ORIGINAL INVESTIGATION

Peroxisome Proliferator–Activated Receptor α L162V Polymorphism in Nonalcoholic Steatohepatitis and Genotype 1 Hepatitis C Virus–Related Liver Steatosis

Hasibe Verdi, Elif Sare Koytak, Oğuz Önder, Ayça Arslan Ergül, Kubilay Cinar, Ramazan Idilman, Esra Erden, Abdurrahman Mithat Bozdayi, Cihan Yurdaydin, Özden Uzunalimoglu, Hakan Bozkaya

ABSTRACT

Background: Peroxisome proliferator–activated receptor α (PPARα) plays important roles in lipid metabolism. A recently discovered L162V polymorphism of the *PPARα* gene is associated with enhanced transcriptional activity. In this study, the frequency of L162V was investigated in nonalcoholic steatohepatitis (NASH) and genotype 1 hepatitis C virus (HCV)-related liver steatosis.

Methods: Seventy-two NASH and 141 HCV-infected patients (54 with steatosis, 87 without steatosis) and 119 healthy controls were included. L162V polymorphism of the *PPARα* gene was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP).

Results: PCR and RFLP analysis of the related gene segment was successful in 93%, 96%, and 100% of NASH and HCV-infected patients and controls, respectively. The frequency of the L162V polymorphism was similar in the NASH and HCV-infected patients and controls (5.9%, 3.6%, and 2.5%, respectively). No difference in the frequency of this polymorphism was observed in HCV-infected patients with or without significant liver steatosis. L162V was not associated with obesity, type 2 diabetes mellitus, hypercholesterolemia, or hypertriglyceridemia.

Conclusions: Neither NASH nor genotype 1 HCV-related liver steatosis seems to be associated with the PPARα L162V polymorphism. This polymorphism may have no association with the presence of type 2 diabetes mellitus, obesity, or various blood lipid alterations in NASH and HCV-infected patients.

From the Institute of Hepatology (H.V., A.A.E., A.M.B., C.Y., O.U., H.B.), Department of Gastroenterology (E.S.K., O.O., K.C., R.I., A.M.B., C.Y., H.B.), School of Medicine, and Department of Pathology (E.E.), School of Medicine, Ankara University, Ankara, Turkey.

Address correspondence to: Dr. Hakan Bozkaya, Department of Gastroenterology, School of Medicine, Ankara University, Tip Fakultesi Hastanesi, 06100 Dikimevi, Ankara, Turkey; e-mail: Hakan.Bozkaya@medicine.ankara.edu.tr.

Key Words: peroxisome proliferator–activated receptor α, L162V, polymorphism, fatty liver, nonalcoholic steatohepatitis, hepatitis C virus

Nonalcoholic steatohepatitis (NASH) represents the progressive form of nonalcoholic fatty liver disease (NAFLD), which is characterized by predominantly macrovesicular steatosis of the liver.[1] NASH may progress to cirrhosis, liver failure, and hepatocellular carcinoma.[2–5] A growing body of evidence supports a multihit hypothesis in the pathogenesis of NASH. Insulin resistance associated with or without obesity and metabolic syndrome is thought to have a central role in the pathogenesis of NAFLD and NASH and to account for the first hit in the development of NASH.[6–9] Insulin resistance leads to accumulation of free fatty acids in the liver by favoring peripheral lipolysis and hepatic uptake of fatty acids. This fatty acid load in the liver is counterbalanced by increased fatty acid oxidation, which produces reactive oxygen species and leads to oxidative stress.[10] Further hits involve several pathogenic stimuli, such as toxic substances produced during increased fatty acid oxidation, endotoxemia, and activation of proinflammatory cytokines, all of which cause oxidative stress and resultant steatohepatitis.[11–14]

Recent studies point to the importance of peroxisome proliferator–activated receptor α (PPARα) in the development of NAFLD and NASH. PPARα is a ligand-activated transcription factor and has an important role in lipid homeostasis. Following activation by its endogenous or exogenous ligands, PPARα forms a heterodimer with the 9-*cis* retinoic acid receptor (RXR), and PPAR/RXR heterodimers bind to deoxyribonucleic acid (DNA) sequences, termed PPAR response elements, present in the 5′-flanking region of target genes.[15–17] PPARα induces fatty acid oxidation in mitochondria, peroxisomes, and microsomes, and there appears to be a crosstalk between these three fatty acid oxidation systems, with PPARα play-

ing a controlling role.[18–21] The findings in animal experiments point to the importance of PPARα-inducible fatty acid oxidation systems in the pathogenesis of NAFLD and NASH.[22–25] Disruption of the *PPAR*α gene in mice causes liver steatosis by reducing mitochondrial fatty acid oxidation, whereas dramatic activation of PPARα by disruption of peroxisomal acyl coenzyme A oxidase leads to steatohepatitis by induction of microsomal oxidation that produces radical oxygen species.[21–25]

Recently, a polymorphism in the *PPAR*α gene, leucine to valine change at codon 162 localized to exon 5 (L162V), has been described. This polymorphism was shown to enhance the transcriptional activity of PPARα in transfection assays.[26] PPARα L162V polymorphism has been reported to be associated with altered lipid and apobetalipoprotein concentrations and with a decreased body mass index, especially in patients with type 2 diabetes mellitus.[26–30]

So far, no study has addressed the importance of the L162V polymorphism in NASH, which is frequently associated with insulin resistance and lipid abnormalities. Another condition associated with hepatic steatosis is chronic hepatitis C virus (HCV) infection. Genotype 3 HCV-associated liver steatosis is likely due to viral factors.[31,32] However, liver steatosis is also observed in HCV infection owing to other genotypes. The body of evidence points to host factors in genotype 1 HCV-associated liver steatosis.[32] In this context, fatty liver may be secondary to the existence of NASH with chronic HCV infection. We therefore decided to also explore the L162V polymorphism in patients infected with genotype 1 HCV, which is the most frequently encountered genotype in Turkish patients with chronic HCV infection.[33] In this group, patients with and without prominent liver steatosis were examined separately.

PATIENTS AND METHODS

Study Samples and Data Collection

Seventy-two biopsy-proven NASH patients and 141 patients with chronic genotype 1–HCV infection were studied. None of the patients were infected with other hepatitis viruses or had any other identifiable causes of transaminase elevation, including drug toxicity and autoimmune or metabolic liver disease. Alcohol intake was absent or less than 20 g per week in all of these patients. In addition, none of the patients had any evidence of systemic disease, including collagen-vascular, neoplastic, cardiopulmonary, or renal disease. The demographic data (age, gender, body mass index); blood chemistry, including liver enzymes, glucose, cholesterol, and triglyceride levels; and the presence of diabetes mellitus, were recorded for each patient. One hundred nineteen healthy subjects served as controls. These control subjects had a normal body mass index and normal blood chemistry and were negative for viral serology.

Histologic Assessment

Liver histology was assessed according to Knodell's scoring system in patients with chronic hepatitis C.[34] The diagnosis of NASH was based on the presence of fat accumulation, lobular inflammation including polymorphonuclear leukocytes, perisinusoidal fibrosis in zone 3, hepatocyte ballooning with or without poorly formed Mallory's hyaline, and glycogenated nuclei. The amount and extent of steatosis, lobular inflammation, ballooning, lobular disarray, portal inflammation, and fibrosis were determined and scored as previously reported.[35] Among patients with chronic hepatitis C, patients with liver steatosis of < 30% were classified as group 1 and those with significant liver steatosis (≥ 30%) were classified as group 2. HCV genotyping was performed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) analysis.[33]

Detection of PPARα L162V Polymorphism

A blood sample was drawn from each patient and from healthy controls for DNA isolation. Cellular DNAs were kept at −20°C until PCR analysis. In the mismatch PCR, the following primers generating HinfI restriction site were used for further RFLP analysis[27]:

Ex 5 F 5′ GAC TCA AGC TGG TGT ATG ACA AGT 3′

Ex 5 R mismatch 5′ CGT TGT GTG ACA TCC CGA CAG AA*T* 3′ (*T* = mismatch nucleotide)

Following preparation of 1.25 U Taq polymerase, 2.5 mM magnesium chloride, and 0.2 mM deoxynucleotide phosphate, forward and reverse primers (10 pmol each) were added. The final volume of the reaction was adjusted to 50 µL. The annealing temperature of the reaction was 61°C. Following application of HinfI endonuclease enzyme to PCR products, while a 117 bp band was visualized for a normal allele, two separate bands of 93 bp and 24 bp were observed for a mutant allele on 2% agarose gel electrophoresis (Figure 1).

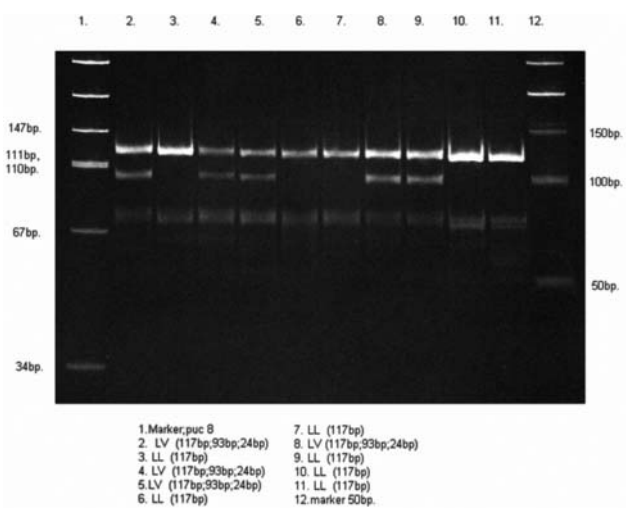

FIGURE 1 Gel electrophoresis showing several cases with a single band of normal alleles (L162V). L = leucine; V = valine.

RESULTS

Patient Characteristics

The patient groups included 72 NASH patients (average age 45 years; 49 males/22 females) and 141 patients with chronic HCV infection (average age 50 years; 60 males/81 females). The control group consisted of 119 healthy subjects (average age 35 years; 56 males/63 females). Eighty-seven (61%) of 141 HCV-infected patients were group 1 (steatosis < 30%), and the remaining 54 patients (39%) were group 2 (steatosis ≥ 30%). Obesity was more frequently found in NASH patients compared with HCV-infected patients (32.8% vs 19.1%, respectively; $p < .05$). Among HCV-infected patients, a comparable number of patients with (23.5%) and without (16.4%) significant liver steatosis had obesity.

Frequency of L162V Polymorphism

In 67 (93%) of 72 NASH patients and 136 of 141 (96%) patients with chronic hepatitis C, the gene segment harboring the polymorphic site at position 162 of PPARα could be amplified by PCR. PCR amplification was successful in all of the 119 control subjects. The frequency of the L162V polymorphism did not differ between patient groups and controls (Table 1): 4 of 67 (5.9%) NASH patients and 5 of 136 (3.6%) chronic hepatitis C patients had the L162V polymorphism. Among the five HCV-infected patients with this polymorphism, three (3.5%) patients were in group 1, and the remaining two (3.9%) were in group 2. Three (2.5%) controls had the L162V polymorphism. Table 2 shows the clinical characteristics of subjects with the L162V polymorphism. None of the patients with this polymorphism were obese, and only one patient (NASH) had hypercholesterolemia and hypertriglyceridemia.

Association between the L162V Polymorphism and Type 2 Diabetes Mellitus

Diabetes mellitus was present in 35 patients, with no difference in frequency between NASH and HCV-infected patients: 10 of 67 (15%) NASH and 25 of 136 (18.3%) HCV-infected patients. Although not significant, a higher number of HCV-infected patients with steatosis had diabetes mellitus compared with those without liver steatosis (25.4% vs 14.1%, respectively). In each patient group, the number of patients with diabetes mellitus was similar in patients with and without the L162V polymorphism (Table 3). In addition, the frequency of this polymorphism was not different in patients with (1/35; 2.8%) and without (8/168; 4.7%) diabetes mellitus when all patients were considered (Table 4).

Association between the L162V Polymorphism and Lipid Abnormalities

Both hypercholesterolemia and hypertriglyceridemia were more frequently found in NASH patients compared with HCV-infected patients (50.7% and 56.7% vs 14.7% and 21.3%, respectively; $p < .0001$ for both comparisons). Among HCV-infected patients, patients with liver steatosis more frequently had hypertriglyceridemia compared with those without liver steatosis (34% vs 21.3%; $p < .05$) (see Table 3). The frequencies of both lipid abnormalities were similar in patients with and without the L162V polymorphism in each patient group (see Table 3). The L162V polymorphism was observed with similar frequency in hypercholesterolemic patients (1/54; 1.85%) versus normocholesterolemic patients (8/149; 5.3%) and in hypertriglyceridemic patients (1/67; 1.5%) versus normotriglyceridemic patients (8/136; 5.8%) (see Table 4).

Association between the L162V Polymorphism and Body Mass Index

Interestingly, none of the patients with the L162V polymorphism were obese. However, in each patient group, the difference in the number of obese patients with and without the L162V polymorphism did not reach statistical significance (see Table 3). When all patients were taken into account, the frequency of this polymorphism was comparable in obese patients (0/48, 0%) versus nonobese patients (9/155; 5.8%) (see Table 4).

DISCUSSION

PPARα is a key regulator of fatty acid oxidation and plays important roles in lipid metabolism. Variations in expression and activity of this molecule can have an impact on the development of NASH, which is frequently associated with abnormalities in lipid metabolism and the existence

TABLE 1 Frequency of Peroxisome Proliferator–Activated Receptor α Polymorphism in Patients and Controls in Whom the *PPARα* Gene Was Successfully Amplified by Polymerase Chain Reaction

Groups	*L162V (%)*
NASH (*n* = 67)	4 (5.9)
HCV (*n* = 136)	5 (3.6)
Without significant steatosis (*n* = 85)	3 (3.5)
With steatosis (*n* = 51)	2 (2.9)
Controls (*n* = 119)	2 (2.5)

HCV = hepatitis C virus; NASH = nonalcoholic steatohepatitis.

TABLE 2 Characteristics of Subjects with L162V Polymorphism

Patient Groups	*Sex*	*Age (yr)*	*Body Mass Index (kg/m²)*	*Diabetes Mellitus*	*Cholesterol (mg/dL)*	*Triglyceride (mg/dL)*	*ALT/AST (U/L)*	*GGT (U/L)*	*Histology*
NASH									
1	Male	42	28	–	223	323	55/27	89	Inflammation: 1
									Fibrosis: 0
2	Male	56	29	–	156	144	97/69	150	Inflammation: 1
									Fibrosis: 1
3	Male	34	28	–	178	88	83/60	28	Inflammation: 1
									Fibrosis: 1
4	Male	41	27	–	150	95	90/55	72	Inflammation: 1
									Fibrosis: 1
HCV									
Steatosis–									
1	Female	43	26	+	108	107	91/121	60	HAI: 10
2	Male	64	25	—	156	118	43/41	11	HAI: 14
3	Female	53	24	–	188	49	40/37	130	HAI: 7
Steatosis+									
1	Female	55	24	–	186	123	46/40	40	HAI: 12
2	Female	63	28	–	99	74	96/65	54	HAI: 11
Controls									
1	Female	29	26	–	180	105	20/15	35	
2	Male	27	23	–	122	80	18/14	28	
3	Male	43	24	–	110	52	22/13	25	

ALT = alanine transaminase; AST = aspartate transaminase; GGT = γ-glutamyltransferase; HAI = histologic activity index (Knodell); HCV = hepatitis C virus; NASH = nonalcoholic steatohepatitis.

of the metabolic syndrome.[6] The L162V polymorphism of the *PPAR*α gene was reported to enhance transactivation function but not increase the expression of this molecule.[26] This polymorphism has been found to be associated with several in vivo lipid and apolipoprotein abnormalities.[26–29] This study is the first to investigate the role of this polymorphism in NASH patients. NASH patients did not differ from healthy controls with respect to the frequency of the PPARα L162V polymorphism, suggesting that the L162V polymorphism has no impact on the development of NASH. A similar conclusion can also be drawn for genotype 1 HCV-related liver steatosis in view of the lack of a difference in mutant allele frequencies in chronic hepatitis C patients with steatosis versus without steatosis and healthy controls.

Insulin resistance is thought to be a key event in the development of NAFLD and NASH, and NASH is frequently observed in patients with type 2 diabetes and metabolic syndrome. In the present study, mutant allele frequencies were similar in patients with type 2 diabetes mellitus versus nondiabetics. In addition, the frequency of diabetes mellitus did not differ in patients with and without the L162V polymorphism. Although a relatively lower number of subjects were included in our study, our findings support the results of previous studies in which the L162V polymorphism was not found to be associated with type 2 diabetes mellitus.[26,27,30,36] Our results are also consistent with the findings of a recent report in which the frequency of the L162V polymorphism was not found to differ between patients with and without metabolic syndrome.[29] Despite the absence of an association between the L162V polymorphism and metabolic syndrome, the L162V polymorphism has been associated with some components of metabolic syndrome, such as abdominal obesity and some lipid metabolism abnormalities.[29]

Hypercholesterolemia, hypertriglyceridemia, and obesity are common abnormalities in NASH patients. In fact, a higher number of NASH patients had increased blood lipid levels and were obese when compared with patients with chronic hepatitis C in the present study. However, overall, neither hypercholesterolemia nor hypertriglyc-

TABLE 3 Frequency of Metabolic Abnormalities in Patients with and without L162V Polymorphism

Groups	*Obesity (> 30 kg/m²) (%)*	*Hypercholesterolemia (> 200 mg/dL) (%)*	*Hypertriglyceridemia (> 200 mg/dL) (%)*	*Diabetes (%)*
NASH (*n* = 67)	22 (32.8)	34 (50.7)	38 (56.7)	10 (15)
L162V + (*n* = 4)	0	1 (25)	1 (25)	0
L162V − (*n* = 63)	22 (35)	33 (52)	37 (58.7)	10 (15.8)
HCV (*n* = 136)	26 (19.1)*	20 (14.7)**	29 (21.3)**	25 (18.3)
HCV without steatosis (*n* = 85)	14 (16.4)	9 (10)**	11 (13)***	12 (14.1)
L162V + (*n* = 3)	0	0	0	1 (33)
L162V − (n = 82)	14 (17)	9 (11)	11 (13.4)	11 (13.4)
HCV with steatosis (*n* = 51)	12 (23.5)	11 (21.5)**	18 (34)	13 (25.4)
L162V + (*n* = 2)	0	0	0	0
L162V − (*n* = 49)	12 (24.4)	11 (22.4)	18 (36.7)	13 (26.5)
Controls (*n* = 119)	0	0	0	0
L162V + (*n* = 3)	0	0	0	0
L162V − (*n* = 116)	0	0	0	0

HCV = hepatitis C virus; NASH = nonalcoholic steatohepatitis.
*$p < .05$ versus NASH.
**$p < .0001$ versus NASH.
***$p < .005$ versus HCV with steatosis.

eridemia was associated with the L162V polymorphism. There was also no association between the L162V polymorphism and obesity. Owing to the low frequency of the L162V polymorphism and the relatively small sample size in our study, it is difficult to make a clear-cut conclusion on relationships between the L162V polymorphism and several metabolic alterations. However, our findings are in accordance with the results of some previous studies in which the L162V polymorphism was not found to be associated with blood lipid abnormalities or with body mass index in healthy controls, diabetics, and patients with coronary artery disease.[27,30,36] In contrast, carriers of L162V were reported to have higher apolipoprotein B and low-density lipoprotein cholesterol levels, even in subjects without a metabolic disease.[26] Although this latter finding suggests that this polymorphism may be associated with some specific lipid abnormalities, these associations may be limited only to diabetic patients.[26,27] Furthermore, a recent study reported an association between the L162V polymorphism and body mass index only in diabetic patients.[30] Variations in the expression and the activity of PPARα, its ligands, and PPARα response elements in different diseases and in different metabolic conditions may determine the weight of this polymorphism in several metabolic alterations.

The mechanism of liver steatosis in patients with chronic hepatitis C is not well understood. In genotype 3–infected patients, viral factors may be responsible for liver steatosis. HCV core protein was shown to cause liver steatosis in transgenic animals and may alter lipid metabolism in humans.[31,37] In our series, all patients were infected with genotype 1 HCV, which is responsible for more than 80% of chronic HCV infection in Turkey.[33] In our hepatitis C patient cohort, no difference in the frequency of the L162V polymorphism was observed among patients with steatosis versus those patients without significant steatosis. In genotype 1 infection, liver steatosis is mostly

TABLE 4 L162V Frequency in Patients with or without Various Metabolic Alterations

Metabolic Alteration	*L162V (%)*
Obesity	
Present (48)	0
Absent (155)	9 (5.8)
Hypercholesterolemia	
Present (54)	1 (1.85)
Absent (149)	8 (5.3)
Hypertriglyceridemia	
Present (67)	1 (1.5)
Absent (136)	8 (5.8)
Diabetes	
Present (35)	1 (2.8)
Absent (168)	8 (4.7)

attributed to some host factors.[31,37,38] In fact, in our study, hypertriglyceridemia was more frequently observed in chronic hepatitis C patients with liver steatosis compared with those without prominent liver steatosis. In addition, although it did not reach statistical significance, diabetes mellitus was more frequent in patients with steatosis compared with those without steatosis. These findings support the idea that development of liver steatosis in genotype 1 HCV infection is mainly dependent on the presence of several host pathologies, such as diabetes mellitus and lipid abnormalities. However, several lines of evidence suggest that such abnormalities can be caused by the virus itself.[31,37] Further studies are needed to understand the mechanism and the weight of host and viral factors in the development of liver steatosis in chronic hepatitis C.

In summary, neither NASH nor genotype 1 HCV-related liver steatosis seems to be associated with the PPARα L162V polymorphism. This polymorphism may not be associated with blood lipid abnormalities, the presence of type 2 diabetes mellitus, or obesity in NASH patients. The role of other host factors in the development of NASH and genotype 1 HCV-related liver steatosis may need to be further investigated.

REFERENCES

1. Brunt EM. Nonalcoholic steatohepatitis: definition and pathology. Semin Liver Dis 2001;21:3–6.
2. Caldwell SH, Oelsner DH, Iezzoni JC, et al. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. Hepatology 1999;29:664–9.
3. Ludwig J, Viggiano TR, McGill DB, Oh BJ. Non-alcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 1980;55:434–8.
4. Falck-Ytter Y, Younossi ZM, Marchesini G, McCullough AJ. Clinical features and natural history of nonalcoholic steatosis syndromes. Semin Liver Dis 2001;21:17–26.
5. Younossi ZM. Non-alcoholic fatty liver disease. Curr Gastroenterol Rep 1999;1:57–61.
6. Marchesini G, Bugianesi E, Forlani G, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology 2003;37:917–23.
7. Chitturi S, Abeygunasekera S, Farrell GC, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology 2002;35:373–9.
8. Neuschwander-Terri BA, Caldwell S. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 2003;37:1202–19.
9. Sanyal AJ, Campbell-Sargent C, Mirshahi F, et al. Nonalcoholic steatohepatitis: association of insulin resistance and mitochondrial abnormalities. Gastroenterology 2001;120:1183–92.
10. Angulo P, Lindor KD. Insulin resistance and mitochondrial abnormalities in NASH: a cool look into a burning issue. Gastroenterology 2001;120:1281–4.
11. Green RM. NASH: hepatic metabolism and not simply the metabolic syndrome. Hepatology 2003;38:14–7.
12. Chitturi S, Farrell GC. Etiopathogenesis of nonalcoholic steatohepatitis. Semin Liver Dis 2001;21:27–41.
13. Rao MS, Reddy JK. Peroxisomal β-oxidation and steatohepatitis. Semin Liver Dis 2001;21:43–55.
14. Younossi ZM, Diehl AM, Ong IP. Nonalcoholic fatty liver disease: an agenda for clinical research. Hepatology 2002;35:746–52.
15. Issemann I, Green S. Activation of a member of the steroid hormone receptor superfamily by peroxisome proliferators. Nature 1990;347:645–50.
16. Desvergne B, Wahli BW. Peroxisome proliferator-activated receptors: nuclear control of metabolism. Endocr Rev 1999;20:649–88.
17. Akbiyik F, Ray DM, Bozkaya H, Demirpence E. Ligand and species dependent activation of PPARα. Cell Physiol Biochem 2004;14:269–76.
18. Hashimoto T, Cook WS, Qi C, et al. Defect in peroxisome proliferator-activated receptor alpha-inducible fatty acid oxidation determines the severity of hepatic steatosis in response to fasting. J Biol Chem 2000;275:28918–28.
19. Johnson EF, Palmer CN, Griffin KJ, Hsu MH. Role of the peroxisome proliferator-activated receptor in cytochrome P450 4A gene regulation. FASEB J 1996;10:1241–8.
20. Lee SS, Pineau T, Drago J, et al. Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol Cell Biol 1995;15:3012–22.
21. Reddy JK. Nonalcoholic steatosis and steatohepatitis III. Peroxisomal β-oxidation, PPAR alpha, and steatohepatitis. Am J Physiol Gastrointest Liver Physiol 2001;281:G1333–9.
22. Fan CY, Pan J, Usuda N, et al. Steatohepatitis, spontaneous peroxisome proliferation and liver tumors in mice lacking peroxisomal fatty acyl-CoA oxidase. J Biol Chem 1998;273:15639–45.
23. Fan CY, Pan J, Chu R, et al. Hepatocellular and hepatic peroxisomal alterations in mice with a disrupted peroxisomal fatty acyl-coenzyme A oxidase gene. J Biol Chem 1996;271:24698–710.
24. Akbiyik F, Cinar K, Demirpence E, et al. Ligand induced expression of peroxisome proliferator-activated receptor alpha and activation of fatty acid oxidation enzymes in fatty liver. Eur J Clin Invest 2004;34:429–35.
25. Hashimoto T, Fujita T, Usuda N, et al. Peroxisomal and mitochondrial fatty acid β-oxidation in mice nullizygous for both peroxisome proliferator-activated receptor alpha and peroxisomal fatty acyl-CoA oxidase. Genotype correlation with fatty liver phenotype. J Biol Chem 1999;274:19228–36.
26. Flavell DM, Pineda Torra I, Jamshidi Y, et al. Variation in the PPAR alpha gene is associated with altered function in vitro and plasma lipid concentrations in type II diabetic subjects. Diabetologia 2000;43:673–80.
27. Vohl MC, Lepage P, Gaudet D, et al. Molecular scanning of the human PPAR alpha gene: association of the L162V mutation with hyperapobetalipoproteinemia. J Lipid Res 2000;41:945–52.
28. Tai ES, Demissie S, Cupples LA, et al. Association between the PPAR alpha L162V polymorphism and plasma lipid levels. The Framingham offspring study. Arterioscler Thromb Vasc Biol 2002;22:805–10.
29. Robitaille J, Brouillette C, Houde A, et al. Association between PPAR alpha-L162V polymorphism and components of the metabolic syndrome. J Hum Genet 2004;49:482–9.

30. Evans D, Aberle J, Wendt D, et al. A polymorphism, L162V, in the PPAR alpha gene is associated with lower body mass index in patients with non-insulin-dependent diabetes mellitus. J Mol Med 2001;79:198–204.
31. Ramalho F. Hepatitis C viral infection and liver steatosis. Antiviral Res 2003;60:125–7.
32. Hezode C, Roudot-Thoraval F, Zafrani ES, et al. Different mechanisms of steatosis in hepatitis C virus genotypes 1 and 3 infections. Viral Hepat 2004;11:455–8.
33. Bozdayi AM, Aslan N, Bozdayi G, et al. Molecular epidemiology of hepatitis B, C and D viruses. Arch Virol 2004;149:2115–29.
34. Knodell RG, Ishak KG, Black WC, et al. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis. Hepatology 1981;1:431–5.
35. Brunt EM, Janney CG, Di Bisceglie AM, et al. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol 1999;94:2467–74.
36. Gouni-Berthold I, Giannakidou E, Muller-Wieland D, et al. Association between the PPAR alpha L162V polymorphism, plasma lipoprotein levels, and atherosclerotic disease in patients with diabetes mellitus type 2 and in non-diabetic controls. Am Heart J 2004;147:1117–24.
37. Lonardo A, Adinolfi LE, Loria P, et al. Steatosis and hepatitis C virus: mechanisms and significance for hepatic and extrahepatic disease. Gastroenterology 2004;126:586–97.
38. Ratziu V, Trabut JB, Poynard T. Fat, diabetes and liver injury in chronic hepatitis C. Curr Gastroenterol Rep 2004;6:22–9.