# PPAR-alpha L162V polymorphism in human hepatocellular carcinoma

## Hepatosellüler kanserde PPARα L162V polimorfizmi

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***Background/aims:*** *Several lines of evidence suggest that peroxisome proliferator-activated receptor alpha may be involved in hepatocarcinogenesis. L162V polymorphism of the peroxisome proliferator-activated receptor alpha gene enhances the transactivation activity of this transcription factor. The aim of this study was to determine the frequency and clinical correlates of peroxisome proliferator-activated receptor alpha L162V polymorphism in hepatitis virus-induced hepatocellular carcinoma.* ***Methods:*** *90 hepatocellular carcinoma patients diagnosed at Ankara University Gastroenterology Clinic between January 2002 and July 2003 and 80 healthy controls with normal body mass index, blood chemistry and with negative viral serology were included. peroxisome proliferator-activated receptor alpha L162V polymorphism was determined by PCR-RFLP.* ***Results:*** *hepatocellular carcinoma etiologies were as follows: 56 HBV, 12 HBV+HDV, 22 HCV. Eighty-seven patients (97%) were cirrhotic, and 60 patients (67.5%) had advanced tumors. In 83 (92%) of 90 hepatocellular carcinoma patients, gene segment including polymorphic region could be amplified by PCR (50 HBV, 12 HBV+HDV, 21 HCV) and 6 of them (7.2%, all infected with HBV) had L162V polymorphism, while 2 (2.5%) of 80 controls had this polymorphism (p=0.162). This trend became more remarkable when only HBV (HBV+HDV)-infected patients were compared with controls (6/62, 9.7% vs. 2/80, 2.5%, respectively, p=0.071). Five of 6 patients with L162V had advanced disease.* ***Conclusions:*** *Peroxisome proliferator-activated receptor alpha L162V polymorphism tends to occur in HBV-induced epatocellular carcinoma and is absent in HCV-related epatocellular carcinoma. These findings may show clues for the existence of different carcinogenesis mechanisms in these two common etiologies. Frequent occurrence of advanced disease in patients with L162V polymorphism suggests a role for this polymorphism in tumor progression.*

**Key words**: PPARα, L162V, polymorphism, hepatitis C virus, hepatitis B virus, hepatocellular carcinoma

***Amaç:*** *Hepatosellüler kanserlerin gelişiminde PPARα'nın rolüne işaret eden birçok kanıt mevcuttur. PPARα geninin L162V polimorfizmi bu transkripsiyon faktörünün transaktivasyonunu arttırır. Bu çalışmanın amacı hepatit virüsü enfeksiyonu zemininde gelişmiş hepatosellüler kanser hastalarında PPAR· L162V polimorfizminin sıklığının ve bu mutasyonun klinik seyir ile ilişkisinin araştırmasıdır.* ***Yöntem:*** *Ankara Üniversitesi Gastroenteroloji Kliniğinde Ocak 2002 – Temmuz 2003 tarihleri arasında tanı almış ve 90 hepatosellüler kanserlerin hastası ve 80 sağlıklı kontrol (normal beden kitle indeksi, kan biyokimyası olan ve viral serolojisi negatif olan) çalışmaya alındı. PPAR· L162V polimorfizmi PZR-RFLP yöntemi ile tespit edildi.* ***Bulgular:*** *Hastaların etyolojileri, 56 HBV, 12 HBV+HDV, 22 HCV olarak tespit edildi. 87 hasta(%97) sirotikti ve 60 hastanın (%67.5) tümörü ileri evredeydi. 83 hastada (%92-50 HBV, 12 HBV+HDV, 21 HCV) PPAR· geninin polimorfik bölgesini PZR ile amplifiye edilebildi. Bu hastaların 6'sında (%7,2- tamamı HBV) L162V polimorfizmi tespit edilirken, kontrol hastalarının 2'sinde (%2,5-p=0,162) bu polimorfizm tespit edildi. Bu trendin HBV+HDV olan hastalar ile kontroller karşılaştırıldığında daha belirgin olduğu (6/62, %9.2 – 2/80 %2.5, p=0.071). L162V polimorfizmi olan hastalardan 5 'inde ileri evre hastalık mevcuttur.* ***Sonuç:*** *PPAR· geninin L162V polimorfizmi HBV ile ilişkili hepatosellüler kanserde görülürken HCV zemininde gelişenlerde görülmemektedir. Bu bulgular farklı iki etyoloji zemininde gelişen hepatosellüler kanserlerin karsinogenetik mekanizmaların farklı olabileceğini düşündürmektedir. L162V polimorfizmi olan hastalardaki Hepatosellüler kanserin ileri evrede olması bu polimorfizmin hastalığın progresyonunu etkilediğini düşündürmektedir.*

**Anahtar kelimeler**: PPARα, L162V, polimorfizm, hepatit C virüsü, Hepatit B virüsü, hepatosellüler kanser

## INTRODUCTION

Peroxisome proliferator-activated receptor alpha (PPARα) is a ligand activated transcription factor and has an important role in lipid homeostasis (1). Following activation by its endogenous/exogenous

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ligands, PPARα forms a heterodimer with the 9-*cis*-retinoic acid receptor (RXR), and PPAR/RXR heterodimers bind to DNA sequences, termed PPAR response elements (PPRE), present in the 5´-flanking region of target genes (2-4). PPARα induces fatty acid oxidation in mitochondria, peroxisomes and microsomes (1, 5), and there appears to be a cross-talk between these three fatty acid oxidation systems, with PPARα playing a controlling role (1, 6-9).

Hepatitis viruses are the main cause of human hepatocellular carcinoma (HCC). Although the mechanism of human tumor development is not completely understood, chronic injury and subsequent hepatocyte regeneration as well as oncogenic potentials of viral proteins are thought to be responsible for tumor development (10, 11). The role of host factors in HCC development is less clear. Several lines of evidences suggest that PPARα may be involved in carcinogenesis (12, 13). Hypolipidemic peroxisome proliferators, well known PPARα activators, have been shown to cause liver tumors in mice and rats (14). Knock-out animal experiments also point to the role of PPARα-inducible fatty acid oxidation systems in the pathogenesis of liver tumor development. Disruption of the PPARα gene in mice causes liver steatosis by reducing mitochondrial fatty acid oxidation, while dramatic activation of PPARα by disruption of peroxisomal acyl CoA oxidase leads to steatohepatitis and liver cell tumors (15-17).

Recently, a polymorphism in the PPARα gene, leucine to valine change at codon 162 localized to exon 5 (L162V), has been described (18, 19). This polymorphism was shown to enhance the transcriptional activity of PPARα in transfection assays (18). PPARα L162V polymorphism has been reported to be associated with altered lipid and apobetalipoprotein concentrations and with an increased body mass index (BMI) (18-21). These associations raised the possibility that this polymorphism may be associated with non-alcoholic steatohepatitis (NASH), which may result in liver cirrhosis and hepatoma. However, our recent study did not show a link between NASH and PPARα L162V polymorphism (22). On the other hand, only a minority of human HCCs are due to NASH, and most are caused by hepatitis viruses.

Thus far, there has been no study addressing an association between PPARα L162V polymorphism and human HCC development. In this study, we aimed to determine the frequency and clinical correlates of PPARα L162V polymorphism in human HCC, which is mainly induced by hepatitis viruses.

## MATERIALS AND METHODS

### Study samples and data collection

Ninety HCC patients (77 M, 13 F) infected with hepatitis viruses diagnosed in Ankara University Gastroenterology Clinic between January 2002 and July 2003 were included. None of the patients had any other identifiable causes of HCC and alcohol intake was absent or less than 20 g per week in all of these patients. None of the patients had a BMI greater than 30. In addition, none of the patients had any evidence of systemic disease including collagen-vascular, neoplastic, cardiopulmonary or renal disease. Eighty healthy subjects (44 M, 36 F) with normal BMI, normal blood chemistry and negative viral serology served as controls.

Presence or absence of cirrhosis and Child score of the cirrhotic patients were recorded. The diagnosis of HCC was based on detection of liver tumors by two different radiological visualizations in the presence of elevated alpha fetoprotein level or cytological/histological assessment of tumor tissue by examination of percutaneous biopsy sample or resection material obtained during resection/transplantation procedures. Advanced tumor was defined as total tumor size ≥ 8 cm or multifocal/diffuse tumors without portal vein thrombosis or distant metastasis. A single solitary tumor <6.5 cm or ≤3 tumors with none >4.5 cm and a total tumor size <8 cm at presentation was accepted as less advanced tumor according to University of California at San Francisco (UCSF) criteria (23).

### 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 polymerase chain reaction (PCR) analysis. In the mismatch PCR, the following primers generating HinfI restriction site were used for further RFLP analysis (18):

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<u>T</u> 3´ (<u>T</u>: mismatch nucleotide).

Following preparation of 1.25 U Taq polymerase, 2.5 mM MgCl and 0.2 mM dNTP, forward and reverse primers (10 pmol each) were added. The fi-

nal 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).

## RESULTS

### Patient characteristics

Table 1 shows the clinical characteristics of HCC patients at the time of the diagnosis. Male predominance was observed in the patient cohort. Eighty-seven patients (97%) were cirrhotic, most of whom (80%) were at Child B or C stage.

Hepatocellular carcinoma etiologies were as follows: 56 (62.2%) HBV, 12 (13.3%) HBV+HDV, and 22 (24.5%) HCV. Sixty patients (67.5%) had advanced tumors (total tumor size ≥ 8 cm or multifocal/diffuse tumors w/o portal vein thrombosis or distant metastasis), while 30 (33.5%) patients had less advanced tumors. Seven patients had distant metastases (2 bone, 5 lung).

### Frequency and clinical correlates of L162V polymorphism

In 83 (92%) of 90 HCC patients, gene segment including polymorphic region could be amplified by PCR. HCC etiologies of these 83 patients were as follows: 50 (60%) HBV, 12 (14.5%) HBV+HDV and 21 (25.5%) HCV. Six of 83 (7.2%) patients had L162V polymorphism, while only 2 (2.5%) of 80 controls had this polymorphism (p=0.162). This trend became more remarkable when only HBV (HBV+HDV)-infected patients were compared with controls (6/62, 9.7% vs. 2/80, 2.5%, respectively, p=0.071) (Table 2). In fact, all of the 6 patients who had L162V polymorphism were infected with HBV (5 HBV alone, 1 HBV+HDV) and none of the HCV-infected patients had this polymorphism (Table 3). Interestingly, 5 of these 6 cases (83.3%) had advanced disease (Table 3) and all of them had a normal BMI.

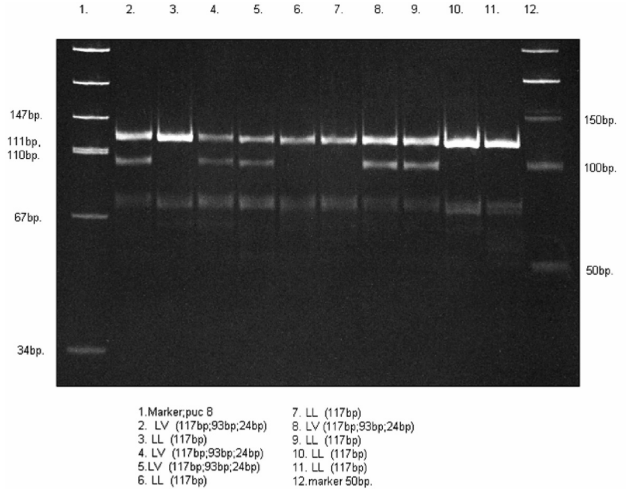


**Figure 1.** Gel electrophoresis showing several cases with a single band of normal alleles (L162L) and with two bands of mutant alleles (L162V)

**Table 1.** Characteristics of HCC patients

| Characteristics | No. of Cases | Percentage |
|---|---|---|
| Sex | | |
| Male | 77 | 85 |
| Female | 13 | 15 |
| Cirrhotic patients | 87 | 97 |
| Child-Pugh A | 17 | 19.5 |
| Child-Pugh B | 34 | 39 |
| Child-Pugh C | 36 | 41.5 |
| Non-cirrhotic patients | 3 | 3 |
| Etiology of HCC | | |
| HBV | 56 | 62.2 |
| HBV+HDV | 12 | 13.3 |
| HCV | 22 | 24.5 |
| Disease stage | | |
| Advanced tumor | 60 | 67.5 |
| - Distant metastasis | 7 | 8 |
| Non-advanced tumor | 30 | 33.5 |

## DISCUSSION

PPARα is a key regulator of fatty acid oxidation and plays important roles in lipid metabolism. Animal experiments suggest that it may also have some roles in liver tumor development, although possible mechanisms for tumor promotion are not well understood. PPARα may induce tumor development by inhibiting apoptosis, interacting with growth control genes and enhancing H2O2 production by stimulation of peroxisome proliferation (12, 13). Genetic or acquired variations in expression and activity of this molecule may have an impact on several physiological and pathological con-

**Table 2.** Frequency of L162V in different etiologies

| Etiology | No. of Patients | No. of patients with L162V | L162V% |
|---|---|---|---|
| HBV | 62 | 6 | 9.7 |
| HBV alone | 50 | 5 | 10 |
| HBV+HDV | 12 | 1 | 8.3 |
| HCV | 21 | 0 | 0 |
| Controls | 80 | 2 | 2.5 |

HBV vs HCV, p=0.163 HBV vs Controls, p=0.076
HBV+HDV vs HCV, p=0.163 HBV+HDV vs Controls, p=0.071

**Table 3.** Characteristics of patients having L162V polymorphism

| Pt. no | Etiology | Tumor size | Met | PVT |
|---|---|---|---|---|
| **1** | **B** | **Diffuse** | **-** | **-** |
| **2** | **B** | **Multiple** (greatest length 8 cm) | **+** | **+** |
| **3** | **B** | **Multiple** (1-2 cm) | **-** | **-** |
| **4** | **B+D** | **Solitary** (5x5x4 cm) | **-** | **-** |
| **5** | **B** | **Multiple** (greatest length 5 cm) | + | + |
| **6** | **B** | **Multiple** (greatest length 4 cm) | **-** | **-** |

PVT: Portal vein thrombosis. Met: Metastasis.

ditions. L162V polymorphism of the PPARα gene was reported to enhance transactivation function but not increase the expression of this molecule (27). This polymorphism has been found to be associated with several in vivo lipid and apolipoprotein abnormalities (18-21), but its role in human cancers has not been explored yet. This study represents the first investigating the role of this polymorphism in human HCC.

PPARα L162V polymorphism tended to more frequently occur in HCC patients compared to healthy controls. Interestingly, this trend was more significant in HBV-related HCC patients. Although the present study included a total of 90 HCC patients, this sample size may not be enough to reach a statistical significance because of the low frequency of L162V polymorphism. In addition, the present study included a heterogeneous patient population consisting of both HBV- and HCV-related HCC cases. In fact, L162V polymorphism was more frequently observed in HBV-related HCC patients, and this trend was not observed in HCV-related HCC patients. Interestingly, all patients carrying L162V polymorphism were infected with HBV and none of the HCV-infected patients had this polymorphism. These findings may be clues for the existence of different carcinogenesis mechanisms in these two common etiologies.

Associations of this polymorphism with several pathological alterations may be limited to some specific conditions. This can be exemplified by the finding that L162V polymorphism may be associated with some specific lipid abnormalities and BMI only in diabetic patients (18-21, 24). In fact, L162V polymorphism was not found to be associated with blood lipid abnormalities nor with BMI in healthy controls and patients with coronary heart disease (18-21, 25). Similarly, L162V and HCC association may be restricted to HBV-induced HCC cases. Variations in the expression and/or the activity of PPARα, its ligands and PPRE and their regulators in different diseases and in different conditions may diversely affect the outcome of these diseases. Presence of a variant PPARα molecule may render HBV-infected patients prone to HCC development.

The potential mechanism(s) of L162V polymorphism and HBV-induced HCC association are not clear. PPARα has been shown to interact with HBV X-associated protein2 (26). In addition, RXR-PPARα heterodimer may transactivate enhancer 1 of HBV (27). Such a viral protein/regulatory sequence and PPARα/PPRE interaction may alter the cellular responses to PPARα and/or HBV viral proteins. Furthermore, variations in PPARα gene (e.g. L162V polymorphism) may change these responses and may facilitate tumor development. In addition, gene activation status and expression of growth control molecules, and malignant cell responses to exogenous/endogenous stimuli may be different in HBV- and HCV-induced HCC. Presence of several variant host proteins (e.g. L162V) may promote cancer development in selected conditions (e.g. HBV-induced disease). Further studies are needed to clarify whether PPARα interacts with several viral and cellular proteins that may result in tumor promotion and progression.

Another interesting finding of the present study was that most patients with L162V polymorphism had advanced tumors. Although this might be related with the small number of patients having this polymorphism, change in transactivation function of PPARα may lead to more aggressive tumor behavior. This latter speculation must be cautiously interpreted and needs to be investigated with further large-scale population studies.

In summary, PPARα L162V polymorphism seems to be selectively associated with HBV-induced HCC but not with HCV-induced HCC. Further studies are needed to determine whether this polymorphism selectively changes cellular responses to exogenous stimuli (e.g. HBV viral protein) and/or cellular factors, thus promoting tumor development.

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