Genetic Background of Japanese Patients with Nonalcoholic Steatohepatitis (NASH)

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Summary. The pathogenesis of nonalcoholic steatohepatitis (NASH) is not understood well. Therefore, it is necessary to examine genetic influences on NASH pathogenesis. Two functional polymorphisms were studied: the –493 G/T polymorphism in the promoter of microsomal triglyceride transfer protein (MTP) and the 1183 T/C polymorphism in the mitochondrial targeting sequence of manganese superoxide dismutase (MnSOD). The G allele in the MTP promoter leads to decreased MTP transcription, less export of triglyceride from hepatocytes, and greater intracellular triglyceride accumulation. In addition, glucose intolerance with hyperinsulinemia, which may be responsible for down-regulating *MTP* mRNA expression, is frequent among NASH patients, as observed in caucasians. The T allele in the MnSOD mitochondrial targeting sequence leads to less transport of MnSOD to the mitochondria. Blood samples from patients with biopsy-proven NASH and healthy controls were analyzed by the polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP). Functional polymorphisms in MTP and MnSOD were revealed to be involved in determining susceptibility to NASH in Japanese.

Key words. Fatty liver, Single-nucleotide polymorphism, Manganese-SOD, Microsomal triglyceride transfer protein, Obesity

Introduction

The term "nonalcoholic steatohepatitis (NASH)" is used to describe a form of liver injury that is indistinguishable from alcoholic hepatitis [1]. The pathological features of NASH reveal hepatic steatosis with hepatocellular injury, focal mixed cell-type inflammation, and fibrosis [2, 3]. In fact, most cases of NASH appear to have a multifactorial etiopathogenesis. Current models propose a "two-hit" hypothesis, whereby lipid first accumulates in hepatocytes, then triggers inflammation by a variety of mechanisms [4]. Accumulated data support this idea. For example, obesity was found in half of a cohort of patients with cryptogenic cirrhosis [5].

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Genetic variation in lipid metabolism may produce differences in the speed and extent of hepatocyte lipid accumulation, the "first hit." One relevant enzyme is the microsomal triglyceride transfer protein (MTP). This protein transfers triglycerides to nascent apolipoprotein B, producing very-low-density lipoprotein (VLDL) and removing lipid from the hepatocyte. Patients with abetalipoproteinemia, an autosomal recessive disease caused by mutations in the MTP coding region, develop marked hepatic steatosis early in life [6]. *MTP* mRNA expression in human hepatocytes is downregulated by insulin (7).

There is considerable evidence for the role of oxidative damage in NASH, one of the "second hits". In a steatotic liver, free fatty acids are diverted to the mitochondria, where they are oxidized by beta-oxidation, and may generate increased amounts of reactive oxygen species (ROS). Lipid peroxidation likely damages plasma and intracellular membranes, leading to apoptosis and necrosis of hepatocytes. Lipid peroxidation end-products (malondialdehyde and 4-hydroxy-2-nonenal [HNE]) may also trigger inflammatory and immune-mediated mechanisms of hepatocyte injury [8]. Animal models of nonalcoholic fatty liver disease and biopsy specimens of patients with alcoholic liver disease have shown that the degree of lipid peroxidation in the liver correlates with the extent of steatosis.

One enzyme that is important in detoxifying mitochondrial ROS is manganese superoxide dismutase (MnSOD). This enzyme is synthesized in the cytosol and modified post-transcriptionally for transport to the mitochondria [9]. A limited number of polymorphisms have been described for MnSOD, including a T/C polymorphism in the mitochondrial targeting sequence. This polymorphism leads to a valine-to-alanine amino- acid change in the mitochondrial targeting sequence. In turn, this amino-acid substitution may alter the helical structure of the mitochondrial targeting sequence, enhancing the transport of MnSOD into the mitochondrial matrix [10].

Here, we review the importance of hyperinsulinemia and functional polymorphisms of MTP and MnSOD in determining an individual's susceptibility to NASH (Fig. 1).

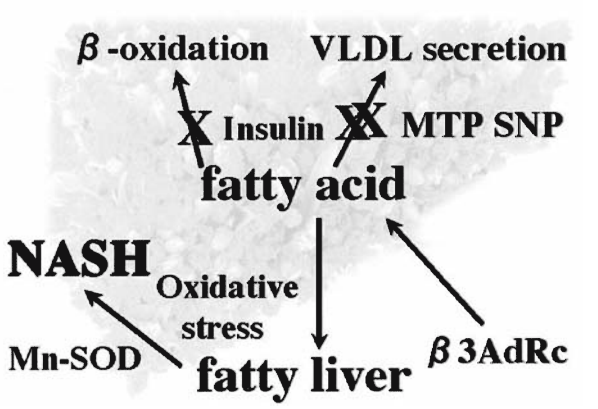

Fig. 1. Fatty acid metabolism pathway in the liver, and the development of hepatic steatosis. *VLDL*, very low density lipoprotein; *MTP*, microsomal triglyceride transfer protein; *SNP*, single-nucleotide polymorphism; *NASH*, nonalcoholic steatohepatitis; *SOD*, superoxide dismutase.

Hyperinsulinemia

NASH is often linked with disorders that are clearly associated with insulin resistance, obesity, hypertriglyceridemia, and type 2 diabetes mellitus. Fasting plasma insulin level is high in two-thirds of NASH patients (Fig. 2). The majority of NASH patients have glucose intolerance when challenged with 75 g oral glucose (Fig. 3). This is a frequent observation in western countries; however, glucose intolerance with hyperinsulinemia is an infrequent observation among Japanese, because the majority of glucose intolerance observed in Japanese is associated with impaired insulin secretion. This unusual frequency of hyperinsulinemia in NASH patients reveals the importance of hyperinsulinemia in the development of NASH. It is important to remember that hyperinsulinemia results in downregulated hepatic fatty-acid beta-oxidation and mRNA expression of MTP [7].

Functional–493 G/T Polymorphism in the Promoter of Microsomal Triglyceride Transfer Protein (MTP)

Recently, hepatic steatosis in patients with chronic hepatitis C has attracted much attention. Hepatitis C virus core protein was proved to inhibit MTP activity and VLDL secretion [11]. The G allele has been previously shown to produce less *MTP* gene transcription than the T allele. Less MTP activity would lead to lower excretion of triglyceride as VLDL, and greater accumulation of lipid in the hepatocytes [12]. Thus, the G allele in the MTP promoter would render a patient more susceptible to steatosis,

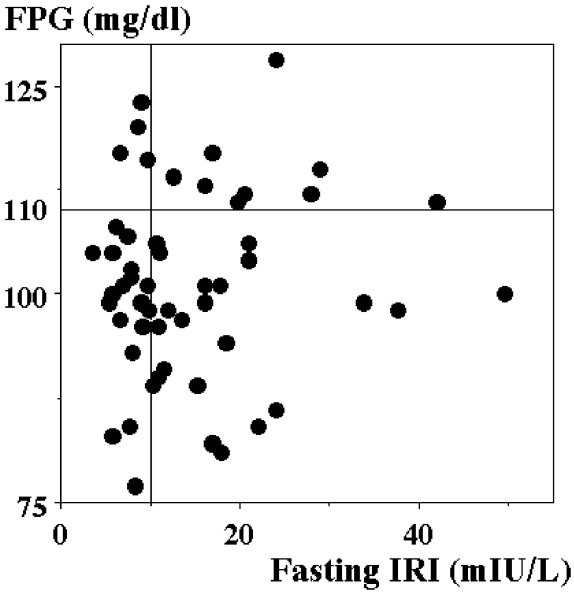

Fig. 2. Levels of fasting plasma glucose (*FPG*) and fasting plasma insulin in NASH patients. *IRI*, immunoreactive insulin.

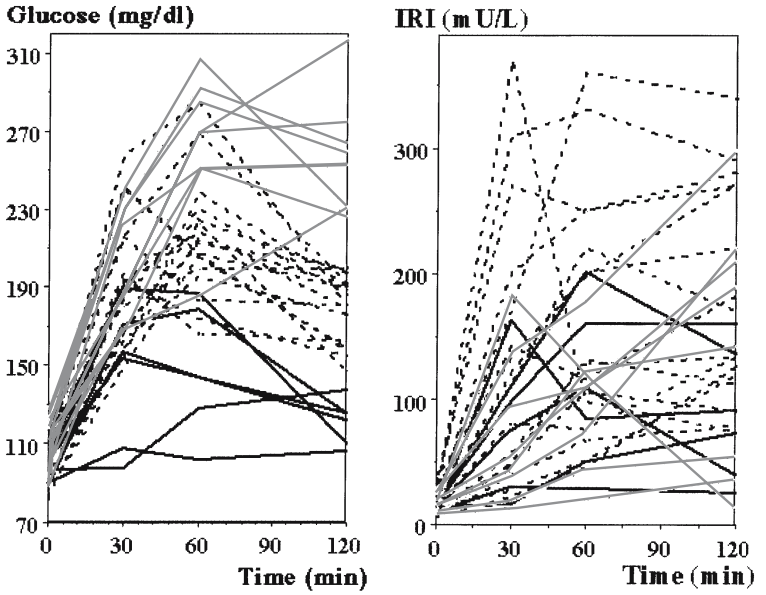

Fig. 3. 75-g Oral glucose tolerance test in NASH patients.

the "first hit" in the "two-hit" hypothesis of NASH pathogenesis. The G/G genotype is observed in 60% of Japanese [13]. The increased susceptibility to steatosis is apparent in a wide population of NASH patients in Japan. The stage of fibrosis, assessed by Brunt's criteria, was significantly higher in NASH patients with the G/G genotype than in patients with the G/T genotype. However, there were no significant differences in terms of age, body mass index (BMI) or levels of alanine aminotransferase (ALT), fasting triglyceride, and total cholesterol between the G/G-genotype and the G/T-genotype groups.

Functional 1183 T/C Polymorphism in the Targeting Sequence of Manganese Superoxide Dismutase (MnSOD)

Excessive oxidative stress has been suggested as the "second hit", by a pathophysiologic molecule, in the development of NASH [14]. MnSOD, a potent scavenger, of oxygen free radicals, localized to mitochondria, has a key role in preventing oxidative stress to hepatocytes. Less effective targeting of MnSOD to mitochondria may affect a patient's susceptibility to NASH, by decreasing the capacity to detoxify superoxide anions produced in mitochondria and increasing the susceptibility to excessive oxidative stress in hepatocytes [15]. In fact, as the T allele results in less effective targeting of MnSOD [16], higher oxidative stress may result in a higher prevalence of NASH. The T/T genotype frequency in NASH patients was found to be significantly higher than that in controls [13]. Unfortunately, the C allele was shown to have a

lower prevalence in Japanese than in Chinese and European populations. This may render Japanese people more susceptible to oxidative stress in hepatocytes than other populations.

Conclusions

Severe insulin resistance with hyperinsulinemia is a characteristic clinical feature in Japanese NASH patients, as well as in NASH patients from other ethnic groups, although the majority of Japanese NASH patients have impaired insulin secretion. However, the frequency of Japanese NASH patients with both the G/G genotype of MTP and the T/T genotype of MnSOD was significantly higher than that in healthy volunteers. This may render Japanese more susceptible to oxidative stress in hepatocytes than other populations in the world.

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