatesan, Subeer S Majundar, Ramesh C Juyal

Perumal Nagarajan, Subeer S Majundar, Ramesh C Juyal, Small Animal Facility, National Institute of Immunology, New Delhi 110067, India
M Jerald Mahesh Kumar, Animal House, Centre for Cellular and Molecular Biology, Hyderabad 500007, India
Ramasamy Venkatesan, Animal House, JNU, New Delhi 110067, India
Author contributions: Nagarajan P drafted and wrote the article; Mahesh Kumar MJ and Venkatesan R collected literature and references; Nagarajan P, Majumdar SS and Juyal RC revised the manuscript critically.
Correspondence to: Dr. Perumal Nagarajan, Small Animal Facility, National Institute of Immunology, New Delhi 110067, India. naga73@yahoo.com
Telephone: +91-11-26703709 Fax: +91-11-26742125
Received: July 6, 2011 Revised: September 19, 2011
Accepted: October 28, 2011
Published online: March 21, 2012

Abstract

Nonalcoholic fatty liver disease (NAFLD) is associated with obesity, insulin resistance, and type 2 diabetes. NAFLD represents a large spectrum of diseases ranging from (1) fatty liver (hepatic steatosis); (2) steatosis with inflammation and necrosis; to (3) cirrhosis. The animal models to study NAFLD/nonalcoholic steatohepatitis (NASH) are extremely useful, as there are still many events to be elucidated in the pathology of NASH. The study of the established animal models has provided many clues in the pathogenesis of steatosis and steatohepatitis, but these remain incompletely understood. The different mouse models can be classified in two large groups. The first one includes genetically modified (transgenic or knockout) mice that spontaneously develop liver disease, and the second one includes mice that acquire the disease after dietary or pharmacological manipulation. Although the molecular mechanism leading to the development of hepatic steatosis in the pathogenesis of NAFLD is complex, genetically modified animal models may be a key for the treatment of NAFLD. Ideal animal models for NASH should closely resemble the pathological characteristics observed in humans. To date, no single animal model has encompassed the full spectrum of human disease progression, but they can imitate particular characteristics of human disease. Therefore, it is important that the researchers choose the appropriate animal model. This review discusses various genetically modified animal models developed and used in research on NAFLD.

Key words: Nonalcoholic fatty liver disease; Steatosis; Steatohepatitis; Knockout; Animal models

Peer reviewer: Po-Shiuan Hsieh, MD, PhD, Department of Physiology and Biophysics, National Defense Medical Center, Taipei 114, Taiwan, China

Nagarajan P, Mahesh Kumar MJ, Venkatesan R, Majundar SS, Juyal RC. Genetically modified mouse models for the study of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; 18(11): 1141-1153 Available from: URL: http://www.wjgnet.com/1007-9327/full/v18/i11/1141.htm DOI: http://dx.doi.org/10.3748/wjg.v18.i11.1141

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) represents a histological spectrum of liver disease associated with obesity, diabetes and insulin resistance that extends from isolated steatosis to steatohepatitis and cirrhosis. Besides being a potential cause of progressive liver disease, steatosis has been shown to be an important cofactor in the pathogenesis of many other liver diseases. Mouse models have been developed and the different mouse models can be classified in two major groups. The first one includes genetically modified (transgenic or knockout) mice that spontaneously develop liver disease, and the second one

includes mice that acquire the disease after dietary or pharmacological manipulation. NAFLD and nonalcoholic steatohepatitis (NASH) are increasing due to the prevalence of the metabolic syndrome linked to visceral adiposity, insulin resistance, dyslipidemia and type two diabetes. In this context, research has been undertaken using animals models for human steatosis and NAFLD to NASH disease progression. Most of the animal models develop a fatty liver and many develop aspects of steatohepatitis. However, spontaneous development of fibrosis is very rare. Because it is highly unlikely that NAFLD in the human population is monogenic, study of animals with deletion or over-expression of a single gene may not mimic etiology of the human disease at the molecular level. Likewise, choice of experimental diet may not mimic the human diets associated with development of NAFLD in man. Although rodent models of hepatic steatosis and/or insulin resistance do not always perfectly reproduce the human pathology of NAFLD, the use of transgenic, knockout, and knockdown mouse models have helped over the past years to better our understanding of the molecular determinants of NAFLD. This literature review describes different genetically modified mouse models that exhibit histological evidence of hepatic steatosis or, more variably, steatohepatitis.

GENETIC MODELS FOR NAFLD

ob/ob mice

The *ob/ob* mice carry a spontaneous mutation in the leptin gene (leptin-deficient). These mice are hyperphagic, inactive, extremely obese and are severely diabetic, with marked hyperinsulinemia and hyperglycemia. *ob/ob* mice develop NASH spontaneously[1], but unlike human NAFLD, *ob/ob* mice do not spontaneously progress from steatosis to steatohepatitis. *ob/ob* mice require a 'second hit' to be administered in order to trigger progression to steatohepatitis. This may be provided by exposure to small doses of lipopolysaccharide (LPS) endotoxin, ethanol exposure or hepatic ischemia-reperfusion challenge which all provoke a severe steatohepatitis and frequently acute mortality[2-5]. *ob/ob* mice require other stimuli such as a methionine choline deficient (MCD) diet or a high fat diet to trigger progression to steatohepatitis. The effects of leptin deficiency on several aspects of physiology increase the complexity of studies while using this strain[6]. Similarly, the limited fibrotic capacity of a leptin-deficient model means that it is best suited to studies investigating the mechanisms behind the development of steatosis and the transition to steatohepatitis. Recent work demonstrates that the apparent flaws in this model can be turned to advantage, providing new insights into stellate cell function and the progression to fibrosis.

db/db mice

The *db/db* mice have a natural mutation in the leptin receptor (*Ob-Rb*) gene[7]. These mice are obese with insulin resistance, and are able to develop macrovesicular hepatic steatosis. These mice readily develop symptoms of NASH upon induction with a second hit, such as feeding with an MCD diet[8]. These mice have normal or elevated levels of leptin but are resistant to its effects. Studies have shown that the *db* gene encodes the leptin receptor (OB-R) which is structurally similar to a class I cytokine receptor[9,10]. There are two isoforms; the short OB-Ra isoform has not been shown to have any signaling activity. In contrast, the OB-Rb isoform has a long intracytoplasmic region that contains signal transduction motifs which activate the JAK/STAT protein kinase signal transduction cascade[11]. *db/db* mice carry a sequence insertion at the 3' end of the mRNA transcript exactly where the OB-Ra and OB-Rb transcripts diverge. This insertion contains a stop codon that leads to the premature termination of the OB-Rb long intracellular signaling domain, loss of function and consequently leptin resistance[12].

Yellow-obese agouti (Ay) mice

KK-A^y mice are a cross-strain of diabetic KK mice[13] and lethal yellow (A^y) mice, which carry mutation of the agouti(a) gene on mouse chromosome 2[14]. KK-A^y mice develop maturity-onset obesity, dyslipidemia, and insulin resistance, in part because of the antagonism of melanocortin receptor-4 by ectopic expression of the agouti protein[14]. Importantly, these mice present hyperleptinemia and leptin resistance without defects in the *Ob*R gene, and the expression of adiponectin is conversely down-regulated[15,16]. The phenotype of KK-A^y mice, including altered adipokine expression, quite resembles metabolic syndrome in humans indicating the potential usefulness of this strain as a model of metabolic syndrome NASH[17,18]. In fact, KK-A^y mice are more susceptible to experimental steatohepatitis induced by MCD diet.

CD36$^{-/-}$ mice

A valuable model for the study of the effects of alteration in fatty acid (FA) utilization on insulin responsiveness is the recently generated CD36-deficient mouse[19,20]. CD36, also known as fatty acid translocase (FAT)[21], is a multispecific, integral membrane glycoprotein[22,23] that has been identified as a facilitator of FA uptake. Its function in binding and transport of FA was documented *in vitro* by affinity labeling with FA derivatives and by cell transfection studies[23,24]. The CD36-deficient mouse exhibits greater than 60% decrease of FA uptake and utilization by heart, skeletal muscle, and adipose tissues and thereby increases FA delivery to liver and exhibits increased plasma free fatty acid (FFA) and triglyceride (TG) levels[20]. The pathogenic role of FAT/CD36 in hepatic steatosis in rodents is well-defined[25].

Phosphoenolpyruvate carboxykinase-sterol regulatory-element binding protein 1a-mice

Sterol regulatory-element binding protein (SREBP) family members have been established as transcription factors regulating the transcription of genes involved in cholesterol and FA synthesis. *In vivo* studies have demonstrated that

SREBP-1 plays a crucial role in the dietary regulation of most hepatic lipogenic genes[26,27]. Physiological changes of SREBP-1 protein in normal mice by dietary manipulation such as placement on high carbohydrate diets, polyunsaturated FA-enriched diets, and fasting-refeeding regimens has been reported[28,29]. SREBP-1a transgenic mice, under the control of liver specific Phosphoenolpyruvate carboxykinase promoter (TgSREBP-1a), show a massively enlarged liver and atrophic peripheral white adipose tissue, and develop steatosis[30].

aP2-NSREBP-1c mice

Leptin has similar effects in lipodystrophic rodents, most notably in aP2-nSREBP-1c transgenic mice. These animals express a truncated, constitutively active form of the SREBP-1c transcription factor under the control of the adipose tissue specific aP2 promoter and develop lipodystrophy with very low plasma leptin levels. These mice are also hyperphagic and have massive fat accumulation in peripheral tissues with hyperglycemia and hyperinsulinemia[31,32]. This mouse model has markedly reduced body fat and develops liver steatosis, profound insulin resistance, and increased levels of triglycerides[31,32].

aP2-diptheria toxin mice

The aP2/DTA mice have low serum leptin levels and are hyperphagic. These mice when fed a control diet are hyperlipidemic, hyperglycemic, and have hyperinsulinemia indicative of insulin-resistant diabetes. These mice are born normally and initially lack any distinguishing phenotypic features, but develop atrophy and necrosis of the adipose tissue at five to six months resulting in the complete absence of subcutaneous or intra-abdominal adipose tissue at eight to nine months of age[33]. This late onset of adipose tissue loss is associated with reduced leptin levels, increased food consumption, hyperlipidemia, hyperglycemia and insulin resistance. Monosodium glutamate-treated aP2/DTA mice develop gross hepatomegaly as a result of severe fatty changes in the liver[33].

A-ZIP/F-1 mice

The A-ZIP/F-1 mice express a dominant negative version of the C/EBPα leucine zipper domain that potently interferes with adipocyte differentiation[34]. The A-ZIP/F-1 mouse (*A-ZIPTg/+*) is a model of severe lipoatrophic diabetes and is insulin resistant, hypoleptinemic, hyperphagic, and shows severe hepatic steatosis. This mouse has essentially no white adipose tissue, reduced brown fat and severe metabolic phenotype with a reduced life span. These mice display massive hepatomegaly causing increased body weight, liver steatosis, severe diabetes (hyperglycemia, hyperinsulinemia, hyperphagic, polydipsia and polyuria), and are hypertensive[35]. They have increased triglycerides and FFA levels, alveolar foam cells and reduced leptin levels. These mice are unable to sustain glucose levels during fasting. The insulin resistance and much of the liver steatosis in the A-ZIP/F-1 mice can be reversed by transgenic over-expression of leptin[36] or by transplanting normal adipose tissue[37]. By contrast, transplantation of adipose tissue from *ob/ob* mice did not reverse the phenotype of the A-ZIP/F-1 mice indicating that leptin deficiency strongly contributes to the metabolic complications in lipodystrophy[38].

Peroxisome proliferator-activated receptor alpha$^{-/-}$ mice

Peroxisome proliferator-activated receptor alpha (PPARα) is expressed in the liver and other metabolically active tissues including striated muscle, kidney and pancreas[39,40]. Many of the genes encoding enzymes involved in the mitochondrial and peroxisomal FA beta-oxidation pathways are regulated by PPARα. In wild-type mice, peroxisome proliferators are compounds that induce lipid catabolism and an associated intracellular increase in peroxisome number and enzymatic activity. PPARα mutated mice exhibit alterations of intracellular lipid processing, particularly in response to peroxisome proliferators. Mice deficient in PPARα exhibit severe hepatic steatosis when subjected to fasting for 24-72 h, indicating that a defect in PPARα-inducible FA oxidation accounts for severe FA overload in liver, causing steatosis, in contrast to the wild-type mice[41,42].

Galactin-3 knockout mice

Galectin-3, a beta-galactoside-binding animal lectin, is a multifunctional protein. Galectin-3 plays a role in the regulation of hepatic stellate cell (HSC) activation *in vitro* and *in vivo,* thereby identifying galectin-3 as a potential therapeutic target in the treatment of liver fibrosis. This model plays a role in investigating liver carcinogenesis based on a natural history of NAFLD[43]. Previous studies have also suggested that galectin-3 may play an important role in inflammatory responses. The livers of *gal3(-/-)* male mice at six months of age displayed mild to severe fatty change. The liver weight per body weight ratio, serum alanine aminotransferase levels, liver triglyceride levels, and liver lipid peroxide in *gal3(-/-)* mice were significantly increased compared with those in *gal3(+/+)* mice. Furthermore, the hepatic protein levels of advanced glycation end-products (AGE), receptor for AGE, and PPARγ were increased in *gal3(-/-)* mice relative to *gal3(+/+)* mice[43,44].

Acetyl CoA oxidase$^{-/-}$ mice

Acyl-coenzyme A oxidase (AOX) is the rate-limiting enzyme in peroxisomal FA β-oxidation for the preferential metabolism of very long-chain FAs. AOX null (*AOX-/-*) mice have defective peroxisomal β-oxidation and exhibit steatohepatitis. Microvesicular fatty change in hepatocytes is evident at 7 d. At 2 mo of age, livers show extensive steatosis and they have clusters of hepatocytes at periportal areas with abundant granular eosinophilic cytoplasm rich in peroxisomes. At 4-5 mo there is increased PPARα, cytochrome P450, Cyp 4a10, and Cyp4a14 expression. By 6 to 7 mo, however, there is a compensatory increase in FA oxidation and reversal of hepatic steatosis resulting from hepatocellular regenera-

tion[45,46]. The *AOX*-/- mice proceed to develop adenomas and carcinomas by 15 mo of age[46,47].

Aromatase (CYP 19)-deficient mice

Aromatase P450 (CYP19) is an enzyme catalysing the conversion of androgens into estrogens[48]. These models present dyslipidemia, central obesity, hypercholesterolemia, hyperinsulinemia, hyperleptinemia, and hypertriglyceridemia[49], and importantly the male mice have hepatic steatosis. Aromatase knockout (ArKO) mice have a similar phenotype to that of estrogen receptor null mice with increased gonadal fat pad weight[50]. Only ArKO males have elevated hepatic triglyceride levels leading to hepatic steatosis partly due to an increase in expression of enzymes involved in *de novo* lipogenesis and transporters involved in FA uptake[51-53].

MTP$^{-/-}$ mice

Mitochondrial β-oxidation of FAs is the major source of energy for skeletal muscle and the heart, and it plays an essential role in intermediary metabolism in the liver and impairment of mitochondrial β-oxidation in pathogenesis of NAFLD. The fetuses of *Mtpa*-/- mice accumulate long chain FA metabolites and have low birth weight compared with the *Mtpa*+/- and *Mtpa*+/+ littermates. *Mtpa*-/- mice suffer neonatal hypoglycemia and sudden death 6-36 h after birth. Analysis of the histopathological changes in the *Mtpa*-/- pups revealed rapid development of hepatic steatosis after birth and, later, significant necrosis and acute degeneration of the cardiac and diaphragmatic myocytes. However, studies by Ibdah *et al*[54] indicated that aged but not young *MTPa*$^{+/-}$ mice developed hepatic steatosis with elevated alanine aminotransferase (ALT), basal hyperinsulinemia, and increased insulin compared with *MTPa*$^{+/+}$ littermates. Significant hepatic steatosis and insulin resistance developed concomitantly in the *MTPa*$^{+/-}$ mice at 9-10 mo of age. The cause resides in heterozygosity for β-oxidation defects that predisposes to NAFLD and insulin resistance in aging mice[55].

Phosphatase and tensin homologue -/- mice

Phosphatase and tensin homologue (PTEN) is a multifunctional phosphatase whose substrate is phosphatidylinositol-3,4,5-triphosphate and which acts as a tumor suppressor gene that downregulates phosphatidyl inositol kinases[56,57]. Hepatocyte-specific PTEN-deficient mice spontaneously develop steatosis, steatohepatitis, and hepatocellular carcinoma[58,59]. By 10 wk of age, these mice have increased concentrations of triglyceride and cholesterol esters, and a histological analysis displays micro- and macrovesicular lipid vacuoles. At 40 wk of age, they have macrovesicular steatosis, mallory bodies, ballooning degeneration, and sinusoidal fibrosis[59-60]. Mice that are homozygous for this allele are viable, fertile, and normal in size and do not display any gross physical or behavioral abnormalities. When crossed to a strain expressing Cre recombinase in liver, this mutant mouse strain may be useful in studies of fatty liver and insulin signaling. Piguet *et al*[61] have investigated the effects of hypoxia in the PTEN-deficient mouse, a mouse model that develops NAFLD. The authors also showed that a short period (7 d) of exposure to hypoxia aggravates the NAFLD phenotype, causing changes in the liver that are in keeping with NASH, with increased lipogenesis and inflammation.

Methionine adenosyl transferase 1A -/- mice

Mice deficient in methionine adenosyl transferase 1A (the enzyme responsible for SAM synthesis in the adult liver) have a decrease in hepatic SAM levels and spontaneously develop steatosis, NASH, and hepatocellular carcinoma (HCC)[62]. By three months of age, these mice have hepatomegaly with macrovesicular steatosis. These mice also have increased mRNA levels of CYP2E1 and UCP2, and levels of glutathione. Also, these mice have changes in the expression of genes involved in cell proliferation of lipid and carbohydrate metabolism[63]. These mice are predisposed to liver injury and have impaired liver regeneration after partial hepatectomy[64].

Adiponectin null mice

Adiponectin is an adipokine abundantly produced from adipocytes[65,66]. Adiponectin is an anti-inflammatory adipocyte-derived plasma protein known to alleviate steatosis and inflammation in NAFLD[65-67]. Two adiponectin receptors (adipoR1 and adipoR2) have been identified and found to be expressed in various tissues[68]. AdipoR1 is abundantly expressed in skeletal muscles, whereas adipoR2 is present predominantly in the liver, suggesting a role of adipoR2 in hepatic adiponectin signaling[68,69]. The physiological roles of adipoR1 and adipoR2 have recently been investigated by several laboratories in *adipoR1/2* knockout mice. Both *adipoR1* and *adipoR2* knockout mice exhibit mild insulin resistance[70]. In *adipoR1/R2* double knockout mice the binding and actions of adiponectin are abolished, resulting in increased tissue triglyceride content, inflammation oxidative stress[70-73] and mice exhibit impaired liver regeneration and increased hepatic steatosis.

Bid null mice

The protein Bid is a participant in the pathway that leads to cell death (apoptosis), mediating the release of cytochrome from mitochondria in response to signals from "death" receptors known as tumor necrosis factor (TNF) receptor 1/Fas on the cell surface. Genetic inactivation of Bid, a key pro-apoptotic molecule that serves as a link between these two cell death pathways, significantly reduced caspase activation, adipocyte apoptosis, prevented adipose tissue macrophage infiltration, and protected against the development of systemic insulin resistance and hepatic steatosis independent of body weight[74,75]. These mice can be used in research based on adipocyte apoptosis which is a key initial event that contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis associated with obesity.

Fas adipocyte-specific (AfasKO) null mice

Fas (CD95), a member of the TNF receptor super family, is a major contributor to apoptosis in many cells. Fas activation may contribute to obesity-induced insulin resistance, since mice lacking Fas in adipocytes were partly protected from developing insulin resistance. In particular, Fas activation led to increased release of pro-inflammatory cytokines, and reduced insulin-stimulated glucose uptake in 3T3-L1 adipocytes[75]. Fas-deficient (Fas-def) mice show increased insulin-stimulated glucose incorporation when compared to wild type (WT) with higher expression levels of Akt[76,77].

Interleukin-6 KO mice

Interleukin-6 (IL-6) is an adipocytokine associated with NALFD and obesity that is secreted in larger amounts by visceral fat compared to subcutaneous fat in obese adults[78]. Increased systemic IL-6 is associated with increased inflammation and fibrosis in NAFLD patients[79]. Expression of IL-6, a major proinflammatory cytokine, is increased in animal models of NAFLD. Hepatic IL-6 production may also play an important role in NASH development, as well as in systemic insulin resistance and diabetes. IL-6 is elevated in the plasma and peripheral blood monocytes of patients with fatty diseases, including alcoholic liver disease and non-alcoholic steatohepatitis, and elevation of IL-6 correlates with the progression and severity of liver disease, suggesting that IL-6 may be involved in the pathogenesis of fatty liver disease[80,81]. Studies using *Il6*-/- mice show these animals display obesity, hepatosteatosis, liver inflammation and insulin resistance when compared with control mice on a standard chow diet[82].

TNF alpha KO mice

TNF-α appears to play a central role in the development of hepatic steatosis. TNF-α, by mechanisms not completely defined, is over expressed in the liver of obese mice and is an important mediator of insulin resistance in both diet-induced and *ob/ob* models of obesity[83,84]. Data from animal and clinical studies indicate that TNF-α mediates not only the early stages of fatty liver disease but also the transition to more advanced stages of liver damage[85,86]. Mice homozygous for the TNF targeted mutation are viable and fertile. Further, male mutant mice at 28 wk old display lower insulin, triglyceride, and leptin levels compared to wild type controls.

NEMO^{LPC-KO} mice

The IκB kinase (IKK) subunit NEMO/IKKγ is essential for activation of the transcription factor nuclear factor kappa B (NF-κB), which regulates cellular responses to inflammation. NEMO-mediated NF-κB activation in hepatocytes has an essential physiological function to prevent the spontaneous development of steatohepatitis and hepatocellular carcinoma. These mice were generated with liver parenchymal cell-specific knockout of these subunits (*NEMO^{LPC-KO}*, *IKK2^{LPC-KO}*) by crossing mice carrying loxP-flanked *Nemo*[87] or *Ikk2*[88] alleles with Alfp-cre transgenic mice that mediate efficient Cre recombination in liver parenchymal cells, including hepatocytes and biliary epithelial cells, but not in endothelial or Kupffer cells[89]. *NEMO^{LPC-KO}* mice were born and reached weaning age at the expected Mendelian frequency[90]. These mice showed efficient ablation of the respective proteins in whole-liver extracts and NF-κB activity in the liver was completely abolished[91,92]. Hepatocytes are LPS sensitive. When fed a high-fat diet, mice had reduced β-oxidation and upregulated PPAR-γ, SReBP1 and FA synthase causing increased *de novo* lipid synthesis and macrovesicular steatosis with increased HCC occurrence[91,92].

Jun N-terminal kinase 1 null mice

Jun N-terminal kinase (JNK) 1 null mice have less hepatic inflammation and fibrosis when fed a choline-deficient, l-amino acid-defined (CDAA) diet due to the absence of JNK1 in immune cells. As JNK is activated by oxidants and cytokines and regulates hepatocellular injury and insulin resistance, this kinase may mediate the development of steatohepatitis. JNK promotes the development of steatohepatitis as MCD diet-fed *JNK* null mice have significantly reduced levels of hepatic triglyceride accumulation, inflammation, lipid peroxidation, liver injury, and apoptosis compared with wild-type and *JNK2* -/- mice[93,94]. Hence JNK1 is responsible for JNK activation that promotes the development of steatohepatitis in the MCD diet model[93]. JNK1 KO produces lean, male JNK1 KO mice which have decreased body weights, fasting blood glucose levels, and fasting blood insulin levels compared to their wild-type controls[94]. This model can be used to study a combination of genetic and dietary challenges that constitute the disease etiology for NASH development and mimic more closely the pathogenesis of human NAFLD/NASH.

Toll-like receptor 9 KO mice

Development of NASH involves the innate immune system and is mediated by Kupffer cells and HSCs. Toll-like receptor 9 (TLR9) is a pattern recognition receptor that recognizes bacteria-derived cytosine phosphate guanine-containing DNA and activates innate immunity. Mice deficient in TLR9 have reduced steatohepatitis and fibrosis[95]. Hence this model can be used to study NAFLD involving innate immunity.

LDLR KO and farnesoid X receptor KO mice

Farnesoid X receptor (FXR) is essential for regulating bile-acid synthesis and transport. Mice with FXR deficiency have severe impairment of bile-acid homeostasis and manifest systemic abnormalities including altered lipid and cholesterol metabolism features known to be associated with the metabolic syndrome and NASH. Kong *et al*[96] studied LDL receptor knockout (*LDLr*-/-) mice fed with a high-fat diet for 5 mo, and checked whether FXR deficiency contributed to NASH development. Both high-fat diet and FXR deficiency increased

serum ALT activity, whereas only FXR deficiency increased bile-acid and ALP levels. FXR deficiency and high-fat feeding increased serum cholesterol and triglycerides. Although high-fat diet led to macrosteatosis and hepatocyte ballooning in livers of mice regardless of genotype, no inflammatory infiltrate was observed in the livers of *LDLr*-/- mice. In contrast, in the livers of *LDLr*-/-/*FXR*-/- mice, foci of inflammatory cells were observed when they were fed with control diet and were greatly increased when fed with the high-fat diet[96,97]. This model can be used to study a combination of genetic and dietary challenges that constitute the disease etiology for NASH development and mimic more closely the pathogenesis of human NAFLD/NASH.

Myd88 KO mice

Chemokines, strongly induced by TLR stimulation, play an important role in the development of metabolic syndrome including NAFLD. TLR4- and MyD88-deficient mice, which are resistant to metabolic syndrome, show reduced chemokine production compared with WT mice[98,99]. MyD88 is a key molecule in the development of metabolic syndrome including NAFLD[98,99]. MyD88, an adaptor protein for all TLRs except for TLR3, is required for the expression of various inflammatory cytokines and chemokines[100]. MyD88-deficient mice are protected from metabolic syndrome as well as atherosclerosis[98,99] and from liver injury induced by bile duct ligation or carbon tetrachloride[101,102]. Miura *et al*[100] demonstrated that MyD88-deficient mice on a CDAA diet show less steatohepatitis with less insulin resistance compared with wild type mice. Inflammatory cytokines and fibrogenic factors are also significantly suppressed in MyD88-deficient mice compared with wild type mice fed a CDAA diet[100].

Fatty liver dystrophy knockout mice

Fatty liver dystrophy (*fld*) is a spontaneous point mutation in *Lpin1* which occurred on C3H/HeJ in 1994. An unstable gait and tremor at 3 wk of age was initially observed in these mice. The pups from these mice have a fatty liver before reaching weaning age. Mice carrying mutations in the *fld* gene have features of human lipodystrophy, a genetically heterogeneous group of disorders characterized by loss of body fat, fatty liver, and hypertriglyceridemia and insulin resistance[103]. Homozygous *fld* mice have an enlarged, fatty liver and hypertriglyceridemia that resolve to normal during the weaning transition. However, decreased overall size, decreased lipid in the fat pads and a peripheral neuropathy persist throughout the lifespan. This peripheral neuropathy manifests as a tremor and an unsteady gait shortly after 10 d of age and worsens with age. As with the original mutation of *fld*, homozygous females will breed and raise their litters but homozygous males do not breed.

Platelet endothelial cell adhesion molecule-1 null mice

Platelet endothelial cell adhesion molecule-1 (PECAM-1) is a 130-kDa transmembrane glycoprotein expressed on blood and vascular cells. Goel *et al*[104] demonstrated that genetic deficiency of PECAM-1 potentiates the development and progression of NASH. After 3 wk on an atherogenic diet, these mice developed mild microvesicular steatosis predominantly in hepatic parenchymal cells in the centrilobular region. At 9 and 18 wk on the atherogenic diet, more severe steatosis with lobular and sinusoidal inflammation developed in the livers, which are consistent with the typical histological features of steatohepatitis[104].

ApolipoproteinB 38.9 mutant mice

Fatty liver is prevalent in apolipoproteinB (apoB)-defective familial hypobetalipoproteinemia (FHBL). Similar to humans, mouse models of FHBL produced by gene targeting (*apoB+/38.9*) manifest low plasma cholesterol and increased hepatic TG even on a chow diet due to impaired hepatic VLDL-TG secretive capacity. These mice will be useful to study the genetic and molecular mechanism of apoB defects and lipid metabolism/liver fat accumulation, the relationship between hepatic steatosis and insulin resistance, and the progression of advanced NAFLD and atherosclerosis[105].

Cystathionine-synthase deficient mice

Cystathionine-synthase (CBS) deficiency causes severe hyperhomocysteinemia, which confers diverse clinical manifestations, notably liver disease. Robert *et al*[106] reported that CBS-deficient mice showed inflammation, fibrosis, and hepatic steatosis. These mice also had pathological resemblance to steatohepatitis and a pattern of perivenous and pericellular hepatic fibrosis around lipid-laden hepatocytes. CBS KO mice develop hepatic steatosis more tardily than inflammation and fibrosis at 8-32 wk old.

In addition to the above KO animals, Postic *et al*[107] has demonstrated a few animal models modulating enzymes in FA synthesis.

Acc2KO mice

Acetyl-CoA carboxylase (ACC) catalyzes the synthesis of malonyl-CoA, the metabolic intermediate between lipogenesis[108] and β-oxidation[109]; this lipogenic enzyme has garnered significant attention over recent years. In mammals, two ACC isoforms exist, each with distinct tissue distribution and physiological roles: ACC1 is highly expressed in liver and adipose tissue, whereas ACC2 is predominantly expressed in heart and skeletal muscle and, to a lesser extent, in liver[110]. It is believed that only ACC1, but not ACC2, is committed to *de novo* lipogenesis in liver. Targeting ACC has beneficial effects on both hepatic steatosis and insulin resistance. ACC1-knockout mice (*Acc1*$^{-/-}$ mice and *ACC2*$^{-/-}$ mice) have been developed to study the effect.

SCD KO mice

SCD1 has recently become a target of interest for the reversal of hepatic steatosis and insulin resistance[111].

Table 1 Potential candidate genes in fatty liver disease

Category of genes	Examples
Genes affecting insulin resistance	*ADIPOQ, AKT2, ENPP1, IRS1, PPARG, HFE, resistin*
Genes affecting hepatic lipid synthesis and uptake	*DGAT2, SLC25A13, ACC, ELOVL6, SCD1, GPAT, SREBP1*
Genes affecting hepatic lipid uptake	*APOC3*
Genes affecting hepatic triglyceride hydrolysis	*PNPLA2, CGI-58, LIPA*
Genes affecting hepatic lipid export	*APOB, MTTP, PEMT*
Genes affecting hepatic oxidative stress	*GCLC, NOS2, SOD2, HFE, UCP2, MAT1A, GST, GSH-Px*
Genes affecting immune regulation	*ADIPOQ, ADIPOR1, ADIPOR2, STAT3, TNFα, IL10, IL6, CTLA-4, IL-4, IL-18*
Genes influencing disease progression and fibrosis	*TGF-β1, 3, PPARα, DDX5, CPT1A, angiotensin Ⅱ*
Genes influencing response to endotoxin	*CD14, TLR4, NOD2*

ADIPOQ: Adiponectin; AKT: Beta serine/threonine-protein kinase; ENPP1: Ectonucleotide pyrophosphatase/phosphodiesterase 1; IRS-1: Insulin receptor substrate 1; PPARG: Peroxisome proliferator-activated receptor gamma; HFE: Hemochromatosis gene; DGAT2: Diacylglycerol acetyltransferase-2; SLC25A13: Solute carrier family 25 Member 13 (citrin); *ACC* gene: Acetyl-CoA carboxylase alpha; ELOVL6: Elongation of very long chain fatty acids; *SCD1* gene: Stearoyl-CoA desaturase gene; GPAT: Glycerol-3-phosphate acyltransferase; SREBP1: Sterol regulatory element-binding transcription factor 1; APOC3: Apolipoprotein C-Ⅲ; PNPLA2: Patatin-like phospholipase domain containing 2; CGI58: Comparative gene identification-58; LIPA: Lipase A; APOB: Apolipoprotein B; MTTP: Microsomal triglyceride transfer protein; PEMT: Phosphatidylethanolamine N-methyltransferase; GCLC: Glutamate-cysteine ligase, catalytic subunit; NOS2: Nitric oxide synthases2; SOD2: Superoxide dismutase-2; UCP2: Uncoupling protein 2; MAT1A: Methionine adenosyltransferase Ⅰ alpha; GST: Glutathione S-transferase; ADH: Alcohol dehydrogenase; ALDH: Aldehyde dehydrogenase; CTGF: Connective tissue growth factor; CTLA-4: Cytotoxic T-cell associated antigen-4; GSH-Px: Glutathione peroxidase; STAT3: Signal transducer and activator of transcription 3; IL: Interleukin; PPAR: Peroxisomal proliferator activated receptor; SCD-1: Stearoyl CoA desaturase-1; TLR: Toll-like receptor; TNFR: TNF-α receptor; DDX5: DEAD box protein 5; CPT1A: Carnitine palmitoyltransferase 1A (liver); NOD2: Nucleotide-binding oligomerization domain containing 2.

SCD1 catalyzes the synthesis of monounsaturated FAs, particularly oleate (C18:1n-9) and palmitoleate (C16:1n-7), which are the major components of membrane phospholipids, TGs, and cholesterol esters. Mice with SCD-1KO (*Scd1*$^{-/-}$ mice) show decreased lipogenic gene expression and increased β-oxidation and are protected from diet-induced obesity and insulin resistance when fed a HC/HF diet[112,113]. Inhibition of SCD1 using an ASO strategy (targeting SCD1 in both liver and adipose tissues) prevents many of the HF/HC-diet metabolic complications, including hepatic steatosis and postprandial hyperglycemia[114,115].

ELOVL6 KO mice

Elovl6$^{-/-}$ mice are protected against the development of hepatic insulin resistance when fed a HF/HC diet, despite the accumulation of palmitate concentrations. Improvement in insulin signaling (as evidenced by the restoration in insulin-mediated Akt phosphorylation) occurred despite hepatic steatosis and marked obesity in *Elovl6*$^{-/-}$ mice[116]. While these results are somewhat surprising given the role of palmitate as a potent inducer of insulin resistance (at least in primary cultures of hepatocytes)[117], they are also interesting since they indicate that the hepatic FA composition, and particularly the conversion of palmitate to stearate, is crucial for insulin sensitivity. It should be noted that the reduced SCD1 expression observed in livers of *Elovl6*$^{-/-}$ mice could have also contributed to the amelioration of insulin resistance in these mice[116].

ChREBP knockdown mice

ChREBP knockdown led to the expected inhibition of L-PK, ACC, FAS and SCD1 as well as GPAT. While a carbohydrate-response element was previously identified in the promoter region of the *GPAT* gene[118], its expression was found to be unaffected in the liver of ChREBP-knockout mice upon refeeding[119]. It is possible that the nutritional regulation of GPAT may be more sensitive to insulin *via* SREBP-1c than to glucose *via* ChREBP. Nevertheless, following ChREBP knockdown, a resultant decrease in lipogenic rates was observed in shChREBP-RNA-treated *ob/ob* mice, leading to a 50% reduction in hepatic and circulating TG concentrations[120]. ChREBP knockdown not only affected the rate of *de novo* lipogenesis but also had consequences for β-oxidation. Therefore, similarly to the liver-specific knockout of SCD1 (LKO mice)[121], the coordinated modulation in FA synthesis and oxidation in liver led to an overall improvement of lipid homeostasis in ChREBP-deficient mice. The decrease in lipogenic rates observed in LKO mice was at least partially attributed to a decrease in ChREBP nuclear protein content[122]. Clearly, ChREBP needs now to be considered as a key determinant of the molecular regulation of the lipogenic pathway.

CLASSIFICATION OF SOME ANIMAL MODELS WITH DISRUPTION OF GENES INVOLVED IN NAFLD

Table 1 presents a number of candidate genes that are involved in the pathogenesis of NAFLD and a few are discussed below.

Genes affecting lipid metabolism

Pemt KO animals: *Pemt-/- mice* have two selectively disrupted alleles of the *Pemt-2* gene at exon 2[123], which encode PEMT, and do not express any PEMT activity in liver. Therefore these mice completely depend on dietary choline intake to meet daily choline requirements. When fed a diet deficient in choline and insufficient in methionine, *Pemt-/-* mice develop decreased PtdCho concentrations in hepatic membranes, leading to severe liver damage and death; a choline supplemented diet prevents this[124] and, if provided early enough, can reverse hepatic damage.

DGAT2 mice: DGAT2, an isoform of the enzyme acylCoA: diacylglycerol acyltransferase, catalyses the final stage of triglyceride synthesis in the liver[125]. Overexpression of DGAT2 in mice led to a 2.4-fold increase in hepatic triglyceride content, but no effect on production of VLDL triglyceride or apoB[126]. In addition, mice on a high-fat diet that overexpress DGAT develop fatty liver but not glucose or insulin intolerance[127], showing that hepatic steatosis can occur independently of insulin resistance. Interestingly, antisense therapy reducing DGAT improves hepatic steatosis, but not insulin sensitivity[128].

Apolipoprotein C-Ⅲ: Apolipoprotein C-Ⅲ (apoC-Ⅲ) is the most abundant C apolipoprotein in human plasma, where it is present as an 8.8-kDa mature protein on chylomicrons, VLDL and HDL. ApoC-Ⅲ is synthesized in the liver and in minor quantities by the intestine[129]. Several lines of evidence have implicated apoC-Ⅲ as contributing to the development of hypertriglyceridemia in the human population. Investigation in *apoC3*-/- mice supports the concept that apoC-Ⅲ is an effective inhibitor of VLDL TG hydrolysis and reveals a potential regulating role for apoC-Ⅲ with respect to the selective uptake of cholesteryl esters[130].

Genes affecting insulin resistance/sensitivity

IRS 1: Studies on mice with targeted disruption of the *Irs* genes lend some support to both situations. *Irs1* knockout (*Irs1*$^{-/-}$) mice show significant embryonic and postnatal growth retardation, suggesting that IRS-1 plays a key role in relaying the growth-stimulating effects of insulin and insulin-like growth factor. IRS-1-deficient mice also have insulin resistance and mild glucose intolerance, but do not develop diabetes[131,132].

Ecto-nucleotide pyrophosphate phosphodiesterase: Ecto-nucleotide pyrophosphate phosphodiesterase (ENPP1) has been shown to negatively modulate insulin receptor and to induce cellular insulin resistance when over-expressed in various cell types. Systemic insulin resistance has also been observed when ENPP1 is over-expressed in multiple tissues of transgenic models and is largely attributed to tissue insulin resistance induced in skeletal muscle and liver. In the presence of a high fat diet, ENPP1 over-expression in adipocytes induces fatty liver, hyperlipidemia and dysglycemia, thus recapitulating key manifestations of the metabolic syndrome[133].

Transcription factor 7-like 2: Transcription factor 7-like 2 (TCF7L2) is a receptor for β-catenin and regulates the expression of a multitude of genes involved in cellular metabolism and growth. Various studies[134-136] have linked TCF7L2 variation with impaired insulin secretion and risk of diabetes, possibly mediated by altered β-cell glucose response. In addition, it regulates adipokine secretion and triglyceride metabolism through effects on PPAR-γ, CCAAT/enhancer-binding protein, and lipoprotein lipase; TCF7L2 SNPs are associated with serum triglyceride concentrations in familial hyperlipidemia[137].

Genes affecting oxidative stress

Glutamate-cysteine ligase: Glutamate-cysteine ligase (GCLC) is the first and rate-limiting enzyme in the synthesis of glutathione, the major antioxidant in the liver. Liver-specific deletion of GCLC in mice rapidly leads to hepatic steatosis and progressive severe parenchymal damage[138].

Nitric oxide synthase: Yoneda *et al*[139], who studied associations of PPARγC1α, also examined the influence of SNPs in the inducible nitric oxide synthase (*NOS2*) gene on their NAFLD cohort. iNOS is expressed as part of the inflammatory response and in the presence of superoxide radicals forms peroxynitrite, which can cause endoplasmic reticulum stress and cell death[140]. iNOS-deficient mice develop NASH with high fat diets[141].

Superoxide dismutase-2: Elevated hepatic reactive oxygen species play an important role in pathogenesis of liver diseases, such as alcohol-induced liver injury, hepatitis C virus infection, and nonalcoholic steatohepatitis. Satoshi *et al*[142] observed significant increases in lipid peroxidation and TG in the liver of *Sod1* KO and double KO mice but not in the liver of *Sod2* KO mice.

Genes affecting immune regulation

Signal transducer and activator of transcription 3: Signal transducer and activator of transcription 3 (STAT3) is an acute-phase transcription factor; after hepatic necrosis it activates pathways associated with liver regeneration and acute inflammation[143]. STAT3 is also implicated in nutrient metabolism and developing metabolic syndrome. Transgenic mice with hepatic deficiency of STAT3 develop insulin resistance and disturbed glucose homeostasis; whereas the constitutive liver specific expression of STAT3 in diabetic mice reduces blood glucose and plasma insulin concentrations and downregulates gluconeogenic gene expression[144].

CONCLUSION

Inbred strains of mice provide convenient tools to study the pathogenesis of NAFLD because they provide the opportunity to control genetic and environmental factors that might influence the natural history of NAFLD. Various genetic alterations or environmental stressors producing a similar phenotype prove that many different immunological, neuronal and hormonal factors are involved in the pathogenesis of NAFLD[145,146]. Transgenic mouse models also represent gene mediation to NAFLD. Therefore, any one of these animal models could be used to clarify how altered cross talk among immune cells, neurons and endocrine cells promote NAFLD. In contrast to human genetic studies, animal studies have found genes that consistently produce disease-like phenotypes, and the underlying genetic basis for the phenotypes in these models have often been

elucidated. Animal studies on NAFLD frequently reveal significant single-locus effects that can be reproduced across species and/or strains. Such "disease genes" in animal models can be found relatively easily using linkage mapping techniques in crossed inbred lines. Similarly, transgenic animals or genetically manipulated animals for NAFLD can reveal significant effects of candidate alleles in well-defined genetic backgrounds. This review has explored some of the advantages and disadvantages of a few genetically modified mouse models of NAFLD that would be useful in understanding the connections between lipid metabolism, host defences, environmental triggers, genetic variability, inflammatory recruitment, and fibrogenesis. These models will also serve as important platforms for assessing therapeutic strategies, which is an essential area of study.

REFERENCES

1 **Brix AE**, Elgavish A, Nagy TR, Gower BA, Rhead WJ, Wood PA. Evaluation of liver fatty acid oxidation in the leptin-deficient obese mouse. *Mol Genet Metab* 2002; **75**: 219-226
2 **Yang SQ**, Lin HZ, Lane MD, Clemens M, Diehl AM. Obesity increases sensitivity to endotoxin liver injury: implications for the pathogenesis of steatohepatitis. *Proc Natl Acad Sci USA* 1997; **94**: 2557-2562
3 **Faggioni R**, Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR, Grunfeld C. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. *Am J Physiol* 1999; **276**: R136-R142
4 **Koteish A**, Diehl AM. Animal models of steatosis. *Semin Liver Dis* 2001; **21**: 89-104
5 **Chavin KD**, Yang S, Lin HZ, Chatham J, Chacko VP, Hoek JB, Walajtys-Rode E, Rashid A, Chen CH, Huang CC, Wu TC, Lane MD, Diehl AM. Obesity induces expression of uncoupling protein-2 in hepatocytes and promotes liver ATP depletion. *J Biol Chem* 1999; **274**: 5692-5700
6 **Diehl AM**. Lessons from animal models of NASH. *Hepatol Res* 2005; **33**: 138-144
7 **Wortham M**, He L, Gyamfi M, Copple BL, Wan YJ. The transition from fatty liver to NASH associates with SAMe depletion in db/db mice fed a methionine choline-deficient diet. *Dig Dis Sci* 2008; **53**: 2761-2774
8 **Hummel KP**, Dickie MM, Coleman DL. Diabetes, a new mutation in the mouse. *Science* 1966; **153**: 1127-1128
9 **Tartaglia LA**, Dembski M, Weng X, Deng N, Culpepper J, Devos R, Richards GJ, Campfield LA, Clark FT, Deeds J, Muir C, Sanker S, Moriarty A, Moore KJ, Smutko JS, Mays GG, Wool EA, Monroe CA, Tepper RI. Identification and expression cloning of a leptin receptor, OB-R. *Cell* 1995; **83**: 1263-1271
10 **Fantuzzi G**, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. *J Leukoc Biol* 2000; **68**: 437-446
11 **Ghilardi N**, Ziegler S, Wiestner A, Stoffel R, Heim MH, Skoda RC. Defective STAT signaling by the leptin receptor in diabetic mice. *Proc Natl Acad Sci USA* 1996; **93**: 6231-6235
12 **Chen H**, Charlat O, Tartaglia LA, Woolf EA, Weng X, Ellis SJ, Lakey ND, Culpepper J, Moore KJ, Breitbart RE, Duyk GM, Tepper RI, Morgenstern JP. Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* 1996; **84**: 491-495
13 **Ikeda H**. KK mouse. *Diabetes Res Clin Pract* 1994; **24** Suppl: S313-S316
14 **Miller MW**, Duhl DM, Vrieling H, Cordes SP, Ollmann MM, Winkes BM, Barsh GS. Cloning of the mouse agouti gene predicts a secreted protein ubiquitously expressed in mice carrying the lethal yellow mutation. *Genes Dev* 1993; **7**: 454-467
15 **Masaki T**, Chiba S, Tatsukawa H, Yasuda T, Noguchi H, Seike M, Yoshimatsu H. Adiponectin protects LPS-induced liver injury through modulation of TNF-alpha in KK-Ay obese mice. *Hepatology* 2004; **40**: 177-184
16 **Okumura K**, Ikejima K, Kon K, Abe W, Yamashina S, Enomoto N, Takei Y, Sato N. Exacerbation of dietary steatohepatitis and fibrosis in obese, diabetic KK-A(y) mice. *Hepatol Res* 2006; **36**: 217-228
17 **Aoyama T**, Ikejima K, Kon K, Okumura K, Arai K, Watanabe S. Pioglitazone promotes survival and prevents hepatic regeneration failure after partial hepatectomy in obese and diabetic KK-A(y) mice. *Hepatology* 2009; **49**: 1636-1644
18 **Fan JG**, Qiao L. Commonly used animal models of non-alcoholic steatohepatitis. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 233-240
19 **Hajri T**, Han XX, Bonen A, Abumrad NA. Defective fatty acid uptake modulates insulin responsiveness and metabolic responses to diet in CD36-null mice. *J Clin Invest* 2002; **109**: 1381-1389
20 **Febbraio M**, Abumrad NA, Hajjar DP, Sharma K, Cheng W, Pearce SF, Silverstein RL. A null mutation in murine CD36 reveals an important role in fatty acid and lipoprotein metabolism. *J Biol Chem* 1999; **274**: 19055-19062
21 **Abumrad NA**, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during preadipocyte differentiation. Homology with human CD36. *J Biol Chem* 1993; **268**: 17665-17668
22 **Tandon NN**, Kralisz U, Jamieson GA. Identification of glycoprotein IV (CD36) as a primary receptor for platelet-collagen adhesion. *J Biol Chem* 1989; **264**: 7576-7583
23 **Greenwalt DE**, Lipsky RH, Ockenhouse CF, Ikeda H, Tandon NN, Jamieson GA. Membrane glycoprotein CD36: a review of its roles in adherence, signal transduction, and transfusion medicine. *Blood* 1992; **80**: 1105-1115
24 **Harmon CM**, Abumrad NA. Binding of sulfosuccinimidyl fatty acids to adipocyte membrane proteins: isolation and amino-terminal sequence of an 88-kD protein implicated in transport of long-chain fatty acids. *J Membr Biol* 1993; **133**: 43-49
25 **Miquilena-Colina ME**, Lima-Cabello E, Sánchez-Campos S, García-Mediavilla MV, Fernández-Bermejo M, Lozano-Rodríguez T, Vargas-Castrillón J, Buqué X, Ochoa B, Aspichueta P, González-Gallego J, García-Monzón C. Hepatic fatty acid translocase CD36 upregulation is associated with insulin resistance, hyperinsulinaemia and increased steatosis in non-alcoholic steatohepatitis and chronic hepatitis C. *Gut* 2011; **60**: 1394-1402
26 **Shimano H**, Yahagi N, Amemiya-Kudo M, Hasty AH, Osuga J, Tamura Y, Shionoiri F, Iizuka Y, Ohashi K, Harada K, Gotoda T, Ishibashi S, Yamada N. Sterol regulatory element-binding protein-1 as a key transcription factor for nutritional induction of lipogenic enzyme genes. *J Biol Chem* 1999; **274**: 35832-35839
27 **Liang G**, Yang J, Horton JD, Hammer RE, Goldstein JL, Brown MS. Diminished hepatic response to fasting/refeeding and liver X receptor agonists in mice with selective deficiency of sterol regulatory element-binding protein-1c. *J Biol Chem* 2002; **277**: 9520-9528
28 **Kim HJ**, Takahashi M, Ezaki O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. A possible mechanism for down-regulation of lipogenic enzyme mRNAs. *J Biol Chem* 1999; **274**: 25892-25898
29 **Worgall TS**, Sturley SL, Seo T, Osborne TF, Deckelbaum RJ.

Polyunsaturated fatty acids decrease expression of promoters with sterol regulatory elements by decreasing levels of mature sterol regulatory element-binding protein. *J Biol Chem* 1998; **273**: 25537-25540
30 **Shimano H**, Horton JD, Hammer RE, Shimomura I, Brown MS, Goldstein JL. Overproduction of cholesterol and fatty acids causes massive liver enlargement in transgenic mice expressing truncated SREBP-1a. *J Clin Invest* 1996; **98**: 1575-1584
31 **Asilmaz E**, Cohen P, Miyazaki M, Dobrzyn P, Ueki K, Fayzikhodjaeva G, Soukas AA, Kahn CR, Ntambi JM, Socci ND, Friedman JM. Site and mechanism of leptin action in a rodent form of congenital lipodystrophy. *J Clin Invest* 2004; **113**: 414-424
32 **Shimomura I**, Hammer RE, Ikemoto S, Brown MS, Goldstein JL. Leptin reverses insulin resistance and diabetes mellitus in mice with congenital lipodystrophy. *Nature* 1999; **401**: 73-76
33 **Ross SR**, Graves RA, Spiegelman BM. Targeted expression of a toxin gene to adipose tissue: transgenic mice resistant to obesity. *Genes Dev* 1993; **7**: 1318-1324
34 **Moitra J**, Mason MM, Olive M, Krylov D, Gavrilova O, Marcus-Samuels B, Feigenbaum L, Lee E, Aoyama T, Eckhaus M, Reitman ML, Vinson C. Life without white fat: a transgenic mouse. *Genes Dev* 1998; **12**: 3168-3181
35 **Takemori K**, Gao YJ, Ding L, Lu C, Su LY, An WS, Vinson C, Lee RM. Elevated blood pressure in transgenic lipoatrophic mice and altered vascular function. *Hypertension* 2007; **49**: 365-372
36 **Ebihara K**, Ogawa Y, Masuzaki H, Shintani M, Miyanaga F, Aizawa-Abe M, Hayashi T, Hosoda K, Inoue G, Yoshimasa Y, Gavrilova O, Reitman ML, Nakao K. Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. *Diabetes* 2001; **50**: 1440-1448
37 **Colombo C**, Cutson JJ, Yamauchi T, Vinson C, Kadowaki T, Gavrilova O, Reitman ML. Transplantation of adipose tissue lacking leptin is unable to reverse the metabolic abnormalities associated with lipoatrophy. *Diabetes* 2002; **51**: 2727-2733
38 **Gavrilova O**, Marcus-Samuels B, Graham D, Kim JK, Shulman GI, Castle AL, Vinson C, Eckhaus M, Reitman ML. Surgical implantation of adipose tissue reverses diabetes in lipoatrophic mice. *J Clin Invest* 2000; **105**: 271-278
39 **Kallwitz ER**, McLachlan A, Cotler SJ. Role of peroxisome proliferators-activated receptors in the pathogenesis and treatment of nonalcoholic fatty liver disease. *World J Gastroenterol* 2008; **14**: 22-28
40 **Mukherjee R**, Jow L, Noonan D, McDonnell DP. Human and rat peroxisome proliferator activated receptors (PPARs) demonstrate similar tissue distribution but different responsiveness to PPAR activators. *J Steroid Biochem Mol Biol* 1994; **51**: 157-166
41 **Auboeuf D**, Rieusset J, Fajas L, Vallier P, Frering V, Riou JP, Staels B, Auwerx J, Laville M, Vidal H. Tissue distribution and quantification of the expression of mRNAs of peroxisome proliferator-activated receptors and liver X receptor-alpha in humans: no alteration in adipose tissue of obese and NIDDM patients. *Diabetes* 1997; **46**: 1319-1327
42 **Reddy JK**. Nonalcoholic steatosis and steatohepatitis. III. Peroxisomal beta-oxidation, PPAR alpha, and steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G1333-G1339
43 **Nakanishi Y**, Tsuneyama K, Nomoto K, Fujimoto M, Salunga TL, Nakajima T, Miwa S, Murai Y, Hayashi S, Kato I, Hiraga K, Hsu DK, Liu FT, Takano Y. Nonalcoholic steatohepatitis and hepatocellular carcinoma in galectin-3 knockout mice. *Hepatol Res* 2008; **38**: 1241-1251
44 **Nomoto K**, Tsuneyama K, Abdel Aziz HO, Takahashi H, Murai Y, Cui ZG, Fujimoto M, Kato I, Hiraga K, Hsu DK, Liu FT, Takano Y. Disrupted galectin-3 causes non-alcoholic fatty liver disease in male mice. *J Pathol* 2006; **210**: 469-477
45 **London RM**, George J. Pathogenesis of NASH: animal models. *Clin Liver Dis* 2007; **11**: 55-74, viii
46 **Cook WS**, Jain S, Jia Y, Cao WQ, Yeldandi AV, Reddy JK, Rao MS. Peroxisome proliferator-activated receptor alpha-responsive genes induced in the newborn but not prenatal liver of peroxisomal fatty acyl-CoA oxidase null mice. *Exp Cell Res* 2001; **268**: 70-76
47 **Fan CY**, Pan J, Chu R, Lee D, Kluckman KD, Usuda N, Singh I, Yeldandi AV, Rao MS, Maeda N, Reddy JK. Hepatocellular and hepatic peroxisomal alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene. *J Biol Chem* 1996; **271**: 24698-24710
48 **Simpson ER**. Genetic mutations resulting in loss of aromatase activity in humans and mice. *J Soc Gynecol Investig* 2000; **7**: S18-S21
49 **Fisher CR**, Graves KH, Parlow AF, Simpson ER. Characterization of mice deficient in aromatase (ArKO) because of targeted disruption of the cyp19 gene. *Proc Natl Acad Sci USA* 1998; **95**: 6965-6970
50 **Jones ME**, Thorburn AW, Britt KL, Hewitt KN, Wreford NG, Proietto J, Oz OK, Leury BJ, Robertson KM, Yao S, Simpson ER. Aromatase-deficient (ArKO) mice have a phenotype of increased adiposity. *Proc Natl Acad Sci USA* 2000; **97**: 12735-12740
51 **Heine PA**, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. *Proc Natl Acad Sci USA* 2000; **97**: 12729-12734
52 **Couse JF**, Korach KS. Estrogen receptor null mice: what have we learned and where will they lead us? *Endocr Rev* 1999; **20**: 358-417
53 **Takeda K**, Toda K, Saibara T, Nakagawa M, Saika K, Onishi T, Sugiura T, Shizuta Y. Progressive development of insulin resistance phenotype in male mice with complete aromatase (CYP19) deficiency. *J Endocrinol* 2003; **176**: 237-246
54 **Ibdah JA**, Paul H, Zhao Y, Binford S, Salleng K, Cline M, Matern D, Bennett MJ, Rinaldo P, Strauss AW. Lack of mitochondrial trifunctional protein in mice causes neonatal hypoglycemia and sudden death. *J Clin Invest* 2001; **107**: 1403-1409
55 **Ibdah JA**, Perlegas P, Zhao Y, Angdisen J, Borgerink H, Shadoan MK, Wagner JD, Matern D, Rinaldo P, Cline JM. Mice heterozygous for a defect in mitochondrial trifunctional protein develop hepatic steatosis and insulin resistance. *Gastroenterology* 2005; **128**: 1381-1390
56 **Maehama T**, Dixon JE. The tumor suppressor, PTEN/MMAC1, dephosphorylates the lipid second messenger, phosphatidylinositol 3,4,5-trisphosphate. *J Biol Chem* 1998; **273**: 13375-13378
57 **Li J**, Yen C, Liaw D, Podsypanina K, Bose S, Wang SI, Puc J, Miliaresis C, Rodgers L, McCombie R, Bigner SH, Giovanella BC, Ittmann M, Tycko B, Hibshoosh H, Wigler MH, Parsons R. PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science* 1997; **275**: 1943-1947
58 **Horie Y**, Suzuki A, Kataoka E, Sasaki T, Hamada K, Sasaki J, Mizuno K, Hasegawa G, Kishimoto H, Iizuka M, Naito M, Enomoto K, Watanabe S, Mak TW, Nakano T. Hepatocyte-specific Pten deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest* 2004; **113**: 1774-1783
59 **Watanabe S**, Horie Y, Kataoka E, Sato W, Dohmen T, Ohshima S, Goto T, Suzuki A. Non-alcoholic steatohepatitis and hepatocellular carcinoma: lessons from hepatocyte-specific phosphatase and tensin homolog (PTEN)-deficient mice. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S96-S100
60 **Brunt EM**. Nonalcoholic steatohepatitis (NASH): further expansion of this clinical entity? *Liver* 1999; **19**: 263-264
61 **Piguet AC**, Stroka D, Zimmermann A, Dufour JF. Hypoxia aggravates non-alcoholic steatohepatitis in mice lacking he-

patocellular PTEN. *Clin Sci* (Lond) 2010; **118**: 401-410
62 **Martínez-Chantar ML**, Corrales FJ, Martínez-Cruz LA, García-Trevijano ER, Huang ZZ, Chen L, Kanel G, Avila MA, Mato JM, Lu SC. Spontaneous oxidative stress and liver tumors in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2002; **16**: 1292-1294
63 **Lu SC**, Alvarez L, Huang ZZ, Chen L, An W, Corrales FJ, Avila MA, Kanel G, Mato JM. Methionine adenosyltransferase 1A knockout mice are predisposed to liver injury and exhibit increased expression of genes involved in proliferation. *Proc Natl Acad Sci USA* 2001; **98**: 5560-5565
64 **Chen L**, Zeng Y, Yang H, Lee TD, French SW, Corrales FJ, García-Trevijano ER, Avila MA, Mato JM, Lu SC. Impaired liver regeneration in mice lacking methionine adenosyltransferase 1A. *FASEB J* 2004; **18**: 914-916
65 **Zhou M**, Xu A, Tam PK, Lam KS, Chan L, Hoo RL, Liu J, Chow KH, Wang Y. Mitochondrial dysfunction contributes to the increased vulnerabilities of adiponectin knockout mice to liver injury. *Hepatology* 2008; **48**: 1087-1096
66 **Wang Y**, Lam KS, Yau MH, Xu A. Post-translational modifications of adiponectin: mechanisms and functional implications. *Biochem J* 2008; **409**: 623-633
67 **Angulo P**. Nonalcoholic fatty liver disease. *N Engl J Med* 2002; **346**: 1221-1231
68 **Kadowaki T**, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; **26**: 439-451
69 **Kadowaki T**, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett* 2008; **582**: 74-80
70 **Yamauchi T**, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; **13**: 332-339
71 **Liu Y**, Michael MD, Kash S, Bensch WR, Monia BP, Murray SF, Otto KA, Syed SK, Bhanot S, Sloop KW, Sullivan JM, Reifel-Miller A. Deficiency of adiponectin receptor 2 reduces diet-induced insulin resistance but promotes type 2 diabetes. *Endocrinology* 2007; **148**: 683-692
72 **Asano T**, Watanabe K, Kubota N, Gunji T, Omata M, Kadowaki T, Ohnishi S. Adiponectin knockout mice on high fat diet develop fibrosing steatohepatitis. *J Gastroenterol Hepatol* 2009; **24**: 1669-1676
73 **Shu RZ**, Zhang F, Wang F, Feng DC, Li XH, Ren WH, Wu XL, Yang X, Liao XD, Huang L, Wang ZG. Adiponectin deficiency impairs liver regeneration through attenuating STAT3 phosphorylation in mice. *Lab Invest* 2009; **89**: 1043-1052
74 **Yin XM**, Wang K, Gross A, Zhao Y, Zinkel S, Klocke B, Roth KA, Korsmeyer SJ. Bid-deficient mice are resistant to Fas-induced hepatocellular apoptosis. *Nature* 1999; **400**: 886-891
75 **Alkhouri N**, Gornicka A, Berk MP, Thapaliya S, Dixon LJ, Kashyap S, Schauer PR, Feldstein AE. Adipocyte apoptosis, a link between obesity, insulin resistance, and hepatic steatosis. *J Biol Chem* 2010; **285**: 3428-3438
76 **Wueest S**, Rapold RA, Schoenle EJ, Konrad D. Fas activation in adipocytes impairs insulin-stimulated glucose uptake by reducing Akt. *FEBS Lett* 2010; **584**: 4187-4192
77 **Wueest S**, Rapold RA, Schumann DM, Rytka JM, Schildknecht A, Nov O, Chervonsky AV, Rudich A, Schoenle EJ, Donath MY, Konrad D. Deletion of Fas in adipocytes relieves adipose tissue inflammation and hepatic manifestations of obesity in mice. *J Clin Invest* 2010; **120**: 191-202
78 **Fontana L**, Eagon JC, Trujillo ME, Scherer PE, Klein S. Visceral fat adipokine secretion is associated with systemic inflammation in obese humans. *Diabetes* 2007; **56**: 1010-1013
79 **van der Poorten D**, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; **48**: 449-457
80 **Park EJ**, Lee JH, Yu GY, He G, Ali SR, Holzer RG, Osterreicher CH, Takahashi H, Karin M. Dietary and genetic obesity promote liver inflammation and tumorigenesis by enhancing IL-6 and TNF expression. *Cell* 2010; **140**: 197-208
81 **Hong F**, Radaeva S, Pan HN, Tian Z, Veech R, Gao B. Interleukin 6 alleviates hepatic steatosis and ischemia/reperfusion injury in mice with fatty liver disease. *Hepatology* 2004; **40**: 933-941
82 **Matthews VB**, Allen TL, Risis S, Chan MH, Henstridge DC, Watson N, Zaffino LA, Babb JR, Boon J, Meikle PJ, Jowett JB, Watt MJ, Jansson JO, Bruce CR, Febbraio MA. Interleukin-6-deficient mice develop hepatic inflammation and systemic insulin resistance. *Diabetologia* 2010; **53**: 2431-2441
83 **Hotamisligil GS**. The role of TNFalpha and TNF receptors in obesity and insulin resistance. *J Intern Med* 1999; **245**: 621-625
84 **Uysal KT**, Wiesbrock SM, Marino MW, Hotamisligil GS. Protection from obesity-induced insulin resistance in mice lacking TNF-alpha function. *Nature* 1997; **389**: 610-614
85 **Lin HZ**, Yang SQ, Chuckaree C, Kuhajda F, Ronnet G, Diehl AM. Metformin reverses fatty liver disease in obese, leptin-deficient mice. *Nat Med* 2000; **6**: 998-1003
86 **Tomita K**, Tamiya G, Ando S, Ohsumi K, Chiyo T, Mizutani A, Kitamura N, Toda K, Kaneko T, Horie Y, Han JY, Kato S, Shimoda M, Oike Y, Tomizawa M, Makino S, Ohkura T, Saito H, Kumagai N, Nagata H, Ishii H, Hibi T. Tumour necrosis factor alpha signalling through activation of Kupffer cells plays an essential role in liver fibrosis of non-alcoholic steatohepatitis in mice. *Gut* 2006; **55**: 415-424
87 **Schmidt-Supprian M**, Bloch W, Courtois G, Addicks K, Israël A, Rajewsky K, Pasparakis M. NEMO/IKK gamma-deficient mice model incontinentia pigmenti. *Mol Cell* 2000; **5**: 981-992
88 **Pasparakis M**, Alexopoulou L, Episkopou V, Kollias G. Immune and inflammatory responses in TNF alpha-deficient mice: a critical requirement for TNF alpha in the formation of primary B cell follicles, follicular dendritic cell networks and germinal centers, and in the maturation of the humoral immune response. *J Exp Med* 1996; **184**: 1397-1411
89 **Coffinier C**, Gresh L, Fiette L, Tronche F, Schütz G, Babinet C, Pontoglio M, Yaniv M, Barra J. Bile system morphogenesis defects and liver dysfunction upon targeted deletion of HNF1beta. *Development* 2002; **129**: 1829-1838
90 **Kellendonk C**, Opherk C, Anlag K, Schütz G, Tronche F. Hepatocyte-specific expression of Cre recombinase. *Genesis* 2000; **26**: 151-153
91 **Luedde T**, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, Roskams T, Trautwein C, Pasparakis M. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2007; **11**: 119-132
92 **Wunderlich FT**, Luedde T, Singer S, Schmidt-Supprian M, Baumgartl J, Schirmacher P, Pasparakis M, Brüning JC. Hepatic NF-kappa B essential modulator deficiency prevents obesity-induced insulin resistance but synergizes with high-fat feeding in tumorigenesis. *Proc Natl Acad Sci USA* 2008; **105**: 1297-1302
93 **Schattenberg JM**, Singh R, Wang Y, Lefkowitch JH, Rigoli RM, Scherer PE, Czaja MJ. JNK1 but not JNK2 promotes the development of steatohepatitis in mice. *Hepatology* 2006; **43**: 163-172
94 **Hirosumi J**, Tuncman G, Chang L, Görgün CZ, Uysal KT, Maeda K, Karin M, Hotamisligil GS. A central role for JNK in obesity and insulin resistance. *Nature* 2002; **420**: 333-336
95 **Brenner DA**, Seki E, Taura K, Kisseleva T, Deminicis S, Iwaisako K, Inokuchi S, Schnabl B, Oesterreicher CH, Paik

YH, Miura K, Kodama Y. Non-alcoholic steatohepatitis-induced fibrosis: Toll-like receptors, reactive oxygen species and Jun N-terminal kinase. *Hepatol Res* 2011; **41**: 683-686
96 **Kong B**, Luyendyk JP, Tawfik O, Guo GL. Farnesoid X receptor deficiency induces nonalcoholic steatohepatitis in low-density lipoprotein receptor-knockout mice fed a high-fat diet. *J Pharmacol Exp Ther* 2009; **328**: 116-122
97 **He S**, McPhaul C, Li JZ, Garuti R, Kinch L, Grishin NV, Cohen JC, Hobbs HH. A sequence variation (I148M) in PNPLA3 associated with nonalcoholic fatty liver disease disrupts triglyceride hydrolysis. *J Biol Chem* 2010; **285**: 6706-6715
98 **Björkbacka H**, Kunjathoor VV, Moore KJ, Koehn S, Ordija CM, Lee MA, Means T, Halmen K, Luster AD, Golenbock DT, Freeman MW. Reduced atherosclerosis in MyD88-null mice links elevated serum cholesterol levels to activation of innate immunity signaling pathways. *Nat Med* 2004; **10**: 416-421
99 **Michelsen KS**, Wong MH, Shah PK, Zhang W, Yano J, Doherty TM, Akira S, Rajavashisth TB, Arditi M. Lack of Toll-like receptor 4 or myeloid differentiation factor 88 reduces atherosclerosis and alters plaque phenotype in mice deficient in apolipoprotein E. *Proc Natl Acad Sci USA* 2004; **101**: 10679-10684
100 **Miura K**, Kodama Y, Inokuchi S, Schnabl B, Aoyama T, Ohnishi H, Olefsky JM, Brenner DA, Seki E. Toll-like receptor 9 promotes steatohepatitis by induction of interleukin-1beta in mice. *Gastroenterology* 2010; **139**: 323-334.e7
101 **Seki E**, De Minicis S, Osterreicher CH, Kluwe J, Osawa Y, Brenner DA, Schwabe RF. TLR4 enhances TGF-beta signaling and hepatic fibrosis. *Nat Med* 2007; **13**: 1324-1332
102 **Imaeda AB**, Watanabe A, Sohail MA, Mahmood S, Mohamadnejad M, Sutterwala FS, Flavell RA, Mehal WZ. Acetaminophen-induced hepatotoxicity in mice is dependent on Tlr9 and the Nalp3 inflammasome. *J Clin Invest* 2009; **119**: 305-314
103 **Péterfy M**, Phan J, Xu P, Reue K. Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat Genet* 2001; **27**: 121-124
104 **Goel R**, Boylan B, Gruman L, Newman PJ, North PE, Newman DK. The proinflammatory phenotype of PECAM-1-deficient mice results in atherogenic diet-induced steatohepatitis. *Am J Physiol Gastrointest Liver Physiol* 2007; **293**: G1205-G1214
105 **Lin X**, Yue P, Xie Y, Davidson NO, Sakata N, Ostlund RE, Chen Z, Schonfeld G. Reduced intestinal fat absorptive capacity but enhanced susceptibility to diet-induced fatty liver in mice heterozygous for ApoB38.9 truncation. *Am J Physiol Gastrointest Liver Physiol* 2005; **289**: G146-G152
106 **Robert K**, Nehmé J, Bourdon E, Pivert G, Friguet B, Delcayre C, Delabar JM, Janel N. Cystathionine beta synthase deficiency promotes oxidative stress, fibrosis, and steatosis in mice liver. *Gastroenterology* 2005; **128**: 1405-1415
107 **Postic C**, Girard J. Contribution of de novo fatty acid synthesis to hepatic steatosis and insulin resistance: lessons from genetically engineered mice. *J Clin Invest* 2008; **118**: 829-838
108 **Wakil SJ**, Stoops JK, Joshi VC. Fatty acid synthesis and its regulation. *Annu Rev Biochem* 1983; **52**: 537-579
109 **McGarry JD**, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. *Eur J Biochem* 1997; **244**: 1-14
110 **Abu-Elheiga L**, Brinkley WR, Zhong L, Chirala SS, Woldegiorgis G, Wakil SJ. The subcellular localization of acetyl-CoA carboxylase 2. *Proc Natl Acad Sci USA* 2000; **97**: 1444-1449
111 **Flowers MT**, Miyazaki M, Liu X, Ntambi JM. Probing the role of stearoyl-CoA desaturase-1 in hepatic insulin resistance. *J Clin Invest* 2006; **116**: 1478-1481
112 **Cohen P**, Miyazaki M, Socci ND, Hagge-Greenberg A, Liedtke W, Soukas AA, Sharma R, Hudgins LC, Ntambi JM, Friedman JM. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. *Science* 2002; **297**: 240-243
113 **Ntambi JM**, Miyazaki M, Stoehr JP, Lan H, Kendziorski CM, Yandell BS, Song Y, Cohen P, Friedman JM, Attie AD. Loss of stearoyl-CoA desaturase-1 function protects mice against adiposity. *Proc Natl Acad Sci USA* 2002; **99**: 11482-11486
114 **Jiang G**, Li Z, Liu F, Ellsworth K, Dallas-Yang Q, Wu M, Ronan J, Esau C, Murphy C, Szalkowski D, Bergeron R, Doebber T, Zhang BB. Prevention of obesity in mice by antisense oligonucleotide inhibitors of stearoyl-CoA desaturase-1. *J Clin Invest* 2005; **115**: 1030-1038
115 **Gutiérrez-Juárez R**, Pocai A, Mulas C, Ono H, Bhanot S, Monia BP, Rossetti L. Critical role of stearoyl-CoA desaturase-1 (SCD1) in the onset of diet-induced hepatic insulin resistance. *J Clin Invest* 2006; **116**: 1686-1695
116 **Matsuzaka T**, Shimano H, Yahagi N, Kato T, Atsumi A, Yamamoto T, Inoue N, Ishikawa M, Okada S, Ishigaki N, Iwasaki H, Iwasaki Y, Karasawa T, Kumadaki S, Matsui T, Sekiya M, Ohashi K, Hasty AH, Nakagawa Y, Takahashi A, Suzuki H, Yatoh S, Sone H, Toyoshima H, Osuga J, Yamada N. Crucial role of a long-chain fatty acid elongase, Elovl6, in obesity-induced insulin resistance. *Nat Med* 2007; **13**: 1193-1202
117 **Mordier S**, Iynedjian PB. Activation of mammalian target of rapamycin complex 1 and insulin resistance induced by palmitate in hepatocytes. *Biochem Biophys Res Commun* 2007; **362**: 206-211
118 **Jerkins AA**, Liu WR, Lee S, Sul HS. Characterization of the murine mitochondrial glycerol-3-phosphate acyltransferase promoter. *J Biol Chem* 1995; **270**: 1416-1421
119 **Cha JY**, Repa JJ. The liver X receptor (LXR) and hepatic lipogenesis. The carbohydrate-response element-binding protein is a target gene of LXR. *J Biol Chem* 2007; **282**: 743-751
120 **Dentin R**, Benhamed F, Hainault I, Fauveau V, Foufelle F, Dyck JR, Girard J, Postic C. Liver-specific inhibition of ChREBP improves hepatic steatosis and insulin resistance in ob/ob mice. *Diabetes* 2006; **55**: 2159-2170
121 **Miyazaki M**, Flowers MT, Sampath H, Chu K, Otzelberger C, Liu X, Ntambi JM. Hepatic stearoyl-CoA desaturase-1 deficiency protects mice from carbohydrate-induced adiposity and hepatic steatosis. *Cell Metab* 2007; **6**: 484-496
122 **Postic C**, Dentin R, Denechaud PD, Girard J. ChREBP, a transcriptional regulator of glucose and lipid metabolism. *Annu Rev Nutr* 2007; **27**: 179-192
123 **Walkey CJ**, Donohue LR, Bronson R, Agellon LB, Vance DE. Disruption of the murine gene encoding phosphatidylethanolamine N-methyltransferase. *Proc Natl Acad Sci USA* 1997; **94**: 12880-12885
124 **Walkey CJ**, Yu L, Agellon LB, Vance DE. Biochemical and evolutionary significance of phospholipid methylation. *J Biol Chem* 1998; **273**: 27043-27046
125 **Yu YH**, Ginsberg HN. The role of acyl-CoA: diacylglycerol acyltransferase (DGAT) in energy metabolism. *Ann Med* 2004; **36**: 252-261
126 **Millar JS**, Stone SJ, Tietge UJ, Tow B, Billheimer JT, Wong JS, Hamilton RL, Farese RV, Rader DJ. Short-term overexpression of DGAT1 or DGAT2 increases hepatic triglyceride but not VLDL triglyceride or apoB production. *J Lipid Res* 2006; **47**: 2297-2305
127 **Monetti M**, Levin MC, Watt MJ, Sajan MP, Marmor S, Hubbard BK, Stevens RD, Bain JR, Newgard CB, Farese RV, Hevener AL, Farese RV. Dissociation of hepatic steatosis and insulin resistance in mice overexpressing DGAT in the liver. *Cell Metab* 2007; **6**: 69-78
128 **Yu XX**, Murray SF, Pandey SK, Booten SL, Bao D, Song XZ, Kelly S, Chen S, McKay R, Monia BP, Bhanot S. Antisense oligonucleotide reduction of DGAT2 expression improves hepatic steatosis and hyperlipidemia in obese mice. *Hepatol-*

ogy 2005; **42**: 362-371
129 **Herbert PN**, Assmann G, Gotto AM JR, Frederickson DS. Disorders of the lipoprotein and lipid metabolism. In: The Metabolic Basis of Inherited Diseases. 5th ed. Stanbury JB, Wyngaarden JB, Frederickson DS, Goldstein JL, Brown MS, editors. New York: McGraw-Hill, 1983: 589-651
130 **Jong MC**, Rensen PC, Dahlmans VE, van der Boom H, van Berkel TJ, Havekes LM. Apolipoprotein C-III deficiency accelerates triglyceride hydrolysis by lipoprotein lipase in wild-type and apoE knockout mice. *J Lipid Res* 2001; **42**: 1578-1585
131 **Araki E**, Lipes MA, Patti ME, Brüning JC, Haag B, Johnson RS, Kahn CR. Alternative pathway of insulin signalling in mice with targeted disruption of the IRS-1 gene. *Nature* 1994; **372**: 186-190
132 **Tamemoto H**, Kadowaki T, Tobe K, Yagi T, Sakura H, Hayakawa T, Terauchi Y, Ueki K, Kaburagi Y, Satoh S. Insulin resistance and growth retardation in mice lacking insulin receptor substrate-1. *Nature* 1994; **372**: 182-186
133 **Pan W**, Ciociola E, Saraf M, Tumurbaatar B, Tuvdendorj D, Prasad S, Chandalia M, Abate N. Metabolic consequences of ENPP1 overexpression in adipose tissue. *Am J Physiol Endocrinol Metab* 2011; **301**: E901-E911
134 **Cauchi S**, El Achhab Y, Choquet H, Dina C, Krempler F, Weitgasser R, Nejjari C, Patsch W, Chikri M, Meyre D, Froguel P. TCF7L2 is reproducibly associated with type 2 diabetes in various ethnic groups: a global meta-analysis. *J Mol Med* (Berl) 2007; **85**: 777-782
135 **Cauchi S**, Meyre D, Dina C, Choquet H, Samson C, Gallina S, Balkau B, Charpentier G, Pattou F, Stetsyuk V, Scharfmann R, Staels B, Frühbeck G, Froguel P. Transcription factor TCF7L2 genetic study in the French population: expression in human beta-cells and adipose tissue and strong association with type 2 diabetes. *Diabetes* 2006; **55**: 2903-2908
136 **Grant SF**, Thorleifsson G, Reynisdottir I, Benediktsson R, Manolescu A, Sainz J, Helgason A, Stefansson H, Emilsson V, Helgadottir A, Styrkarsdottir U, Magnusson KP, Walters GB, Palsdottir E, Jonsdottir T, Gudmundsdottir T, Gylfason A, Saemundsdottir J, Wilensky RL, Reilly MP, Rader DJ, Bagger Y, Christiansen C, Gudnason V, Sigurdsson G, Thorsteinsdottir U, Gulcher JR, Kong A, Stefansson K. Variant of transcription factor 7-like 2 (TCF7L2) gene confers risk of type 2 diabetes. *Nat Genet* 2006; **38**: 320-323
137 **Huertas-Vazquez A**, Plaisier C, Weissglas-Volkov D, Sinsheimer J, Canizales-Quinteros S, Cruz-Bautista I, Nikkola E, Herrera-Hernandez M, Davila-Cervantes A, Tusie-Luna T, Taskinen MR, Aguilar-Salinas C, Pajukanta P. TCF7L2 is associated with high serum triacylglycerol and differentially expressed in adipose tissue in families with familial combined hyperlipidaemia. *Diabetologia* 2008; **51**: 62-69
138 **Chen Y**, Yang Y, Miller ML, Shen D, Shertzer HG, Stringer KF, Wang B, Schneider SN, Nebert DW, Dalton TP. Hepatocyte-specific Gclc deletion leads to rapid onset of steatosis with mitochondrial injury and liver failure. *Hepatology* 2007; **45**: 1118-1128
139 **Yoneda M**, Hotta K, Nozaki Y, Endo H, Tomeno W, Watanabe S, Hosono K, Mawatari H, Iida H, Fujita K, Takahashi H, Kirikoshi H, Kobayashi N, Inamori M, Kubota K, Shimamura T, Saito S, Maeyama S, Wada K, Nakajima A. Influence of inducible nitric oxide synthase polymorphisms in Japanese patients with non-alcoholic fatty liver disease. *Hepatol Res* 2009; **39**: 963-971
140 **Dickhout JG**, Hossain GS, Pozza LM, Zhou J, Lhoták S, Austin RC. Peroxynitrite causes endoplasmic reticulum stress and apoptosis in human vascular endothelium: implications in atherogenesis. *Arterioscler Thromb Vasc Biol* 2005; **25**: 2623-2629
141 **Chen Y**, Hozawa S, Sawamura S, Sato S, Fukuyama N, Tsuji C, Mine T, Okada Y, Tanino R, Ogushi Y, Nakazawa H. Deficiency of inducible nitric oxide synthase exacerbates hepatic fibrosis in mice fed high-fat diet. *Biochem Biophys Res Commun* 2005; **326**: 45-51
142 **Uchiyama S**, Shimizu T, Shirasawa T. CuZn-SOD deficiency causes ApoB degradation and induces hepatic lipid accumulation by impaired lipoprotein secretion in mice. *J Biol Chem* 2006; **281**: 31713-31719
143 **Moh A**, Iwamoto Y, Chai GX, Zhang SS, Kano A, Yang DD, Zhang W, Wang J, Jacoby JJ, Gao B, Flavell RA, Fu XY. Role of STAT3 in liver regeneration: survival, DNA synthesis, inflammatory reaction and liver mass recovery. *Lab Invest* 2007; **87**: 1018-1028
144 **Inoue H**, Ogawa W, Ozaki M, Haga S, Matsumoto M, Furukawa K, Hashimoto N, Kido Y, Mori T, Sakaue H, Teshigawara K, Jin S, Iguchi H, Hiramatsu R, LeRoith D, Takeda K, Akira S, Kasuga M. Role of STAT-3 in regulation of hepatic gluconeogenic genes and carbohydrate metabolism in vivo. *Nat Med* 2004; **10**: 168-174
145 **Hebbard L**, George J. Animal models of nonalcoholic fatty liver disease. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 35-44
146 **Anstee QM**, Goldin RD. Mouse models in non-alcoholic fatty liver disease and steatohepatitis research. *Int J Exp Pathol* 2006; **87**: 1-16

S- Editor Tian L **L- Editor** Logan S **E- Editor** Li JY