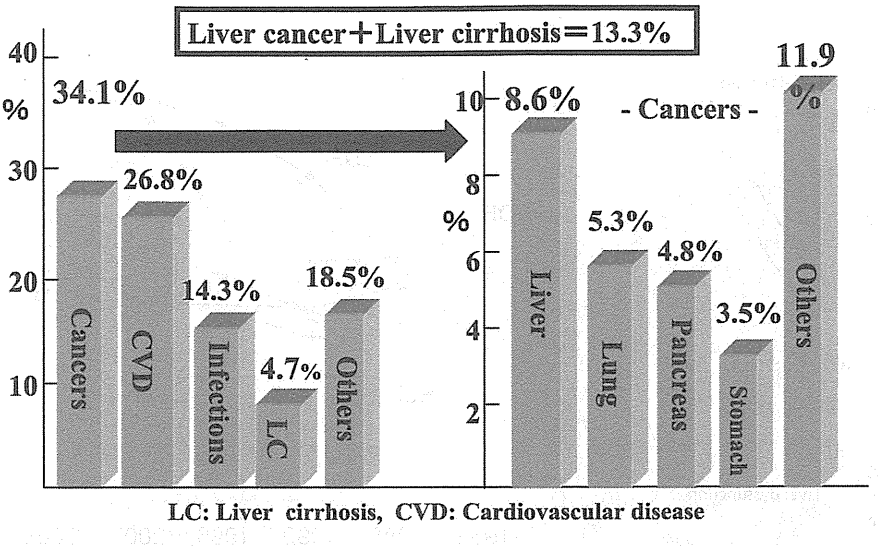

Figure 1 Cause of death in diabetes mellitus patients in Japan. The leading cause of death in 18 385 diabetes mellitus patients, who died from 1991 to 2000, was cancer, the second was cardiovascular diseases, the third was infection and the fourth was liver cirrhosis. Among the cancer deaths, the highest rate was of hepatocellular carcinoma (8.6%; males: 10.5% and females: 5.2%). In the general Japanese population, the primary cause of cancer death is lung cancer, the next is gastric cancer, the third is colon cancer and the fourth is HCC in males.

lifestyle-related diseases, such as obesity, diabetes and dyslipidemia. The Japan Society of Ningen Dock (health check-up organization) reported in 2008[2] that the prevalence of liver dysfunction, including fatty liver, was 31.9% in men and 17.1% in women, based on a study carried out on 1 814 864 adult men and 1 136 903 adult women. The prevalence of obesity, liver dysfunction, and high levels of cholesterol and triglyceride showed no significant differences in distribution with age in men, but the prevalence increased with age in women; for those in their 60s, it reached a high level comparable to that in men. Glucose intolerance and high blood pressure increased with age in both men and women (Fig. 3a/3b). A comparison of annual variations showed increase of all these factors, but the increase was especially marked in the incidence of liver dysfunction, obesity, and hypercholesteremia, and these became prominent in the late 1990s (Fig. 4).

Kojima *et al.* reported that the prevalence of fatty liver detected by medical health checks increased year after year, from 12.6% in 1989 to 30.3% in 1998.[3] According to the report by the Japan Society of Ningen Dock in 2008, 26.2% of subjects who underwent health check-ups showed fatty liver by abdominal ultrasonography.[2]

The majority of fatty liver disease comprises alcoholic fatty liver and NAFLD, including NASH. Tanaka *et al.* reported that approximately 25% of the health check-up examinees had fatty liver.[4] Hamaguchi *et al.* reported that the prevalence of NAFLD was 23.3% in Japanese adults.[5] There is a gender difference in the incidence of NAFLD; men are more likely to develop fatty liver. There is also a gender difference in the age distribution; in men, the incidence of fatty liver is about 25% and remains unchanged from the 30s to the 60s, whereas in women, the prevalence of fatty liver increases gradually with age and, in the 60s and beyond, reaches nearly the same level as in men. According to previous reports, the number of NAFLD patients is estimated to be 10 million (the population in Japan is around 130 million), and, from recent studies around 2% of them are considered to have NASH.

In 2008, a fact-finding survey was conducted on the causes of cirrhosis at the 44th Annual Meeting of the Japan Society of Hepatology; 33 379 cirrhotic patients were enrolled in 58 hospitals, and 2.1% were diagnosed with NASH-induced cirrhosis (Fig. 5). According to that survey, the proportion of NASH cirrhosis is 1.4% in males and 3.4% in females, and there is a significant gender difference ($P < 0.005$). In that study, obese subjects were few and, at that time, the concept of NASH was not yet commonly accepted by many Japanese doctors. Furthermore, many cases of advanced stage NASH show no fatty deposit, so-called "burn-out NASH", resulting in the diagnosis of cryptogenic liver cirrhosis. Therefore, the actual incidence of NASH-related cirrhosis might be higher than was reported.

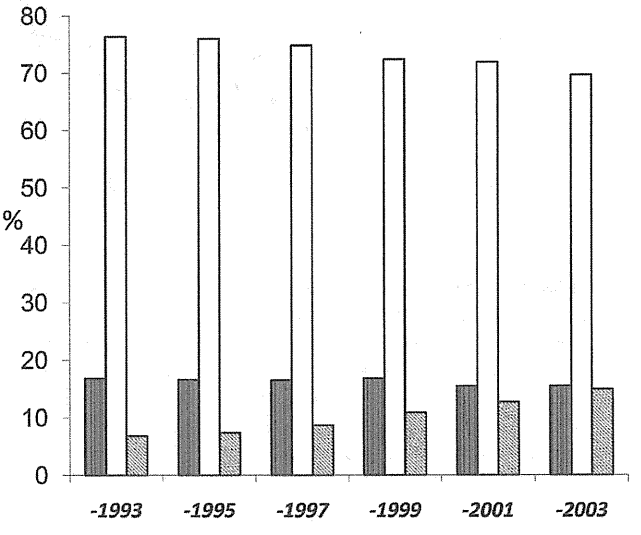

Figure 2 Change of the etiology of hepatocellular carcinoma (HCC) in Japan from 1993 to 2003. Among the causes of HCC, the HCV infection rate was decreasing gradually and HBV infection showed no significant change; however, the prevalence of non B, non C HCC has doubled over the past 10 years. HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non B, non C. ▥, HBV; □, HCV; ▨, NBNC.

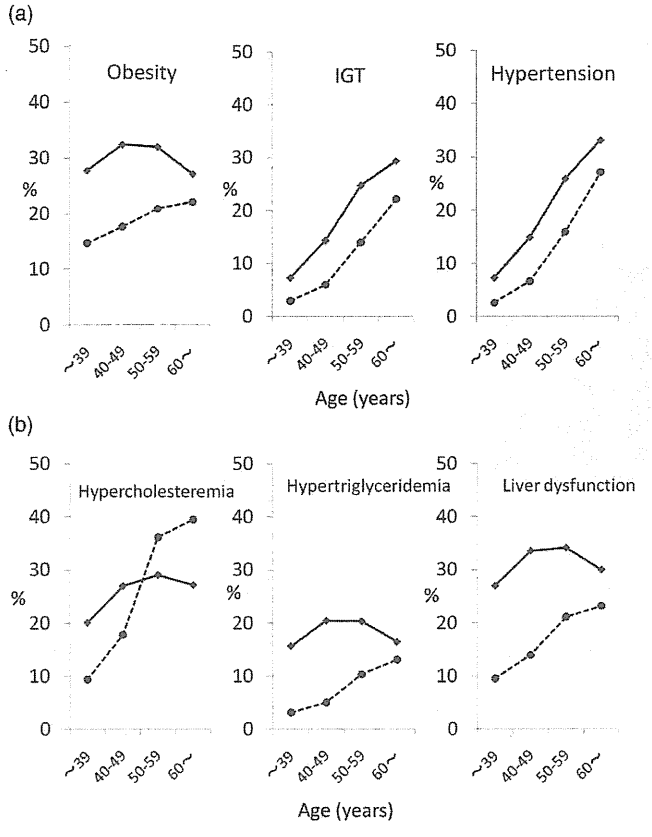

Figure 3a/3b The complication rates of life-style related diseases in the general population receiving health check-ups in 2008. In women, the prevalence of obesity, hypercholesteremia, hypertriglyceridemia, and liver dysfunction increased with age and, for women in their 60s, these reached a high level comparable to those in men. Glucose intolerance and high blood pressure increased with age in both sexes. IGT, impaired glucose tolerance. —◆—, Male; --●--, Female.

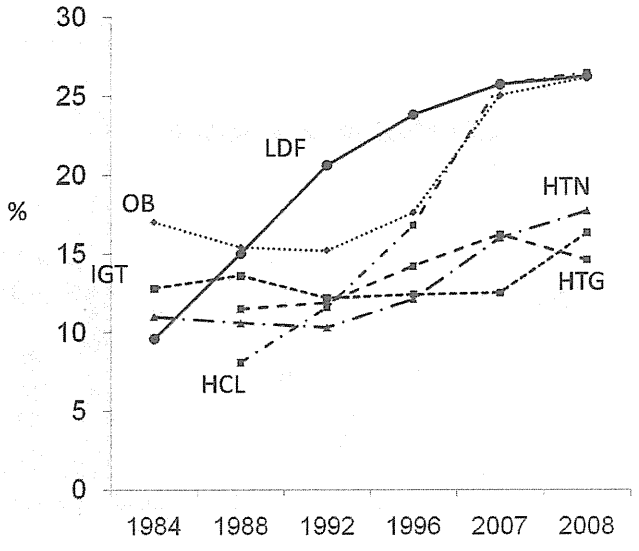

Figure 4 Annual variation of life-style related diseases in the general population. During the past 20 years all diseases significantly increased year by year. ···◆···, Obesity(OB); --■--, Impaired Glucose Tolerance (IGT); —▲—, Hypertension(HTN); —■—, Hypercholesteremia(HCL); —■—, Hypertriglyceridemia(HTG); —●—, Liver dysfunction(LDF).

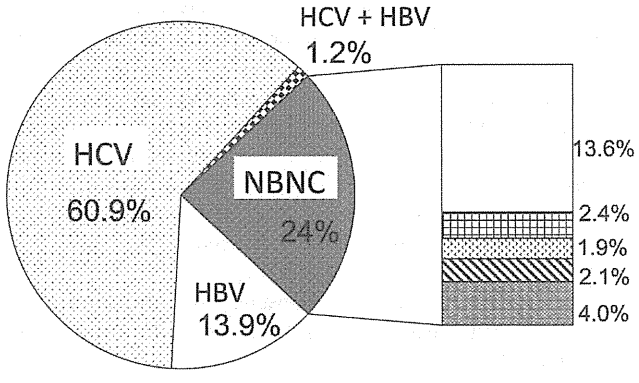

Figure 5 Etiology of liver cirrhosis in Japan. A nationwide survey was carried out in 2008 at the 44th Annual Meeting of the Japan Society of Hepatology. The non B, non C group occupied 24%, of which 2.1% were diagnosed as definite NASH. HBV, hepatitis B virus; HCV, hepatitis C virus; NBNC, non B, non C; PBC, primary biliary cirrhosis; AIH, autoimmune hepatitis; NASH, nonalcoholic steatohepatitis. □, Alcohol; ▦, PBC; ▨, AIH; ▧, NASH; ■, Others.

Metabolic syndrome

Patients with metabolic syndrome are increasing in number in Japan (Figs. 4,6). Visceral fat accumulation and insulin resistance are usual in these patients. The enhanced insulin resistance caused by the excessive accumulation of body fat (especially visceral fat) is considered to be important in the pathogenesis of fatty liver.

The diagnostic criteria for metabolic syndrome established by the Japanese Society of Internal Medicine are as follows:[6] an umbilical abdominal circumference (men: 85 cm or more; women: 90 cm or more) which reflects visceral fat accumulation (a visceral fat area of 100 cm^2 or more), and any two of the following four criteria: (i) elevated serum triglyceride level; (ii) reduced HDL cholesterol; (iii) elevated blood pressure; and (iv) elevated fasting plasma glucose. According to the National Health and Nutrition Examination Survey conducted in Japan in 2008, the prevalence of patients afflicted by metabolic syndrome was 25.3% among men and 10.6% among women, whereas patients with pre-metabolic syndrome (patients with an abdominal circumference of ≥85 cm in men and 90 cm in women, and who fulfill one other criterion) accounted for 21.9% of the men and 8.3% of the women. Therefore, approximately half of Japanese men and about 20% of Japanese women might have metabolic syndrome or be predisposed to metabolic syndrome.[7]

The criteria for metabolic syndrome are useful for the screening of NAFLD. The previous report by Ishibashi *et al.* stated that abdominal circumference was well correlated with NAFLD in men, but not in women.[8] Waist circumference has been reported to be smaller in men than women and there has been considerable debate regarding whether this criterion is appropriate or not.[9] There is the possibility that the amount of visceral fat might be underestimated and that the estimate may detect fewer than the actual number of

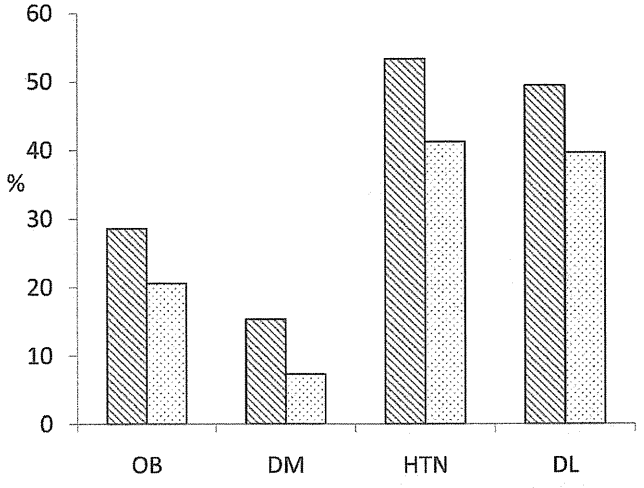

Figure 6 The complication rate of lifestyle-related diseases in Japan in 2007 to 2008. OB, obesity; DM, diabetes mellitus; HTN, hypertension; DL, dyslipidemia. ▨, Male; ▩, Female.

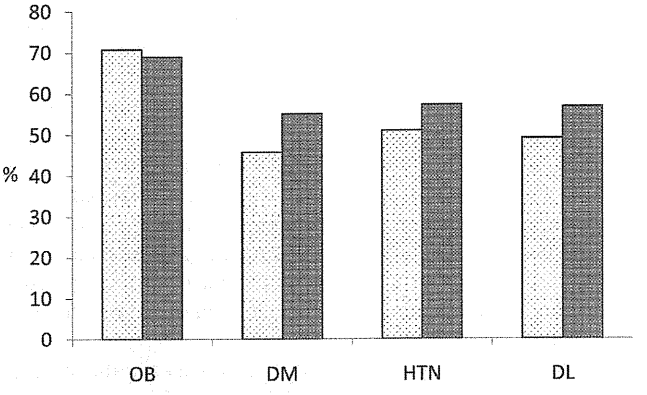

Figure 7 The complication rates of life-style related diseases among 283 NAFLD patients subjected to liver biopsy at Saiseikai Suita Hosptital from 2008 to 2010. SS, simple steatosis; NASH, nonalcoholic steatohepatitis; OB, obesity; DM, diabetes mellitus; HTN, hypertension; DL, dyslipidemia. ▨, SS n=96; ▩, NASH n=187.

women with NAFLD. In women, caution is required when the abdominal circumference is used instead of the visceral fat area.

Epidemiologically, it is clear that the risk of cardiovascular diseases is increased markedly in people with multiple risk factors for life-style related diseases. In addition, Hamaguchi *et al.* showed that NAFLD patients were at high risk of cardiovascular diseases and NAFLD, but not metabolic syndrome (MS), and showed a statistically significant correlation with cardiovascular disease in a multivariate model (OR = 4.12; 95% CI, 1.58–10.75).[10] Having multiple life-style related diseases is considered to be a risk factor for developing NASH.

The incidence of complications of life-style related diseases among 283 biopsy-proven NAFLD patients in Saiseikai Suita Hospital from April 2007 to March 2010 was high. However, no significant difference was seen in the incidence of individual factors between 187 NASH patients and 96 SS patients; obesity: NASH 69.0% *versus* SS 70.8%, diabetes: NASH 55.1% *versus* SS 45.8%, hypertension: NASH 57.2% *versus* SS 51.0%, and dyslipidemia: NASH 56.7% *versus* SS 49.0% (Fig. 7). Most NASH-cirrhotic patients have been reported to be obese with an average BMI of 27.6 ± 4.5 kg/m^2; the prevalence of the complications of diabetes and hypertension were 66.6% and 50.2%, respectively.[11]

Hamaguchi *et al.* also showed that the presence of metabolic syndrome was related to the new onset of NAFLD, with a 4-fold increase in men and an 11-fold increase in women compared to non-metabolic syndrome subjects.[5] Mitsumune *et al.* reported that obesity (6.3 fold), dyslipidemia (2.4 fold), hyperglycemia (1.8 fold), and hypertension (1.4 fold) all increased the odds of having NAFLD.

Obesity

The National Health and Nutrition Examination Survey[7] conducted in 2008 showed that the proportion of obese subjects with a BMI of 25 kg/m^2 or more was 28.6% of men and 20.6% of women. Classified by age, this category accounted for over 29% of men aged 30–69 years, whereas, for women, the obesity rate increased with age: 11.8% in their 30s, 18.0% in their 40s, 21.1% in their 50s, 24.4% in their 60s, and 26.8% in their 70s. The prevalence of fatty liver according to age shows the same tendency as obesity. Fatty liver was noted in only 2.7% of non-obese subjects with a BMI less than 23 kg/m^2 and was 10.5% in those with a BMI ≥ 23 but < 25 kg/m^2, 34.6% in those with a BMI ≥ 25 but < 30 kg/m^2, and 77.6% in highly obese subjects (BMI > 30 kg/m^2).[12] Kojima *et al.* carried out an analysis of 39 151 people who underwent a health check-up over a period of 12 years from 1989 to 2000. They reported that the grade of obesity correlated with the development of fatty liver. The prevalence was 12.8% in non-obese subjects (BMI < 25 kg/m^2), 51.4% in subjects with BMI ≥ 25 but < 30 kg/m^2, and 80.4% in highly obese subjects (BMI > 30 kg/m^2). In subjects with BMI values of 25 kg/m^2, the odds ratio for fatty liver was 6.3 compared with of non-obese subjects.[3]

Hamaguchi *et al.* reported that, in a group receiving a health check-up, 18% showed NAFLD at the time of the initial health check-up, and 10% (14% of men and 5% of women) developed NAFLD during the follow-up period of an average 414-days.[5] Patients with newly developed NAFLD showed weight gain of 1.7 ± 1.7 kg for men and 1.3 ± 1.4 kg for women. Logistic regression analysis showed that weight gain was an independent risk factor for the newly developed NAFLD, with an OR = 1.51 (95% CI, 1.40–1.63) for men and OR = 1.62 (95% CI, 1.39–1.89) for women.

Diabetes/impaired glucose tolerance

In the National Health and Nutrition Examination Survey conducted in Japan in 2007, 8 900 000 people were strongly suspected of diabetes (HbA1c ≥ 6.1%, or currently under treatment); the number of people with an undeniable possibility of diabetes (HbA1c ≥ 5.6% but < 6.1%) was 13 200 000, in total, the number of people possibly with diabetes was 22 100 000, which was 1.6-fold higher than 10 years earlier.[13] Kojima *et al.* reported that the prevalence of fatty liver was 18.6% in subjects with normal glucose metabolism (FBS < 110 mg/dL), 43.7% in borderline subjects (FBS ≥ 110 but < 126 mg/dL), and 53.3% in diabetic

patients (FBS ≥ 126 mg/dL). FBS ≥ 110 mg/dL was an independent risk factor for fatty liver (OR = 3.1).[3] Likewise, Jimba *et al.* reported that the overall prevalence of NAFLD was 29% among 1950 Japanese people receiving a health check-up; the prevalence was 27% in the normal glucose metabolism group (FBG < 6.1 mmol/L) and rose to 43% for the borderline type (FPG ≥ 6.1 but < 7.0 mmol/L) and 62% for the diabetic type (FBG ≥ 7.0 mmol/L or a medical history of diabetes). In addition, the incidence of complications with abnormal glucose metabolism (borderline type and diabetic type) was 19.1% in NAFLD patients, which was higher than the 5.6% of patients without NAFLD ($P < 0.001$).[14]

Miyaaki *et al.* examined the relationship between the stage of hepatic fibrosis and the prevalence of diabetes in Japanese patients. In the mild fibrosis group, 42% were complicated with diabetes, whereas in the severe fibrosis (bridging fibrosis or cirrhosis) group, the prevalence was as high as 71% ($P = 0.020$). Diabetes might be a factor responsible for the development of hepatic fibrosis in NAFLD.[15] Shiga *et al.* performed a 75-g oral glucose tolerance test on the participants of a health check-up. They found that blood glucose levels at one and two hours after glucose load showed a closer relationship with NAFLD than the fasting blood glucose level. Therefore, they stated the importance of the evaluation of impaired glucose tolerance (IGT) in detecting NAFLD.[16]

Hypertension

According to the criteria of the Japanese Society of Hypertension, systolic blood pressure under 130 mm Hg/diastolic blood pressure under 85 mm Hg is normal, pressure higher than 140/90 mm Hg is diagnosed as hypertension, and pressure 130–139/85–89 mm Hg is high-normal blood pressure.

In the National Health and Nutrition Examination Survey conducted in 2007, the prevalence of subjects with hypertension (including 24.0% currently under treatment) was 46.2%, the prevalence of high-normal blood pressure was 13.8%, and the normal pressure group was 40.0%.

Hypertension is frequently seen in NASH/NAFLD patients, but there are no reports describing the prevalence of NAFLD among hypertensive patients in Japan. Reports from overseas stated that systolic and diastolic blood pressures were correlated with liver fat content, and that the risk of developing NAFLD was 2-fold higher in patients with systolic hypertension, and 1.7-fold higher in patients with diastolic hypertension.[17,18] In addition, Donati *et al.* reported that even in non-obese, non-diabetic high blood pressure patients, the prevalence of fatty liver was three times higher than in healthy individuals. Further such patients showed high levels of HOMA-IR, indicating insulin resistance.[19] The pathogenesis of hypertension is influenced by various factors, such as salt intake, and also is associated with insulin resistance. It is important to know that even non-obese high blood pressure patients with no other lifestyle-related diseases are likely to develop NAFLD if they have insulin resistance. In Japan, large-scale studies on hypertension and NAFLD are currently underway, including among subjects with chronic kidney disease (CKD).

Recently, we reported the prevalence of CKD in 174 NAFLD patients. The prevalence of CKD was significantly higher in NASH patients (19 of 92; 21%) than SS patients (5 of 82; 6%), and associated with a higher body mass index and the presence of hypertension.[20]

Dyslipidemia

Dyslipidemia is a generic term describing a clinical condition in which the levels of cholesterol esters or triglycerides increase in the blood: high levels of triglycerides (150 mg/dL or higher) and LDL cholesterol (140 mg/dL or higher), with decreased levels of HDL cholesterol (less than 40 mg/dL) are each risk factors for other diseases. In the National Health and Nutrition Examination Survey conducted in 2007, the percentage of subjects suspected of dyslipidemia (including 9.7% currently under treatment) was 44.1%, and that of normal subjects was 55.9%.

Dyslipidemia in NAFLD often involves hypertriglyceridemia and decreased blood levels of HDL cholesterol. This is due to the insufficient effects of lipoprotein lipase (LPL), which leads to a decreased metabolism of triglyceride-rich lipoproteins into HDL cholesterol. In addition, there is also an increased synthesis of very-low density lipoprotein (VLDL).

Pediatric NASH/NAFLD

The incidence of pediatric NAFLD in Japan is increasing in proportion to the increase in the prevalence of childhood obesity. In a previous study conducted on children aged 6–15 years, Tominaga *et al.* reported that the prevalence of NAFLD was 3.4% in children aged 6–10 years and 5.2% in those aged 11–15 years.[21] In addition, the prevalence of NAFLD in children who met the diagnostic criteria for pediatric metabolic syndrome was 40.0% in those diagnosed with pre-metabolic syndrome, and 76.8% in those who fulfilled the criteria for metabolic syndrome. Tsuruta *et al.* also reported that, in a similar study conducted in 2007 on 288 junior high school students (13–15 years old), 5.9% were obese, the prevalence of NAFLD was 4.5%, and obesity and ALT levels of 30 IU/L or higher were independent risk factors for NAFLD in children.[22] In addition, the prevalence of complications with obesity (degree of obesity ((body weight—standard body weight)/standard body weight × 100) ≥ 20%) showed a higher frequency in NAFLD patients (58.3%) than those without NAFLD (5.7%, $P < 0.001$).

Pathophysiology and genetic background

Racial differences might affect the onset and pathophysiology of NAFLD. Weston *et al.* reported that the prevalence of obesity, dyslipidemia, and diabetes in NAFLD was similar among racial and ethnic groups, except that body mass index was lower in Asians compared to Whites, Hispanics, and African Americans ($P < 0.001$). Compared with the base population, Hispanics with NAFLD were overrepresented and Whites were underrepresented.[23] In addition, Mohanty *et al.* reported that African Americans showed a lower degree of steatosis than Whites. In contrast, it has been considered that Asians showed higher grades of ballooning and Hispanics showed higher grades of Mallory-Denk bodies, than Whites and other ethnicities combined.[24] These findings indicate the importance of racial differences for the development and progression of NAFLD.

There are many reports concerning the genetic predisposition to the development of NASH and NAFLD, and most of them refer to functional genetic polymorphisms. Tumor necrosis factor-alpha (TNF-α) is known to be produced by adipocytes in visceral fat and Kupffer cells in the liver. It inhibits insulin receptor substrate-1 (IRS-1) of target cells, and insulin receptor kinase in skeletal muscles and adipocytes, thereby cause or exacerbating insulin resistance. Increased blood levels of TNF-α have been reported in NAFLD and NASH patients whose BMI and insulin resistance were matched, thereby suggesting a relationship between increased levels of TNF-α and the development of NAFLD or the progression of NASH.[25] It has been reported in Japanese subjects that functional genetic polymorphisms of TNF-α are present at positions T-1031C and C-856A in the promoter region, and these were more frequent in patients with NASH, potentially mediating progression of the disease.[26]

Adiponectin has an insulin sensitivity effect by opposing fatty acid accumulation which causes insulin-resistance, an anti-atheriosclerotic effect, and an anti-inflammatory effect. Therefore, hypoadiponectinemia associated with obesity has been considered to play a crucial role in the development of metabolic syndromes. In addition, the serum adiponectin level has been shown to be lower in NASH patients than in healthy groups and simple fatty liver groups.[27] The presence of functional polymorphisms G45T and G276T is the adiponectin gene have been reported to be associated with diabetes.[28,29] Regarding Japanese subjects with NASH, it has been reported that the G/G homo-allele at the 45th base of the exon of adiponectin was more frequent in NASH with advanced fibrosis than that in mild fibrosis, and that insulin resistance was distinctly more prominent.[30] Yoneda *et al.* reported that genetic variations in angiotensin II type1 receptor (ATGR1) may influence the risk of NAFLD and liver fibrosis in NAFLD.[31]

Functional polymorphisms in the β3-adrenergic receptor gene, microsomal triglyceride transfer protein (MTP), phosphatidylethanolamine *N*-methyltransferase (PEMT), interleukin-1β (IL-1β), and manganese superoxide dismutase (MnSOD) have also been reported in Japan.[32–34] MTP and PEMT are important factors for the metabolism in triglyceride.

In addition, sex hormones are involved in gender differences in the incidence of NAFLD, and in postmenopausal women the decreased level of estrogen results in the accumulation of visceral fat and insulin resistance.[35] This may explain why postmenopausal women appear to be at a higher risk for the development of NAFLD.

Pathological diagnosis

NAFLD can be diagnosed in patients from whom hepatitis virus infection, alcoholic liver disease and autoimmune hepatitis have been excluded when over 5% of hepatocytes contain fatty droplets.

NAFLD encompasses a histological spectrum ranging from simple steatosis (SS) to NASH, the latter showing hepatocyte degeneration (ballooning hepatocyte), necrosis, inflammation and fibrosis.[36]

Recently, Matteoni *et al.* categorized NAFLD into four types; type 1 (simple fatty liver), type 2 (steatohepatitis), type 3 (steatonecrosis) and type 4 (steatonecrosis + Mallory-Denk body (MDB) or fibrosis). They proposed that types 1 and 2 should be categorized as SS, and types 3 and 4 as NASH, according to the prognosis based on their follow-up study.[37] Actually we sometimes encounter difficulty in the differential diagnosis between type 2 and type 3 NAFLD, and between type 3 and type 4 NAFLD. This is because the criteria of ballooning hepatocytes and presence of pericentral and pericelluar fibrosis are unclear when these morphological changes are very mild.

In 2005, Kleiner *et al.* proposed a new scoring system, the so-called NAFLD activity score (NAS), according to the extent of the three features: steatosis, hepatocellular ballooning and lobular inflammation. By the NAS, NASH is defined as having a score of five or more.[38] This score is based on disease activity and the evaluation of fibrosis is excluded; this might be not suitable for the diagnosis of advanced staged NASH. Brunt and others proposed a grading and staging system according to the grade of inflammation and fibrosis,[39] and this method is widely accepted in Japan.

Ten to 30% of NASH cases have the potential to develop to cirrhosis within 10 years. However, much attention should be paid to so-called "burn-out NASH", in which fatty droplets have disappeared during the progression of hepatic fibrosis, resulting in difficulty making a precise diagnosis of NASH. In such a case, we must make an effort to collect the detailed background and previous patient history. This difficulty could lead to an underestimation of the prevalence of NASH-cirrhosis the Mallory-Denk bodies (MDB) are one of the morphological hallmarks for the diagnosis of type 4 NAFLD: they are an abnormal flocculent producter in degenerated hepatocytes and are comprised of intermediate filaments (IF).[40] We consider that the frequency of MDB in Japanese NASH is lower than in western countries.

Biochemical markers: non-invasive score assessment

Liver histology is the gold standard for the diagnosis of NASH; however, it is invasive and there is a risk of sampling errors in some cases. It has been anticipated that it should be possible to use serum biochemical markers to diagnose NASH, and various parameters reflecting oxidative stress, insulin resistance, inflammation, apoptosis, and fibrosis have been proposed to discriminate between SS and NASH. A NASH test that allows prediction on the basis of 13 parameters has been reported in Europe but, in recent years, Gholam *et al.* designed a more convenient differential formula based on only two criteria: the AST level and the presence or absence of diabetes mellitus (DM).[41] Campos *et al.* proposed a clinical scoring system for NASH[42] in which the scored criteria consist of hypertension (HTN), type 2 DM, AST, ALT, sleep apnea syndrome, and race (exception for blacks). However, these reports are from Europe and the USA. Recently, it was reported that the serum level of soluble fraction in cytokeratin 18 (soluble CK-18) was able to discriminate between SS and NASH,[43] and this has been adopted for our Japanese patients (unpublished data).

We reported previously the importance of serum ferritin and thioredoxin levels, reflecting status of oxidative stress, in the differential diagnosis between SS and NASH.[44,45] Recently, Sumida *et al.* proposed the NAFIC (NASH, Ferritin, Insulin, Collagen) scoring using Japanese patients. This comprises three measurements: serum ferritin, insulin, and type-4 collagen 7s.[46] To determine the utility of this score, we conducted a validation study in collaboration with ten centers all over Japan (Japan Study Group of NAFLD; JSG-NAFLD).[46]

Various indicators have been proposed for the evaluation of the degree of fibrosis in NASH. From a study based on the analysis of 50 NASH patients including nine with cirrhosis, Fujii *et al.* reported that the cirrhosis determinant score (CDS) and the hepatitis C antiviral long-term treatment against cirrhosis (HALT-C) model were valuable for the differentiation of cirrhosis induced by NASH and HCV infection.[47] A French group proposed the BAAT score[48] and Fibrotest,[49] which assign one point to each of the following items: BMI, ALT, age, and triglycerides. Angulo *et al.* proposed the NAFLD fibrosis score which can be calculated from parameters such as age, platelet count, albumin, AST/ALT ratio, fasting hyperglycemia/DM, and BMI.[50] The NAFLD fibrosis score is simple and has advantages. However, the major problem is that liver biopsy cannot be avoided in around 25% cases, which are classified as intermediate because of scores halfway between the high cut-off level and the low cut-off level. Harrison *et al.* proposed the simple and easy BARD score based on BMI $\geq$ 28 kg/m^2, AST/ALT ratio, and DM; and reported that the odds ratio increased 17-fold for cases with scores of two points or higher, associated with F3 or higher stages of fibrosis.[51] However, Fujii *et al.* suggested that the BARD score was not useful in Japanese patients because the average BMI is significantly lower than western people.[52] The N (Nippon) score[15] is very simple; it can be calculated on the basis of only gender, age, and the presence or absence of type 2 DM and HTN, and has been evaluated by a multicenter study in Japan.[16] Recently we showed that senescence marker protein 30 (SMP-30), which has an antiapoptotic activity and an effect on Ca^{++} efflux, was significantly decreased in NASH compared to SS. Thus, SMP-30 is a useful marker for the differential diagnosis between SS and NASH. However, at present we cannot detect it in serum.[53]

Prognosis

It has been reported that cardiovascular-related death and liver-related death are significantly higher in NAFLD patients than with the general population.[54] A cohort study conducted in 2006, reported a development of cancers among 97 771 individuals in the general Japanese population; 6.7% of men and 3.1% of women had DM, in diabetes patients, the hazard ratio of developing liver cancer was 2.24 (95% CI, 1.64–3.04) in men, and 1.94 (95% CI, 1.00–3.73) in women during an average follow-up period of 10.7-years.[55]

In a comparative study between HCV and NASH cirrhosis matched by gender and age, obesity, diabetes, and dyslipidemia were significantly more frequent in NASH cirrhosis. The 5-year cancer rate was 11.3% in NASH cirrhosis and 30.5% in HCV cirrhosis.[55] The leading cause of death in these two types of cirrhosis was HCC, 47% in NASH and 68% in HCV, and the second cause was hepatic failure, 32% in NASH and 25% in HCV.[56,57] The annual incidence of HCC in Japan is 2.2% in NASH cirrhosis and 6.1% in HCV cirrhosis. Meanwhile, Ascha *et al.* reported that the annual incidence of HCC was 2.6% in patients with NASH cirrhosis, compared to 4.0% in HCV cirrhosis in the USA.[58]

Treatment

Diet and exercise therapy

Weight loss achieved by diet and exercise is the most important aspect of treatment in obese patients with NAFLD, including NASH. In those treated weight, blood biochemical data such as ALT, albumin, cholinesterase, total cholesterol and fasting blood glucose values, and steatosis decreased significantly after significant weight loss.[59] The recommended daily energy intake is 25–35 kcal/kg, daily protein intake is 1.0–1.5 g/kg and fat should be less than 20% of total calories.

Antihyperlipidemic drugs

Saibara *et al.* showed that bezafibrate for tamoxifen-induced NASH resulted in biochemical and histological improvement.[60] Dohmen *et al.* reported that administration of fenofibrate for fatty liver complicated with dyslipidemia improved dyslipidemia and led to a decrease in the levels of ALP, whereas the levels of ALT showed no significant change.[61] Hyogo *et al.* reported that atorvastatin led to an improvement in liver function, fibrosis marker, adipocytokine, and improvement of fatty liver and hepatic inflammation.[62] Nozaki *et al.* reported the utility of ezetimibe and acarbose in mouse models of NAFLD.[63] Recently, we also demonstrated the histological improvement using 96 weeks of ezetimibe monotherapy in the 45 biopsy-proven NAFLD patients.[64]

Angiotensin II type-1 receptor blockers (ARB)

There also have been a number of reports from Japan regarding the utility of angiotensin II type-1 blockers (ARB) in NASH. This application is derived from basic studies which showed the inhibitory effect of ARB on the progression of fibrosis via inhibition of the activation of hepatic stellate cells.[65–67]

Antidiabetic drugs

Morita *et al.* demonstrated the effect of nateglinide on glucose metabolism, liver function, and liver histology in NASH patients with type 2 diabetes.[68] The effects of metformin and thiazolidine derivatives such as pioglitazone and rosiglitazone on NASH were reported in Japan, however, the numbers were small and the trials were uncontrolled.

Other drugs

There is the possibility that combination therapies using pantethine and probucol,[69] colestimide[70] and α-tocopherol[71] are useful for NASH; however, the subjects were in small numbers and there was no histological analysis after treatment. Recently, Sanyal *et al.* reported that administration of vitamin E for 96 weeks administration for non-DM NASH patients significantly improved liver histology compared to placebo, this result being more promising than pioglitazone administration.[72] Phlebotomy might be effective in NASH with excessive iron deposition in the liver.[73]

Group survey in NASH in Japan

As mentioned above, the Japan NASH Study Group founded in April 2008 (the representative: Takeshi Okanoue, Table 1), has started the following research projects: (i) nationwide study of 5000 cases of diabetes mellitus; (ii) SNP study of 1000 cases of SS and NASH; (iii) long-term follow-up study of 1000 cases of SS

Table 1 The members of Japan NASH study group

The representative of this study group:
Takeshi Okanoue MD, PhD., Hepatology Unit, Saiseikai Suita Hospital
The members of this study group:
Yutaka Kohgo M.D, PhD., Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College,
Sumio Kawata M.D, PhD., Department of Gastroenterology, Yamagata University Faculty of Medicine,
Kazuhiko Koike, M.D., PhD, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo,
Kohjiro Ueki, M.D., PhD., Department of Diabetes and Metabolic Diseases, Graduate School of Medicine, the University of Tokyo,
Shigeki Arii, M.D., PhD., Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University,
Etsuko Hashimoto, M.D., PhD., Department of Internal Medicine and Gastroenterology, Tokyo Women's Medical University,
Sumio Watanabe, M.D., PhD., Department of Gastroenterology, Juntendo University School of Medicine,
Toshinari Takamura, M.D., PhD., Department of Disease Control and Homeostasis, Kanazawa University Graduate School of Medical Science
Kohichiroh Yasui, M.D., PhD., Department of Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine,
Fumihiko Matsuda, PhD, Center for Genomic Medicine, Kyoto University Graduate School of Medicine,
Tetsuo Takehara, M.D., PhD., Gastroenterology and Hepatology, Osaka University Graduate School of Medicine,
Toshiji Saibara, M.D., PhD., Department of Gastroenterology and Hepatology, Kochi Medical School,
Hirofumi Uto, M.D., PhD., Department of Digestive and Life-Style Related Disease, Kagoshima University Graduate School of Medical and Dental Sciences

and NASH; (iv) collection of 100 cases of NASH-HCC; (v) biochemical markers of differential diagnosis between SS and NASH; and (vi) therapeutic guidelines based on the individual pathophysiology. Projects i, ii, iii, and iv are going well and we are expecting to present these results, including SNPs, in the near future.

Conclusion

Recently, much attention has been paid to NAFLD in Japan because the number of NAFLD patients has been increasing, while non-B, non-C HCC also is increasing gradually. We suspect that NASH might be responsible for this increase in HCC in Japan; however, the precise cause of the increased non B, non C HCC has not yet been established. In this review, we have described the epidemiology and the present status of clinical and basic aspects of NASH/NAFLD in Japan.

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Original Article

Accumulation of refractory factors for pegylated interferon plus ribavirin therapy in older female patients with chronic hepatitis C

Kazuaki Chayama,[1] C. Nelson Hayes,[1] Kentaro Yoshioka,[2] Hisataka Moriwaki,[3] Takeshi Okanoue,[4] Shotaro Sakisaka,[5] Tetsuo Takehara,[6] Makoto Oketani,[7] Joji Toyota,[8] Namiki Izumi,[9] Yoichi Hiasa,[10] Akihiro Matsumoto,[11] Hideyuki Nomura,[12] Masataka Seike,[13] Yoshiyuki Ueno,[14] Hiroshi Yotsuyanagi[15] and Hiromitsu Kumada[16]

[1]*Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima,* [2]*Division of Liver, Biliary Tract and Pancreas Diseases, Department of Internal Medicine, Fujita Health University, Aichi,* [3]*Department of Gastroenterology, Gifu University Graduate School of Medicine, Gifu,* [4]*Department of Gastroenterology and Hepatology, Saiseikai Suita Hospital,* [6]*Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine, Osaka,* [5]*Department of Gastroenterology and Medicine, Fukuoka University School of Medicine, Fukuoka,* [7]*Department of Digestive and Lifestyle Related Disease, Health Research Course, Human and Environmental Sciences, Kagoshima University Graduate School of Medical and Dental Sciences, Kagoshima,* [8]*Department of Gastroenterology, Sapporo Kosei General Hospital, Sapporo, Hokkaido,* [9]*Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital,* [15]*Department of Internal Medicine, Graduate School of Medicine, University of Tokyo,* [16]*Department of Hepatology, Toranomon Hospital, Tokyo,* [10]*Department of Gastroenterology and Metabology, Ehime University Graduate School of Medicine, Matsuyama, Ehime,* [11]*Department of Medicine, Shinshu University School of Medicine, Matsumoto, Nagano,* [12]*The Center for Liver Diseases, Shin-Kokura Hospital, Kitakyushu City,* [13]*Department of Internal Medicine 1, Faculty of Medicine, Oita University, Oita, and* [14]*Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai, Miyagi, Japan*

Aim: Several host and viral factors have been reported to influence the effectiveness of pegylated interferon plus ribavirin combination therapy for chronic hepatitis C. In Japan, where the age of treated patients is comparatively high, recent studies have reported poor response to treatment in older female patients, but little is known about the relationship between advanced age in women and previously reported factors.

Methods: Using a database of 1167 patients chronically infected with hepatitis C virus (HCV) genotype 1b, we analyzed the amino acid sequences of the HCV core protein and interferon sensitivity determining region (ISDR) and examined the relationships among predictive factors.

Results: The proportion of patients with substitutions at core 70, which is associated with poor response to pegylated interferon plus ribavirin therapy, increased with age only in female patients. A similar trend was observed for ISDR wild type (wt). We also found that core 70 wt is associated with core 91 wt ($P = 5.4 \times 10^{-9}$) as well as ISDR wt ($P = 0.025$). HCV RNA levels were higher in patients with core and ISDR wt ($P < 0.001$). Furthermore, core amino acid mutations were associated with advanced fibrosis and higher inflammatory activity ($P = 0.028$ and 0.048, respectively) as well as higher gamma-glutamyltranspeptidase, alanine aminotransferase and low-density lipoprotein cholesterol levels ($P < 0.001$, 0.006 and 0.001, respectively).

Conclusion: A combination of factors account for poor response rate in older female patients in Japan. Elucidating the relationship between amino acid substitutions and metabolic alteration is an important step in understanding the mechanism of HCV interferon resistance.

Key words: combination therapy, core protein, genotype 1b, interferon sensitivity determining region, low-density lipoprotein cholesterol

Correspondence: Professor Kazuaki Chayama, Department of Medical and Molecular Science, Division of Frontier Medical Science Programs for Biomedical Research, Graduate School of Biomedical Science Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan.
Email: chayama@hiroshima-u.ac.jp
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INTRODUCTION

HEPATITIS C VIRUS (HCV) is a causative agent of acute and chronic hepatitis as well as liver cirrhosis and hepatocellular carcinoma.[1–3] The single stranded RNA genome encodes one large open reading frame that is processed into at least 10 proteins by host and viral enzymes.[4,5] Some viral proteins are known to affect the outcome of pegylated interferon (PEG IFN) plus ribavirin combination therapy, the current standard of care for chronic hepatitis.[6–8] The number of amino acid substitutions in the IFN sensitivity determining region (ISDR) of the NS5A protein, which was initially reported to affect IFN monotherapy,[9,10] has recently been reported to affect PEG IFN plus ribavirin combination therapy as well.[11–14]

NS5A PKR binding domain (PKRBD),[15–19] variable region 3 (V3),[20–23] IFN/ribavirin resistance determining region (IRRDR),[24,25] and E2 PKR-eIF2α phosphorylation homology domain (PePHD)[26] have also been reported to affect therapy outcome, although these results need to be confirmed. More recently, amino acid (a.a.) substitutions in the core protein have been reported to negatively affect IFN plus ribavirin therapy.[27,28] Substitution at a.a. 70 of the core protein (core 70) has been reported to be associated with non-virological response (NVR), and this finding was confirmed by several groups.[29–31]

Several cytokines and adipokines have also been reported to be associated with the effectiveness of therapy. For instance, tumor necrosis factor (TNF)-α expression has been reported to be elevated in patients with HCV infection, and high expression levels are associated with poor response to IFN therapy.[32] IP-10 has also been reported to associate with response to therapy in patients with HCV and HIV co-infection.[33] Leptin and adiponectin levels are also reportedly associated with the effect of combination therapy.[34,35] In addition to these factors, there are many studies reporting relationships between common polymorphisms in the human genome and outcome of IFN therapy.[36–44] Among them, single nucleotide polymorphisms (SNP) in the interleukin (IL)-28B locus discovered through genome-wide association studies appear to have a large effect on outcome of PEG IFN plus ribavirin combination therapy[42–44] as well as spontaneous eradication of HCV.[45]

In addition to the above viral and host genetic factors, several metabolic factors such as obesity,[34] insulin resistance[46] and low-density lipoprotein (LDL) cholesterol levels[28,47] have been reported to be correlated with the effect of combination therapy. Furthermore, higher gamma-glutamyltranspeptidase (γ-GTP) levels, often associated with fatty liver, have also been reported to be associated with treatment outcome.[48,49] Although these factors may be mutually interdependent, their relationships with viral factors have not yet been analyzed.

Recent papers have reported poor response to therapy in older female patients,[50–52] but little is known about the relationship between age, sex and other predictive factors. To analyze these associations, we constructed a database consisting of 1425 patients with chronic hepatitis C. Using this database, we analyzed the relationship between viral and metabolic data and found that a.a. substitutions in the core and ISDR are associated with metabolic change, which may be related to disease progression and response to therapy.

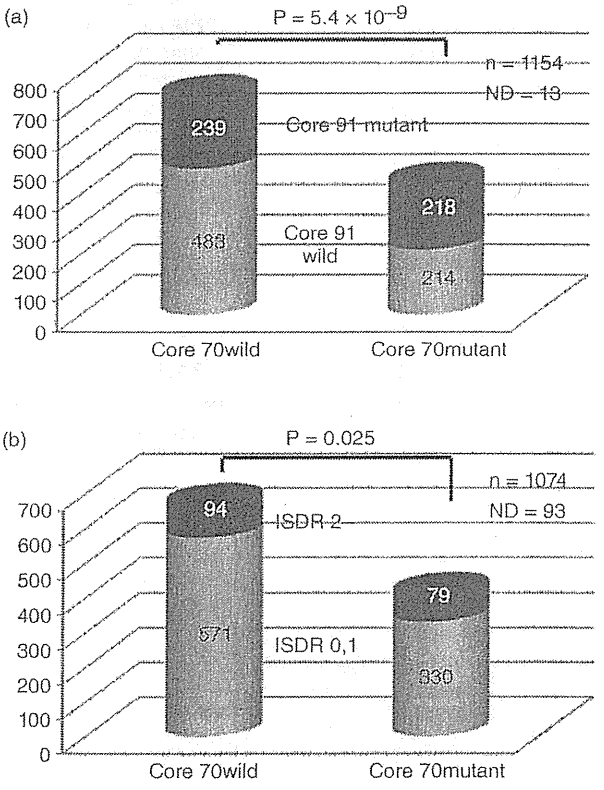

Figure 1 Association of core amino acid 70, amino acid 91 and interferon sensitivity determining region (ISDR). The relationship between hepatitis C virus core 70 and core 91 wild type and mutant amino acids (a) and the ISDR (b) were examined. Statistical significance was assessed using the χ^2-test. ND, not determined due to polymerase chain reaction or sequence calling failure.

Table 1 Clinical profile of 1167 patients

	All patients $n = 1167$	Tx naive $n = 570$ (48.84%)	Prev. tx $n = 597$ (51.16%)	*P*-value
Sex (male/female)	606/561	259/311	347/250	1.45E-05
Age	55.1 ± 10.7	55.2 ± 11.0	55.0 ± 10.5	0.604
Body weight	60.6 ± 10.8	59.5 ± 10.5	61.7 ± 11.0	0.001
BMI	27.0 ± 7.38	24.3 ± 5.46	29.6 ± 8.02	0
Fibrosis stage (0–2/3–4/ND)	815/192/160	422/78/70	393/114/90	0.005
Activity stage (0–1/2–3/ND)	531/465/171	263/234/73	268/231/98	0.803
Steatosis (present/absent/ND)	207/428/532	103/175/292	104/253/240	0.034
White blood cells (/mm^3)	4808 ± 1428	4871 ± 1395	4748 ± 1457	0.127
Hemoglobin (g/dL)	14.1 ± 1.88	14.0 ± 1.39	14.3 ± 2.23	0.001
Platelets (×10^4/mm^3)	16.6 ± 5.06	16.5 ± 5.31	16.7 ± 4.82	0.288
ALT (IU/L)	66 ± 52	67 ± 48	65 ± 55	0.265
AST (IU/L)	65 ± 54	58 ± 37	71 ± 66	0.001
γ-GTP (IU/L)	56 ± 58	57 ± 62	55 ± 54	0.942
Albumin (g/dL)	4.00 ± 0.375	4.04 ± 0.402	3.97 ± 0.347	0.001
Total cholesterol (mg/dL)	173 ± 32.1	175 ± 32.7	172 ± 31.6	0.206
Fasting blood sugar (mg/dL)	101 ± 24.9	102 ± 27.2	99.8 ± 22.2	0.715
HCV RNA (KIU/mL: amp)	2999 ± 4523	2822 ± 4365	3169 ± 4668	0.048
ISDR (0–1/≥2/ND)	908/178/81	440/85/45	468/93/36	0.863
Core 70 (wild/mutant/ND)	722/433/12	349/218/3	373/215/9	0.509
Core 91 (wild/mutant/ND)	697/457/13	349/217/4	348/240/9	0.39

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; ND, not determined; tx., treatment.

METHODS

Study subjects

WE COLLECTED DATA from 1425 participating patients with chronic hepatitis C from 16 centers in Japan. Inclusion criteria included testing positive for HCV RNA over a period of more than 6 months and testing negative for both hepatitis B virus surface antigen and anti-HIV antibody. Patients with confounding liver conditions were excluded, as well as patients who were lost to follow up or who did not have high viral load (≥5 log IU/mL) for HCV genotype 1b (Fig. 1). Patient data was not used when we failed to determine core 70, core 90 and ISDR sequences. In total, data from 1167 patients were included in the analysis. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

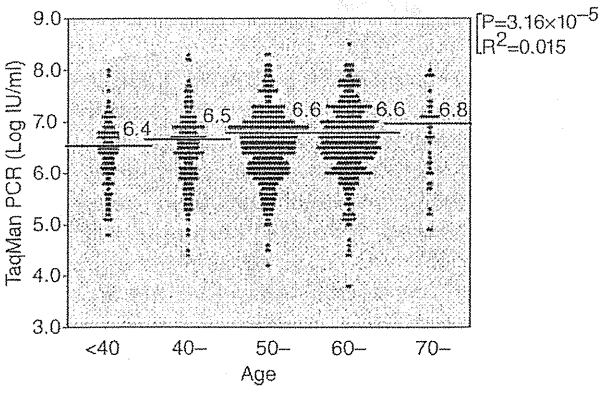

Figure 2 Relationship between age and virus titer. Virus titers were plotted according to age. The median titer within each 10-year age group is shown as horizontal bars.

Patients received weekly injections of PEG IFN-α-2b for either 48 or 72 weeks using the following doses: 60 μg for 35–45 kg bodyweight; 80 μg for 46–60 kg; 100 μg for 61–75 kg; 120 μg for 76–90 kg; and 150 μg for 91–120 kg. Ribavirin was administrated p.o., and the dose was determined based on the patient's bodyweight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Ribavirin dosage was reduced when hemoglobin levels reduced to 10.0 g/dL and stopped if hemoglobin levels reached 8.5 g/dL. Bio-

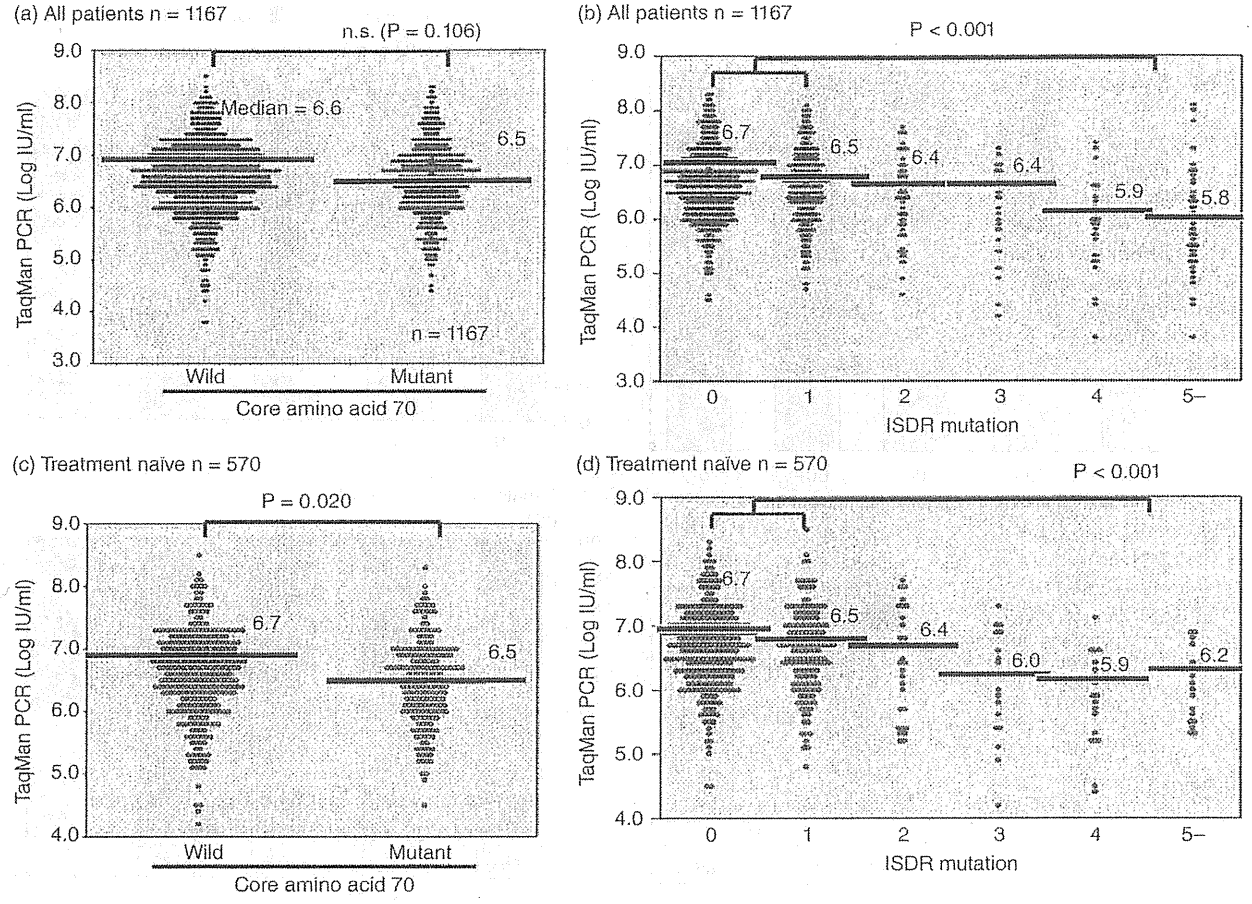

Figure 3 Analysis of virus load by core amino acid 70 substitution and number of amino acid substitutions in the interferon sensitivity determining region (ISDR). Virus titers of all 1167 patients were classified according to core 70 wild type and mutant amino acids (a) or by the number of substitutions in the ISDR (b). The 570 interferon therapy naive patients were also examined separately (c,d).

chemical tests were performed by center, and pathological diagnosis was made according to the criteria of Desmet *et al.*[53] Successful treatment was ascertained based on sustained virological response (SVR), defined as HCV RNA negative 6 months after cessation of therapy.

Analysis of viral titer and a.a. sequences in the core and ISDR region

The HCV RNA level was analyzed using reverse transcription polymerase chain reaction (RT–PCR)-based methods (Amplicor Hepatitis C Virus test: Roche Diagnostics, Basel, Switzerland; high range test: Cobas Amplicor, Roche Diagnostics, Basel, Switzerland; or TaqMan RT–PCR test: Applied Biosystems, Foster city, CA, USA). The measurement ranges of these assays were 5–5000 KIU/mL and 1.2–7.8 log IU, respectively. For values exceeding the measurable range, the titer was determined after dilution of the serum samples.

Sequences were determined by direct sequencing of PCR fragments following extraction and RT of serum HCV RNA. For core 70 and 91, arginine and leucine were considered wild type (wt) according to Akuta *et al.*[27,28] The number of a.a. substitutions in the ISDR was determined as described previously.[9,10,53]

Statistical analysis

The χ^2-test and Mann–Whitney *U*-test were applied to detect significant associations using PASW ver. 18

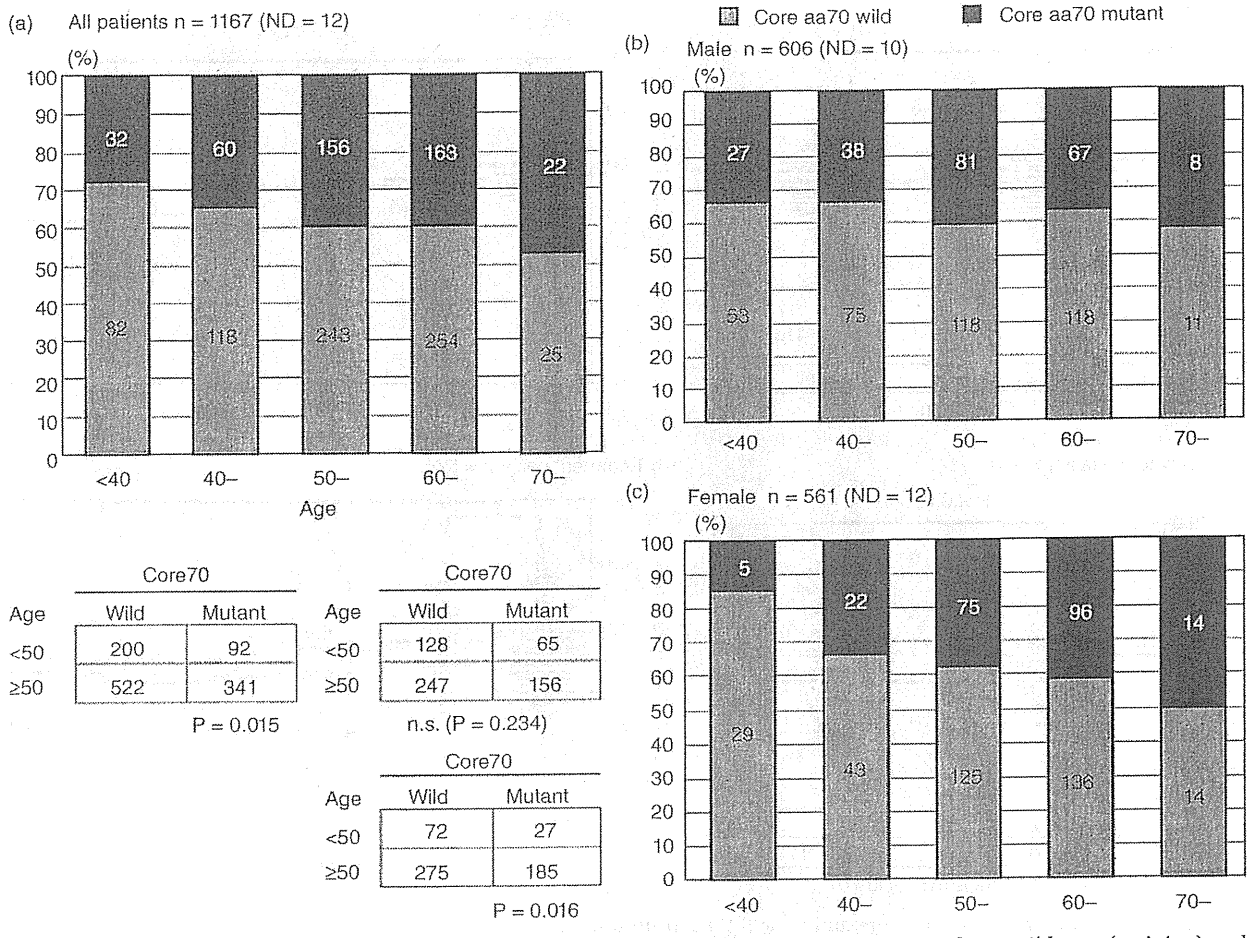

Figure 4 Age-dependent increase in core amino acid 70 mutants in female patients. Percentages of core wild type (arginine) and mutant amino acids for all patients (a), as well as for male (b) and female (c) patients are shown. Note that the age-dependent increase in mutant frequency was observed only in female patients. Statistical analysis was performed by χ^2-test. ND, not determined.

(SPSS, Chicago, IL, USA). All statistical analyses were two sided, and $P < 0.05$ was considered significant. Simple and multiple regression analyses were used to examine the association between viral substitutions and clinical factors using $P < 0.05$ as the criterion for inclusion in the multivariate model. Continuous variables were split into indicator variables based on the median, except for age which was divided into 10-year intervals. Multivariate logistic regression analysis was performed using the Design package in R (www.r-project.org) with fast backward elimination and validation based on AIC score.

RESULTS

Patient characteristics

PATIENT PROFILES ARE shown in Table 1. Results are presented separately for patients who were naive to IFN therapy and those who had had previous IFN therapy but failed to eradicate the virus.

Virus titer and a.a. substitutions in the core and the ISDR

We found a significant positive correlation between patient age and virus titer ($P = 3.16 \times 10^{-5}$, $R^2 = 0.015$,

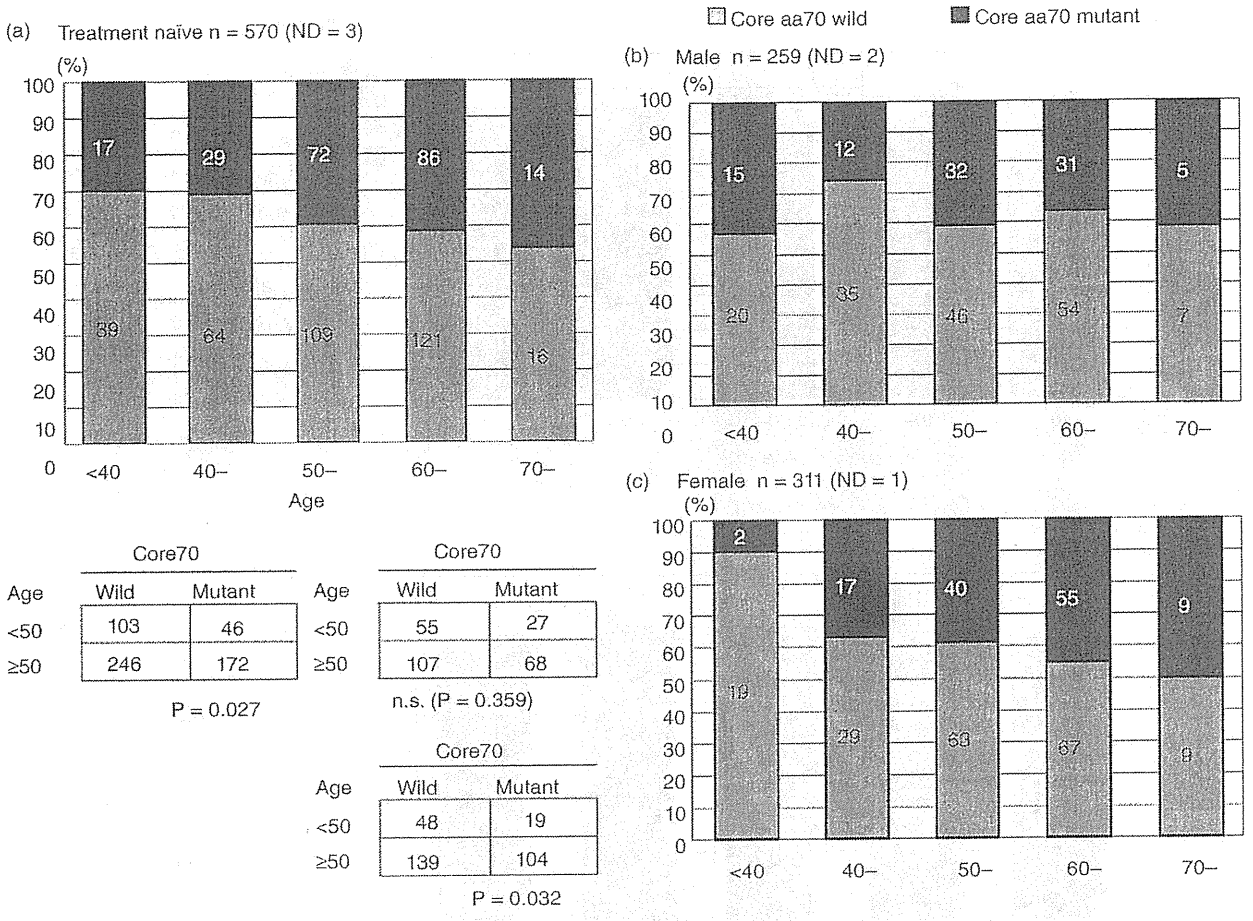

Figure 5 Age-dependent increase in core amino acid 70 mutants in treatment-naive female patients. Percentage of core wild type (arginine) and mutant amino acid were analyzed as in Figure 5 using only interferon treatment-naive patients. Results for all 561 patients (a), as well as for male (b) and female (c) patients are shown. ND, not determined.

Fig. 2). Wt core 70 was associated with wt core 91, with 40% of patients wt for both core 70 and core 91 and 20% of patients non-wt for both (Fig. 1, $P = 5.4 \times 10^{-9}$). Virus titer did not differ in patients with wt core 70 compared to non-wt when all patients were included (Fig. 3a), but when treatment-naive patients were analyzed separately, virus titer was significantly higher in patients with core 70 wt ($P = 0.02$, Fig. 3c). We found a significant negative linear relationship between virus titer and the number of substitutions in the ISDR ($P < 0.001$, Fig. 3b), regardless of treatment history ($P < 0.001$, Fig. 3d).

Amino acid substitution and age

The proportion of patients with core 70 substitutions increased with age among female patients (Figs 4,5), and the proportion of patients without substitutions in the ISDR tended to increase with age among treatment-naive females ($P = 0.0581$, Fig. 6).

Core 70 a.a. substitution and histological findings

Fibrosis stage and activity were higher in patients with core 70 mutants ($P = 0.028$ and $P = 0.048$, respectively; Fig. 7). There was no apparent correlation between his-

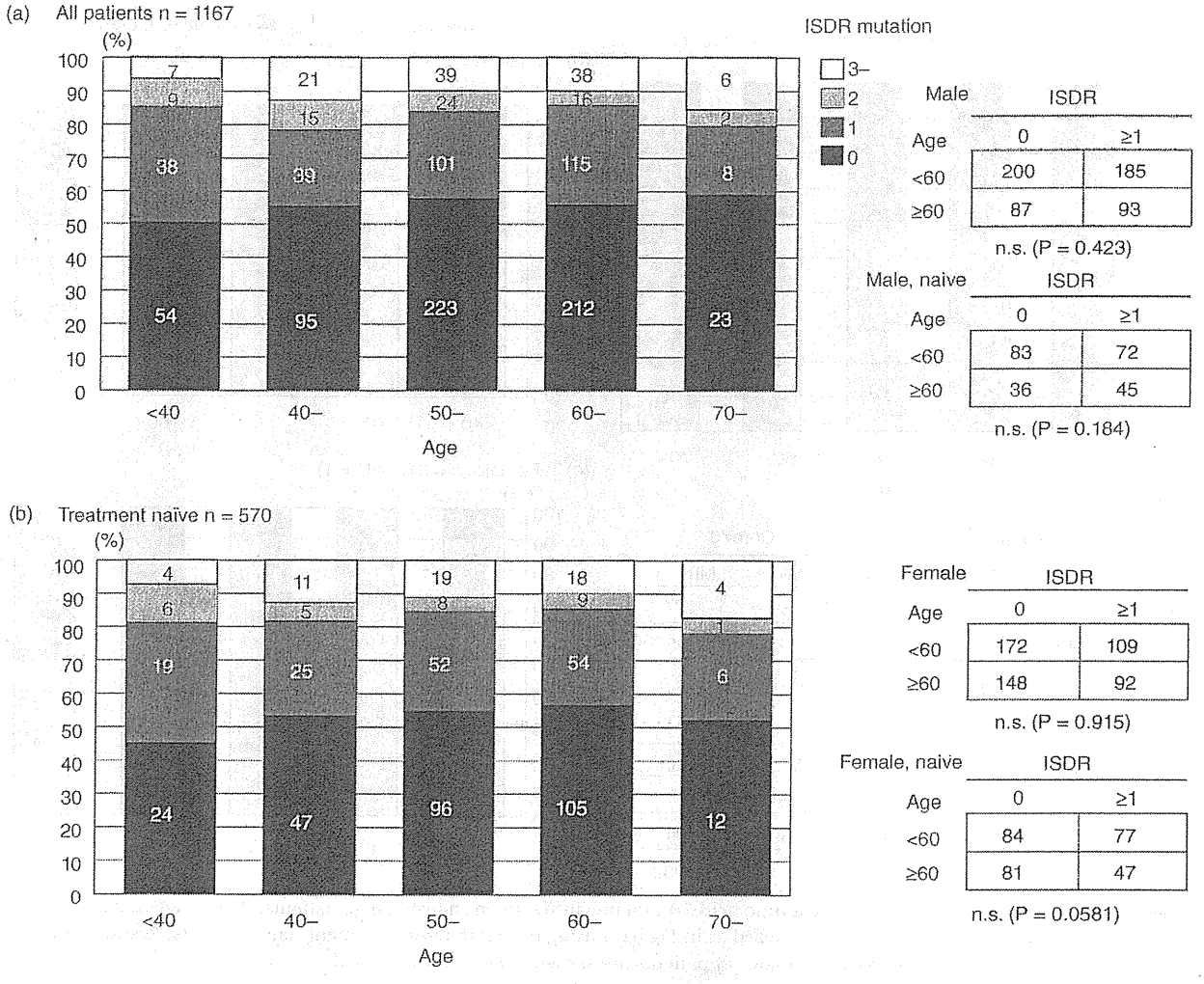

Figure 6 Age-dependent increase in number of amino acid substitutions in the interferon sensitivity determining region (ISDR). The relationship between age and the number of amino acid substitutions in the ISDR was examined. All patients (a) and only naive patients (b) were analyzed. Statistical analysis was performed using the χ^2-test.

tological findings and the number of a.a. substitutions in the ISDR (data not shown).

Correlation between viral a.a. substitutions and clinical conditions

We compared γ-GTP, ALT, LDL cholesterol levels and other clinical conditions between patients with core 70 wild and mutant types (Fig. 8). ALT and γ-GTP levels were significantly higher in patients with core 70 substitutions (Fig. 8a,b). In contrast, LDL cholesterol levels and platelet counts were significantly higher in patients with core 70 wt (Fig. 8c,d). However, only sex, fibrosis, γ-GTP and core 91 substitution were independently associated with core 70 substitution (Table 2). Only viral load and core 70 substitutions are independent predictive factors for the presence of two or more ISDR substitutions (Table 3).

DISCUSSION

WE FOUND THAT factors previously reported to be associated with poor response to IFN-based treatment for chronic hepatitis C tended to be most strongly associated with older female patients. Studies on difficult-to-treat older female patients have so far only been reported in Japan, probably due to the rela-

Table 2 Factors associated with HCV core protein amino acid 70 substitutions

Variable	Simple			Multiple			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age (in 10-year increments)	331	1.1	0.3536				
Sex (male vs female)	365	1.58	0.04178	214	2.09	(1.11–3.95)	0.0234
BMI (kg/m^2)	363	0.763	0.2229				
Diabetes	312	1.77	0.08053				
Fibrosis (F0–1 vs F2–4)	252	2.12	0.007444	214	2.18	(1.15–4.13)	0.017
Activity (A0–1 vs A2–4)	246	1.73	0.04849				
ALT (IU/L)	329	0.866	0.5461				
Platelets (×104/mm^3)	329	0.937	0.7836				
γ-GTP (IU/L)	305	1.69	0.03427	214	1.59	(0.841–3.02)	0.153
Albumin (g/dL)	190	0.765	0.3981				
Fasting blood sugar (mg/dL)	250	0.898	0.6878				
TaqMan PCR (log IU/mL)	327	0.748	0.2232				
HDL cholesterol (mg/dL)	202	1.64	0.1025				
LDL cholesterol (mg/dL)	165	1.25	0.5085				
Total cholesterol (mg/dL)	321	0.907	0.6847				
Core 91 (wild vs others)	365	2.22	0.000393	214	2.68	(1.43–5.02)	0.002
ISDR (0,1 vs >1)	343	1.82	0.03102	214	1.85	(0.853–4)	0.1197

Simple and multiple logistic regression were used to examine the association between substitution at core amino acid 70 and patient and viral factors.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; HDL, high-density lipoprotein; ISDR, interferon sensitivity determining region; LDL, low-density lipoprotein; ND, not determined; OR, odds ratio.

Table 3 Factors associated with viral ISDR substitutions (0–1 vs >1 mutations)

Variable	Simple			Multiple			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age (in 10-year increments)	311	1	0.9735				
Sex (male vs female)	345	0.644	0.1247				
BMI (kg/m^2)	343	1.14	0.6254				
Diabetes	293	0.818	0.6509				
Fibrosis (F0–1 vs F2–4)	235	1.28	0.4545				
Activity (A0–1 vs A2–4)	229	1.3	0.4281				
ALT (IU/L)	309	1.15	0.646				
Platelets (×104/mm^3)	309	0.668	0.1707				
γ-GTP (IU/L)	287	1.47	0.2115				
Albumin (g/dL)	172	0.979	0.9622				
Fasting blood sugar (mg/dL)	233	1.36	0.3641				
TaqMan PCR (log IU/mL)	307	0.517	0.02527	305	0.529	(0.30–0.95)	0.03223
HDL cholesterol (mg/dL)	189	1.23	0.617				
LDL cholesterol (mg/dL)	152	0.463	0.1199				
Total cholesterol (mg/dL)	303	0.656	0.1537				
Core 70 (wild vs others)	343	1.82	0.03102	305	1.82	(1.01–3.3)	0.04763
Core 91 (wild vs others)	344	0.699	0.2038				

Simple and multiple logistic regression was used to examine the association between the number of substitutions in the ISDR region and patient and viral factors.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; HDL, high-density lipoprotein; ISDR, interferon sensitivity determining region; LDL, low-density lipoprotein; ND, not determined; OR, odds ratio.

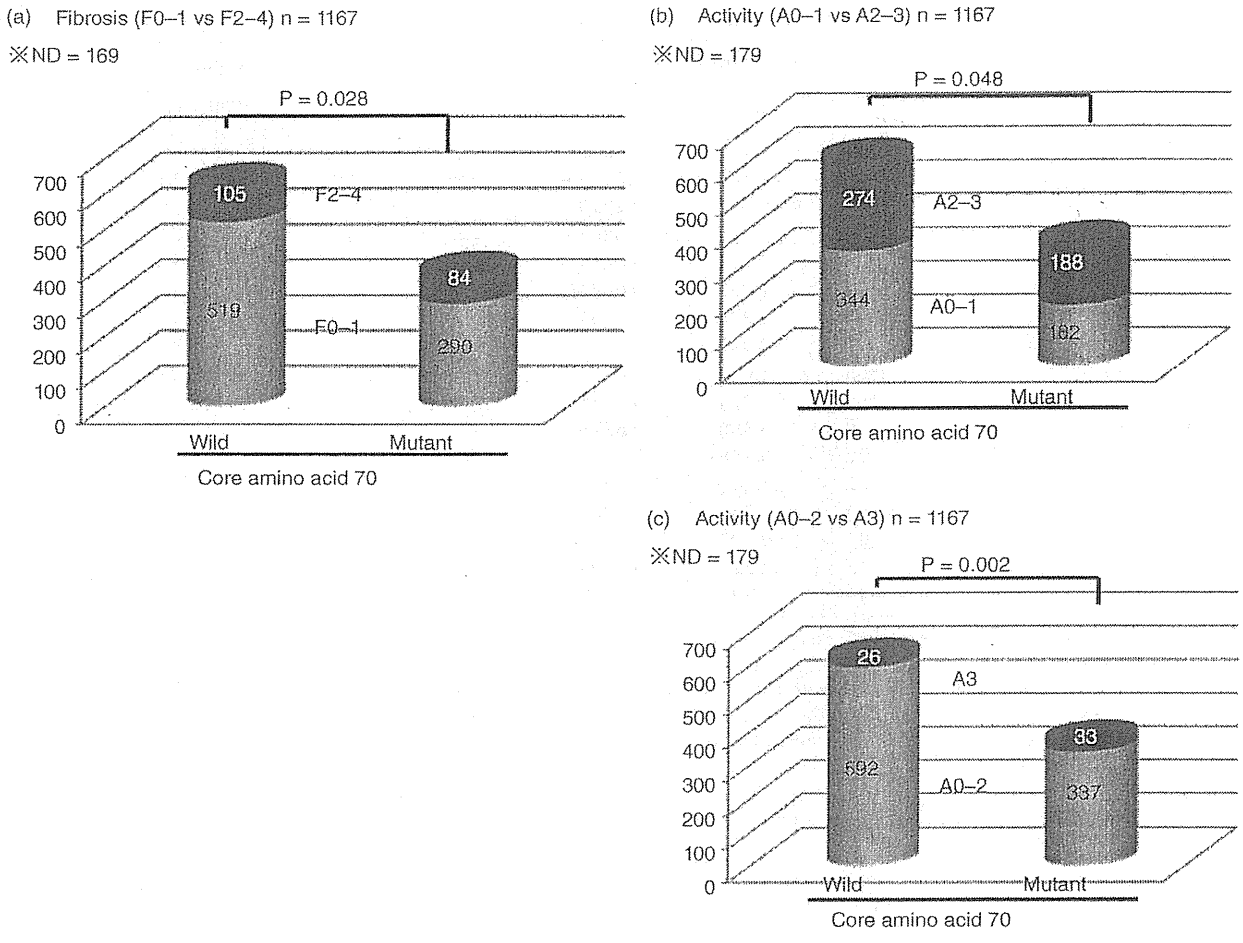

Figure 7 Histological findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and degree of fibrosis (F0–1 and F2–4) (a) and activity (b,c) were examined. Activity was divided into A0–1 and A2–3 (b) or A0–2 and A3 (c) and compared with amino acid 70. ND, not determined.

tively higher age at treatment. The mechanism underlying this association is unknown. Recently, SNP in the IL-28B locus were found to be associated with response to combination therapy as well as to spontaneous eradication of the virus,[42–44] although differences in the eradication rate between men and women have not been reported so far. We have previously reported that incidence of wt core 70 is significantly higher in patients with the IL-28 protective allele.[54] Therefore, it seems reasonable that the wt core 70 confers a selective advantage for the virus in patients with the IL-28 protective allele. During the time when IFN monotherapy was still the standard treatment, female sex, or perhaps the lower iron concentration associated with female sex, had been reported as one of the predictive factors for a favorable response to monotherapy.[55–57] It is possible that spontaneous eradication of the virus occurs during the natural course of chronic hepatitis through IFN produced naturally as a result of liver inflammation in young female patients with wt core 70, resulting in accumulation of core mutant viruses as the patient ages. Further prospective observations are necessary to address this issue.

In this study, we found that each of the previously reported predictive factors that we examined also correlated with HCV a.a. substitutions. Interestingly, a.a. substitutions in the virus are associated with metabolic factors such as LDL and high-density lipoprotein cholesterol and fatty liver-related γ-GTP, and in particular, we found that substitution in the core protein (and possibly ISDR) is correlated with LDL cholesterol. The virus appears to influence expression of genes involved

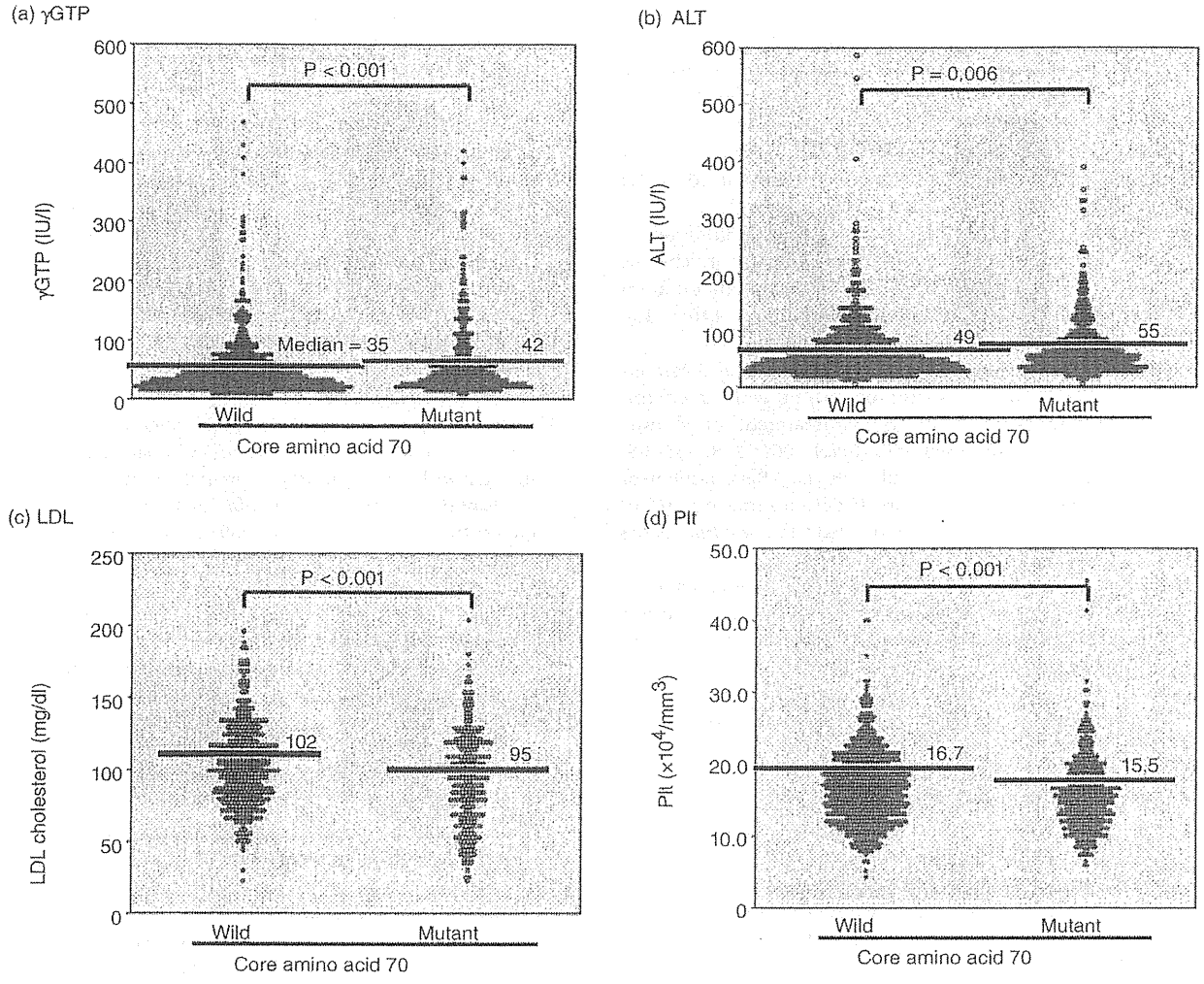

Figure 8 Relationship between blood test findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and gamma-glutamyltranspeptidase (γ-GTP) (a), alanine aminotransferase (ALT) (b), low-density lipoprotein (LDL) cholesterol (c) and platelet count (Plt) (d) were examined. Bars represent the median.

in host cell lipid metabolism to enhance its own replication and secretion.[58] Consequently, metabolic changes induced by infection by different strains of HCV should be investigated further to understand viral mechanisms of IFN resistance and to develop effective personalized therapies.

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