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*BRIEF ARTICLE*

# Association between inherited monogenic liver disorders and chronic hepatitis C

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## Abstract

**AIM:** To determine the frequencies of mutations that cause inherited monogenic liver disorders in patients with chronic hepatitis C.

**METHODS:** This study included 86 patients with chronic hepatitis C (55 men, 31 women; mean age at diagnosis, 38.36 ± 14.52 years) who had undergone antiviral therapy comprising pegylated interferon and ribavirin. Viral load, biochemical parameter changes, and liver biopsy morphological data were evaluated in all patients. The control group comprised 271 unrelated individuals representing the general population of Latvia for mutation frequency calculations. The most frequent mutations that cause inherited liver disorders [gene (mutation): *ATP7B* (H1069Q), *HFE* (C282Y, H63D), *UGT1A1* (TA)7, and *SERPINA1* (PiZ)] were detected by polymerase chain reaction (PCR), bidirectional PCR allele-specific amplification, restriction fragment length polymorphism analysis, and sequencing.

**RESULTS:** The viral genotype was detected in 80 of the 86 patients. Viral genotypes 1, 2, and 3 were present in 61 (76%), 7 (9%), and 12 (15%) patients, respectively. Among all 86 patients, 50 (58%) reached an early viral response and 70 (81%) reached a sustained viral response. All 16 patients who did not reach a sustained viral response had viral genotype 1. Case-control analysis revealed a statistically significant difference in only the H1069Q mutation between patients and controls (patients, 0.057; controls, 0.012; odds ratio, 5.514; 95%CI: 1.119-29.827, $P$ = 0.022). However, the H1069Q mutation was not associated with antiviral treatment outcomes or biochemical indices. The (TA) 7 mutation of the *UGT1A1* gene was associated with decreased ferritin levels (beta regression coefficient = -295.7, $P$ = 0.0087).

**CONCLUSION:** Genetic mutations that cause inherited liver diseases in patients with hepatitis C should be studied in detail.



**Key words:** Hepatitis C; Hepatolenticular degeneration (Wilson's disease); *ATP7B*; *SERPINA1*; *UGT1A1*; *HFE*

**Core tip:** This is the first study to evaluate the association between hepatitis C and the most frequently inherited monogenic liver diseases (hereditary hemochromatosis, alpha-1 antitrypsin deficiency, Gilbert's syndrome, and Wilson's disease) and their causative mutations. This case-control study revealed an association between hepatitis C and the mutation that causes Wilson's disease. In addition, biochemical data analysis

revealed an association between hepatitis C and the mutation that causes Gilbert's syndrome.

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## INTRODUCTION

Hepatitis C is an infectious disease caused by the hepatitis C virus (HCV), which primarily affects the liver. An estimated 130 to 200 million people worldwide are infected with HCV[1]. The most common monogenic inborn errors of metabolism associated with liver disease are hereditary hemochromatosis, alpha-1 antitrypsin deficiency, Wilson's disease, and Gilbert's syndrome. These diseases have a particularly high frequency in Northern Europe and Latvia[2-4]. Hereditary hemochromatosis is characterized by excessive iron overload and is most commonly caused by *HFE* gene mutations[2]. The frequency of the most common *HFE* mutation, C282Y, is 0.035 in Latvia and 0.026 Lithuania; however, the frequency of hereditary hemochromatosis is lower at 0.013[5]. Alpha-1 antitrypsin deficiency is caused by the absence of the proteinase inhibitor alpha-1 antitrypsin, and affected patients develop liver disease and emphysema in the third or fourth decade of life[3]. Wilson's disease is a progressive autosomal recessive disorder of copper metabolism. The carrier frequency of the causative mutation is 1:80 in Latvia and 1:90 in Europe[4]. Finally, Gilbert's syndrome is characterized by benign unconjugated hyperbilirubinemia with a frequency of 5.0% to 14.8% in Europe[6]. Most reports on the coexistence of monogenic liver diseases and HCV infection have focused primarily on hereditary hemochromatosis[7] because elevated iron levels are necessary for viral replication[8,9]. Although the associations of HCV infection with alpha-1 antitrypsin deficiency[10,11] and Gilbert's syndrome[12-14] have been investigated, the association of HCV infection with Wilson's disease remains unclear. Copper reportedly plays a potential role in the development of HCV infection[15,16].

The aim of the present study was to determine the frequency of mutations that cause inherited monogenic liver disorders in patients with chronic HCV infection who have undergone antiviral therapy and in whom the viral response status is known.

## MATERIALS AND METHODS

### Ethics

This study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by the *Central Medical Ethics Committee* of *Latvia*. All study participants signed an informed consent form that was issued according to the regulations of the *Central Medical Ethics Committee* of *Latvia*.

### Subjects

Eighty-six patients with HCV infection who had undergone antiviral treatment with ribavirin and pegylated interferon were included in this study. These patients comprised 55 men and 31 women with a mean age at diagnosis of 38.36 ± 14.52 years (men, 37.27 ± 15.69 years; women, 40.25 ± 12.26 years). All patients were of European descent. The pretherapeutic alanine transaminase level, iron level, ferritin level, viral load, and HCV genotype were evaluated in all patients. The Knodell histology activity index was used for morphological examination.

The control group comprised 271 unrelated individuals chosen to represent the general population of Latvia. Participants in the control group underwent polymorphism frequency determination only. Biochemical association analysis, clinical examination, and exclusion of HCV infection were not performed in this group.

### Genotyping methods

Peripheral blood genomic DNA was purified by standard phenol: chloroform extraction and ethanol precipitation with slight modification as described elsewhere[17] using reagents from Sigma Aldrich, Inc. (St. Louis, MO, United States). A summary of the methods used in this study is presented in Table 1[4,18-20]. Reagents used for polymerase chain reaction (PCR) (buffers, dNTP mix, Taq polymerase, and agarose) were obtained from Thermo Fisher Scientific (Waltham, MA, United States). Synthetic oligonucleotides, the sequences of which have been previously published[4,18-20], were obtained from Metabion GmbH (Martinsried, Germany). Fluorescent PCR products were analyzed with an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, United States) using the reagents described in the manufacturer's protocol.

### Statistical analysis

PLINK software[21] was used for genotyping data analysis and quality control. Analysis adhered to a call rate of < 98% and Hardy-Weinberg equilibrium $P$ value of $\leqslant$ 0.05. The chi-square test was used to compare the patient and control groups with a significance threshold of $P < 0.05$. SPSS software v.16.0 (SPSS Inc., Chicago, IL, United States) was used to compare mean biochemical marker values between the patient and control groups and between the two patient groups [with and without a sustained viral response (SVR), defined as the inability to detect viral RNA six months after therapy[22]. Parametric values were compared using ANOVA, and nonparametric data were evaluated with the Mann-Whitney test. Genotype association analysis with biochemical markers was conducted using a full linear model comprising

**Table 1 Genotyping methods used in the present study**

| Disease | Gene | Mutation | rs[1] | Analysis method |
|---|---|---|---|---|
| Hereditary hemochromatosis | *HFE* | C282Y | rs1800562 | PCR-RFLP with restrictase *RsaI*[18] |
| | | H63D | rs1799945 | PCR-RFLP with restrictase *MboI*[18] |
| Gilbert's syndrome | *UGT1A1* | (TA)7, UGT1A1*28 | rs8175347 | Fluorescent PCR[20] |
| Alpha-1 antitrypsin deficiency | *SERPINA1* | PIZ | rs28929474 | Bi-PASA[19] |
| Wilson's disease | *ATP7B* | H1069Q | rs76151636 | Bi-PASA[4] |

[1]Single nucleotide polymorphism database number (http://www.ncbi.nlm.nih.gov/snp/). PCR: Polymerase chain reaction; RFLP: Restriction fragment length polymorphism analysis; Bi-PASA: Bidirectional PCR allele-specific amplification.

**Table 2 Allelic frequencies in patient and control groups**

| Gene | rs[1] | Mutation | Patients ($n$ = 86) | Controls ($n$ = 271) | OR | 95%CI | $P$ value |
|---|---|---|---|---|---|---|---|
| *UGT1A1* | rs8175347 | (TA)7 | 0.371 | 0.350 | 1.098 | 0.643-1.871 | 0.796 |
| *HFE* | rs1799945 | C282Y | 0.048 | 0.035 | 1.420 | 0.358-5.218 | 0.522 |
| | rs1800562 | H63D | 0.096 | 0.121 | 0.740 | 0.301-1.760 | 0.563 |
| *ATP7B* | rs76151636 | H1069Q | 0.057 | 0.012 | 5.514 | 1.119-29.827 | 0.022 |
| *SERPINA1* | rs28929474 | PIZ | 0.012 | 0.016 | 0.363 | 0.013-5.158 | 0.576 |

[1]Single nucleotide polymorphism database number (http://www.ncbi.nlm.nih.gov/snp/). OR: Odds ratio.

three genetic effects: additive effects of allele dosage, dominance deviation from additivity (a negative value indicates a recessive allele), and the 2-df joint test of both additive and dominance. Beta was evaluated as the regression coefficient. Data were accepted as statistically significant at a $P$ value of $< 0.05$. Sex and age (with confirmed HCV infection) were used separately as covariates. The adjusted beta coefficient and $P$ value were applied for each covariate. The association of the HCV genotype with the therapy response was assessed using the $\chi^2$ test.

## RESULTS

Viral RNA was not detectable in 50 (58%) of the 86 patients (30 men, 20 women) in the third month of antiviral therapy. Sixteen (19%) patients (13 men, 3 women) did not reach an SVR. The HCV genotype was determined in 80 patients; genotypes 1, 2, and 3 were present in 61, 7, and 12 patients, respectively. All patients who did not reach an SVR had viral genotype 1; for this reason, the odds ratio (OR) and 95%CI were not calculated ($P = 0.015$).

Genetic marker analysis revealed a significantly higher frequency of the *ATP7B* H1069Q mutation in patients than in controls (0.057 *vs* 0.012, respectively; OR = 5.514; 95%CI: 1.119-29.827, $P = 0.022$). Further results of the genetic marker analysis are shown in Table 2.

The presence of inherited liver disease was confirmed in nine patients (eight had Gilbert's syndrome with genotype (TA)7/(TA)7, and one had hereditary hemochromatosis with genotype C282Y/H63D). The presence of inherited liver disease was confirmed in 30 (11%) controls; all had Gilbert's syndrome.

In the comparison of patients who had reached an SVR with those who had viral persistence (*i.e.*, negative response to treatment), a significant association was found between the iron level and the presence of viral persistence (Table 3). Neither the other biochemical markers nor the histology activity index showed statistically significant differences between the patient and control groups.

In the patient group, association analysis was performed between genetic markers and the biochemical markers alanine transaminase level, ferritin level, iron level, and viral load. A statistically significant association was found only between the ferritin level and the (TA)7 allele of the *UGT1A1* gene. The strongest model for the association of the *UGT1A1* gene with the ferritin level was dominance deviation from additivity (beta = -295.7, $P = 0.0087$), and the statistical significance remained after adjusting for age ($\text{beta}_{\text{adjusted}} = -264.4$, $P_{\text{adjusted}} = 0.0219$) and sex ($\text{beta}_{\text{adjusted}} = -249.3$, $P_{\text{adjusted}} = 0.0305$). The associations between the other biochemical indices and genetic markers were not statistically significant for any of the analyzed models.

## DISCUSSION

Numerous studies have been conducted to identify host and viral factors that influence antiviral therapy efficiency in patients with HCV infection. Approximately 40% to 50% of individuals with viral genotype 1 and 80% with genotypes 2 and 3 reach an SVR[1]. Compared with these previously reported rates, a higher number of patients with genotype 1 in the present study reached SVR. In addition, all patients with genotypes 2 and 3 reached an SVR. These differences between our study results and those in the literature are likely due to our small patient group and relatively young patient age (38.36 ± 14.52 years) because increasing age is a risk factor for ineffective therapy[22]. Various risk factors are reportedly

**Table 3 Characterization of the patient group**

| | Result of antiviral therapy | Mean | 95%CI of mean | | *P* value |
|---|---|---|---|---|---|
| | | | Lower bound | Upper bound | |
| Age in year at diagnosis | Sustained viral response | 38.76 | 34.88 | 42.64 | 0.788 |
| | Viral persistence | 37.87 | 32.07 | 43.66 | |
| | Total | 38.43 | 35.24 | 41.61 | |
| Alanine transaminase level | Sustained viral response | 106.45 | 81.73 | 131.17 | 0.056 |
| | Viral persistence | 153.75 | 104.27 | 203.23 | |
| | Total | 123.65 | 99.86 | 147.44 | |
| Iron level | Sustained viral response | 20.84 | 18.19 | 23.49 | 0.015 |
| | Viral persistence | 29.92 | 22.05 | 37.80 | |
| | Total | 24.71 | 20.98 | 28.45 | |
| Ferritin level | Sustained viral response | 298.67 | 185.52 | 411.82 | 0.354 |
| | Viral persistence | 397.79 | 197.96 | 597.62 | |
| | Total | 336.09 | 231.98 | 440.20 | |
| Viral load | Sustained viral response | 1.91E + 06 | 1.12E + 06 | 2.70E + 06 | 0.115 |
| | Viral persistence | 4.21E + 06 | 5.15E + 05 | 7.90E + 06 | |
| | Total | 2.75E + 06 | 1.35E + 06 | 4.14E + 06 | |

associated with an individual patient's response to antiviral treatment, including the homocysteine level, vitamin D level, and many other parameters[23,24]. However, only sex, age, liver disease progression, viral genotype, and insulin resistance are included in the clinical guidelines as possible risk factors[22]. In the present study, the only markers that significantly influenced the efficacy of antiviral therapy were the alanine transaminase level ($P$ = 0.056) and the iron level ($P$ = 0.015). Iron is necessary for the replication of HCV; however, iron depletion therapy before antiviral therapy has not been proven to be effective[25]. The small size of our patient group is the main reason why the other data did not show a statistically significant impact on the efficacy of antiviral therapy.

Of all mutations that cause inherited liver diseases, the most extensively studied are those that cause hereditary hemochromatosis[26]. Although we did not detect a statistically significant association between HCV infection and the C282Y or H63D mutation in our study, the C282Y mutation was found to be more common in the patient group than in the control group (frequency of 0.048 *vs* 0.035, respectively) (Table 2). We also failed to detect an association between the iron or ferritin level with either the C282Y or H63D mutation. This result may have been due to our small patient group and/or the ages of our patients (men, 37.27 ± 15.69 years; women, 40.25 ± 12.26 years). Our patients may have been too young to manifest the symptoms characteristic of hereditary hemochromatosis because symptoms related to iron overload usually appear between the ages of 40 and 60 years in men and after menopause in women[27]. In addition, the higher serum iron levels seen in our patients with HCV infection may have been caused by various factors other than *HFE* gene mutations; *e.g.*, hepatocyte necrosis or increased intestinal iron uptake[28].

In contrast to previous reports[10,11], we did not detect an association between HCV infection and alpha-1 antitrypsin deficiency. Again, this may have been due to the small number of patients in our study and/or the fact that liver symptoms in patients with alpha-1 antitrypsin deficiency more commonly manifest in childhood or late adulthood. Advanced liver disease generally occurs around the age of 66 years in individuals heterozygous for the PIZ mutation[29]. The mean age of our patients was 38.43 years at the completion of analysis.

Gilbert's syndrome, also termed benign hyperbilirubinemia[30], was included in our study because an estimated 10% to 15% of European descent individuals are affected by this syndrome and because previous data have demonstrated anti-inflammatory and antioxidant functions of bilirubin[31,32]. The (TA)7 polymorphism of the *UGT1A1* gene was shown to be significantly associated with the ferritin level (beta = -295.7, $P$ = 0.0087). Some studies have proposed that ferritin, being an acute-phase reactant, behaves as a marker of more active and advanced liver disease. Patients with chronic HCV infection and high serum ferritin levels reportedly have significantly more severe liver inflammation and fibrosis than do patients with normal serum ferritin levels[7,33]. In our study, patients with viral persistence had slightly elevated ferritin levels. Based on the analysis of the association of the ferritin level with genetic markers, the (TA)7 polymorphism could be associated with less prominent liver inflammation and lower ferritin levels in patients with HCV infection. This may in turn lead to a better antiviral treatment response, and future studies should address this notion. Our results also support the idea that more extensive liver inflammation can lead to viral persistence as evidenced by the fact that alanine transaminase levels were higher in patients with viral persistence.

Interestingly, the H1069Q mutation of the *ATP7B* gene was found to be associated with chronic HCV infection. We included this mutation in our analysis because a high rate of Latvians reportedly carry this mutation[4]. The *ATP7B* gene is involved in copper metabolism. Copper is well known to be critical for the proper functioning of both the humoral and innate immune systems; however, its precise mechanisms of action are unknown[34]. The spontaneous elimination or

persistence of HCV infection depends on the host's immune status[35]. Previous reports have stated that the host response to HCV infection may be primarily dependent on the human leukocyte antigen system. However, other factors, such as copper, may also influence the host response because changes in copper levels in patients with HCV infection have been reported[15,36,37]. We propose that the H1069Q mutation of the *ATP7B* gene may be an important modifier in patients with HCV infection. Future studies should investigate this in detail, especially considering the fact that Wilson's disease is treatable.

The main limitation of our study was the small number of patients and controls. No analysis was performed to exclude HCV infection in the control group. However, this was a pilot study. Research involving larger numbers of patients and controls in whom HCV infection has been excluded is warranted.

## COMMENTS

### Background

Inherited monogenic liver diseases and their causative mutations may represent genetic factors responsible for changing the host response to hepatitis C virus (HCV) infection. Although the association between HCV infection and hereditary hemochromatosis has been extensively studied, only a few studies on the associations between HCV and the mutations causing alpha-1 antitrypsin deficiency, Gilbert's syndrome, and Wilson's disease have been performed.

### Research frontiers

Mutations that cause inherited liver diseases are highly distributed and associated with chronic inflammation and liver damage. This is one of the critical points in HCV infection.

### Innovations and breakthroughs

Many studies have been performed in an attempt to identify host genetic factors that can influence the efficacy of antiviral therapy in patients with chronic HCV infection. This is first study to analyze all of the most common genetic disorders in one patient group. The results of this pilot study show that this research should be continued with a larger group of patients.

### Applications

Therapy for inherited liver disorders is either already available or is currently under investigation. If the importance of such therapies with respect to alleviating liver damage in HCV infection is proven, the efficacy of antiviral therapy may be improved by establishing treatment that is more specifically targeted not only to the viral life cycle, but also to factors directly associated with the development of liver damage. Alpha-1 antitrypsin deficiency is a liver disease caused by the absence of the proteinase inhibitor alpha-1 antitrypsin. Wilson's disease is a progressive autosomal recessive disorder of copper metabolism. Gilbert's syndrome is characterized by benign unconjugated hyperbilirubinemia.

### Terminology

Hepatitis C is an infectious liver disease caused by the HCV, that affects an estimated 130 to 200 million people worldwide. Inherited monogenic liver disorders are inherited diseases, caused by mutations in one gene (autosomal recessive inheritance), in which the primary manifestation is liver damage.

### Peer review

This case-control study is the first to examine the association between HCV and frequently inherited monogenic liver diseases (hereditary hemochromatosis, alpha-1 antitrypsin deficiency, Gilbert's syndrome, and Wilson's disease) and their causative mutations. This study revealed an association between HCV and the mutation responsible for Wilson's disease. Biochemical experiments revealed an association between HCV and the mutation that causes Gilbert's syndrome. This is a well-designed study that brings new insight into the association between inherited liver diseases and HCV.

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