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Streszczenie w języku angielskim ·

The impact of selected genetic polymorphisms on the progression of liver fibrosis in cholestatic liver disease

Introduction: The data presented experts of the European Association of Hepatology (EASL) clearly demonstrate the significant burden of liver disease in Europe. Progressive fibrosis is common in individuals with chronic liver diseases (CLD) and can be induced by a wide variety of causes including non-alcoholic fatty liver disease (NAFLD), viral infections (mainly hepatitis B and C, i.e., HBV and HCV), alcoholic liver disease (ALD), cholestatic diseases (PBC - primary biliary cholangitis; PSC - primary sclerosing cholangitis) and autoimmune hepatitis (AIH). Excessive hepatic aggregation of collagen (liver fibrosis) is caused by persistent liver injury and sustained wound healing. Early stages of hepatic fibrosis are asymptomatic in most patients. The signs of liver scarring are clinically apparent in late stage of the disease, i.e., when patients develop cirrhosis. Transplantation is the only therapeutic option at the stage of decompensated cirrhosis, which due to limited access to organs is possible only in some patients. Complications associated with liver fibrosis and cirrhosis are the main limiting factors in the survival of patients with chronic liver diseases (CLD). Additionally, more than 30% of patients with cirrhosis will develop hepatocellular carcinoma (HCC).

Progression of liver fibrosis is the result of an interaction between genetic predisposition and external factors. Fibrogenesis is one of the so-called complex traits, from a genetic standpoint. This means that progress is regulated by numerous genes, their polymorphisms and mutations, which interact to produce the phenotype. A significant part of the genetic component of the progression of viral and metabolic liver diseases has been detected but less is known about genetic modifiers of other chronic liver diseases.

Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) represent two rare chronic cholestatic liver diseases. They can progress to liver fibrosis and cirrhosis and the pathogenesis of both of them is not clear. PBC affects the interlobular bile ducts, causing ductopenia and progressive cholestasis. It is characterized by immune-mediated destruction of intrahepatic small bile ducts and lymphocyte-mediated portal inflammation. PSC is characterized by progressive, chronic inflammation and destruction of intrahepatic and extrahepatic bile ducts, liver cell damage and fibrosis. The role of

genetic predisposition in PBC and PSC has been suspected, but so far genetic studies have been inconclusive. Liver scarring and its consequences - impaired liver function and portal hypertension - affect the life expectancy of patients with chronic liver disease. To date, major genetic modifiers of PBC and PSC have not been identified.

Materials and Methods: This doctoral dissertation consists of a series of three original papers. This study examined the effect of selected genetic polymorphisms on the progression of liver fibrosis in cholestatic liver disease (PSC, PBC, AIH and PSC-AIH, PBC-AIH). Following polymorphisms that could potentially be associated with liver disease progression were genotyped using TaqMan assays: *PNPLA3* (rs738409), *TM6SF2* (rs58542926), *MARC1* (rs2642438), *HSD17B13* (rs72613567), *MBOAT7* (rs641738), *ABCB4* (rs2109505). Genetic analysis using the TaqMan methodology utilizes DNA amplification (PCR) and DNA labeling with fluorescent probes.

In the first study, we prospectively included 178 patients with PSC (66 women, age range 17-75 years, 55 with cirrhosis).

In the second study, 313 patients with AIH were prospectively enrolled including AIH and AIH-PSC and AIH-PBC variants of 206 (66%), 77 (25%) and 30 (9%) patients, respectively (218 women, age range 18-84 years, 130 with cirrhosis).

In the third study, 867 patients with PBC and PSC were included. The first group of patients recruited in Szczecin consisted of 196 patients with PBC (174 women, age range 23-87 years, 82 with cirrhosis) and 135 patients with PSC (39 women, age range 17-69 years, 53 with cirrhosis). The second group of patients recruited in Warsaw consisted of 260 patients with PBC (241 women, age range 29-86 years, 114 with cirrhosis) and 276 patients with PSC (97 women, age range 22-81 years, 92 with cirrhosis). Two control groups, 150 healthy blood donors and 318 patients without liver disease, were recruited in Szczecin and Warsaw, respectively.

Results: Statistical analysis of the results of first study showed that both *PNPLA3* and *TM6SF2* polymorphisms had no effect on liver fibrosis progression. Serum liver enzyme activities were not modified by the presence of *PNPLA3* (ALT $P = 0.88$, AST $P = 0.77$) or *TM6SF2* (ALT $P = 0.92$, AST $P = 0.49$) risk variants. Increasing number of risk alleles had no impact on serum liver enzyme activities, as demonstrated by a separate analysis of patients carrying 0 ($n = 99$), 1 ($n = 64$), 2 ($n = 12$) or 3 ($n = 3$) risk alleles ($P > 0.05$). No significant effect of the polymorphisms was observed in the group of patients before and

after transplantation, as well as in patients with ulcerative colitis, which is a common comorbidity in PSC. We did not observe any correlation between the polymorphisms studied and gender, liver fibrosis and liver enzyme activity.

In the second study we investigated the effect of *MARC1* (rs2642438), *HSD17B13* (rs72613567), *PNPLA3* (rs738409), *TM6SF2* (rs58542926) and *MBOAT7* (rs641738) polymorphisms in AIH patients with cholestatic variants of PBC (AIH-PBC) and PSC (AIH-PSC). Carriers of the protective *MARC1* allele had lower ALT and AST values (P = 0.04, P = 0.02, respectively). Patients with AIH-PSC and AIH-PBC variants carrying the *HSD17B13* polymorphism had lower serum GGT (P = 0.02). The *MBOAT7* polymorphism was associated with an increased risk of developing HCC cancer (OR = 3.71, P = 0.02). The *HSD17B13*, *TM6SF2*, and *MBOAT7* variants were not associated with liver function test scores, LSM, FIB-4, APRI, or MELD (all P > 0.05). In addition, none of the polymorphisms studied was associated with cirrhosis at the time of diagnosis. Also, none of the five genetic variants studied were associated with transplantation or death (all P > 0.05).

In the third and final paper we investigated the effect of the *ABCB4* c.711A>T polymorphism on fibrosis progression in PBC and PSC patients. In this study we demonstrated that in two studied PBC cohorts, carriers of the risk variant presented more frequently with cirrhosis (Szczecin: OR = 1.841, P = 0.025; Warsaw: OR = 1.528, P = 0.039). The risk allele was associated with increased serum AST (Szczecin P = 0.018), ALP (Szczecin P < 0.001; Warsaw P = 0.021) and GGT (Szczecin P = 0.003; Warsaw P = 0.045) at inclusion. During the follow-up, patients with PBC significantly improved their laboratory results, regardless of *ABCB4* c.711A>T genotype (P > 0.05). During 8±4 years of follow-up, 22 patients in the Szczecin PBC group developed cirrhosis, and this risk was higher among risk variant carriers (OR = 5.65, P = 0.04). In contrast to PBC, we did not detect any association of *ABCB4* c.711A>T with liver phenotype in the PSC cohorts.

Conclusions: Neither *PNPLA3* nor *TM6SF2* polymorphisms appear to significantly contribute to the risk of liver function decline in patients with PSC (paper 1). *MARC1* polymorphism has a protective effect in patients with AIH, AIH-PSC and AIH-PBC variants (paper 2). The *MBOAT7* polymorphism was associated with an increased risk of developing HCC cancer. The common procholestatic variant *ABCB4* c.711A>T modulates liver damage in PBC but not in PSC. In particular, carriers of the major allele have an increased risk of progressive hepatic scarring (paper 3).