Nakanishi H, Itakura J, Takahashi Y, Asahina Y, Matsuura K, Sugauchi F, Enomoto N, Nishida N, Tokunaga K, Mizokami M, Izumi N (2011) Relationship between polymorphisms of the inosine triphosphatase gene and anaemia or outcome after treatment with pegylated interferon and ribavirin. Antivir Ther 16:685–694. doi:10.3851/IMP1796

Matsuura K, Tanaka Y, Watanabe T, Fujiwara K, Orito E, Kurosaki M, Izumi N, Sakamoto N, Enomoto N, Yatsuhashi H, Kusakabe A, Shinkai N, Nojiri S, Joh T, Mizokami M (2014) ITPA genetic variants influence efficacy of PEG-IFN/RBV therapy in older patients infected with HCV genotype 1 and favourable IL28B type. J Viral Hepat 21:466–474. doi:10.1111/jvh.12171

McHutchison JG, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK (2002) Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. Gastroenterology 123:1061–1069

Nagata S, Tsuchiya M, Asano S, Kaziro Y, Yamazaki T, Yamamoto O, Hirata Y, Kubota N, Oheda M, Nomura H et al (1986) Molecular cloning and expression of cDNA for human granulocyte colony-stimulating factor. Nature 319:415–418. doi:10.1038/319415a0

Nishida N, Tanabe T, Takasu M, Suyama A, Tokunaga K (2007) Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. Anal Biochem 364:78–85. doi:10.1016/j.ab.2007.02.005

Ochi H, Maekawa T, Abe H, Hayashida Y, Nakano R, Kubo M, Tsunoda T, Hayes CN, Kumada H, Nakamura Y, Chayama K (2010) ITPA polymorphism affects ribavirin-induced anemia and outcomes of therapy—a genome-wide study of Japanese HCV virus patients. Gastroenterology 139:1190–1197. doi:10.1053/j.gastro.2010.06.071

Okada Y, Kamatani Y, Takahashi A, Matsuda K, Hosono N, Ohmiya H, Daigo Y, Yamamoto K, Kubo M, Nakamura Y, Kamatani N (2010) Common variations in PSMD3-CSF3 and PLCB4 are associated with neutrophil count. Hum Mol Genet 19:2079–2085. doi:10.1093/hmg/ddq080

Poordad F, Bronowicki JP, Gordon SC, Zeuzem S, Jacobson IM, Sulkowski MS, Poynard T, Morgan TR, Molony C, Pedicone LD, Sings HL, Burroughs MH, Sniukiene V, Boparai N, Goteti VS, Brass CA, Albrecht JK, Bacon BR (2012) Factors that predict response of patients with hepatitis C virus infection to boceprevir. Gastroenterology 143(608–18):e1–e5. doi:10.1053/j.gastro.2012.05.011

Reiner AP, Lettre G, Nalls MA, Ganesh SK, Mathias R, Austin MA, Dean E, Arepalli S, Britton A, Chen Z, Couper D, Curb JD, Eaton CB, Fornage M, Grant SF, Harris TB, Hernandez D, Kamatini N, Keating BJ, Kubo M, LaCroix A, Lange LA, Liu S, Lohman K, Meng Y, Mohler ER 3rd, Musani S, Nakamura Y, O'Donnell CJ, Okada Y, Palmer CD, Papanicolaou GJ, Patel KV, Singleton AB, Takahashi A, Tang H, Taylor HA Jr, Taylor K, Thomson C, Yanek LR, Yang L, Ziv E, Zonderman AB, Folsom AR, Evans MK, Liu Y, Becker DM, Snively BM, Wilson JG (2011) Genome-wide association study of white blood cell count in 16,388 African Americans: the continental origins and genetic epidemiology network (COGENT). PLoS Genet 7:e1002108. doi:10.1371/journal.pgen.1002108

Sakamoto N, Tanaka Y, Nakagawa M, Yatsuhashi H, Nishiguchi S, Enomoto N, Azuma S, Nishimura-Sakurai Y, Kakinuma S, Nishida N, Tokunaga K, Honda M, Ito K, Mizokami M, Watanabe M (2010) ITPA gene variant protects against anemia induced by pegylated interferon-alpha and ribavirin therapy for Japanese patients with chronic hepatitis C. Hepatol Res 40:1063–1071. doi:10.1111/j.1872-034X.2010.00741.x

Soranzo N, Spector TD, Mangino M, Kuhnel B, Rendon A, Teumer A, Willenborg C, Wright B, Chen L, Li M, Salo P, Voight BF, Burns P, Laskowski RA, Xue Y, Menzel S, Altshuler D, Bradley JR, Bumpstead S, Burnett MS, Devaney J, Doring A, Elosua R, Epstein SE, Erber W, Falchi M, Garner SF, Ghori MJ, Goodall AH, Gwilliam R, Hakonarson HH, Hall AS, Hammond N, Hengstenberg C, Illig T, Konig IR, Knouff CW, McPherson R, Melander O, Mooser V, Nauck M, Nieminen MS, O'Donnell CJ, Peltonen L, Potter SC, Prokisch H, Rader DJ, Rice CM, Roberts R, Salomaa V, Sambrook J, Schreiber S, Schunkert H, Schwartz SM, Serbanovic-Canic J, Sinisalo J, Siscovick DS, Stark K, Surakka I, Stephens J, Thompson JR, Volker U, Volzke H, Watkins NA, Wells GA, Wichmann HE, Van Heel DA, Tyler-Smith C, Thein SL, Kathiresan S, Perola M, Reilly MP, Stewart AF, Erdmann J, Samani NJ, Meisinger C, Greinacher A, Deloukas P, Ouwehand WH, Gieger C (2009) A genome-wide meta-analysis identifies 22 loci associated with eight hematological parameters in the HaemGen consortium. Nat Genet 41:1182–1190. doi:10.1038/ng.467

Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Muller T, Bahlo M, Stewart GJ, Booth DR, George J (2009) IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 41:1100–1104. doi:10.1038/ng.447

Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M (2009) Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 41:1105–1109. doi:10.1038/ng.449

Tanaka Y, Kurosaki M, Nishida N, Sugiyama M, Matsuura K, Sakamoto N, Enomoto N, Yatsuhashi H, Nishiguchi S, Hino K, Hige S, Itoh Y, Tanaka E, Mochida S, Honda M, Hiasa Y, Koike A, Sugauchi F, Kaneko S, Izumi N, Tokunaga K, Mizokami M (2011) Genome-wide association study identified ITPA/DDRGK1 variants reflecting thrombocytopenia in pegylated interferon and ribavirin therapy for chronic hepatitis C. Hum Mol Genet 20:3507–3516. doi:10.1093/hmg/ddr249

Thompson AJ, Clark PJ, Singh A, Ge D, Fellay J, Zhu M, Zhu Q, Urban TJ, Patel K, Tillmann HL, Naggie S, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, King JW, Kwo PY, Shianna KV, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS, Goldstein DB, McHutchison JG, Muir AJ (2012) Genome-wide association study of interferon-related cytopenia in chronic hepatitis C patients. J Hepatol 56:313–319. doi:10.1016/j.jhep.2011.04.021

Yang TP, Beazley C, Montgomery SB, Dimas AS, Gutierrez-Arcelus M, Stranger BE, Deloukas P, Dermitzakis ET (2010) Genevar: a database and Java application for the analysis and visualization of SNP-gene associations in eQTL studies. Bioinformatics 26:2474–2476. doi:10.1093/bioinformatics/btq452

Yoshida H, Tateishi R, Arakawa Y, Sata M, Fujiyama S, Nishiguchi S, Ishibashi H, Yamada G, Yokosuka O, Shiratori Y, Omata M (2004) Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. Gut 53:425–430

Zeuzem S, Andreone P, Pol S, Lawitz E, Diago M, Roberts S, Focaccia R, Younossi Z, Foster GR, Horban A, Ferenci P, Nevens F, Mullhaupt B, Pockros P, Terg R, Shouval D, van Hoek B, Weiland O, Van Heeswijk R, De Meyer S, Luo D, Boogaerts G, Polo R, Picchio G, Beumont M (2011) Telaprevir for retreatment of HCV infection. N Engl J Med 364:2417–2428. doi:10.1056/NEJMoa1013086

Elmer Press

Case Report

J Med Cases. 2014;5(4):227-231

Transmission of Hepatitis C Virus From a Mother to a Child Carrying IL28B Heterozygote rs8099917 Among Three Brothers: A Long-Term Follow-Up

Takafumi Saito[a, c], Kei Mizuno[a], Tomohiro Katsumi[a], Kyoko Tomita[a], Chikako Sato[a], Kazuo Okumoto[a], Yuko Nishise[a], Hisayoshi Watanabe[a], Li Shao[b], Yoshiyuki Ueno[a]

Abstract

Three male children were born every 2 years by spontaneous delivery from a mother infected with hepatitis C virus (HCV) genotype 2b, and all have been followed up after birth. The viral load in the serum of the mother was high before their deliveries, and anti-HCV antibody immunoglobulin G, which is allowed to pass through placenta, was positive in the umbilical blood of all the children. Mother-to-child transmission of HCV was confirmed in the second son, who was positive for both anti-HCV antibody and serum HCV RNA when first examined 108 days after birth, but not in the other siblings. Persistent HCV genotype 2b infection with mild elevation of the serum alanine aminotransferase level has been established in the second son for more than 14 years. The *interleukin 28B (IL28B)* genotype (rs8099917) of the second son showed the TG heterozygote, which is unfavorable for viral clearance, and this may predict persistent HCV infection. Among the three brothers sharing the same delivery conditions with exposure to the same virus, as well as sharing the same environment after birth, HCV infection has not been consistent, and one of them possessing the TG genotype of the *IL28B* gene (rs8099917) has had chronic HCV infection. These cases suggest that maternal HCV transmission does not occur so often, even among multiple children who are exposed to the same HCV with a high viral load, and that this variation might be attributable to very minor events that can impact on viral exposure in the perinatal period.

Keywords: Hepatitis; HCV; Perinatal infection; Vertical infection; Interleukin 28B

Manuscript accepted for publication March 4, 2014

[a]Department of Gastroenterology, Yamagata University School of Medicine, Yamagata 990-9585, Japan
[b]Public Health, Yamagata University School of Medicine, Yamagata 990-9585, Japan
[c]Corresponding author: Takafumi Saito, Department of Gastroenterology, Yamagata University School of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan.
Email: tasaitoh@med.id.yamagata-u.ac.jp

doi: http://dx.doi.org/10.14740/jmc1736w

Introduction

Mother-to-child transmission of hepatitis C virus (HCV) is a serious health problem, and no effective preventive vaccine has yet been developed. The mechanism and timing of mother-to-child HCV transmission are not understood, nor is the natural history of the infection in mothers and their children. The rate of HCV transmission from HCV-infected mother to child is reported to be approximately 4-10% [1-7], but the associated factors are not fully defined [8].

HCV infection leads to development of chronic hepatitis with a risk of progression to cirrhosis and liver cancer, but some individuals clear the virus spontaneously and the hepatitis resolves in a self-limiting manner in the acute phase of infection [9-11]. Recently, it has been reported that the host genetic single nucleotide polymorphism (SNP) in the region of the *interleukin 28B (IL28B)* gene encoding interferon-λ-3, rs12979860, is associated with spontaneous HCV clearance in adults [12, 13], and even in infants vertically infected with HCV from their mothers [14]. This SNP is in high linkage disequilibrium with rs8099917, as reported for Japanese subjects [15, 16]. Furthermore, upstream of the *IL28B* gene, a dinucleotide variant ss469415590 in the *IFNL4* gene encoding interferon-λ-4 protein has been reported to be more strongly associated with HCV clearance, and this variant is in high linkage disequilibrium with the SNPs of *IL28B* [17]. Thus, these genetic markers are worth investigating further for their possible usefulness in predicting the outcome of maternal HCV infection in children.

The pattern of maternal HCV transmission in multiple children from the same mother is still unclear. There is little evidence to indicate whether mother-to-child transmission of HCV occurs evenly or unevenly in this situation. Such cases would be informative for understanding the risk of perinatal HCV infection. We have experienced three deliveries from the same HCV-monoinfected mother, and prospectively followed up the three children after birth. In all of them, the factors possibly related to maternal HCV transmission, namely, the gender of the children, birth weight, delivery conditions, a high viral titer in the mother before their births, positivity for anti-HCV antibody in their umbilical blood and their en-

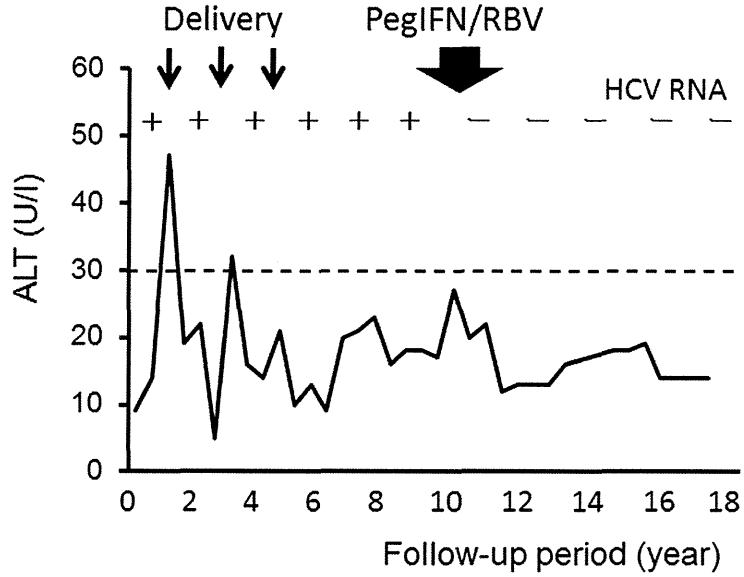

Figure 1. Clinical course and serum alanine aminotransferase (ALT) levels in the mother with chronic HCV genotype 2b infection. Positivity and negativity for serum HCV RNA are represented as plus (+) and minus (-), respectively. PegIFN/RBV: pegylated interferon plus ribavirin treatment.

vironment after birth were uniform.

Here, we report the results of HCV transmission in these three children born from the same mother infected with HCV, with special reference to both the natural course of HCV infection in the children and their genotypes of *IL28B* (rs8099917) and *IFNL4* (ss469415590) associated with the outcome of infection.

Case Report

The mother has been followed up for hepatitis C at our hospital for 18 years. The long-term course of her HCV infection is shown in Fig. 1. The serum alanine aminotransferase (ALT) level was low, at under 30 U/L, during the follow-up period except for two occasions when it exceeded 30 U/L just after delivery of the first and second sons. She had been infected with HCV genotype 2b, and the viral load was always high before she received antiviral therapy. Both hepatitis B surface antigen and anti-immunodeficiency virus antibody were negative in her serum. Six years after the third delivery, she received antiviral therapy with pegylated interferon plus ribavirin for chronic hepatitis C according to the Japanese standard protocol [18], and the therapy was successful in achieving a sustained virologic response.

The characteristics of the three children are summarized in Table 1. All were boys who were born by normal spontaneous delivery 40 weeks after the start of pregnancy. All were healthy and their birth weight was almost the same, at approximately 3,400 g. Nothing in their history suggested any concern about contamination with HCV during their perinatal periods, namely, wounds, surgery or transfusion of blood products. Anti-HCV antibody in the umbilical blood was tested for at birth, and it was positive in all of them. The first and third sons had no evidence of HCV infection because neither anti-HCV antibody nor HCV RNA in serum was positive after birth. However, the second son was confirmed to be infected with HCV 108 days after the delivery showing positivity for both anti-HCV antibody and HCV RNA in serum by the first postnatal assay. HCV transmission to this child resulted in persistent infection. The long-term follow-up and clinical course of this HCV-infected child are shown in Fig. 2. The serum ALT level had been low, at under 30 U/L, for approximately the first 2 years after birth, but thereafter fluctuated above and below 30 U/L until approximately 14 years of age. The HCV genotype of this child was 2b, which was the same as the mother's, and the high-level viremia has continued during the follow-up period. Although the factors possibly influencing maternal HCV transmission, namely, gender, birth weight, delivery conditions, the mother's high viral titer and positivity for anti-HCV antibody in the umbilical blood, were the same among the three siblings, HCV transmission from the mother occurred only in the second child. The genetic polymorphisms of the *IL28B* gene (rs8099917) and *IFNL4* gene (ss469415590) associated with spontaneous viral clearance were examined after obtaining written informed consent from their mother. The genotypes of the *IL28B* gene (rs8099917) and *IFNL4* gene (ss469415590) in the second son, in whom persistent HCV infection had become established, were TG and ΔG/TT, re-

Table 1. Characteristics of Children Born From the HCV-Infected Mother

	First child	Second child	Third child
Sex	Male	Male	Male
Delivery	NSD (39 weeks)	NSD (40 weeks)	NSD (40 weeks)
Body weight at birth	3,364 g	3,492 g	3,400 g
HCV RNA in mother's serum before birth	8.2 Meq/mL*	6.0 log IU/mL**	40.0 Meq/mL*
Anti-HCV antibody in umbilical blood	Positive (CI > 5.0)	Positive (CI > 5.0)	Positive (CI > 5.0)
HCV RNA in umbilical blood	n.t.	< 0.5 Meq/mL*	n.t.
Anti-HCV antibody in serum after birth	Negative	Positive (CI > 5.0)	Negative
HCV RNA in serum after birth	Negative***	Positive***	Negative***
Persistent HCV viremia (period)	Not applicable	15 years	Not applicable
IL28B polymorphism (rs8099917)	TG	TG	TT
IFNL4 polymorphism (ss469415590)	ΔG/TT	ΔG/TT	TT/TT

NSD: normal spontaneous delivery; n.t.: not tested; CI: cutoff index. **This assay was done using the preserved sample. HCV RNA was measured by *branched DNA assay, **real time PCR, ***qualitative PCR.

spectively. Those in the other children who were not infected with HCV varied, being TG and ΔG/TT in the first child, and TT and TT/TT in the third child.

Discussion

In this study, we observed different outcomes of HCV transmission among three brothers who had been exposed to the same HCV strain from the mother during the perinatal period. Interestingly, maternal HCV genotype 2b transmission did not occur evenly in these three siblings, despite the fact that all had the same HCV exposure as well as upbringing environment, and only the second child was infected with HCV genotype 2b. This HCV transmission led to persistent infection in this child, in whom the genotypes of the *IL28B* gene (rs8099917) and *IFNL4* gene (ss469415590) were TG and ΔG/TT, respectively, which are unfavorable for spontaneous viral clearance after the establishment of infection [13, 19].

It is still unclear how mother-to-child HCV transmission occurs in the perinatal period, and our present findings may help to shed some light on the route of infection. During the perinatal period, there are two possible major routes of HCV transmission from mother to child: placental infection and birth canal infection. Placental infection results from active transport of virus from mother to child, or from micro-transfusion of virus due to placental membrane damage. In all of the three brothers, blood exchange between the fetus and the mother through the placenta had been good, because anti-HCV antibody (immunoglobulin G) had been transferred to all fetuses and was detected in the umbilical blood at birth in all cases. However, only the second child had been infected with HCV. Negativity for HCV RNA in the umbilical blood of this child was different from the viral titer in the serum of his mother, which indicated a very high level of HCV RNA, as shown in Table 1. Thus placental infection by active transport of HCV from mother to fetus appeared to have been negligible. Placental membrane damage possibly induced by a small wound that goes unnoticed at delivery may result in maternal HCV infection. In cases of twin delivery, HCV transmission is more likely to affect the second child because possible partial placental separation upon delivery of the first baby increases the chance of exposing the second

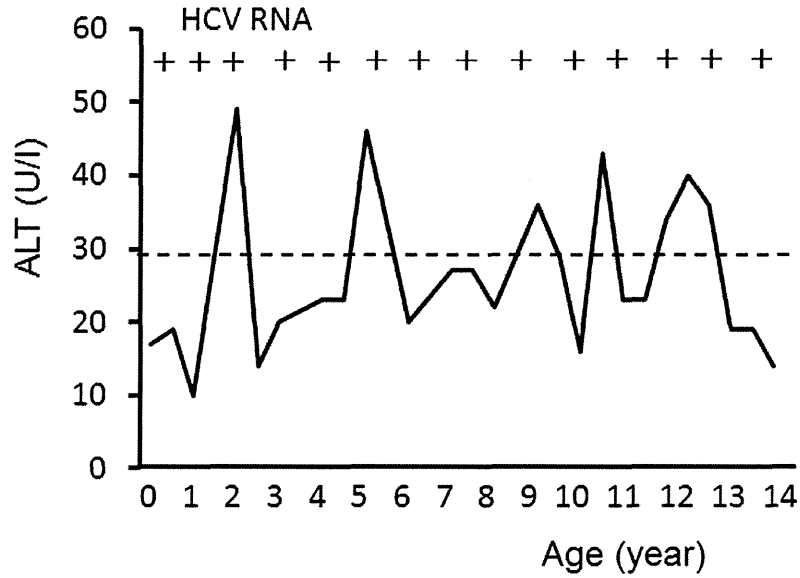

Figure 2. Long-term follow-up of serum alanine aminotransferase (ALT) levels in the second child with maternal HCV genotype 2b infection. Positivity for serum HCV RNA is represented as plus (+).

child to maternal blood [20]. Otherwise, any small wound in child, such as on the skin or in the mucosa of the oral cavity, or the bulbar conjunctiva, may allow micro-transfusion of HCV from mother to a child, and this may result in maternal HCV transmission in the birth canal. After birth, the present three brothers were brought up in the same environment, and were breast-fed. This kind of background carries little risk of HCV infection [21]. These findings suggest that maternal HCV transmission does not usually occur in the perinatal period, with only rare exceptions due to accidental exposure such as minor placental damage or micro-wounds in the child. The prevalence of maternal HCV infection seems to be relatively low even among children exposed to the same virus, although serologic tests for HCV in children should be carefully conducted during follow-up.

Most adults with HCV infection fail to clear the virus and develop chronic hepatitis, but some are known to show resolution of the infection in a self-limiting manner. The rate of spontaneous viral clearance in the acute phase of infection is reported to be approximately 15-40% of all HCV-infected adults [9-11], and a systematic review of 31 studies has estimated this rate to be 26% [11]. In a recent genome-wide association study, the SNPs in the region of the *IL28B* gene encoding interferon-λ-3 were shown to be associated with the virologic response of HCV to antiviral therapy [15, 22]. Patients carrying an *IL28B* homozygote for the major alleles of rs12979860 (CC genotype) [22] or rs8099917 (TT genotype) [15] show a greater propensity for achieving a sustained virologic response to pegylated interferon-α and ribavirin therapy than those carrying an *IL28B* heterozygote or homozygote for its minor allele. This SNP (rs12979860) also influences the outcome of HCV infection in the context of natural history; the CC genotype of rs12979860, which is in high linkage disequilibrium with the TT genotype of rs8099917, enhances the resolution of HCV infection with spontaneous clearance [12, 13]. Furthermore, upstream of the *IL28B* gene, a dinucleotide variant ss469415590 (TT or ΔG) has been reported to be more strongly associated with HCV clearance in individuals of African ancestry than the SNP of *IL28B* (rs12979860) [17].

The genotype of *IL28B* is also associated with the outcome of infection in maternal HCV transmission; self-limiting hepatitis or persistent hepatitis has been reported in a child infected with HCV genotype 1 during the perinatal period [14]. The three children in the present report were checked for the SNPs of both the *IL28B* (rs8099917) and *IFNL4* (ss469415590) genes, and the second son in whom HCV genotype 2b infection had been established and led to persistent infection with mild fluctuation of the ALT levels was found to carry the TG (rs8099917) type and ΔG/TT (ss469415590) type in the *IL28B* and *IFNL4* genes, respectively. Thus the SNPs in these two genes predicted the outcome of infection in the second son with chronic HCV genotype 2b infection.

In conclusion, our present findings suggest that the chance of maternal HCV transmission is not so high even in multiple children exposed to the same HCV strain with a high viral load, and that maternal HCV transmission might be attributable to very minor events that can potentially result in viral exposure during the perinatal period. Here, the multiple deliveries did not largely affect the serum levels of ALT and HCV RNA in the mother. The genotype defined by

SNPs in the *IL28B* gene (rs8099917) and the *IFNL4* gene (ss469415590) was able to predict the development of persistent infection in the child with established maternal HCV genotype 2b transmission.

Conflict of Interest

The authors have no conflict of interest.

References

1. Ohto H, Terazawa S, Sasaki N, Hino K, Ishiwata C, Kako M, Ujiie N, et al. Transmission of hepatitis C virus from mothers to infants. The Vertical Transmission of Hepatitis C Virus Collaborative Study Group. N Engl J Med. 1994;330(11):744-750.
2. Matsubara T, Sumazaki R, Takita H. Mother-to-infant transmission of hepatitis C virus: a prospective study. Eur J Pediatr. 1995;154(12):973-978.
3. Zanetti AR, Tanzi E, Paccagnini S, Principi N, Pizzocolo G, Caccamo ML, D'Amico E, et al. Mother-to-infant transmission of hepatitis C virus. Lombardy Study Group on Vertical HCV Transmission. Lancet. 1995;345(8945):289-291.
4. Resti M, Azzari C, Mannelli F, Moriondo M, Novembre E, de Martino M, Vierucci A. Mother to child transmission of hepatitis C virus: prospective study of risk factors and timing of infection in children born to women seronegative for HIV-1. Tuscany Study Group on Hepatitis C Virus Infection. BMJ. 1998;317(7156):437-441.
5. Gibb DM, Goodall RL, Dunn DT, Healy M, Neave P, Cafferkey M, Butler K. Mother-to-child transmission of hepatitis C virus: evidence for preventable peripartum transmission. Lancet. 2000;356(9233):904-907.
6. Dal Molin G, D'Agaro P, Ansaldi F, Ciana G, Fertz C, Alberico S, Campello C. Mother-to-infant transmission of hepatitis C virus: rate of infection and assessment of viral load and IgM anti-HCV as risk factors. J Med Virol. 2002;67(2):137-142.
7. Steininger C, Kundi M, Jatzko G, Kiss H, Lischka A, Holzmann H. Increased risk of mother-to-infant transmission of hepatitis C virus by intrapartum infantile exposure to maternal blood. J Infect Dis. 2003;187(3):345-351.
8. Cottrell EB, Chou R, Wasson N, Rahman B, Guise JM. Reducing risk for mother-to-infant transmission of hepatitis C virus: a systematic review for the U.S. Preventive Services Task Force. Ann Intern Med. 2013;158(2):109-113.
9. Di Bisceglie AM. Natural history of hepatitis C: its impact on clinical management. Hepatology. 2000;31(4):1014-1018.
10. Gerlach JT, Diepolder HM, Zachoval R, Gruener NH, Jung MC, Ulsenheimer A, Schraut WW, et al. Acute hepatitis C: high rate of both spontaneous and treatment-induced viral clearance. Gastroenterology. 2003;125(1):80-88.
11. Micallef JM, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. J Viral Hepat. 2006;13(1):34-41.
12. Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature. 2009;461(7265):798-801.
13. Hung CH, Chang KC, Lu SN, Wang JH, Chen CH, Lee CM, Hu TH. Spontaneous clearance of hepatitis C virus in an interleukin 28B favorable genotype highly prevalent area. Hepatology. 2013;57(5):2089-2090.
14. Ruiz-Extremera A, Munoz-Gamez JA, Salmeron-Ruiz MA, de Rueda PM, Quiles-Perez R, Gila-Medina A, Casado J, et al. Genetic variation in interleukin 28B with respect to vertical transmission of hepatitis C virus and spontaneous clearance in HCV-infected children. Hepatology. 2011;53(6):1830-1838.
15. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet. 2009;41(10):1105-1109.
16. Balagopal A, Thomas DL, Thio CL. IL28B and the control of hepatitis C virus infection. Gastroenterology. 2010;139(6):1865-1876.
17. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, et al. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet. 2013;45(2):164-171.
18. Guidelines for the Management of Hepatitis C Virus Infection: First edition, May 2012, The Japan Society of Hepatology. Hepatol Res. 2013;43(1):1-34.
19. Saito T, Ueno Y. Transmission of hepatitis C virus: self-limiting hepatitis or chronic hepatitis? World J Gastroenterol. 2013;19(41):6957-6961.
20. Boxall E, Baumann K, Price N, Sira J, Brown M, Kelly D. Discordant outcome of perinatal transmission of hepatitis C in twin pregnancies. J Clin Virol. 2007;38(2):91-95.
21. Indolfi G, Resti M. Perinatal transmission of hepatitis C virus infection. J Med Virol. 2009;81(5):836-843.
22. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature. 2009;461(7262):399-401.

Hepatology Research

Hepatology Research 2014; 44: E26–E31 doi: 10.1111/hepr.12202

Original Article

Clinical manifestations of liver injury in patients with anorexia nervosa

Kyoko Tomita,[1] Hiroaki Haga,[1] Genki Ishii,[3] Tomohiro Katsumi,[1] Chikako Sato,[1,2] Rika Aso,[1] Kazuo Okumoto,[1] Yuko Nishise,[1] Hisayoshi Watanabe,[1,2] Takafumi Saito,[1] Koichi Otani[3] and Yoshiyuki Ueno[1,2]

[1]Department of Gastroenterology, [2]CREST, and [3]Department of Psychiatry, Yamagata University Faculty of Medicine, Yamagata, Japan

Aim: The number of Japanese patients with anorexia nervosa (AN) is increasing as society changes. Mild liver injury is a complication of AN in around 30% of cases. In some rare instances, patients present with severe liver injury similar to acute liver failure. However, there are numerous uncertainties over the clinical characteristics of this condition. The objective of the present study was to clarify the clinical characteristics of AN complicated by liver injury and to investigate the factors related to hepatic complications.

Methods: Thirty-seven patients hospitalized at our institution with a diagnosis of AN were enrolled as the study subjects. The study used clinical data obtained at the time of hospitalization. The enrolled patients underwent subgroup analysis and were categorized into three groups: (i) normal alanine aminotransferase (ALT), (ii) moderately elevated ALT, and (iii) highly elevated ALT.

Results: All of the study subjects were female with a median age of 24 years and presenting with marked weight loss (mean body mass index, 13 kg/m^2). Thirteen of the subjects had liver injury. We found that patients in the highly elevated ALT group had a significantly high blood urea nitrogen (BUN)/creatinine ratio, and a low blood sugar level.

Conclusions: Our present findings indicate that AN patients with highly elevated ALT have a severe dehydration. This suggests that dysfunction of hepatic circulation accompanying severe dehydration due to malnutrition may be an important factor in the development of liver injury in AN patients.

Key words: dehydration, eating disorder, emaciation, hypoxic hepatitis, liver enzyme

INTRODUCTION

ANOREXIA NERVOSA (AN) affects mainly adolescent females in developed countries including the USA, Europe and Japan. The majority of patients develop AN due to abnormal eating habits resulting from a desire to be lean or fear of becoming obese after exposure to psychiatric stress. In Japan, the prevalence of AN has been increasing rapidly;[1] according to annual reports issued by the Ministry of Health, Labour and Welfare, the incidence of eating disorders increased 10-fold in the 20 years since 1980, and the number of AN cases in particular increased fourfold during the 5 years since the mid 1990s. The prevailing explanation for this increase is the change of lifestyle in Japan including the increased variety of social circumstances. AN is associated with a number of complications including liver injury, especially elevation of the serum alanine aminotransferase (ALT) level in more than 30% of cases.[2] Furthermore, rare cases of severe liver injury resulting in acute liver failure have been reported.[3–5] However, the precise mechanism involved in the pathogenesis of liver injury associated with AN remains unclear. Moreover, few reports have documented the clinical features of AN complicated by liver injury. Some have indicated an association with low body mass index (BMI),[6,7] although the roles of other clinical surrogate markers are unclear.

The aim of the present study was to clarify the clinical features of AN complicated by liver injury and the

Correspondence: Dr Kyoko Tomita, Department of Gastroenterology, Yamagata University Faculty of Medicine, 2-2-2 Iida-nishi, Yamagata 990-9585, Japan. Email: mailto:to.kyoko@med.id.yamagata-u.ac.jp
Received 25 March 2013; revision 12 June 2013; accepted 7 July 2013.

clinical factors influencing hepatic complications. In clinical settings, it is important to predict the onset of severe liver injury associated with AN, which could be potentially life-threatening, and therefore it was anticipated that the information obtained from the present study would be of value to clinicians in assessing the risk of developing this serious complication.

METHODS

Study population

THIS RETROSPECTIVE OBSERVATION study was conducted between January 2010 and December 2011 at the Department of Gastroenterology and Department of Neuropsychiatry, Yamagata University Hospital. During this 2-year period, a total of 37 patients were admitted under a diagnosis of AN. These patients comprised both newly referred patients and established outpatients with exacerbation. There were also first admissions and repeat admissions due to deterioration of the patients' condition. The diagnosis of AN was made by a psychiatrist in accordance with the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (DSM-IV) on the basis of information obtained by interview from the patients and their families. The exclusion criteria were: (i) a history of hepatic disease, (ii) established infection with hepatitis viruses (HBV or HCV), (iii) drug abuse, (iv) excessive alcohol intake, and (v) presence of autoimmune liver disease. Especially for patients with highly elevated ALT, imaging studies (both ultrasound and dynamic CT scan) were performed to exclude other hepatobiliary diseases.

Study design

Clinical physiological parameters such as age, gender, BMI, body temperature, pulse rate, and blood pressure were evaluated, as well as routine laboratory data obtained on admission. Routine laboratory data included a complete blood cell count (CBC), hepatobiliary enzyme levels, renal function and blood sugar (BS) levels. The enrolled patients were subjected to subgroup analysis and categorized into three groups: (i) normal ALT defined as a serum ALT level of <42 IU/L, (ii) moderately elevated ALT defined as a serum ALT level between 42 IU/L and <840 IU/L (20 times above the institutional upper normal limit), and (iii) highly elevated ALT defined as a serum ALT level of >840 IU/L.

Statistical analysis

The above three subgroups were evaluated statistically by analysis of variance (ANOVA). If a significant difference was found, multiple comparisons (post hoc test) were performed with Tukey–Kramer and Steel–Dwass test. The risk related to elevated ALT was analyzed by univariate and multivariate logistic regression. The results of logistic regression analysis were expressed as odds ratio with 95% confidence interval. Differences after these modifications were considered significant at $P < 0.05$. Analyses were performed by using Excel Statistics (2010, Social Survey Research Information, Tokyo, Japan).

RESULTS

Patient features

THE BACKGROUNDS OF the 37 enrolled patients are listed in Table 1. The ages of the patients ranged from 12 to 67 years (median age 24 years), and all were lean females with a mean BMI on admission of 13 kg/m^2. The serum ALT level ranged widely from 11 to 2321 IU/L, with a median of 27 IU/L. Besides liver injury, physiological and laboratory abnormalities frequently associated with AN, such as bradycardia, hypothermia, hypotension, anemia, leukopenia, thrombocytopenia, hyponatremia, hypokalemia, and hypoglycemia were present in some of the enrolled patients.

Comparison of each clinical parameter according to the ALT level

Elevated liver enzyme (serum ALT level ≥42 IU/L) was observed in 13 (35%) of the 37 cases. Highly elevated ALT was evident in four cases (11%), the median ALT level being 1986.5 IU/L. Patients in the moderately elevated ALT group accounted for 24% of the subjects overall (9/37), and the median ALT level was 71 IU/L. The median serum ALT level in the normal ALT group was 20.5 IU/L. The clinical parameters in these three groups are detailed in Table 2. Among the clinical parameters evaluated, body temperature, pulse rate, blood urea nitrogen (BUN), BUN/creatinine ratio, BS, and platelet count differed significantly among the groups ($P < 0.05$). These six parameters were further analyzed statistically, and this revealed that both BUN and the BUN/creatinine ratio were significantly higher in the high ALT group than in the normal ALT ($P < 0.05$) and moderate ALT ($P < 0.05$) groups, respectively (Fig. 1). Body temperature, BS and platelet count were

Table 1 Characteristics of study population ($n = 37$)

Parameter	Mean ± SD	Range
Age (years)†	24	12–67
BMI (kg/m^2)	13.0 ± 2.2	9.5–17.9
Body temperature (°C)	36.5 ± 0.7	34.5–38.0
Pulse rate (bpm)	72.1 ± 19.8	46–111
Systolic blood pressure (mmHg)	95.8 ± 18.0	67–153
Diastolic blood pressure (mmHg)	63.2 ± 15.3	33–101
Albumin (g/dL)	4.4 ± 0.9	2.6–5.7
Total bilirubin (mg/dL)†	0.9	0.2–7.3
AST (IU/L)†	29	12–2628
ALT (IU/L)†	27	11–2321
BUN (mg/dL)	19.8 ± 11.3	4–47
Creatinine (mg/dL)†	0.66	0.16–1.57
BUN/creatinine (ratio)†	24.3	8–104.4
Na (mEq/L)†	140	121–146
K (mEq/L)	3.7 ± 0.6	2.4–5.1
Cl (mEq/L)	99.8 ± 6.8	76–110
Blood sugar (mg/dL)†	81	12–150
White blood cells (/μL)	5132.9 ± 2564.7	1 820–15 600
Hemoglobin (g/dL)	12.6 ± 2.0	5.7–16.5
Platelet count (/μL)	23.6 ± 9.2	8.1–41.1

†Median.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; SD, standard deviation.

Table 2 Comparison of each clinical parameter according to the alanine aminotransferase (ALT) level

Parameter	Highly elevated ALT group (ALT ≥ 840) $n = 4$	Moderately elevated ALT group (840 > ALT ≥ 42) $n = 9$	Normal ALT group (ALT < 42) $n = 24$	*P*-value
Age (years); median [range]	17 [16–31]	29 [13–50]	22 [12–67]	0.495
BMI (kg/m^2); mean ± SD	10.7 ± 1.1	13.4 ± 3.0	13.3 ± 1.8	0.070
Body temperature (°C); mean ± SD	35.3 ± 0.9	36.8 ± 0.5	36.7 ± 0.7	<0.05
Pulse rate (bpm); mean ± SD	50.5 ± 12.2	67.7 ± 15.7	75.9 ± 20.1	<0.05
Systolic blood pressure (mmHg); mean ± SD	92.3 ± 12.1	97.7 ± 11.6	95.7 ± 20.9	0.890
Albumin (g/dL); mean ± SD	4.4 ± 0.6	3.9 ± 1.0	4.6 ± 0.8	0.110
AST (IU/L); median [range]	2249.5 [1684–2628]	68 [39–512]	22 [12–53]	<0.05
ALT (IU/L); median [range]	986.5 [871–2321]	71 [48–215]	20.5 [11–36]	<0.05
BUN (mg/dL); mean ± SD	41.0 ± 6.1	17.4 ± 9.9	17.1 ± 8.6	<0.05
Creatinine (mg/dL); median [range]	0.64 [0.45–0.67]	0.56 [0.35–0.7]	0.71 [0.16–1.57]	0.062
BUN/creatinine; median [range] (ratio)	63.6 [53.2–104.4]	28.3 [11.6–56.9]	19.4 [8–68.6]	<0.05
Na (mEq/L); mean ± SD	141.0 ± 5.0	138.2 ± 3.6	138.4 ± 5.3	0.592
K (mEq/L); mean ± SD	3.7 ± 0.3	3.7 ± 0.6	3.7 ± 0.7	0.998
Cl (mEq/L); mean ± SD	102.3 ± 7.4	100.3 ± 4.0	99.3 ± 7.6	0.89
Blood sugar (mg/dL); median [range]	26 [12–46]	80 [57–109]	84.5 [62–159]	<0.05
White blood cells (/μL) mean ± SD;	6067.5 ± 2880.3	4503 ± 1749.3	5213.3 ± 2799.2	0.591
Hemoglobin (g/dL); mean ± SD	12.1 ± 4.3	12.1 ± 3.9	12.8 ± 1.7	0.706
Platelet count (/μL); mean ± SD	10.4 ± 2.1	24.9 ± 7.9	25.3 ± 8.7	<0.05

AST, aspartate aminotransferase; BMI, body mass index; BUN, blood urea nitrogen; SD, standard deviation.

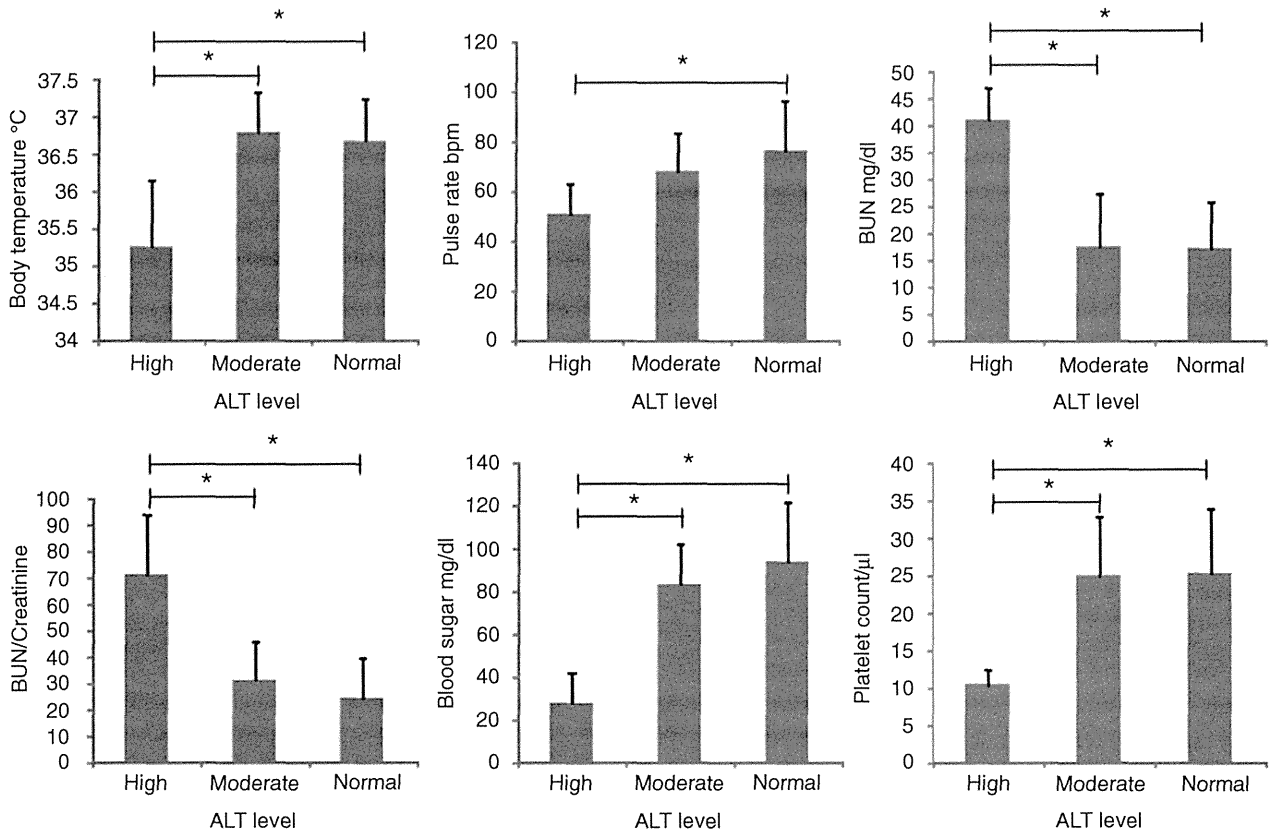

Figure 1 Statistically significant clinical parameters (body mass index [BMI], pulse rate, blood urea nitrogen [BUN], BUN/creatinine ratio, blood sugar, platelets count) among the three groups divided according to the severity of liver injury. Multiple comparisons (post hoc test) were performed with Tukey–Kramer and Steel–Dwass test. *$P < 0.05$.

significantly lower in the high ALT group than in the normal ALT ($P < 0.05$) and moderate ALT ($P < 0.05$) groups. Pulse rate in the high ALT group was significantly decreased than in the normal ALT group ($P < 0.05$), but the difference between the high ALT and moderate ALT groups was not significant.

Risk factors related to elevated ALT levels in patients with AN

Among the six parameters (body temperature, pulse rate, BUN, BUN/creatinine, BS, platelet count) initially demonstrating significant differences among the three groups, risk factors relating to elevated ALT levels were examined. BUN/creatinine and BS were significantly associated with the incidence of elevated ALT by univariate analyses (Table 3). In further analysis with multiple logistic regression, there was not a significant association between the six parameters (body temperature, pulse rate, BUN, BUN/creatinine, BS, platelet count). However, there was a trend with BUN/creatinine (odds ratio [OR] = 1.051; 95% confidence interval [CI]; 0.999–1.105, $P = 0.054$) and BS (OR = 0.967; 95% CI; 0.933–1.002, $P = 0.066$ (Table 3).

DISCUSSION

WE FOUND THAT AN patients with highly elevated ALT had a significantly high BUN level and BUN/creatinine ratio, and a low body temperature, low blood sugar level, and low platelet count. Moreover, BUN/creatinine and BS had trends associated with the incidence of elevated ALT by multivariate analyses.

Clinical parameters in patients with AN demonstrating liver injury have been reported previously, especially the relationship between elevation of serum liver enzyme levels and low BMI.[6,7] However, in the present study, we found no significant correlation of serum liver

Table 3 Multiple logistic regression analysis related to elevated alanine aminotransferase (ALT) levels in anorexia nervosa (AN) patients

	Univariate		Multivariate	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
BUN/Creatinine (ratio)	1.051 (1.008–1.096)	0.019	1.051 (0.999–1.105)	0.054
Blood sugar (mg/dL)	0.962 (0.930–0.995)	0.025	0.967 (0.933–1.002)	0.066
Body temperature (°C)	0.509 (0.192–1.347)	0.174		
Pulse rates (bpm)	0.958 (0.916–1.002)	0.061		
BUN (mg/dL)	1.064 (0.997–1.135)	0.062		
Platelet count (/μL)	0.939 (0.866–1.019)	0.131		

Stepwise method was used to select the variables in multiple logistic regression analysis.
BUN, blood urea nitrogen; CI, confidence interval; OR, odds ratio.

enzyme levels with BMI. We speculate that this may have been attributable to the inclusion criteria we used for our AN patients. The present study recruited only AN patients who required hospitalization, so our study patients tended to have lower BMI values than outpatient studies, thus possibly masking any statistically significant differences.

Among several clinical parameters, we found that the serum BUN level and BUN/creatinine ratio were significantly high in the high ALT group. We speculate that this phenomenon could have been attributable to the presence of severe dehydration in this group, where a high BUN level and a high BUN/creatinine ratio (so-called "hypoxic hepatitis") were also observed. This is in accord with the fact that even patients with severe liver injury usually recover after conservative treatment such as drip infusion or bed rest, as seen in cases of hypoxic hepatitis due to circulatory failure occasionally encountered in various clinical settings.

We also observed that the high ALT group had significantly lower values of pulse rate. It seems paradoxical that AN patients with severe liver injury often have bradycardia despite the presence of severe dehydration. We speculate that this phenomenon may be due to the fact that patients with AN usually have hypertonic parasympathetic nervous conditions and hypotonic sympathetic nervous conditions, which lead to failure to respond to the stimulation of the sympathetic nervous system resulting from dehydration.[8] Also, among our AN patients in the high ALT group, hypoglycemia (median value as low as 26 mg/dL) was observed in four, and this led to consciousness disturbance in two. Up to now, there have been no convincing explanations for the hypoglycemia and liver injury associated with AN, although hypoglycemia could affect the systemic circulation, in turn influencing the hepatic circulation and resulting in liver injury. In fact, it has been reported that patients with hypoxic hepatitis are often complicated by hypoglycemia.[9]

In patients with AN, various complications known as "refeeding syndrome"can occur after initiation of food intake or hyperalimentation on admission and hypoglycemia is one of its symptoms.[10] Although some reports have described the presence of liver injury during hypoglycemia in refeeding syndrome,[11,12] its precise mechanism remains unknown.

Our present findings suggested a close relationship of dehydration in the pathogenesis of elevated liver enzyme in AN, with features clinically reminiscent of hypoxic hepatitis. However, we were not able to exclude the opposite possibility that high BUN and BUN/creatinine ratio could be caused by elevated ALTs. Unfortunately this is the limitation of this retrospective study. Our study was also limited in that it did not evaluate liver pathology. Hepatic histological findings in AN with liver insufficiency include centrilobular lesions with fibrosis or atrophy, hepatocytes swelling, glycogen depletion, and ceroid pigmentation.[13] Since almost all patients with AN are young females, who often have accompanying mood disorder and/or obsessive-compulsive disorder,[14] and liver injury rapidly improves after hospitalization,[15] invasive procedures such as liver biopsy are rarely performed at an early stage after admission when patients are psychiatrically unstable. Accordingly, future studies will need to evaluate liver histology or use an appropriate animal model.

In conclusion, the present study has demonstrated that AN patients with severe liver injury have significantly increased in the serum BUN level and BUN/creatinine ratio. This could account for failure of the

hepatic circulation due to severe dehydration based on malnutrition, being a potentially important factor in the development of severe liver injury in AN patients, mimicking hypoxic hepatitis. These factors offer an interesting insight into the pathogenesis of AN.

ACKNOWLEDGMENT

WE THANK MEMBERS of the Department of Psychiatry, Yamagata University Faculty of Medicine for their support and encouragement in this research.

REFERENCES

1 Kuboki T, Nomura S, Ide M, Suematsu H, Araki S. Epidemiological data on anorexia nervosa in Japan. *Psychiatry Res* 1996; 62: 11–16.
2 Fong HF, DiVasta AD, DiFabio D, Ringelheim J, Jonas MM, Gordon CM. Prevalence and predictors of abnormal liver enzymes in young women with anorexia nervosa. *J Pediatr* 2008; 153: 247–53.
3 De Caprio C, Alfano A, Senatore I, Zarrella L, Pasanisi F, Contaldo F. Severe acute liver damage in anorexia nervosa: two case reports. *Nutrition* 2006; 22: 572–5.
4 Furuta S, Ozawa Y, Maejima K *et al.* Anorexia nervosa with severe liver dysfunction and subsequent critical complications. *Intern Med* 1999; 38: 575–9.
5 Dowman J, Arulraj R, Chesner I. Recurrent acute hepatic dysfunction in severe anorexia nervosa. *Int J Eat Disord* 2010; 43: 770–2.
6 Tsukamoto M, Tanaka A, Arai M *et al.* Hepatocellular injuries observed in patients with an eating disorder prior to nutritional treatment. *Intern Med* 2008; 47: 1447–50.
7 Ozawa Y, Shimizu T, Shishiba Y. Elevation of serum aminotransferase as a sign of multiorgan-disorders in severely emaciated anorexia nervosa. *Intern Med* 1998; 37: 32–9.
8 Petretta M, Bonaduce D, Scalfi L *et al.* Heart rate variability as a measure of autonomic nervous system function in anorexia nervosa. *Clin Cardiol* 1997; 20: 219–24.
9 Fuhrmann V, Kneidinger N, Herkner H *et al.* Hypoxic hepatitis: underlying conditions and risk factors for mortality in critically ill patients. *Intensive Care Med* 2009; 35: 1397–405.
10 Crook MA, Hally V, Panteli JV. The importance of the refeeding syndrome. *Nutrition* 2001; 17: 632–7.
11 Sakurai-Chin C, Ito N, Taguchi M, Miyakawa M, Takeshita A, Takeuchi Y. Hypoglycemic coma in a patient with anorexia nervosa coincident with acute exacerbation of liver injury induced by oral intake of nutrients. *Intern Med* 2010; 49: 1553–6.
12 Narayanan V, Gaudiani JL, Harris RH, Mehler PS. Liver function test abnormalities in anorexia nervosa-cause or effect. *Int J Eat Disord* 2010; 43: 378–81.
13 Rautou P-E, Cazals-Hatem D, Mareau R *et al.* Acute liver cell damage in patients with anorexia nervosa: a possible role of starvation-induced hepatocyte autophagy. *Gastroenterology* 2008; 135: 840–8.
14 Salbach-Andrae H, Lenz K, Simmendinger N, Klinkowski N, Lehmkuhl U, Pfeiffer E. Psychiatric comorbidities among female adolescents with anorexia nervosa. *Child Psychiatry Hum Dev* 2008; 39: 261–72.
15 Giordano F, Arnone S, Santeusanio F, Pampanelli S. Brief elevation of hepatic enzymes due to liver ischemia in anorexia nervosa. *Eat Weight Disord* 2010; 15: E294–7.

HEPATOLOGY
Official Journal of the American Association for the Study of Liver Diseases

Impaired Interferon Signaling in Chronic Hepatitis C Patients With Advanced Fibrosis via the Transforming Growth Factor Beta Signaling Pathway

Takayoshi Shirasaki,[1,2] Masao Honda,[1,2] Tetsuro Shimakami,[1] Kazuhisa Murai,[1,2] Takayuki Shiomoto,[1,2] Hikari Okada,[1] Riuta Takabatake,[1] Akihiro Tokumaru,[1] Yoshio Sakai,[1] Taro Yamashita,[1] Stanley M. Lemon,[3] Seishi Murakami,[1] and Shuichi Kaneko[1]

Malnutrition in the advanced fibrosis stage of chronic hepatitis C (CH-C) impairs interferon (IFN) signaling by inhibiting mammalian target of rapamycin complex 1 (mTORC1) signaling. However, the effect of profibrotic signaling on IFN signaling is not known. Here, the effect of transforming growth factor (TGF)-β signaling on IFN signaling and hepatitis C virus (HCV) replication was examined in Huh-7.5 cells by evaluating the expression of forkhead box O3A (Foxo3a), suppressor of cytokine signaling 3 (Socs3), c-Jun, activating transcription factor 2, ras homolog enriched in brain, and mTORC1. The findings were confirmed in liver tissue samples obtained from 91 patients who received pegylated-IFN and ribavirin combination therapy. TGF-β signaling was significantly up-regulated in the advanced fibrosis stage of CH-C. A significant positive correlation was observed between the expression of TGF-β2 and mothers against decapentaplegic homolog 2 (Smad2), Smad2 and Foxo3a, and Foxo3a and Socs3 in the liver of CH-C patients. In Huh-7.5 cells, TGF-β1 activated the Foxo3a promoter through an AP1 binding site; the transcription factor c-Jun was involved in this activation. Foxo3a activated the Socs3 promoter and increased HCV replication. TGF-β1 also inhibited mTORC1 and IFN signaling. Interestingly, c-Jun and TGF-β signaling was up-regulated in treatment-resistant IL28B minor genotype patients (TG/GG at rs8099917), especially in the early fibrosis stage. Branched chain amino acids or a TGF-β receptor inhibitor canceled these effects and showed an additive effect on the anti-HCV activity of direct-acting antiviral drugs (DAAs). *Conclusion:* Blocking TGF-β signaling could potentiate the antiviral efficacy of IFN- and/ or DAA-based treatment regimens and would be useful for the treatment of difficult-to-cure CH-C patients. (HEPATOLOGY 2014;60:1519-1530)

A human liver infected with hepatitis C virus (HCV) develops chronic hepatitis, cirrhosis, and in some instances, hepatocellular carcinoma (HCC). HCC develops frequently in the advanced fibrosis stage, and the annual incidence of HCC in patients with HCV-related liver cirrhosis is ~6-8%.[1] The eradication of HCV infection has been a promising prophylactic therapy for preventing the occurrence of HCC.

Interferon (IFN) and ribavirin (RBV) combination therapy has been a popular modality for eliminating HCV; however, its efficacy is limited in patients with advanced liver fibrosis.[2] The use of the recently developed direct-acting antiviral drugs (DAAs) telaprevir or

Abbreviations: AMPK, protein kinase, AMP-activated, alpha 1 catalytic subunit; CH-C, chronic hepatitis C; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; IL28B, interleukin 28B; ISG-20, interferon-stimulated exonuclease gene 20; MX1, myxovirus resistance 1; NR, no response; RBV, ribavirin; RHEB, ras homolog enriched in brain; RIG-I, retinoic acid inducible gene I; SMAD, mothers against decapentaplegic homolog; TGF, transforming growth factor; TGF-RI, transforming growth factor-receptor inhibitor.

From the [1]Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Kanazawa, Japan; [2]Department of Advanced Medical Technology, Kanazawa University Graduate School of Health Medicine, Kanazawa, Japan; [3]Division of Infectious Diseases, School of Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA.

Received February 1, 2014; accepted June 20, 2014.

boceprevir, combined with pegylated (PEG)-IFN plus RBV, significantly improved the sustained virologic response (SVR) rates; however, the SVR rate is reduced in patients with advanced liver fibrosis and the treatment-resistant interleukin 28B (IL28B) genotype,[3-5] in whom HCC can develop at a high frequency. Moreover, extended therapy should be avoided in these patients in terms of the high frequency of adverse effects.

The mechanism of treatment resistance in patients with advanced liver fibrosis has not yet been clarified completely. Previously, we reported that the malnutrition status of patients with advanced chronic hepatitis C (CH-C) is associated with IFN resistance, and Fischer's ratio (branched chain amino acids [BCAAs] / aromatic amino acids) is an independent predictor of treatment outcome of PEG-IFN plus RBV combination therapy. Furthermore, we showed that malnutrition impaired IFN signaling by inhibiting mammalian target of rapamycin complex 1 (mTORC1) and activating suppressor of cytokine signaling 3 (Socs3)-mediated IFN inhibitory signaling through the nutrition-sensing transcriptional factor forkhead box protein O3a (Foxo3a).[6] This report represented the first clue to disentangling the molecular links between advanced CH-C and poor treatment response; however, the association of profibrotic signaling and IFN signaling was not evaluated in detail.

In the present study, we investigated the interaction between the signaling of the profibrotic gene transforming growth factor (TGF)-β and IFN signaling in the liver of CH-C patients. We showed that blocking TGF-β signaling as well as improving the nutritional status of patients by using BCAAs restored IFN signaling and increased the treatment efficacy of anti-HCV therapy.

Materials and Methods

Cell Lines. A reversibly immortalized human hepatocyte cell line (TTNT) was established by transduction with a retroviral vector containing cDNA expressing hTERT for immortalization.[7] TTNT, Huh-7, and Huh-7.5 cells (kindly provided by Professor C.M. Rice, Rockefeller University, New York, NY) were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Gaithersburg, MD) containing 10% fetal bovine serum and 1% penicillin/streptomycin. Primary human hepatocytes (PHH) were isolated from chimeric mice with a humanized liver (PXB-mice; PhoenixBio, Hiroshima, Japan).

Amino Acid-Free Medium and BCAAs. Amino acid-free medium and BCAAs were prepared as described previously. Details are given in the Supporting Materials and Methods.

TGF-β and IFN Treatment. Huh-7.5 cells or HCV-RNA-transfected Huh-7.5 cells were seeded at 1.0×10^5 cells/well in 12-well plates. After 24 hours, the cells were treated with TGF-β (Millipore, Billerica, MA). At 24 hours later, the cells were treated with the indicated international units of IFN-α for 24 hours (Schering-Plough, Tokyo, Japan).

BCAA Treatment. HCV-RNA-transfected Huh-7.5 cells were seeded at 1.0×10^5 cells/well in 12-well plates. After 24 hours, the cells were treated with TGF-β in low-amino-acid medium and the indicated concentration of BCAAs. At 48 hours after treatment, real-time detection, polymerase chain reaction (RTD-PCR), western blotting, and *Gaussia* luciferase assays were carried out as described previously.

TGF-β Receptor Inhibitor Treatment. HCV-RNA-transfected Huh-7.5 cells were seeded at 1.0×10^5 cells/well in 12-well plates. After 24 hours, the cells were treated with TGF-β in low-amino-acid medium and TGF-β Receptor Inhibitor (TGF-β RI; Millipore). At 24 hours after treatment, RTD-PCR, western blotting, and *Gaussia* luciferase assays were carried out as described previously.

DAA Treatment. DAAs (boceprevir and BMS-790052) were purchased from AdooQ Bioscience (Irvine, CA). HCV-RNA-transfected Huh-7.5 cells were seeded at 1.0×10^5 cells/well in 12-well plates. After 24 hours, the cells were treated with TGF-β in low-amino-acid medium and BCAAs and DAAs. At 24 hours after treatment, the *Gaussia* luciferase assay was carried out as described previously.

Patients' characteristics, HCV replication analysis, western blotting, quantitative RTD-PCR, and promoter analysis are described in the Supporting Materials and Methods.

Address reprint requests to: Masao Honda, M.D., Ph.D., Department of Gastroenterology, Graduate School of Medicine, Kanazawa University, Takara-Machi 13-1, Kanazawa 920-8641, Japan. E-mail: mhonda@m-kanazawa.jp; fax: +81-76-234-4250.

View this article online at wileyonlinelibrary.com.
DOI 10.1002/hep.27277
Potential conflict of interest: Nothing to report.

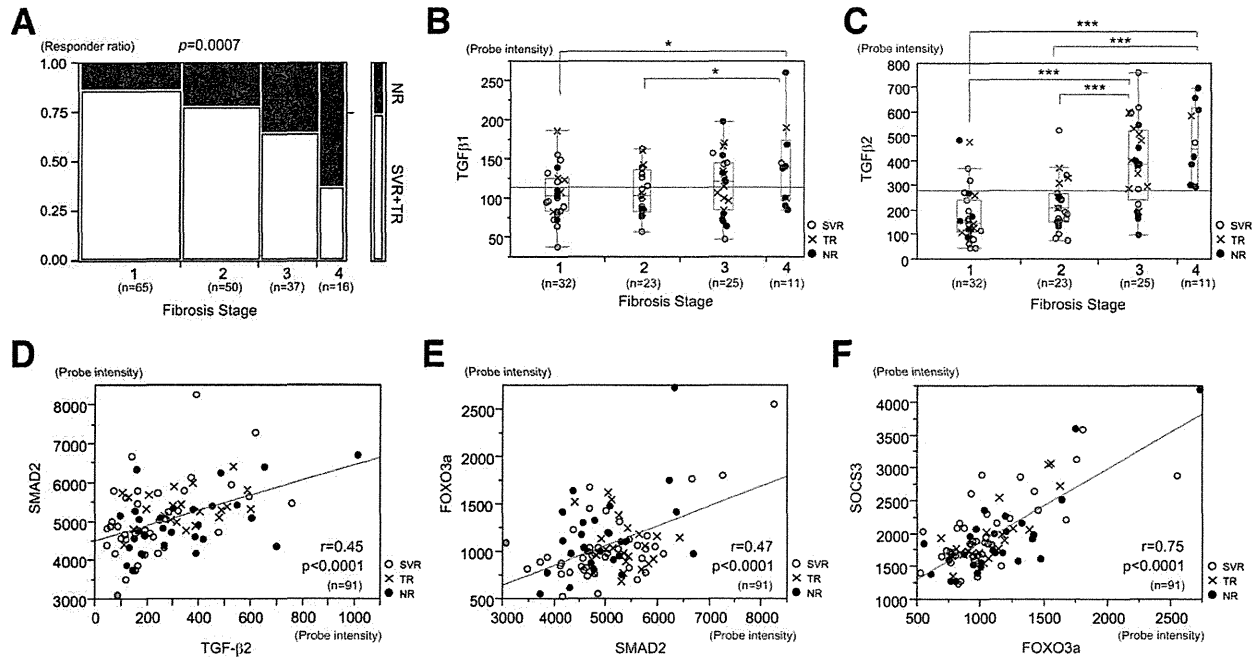

Fig. 1. Activation of TGF-β signaling in the liver of patients at the advanced fibrosis stage of CH-C. A: Significant increase in the NR ratio with the progression of fibrosis stage. B,C: Expression of TGF-β1 (B) and TGF-β2 (C) with the progression of fibrosis stage. D-F: Significant correlations of TGF-β2 and Smad2 (D), Smad2 and Foxo3a (E), and Foxo3a and Socs3 (F) expression in the liver of CH-C patients.

Statistical Analysis. The results are expressed as the mean value ± standard deviation. At least three samples were tested in each assay. Significance was tested by one-way analysis of variance with Bonferroni methods, and differences were considered statistically significant at $P < 0.05$.

Results

Up-Regulated TGF-β Signaling and Low Treatment Response in CH-C Patients With Advanced Liver Fibrosis Who Received PEG-IFN Plus RBV Combination Therapy. Previously, using a cohort of 168 CH-C patients who received PEG-IFN plus RBV combination therapy, we demonstrated that liver fibrosis stage and Fischer's ratio as well as IL28B genotype were independent significant factors associated with no response (NR) to treatment (Supporting Table 1).[6] The NR rate was significantly increased according to the increase in fibrosis stage ($P = 0.007$) (Fig. 1A). To reveal the molecular mechanism between profibrotic signaling and treatment resistance, we focused on TGF-β signaling in the liver of CH-C patients. The expression of TGF-β1 and TGF-β2, deduced from 91 CH-C patients whose liver tissues were analyzed previously using an Affymetrix GeneChip (Supporting Table 2),[6,8] was significantly up-regulated in the advanced fibrosis stage (Fig. 1B,C). In particular, the up-regulation of TGF-β2 in patients with stage 3 and 4 fibrotic livers was more prominent (Fig. 1C). There was a significant correlation between the expression of TGF-β2 and mothers against decapentaplegic homolog 2 (Smad2), a downstream signaling molecule of the TGF-β receptor, showing the activation of TGF-β signaling in the liver of CH-C patients. Interestingly, Smad2 expression was significantly correlated with Foxo3a expression, a nutrition-sensing transcription factor. Previously, we reported that Foxo3a increases the transcription of Socs3, an inhibitor of IFN signaling, through binding to the Socs3 promoter (Foxo3a-Socs3 signaling).[6] Foxo3a expression was significantly correlated with Socs3 expression in the CH-C patients (Fig. 1F).

TGF-β Signaling Activates Foxo3a-Socs3 Signaling in the Huh-7.5 Human Hepatoma Cell Line and PHH. The relationship between TGF-β and Foxo3a-Socs3 signaling was evaluated in PHH and the Huh-7.5 human hepatoma cell line without HCV replication (Huh-7.5 HCV (–)). This signaling was also evaluated in Huh-7.5 cells in which the infectious HCV clone H77Sv3 GLuc2A[6] was replicating (Huh-7.5 HCV (+)) (Fig. 2A). Treatment of these cells with TGF-β1 substantially increased the levels of phosphorylated (p)-Smad2 and p-Smad3. In this condition, the levels of p-Foxo3a, which is degraded through the proteasomal pathway,

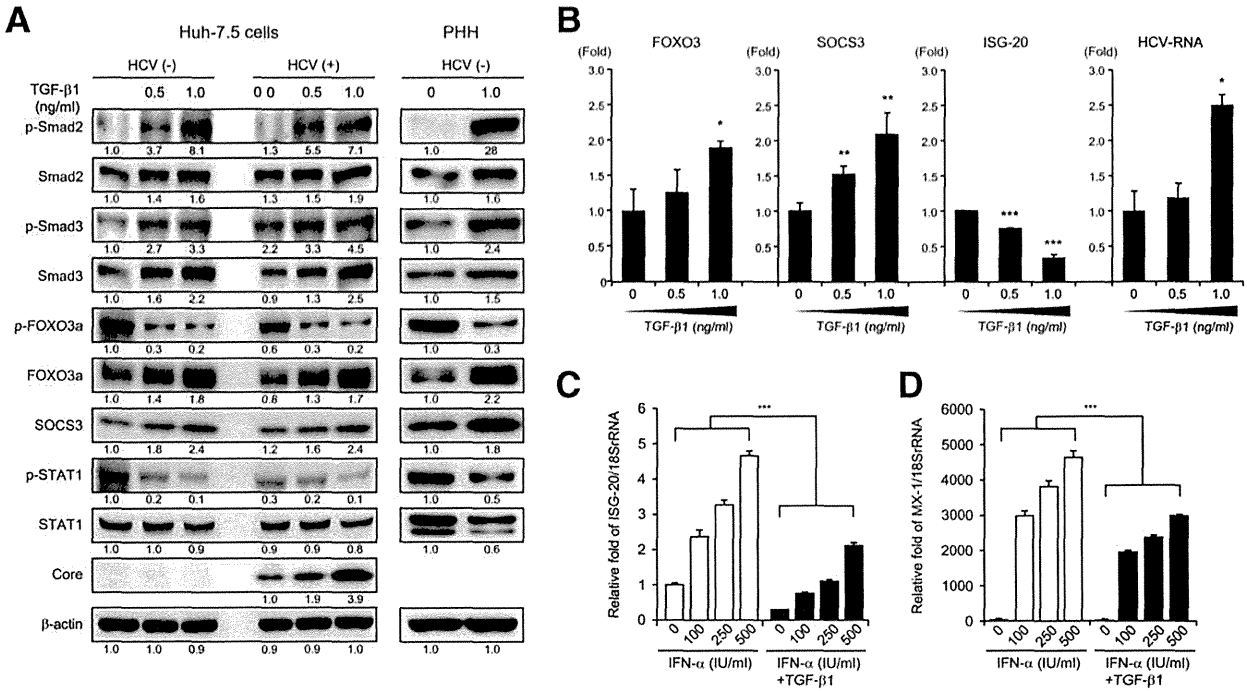

Fig. 2. Effect of TGF-β1 on IFN signaling in Huh-7.5 cells and PHH. A: Western blotting of TGF-β, Foxo3a-Socs3, and IFN signaling in Huh-7.5 cells and PHH treated with TGF-β1. Huh-7.5 cells were transfected with infectious HCV RNA, H77Sv3 GLuc2A prior to TGF-β1 treatment (Huh-7.5 HCV (+)). The experiments were repeated 3 times. B: RTD-PCR results for Foxo3a, Socs3, ISG-20, and HCV-RNA expression in Huh-7.5 HCV (+) treated with TGF-β1. C,D: Inhibition of IFN-α-induced ISG induction (ISG-20 [C] and MX1 [D]) by TGF-β1 in Huh-7.5 HCV (+). B-D: The experiments were performed in triplicate and repeated 3 times ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

decreased and total Foxo3a expression increased, and then Socs3 expression increased. Subsequently, the levels of phosphorylated signal transducer and activator of transcription 1 (p-STAT1) were decreased and the amount of HCV core protein increased in Huh-7.5 HCV (+). Thus, TGF-β signaling activated Foxo3a-Socs3 signaling and inhibited IFN signaling in hepatocytes, regardless of HCV replication and a loss-of-function mutation in retinoic acid inducible gene I (RIG-I).

These findings were also confirmed at the mRNA level in Huh-7.5 HCV (+). RTD-PCR showed that TGF-β1 treatment significantly increased Foxo3a and Socs3 expression, and decreased the expression of interferon-stimulated exonuclease gene 20 (ISG-20) in a dose-dependent manner. HCV-RNA was significantly increased in this condition (Fig. 2B). Moreover, the induction of interferon-stimulated genes (ISG-20 and myxovirus-resistance 1 [MX1]) by IFN-α treatment was significantly reduced in the presence of TGF-β1 (Fig. 2C,D).

When endogenous TGF-β signaling was compared between Huh-7.5 HCV (–) and Huh-7.5 HCV (+), TGF-β signaling was preactivated in Huh-7.5 HCV (+) before TGF-β1 treatment (Fig. 2A). To examine the role of endogenous TGF-β1 signaling on Foxo3a-Socs3 signaling and HCV replication, a small interfering (si) RNA specific to TGF-β1 was introduced to Huh-7 cells in which cell culture-derived infectious HCV HJ3-5 (HCVcc HJ3-5)[9] (Supporting Materials and Methods) was replicating. With the repression of TGF-β1, the levels of p-Smad2, p-Smad3, Foxo3a, and Socs3a decreased, while the levels of p-STAT1 increased. As a result, HCV replication deceased in both the amino acid-depleted (1/5 DMEM) and non-depleted (DMEM) conditions (Supporting Fig. 1).

AP1 Binding Site in the Foxo3a Promoter Is Responsible for the Induction of Foxo3a by TGF-β Signaling. To identify which transcription factors were involved in the induction of Foxo3a by TGF-β1, we cloned the upstream promoter region of Foxo3a and generated Foxo3a promoter-luciferase reporter constructs with various lengths of 5′-end deletions (−1780, −1340, and −801 nucleotides [nt]) (Fig. 3A). Luciferase activity deduced from pGL4-FOXO3a (−1780) increased by ~1.5-fold in the amino acid-depleted condition (1/5 DMEM) compared with the nondepleted condition (DMEM). TGF-β1 further stimulated the promoter activity of pGL4-FOXO3a (−1780) (Fig. 3B). A TGF-β1 RI canceled this stimulation (Fig. 3B). pGL4-FOXO3a (−1340) retained the regulation of promoter activity by amino acid depletion (1/5 DMEM) and

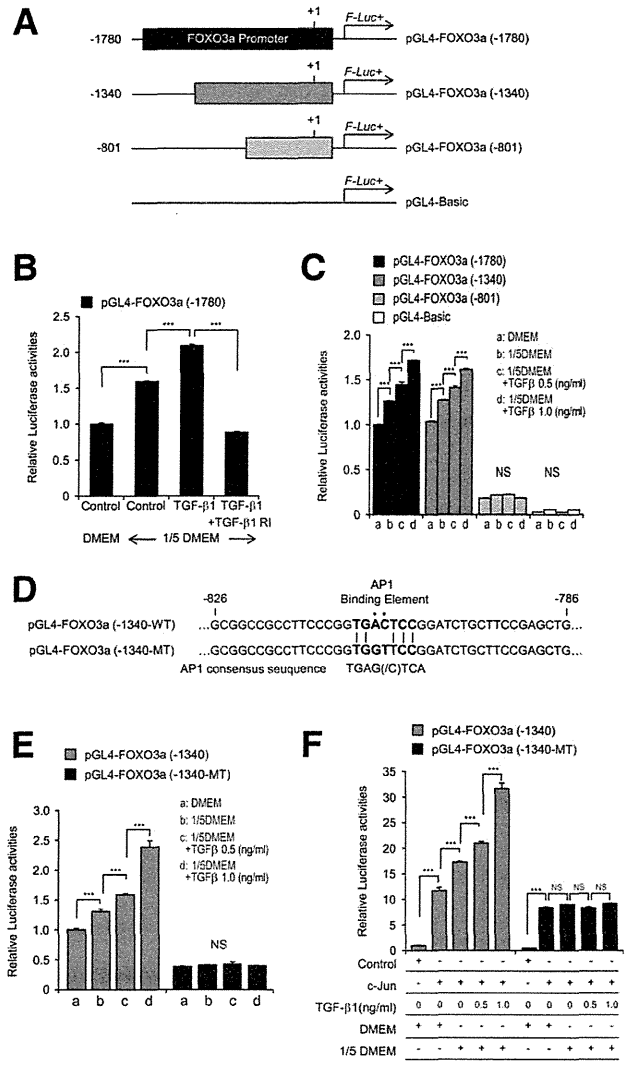

Fig. 3. Foxo3a promoter analysis. A: Foxo3a promoter-luciferase reporter constructs. B: Promoter activity of pGL4-FOXO3a (−1780) following amino acid depletion (1/5 DMEM), TGF-β1 treatment, and TGF-β1 RI treatment. C: Abolished regulation of the promoter activity of pGL4-FOXO3a (−801) by amino acid depletion (1/5 DMEM) and TGF-β1 treatment. D: Alignment of the AP1 binding element of pGL4-FOXO3a (−1340) and pGL4-FOXO3a (−1340-MT), in which the AP1 site was mutated. E: Abolished regulation of the promoter activity of pGL4-FOXO3a (−1340-MT) by amino acid depletion (1/5 DMEM) and TGF-β1 treatment. F: Overexpression of c-Jun, amino acid depletion (1/5 DMEM), and TGF-β1 treatment increased the promoter activity of pGL4-FOXO3a (−1340) by up to 32-fold, while these had less of an effect on the promoter activity of pGL4-FOXO3a (−1340-MT). The experiments were performed in triplicate and repeated 3 times ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

TGF-β1 treatment; however, pGL4-FOXO3a (−801) lost this regulation (Fig. 3C), suggesting the presence of a response element between −1340 and −801 nt. We identified an activator protein (AP) 1 transcription factor binding site at −810 to −804 nt. (Fig. 3D). We introduced two nucleotide mutations (AC to GT) in the AP1 consensus binding sequence, and the mutant construct, pGL4-FOXO3a (−1340-MT), lost the response to amino acid depletion (1/5 DMEM) and TGF-β1 treatment (Fig. 3E). These results were confirmed by using three different hepatocyte-derived cell lines (TTNT, Huh-7, and Huh-7.5 cells; Supporting Fig. 2A-C). Although RIG-I-dependent IFN signaling was active in TTNT cells (Supporting Fig. 2D), Foxo3a promoter activity in response to amino acid depletion (1/5 DMEM) and TGF-β1 treatment was not significantly different between these cell lines.

To confirm these findings further, we overexpressed c-Jun, a component of AP1, and evaluated Foxo3a promoter activity. The overexpression of c-Jun increased the promoter activity of pGL4-FOXO3a (−1340) to 12-fold, and amino acid depletion (1/5 DMEM) and TGF-β1 treatment further increased promoter activity up to 32-fold (Fig. 3F). Conversely, pGL4-FOXO3a (−1340-MT) lost the response to amino acid depletion (1/5 DMEM) and TGF-β1 treatment (Fig. 3F). These results confirmed that AP1 plays an important role in the induction of Foxo3a by these stimulatory factors.

Transcription Factor c-Jun Is Involved in the Induction of Foxo3a in the Liver of CH-C Patients. The AP1 transcription factor is mainly composed of Jun, Fos, and activating transcription factor (ATF) protein dimers.[10] Therefore, we evaluated the expression of c-Jun, ATF2, and c-Fos in Huh-7.5 cells and PHH under amino acid depletion (1/5 DMEM) and TGF-β1 treatment. Western blotting analysis showed that the levels of p-c-Jun and p-ATF2 were increased under these conditions, although the induction of p-c-Jun by amino acid depletion (1/5 DMEM) was not obvious in PHH (Fig. 4A). These findings were also confirmed by RTD-PCR. The mRNA expression of c-Jun and ATF2 increased significantly, while the expression of c-Fos decreased (Supporting Fig. 3A-C). The overexpression of c-Jun in Huh-7.5 cells induced Foxo3a and Socs3 expression at the protein and mRNA levels (Supporting Fig. 3D,E). In the liver of CH-C patients, there were significant correlations between the expression of Smad2 and c-Jun, and c-Jun and Foxo3a (Fig. 4B,C). ATF2 expression was significantly correlated with c-Jun expression (Fig. 4D). Similarly, there were significant correlations between the expression of Smad2 and ATF2, and ATF2 and Foxo3a (Fig. 4E,F). These results suggested that c-Jun and possibly ATF2, but not c-Fos, might be involved in TGF-β-Foxo3a signaling.

TGF-β Signaling Induces Socs3 Through the Induction of Foxo3a. Previously, we reported that Foxo3a increases the transcription of Socs3 through its binding to the Socs3 promoter region.[6] We confirmed

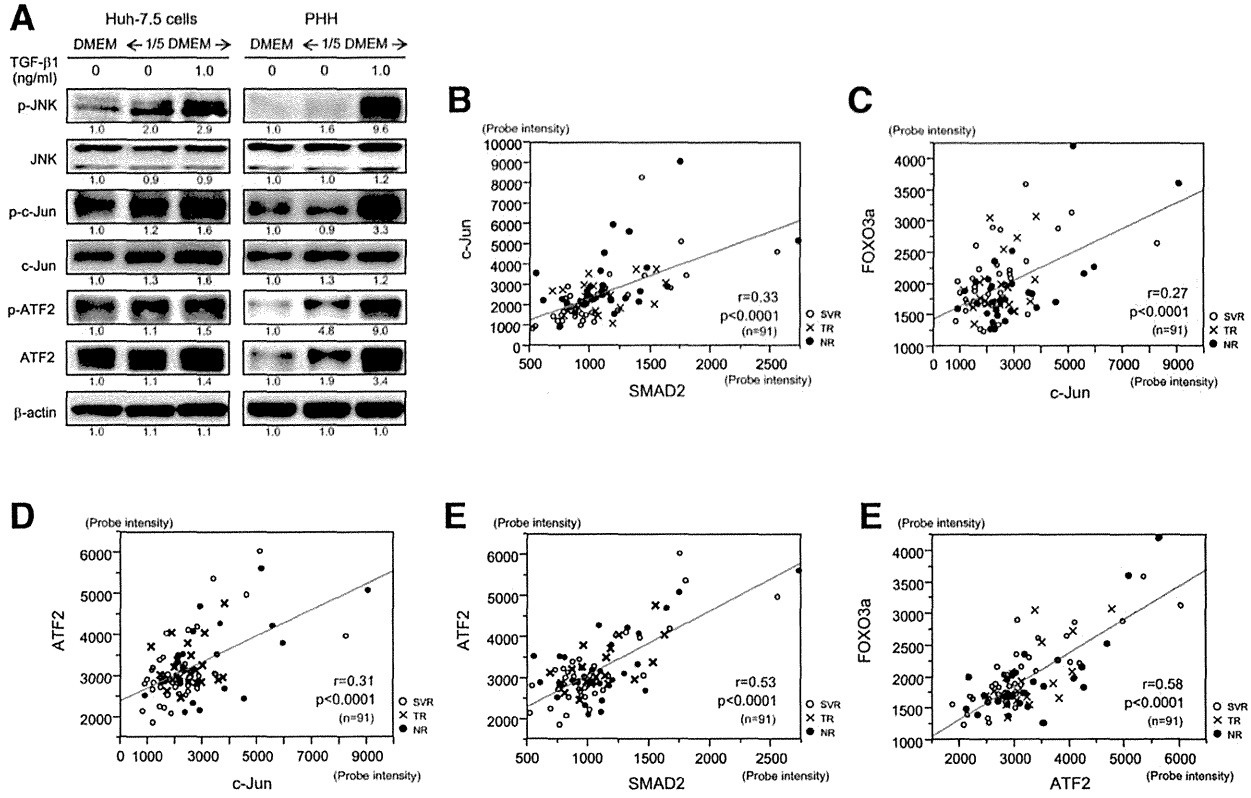

Fig. 4. TGF-β signaling up-regulates the expression of the transcription factors c-Jun and ATF2 in Huh-7.5 cells, PHH, and the liver of CH-C patients. A: Western blotting of JNK, c-Jun, and ATF2 in Huh-7.5 cells and PHH treated with amino acid depletion (1/5 DMEM) and TGF-β1. The experiments were repeated 3 times. B-F: Significant correlations of Smad2 and c-Jun (B), Foxo3a and c-Jun (C), c-Jun and ATF2 (D), Smad2 and ATF2 (E), and ATF2 and Foxo3a (F) expression in the liver of CH-C patients.

these findings in more detail in conjunction with TGF-β signaling. The overexpression of Foxo3a increased Socs3 expression in the nonamino acid-depleted condition (DMEM), and Socs3 was further induced in the amino acid-depleted condition (1/5 DMEM) and by TGF-β1 treatment (Supporting Fig. 4A). HCV-RNA was similarly increased in these conditions (Supporting Fig. 4B). Foxo3a mRNA expression, as deduced from RTD-PCR, was increased up to 7-fold in the combination of amino acid depletion (1/5 DMEM), c-Jun overexpression, and TGF-β1 treatment (Supporting Fig. 4C). Socs3 mRNA expression was up-regulated by 8-fold in the same conditions (Supporting Fig. 4D). The promoter activity of Socs3 was significantly increased by amino acid depletion (1/5 DMEM) and TGF-β1 treatment (pGL4-SOCS3-WT, Supporting Fig. 4E), while mutation of the Foxo3a binding site in the Socs3 promoter (pGL4-SOCS3-MT) abrogated this regulation. These results confirmed that TGF-β signaling up-regulated the expression of Socs3 through the induction of Foxo3a.

TGF-β Signaling Suppresses mTORC1 Signaling. Previously, we demonstrated that malnutrition decreased mTORC1 and IFN signaling using Huh-7 cells and clinical samples.[6] In the present study, we examined the effect of TGF-β signaling on mTORC1 signaling. In Huh-7.5 cells and PHH, amino acid depletion (1/5 DMEM) repressed mTORC1 signaling, as demonstrated by the decreased expression of ras homolog enriched in brain (RHEB),[11] a stimulator of mTORC1 signaling, p-mTOR, and p-p70S6K (Fig. 5A). Interestingly, TGF-β1 further decreased this expression. The decreased mTORC1 signaling was independent of AMP-activated, alpha 1 catalytic subunit (AMPK), a suppressor of mTORC1 signaling, as the levels of p-AMPK were rather decreased by amino acid depletion (1/5 DMEM) and TGF-β1 treatment in Huh-7.5 cells and PHH (Fig. 5A). It could be speculated that TGF-β signaling, combined with malnutrition, repressed the expression of RHEB and induced the expression of Foxo3a, which leads to the impaired IFN signaling observed in the advanced fibrosis stage of CH-C (Fig. 5B). In the liver of CH-C

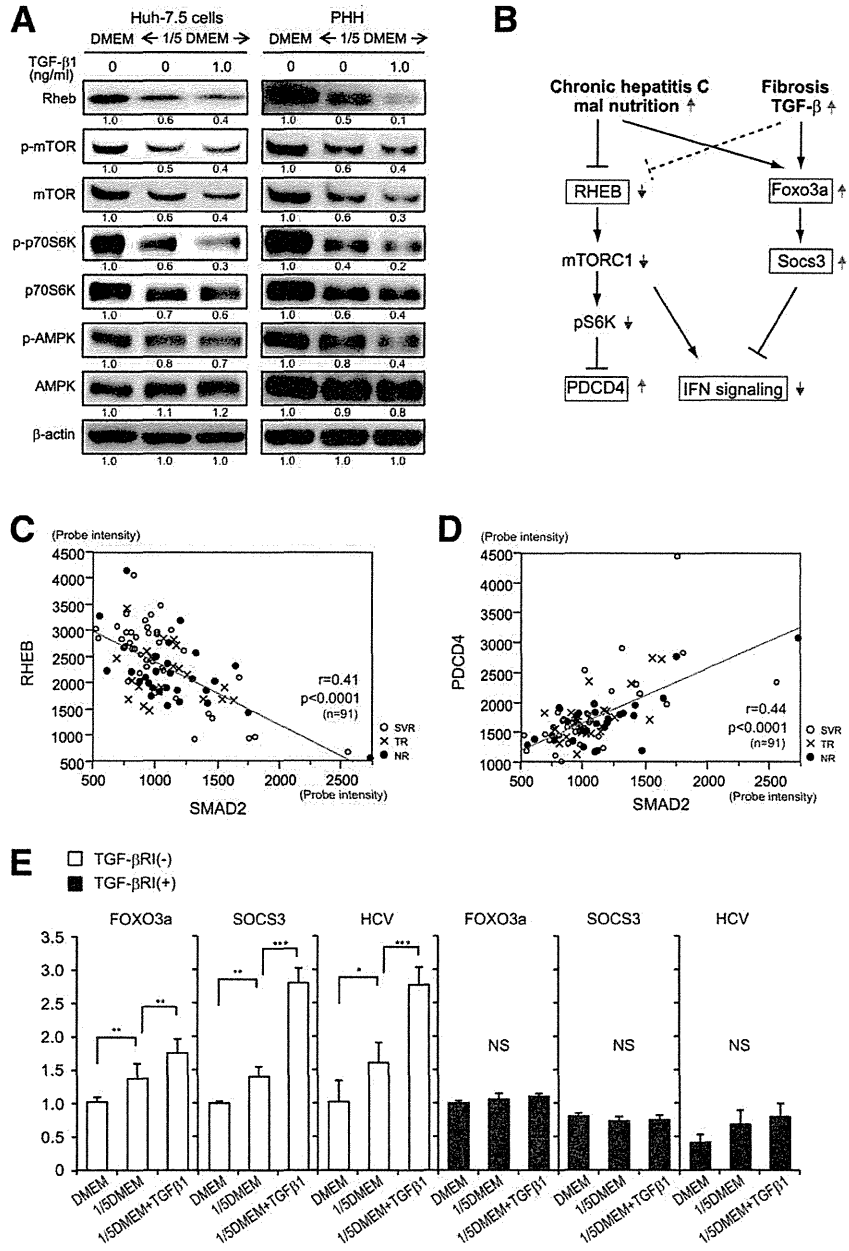

Fig. 5. TGF-β signaling represses mTORC1 signaling in Huh-7.5 cells, PHH, and the liver of CH-C patients. A: Western blotting of RHEB, mTOR, p70S8K, and AMPK in Huh-7.5 cells and PHH treated with amino acid depletion (1/5 DMEM) and TGF-β1. The experiments were repeated 3 times. B: Schematic representation of the effects of malnutrition and TGF-β signaling on IFN signaling. C,D: Significant correlations of Smad2 and RHEB (C), and Smad2 and PDCD4 (D) expression in the liver of CH-C patients. E: Blocking TGF-β signaling by TGF-β1 RI treatment abolishes the increase in Foxo3a, Socs3, and HCV replication by amino acid depletion (1/5 DMEM) and TGF-β1 treatment. The experiments were performed in triplicate and repeated 3 times ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

patients, Smad2 expression was significantly negatively correlated with RHEB expression. The expression of programmed cell death 4 (PDCD4), which is negatively regulated by mTORC1 signaling at the transcriptional level (Fig. 5C),[12] was significantly positively correlated with Smad2 expression (Fig. 5D).

We further examined the effect of TGF-β1 on IFN signaling by using TGF-β RI. TGF-β RI substantially repressed the levels of p-Smad2 and p-Smad3 (Supporting Fig. 5). TGF-β RI abolished the induction of Foxo3a expression and the subsequent induction of Socs3 by amino acid depletion (1/5 DMEM) and TGF-β1 treatment (Fig. 5E). HCV replication in normal medium (DMEM), as deduced from *Gaussia* luciferase activity, was repressed by TGF-β RI, and the increase in HCV replication by amino acid depletion (1/5 DMEM) and TGF-β1 treatment was abrogated (Fig. 5E).

c-Jun Is Up-Regulated in the Liver of NR and Treatment-Resistant IL28B Minor Genotype Patients. We evaluated the clinical significance of c-Jun for treatment response. The expression of c-Jun was significantly higher in NR patients than in responder patients (SVR+TR) (Fig. 6A). Furthermore, c-Jun expression was significantly higher in patients with the treatment-resistant IL28B minor genotype

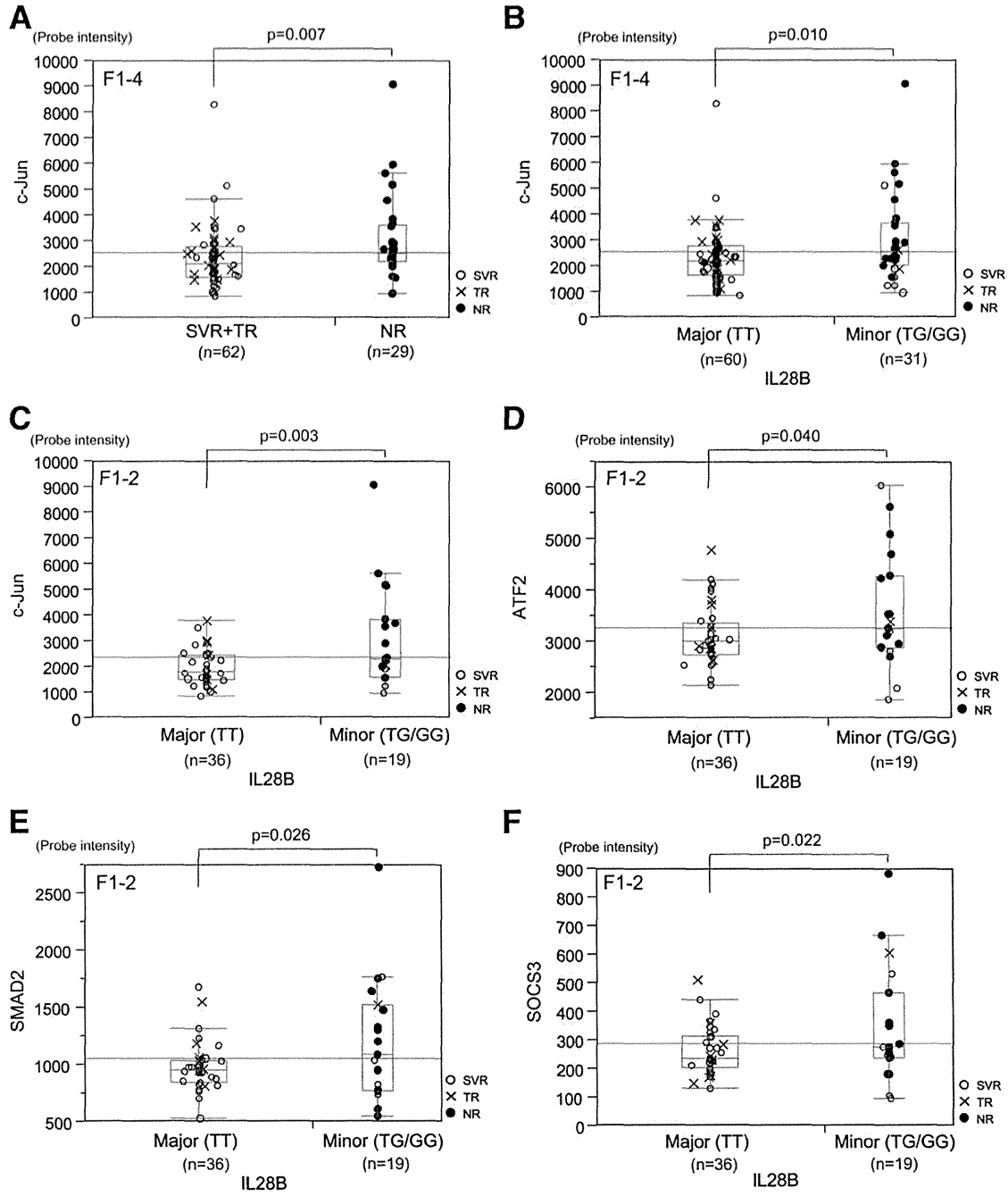

Fig. 6. Relationship between TGF-β signaling and treatment response and the IL28B genotype. The expression of c-Jun was up-regulated in NR (A) and IL28B minor genotype (TG/GG at rs8099917) (B) patients in all fibrosis stages (F1-4). The expression of ATF2 (D), Smad2 (E), and Socs3 (F) was up-regulated in IL28B minor genotype (TG/GG at rs8099917) patients at early fibrosis stages (F1-2).

(TG/GG at rs8099917) than in those with the treatment-sensitive IL28B major genotype (TT) (Fig. 6B).[5] Interestingly, TGF-β signaling was more activated in patients with the treatment-resistant IL28B minor genotype at an early stage of liver fibrosis (F1 and F2). The expression of c-Jun, ATF2, Smad2, and Socs3 was significantly higher in IL28B minor genotype patients (Fig. 6C-F).

BCAAs Inhibit TGF-β Signaling and Restore IFN Signaling. Previously, we reported that BCAAs restored IFN signaling in the amino acid-depleted condition (1/5 DMEM) by activating mTORC1 signaling and suppressing Foxo3a-Socs3 signaling.[6] In the present study, we examined whether BCAAs could inhibit TGF-β signaling and restore IFN signaling. Western