

Pro12Ala polymorphism of the peroxisome proliferator-activated receptor γ 2 in patients with fatty liver diseases

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RESULTS: In the NAFLD and the AFLD collective, 3% of the patients showed homozygous occurrence of the Ala12 PPAR γ 2-allele, differing from only 1.5% cases in the healthy population. In NAFLD patients, a high incidence of the Ala12 mutant was not associated with the progression of fatty liver disease. However, we observed a significantly higher risk (odds ratio = 2.50, CI: 1.05-5.90, $P = 0.028$) in AFLD patients carrying the mutated Ala12 allele to develop inflammatory alterations. The linkage of the malfunctioning Ala12-positive PPAR γ 2 isoform to an increased risk in patients with AFLD to develop severe steatohepatitis and fibrosis indicates a more prominent anti-inflammatory impact of PPAR γ 2 in progression of AFLD than of NAFLD.

CONCLUSION: In AFLD patients, the Pro12Ala single nuclear polymorphism should be studied more extensively in order to serve as a novel candidate in biomarker screening for improved prognosis.

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Key words: Single nucleotide polymorphism; Peroxisome proliferator-activated receptor γ ; Non-alcoholic steatohepatitis; Alcoholic steatohepatitis; Inflammation; Fibrosis; Hepatitis; Steatosis; Steatohepatitis

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Abstract

AIM: To test the occurrence of the Pro12Ala mutation of the peroxisome proliferator-activated receptor- γ (PPAR γ)2-gene in patients with non-alcoholic fatty liver disease (NAFLD) or alcoholic fatty liver disease (AFLD).

METHODS: DNA from a total of 622 specimens including 259 blood samples of healthy blood donors and 363 histologically categorized liver biopsies of patients with NAFLD ($n = 263$) and AFLD ($n = 100$) were analyzed by Real-time polymerase chain reaction using allele-specific probes.

INTRODUCTION

Fatty liver diseases are becoming a common cause of chronic liver diseases in the Western countries encountering in about 20% of the general adult and child population^[1-5]. Excessive accumulation of triglycerides in hepatocytes occurring in etiologically diverse conditions causes hepatic steatosis characterized by more than 5%-10% fat stored either in macrovesicles or in microvesicles of hepatocytes^[6,7]. Whereas in the past, regular and excess alcohol consumption was the most common reason for hepatic steatosis^[8,9], fatty liver diseases are now most frequently associated with obesity, insulin resistance and type 2 diabetes due to an unbalanced and rich diet in industrial nations^[3,5].

The spectrum of fatty liver diseases (FLD) independent of causative agents ranges from simple steatosis to steatohepatitis, which can progress to liver fibrosis ending up in cirrhosis or hepatocellular carcinoma^[3,5,10-13]. Thus, 5%-10% of non-alcoholic fatty liver disease (NAFLD) patients with steatosis develop a steatohepatitis accompanied by a high risk of progression to fibrosis^[3,4]. Although fatty liver diseases can have various causes, features of steatohepatitis in NAFLD and alcoholic fatty liver disease (AFLD) are difficult to distinguish histologically^[6,7]. Both are characterized by foci of liver cell necrosis and lobular inflammatory infiltrates with polymorphonuclear leukocytes. Furthermore, the onset of steatohepatitis is accompanied by ballooned hepatocytes, often harboring Mallory's hyaline and megamitochondria or undergoing apoptosis^[14]. Whereas steatosis seems to be more pronounced in non-alcoholic steatohepatitis (NASH) than in alcoholic steatohepatitis (ASH), features of necroinflammatory and cholestatic activity are more prominent in ASH liver biopsies^[6]. Progression of steatohepatitis then results in pericellular fibrosis^[6,7,15] involving myofibroblastic activation of sinusoidal hepatic stellate cells responsible for elevated extracellular matrix deposition^[16].

Members of the peroxisome proliferator-activated receptors (PPAR) seem to play a key role in the pathophysiology of FLD by modulating increased glucose uptake and hepatic triglyceride accumulation, but also perform anti-inflammatory signals when steatohepatitis has occurred^[17-19]. The PPAR family consists of PPAR α , PPAR γ , and PPAR δ nuclear receptors, functioning as transcription factors, that mediate transcriptional response to insulin resulting in glucose uptake, increased fatty acid oxidation, lipogenesis and lipid storage, respectively^[17]. Whereas the PPAR α is highly present in hepatocytes, the splice variants PPAR γ 1 and 2 triggering adipogenesis are mainly expressed in adipose tissues and only to a minor extent in the liver. PPAR γ increases the expression of genes that promote fatty acid storage, whereas it represses genes that induce lipolysis in adipocytes. In patients suffering from FLD, hepatic expression of PPAR γ is shown to be involved in insulin sensitivity, triglyceride clearance and hepatic steatosis^[20].

Due to its high impact as an insulin-sensitising transcription factor involved in adipogenesis and lipogenesis, the occurrence of single nucleotide polymorphisms (SNP) in the PPAR γ gene was recently addressed by numerous

reports studying subjects with insulin resistance, type 2 diabetes, arteriosclerosis, and hypertension^[21-23]. A prevalent SNP association with impaired lipid homeostasis was observed in terms of the N-terminal proline alanine exchange (Pro12Ala) of the extra domain in the PPAR γ 2 variant. This PPAR γ splice form includes 30 additional amino acids^[24], which are responsible for a 5-6-fold increase of PPAR γ 's transcriptional activity. The Pro12Ala exchange in the activating extra region of the PPAR γ 2 is the result of a cytosine to guanine substitution in the PPAR γ gene, as a consequence encoding the Ala-allele form with a heavily reduced function^[23]. In several populations, the association of the Pro12Ala polymorphism with insulin-sensitivity, type 2 diabetes, obesity and adipositas have been shown^[25-27]. However, the role of the Pro12Ala polymorphism of PPAR γ gene in occurrence and progression of fatty liver diseases is not yet defined.

In the present study, we analyzed the frequency of the Pro12Ala polymorphism in the PPAR γ gene by a highly sensitive LNA-probe based polymerase chain reaction (PCR) approach in a total of 622 subjects of a Caucasian population, suffering from fatty liver disease ($n = 359$) or being healthy blood donors ($n = 263$). In agreement with reports showing a high Ala allele prevalence in patients with impaired lipid metabolism in obese and adipose patients^[26,28], in FLD patients the Ala allele also occurs more often than in the healthy control group. Interestingly, the interpretation and linkage of the allele frequency to histological evaluation and clinical data demonstrates a prominent risk in AFLD patients bearing the Ala allele to develop severe steatohepatitis and fibrosis. Furthermore, our data revealed for the first time a higher anti-inflammatory impact of PPAR γ in progression of human AFLD than NAFLD.

MATERIALS AND METHODS

Patients, biopsies and liver disease classification

From a total of 622 cases, 259 blood samples and 363 biopsies were studied for occurrence of the Pro12Ala exchange in the PPAR γ gene. Local research ethics guidelines were followed. We collected 363 cases from the files of the Department of Gastroenterology and Hepatology, University Hospital of Essen (GER) and the Institute for Pathology, University Hospital of Cologne (GER) according to their histological criteria of fatty liver disease (Table 1). 263 tissue specimens from patients were classified as NAFLD according to the clinical information about alcohol consumption (less than 20 g alcohol per day). One hundred specimens of patients who consume more than 20 g alcohol per day met the definition of AFLD as described by Neuschwander-Tetri *et al.*^[29]. Clinical data, such as GOT, GPT, and γ GT, were compiled along with the state of diabetes. There was no appreciable difference between the mean age of AFLD (53.93 ± 10.63 , range 20-81 years) and NAFLD (50.48 ± 15.25 , range 16-80 years). All specimens, stained with haematoxylin and eosin (HE) and by the Gomori method for visualization of reticular fibers, were independently classified by three experienced liver pathologists (Hardt A, Drebber U, Dienes HP), according to

Table 1 Scoring according to the histological features described by Kleiner *et al.*^[15]

Definition	Score
Steatosis	
Grade	
< 5%	0
5%-33%	1
33%-66%	2
> 66 %	3
Localization	
Zone 3	0
Zone 2	1
Zone 1	2
Azonal	3
Panacinar	4
Type	
Macrovesicles	In %
Microvesicles	In %
Mixed	
Inflammation	
Lobular	
No foci	0
< 2 foci	1
2-4 foci	2
> 4 foci	3
Portal	
No inflammation	0
Minimal	1
Mild	2
Moderate	3
Severe	4
Fibrosis	
None	0
Mild/moderate	1
Periportal or perisinusoidal	2
Bridging fibrosis	3
Cirrhosis	4
Liver cell damage	
Ballooning	
None	0
Moderate	1
Severe	2
Mallory bodies	
None	0
Moderate	1
Severe	2

the histological score described by Kleiner *et al.*^[15] (Table 1). Additionally, 259 DNA extracts from blood samples of healthy blood donors were taken as references for local gene distribution.

Automatic DNA extraction from formalin fixed and paraffin embedded biopsies

Extraction of DNA from 363 formalin fixed and paraffin embedded (FFPE) biopsies was performed from three 7 μm-microtome sections after deparaffinization and proteinase K treatment, as previously described^[30]. Then, DNA was purified by means of magnetic bead technology (FormaPure™ Kit of Agentcourt, Beverly MA, USA). All DNA purification steps were carried out by the BioMek FX laboratory automatic workstation (Beckman Coulter, USA) according to the work file and recommendations of Agentcourt.

Furthermore, DNA from 259 blood samples was pre-

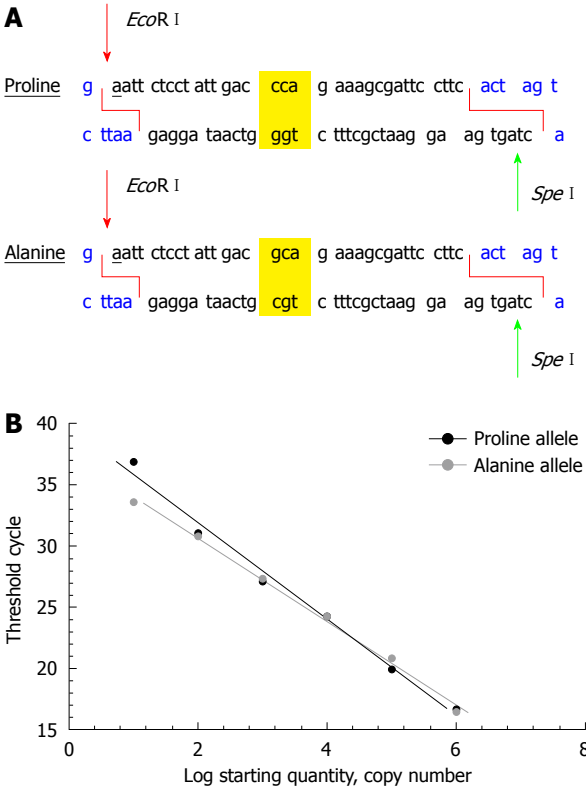


Figure 1 Cloning strategy and validation of a reference system. A: Cloning strategy for the generation of a reference system. The Pro12Ala mutation locus of the peroxisome proliferator-activated receptor-γ gene was synthesized as the proline or the alanine encoding oligonucleotides sequence. The proline or the alanine codon is indicated in yellow. The chemically synthesized oligonucleotide dimers, flanked by the overhangs of the *EcoR* I and the *Spe* I restriction sites (red/green), respectively, were used for insertion into pBluescript SKII plasmids; B: Real-time polymerase chain reaction (PCR) of the Pro12Ala locus depending on different copy numbers of reference sequences. Real-time PCR was performed using the LNA probes (Table 2) specific for the proline encoding sequence or the alanine encoding allele, respectively. 10⁶, 10⁵, 10⁴, 10³, 10², 10 copies of the plasmid reference sequences encoding either the Ala12 or the Pro12 locus were each diluted in 10 ng salmon sperm DNA and used for LNA probe based real-time PCR assays. Up to 10 copies per ng total DNA of both reference sequences were efficiently detected by the corresponding LNA probe labelled either by Hex or Fam fluorochrome.

pared by the robotic workstation using the Genfind™ Kit of Agentcourt according to the manufacturer's instructions.

Cloning of reference sequences into pBluescript

For construction of a reference system with sequences of the Pro12Ala locus (rs1805192) carrying either the mutation or the wild type sequence of PPARγ2 gene we used oligonucleotides comprising the proline or alanine encoding sequences (Figure 1). These oligonucleotides were dimerized in 150 mmol/L NaCl, 5 mmol/L EDTA, 50 mmol/L Tris pH 7.4, creating *EcoR* I and *Spe* I compatible overhangs and inserted into pBluescript SKII (+) (Stratagene, Texas, USA) by the respective restriction sites (Figure 1).

Allelic discrimination of the PPARγ2 by real-time PCR

The Pro12Ala exchange of the PPARγ gene (rs1801282) was examined in DNA from normal blood donors and patients by a Taqman probe associated real-time PCR. Genomic DNA was amplified by real-time PCR in a total vol-

Table 2 Real-time polymerase chain reaction primers and probes

Name	Oligonucleotide sequence	PCR application
Plas A primer	5'-CCGCTCTAGAACTAGTGAAGGAA-3'	Reference
Plas S primer	5'-ACTCACTATAGGGCGAATTGG-3'	DNA
PPAR γ A primer	5'-TTACCTTGTGATATGTTGCAGAC A-3'	Target
PPAR γ mis primer	5'-GTTATGGGTGAAACTCTTGGAGA-3'	DNA
TM LNA probe wt	5'-6FAM-CTATTG <u>CCC</u> AGAAAGC-- BHQ1	Target and reference
TM LNA probe mut	5'-YAK-CTATTG <u>ACG</u> AGAAAGC-- BHQ1	DNA

Bold letters indicate the single nucleic polymorphism and underlined letters indicate the insertion of locked nucleotides (LNA). PPAR γ : Peroxisome proliferator-activated receptor- γ ; PCR: Polymerase chain reaction.

ume of 10 μ L using the Eppendorf MasterMix (Hamburg, Germany) and 0.3 μ L of each primer (10 μ mol/L). The Plas A and Plas S primer set was used for amplification of reference plasmids; the PPAR γ A and PPAR γ mis primer set was used for genomic DNA samples (Table 2).

Allelic discrimination was achieved by adding 0.4 μ L of 2.5 μ mol/L LNA probes (TIB Molbiol, Berlin, Germany) recognizing the wild type and the mutant variant of the Pro12Ala locus of the PPAR γ 2 gene (Table 2). In parallel to the allelic Pro12Ala discrimination, plasmid reference sequences diluted from 10^5 to 10 copies in herring's sperm DNA (1 ng/mL) were applied to all assays as positive controls. Amplification and analyses were accomplished by the following cycling conditions using a MX3000P qPCR System of Stratagene (Texas, USA): initial denaturation at 95°C for 2 min, following 50 cycles 95°C for denaturation, 55°C for annealing, 65°C for extension, each step lasting 20 s.

Statistical analysis

Pro12Ala distribution was evaluated using the SPSS software 17 of IBM® (Chicago, USA). Significance of cross-classification was calculated by the Fisher's exact test. Odds ratios were used to describe the risk of disease progression.

RESULTS

Prominent occurrence of the PPAR γ alanine variant in patients with fatty liver disease

In order to detect the Ala12Pro polymorphism in patients with fatty liver disease we established an assay using locked nucleotide acid (LNA) probes for allelic discrimination. For this purpose, both variants of the Ala12Pro locus were cloned (Figure 1A) and the sequences, encoding either the proline or the alanine variant, were used as reference sequences for efficient allele detection and discrimination. The LNA probe hybridization assay linked to real-time PCR efficiently detected both, the proline wild type and the alanine mutated variant of the PPAR γ gene (Figure 1B). Up to 10 copies of each reference sequence were successfully proven. In addition to the high sensitivity, application of the reference sequences attested that the LNA probe

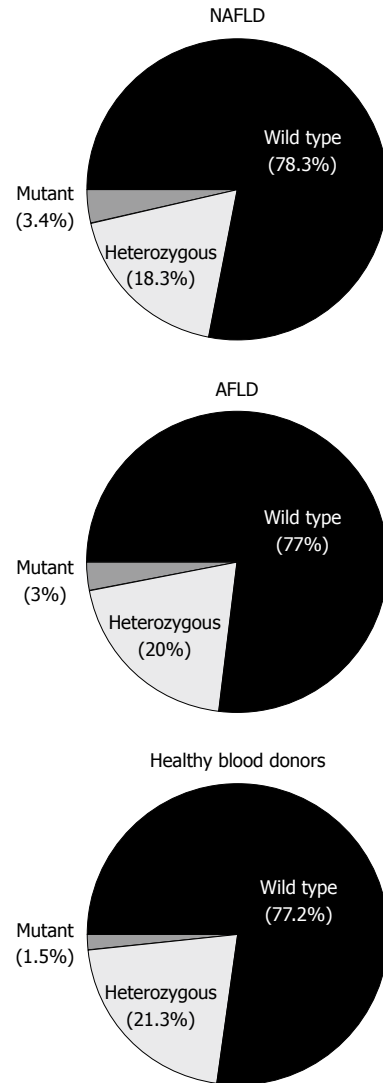


Figure 2 Frequency of the Pro12Ala polymorphism in patients with non-alcoholic fatty liver disease ($n = 263$), alcoholic fatty liver disease ($n = 100$) and in healthy blood donors ($n = 259$). The wild type allele, which is the Pro allele, is indicated in grey, the heterozygous genotype (Pro/Ala) in black and the homozygous Ala/Ala mutant in pale-grey. Genotype analyses revealed a higher prevalence of the homozygous Ala/Ala genotype in non-alcoholic fatty liver disease (NAFLD) and alcoholic fatty liver disease (AFLD) patients.

based PCR assay was highly specific, enabling the differentiation of the alanine and the proline encoding sequence.

In the 259 healthy blood donors the assay accounted for 1.5% homozygous variants carrying the alanine encoding sequence (Figure 2). Analyses of the Pro12Ala distribution in the collective of patients with fatty liver disease ($n = 363$) revealed an increased incidence of the alanine mutant (3%) compared to the healthy population (1.5%). However, the difference was not statistically significant.

In order to identify the association of enhanced alanine occurrence, clinical data of patients with fatty liver disease were considered, revealing that 263 patients suffered from NAFLD, whereas fatty liver disease was linked to alcohol consumption in 100 cases (Table 3). In both cohorts, 12%–14% of the subjects were diabetes positive.

Furthermore, we analyzed the transaminase values in the two cohorts, showing a GPT/GOT ratio of 2 in

Table 3 Occurrence of diabetes mellitus in patients with fatty liver disease *n* (%)

	No C2 (NAFLD)	C2 (AFLD)
Diabetes mellitus positive	33 (12.5)	14 (14)
Diabetes mellitus negative	144 (54.8)	51 (51)
ND	86 (32.7)	35 (35)
Total	263 (100)	100 (100)

NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease; ND: Not determined.

Table 4 Histological features in the fatty liver diseases cohort

Cases of fatty liver disease (<i>n</i> = 363)	<i>n</i> (%)
Steatosis	
S0	9 (2.5)
S1	169 (46.6)
S2	84 (23.1)
S3	101 (27.8)
Ballooning	
No ballooning	164 (45.2)
Stage 1	124 (34.2)
Stage 2	75 (20.7)
Inflammation	
G0	4 (1.1)
G1	217 (59.8)
G2	113 (31.1)
G3	29 (8)
Fibrosis	
F0	97 (26.7)
F1	116 (32)
F2	66 (18.2)
F3	56 (15.4)
F4	28 (7.7)

NAFLD patients, but a GPT/GOT ratio of 1 in sera of AFLD patients (Figure 3). The high GPT values in NAFLD patients are in accordance with numerous reports, characterizing the progress of steatohepatitis due to non-alcoholic steatosis in comparison to alcoholic steatosis^[31]. With respect to the distribution of the Pro12 and the Ala12 alleles in these two cohorts, we found that in both the AFLD and the NAFLD collective the frequency of the alanine genotype was higher (about 3%) compared to the healthy population (Figure 2).

Association of the alanine allele with inflammation and fibrosis in fatty liver disease

We next addressed the question whether the elevated incidence of the alanine allele in the population of fatty liver diseases is associated with the grade of steatosis, ballooning, steatohepatitis, or liver fibrosis (Table 4).

The degree of steatosis is traditionally classified into mild (< 30%), moderate (30%-60%), and severe (> 60%). More than 50% of the patients with NAFLD and AFLD had developed severe steatosis, however, in patients carrying the alanine allele severe steatosis occurred slightly more often. Pronounced ballooning was observed likewise often in NAFLD patients with the mutant or the wild type isoform of the PPAR γ 2 (Table 5).

Histological scoring for inflammatory alterations and

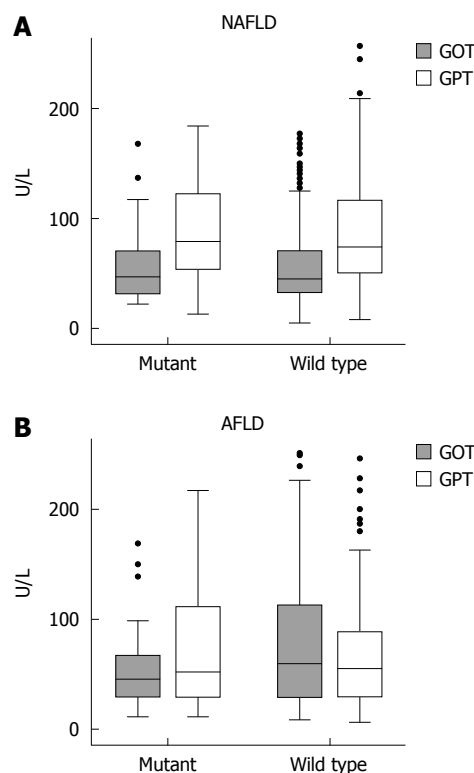


Figure 3 GOT and GPT levels in sera of non-alcoholic fatty liver disease (A) or alcoholic fatty liver disease (B) patients carrying the Ala mutated allele or the Pro wild type allele of the peroxisome-proliferator-activated receptor- γ 2 gene. NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease. Dots (•) indicate outliers that are not included between the whiskers.

fibrosis according to the recommendations described by Kleiner *et al.*^[15] (Table 1) revealed that in most of the patients steatosis was accompanied by moderate, mild or severe steatohepatitis. In particular, in patients suffering from AFLD steatohepatitis has passed over to fibrosis in 71% of the cases.

Whereas in NAFLD patients inflammation was not significantly associated with the allelic incidence, in AFLD patients the frequency of the Ala12 variant of the PPAR γ 2 gene was significantly increased when prominent inflammation had occurred ($P = 0.028$). The higher risk of AFLD patients developing several inflammatory processes ending in liver fibrosis was also shown by elevated Odds ratios (Odd_{inflammation} = 2.50, CI: 1.05-5.90 and Odd_{fibrosis} = 2.48, CI: 0.81-7.53) (Table 6).

DISCUSSION

FLD has a high incidence of approximately 20% worldwide and is regarded as a major cause of liver-related morbidity and mortality due to its risk of progression into cirrhosis or hepatocellular carcinoma. Since the transcription factor PPAR γ has been shown to be markedly involved in adipogenesis, hepatic lipid storage and metabolism, we first analyzed the frequency of the Pro12Ala polymorphism of the PPAR γ gene in a German cohort of patients with FLD compared to German healthy blood donors. A highly sensitive and robust test was established which was certain to distinguish the Ala and the Pro alleles, even

Table 5 Occurrence of the wt and the mutated form of peroxisome proliferator-activated receptor- γ 2 depending on the grade of steatosis and ballooning *n* (%)

	Steatosis						Ballooning					
	NAFLD			AFLD			NAFLD			AFLD		
	Total	Moderate (0-1)	Severe (2-3)	Total	Moderate (0-1)	Severe (2-3)	Total	-	+	Total	-	+
Allelic discrimination												
Mutation	66 (100)	27 (41)	39 (59)	26 (100)	12 (46)	14 (54)	66 (100)	28 (42)	38 (58)	26 (100)	19 (73)	7 (27)
Wild type	460 (100)	229 (50)	231 (50)	174 (100)	88 (51)	86 (49)	460 (100)	206 (45)	254 (55)	174 (100)	121 (70)	53 (30)
<i>P</i> value		0.112			0.417			0.411			0.454	
Odds ratio (CI)		1.43 (0.85-2.42)			1.19 (0.52-2.73)			1.10 (0.65-1.85)			0.84 (0.33-2.12)	

NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease.

Table 6 Occurrence of the wt and mutated form of the peroxisome proliferator-activated receptor- γ 2 depending on the grade of inflammation and fibrosis *n* (%)

	Inflammation						Fibrosis					
	NAFLD			AFLD			NAFLD			AFLD		
	Total	Moderate (0-1)	Severe (2-3)	Total	Moderate (0-1)	Severe (2-3)	Total	F0-F1	F2-F4	Total	F0-F1	F2-F4
Allelic discrimination												
Mutation	66 (100)	38 (58)	28 (42)	26 (100)	9 (35)	17 (65)	66 (100)	47 (71)	19 (29)	26 (100)	4 (15)	22 (85)
Wild type	460 (100)	296 (64)	164 (36)	174 (100)	99 (57)	75 (43)	460 (100)	327 (71)	133 (29)	174 (100)	54 (31)	120 (69)
<i>P</i> value		0.175			0.028			0.555			0.075	
Odds ratio (CI)		1.33 (0.79-2.25)			2.50 (1.05-5.90)			0.99 (0.56-1.76)			2.48 (0.81-7.53)	

NAFLD: Non-alcoholic fatty liver disease; AFLD: Alcoholic fatty liver disease.

though only low copy numbers from some FFPE biopsies might be available. The genotype distribution (77.5% wt, 21.2% heterozygous, and 1.5% homozygous Ala/Ala mutants) in the collective of the healthy blood donors resembles previous data collected on more than 600 Caucasians by Yen *et al*^[32] and Ghossaini *et al*^[33]. This genotype distribution in Caucasians, however, differs from the Asian or African frequency, in which less Ala alleles of the PPAR γ occur^[23,32]. In contrast to the data of healthy blood donors, PCR analyses of DNA from subjects with FLD revealed an increased frequency of the homozygous Ala-subtype up to almost 3.5% in both the AFLD and the NAFLD collectives. Recent meta-analyses summarized data of the Pro12Ala polymorphism in patients with diabetes and identified the mutated Ala variant as a protection factor of diabetes type 2^[34-36]. The malfunctioning Ala variant was also shown to be associated with coronary heart disease^[37] and with obesity indicated by significant higher BMI in homozygous Ala carriers than in subjects expressing the heterozygous or the wild type PPAR γ 2 form^[38,39]. Although in some reports higher insulin sensitivity and BMI could not be confirmed^[40,41], a comprehensive study on 1170 British patients with coronary heart disease^[42] and a meta-analysis including 19 136 subjects clearly identified the Ala carriers as individuals with significant higher BMI^[43]. Additionally, cholesterol, LDL-cholesterol and apolipoprotein B concentrations are elevated in Ala carriers^[42,44]. Therefore, this Ala-associated hyperlipidemia is assumed to be a reason for the 2-fold higher incidence of the Ala genotype in patients with FLD compared to

healthy blood donors. Since free fatty acids are shown to be involved in upregulation of Fas/CD-95 death receptor^[45], enhanced levels of circulating fatty acids due to impaired PPAR γ function in Ala/Ala patients may result in apoptosis and inflammatory processes. In contrast to NAFLD, where no or only a moderate link of inflammatory progression to the Pro12Ala polymorphism was shown, a prominent risk of developing steatohepatitis was observed in AFLD patients carrying the Ala allele. This difference in the associated frequency of the Ala variant encoding the minor active PPAR γ 2 form^[23] argues for a divergent role of the PPAR γ 2 in mechanisms of AFLD and NAFLD progression. The PPAR γ 2 isoform is up-regulated by phosphatidylinositol 3-kinase activation in response to free fatty acids or by insulin^[46]. In post-ischemic liver injury and also in alcohol-induced fibrosis, the PPAR γ 1 and γ 2 variants were shown to be downregulated and to function protectively^[47,48]. Therefore, the 2-3-fold higher risk of AFLD patients, but not of NAFLD patients, to develop inflammatory and fibrotic progression if they carry the malfunctioning Ala variant of PPAR γ 2, emphasizes a more prominent anti-inflammatory impact of the PPAR γ 2 in AFLD than in NAFLD wt-carriers.

The anti-inflammatory action of PPAR γ was also demonstrated by previous studies on hepatic stellate cells^[49,50], which take centre stage of sinusoidal liver fibrosis due to their tremendous matrix production and secretion of pro-inflammatory and pro-fibrotic mediators after myofibroblastic transition in chronic liver injury^[16]. The authors show that PPAR γ s repressed in myofibroblastic hepatic

stellate cells. Additionally, the inflammatory chemokine expression by hepatic stellate cells is markedly inhibited in response to the activation of PPAR γ by the agonistic ligand glitazone^[49,50]. Taken into account that the malfunctioning Ala variant is associated with a higher risk of progression into steatohepatitis in AFLD patients these results lead to the suggestion that in particular inflammation and fibrosis of AFLD wt-patients can be attenuated by a treatment with PPAR γ thiazolidinedione ligands such as rosiglitazone and pioglitazone. Patients with NASH, however, may benefit from glitazone therapy by other mechanisms like improved insulin sensitivity, decreased hyperlipidemia and impeded steatosis as a result of the therapeutic approach^[51,52].

In conclusion, our data of a comprehensive study of the Pro12Ala polymorphism on biopsies with FLD, well classified concerning inflammatory and fibrotic alterations, revealed for the first time an association of the Pro12Ala polymorphism with the risk of developing ASH and suggests a more prominent anti-inflammatory influence of the PPAR γ 2 on progression of human AFLD than on NAFLD. Therefore, the Pro12Ala polymorphism should be studied on an expanded cohort of AFLD patients, in order to be later integrated in a panel of genetic markers applied for future improved prognosis of disease progression.

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COMMENTS

Background

Fatty liver diseases are becoming a common cause of chronic liver diseases in the Western countries encountered in about 20% of the general adult and child population. The spectrum of fatty liver diseases (FLD) independently of causative agents ranges from simple steatosis to steatohepatitis, which can progress to liver fibrosis ending up in cirrhosis or hepatocellular carcinoma. Members of the peroxisome proliferator-activated receptors (PPAR) seem to play a key role in the pathophysiology of FLD by modulating increased glucose uptake and hepatic triglyceride accumulation, but also perform anti-inflammatory signals when steatohepatitis has occurred. Since the transcription factor PPAR γ has been shown to be markedly involved in adipogenesis, hepatic lipid storage and metabolism, we analyzed the frequency of the Pro12Ala polymorphism of the PPAR γ gene in a German cohort of patients with FLD compared to German healthy blood donors.

Research frontiers

Due to its high impact as an insulin-sensitising transcription factor involved in adipogenesis and lipogenesis, the occurrence of single nucleotide polymorphisms (SNP) in the PPAR γ gene was recently addressed by numerous reports studying subjects with insulin resistance, type 2 diabetes, arteriosclerosis, and hypertension. A prevalent SNP association with impaired lipid homeostasis was observed in terms of the N-terminal proline alanine exchange (Pro12Ala) of the extra domain in the PPAR γ 2 variant. The authors analyzed the frequency of the Pro12Ala polymorphism in the PPAR γ gene by a highly sensitive LNA-probe based polymerase chain reaction approach in a total of 622 subjects of a Caucasian population, suffering from fatty liver disease ($n = 359$) or healthy blood donors ($n = 263$). In agreement with reports showing a high Ala allele prevalence in patients with impaired lipid metabolism in obese and adipose patient, the Ala allele also

occurs more often in FLD patients than in the healthy control group. Interestingly, the interpretation and linkage of the allele frequency to histological evaluation and clinical data demonstrates a prominent risk in alcoholic fatty liver disease (AFLD) patients bearing the Ala allele to develop severe steatohepatitis and fibrosis.

Innovations and breakthroughs

In order to detect the Ala12Pro polymorphism in patients with fatty liver disease the authors established an assay using locked nucleotide acid probes for allelic discrimination. The authors' data of a comprehensive study of the Pro12Ala polymorphism on biopsies with FLD, well classified concerning inflammatory and fibrotic alterations, revealed in patients with AFLD, but not with non-alcoholic fatty liver disease (NAFLD), an significant association of the Pro12Ala polymorphism with the risk to develop steatohepatitis. Therefore, PPAR γ is suggested to exert a higher anti-inflammatory impact in progression of human AFLD than NAFLD.

Applications

The association of the Pro12Ala polymorphism with the risk of developing inflammatory progression in patients with AFLD suggests a more prominent influence of PPAR γ 2 on progression of human AFLD than on NAFLD. Therefore, the Pro12Ala polymorphism should be studied on an expanded cohort of AFLD patients, in order to be later integrated in a panel of genetic markers applied for future improved prognosis of disease progression and therapy planning.

Peer review

This review article provides an overview of SLE-related gastrointestinal system involvements, and there are only few review article in the international literatures in recent years.

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