Familial Aggregation in Patients with Non-Alcoholic Steatohepatitis

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Abstract

We encountered three families that showed NASH accumulation. In family #1, a 21-year-old son and 10-year-old daughter were diagnosed with nonalcoholic steatohepatitis (NASH). They shared two adiponectin-gene single nucleotide polymorphisms (SNP). In family #2, a 51-year-old mother and 27-year-old son were diagnosed with NASH and shared the SNPs of other genes. In family #3, a 66-year-old mother and 34-year-old son were diagnosed with NASH and shared the SNPs of other genes. SNP sites differed among the three families, suggesting that the genes associated with the occurrence of NASH might be different in each patient.

Key words: NASH, SNP, familial aggregation

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Introduction

In lifestyle-related diseases such as type 2 diabetes mellitus (DM/) hypertension, accumulation in a family is often observed (1, 2). Hara et al reported that two single nucleotide polymorphism (SNP) sites, Intron 2 and Exon 2 of the adiponectin gene, are associated with DM onset and insulin resistance (3). In addition, SNPs of the PPAR γ 2 gene, calpains 10 (intron polymorphism) gene and adrenergic receptor (Trp64Ayg polymorphism) gene have been reported to be associated with the onset of DM, suggesting that genetic background might be associated with DM (4-6). In hypertension, a polymorphism of the angiotensinogen gene (M235 T) is reported to be correlated with serum renin levels and to related to hypertension (7). It is reported that insertion/deletion (I/D) polymorphisms of the angiotensin-converting enzyme (ACE) gene are correlated with the serum ACE level, which is related to cardiovascular disease and essential hypertension (8). These results suggested that the occurrence and progression of lifestyle-related diseases are associated with genetic background as well.

Non-alcoholic fatty liver disease (NAFLD) has recently been recognized as a leading cause of abnormal liver func-

tion tests. Its spectrum ranges from simple fatty liver, which is usually a benign and non-progressive condition, to non-alcoholic steatohepatitis (NASH), which may progress to cirrhosis (9, 10). Patients with NASH usually have insulin resistance syndrome as well. In addition, NASH is increasingly being recognized as a major cause of cryptogenic cirrhosis and as an indication for liver transplantation. Its etiology remains unclear, but most investigators agree that development of NASH requires underlying steatosis followed by a "second hit" that induces inflammation, fibrosis, or necrosis (10). As NASH is included in metabolic syndrome, familial aggregation is suggested (11-13).

Genetic SNPs of the β 3 adrenergic receptor gene, microsomal triglyceride transfer protein (MTP) gene, MnSOD gene, and interleukin 1 β gene are reported to be associated with NASH (14, 15). We also demonstrated that SNPs of the TNF promoter region might be associated with the "second hit" of NASH (16). Here, we report three families in which NASH accumulation was observed, and analyze the disease course and gene polymorphisms of these patients.

Case Report

Between January 1991 and December 2005, 249 patients

Table 1. Laboratory Data

	Case1	Case2	Case3	Case4	Case5	Case 6	Unit
T-bil	0.7	1.5	0.3	0.8	0.6	0.8	(mg/dl)
AST	280	61	43	134	66	30	(IU/l)
ALT	504	154	98	292	69	65	(IU/l)
γ -GTP	54	23	41	184	74	36	(IU/l)
T-chol	205	139	256	211	249	186	(mg/dl)
TG	85	79	83	368	246	224	(mg/dl)
Plt	33.7	24.5	27.2	19.4	20.3	20.9	($10^4/\text{mm}^3$)
PT%	85.3	82.5	100	89.4	93.8	100	(%)
FBS	99	95	115	91	140	137	(mg/dl)
HbA1c	4.9	4.9	6.2	5.6	8.0	7.3	(%)
IRI	10.5	3.3	9.1	16.2	16.6	11.2	($\mu\text{U/ml}$)
HOMA-R	2.57	0.77	2.58	3.64	5.74	3.79	
HBsAg	(-)	(-)	(-)	(-)	(-)	(-)	
HCV Ab	(-)	(-)	(-)	(-)	(-)	(-)	
DM	(-)	(-)	(+)	(-)	(+)	(+)	
HT	(-)	(-)	(-)	(-)	(-)	(+)	
Hyperlipidemia(-)	(-)	(-)	(+)	(+)	(-)	(+)	

were diagnosed as having biopsy-proven NASH at Tokyo Women's Medical University Hospital. Three families were found to have NASH between siblings or between mother and child. The diagnosis of NASH was based on the following criteria: 1) the presence of steatosis (>10%), lobular inflammation, and ballooning, with or without Mallory bodies and perivenular or pericellular fibrosis; 2) intake of less than 40 g of ethanol per week, as confirmed by physicians and family members in close contact with the patient; and 3) appropriate exclusion of other liver diseases such as alcoholic liver disease, viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, primary sclerosing cholangitis, biliary obstruction, and metabolic liver diseases.

Family #1 (Case 1, 10-year-old girl; Case 2, 21-year-old man)

In the summer of 1999, Case 1, a 10-year-old girl had eaten ice cream every day and had gained 3 kg of body weight in one month. Because her mother had type 2 DM, a parent-and-child medical examination for DM was performed, revealing hepatic dysfunction. Laboratory and physical data show BMI 21.8 kg/m^2 , AST 280, ALT 504 (Table 1). Liver biopsy showed steatohepatitis (Stage 1, Grade 2-3) according to Brunt's NASH classification (17). Thus, she was diagnosed with NASH. Diet and exercise therapy was started, and her serum AST/ALT levels normalized. Her older brother (Case 2) had had mild liver dysfunction since 1999. His body weight had increased by 5 kg from 2002 to 2003, and his serum AST/ALT levels were markedly increased. Laboratory and physical data showed BMI 23.3 kg/m^2 , AST 61, and ALT 154 (Table 1). Liver biopsy showed steatohepatitis (Stage 1, Grade 2), and he was diagnosed with NASH. After diet and exercise therapy, his

serum AST/ALT levels improved to the normal range. We investigated the lifestyle and food preference by questionnaires. In cases 1 and 2, both liked to eat snacks and ice cream. Figure 1 shows the family tree of these cases. Their mother had DM. Both her children (cases 1 and 2) were diagnosed with NASH at relatively young ages. In addition, we investigated 10 SNP sites reported to be associated with NASH or DM (3, 14-16), and found that both cases shared two adiponectin-gene SNP sites that have been suggested to be related to DM (Table 2).

Family #2 (Case 3, 51-year-old woman; Case 4, 27-year-old man)

Case 3 had been treated for DM from 1990. Around 2000, liver dysfunction was discovered, and she was referred to our hospital in 2001. Laboratory and physical data showed BMI 26.4 kg/m^2 , AST 43, ALT 98 (Table 1). Liver biopsy showed steatohepatitis (Stage 0-1, Grade 2), and she was diagnosed with NASH. Figure 2 shows her clinical course. We recommended diet and exercise therapy, but her body weight did not decrease. Serum AST/ALT levels increased and decreased in direct proportion to the increase and decrease in her body weight. In 2004, her serum AST/ALT levels were markedly increased. A second biopsy was performed, showing steatohepatitis (Stage 2, Grade 2-3), and the fibrosis was observed to have progressed during the four years since diagnosis. Her son (Case 4) had had hepatic dysfunction since his late teens. Serum AST/ALT levels, which at one time were normalized, had increased again in 2004, prompting a medical examination at that time. In 2005, his serum AST/ALT levels were increased to 134/292 when his body weight increased from 78 kg to 82 kg, as shown in Fig. 2. Liver biopsy showed steatohepatitis (Stage 1, Grade 3). With his weight loss, liver function improved. In cases 3

Figure 1. The family tree of family #1. Cases 1 and 2 were diagnosed with NASH. Their mother had type 2 diabetes mellitus (DM).

Table 2. SNP Analysis

	TNF- α -238	TNF- α -308	TNF- α -863	TNF- α -1031	Adipo +45	Adipo +276	IL-1 β +511	β 3-Ad	MTP	MnSOD
Case1	G/G	G/G	C/C	T/T	G/T	G/G	T/T	Trp/Arg	T/G	T/T
Case2	G/G	G/G	C/A	C/T	G/T	G/G	C/T	Trp/Trp	G/G	C/T
Case3	G/G	G/G	C/A	C/T	T/T	G/G	T/T	Trp/Arg	G/G	C/T
Case4	G/G	G/G	A/A	C/C	G/T	G/G	C/T	Arg/Arg	G/G	T/T
Case5	G/G	G/G	C/C	T/T	G/T	T/G	C/T	Trg/Trg	G/G	T/T
Case6	G/G	G/G	C/C	T/T	T/T	T/T	T/T	Trp/Arg	G/G	T/T

Shaded genotypes indicate those thought to contribute to the occurrence or progression of NASH or DM.

TNF, tumor necrosis factor

Adipo, adiponectin

IL-1, interleukin-1

β 3-Ad, β 3-adrenergic receptor

MTP, microsomal triglyceride transfer protein

and 4, there was no common tendency of lifestyle or food preference. Figure 3 shows the family tree of Cases 3 and 4. DM and hypertension were found in 3 other relatives. In the SNP analysis, both cases shared the SNPs of TNF- α promoter regions -1,031 and -863, which have been suggested to be related to TNF- α production, and the SNPs of adiponectin gene, the β 3-adrenergic receptor and MTP genes, reportedly related to NASH (Table 2).

Family #3 (Case 5, 34-year-old man; Case 6, 66-year-old woman)

Case 5 had been obese since primary school age. At age 24, his serum AST/ALT levels were slightly increased and his body weight had increased to 90 kg (BMI 28.7 kg/m²). He was diagnosed with DM at age 29. As his control of DM was poor, medical treatment was started. In 2004, he was referred for hepatic dysfunction to our hospital. Laboratory and physical data showed BMI 33.5 kg/m², AST 66, ALT 69 (Table 1). Liver biopsy showed steatohepatitis

(Stage 3, Grade 3). We recommended diet and exercise therapy, after which his serum AST/ALT levels decreased. His mother (Case 6) had had hypertension from about age 50. At about age 60, hyperlipemia, hyperuricemia, and fatty liver were discovered. At age 63, therapy against DM was started. She was referred to our hospital for continuous hepatic dysfunction in 2005. Laboratory and physical data showed BMI 26.6 kg/m², AST 30, ALT 65 (Table 1). Liver biopsy showed steatohepatitis (Stage 3, Grade 3). In cases 5 and 6, there was no common tendency of lifestyle or food preference. Figure 4 shows the family tree of Cases 5 and 6. DM and arteriosclerotic disease were found in 3 other relatives. In SNP analysis, both cases shared the SNPs of the MTP and MnSOD genes, which are reportedly related to NASH (Table 2).

Case 3 51yr. Woman **Case 4 27yr. Man**

Figure 2. Clinical courses of cases 3 and 4 as well as the serum AST/ALT levels and body weight are shown. In case 3, the fibrosis stage progressed for 4 years. BW: body weight (kg).

Figure 3. The family tree of family #2. Cases 3 and 4 were diagnosed with NASH. The mother of case 3 and the father of case 4 had type 2 diabetes mellitus (DM).

Discussion

We encountered three families in which NASH occurred in two siblings, or in both parent and child, similar to other lifestyle-related diseases. In these families, DM and other lifestyle-related diseases were frequently observed. The NASH-associated SNP sites that were found in each family were different, but all patients had some SNPs that are reportedly related to NASH or DM. For example, in family #1, two SNP sites of adiponectin were shared between siblings, and each patient had other genetic SNPs. In family #

2, both patients shared an adiponectin gene SNP associated with DM, an economizing genotype of the $\beta 3$ -adrenergic receptor gene, a genotype of MTP that decreases the release capacity of very low-density lipoprotein (VLDL), and SNPs related to high TNF- α production. In family #3, both patients shared SNPs of the MnSOD gene and MTP gene, which are associated with oxidative stress. It was not confirmed whether these SNPs actually contributed to the occurrence and progression of NASH in our patients. For example, case 2 did not show insulin resistance, raising suspicion as to whether the adiponectin gene contributed to the pathogenesis of NASH in this case. The biological function

Figure 4. The family tree of family #3. Cases 5 and 6 were diagnosed with NASH. The mother and brothers of case 6 had type 2 diabetes mellitus (DM) or atherosclerotic diseases.

of adiponectin has been associated with not only insulin resistance, but also anti-inflammatory and anti-liver fibrosis, and it has been demonstrated that adiponectin is associated with the occurrence and progression of NASH (18, 19). In addition, Hara et al reported that adiponectin SNP might influence the production of adiponectin (3). These data suggest that adiponectin and these SNPs are associated with NASH. Among the SNPs which are reported to be related to the pathogenesis and progression of NASH, only adiponectin SNPs were shared in Cases 1 and 2. Therefore, we suggest that adiponectin SNPs are associated with NASH. In the future, SNP analysis and investigation of lifestyles in other family members who have not developed NASH will clarify which factors are more important.

Abdelmalek et al reported that NAFLD patients were more likely to have DM and that insulin resistance and DM occurred frequently in their first-degree relatives. In addition, a trend toward insulin resistance was noted in the mothers, but not the fathers, of patients with NAFLD (11). In our three families, all mothers had DM and/or NASH. In addition, in families #2 and #3, the grandmothers had DM or atherosclerotic diseases. Yatsuji et al reported that DM was more prevalent in older female NASH patients and suggested the possibility that female NASH patients might have stronger genetic factors (20). At any rate, it is necessary to pay close attention to children of mothers with DM or NASH.

Familial aggregation has been reported for DM, insulin

resistance, and arteriosclerotic diseases (1, 2, 11). Here, in three family members, DM, HT, and arteriosclerotic diseases were frequently observed. These data support the hypothesis that NASH is associated with genetic background of these diseases and is part of the metabolic syndrome (12).

Case 5 was at stage 3 in young adulthood. In addition, the fibrosis of Case 3 progressed in the four years after diagnosis. Genetic background and similar eating habits or lifestyles might accelerate the progress of NASH. Considering the rapid progression of NASH in these patients, in addition to their family histories, amelioration of living habits and pharmacotherapy should be started from a young age.

Even when NASH patients had a genetic background for the disease, their liver function improved when body weight was controlled. These results suggested that adiposis based on living habits is the origin of NASH. We investigated the lifestyle and food preference by questionnaires. In cases 1 and 2, both liked to eat snacks and ice cream. In cases 3 and 4, there was no common tendency of lifestyle or food preference. Cases 5 and 6 also showed no common tendency. More detailed investigations will be necessary, because the possibility could not be denied that a similar lifestyle might be the cause of NASH among members of the same family. As overeating is encouraged in contemporary society, it is believed that the incidence of NASH will increase in the future. It is also recommended for the entire family of a NASH patient to undergo medical examinations.

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