**Name of Journal: World Journal of Gastroenterology**

**ESPS Manuscript NO: 23874**

**Manuscript Type: ORIGINAL ARTICLE**

***Basic Study***

**phosphatase and tensin homolog is a differential diagnostic marker between nonalcoholic and alcoholic fatty liver disease**

Sanchez-Pareja A *et al*. PTEN and alcoholic fatty liver disease

Andrea Sanchez-Pareja, Sophie Clément, Marion Peyrou, Laurent Spahr, Francesco Negro, Laura Rubbia-Brandt, Michelangelo Foti

**Andrea Sanchez-Pareja, Sophie Clément, Francesco Negro, Laura Rubbia-Brandt,** Division of clinical Pathology, University Hospital of Geneva, 1211 Geneva, Switzerland

**Laurent Spahr, Francesco Negro,** Division of Gastroenterology and Hepatology, University Hospital of Geneva, 1211 Geneva, Switzerland

**Marion Peyrou, Michelangelo Foti,** Department of Cellular Physiology and Metabolism, Faculty of Medicine, University of Geneva, 1211 Geneva, Switzerland

**Marion Peyrou, Michelangelo Foti,** Diabetes Center, Faculty of Medicine, University of Geneva, 1211 Geneva, Switzerland

**Author contributions**: Rubbia-Brandt L and Foti M contributed equally to this work; Sanchez-Pareja A and Clément S acquired the data; Sanchez-Pareja A, Clément S, Peyrou M, Negro F, Rubbia-Brandt L and Foti M analyzed and interpreted the data; Spahr L and Rubbia-Brandt L provided material; Sanchez-Pareja A, Clément S and Foti M wrote the manuscript; Peyrou M, Spahr L, Negro F, Rubbia-Brandt L and Foti M critically revised the manuscript for important intellectual content; Rubbia-Brandt L and Foti M designed the study; Negro F and Foti M obtained funding.

**supported by** the Swiss National Science Foundation, No.314730-146991 (to Negro F); and the Swiss National Science Foundation, No. 310030-152618 and No. CRSII3-160717 (to Foti M).

**Institutional review board statement:** This work was approved by the Ethics Committee of the Geneva University Hospital, Geneva, Switzerland.

**Institutional animal care and use committee statement:** No animal experimentation was performed in this work.

**Conflict-of-interest statement:** None related to the content of this article.

**Data sharing statement:** No additional unpublished data are available.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: http://creativecommons.org/licenses/by-nc/4.0/

**correspondence to**: **Michelangelo Foti, Professor,** Department of Cellular Physiology and Metabolism, Faculty of Medicine, University of Geneva, Centre Médical Universitaire, 1 rue Michel-Servet, 1211 Geneva, Switzerland. michelangelo.foti@unige.ch

**Telephone:** +41-22-3795204

**Fax:** +41-22-3795260

**Received:** December 21, 2015

**Peer-review started:** December 22, 2015

**First decision:** January 13, 2016

**Revised:** January 28, 2016

**Accepted:**March 1, 2016

**Article in press:**

**Published online:**

**Abstract**

**AIM:** To investigate the protein expression of phosphatase and tensin homolog (PTEN) in human liver biopsies of patients with alcoholic and non-alcoholic liver disease.

**METHODS:** PTEN protein expression was assessed by immunohistochemistry in formalin-fixed, paraffin-embedded liver sections of patients with non-alcoholic fatty liver disease (NAFLD) (*n =* 43) or alcoholic liver disease (ALD) (*n =* 25). Liver resections obtained from 3 healthy subjects candidate for partial liver donation served as controls. Histological evaluations were performed by two experienced pathologists, and diagnoses established based on international criteria. The intensity of the PTEN staining in nuclei was compared between steatotic and non-steatotic areas of each liver fragment analyzed. For each liver specimen, the antibody-stained sections were examined and scored blindly by three independent observers, who were unaware of the patients’ clinical history.

**RESULTS:** In healthy individuals, PTEN immunostaining was intense in both the cytoplasm and nuclei of all hepatocytes. However, PTEN was strongly downregulated in both the nucleus and the cytoplasm of hepatocytes from steatotic areas in patients with NAFLD, independently of the disease stage. In contrast, no changes in PTEN protein expression were observed in patients with ALD, regardless of the presence of steatosis or the stage of the disease. The degree of PTEN downregulation in hepatocytes of patients with NAFLD correlated with the percentage of steatosis (*r =* 0.3061; *p =* 0.0459) and the BMI (*r =* 0.4268; *p =* 0.0043. Hovewer, in patients with ALD, PTEN expression was not correlated with the percentage of steatosis with or without obesity as a confounding factor (*p =* 0.5574). Finally, PTEN expression level in steatotic areas of ALD patients was significantly different from that seen in steatotic areas of NAFLD patients (*p <* 0.0001).

**CONCLUSION:** PTEN protein expression is downregulated early in NAFLD, but not in ALD. PTEN immunohistochemical detection could help in the differential diagnosis of NAFLD and ALD.

**Key words**: Phosphatase and tensin homolog; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Alcoholic liver disease; Steatosis; Fibrosis; Cirrhosis; Hepatocellular carcinoma

**© The Author(s) 2016.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip:**Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) display similar histopathological features making difficult to discriminate between them apart from patient history.In this report, we assessed the phosphatase and tensin homolog (PTEN) expression level by immunohistochemistry and observed that while PTEN was downregulated in steatotic hepatocytes from patients with NAFLD, its expression remained unchanged in patients with ALD.We therefore propose that the evaluation of PTEN expression could be a useful tool for the differential diagnosis of NAFLD and ALD.

Sanchez-Pareja A, Clément S, Peyrou M, Spahr L, Negro F, Rubbia-BrandtL, Foti M. phosphatase and tensin homolog is a differential diagnostic marker between nonalcoholic and alcoholic fatty liver disease. *World J Gastroenterol* 2016; In press

**Introduction**

Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) are two major causes of chronic liver disease worldwide[1]. Both conditions display very similar histopathological features ranging from excessive accumulation of fat in the liver (steatosis) to steatohepatitis, fibrosis and cirrhosis[2].Development of hepatocellular carcinoma (HCC) is a severe complication of both liver diseases.Liver biopsy is the gold standard method to confirm the diagnosis and to evaluate the degree of necroinflammatory activity and the stage of fibrosis. For the NAFLD, a scoring system (NAFLD activity score or NAS) may be useful to identify patients who are at risk to develop cirrhosis[3]. However, the differential diagnosis between ALD and NAFLD cannot be made on the basis of histological criteria alone, as it relies on patient’s reported alcohol consumption, which is subject to the well-known self-reporting biases.

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a major tumor suppressor frequently mutated/deleted or downregulated in human cancers including HCC[4,5]. The tumor suppressive activity of PTEN appears to rely on multiple functions of this protein, including antagonism of PI3K signaling in response to growth factors[6], modulation of the activity of critical factors regulating cell adhesion, migration and invasion[7-9], and maintenance of chromosome stability and DNA repair[10]. In addition, the expression or activity of a whole network of other tumour suppressors frequently altered in human cancers can be modulated by dysregulated PTEN expression or activity[11]. Importantly, recent evidence indicated that in contrast to the classical “two hits” Knudson’s hypothesis for tumour suppressors, PTEN haploinsufficiency, or even a partial loss of its activity/expression through post-transcriptional alterations, is sufficient to promote carcinogenesis in specific organs[12].

Conditional knockout of PTEN specifically in hepatocytes was shown to induce the sequential development of steatosis, fibrosis and HCC with ageing in mice[13]. Conversely, we previously demonstrated that PTEN expression/activity was downregulated in the liver of obese human and rodent models developing hepatic steatosis[14]. Further analyses revealed that PTEN expression was downregulated in cultured hepatocytes exposed to free fatty acids[15]. Together, these data indicated that alterations of PTEN expression/activity represent an early event in NAFLD likely contributing to steatosis development. Surprisingly, studies in rodents, or using *in vitro* cell cultures, indicated that hepatic PTEN expression might be not decreased, but rather increased, with abusive alcohol consumption although histopathological alterations of the liver are very similar to those occurring in NAFLD[16-19]. However, whether these findings are relevant for human diseases is still unclear and the PTEN expression and activity status in the liver of patients with ALD is unknown.

Based on the above observations, we hypothesized that PTEN expression level in the liver may allow discriminating between NAFLD and ALD. Thus, we investigated the expression of PTEN by immunohistochemistry in liver biopsy samples from patients displaying all histopathological stages of NAFLD and ALD. Our results demonstrate that assessing PTEN protein expression in human livers may represent an important tool for the differential diagnosis of NAFLD and ALD.

**Materials and Methods**

***Human samples***

Liver biopsy samples were collected between 2006 and 2015 from patients consecutively admitted at the Division of Gastroenterology and Hepatology of Geneva University Hospitals for investigation of abnormal liver function tests and fulfilling the diagnostic criteria of NAFLD or ALD[20]. Overall, a total of 69 patients were studied, including 43 obese patients with NAFLD, 17 non-obese patients with ALD and 8 obese patients with ALD. All patients had a negative serology for hepatitis B and C, tested negative for autoantibodies, have normal -antitrypsin and ceruloplasmin serum levels and normal transferrin saturation. No patients were under medications that may induce steatohepatitis-like lesions. Patients with NAFLD were obese (Median BMI 41) and consume less than 10 gr of alcohol per day. Patients with ALD had a median BMI of 26 and declared daily alcohol intake above or equal to 40 gr for women and 60 gr for men at the time of liver biopsy. Liver resections obtained from 3 healthy subjects candidate for partial liver donation served as controls. Demographic data, including age, sex, body mass index (BMI) and alcohol consumption, were collected at the time of liver biopsy.

***Histology and immunohistochemistry***

Liver biopsies were formalin-fixed, paraffin-embedded and processed for histological staining. Histological evaluations were performed by two experienced pathologists, and diagnoses established based on international criteria[20]. NAFLD activity and fibrosis were graded using the NAS score[21]. Steatosis severity was scored as follows: 0 (none) ≤ 2%, 1 (mild) = 2%-30%, 2 (moderate) = 30%-60% and 3 (severe) ≥ 60% of hepatocytes affected.

All biopsy samples were processed together for the HE staining and PTEN immunohistological staining. Immunohistochemical analysis of PTEN expression was performed as previously described[14].

The intensity of the PTEN staining in nuclei was compared between steatotic (S) and non-steatotic (NS) areas of each liver fragment analyzed. Both S and NS areas from all slides were scored on a staining intensity scale from 0 to 4 with score = 0 for lack of staining, score = 1 for weak staining, score = 2 for moderate staining, score = 3 for strong staining and score = 4 representing the highest intensity. Then, the difference between NS and S areas (NS-S) was calculated. For each liver specimen, the antibody-stained sections were examined and scored blindly by three independent observers, who were unaware of the patients’ clinical history.

***Statistical analyses***

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Turkey test for continuous variables and nonparametric Spearman correlation for categorical variables.

**Results**

***PTEN expression is downregulated in steatotic hepatocytes from patients with NAFLD***

PTEN expression was investigated in 43 patients with NAFLD at different stages of disease (steatosis *n =* 10, steatohepatitis without fibrosis *n =* 14, steatohepatitis with fibrosis *n =* 12 and cirrhosis *n =* 7) and compared to liver biopsy specimens from 3 healthy donors. In healthy individuals (Figure 1a and b), PTEN immunostaining was intense in both the cytoplasm and nuclei of all hepatocytes (Figure 1a and b). In the liver biopsies of patients with NAFLD, a significant decrease of PTEN expression was observed in steatotic hepatocytes, or in those in their close vicinity. Importantly, non-steatotic areas within the same specimen displayed similar immunostaining intensity than those of healthy controls, suggesting that PTEN expression was altered only in steatotic areas of NAFLD patients. Decrease of PTEN expression occurred in both the nucleus and the cytoplasm of hepatocytes. Nevertheless, only the nuclear staining was used to establish the PTEN score in order to prevent misevaluation of cytoplasmic PTEN staining potentially corrupted by the presence of large and unstained cytoplasmic lipid droplets in steatotic areas. When patients were divided according to the NAFLD stage, the expression of PTEN in steatotic areas was decreased in 9/10 patients with steatosis only (Figure 1c and d, Table 1), in 12/14 patients with steatosis and inflammatory infiltrate (Figure 1e and f, Table 1), in 11/12 patients with fibrosis (Figure 1g and h, Table 1) and 6/8 patients with cirrhosis (Figure 1i and j, Table 1). The degree of PTEN downregulation in hepatocytes of patients with NAFLD only weakly correlated with the percentage of steatosis (*r =* 0.3061; *p =* 0.0459) and the BMI (*r =* 0.4268; *p =* 0.0043), but not with the NAS score (*p =* 0.3061), sex (*p =* 0.3583), age (*p =* 0.7931), fibrosis stage (*p =* 0.7235), degree of lobular inflammation (*p =* 0. 9822) or hepatocyte ballooning (*p =* 0.9728).

***Steatosis in patients with ALD does not alter PTEN expression in hepatocytes***

PTEN expression was then performed on paraffin-embedded liverspecimens from 25 patients with ALD at different stages of disease (all had steatosis, 15 had inflammation, 13 had different degrees of fibrosis and 21 had cirrhosis) (Table 2). In contrast to NAFLD patients, the presence of steatosis in liver samples of patients with ALD was not associated with a significant decrease of PTEN expression. Indeed, the intensity of PTEN immunostaining was similar in steatotic and non-steatotic areas of each liver biopsy, unaffected by the stage of the disease or obesity, and always comparable to the PTEN staining of healthy liver controls (Figure 2). PTEN expression was therefore not correlated with the percentage of steatosis in patients with ALD with or without obesity as a confounding factor (*p =* 0.5574). Finally, the PTEN expression level in steatotic areas of ALD patients was significantly different from that seen in steatotic areas of NAFLD patients (*p <* 0.0001).

**Discussion**

NAFLD and ALD are two frequent liver diseases that require each a specific clinical management[22]. Non-alcoholic steatohepatitis is primarily managed by lifestyle interventions, *i.e.,* body weight loss combined to increased physical activity[22-24] associated with selected insulin sensitizers[22,25] and vitamin E administration[22,26]. Patients with ALD require complete alcohol abstinence, and, in severe forms of steatohepatitis, a short course of steroids, which has been proven effective in reducing mortality[27]. Unfortunately, the differential diagnosis between NAFLD and ALD may be difficult, owing to the bias intrinsic to the self-reported nature of alcohol consumption. In addition, both NAFLD – in its steatohepatitis form – and ALD share similar histological features. However, macrovesicular steatosis is reportedly more frequent and severe in NAFLD than in ALD, whereas microvesicular steatosis, lobular inflammation, Mallory-Denk bodies, satellitosis, acute cholestasis, sclerosing hyaline necrosis and veno-occlusive disease occur more frequently in ALD than in non-alcoholic steatohepatitis[28]. Nevertheless, all these histological features remain non-specific. Although one study suggested a disease-specific pattern of fibrosis silver impregnation (*i.e.,* lattice-like in non-alcoholic steatohepatitis and solid in ALD[29], a molecular marker easy to use and able to discriminate between NAFLD and ALD has not been identified yet. Here, we show the potential role of PTEN immunostaining as a differential diagnostic marker to discriminate between ALD and NAFLD. The PTEN expression was found to be decreased in steatotic areas of the liver of patients with NAFLD, while being preserved in patients with ALD, irrespectively of the presence of obesity, steatosis and/or the liver disease stage. However, it has to be acknowledged that our group of patients with ALD is small and somewhat heterogeneous with regards to fibrosis and inflammation. Nevertheless, if our results are confirmed at a largest scale, PTEN immunostaining could be a valuable routine diagnostic tool for differentiating ALD and NAFLD therefore helping the decision making process in the clinical management of these patients.

The mechanisms of lipid accumulation in NAFLD and ALD are only partially known: some appear to be shared, while others seem to follow disease-specific pathways. The unfolded protein response is activated in both NAFLD and ALD by oxidative stress, and leads to the increase of transcriptional factors, such as the sterol regulatory element binding proteins, involved in *de novo* fatty acid synthesis[30,31]. Adiponectin, an adipokine produced by adipocytes that stimulates fatty acid oxidation, is downregulated in both NAFLD[32] and ALD[33]. On the other hand, some mechanisms contributing to the development of steatosis might be disease-specific. For example, an augmented lipolysis in the adipose tissue leading to increased circulating free fatty acids has been reported to occur mainly in NALFD but not in ALD[30,34-36], although this difference has been recently challenged in rodent models[37]. Dysregulated immune mechanisms may also play an important role in the early events and progression of alcohol-related liver injuries[38].

Increasing evidence currently supports a key role for PTEN in the development of steatosis and fibrosis with distinct etiologies. First, mice bearing a hepatocyte-specific deletion of PTEN develop sequentially an extended hepatic steatosis, inflammation, fibrosis and HCC with ageing[13,39]. *In vitro* studies also showed that PTEN downregulation triggers lipid accumulation in hepatoma cell lines[14,40].Consistent with these studies, PTEN expression is decreased in steatotic livers of genetic and diet-induced obese rat models and in obese human subjects[14]. Hepatic PTEN downregulation was further reported in three different rodent models of liver inflammation/fibrosis induced either by a methionine/choline deficient diet[41], or bile-duct ligation[42] or CCL4 administration[43]. In hepatitis C virus (HCV) infection, PTEN expression and/or activity were also reported to be altered in hepatocytes and to promote the development of steatosis[44]. PTEN downregulation was even reported to represent an independent prognostic factor for the survival of HCV-infected patients developing a cirrhotic hepatocellular carcinoma (HCC)[45]. It thus appears that steatosis and its progression towards more severe hepatic disorders are associated with PTEN protein downregulation in the case of the metabolic syndrome and HCV infection.

The observation linking PTEN downregulation with liver disease progression in NAFLD and hepatitis C may have implications for treatment. Regular statin use has been shown to upregulate PTEN in heart tissue[46,47], skeletal muscles[48] and cancer cells[49-51] through various mechanisms including decreased expression of PTEN-targeting microRNAs[52], NFκB inactivation[49] and PPAR activation[50,51]. Regarding the relationship between statins and liver disease progression, the few data in NAFLD suggests some beneficial effect, at least on steatosis[53]. More convincing evidence has been reported in patients with chronic hepatitis C, where statin use was associated with a significantly lower liver fibrosis progression, independently of inflammation and viral load changes over time[54-56]. Thus, one cannot exclude that the beneficial effects of statins may be mediated by PTEN upregulation in the liver with NAFLD or HCV infection, however direct evidence are currently lacking. Obviously, the long-term use of statins in these conditions should also be weighed against the risk of toxicity, in particular concerning the increased risk of insulin resistance and type 2 diabetes development, which can results from PTEN upregulation in skeletal muscles[48,57,58].

Surprisingly, PTEN protein expression was reported increased in hepatoma cells exposed to ethanol[18] and in the liver of rats chronically fed with ethanol[16]. However, although PTEN expression is upregulated in the liver of ethanol-fed mice, the enzyme was shown to be highly carbonylated, a post-translational modification that decreases its phosphatase activity[59]. From these data, it results that steatosis in ALD is also associated in final with an impaired PTEN activity, although this defect is mediated by inhibition of its enzymatic activity and not by repression of its expression as it is the case with obesity and HCV infection.

The mechanisms leading to PTEN protein upregulation in hepatocytes exposed to alcohol are currently unknown. One attractive hypothesis is that PTEN transcription is stimulated by the transcription factor Egr1 in response to alcohol[60,61]. However, assessing whether PTEN expression is modulated at the mRNA level in human livers is technically challenging. Indeed, isolation of good quality mRNA from human liver biopsies processed for histological analysis is still poorly effective and cannot be used in routine diagnostic procedures. In addition, the high expression of a PTEN pseudogene (PTENP1) in human tissues can lead to misinterpretation of analyses aiming at assessing PTEN mRNA expression in pathological situations[62]. This is the reason why immunohistochemical detection of PTEN protein expression in clinical samples remains likely the gold standard method to assess PTEN expression in liver biopsies.

Our data and previous reports discussed here strongly support the concept that downregulation of either the expression, or the activity, of PTEN represents an important pathological mechanism contributing to the development of fatty liver diseases with distinct etiologies. However the mechanism affecting PTEN functions are different depending on the etiologies of the diseases. In this study, we show that we can take advantage of the different molecular mechanisms affecting PTEN activity or expression in liver metabolic disorders to identify their etiology. Thus, theimmunohistochemical detection of PTEN protein expression should be added to the diagnostic armamentarium of pathologists and clinicians in the differential diagnosis of NAFLD and ALD in humans.

**comments**

***Background***

Similar histopathological alterations occur in patients suffering from non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). Therefore, the differential diagnosis between ALD and NAFLD cannot be made on the basis of histological criteria alone, and it must rely on patient’s reported alcohol consumption, which is subject to the well-known self-reporting biases.Studies in rodents, or *in vitro*, indicated that hepatic phosphatase and tensin homolog (PTEN) expression is decreased with obesity-associated steatosis, whereas it is increased with abusive alcohol consumption, although histopathological alterations of the liver are very similar. However, whether these findings are relevant for human diseases is still unclear and the PTEN expression and activity status in the liver of patients with ALD is unknown.

***Research frontiers***

Biomarker research in liver diseases is of growing importance not only to provide reliable differential diagnostic markers for therapeutic choices, but also as prognostic factors for progression of early stages of these diseases toward more severe pathologies such as cirrhosis and cancer.

***Innovations and breakthroughs***

Studies in rodents, or *in vitro*, indicate that alteration of PTEN function is a common event occurring at early stage of NAFLD, hepatitis C virus (HCV) infection and ALD. However, the molecular mechanisms underlining the loss of PTEN function are different, since they consist of the PTEN protein downregulation in NAFLD and HCV infection, whereas in ALD only PTEN activity is impaired but not its expression. In this study, the authors reported the differential expression of the PTEN protein in the liver of patients with NAFLD or ALD at various stages of these diseases, a finding of diagnostic significance.

***Applications***

Herein, the authors show that the authors can take advantage of the different molecular mechanisms affecting PTEN expression in liver metabolic disorders to identify their etiology. Thus, theimmunohistochemical detection of PTEN protein expression should be added to the diagnostic armamentarium of pathologists and clinicians in the differential diagnosis of NAFLD and ALD in humans.

***Terminology***

The “two hits” Knudson’s hypothesis for tumour suppressors suggests that each allele of a tumour suppressor needs to be mutated, or deleted, to contribute to carcinogenesis, since one copy of a tumour suppressor is sufficient to ensure its function.Mallory-Denk bodies are damaged eosinophilic filamentous material forming inclusions in the cytoplasm of hepatocytes, in both ALD and NAFLD.

***Peer-review***

The article is very interesting and useful for clinicians. The small number of patients included in the study is a limit recognized by the authors, but most studies requiring liver biopsy in these types of pathology are difficult to achieve in a single center. A multicenter future study would be useful in this regard. Authors must underline the clinical implications of their research and add to discussion a comment on the beneficial role of statins, with regard to PTEN expression, in nonalcoholic fatty liver disease and in chronic hepatitis C.

**References**

1 **Hellerbrand C**. Pathophysiological similarities and synergisms in alcoholic and non-alcoholic steatohepatitis. *Dig Dis* 2010; **28**: 783-791 [PMID: 21525763 DOI: 10.1159/000324286]

2 **Tannapfel A**, Denk H, Dienes HP, Langner C, Schirmacher P, Trauner M, Flott-Rahmel B. [Histopathological diagnosis of non-alcoholic and alcoholic fatty liver disease. Grade 2 consensus-based guidelines]. *Pathologe* 2010; **31**: 225-237 [PMID: 20221762 DOI: 10.1007/s00292-010-1298-x]

3 **Brunt EM**, Kleiner DE, Wilson LA, Belt P, Neuschwander-Tetri BA. Nonalcoholic fatty liver disease (NAFLD) activity score and the histopathologic diagnosis in NAFLD: distinct clinicopathologic meanings. *Hepatology* 2011; **53**: 810-820 [PMID: 21319198 DOI: 10.1002/hep.24127]

4 **Peyrou M**, Bourgoin L, Foti M. PTEN in liver diseases and cancer. *World J Gastroenterol* 2010; **16**: 4627-4633 [PMID: 20872961 DOI: 10.3748/wjg.v16.i37.4627]

5 **Peyrou M**, Bourgoin L, Foti M. PTEN in non-alcoholic fatty liver disease/non-alcoholic steatohepatitis and cancer. *Dig Dis* 2010; **28**: 236-246 [PMID: 20460918 DOI: 10.1159/000282095]

6 **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 [PMID: 9593664 DOI: 10.1074/jbc.273.22.13375]

7 **Zhang XC**, Piccini A, Myers MP, Van Aelst L, Tonks NK. Functional analysis of the protein phosphatase activity of PTEN. *Biochem J* 2012; **444**: 457-464 [PMID: 22413754 DOI: 10.1042/BJ20120098]

8 **Davidson L**, Maccario H, Perera NM, Yang X, Spinelli L, Tibarewal P, Glancy B, Gray A, Weijer CJ, Downes CP, Leslie NR. Suppression of cellular proliferation and invasion by the concerted lipid and protein phosphatase activities of PTEN. *Oncogene* 2010; **29**: 687-697 [PMID: 19915616 DOI: 10.1038/onc.2009.384]

9 **Tamura M**, Gu J, Matsumoto K, Aota S, Parsons R, Yamada KM. Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN. *Science* 1998; **280**: 1614-1617 [PMID: 9616126 DOI: 10.1126/science.280.5369.1614]

10 **Planchon SM**, Waite KA, Eng C. The nuclear affairs of PTEN. *J Cell Sci* 2008; **121**: 249-253 [PMID: 18216329 DOI: 10.1242/jcs.022459]

11 **Leslie NR**, Foti M. Non-genomic loss of PTEN function in cancer: not in my genes. *Trends Pharmacol Sci* 2011; **32**: 131-140 [PMID: 21236500 DOI: 10.1016/j.tips.2010.12.005]

12 **Berger AH**, Knudson AG, Pandolfi PP. A continuum model for tumour suppression. *Nature* 2011; **476**: 163-169 [PMID: 21833082 DOI: 10.1038/nature10275]

13 **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 [PMID: 15199412 DOI: 10.1172/JCI20513]

14 **Vinciguerra M**, Veyrat-Durebex C, Moukil MA, Rubbia-Brandt L, Rohner-Jeanrenaud F, Foti M. PTEN down-regulation by unsaturated fatty acids triggers hepatic steatosis via an NF-kappaBp65/mTOR-dependent mechanism. *Gastroenterology* 2008; **134**: 268-280 [PMID: 18166358 DOI: 10.1053/j.gastro.2007.10.010]

15 **Vinciguerra M**, Sgroi A, Veyrat-Durebex C, Rubbia-Brandt L, Buhler LH, Foti M. Unsaturated fatty acids inhibit the expression of tumor suppressor phosphatase and tensin homolog (PTEN) via microRNA-21 up-regulation in hepatocytes. *Hepatology* 2009; **49**: 1176-1184 [PMID: 19072831 DOI: 10.1002/hep.22737]

16 **Yeon JE**, Califano S, Xu J, Wands JR, De La Monte SM. Potential role of PTEN phosphatase in ethanol-impaired survival signaling in the liver. *Hepatology* 2003; **38**: 703-714 [PMID: 12939597 DOI: 10.1053/jhep.2003.50368]

17 **Yao XH**, Grégoire Nyomba BL. Abnormal glucose homeostasis in adult female rat offspring after intrauterine ethanol exposure. *Am J Physiol Regul Integr Comp Physiol* 2007; **292**: R1926-R1933 [PMID: 17218436 DOI: 10.1152/ajpregu.00822.2006]

18 **Shulga N**, Hoek JB, Pastorino JG. Elevated PTEN levels account for the increased sensitivity of ethanol-exposed cells to tumor necrosis factor-induced cytotoxicity. *J Biol Chem* 2005; **280**: 9416-9424 [PMID: 15623531 DOI: 10.1074/jbc.M409505200]

19 **He J**, de la Monte S, Wands JR. Acute ethanol exposure inhibits insulin signaling in the liver. *Hepatology* 2007; **46**: 1791-1800 [PMID: 18027876 DOI: 10.1002/hep.21904]

20 **Chalasani N**, Younossi Z, Lavine JE, Diehl AM, Brunt EM, Cusi K, Charlton M, Sanyal AJ. The diagnosis and management of non-alcoholic fatty liver disease: practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. *Hepatology* 2012; **55**: 2005-2023 [PMID: 22488764 DOI: 10.1002/hep.25762]

21 **Kleiner DE**, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, Ferrell LD, Liu YC, Torbenson MS, Unalp-Arida A, Yeh M, McCullough AJ, Sanyal AJ. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. *Hepatology* 2005; **41**: 1313-1321 [PMID: 15915461 DOI: 10.1002/hep.20701]

22 **Spahr L**, Hadengue A. [Alcoholic and nonalcoholic steatohepatitis: the same disease! II. Management]. *Rev Med Suisse* 2005; **1**: 2032-2034 [PMID: 16212005]

23 **Huang MA**, Greenson JK, Chao C, Anderson L, Peterman D, Jacobson J, Emick D, Lok AS, Conjeevaram HS. One-year intense nutritional counseling results in histological improvement in patients with non-alcoholic steatohepatitis: a pilot study. *Am J Gastroenterol* 2005; **100**: 1072-1081 [PMID: 15842581 DOI: 10.1111/j.1572-0241.2005.41334.x]

24 **Hickman IJ**, Jonsson JR, Prins JB, Ash S, Purdie DM, Clouston AD, Powell EE. Modest weight loss and physical activity in overweight patients with chronic liver disease results in sustained improvements in alanine aminotransferase, fasting insulin, and quality of life. *Gut* 2004; **53**: 413-419 [PMID: 14960526 DOI: 10.1136/gut.2003.027581]

25 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Zoli M, Melchionda N. Metformin in non-alcoholic steatohepatitis. *Lancet* 2001; **358**: 893-894 [PMID: 11567710 DOI: 10.1016/S0140-6736(01)06042-1]

26 **Harrison SA**, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 2485-2490 [PMID: 14638353 DOI: 10.1111/j.1572-0241.2003.08699.x]

27 **Mathurin P**, Mendenhall CL, Carithers RL, Ramond MJ, Maddrey WC, Garstide P, Rueff B, Naveau S, Chaput JC, Poynard T. Corticosteroids improve short-term survival in patients with severe alcoholic hepatitis (AH): individual data analysis of the last three randomized placebo controlled double blind trials of corticosteroids in severe AH. *J Hepatol* 2002; **36**: 480-487 [PMID: 11943418 DOI: 10.1016/S0168-8278(01)00289-6]

28 **Tannapfel A**, Denk H, Dienes HP, Langner C, Schirmacher P, Trauner M, Flott-Rahmel B. Histopathological diagnosis of non-alcoholic and alcoholic fatty liver disease. *Virchows Arch* 2011; **458**: 511-523 [PMID: 21442288 DOI: 10.1007/s00428-011-1066-1]

29 **Nakano M**, Fukusato T. Histological study on comparison between NASH and ALD. *Hepatol Res* 2005; **33**: 110-115 [PMID: 16219486 DOI: 10.1016/j.hepres.2005.09.016]

30 **Sozio MS**, Liangpunsakul S, Crabb D. The role of lipid metabolism in the pathogenesis of alcoholic and nonalcoholic hepatic steatosis. *Semin Liver Dis* 2010; **30**: 378-390 [PMID: 20960377 DOI: 10.1055/s-0030-1267538]

31 **Foufelle F**, Ferré P. New perspectives in the regulation of hepatic glycolytic and lipogenic genes by insulin and glucose: a role for the transcription factor sterol regulatory element binding protein-1c. *Biochem J* 2002; **366**: 377-391 [PMID: 12061893 DOI: 10.1042/bj20020430]

32 **Tsochatzis EA**, Papatheodoridis GV, Archimandritis AJ. Adipokines in nonalcoholic steatohepatitis: from pathogenesis to implications in diagnosis and therapy. *Mediators Inflamm* 2009; **2009**: 831670 [PMID: 19753129]

33 **Tang H**, Sebastian BM, Axhemi A, Chen X, Hillian AD, Jacobsen DW, Nagy LE. Ethanol-induced oxidative stress via the CYP2E1 pathway disrupts adiponectin secretion from adipocytes. *Alcohol Clin Exp Res* 2012; **36**: 214-222 [PMID: 21895711 DOI: 10.1111/j.1530-0277.2011.01607.x]

34 **Tamura S**, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1139-1142 [PMID: 15864343 DOI: 10.1172/JCI24930]

35 **Sozio M**, Crabb DW. Alcohol and lipid metabolism. *Am J Physiol Endocrinol Metab* 2008; **295**: E10-E16 [PMID: 18349117 DOI: 10.1152/ajpendo.00011.2008]

36 **Westerbacka J**, Lammi K, Häkkinen AM, Rissanen A, Salminen I, Aro A, Yki-Järvinen H. Dietary fat content modifies liver fat in overweight nondiabetic subjects. *J Clin Endocrinol Metab* 2005; **90**: 2804-2809 [PMID: 15741262 DOI: 10.1210/jc.2004-1983]

37 **Zhong W**, Zhao Y, Tang Y, Wei X, Shi X, Sun W, Sun X, Yin X, Sun X, Kim S, McClain CJ, Zhang X, Zhou Z. Chronic alcohol exposure stimulates adipose tissue lipolysis in mice: role of reverse triglyceride transport in the pathogenesis of alcoholic steatosis. *Am J Pathol* 2012; **180**: 998-1007 [PMID: 22234172 DOI: 10.1016/j.ajpath.2011.11.017]

38 **Bode C**, Bode JC. Activation of the innate immune system and alcoholic liver disease: effects of ethanol per se or enhanced intestinal translocation of bacterial toxins induced by ethanol? *Alcohol Clin Exp Res* 2005; **29**: 166S-171S [PMID: 16344604 DOI: 10.1097/01.alc.0000189280.19073.28]

39 **Stiles B**, Wang Y, Stahl A, Bassilian S, Lee WP, Kim YJ, Sherwin R, Devaskar S, Lesche R, Magnuson MA, Wu H. Liver-specific deletion of negative regulator Pten results in fatty liver and insulin hypersensitivity [corrected]. *Proc Natl Acad Sci USA* 2004; **101**: 2082-2087 [PMID: 14769918 DOI: 10.1073/pnas.0308617100]

40 **Peyrou M**, Clément S, Maier C, Bourgoin L, Branche E, Conzelmann S, Kaddai V, Foti M, Negro F. PTEN protein phosphatase activity regulates hepatitis C virus secretion through modulation of cholesterol metabolism. *J Hepatol* 2013; **59**: 420-426 [PMID: 23623999 DOI: 10.1016/j.jhep.2013.04.012]

41 **Wang B**, Majumder S, Nuovo G, Kutay H, Volinia S, Patel T, Schmittgen TD, Croce C, Ghoshal K, Jacob ST. Role of microRNA-155 at early stages of hepatocarcinogenesis induced by choline-deficient and amino acid-defined diet in C57BL/6 mice. *Hepatology* 2009; **50**: 1152-1161 [PMID: 19711427 DOI: 10.1002/hep.23100]

42 **Hao LS**, Zhang XL, An JY, Karlin J, Tian XP, Dun ZN, Xie SR, Chen S. PTEN expression is down-regulated in liver tissues of rats with hepatic fibrosis induced by biliary stenosis. *APMIS* 2009; **117**: 681-691 [PMID: 19703128 DOI: 10.1111/j.1600-0463.2009.02515.x]

43 **Zheng L**, Chen X, Guo J, Sun H, Liu L, Shih DQ, Zhang X. Differential expression of PTEN in hepatic tissue and hepatic stellate cells during rat liver fibrosis and its reversal. *Int J Mol Med* 2012; **30**: 1424-1430 [PMID: 23041795 DOI: 10.3892/ijmm.2012.1151]

44 **Clément S**, Peyrou M, Sanchez-Pareja A, Bourgoin L, Ramadori P, Suter D, Vinciguerra M, Guilloux K, Pascarella S, Rubbia-Brandt L, Negro F, Foti M. Down-regulation of phosphatase and tensin homolog by hepatitis C virus core 3a in hepatocytes triggers the formation of large lipid droplets. *Hepatology* 2011; **54**: 38-49 [PMID: 21465511 DOI: 10.1002/hep.24340]

45 **Rahman MA**, Kyriazanos ID, Ono T, Yamanoi A, Kohno H, Tsuchiya M, Nagasue N. Impact of PTEN expression on the outcome of hepatitis C virus-positive cirrhotic hepatocellular carcinoma patients: possible relationship with COX II and inducible nitric oxide synthase. *Int J Cancer* 2002; **100**: 152-157 [PMID: 12115563 DOI: 10.1002/ijc.10458]

46 **Mensah K**, Mocanu MM, Yellon DM. Failure to protect the myocardium against ischemia/reperfusion injury after chronic atorvastatin treatment is recaptured by acute atorvastatin treatment: a potential role for phosphatase and tensin homolog deleted on chromosome ten? *J Am Coll Cardiol* 2005; **45**: 1287-1291 [PMID: 15837263 DOI: 10.1016/j.jacc.2005.01.021]

47 **Ye Y**, Lin Y, Atar S, Huang MH, Perez-Polo JR, Uretsky BF, Birnbaum Y. Myocardial protection by pioglitazone, atorvastatin, and their combination: mechanisms and possible interactions. *Am J Physiol Heart Circ Physiol* 2006; **291**: H1158-H1169 [PMID: 16603698 DOI: 10.1152/ajpheart.00096.2006]

48 **Birnbaum Y**, Nanhwan MK, Ling S, Perez-Polo JR, Ye Y, Bajaj M. PTEN upregulation may explain the development of insulin resistance and type 2 diabetes with high dose statins. *Cardiovasc Drugs Ther* 2014; **28**: 447-457 [PMID: 25106875 DOI: 10.1007/s10557-014-6546-5]

49 **Ghosh-Choudhury N**, Mandal CC, Ghosh-Choudhury N, Ghosh Choudhury G. Simvastatin induces derepression of PTEN expression via NFkappaB to inhibit breast cancer cell growth. *Cell Signal* 2010; **22**: 749-758 [PMID: 20060890 DOI: 10.1016/j.cellsig.2009.12.010]

50 **Teresi RE**, Shaiu CW, Chen CS, Chatterjee VK, Waite KA, Eng C. Increased PTEN expression due to transcriptional activation of PPARgamma by Lovastatin and Rosiglitazone. *Int J Cancer* 2006; **118**: 2390-2398 [PMID: 16425225 DOI: 10.1002/ijc.21799]

51 **Teresi RE**, Planchon SM, Waite KA, Eng C. Regulation of the PTEN promoter by statins and SREBP. *Hum Mol Genet* 2008; **17**: 919-928 [PMID: 18065496 DOI: 10.1093/hmg/ddm364]

52 **Tu Y**, Wan L, Bu L, Zhao D, Dong D, Huang T, Cheng Z, Shen B. MicroRNA-22 downregulation by atorvastatin in a mouse model of cardiac hypertrophy: a new mechanism for antihypertrophic intervention. *Cell Physiol Biochem* 2013; **31**: 997-1008 [PMID: 23860036 DOI: 10.1159/000350117]

53 **Musso G**, Cassader M, Rosina F, Gambino R. Impact of current treatments on liver disease, glucose metabolism and cardiovascular risk in non-alcoholic fatty liver disease (NAFLD): a systematic review and meta-analysis of randomised trials. *Diabetologia* 2012; **55**: 885-904 [PMID: 22278337 DOI: 10.1007/s00125-011-2446-4]

54 **Simon TG**, King LY, Zheng H, Chung RT. Statin use is associated with a reduced risk of fibrosis progression in chronic hepatitis C. *J Hepatol* 2015; **62**: 18-23 [PMID: 25135867 DOI: 10.1016/j.jhep.2014.08.013]

55 **Yang YH**, Chen WC, Tsan YT, Chen MJ, Shih WT, Tsai YH, Chen PC. Statin use and the risk of cirrhosis development in patients with hepatitis C virus infection. *J Hepatol* 2015; **63**: 1111-1117 [PMID: 26196278 DOI: 10.1016/j.jhep.2015.07.006]

56 **Butt AA**, Yan P, Bonilla H, Abou-Samra AB, Shaikh OS, Simon TG, Chung RT, Rogal SS. Effect of addition of statins to antiviral therapy in hepatitis C virus-infected persons: Results from ERCHIVES. *Hepatology* 2015; **62**: 365-374 [PMID: 25847403 DOI: 10.1002/hep.27835]

57 **Sattar N**, Preiss D, Murray HM, Welsh P, Buckley BM, de Craen AJ, Seshasai SR, McMurray JJ, Freeman DJ, Jukema JW, Macfarlane PW, Packard CJ, Stott DJ, Westendorp RG, Shepherd J, Davis BR, Pressel SL, Marchioli R, Marfisi RM, Maggioni AP, Tavazzi L, Tognoni G, Kjekshus J, Pedersen TR, Cook TJ, Gotto AM, Clearfield MB, Downs JR, Nakamura H, Ohashi Y, Mizuno K, Ray KK, Ford I. Statins and risk of incident diabetes: a collaborative meta-analysis of randomised statin trials. *Lancet* 2010; **375**: 735-742 [PMID: 20167359 DOI: 10.1016/S0140-6736(09)61965-6]

58 **Wijesekara N**, Konrad D, Eweida M, Jefferies C, Liadis N, Giacca A, Crackower M, Suzuki A, Mak TW, Kahn CR, Klip A, Woo M. Muscle-specific Pten deletion protects against insulin resistance and diabetes. *Mol Cell Biol* 2005; **25**: 1135-1145 [PMID: 15657439 DOI: 10.1128/MCB.25.3.1135-1145.2005]

59 **Shearn CT**, Smathers RL, Backos DS, Reigan P, Orlicky DJ, Petersen DR. Increased carbonylation of the lipid phosphatase PTEN contributes to Akt2 activation in a murine model of early alcohol-induced steatosis. *Free Radic Biol Med* 2013; **65**: 680-692 [PMID: 23872024 DOI: 10.1016/j.freeradbiomed.2013.07.011]

60 **Donohue TM**, Osna NA, Trambly CS, Whitaker NP, Thomes PG, Todero SL, Davis JS. Early growth response-1 contributes to steatosis development after acute ethanol administration. *Alcohol Clin Exp Res* 2012; **36**: 759-767 [PMID: 22141421 DOI: 10.1111/j.1530-0277.2011.01681.x]

61 **Baron V**, Adamson ED, Calogero A, Ragona G, Mercola D. The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer Gene Ther* 2006; **13**: 115-124 [PMID: 16138117 DOI: 10.1038/sj.cgt.7700896]

62 **Dahia PL**, FitzGerald MG, Zhang X, Marsh DJ, Zheng Z, Pietsch T, von Deimling A, Haluska FG, Haber DA, Eng C. A highly conserved processed PTEN pseudogene is located on chromosome band 9p21. *Oncogene* 1998; **16**: 2403-2406 [PMID: 9620558 DOI: 10.1038/sj.onc.1201762]

**P-Reviewer:** Fierbinteanu-Braticevici C, Mihaila rg, Wisse e **S-Editor:** Ma YJ **L-Editor:** **E-Editor:**

**Table 1 Demographic data, histological diagnosis of liver biopsies and phosphatase and tensin homolog expression in patients with non-alcoholic fatty liver disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Case** | **Age/sex** | **BMI (kg/m2)** | **Lobular inflammation** | **Hepatocyte ballooning** | **Steatosis score** | **Steatosis %** | **Fibrosis** | **NAS score** | **Scores for PTEN expression** | | | | |
| **NS** | **S** | | **NS-S** | |
| Ctrl | 1 | 86/M | ND | 0 | 0 | 0 | 0% | 0 | 0 | 4 | | / | | / |
| 2 | 32/F | ND | 0 | 0 | 0 | 0% | 0 | 0 | 4 | | / | | / |
| 3 | 36/M | ND | 0 | 0 | 0 | 0% | 0 | 0 | 3 | | / | | / |
| Pure Steatosis | 4 | 26/M | 39 | 0 | 0 | 1 | 10% | 0 | 1 | 4 | | 2 | | 2 |
| 5 | 46/M | 43 | 0 | 0 | 1 | 30% | 0 | 1 | 3 | | 2 | | 1 |
| 6 | 38/F | 55 | 0 | 0 | 1 | 20% | 0 | 1 | 4 | | 2 | | 2 |
| 7 | 36/F | 34 | 0 | 0 | 1 | 10% | 0 | 1 | 4 | | 4 | | 0 |
| 8 | 37/F | 35 | 0 | 0 | 2 | 60% | 0 | 2 | 3 | | 1 | | 2 |
| 9 | 45/M | 54 | 0 | 0 | 2 | 50% | 0 | 2 | 4 | | 1 | | 3 |
| 10 | 29/M | 41 | 0 | 0 | 2 | 40% | 0 | 2 | 4 | | 1 | | 3 |
| 11 | 60/F | 39 | 0 | 0 | 3 | 80% | 0 | 3 | 4 | | 2 | | 2 |
| 12 | 43/M | 40 | 0 | 0 | 3 | 70% | 0 | 3 | 4 | | 1 | | 3 |
| 13 | 45/M | 42 | 0 | 0 | 3 | 70% | 0 | 3 | 4 | | 1 | | 3 |
| Steatohepatitis without fibrosis | 14 | 49/F | 36 | 0 | 1 | 1 | 30% | 0 | 2 | 2 | | 2 | | 0 |
| 15 | 48/F | 58 | 0 | 1 | 2 | 50% | 0 | 3 | 4 | | 1 | | 3 |
| 16 | 57/M | 34 | 1 | 1 | 2 | 60% | 0 | 4 | 3 | | 3 | | 0 |
| 17 | 56/F | 47 | 0 | 1 | 3 | 70% | 0 | 4 | 4 | | 2 | | 2 |
| 18 | 40/M | 40 | 0 | 1 | 3 | 70% | 0 | 4 | 3 | | 2 | | 1 |
| 19 | 38/F | 45 | 1 | 2 | 3 | 80% | 0 | 6 | 3 | | 1 | | 2 |
| 20 | 59/F | 41 | 1 | 1 | 3 | 70% | 0 | 5 | 4 | | 1 | | 3 |
| 21 | 50/M | 50 | 1 | 1 | 3 | 70% | 0 | 5 | 4 | | 1 | | 3 |
| 22 | 35/F | 51 | 1 | 1 | 3 | 80% | 0 | 5 | 3 | | 2 | | 1 |
| 23 | 54/F | 44 | 1 | 1 | 2 | 50% | 0 | 4 | 3 | | 2 | | 1 |
| 24 | 42/F | 39 | 1 | 1 | 3 | 80% | 0 | 5 | 4 | | 1 | | 3 |
| 25 | 51/F | 43 | 1 | 2 | 2 | 50% | 0 | 5 | 4 | | 2 | | 2 |
| 26 | 56/M | 39 | 1 | 1 | 3 | 80% | 0 | 5 | 3 | | 2 | | 1 |
| 27 | 39/F | 40 | 1 | 1 | 3 | 70% | 0 | 5 | 3 | | 2 | | 1 |
| Steatohepatitis with fibrosis | 28 | 62/M | 39 | 0 | 0 | 3 | 80% | 1a | 3 | 4 | | 1 | | 3 |
| 29 | 47/F | 47 | 0 | 0 | 2 | 50% | 1a | 2 | 4 | | 1 | | 3 |
| 30 | 21/M | 40 | 0 | 0 | 3 | 80% | 1a | 3 | 3 | | 2 | | 1 |
| 31 | 50/F | 42 | 0 | 0 | 3 | 70% | 1a | 3 | 4 | | 2 | | 1 |
| 32 | 54/M | 32 | 0 | 0 | 1 | 10% | 1a | 1 | 3 | | 2 | | 1 |
| 33 | 57/M | 51 | 0 | 0 | 2 | 50% | 1b | 2 | 4 | | 1 | | 3 |
| 34 | 60/M | 40 | 0 | 0 | 1 | 20% | 1b | 1 | 3 | | 3 | | 0 |
| 35 | 48/F | 63 | 0 | 0 | 3 | 80% | 1c | 3 | 4 | | 1 | | 3 |
| 36 | 55/M | 35 | 0 | 0 | 2 | 50% | 2 | 2 | 4 | | 1 | | 3 |
| 37 | 40/F | 45 | 0 | 0 | 3 | 70% | 2 | 3 | 2 | | 1 | | 1 |
| 38 | 36/M | 41 | 1 | 2 | 3 | 90% | 3 | 6 | 4 | | 1 | | 3 |
| 39 | 59M | 36 | 1 | 2 | 2 | 60% | 3 | 5 | 4 | | 1 | | 3 |
| Cirrhosis | 40 | 61/M | ND | 0 | 1 | 1 | 20% | 4 | 2 | 3 | | 3 | | 0 |
| 41 | 51/F | 38 | 1 | 0 | 1 | 20% | 4 | 2 | 3 | | 3 | | 0 |
| 42 | 54/M | 58 | 1 | 1 | 2 | 50% | 4 | 4 | 4 | | 1 | | 3 |
| 43 | 64/F | 35 | 3 | 2 | 1 | 20% | 4 | 6 | 3 | | 2 | | 1 |
| 44 | 60/M | 36 | 1 | 2 | 2 | 50% | 4 | 5 | 2 | | 1 | | 1 |
| 45 | 68/F | 48 | 2 | 1 | 2 | 50% | 4 | 5 | 3 | | 1 | | 2 |
| 46 | 65/F | 47 | 1 | 1 | 3 | 70% | 4 | 5 | 4 | | 1 | | 3 |
| 47 | 54/F | 42 | 2 | 1 | 3 | 70% | 4 | 6 | 4 | | 1 | | 3 |

The intensity of the PTEN nuclear staining was scored in steatotic (S) and non-steatotic (NS) areas of biopsies. All slides from healthy (ctrl) and obese patients were scored on a staining intensity scale from 0 to 4 with score = 4 representing the highest intensity. Difference between NS and S area (NS-S) was calculated. ND: Not determined; Ctrl: controls; PTEN: phosphatase and tensin homolog.

**Table 2 Demographic data, histological diagnosis of liver biopsies and phosphatase and tensin homolog expression in patients with alcoholic liver disease**

|  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Case** | **Age/sex** | **BMI (kg/m2)** | **Inflammation** | **Fibrosis** | **Cirrhosis** | **Steatosis** | **Score for PTEN expression** | | | | |
| **NS** | **S** | | **NS-S** | |
| Non-obese patients | 1 | 56/F | 26 | - | p | - | 100% | 3 | | 2 | | 1 |
| 2 | 49/F | 25 | - | psn | - | 40% | 4 | | 3 | | 1 |
| 3 | 59/M | 29 | + | - | + | 60% | 3 | | 3 | | 0 |
| 4 | 57/F | 27 | + | - | + | 80% | 3 | | 3 | | 0 |
| 5 | 66/M | 25 | + | - | + | 90% | 4 | | 3 | | 1 |
| 6 | 55/F | 28 | + | - | + | 90% | 3 | | 3 | | 0 |
| 7 | 61/M | 25 | + | psn | + | 70% | 4 | | 4 | | 0 |
| 8 | 43/M | 23 | + | psn | + | 80% | 4 | | 3 | | 1 |
| 9 | 36/M | 25 | + | - | + | 60% | 3 | | 2 | | 1 |
| 10 | 41/M | 24 | + | psn | + | 40% | 4 | | 3 | | 1 |
| 11 | 64/M | 21 | + | - | + | 10% | 4 | | 4 | | 0 |
| 12 | 46/F | 27 | - | - | + | 90% | 3 | | 3 | | 0 |
| 13 | 28/F | 22 | + | - | + | 80% | 4 | | 4 | | 0 |
| 14 | 51/M | 25 | - | p | + | 90% | 4 | | 3 | | 1 |
| 15 | 43/M | 20 | + | psn | + | 70% | 3 | | 3 | | 0 |
| 16 | 54/M | 20 | - | psn | + | 80% | 4 | | 4 | | 0 |
| 17 | 36/M | 23 | - | psn | + | 70% | 3 | | 2 | | 1 |
| Obese patients | 18 | 60/M | 30 | - | psn | - | 70% | 3 | | 3 | | 0 |
| 19 | 55/F | 41 | - | p | - | 70% | 3 | | 3 | | 0 |
| 20 | 57/M | 33 | + | - | + | 80% | 3 | | 3 | | 0 |
| 21 | 55/M | 30 | + | - | + | 70% | 3 | | 2 | | 1 |
| 22 | 51/M | 32 | - | - | + | 20% | 4 | | 4 | | 0 |
| 23 | 65/M | 31 | - | psn | + | 5% | 4 | | 4 | | 0 |
| 24 | 72/M | 34 | + | psn | + | 90% | 4 | | 4 | | 0 |
| 25 | 39/M | 31 | + | - | + | 20% | 4 | | 4 | | 0 |

The intensity of the PTEN staining was scored in steatotic (S) and non-steatotic (NS) areas of liver sections. All liver sections from ALD patients were scored on a staining intensity scale from 0 to 4 with score = 4 representing the highest intensity. Difference between NS and S area (NS-S) was calculated. ND: Not determined; Ctrl: Controls; psn: Perisinusoidal; p: Portal; PTEN: phosphatase and tensin homolog; ALD: alcoholic liver disease.

C:\Users\Administrator\Desktop\23874\23874\23874-Figures\23874-Figure 1.tif

**Figure 1 General histology and immunohistochemical detection of phosphatase and tensin homolog protein expression in the liver of healthy donor (hepatic resections) or patients with different stages of non-alcoholic fatty liver disease (liver biopsies).** Liver sections of healthy donors (a and b) or of obese patients with steatosis (*n =* 10, c and d), nonalcoholic steatohepatitis (*n =* 14, e and f), fibrosis (*n =* 12, g and h) and cirrhosis (*n =* 8, i and j) were either stained with hematoxylin eosin (a, c, e), Masson’s trichrome (g and i) or immunostained with anti-PTEN antibody (b, d, f, h and j). The inset in image (e) shows hepatic intralobular inflammation characterized by neutrophils (arrow) and ballooning hepatocyte (star). Scale bar = 100 µm. PTEN: phosphatase and tensin homolog.

**C:\Users\Administrator\Desktop\23874\23874\23874-Figures\23874-Figure 2.tif**

**Figure 2 General histology and immunohistochemical detection of phosphatase and tensin homolog protein expression in the liver of healthy donor (hepatic resections) or patients with different stages of alcoholic liver disease (liver biopsies).** Liver sections of healthy donors (a and b) or of patients with alcoholic steatosis (c and d), alcoholic steatohepatitis (e and f) and cirrhotic alcoholic disease (g and h) were stained with hematoxylin eosin (a, c and e), Massson’s trichrome (g) or immunostained with anti-PTEN antibody (b, d, f and h). Scale bar = 100 µm. The inset in image (e) shows hepatic intralobular inflammation characterized by neutrophils (star). Scale bar = 100 µm. PTEN: phosphatase and tensin homolog.