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# A functional variation in *CHI3L1* is associated with severity of liver fibrosis and YKL-40 serum levels in chronic hepatitis C infection☆

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***Background/Aims*: YKL-40 is a chitinase-like protein involved in matrix remodelling and a non-invasive fibrosis marker. We assessed whether a functional promoter polymorphism in *CHI3L1*, encoding YKL-40, is associated with HCV-induced liver fibrosis and influences YKL-40 serum concentrations.**

***Methods*: The *CHI3L1* −131G → C promoter polymorphism was genotyped in two cohorts of HCV infected patients ($n = 440$) by 5′-endonuclease assays. Histological fibrosis scores and YKL-40 serum levels (ELISA) were associated with *CHI3L1* −131G → C by quantitative and qualitative genetic analyses and corrected by multivariate analysis.**

***Results*: *CHI3L1* −131G → C genotype was strongly associated with the stage of liver fibrosis in the screening ($n = 265$, $P = 0.001$) and validation cohort ($n = 175$, $P = 0.009$). Homozygous carriers of the G allele were protected from severe fibrosis (F3/ F4). This association was confirmed after correction for age and gender. Functionally, the G allele was associated with reduced serum levels of YKL-40 in HCV infected patients ($P = 0.002$).**

***Conclusions*: The *CHI3L1* promoter polymorphism −131G → C determines YKL-40 serum levels and is associated with the severity of HCV-induced liver fibrosis. These results suggest a functional role of YKL-40 in liver fibrogenesis and should be taken into account when using YKL-40 as a non-invasive fibrosis marker.**



***Keywords*: Liver fibrosis; YKL-40; Gene polymorphisms**

## 1. Introduction

Chronic hepatitis C (HCV) is one of the most common causes of liver cirrhosis and hepatocellular carcinoma in the US and Europe. In recent years it has become apparent that only about half of all infected patients progress to end-stage liver disease and are at increased risk of dying from complications directly related to HCV [1]. These individuals are considered as primary candidates for antiviral therapies which are still only successful in a proportion of all patients and are limited by side effects and costs [2]. Numerous studies have identified non-modifiable (e.g. age, gender, age

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*Abbreviations:* HCV, hepatitis C virus; SNP, single nucleotide polymorphism.

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at infection) and potentially modifiable factors (e.g. alcohol consumption, cigarette smoking, iron overload) which are associated with progressive liver disease in HCV infection [3]. However, even when studies were controlled for these parameters, there is still a great variation between individuals who develop severe liver fibrosis and cirrhosis. Therefore, genetic predisposition to HCV-induced liver fibrosis is considered to play an important role for an individual's risk to develop progressive liver disease [4,5]. The identification of genetic variants which determine the development of liver fibrosis has been the focus of numerous studies within the last decade but results have been difficult to reproduce and some associations have uncertain underlying biological mechanisms. Therefore, in contrast to genome-wide association studies [6,7], hypothesis driven genetic studies which investigate limited numbers of polymorphisms should concentrate on variants which have substantial probability of affecting the function of the relevant gene product [8,9].

YKL-40 (chondrex, cartilage glycoprotein-39) belongs to the chitinase family and is strongly expressed in human liver and arthritic articular cartilage [10,11]. Although YKL-40's physiological function has not been fully elucidated, it is thought to contribute to tissue remodelling and degradation of extracellular matrix (ECM) [12]. YKL-40 has also been shown to act as a growth-factor for fibroblasts [13] and acts as a chemoattractant for endothelial cells, thereby modulating angiogenesis during tissue damage [14]. Based on this background, serum levels of YKL-40 have been repeatedly used as a non-invasive marker of chronic inflammatory and fibrotic diseases. With respect to liver disease, YKL-40 serum levels are reproducibly increased in alcoholic liver disease [15] and in HCV-induced liver fibrosis [10,16–18]. Notably, YKL-40 serum levels closely correlate with hepatic mRNA levels, suggesting that the serum levels are a reflection of ECM remodelling in the liver. Furthermore, YKL-40 serum concentrations were able to differentiate progression rates between slow and rapid fibrosing patients [10].

The YKL-40 protein is encoded by the chitinase 3-like 1 gene *CHI3L1* located on human chromosome 1q32.1 [19]. Variations within *CHI3L1* have recently been associated with the risk of asthma and bronchial hyperresponsiveness. Furthermore, a promoter polymorphism of *CHI3L1* ($-131G \rightarrow C$) has been strongly correlated with the serum levels of YKL-40 with a *P*-value of $1.1 \times 10^{13}$, a difference which is already present at birth [20].

Based on these results we performed a genetic study in two large cohorts of patients with HCV infection and hypothesized that the functional promoter polymorphism *CHI3L1* $-131\ G \rightarrow C$ is associated with the severity of liver fibrosis and the serum levels of YKL-40 in HCV infection.

## 2. Patients and methods

### 2.1. Patients

Overall, 440 patients with chronic HCV infection were included in the study. All included individuals were of Caucasian origin. The patients were recruited from the outpatients departments of the Universities in Aachen, Regensburg and Berlin. Chronic HCV infection was diagnosed based on a positive anti-HCV assay and a positive HCV-RNA test (TaqMan assay with a lower limit of detection of 50 IU/ml). In all patients other chronic liver diseases were excluded by appropriate serological tests. Alcohol intake was below 20 g/day in all patients included. All subjects tested negative for HBsAg and anti-HIV (both Abbott).

Prior to antiviral therapy, all included patients underwent percutaneous liver biopsy for grading and staging of their liver damage according to the score proposed by Desmet et al. [21]. Of note, the liver biopsies taken in Aachen and Regensburg ($n = 265$) were retrospectively scored by the same pathologist to avoid interobserver variation and to homogenize the phenotypic data. In Berlin the histology samples ($n = 175$) were also scored by one experienced pathologist. Thus, two independent and homogeneous cohorts were generated which fully fulfil current criteria for genetic association studies [22].

The study protocol was approved at all three locations by the medical ethics committees and informed consent was obtained from patients prior to inclusion.

### 2.2. Genotyping of *CHI3L1* $-131\ G \rightarrow C$

Genotyping of patients for the single nucleotide polymorphism (SNP) rs4950928 (*CHI3L1* $-131G \rightarrow C$) was performed with genomic DNA with 5′-endonuclease (TaqMan) assays. Primers, probes and assay conditions can be obtained from www.appliedbiosystems.com. Each 25 μl of PCRs contained 20 ng of genomic DNA, 900 nM primers, 250 nM probes, and 12.5 μl of TaqMan Universal PCR master mix (Applied Biosystems). The cycling conditions were as follows: 50 °C for 2 min, 95 °C for 10 min, and 40 cycles at 94 °C for 15 s and 60 °C for 1 min. Fluorescence in each well was measured using an ABI PRISM 7000 Sequence Detection System (Applied Biosystems). Additionally, we confirmed the TaqMan assay findings by direct sequencing in representative samples. The *CHI3L1 −131* genotypes were classified as CC (homozygous for the major allele), GC (heterozygous) or GG (homozygous for the minor allele).

### 2.3. YKL-40 serum levels

The serum concentration of YKL-40 was determined in 74 patients by a validated ELISA system according to the manufacture's instructions (Quidel, San Diego, USA). Serum samples were taken at the time of liver biopsy.

### 2.4. Statistical analysis

Continuous data is given as mean ± standard error of the mean. Genetic data were analyzed on the genotypic and allelic level after accordance of genotype distribution with Hardy–Weinberg equilibrium was assessed with an exact test.

For quantitative genetic analysis the stage of fibrosis was compared between the *CHI3L1 −131* genotypes CC, GC and GG by one-way ANOVA with Kruskal–Wallis tests. For qualitative analysis, patients were separated in two groups according to the severity of histological fibrosis (group 1 with histological stages 0–2 representing mild to moderate fibrosis, and group 2 with stages 3 and 4 representing severe fibrosis and cirrhosis). The groups with mild and severe fibrosis consisted of 314 (192 from Aachen/Regensburg, 122 from Berlin) and 126 (73 from Aachen/Regensburg, 53 from Berlin) patients, respectively.

For all markers the effect of the allele with the lower minor allele frequency (risk allele) was tested. The groups with mild and severe fibrosis were analyzed for difference in allele frequency by chi-square