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ORIGINAL ARTICLE

Association of autoimmune hepatitis with *Src homology 2 adaptor protein 3* gene polymorphisms in Japanese patients

Takeji Umemura[1], Satoru Joshita[1], Hideaki Hamano[1,2], Kaname Yoshizawa[3], Shigeyuki Kawa[4], Eiji Tanaka[1] and Masao Ota[1,5]

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized by an autoimmune reaction to hepatocytes. The *Src homology 2 adaptor protein 3* (*SH2B3*) gene is a member of the SH2B family of adaptor proteins that has been implicated in the integration and regulation of multiple signaling events. SH2B3 is involved in cytokine signaling pathways and serves as a negative mediator of T-cell receptor signaling. Genome-wide association analyses in Caucasians have linked a missense mutation at rs3184504 in *SH2B3* with AIH. Accordingly, four selected single-nucleotide polymorphisms (SNPs) in the *SH2B3* gene were genotyped in 158 patients with AIH, 327 patients with primary biliary cholangitis, 160 patients with autoimmune pancreatitis, and 325 healthy subjects of Japanese descent. Although the functional rs3184504 was non-polymorphic in 952 subjects, the frequency of the minor rs11065904 T allele was significantly decreased in AIH patients compared with healthy controls (odds ratio (OR) = 0.68; corrected $P = 0.025$). Haplotype 2 (rs2238154 A, rs11065904 T and rs739496 G) was associated with resistance to AIH (OR 0.67, $P = 0.021$) as well as to autoimmune pancreatitis (OR = 0.70, $P = 0.035$). Our findings suggest that an SNP and haplotype in *SH2B3* are associated with AIH.
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INTRODUCTION

Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease characterized by an autoimmune reaction to hepatocytes and a favorable response to immunosuppressive treatment.[1–3] Although the pathogenesis of AIH has long been studied, a coherent explanation on the mechanisms involved in disease development and progression remains elusive. The HLA *DRB1*04:05-DQB1*04:01* haplotype was reported to be associated with AIH in Japanese populations,[4,5] but no non-HLA susceptibility loci identified using a genome-wide association study (GWAS) have been uncovered to date in Japan. A recent GWAS of Caucasian patients with type 1 AIH revealed *Src homology 2 adaptor protein 3* (*SH2B3*) as a disease susceptibility gene.[6] However, this association has not been validated in other ethnicities.

Located on chromosome 12q24, the *SH2B3* gene is a member of the SH2B family of adaptor proteins that has been implicated in the integration and regulation of multiple signaling events.[7] SH2B3 is involved in cytokine and Janus kinase 2 and 3 signaling pathways, functions as a negative regulator of T-cell activation and is required for normal hematopoiesis.[8] Recent genetic studies have associated *SH2B3* single-nucleotide polymorphisms (SNPs) with several autoimmune disorders in Caucasians, including Celiac disease, type 1 diabetes, primary sclerosing cholangitis and primary biliary cholangitis (PBC),[9–13] in which a missense SNP known as rs3184504 produced an R262W amino-acid substitution in the pleckstrin homology domain. This study investigated whether *SH2B3* SNPs were also associated with AIH in a Japanese population.

MATERIALS AND METHODS

Subjects

A total of 952 Japanese individuals participated in this study. One hundred fifty-eight patients with type 1 AIH were enrolled between January 2001 and August 2015. Their clinical and laboratory data at the time of diagnosis are summarized in Table 1. Our series also included 327 patients with PBC and 160 patients with autoimmune pancreatitis (AIP) as disease controls along with 325 volunteer healthy subjects. The diagnosis of type 1 AIH was determined based on the scoring system of the International Autoimmune Hepatitis Group.[14] The diagnoses of PBC and AIP were made according to criteria from the American Association for the Study of Liver Diseases[15] and the Japan Pancreas Society in 2006,[16] respectively. All patients were seronegative for the hepatitis B surface antigen anti-HBc and the anti-hepatitis C virus. AIH–PBC overlap syndromes were not included. This study was approved by the ethics committee of Shinshu University School of Medicine. Written informed consent was obtained from all subjects.

[1]Division of Hepatology and Gastroenterology, Department of Medicine, Shinshu University School of Medicine, Matsumoto, Japan; [2]Medical Informatics Division, Shinshu University Hospital, Matsumoto, Japan; [3]Department of Gastroenterology, NHO Shinshu Ueda Medical Center, Ueda, Japan; [4]Center for Health, Safety, and Environmental Management, Shinshu University, Matsumoto, Japan and [5]Department of Legal Medicine, Shinshu University School of Medicine, Matsumoto, Japan
Correspondence: Dr T Umemura, Division of Hepatology and Gastroenterology, Department of Medicine, Shinshu University School of Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan.
E-mail: tumemura@shinshu-u.ac.jp
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Table 1 Demographic and clinical characteristics of 158 patients with type 1 AIH

Clinical feature	
Age at diagnosis (years)	59 (51–66)
Female, *n* (%)	141 (89)
AST (12–37 IU l^{-1})	402 (144–823)
ALT (7–45 IU l^{-1})	420 (153–967)
Bilirubin (0.3–1.2 mg dl^{-1})	1.7 (0.9–6.4)
IgG (870–1700 mg dl^{-1})	2698 (2053–3466)
ANA ($<\times40$), *n* (%)	150 (95)
SMA ($<\times40$), *n* (%)	71/123 (58)

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, antinuclear antibody; AST, aspartate aminotransferase; IgG, immunoglobulin G; SMA, anti-smooth muscle antibody.
Values are expressed as median (interquartile range) unless otherwise noted.

Genotyping of SH2B3 SNPs

Genomic DNA was extracted from whole-blood samples of all subjects using QuickGene-800 assays (Fujifilm, Tokyo, Japan). The four SNPs in the *SH2B3* gene examined (rs3184504, rs2238154, rs11065904 and rs739496) were genotyped using a TaqMan 5′ exonuclease assay (Applied Biosystems, Tokyo, Japan). The rs3184504 SNP has been associated with various autoimmune diseases, while the other SNPs were selected from a previous report[9] and had minor allele frequencies of $>5\%$. The PCR was performed with the StepOne Plus Real-Time PCR System (Applied Biosystems) following the manufacturer's instructions.

Statistical analysis

Allele, genotype and haplotype frequencies along with Hardy–Weinberg equilibrium and linkage disequilibrium (LD) were assessed using the SNPStats software (Catalan Institute Oncology, Barcelona, Spain; http://bioinfo.iconcologia.net/SNPstats).[17] Akaike's information criterion was used to determine the most suitable inheritance model.[18] The significance of an association was evaluated using chi-square analysis or Fisher's exact test. *P*-values were subjected to Bonferroni's correction by multiplication by the number of different alleles observed in each locus (*Pc*). Mann–Whitney *U*-test was used to analyze continuous variables. A two-sided *P*-value of <0.05 was considered to be statistically significant. Association strength was estimated by calculating the odds ratio (OR) and 95% confidence interval (CI). Genetic power was calculated using the EZR program of R commander software.[19] This study had 80% statistical power to detect associations when the genotype relative risk was >1.20.[20]

RESULTS

The functional rs3184504 SNP was not polymorphic in 952 subjects, which was in agreement with established HapMap data of Japanese populations, and therefore excluded from further analyses. Hardy–Weinberg equilibrium was observed for the remaining three SNPs in patients with AIH, PBC or AIP and in controls (Table 2). The frequency of the minor T allele at rs11065904 was significantly lower in AIH patients than in healthy subjects (OR = 0.68, 95% CI: 0.51–0.91; *Pc* = 0.025; Table 2). The frequency of the TA or AA genotypes of rs11065904 also differed significantly between AIH patients and controls (OR = 0.37, 95% CI: 0.20–0.70; *Pc* = 0.0027; Table 3). There were no significant differences observed for rs2238154 or rs739496 with AIH. None of the three SNPs examined were associated with PBC or AIP.

Pairwise LD mapping confirmed that the three tested alleles were in strong LD over a narrow range, with an LD index of >0.9. Strong LD was indicated in the same block for AIH, PBC and AIP patients and controls (Figure 1). Six SNP haplotypes were found, of which three had a frequency of $>5\%$ as determined by expectation-maximization algorithms (Table 4). Haplotype 2 (ATG) was significantly associated with AIH resistance (OR = 0.67, 95% CI: 0.48–0.94; *P* = 0.013) and AIP resistance (OR = 0.70, 95% CI 0.50–0.97; *P* = 0.035). No other haplotypes were associated with either susceptibility or resistance to AIH, PBC or AIP.

As HLA *DRB1*04:05-DQB1*04:01* has been linked to AIH susceptibility,[5] the genetic association between this haplotype and rs11065904 was assessed. Analysis of allelic frequencies revealed no significant difference between patients with or without the *DRB1*04:05-DQB1*04:01* haplotype for rs11065904 (*P* = 0.385). Moreover, no remarkable differences were found for the clinical parameters of aspartate aminotransferase, alanine aminotransferase, bilirubin, immunoglobulin G, gender, elevated antinuclear antibody or anti-smooth muscle antigen compared with AIH patients under the recessive model of the T allele for rs11065904 (Table 5).

DISCUSSION

Several GWAS have associated an rs3184504 missense substitution SNP on the *SH2B3* gene with AIH, PBC and primary sclerosing cholangitis in the populations of European descent.[6,12,13] Expression quantitative trait locus analyses established the risk allele to be linked to the increased expression of several genes involved in interferon-gamma responses.[9] Hence, rs3184504 variants may lead to an increased adaptive immune response.[21] Our findings revealed that this critical SNP was non-polymorphic in 952 Japanese subjects, suggesting that rs3184504 was not related with autoimmune liver disease or AIP.

Our data uncovered a striking association between AIH protection and rs11065904 in the *SH2B3* gene. We observed that *SH2B3* haplotypes might also be important determinants of AIH protection as the haplotype containing the rs11065904 T allele was significantly associated with a 0.37 times less likelihood of developing AIH. Thus our findings suggest that rs11065904 may be involved in resistance to AIH, although it is possible that the *SH2B3* locus contains another, undefined functional variant in LD with rs3184504. SH2B3 regulates T-cell receptor, growth factor and cytokine receptor-mediated signaling.[22,23] Moreover, *Sh2b3*$^{-/-}$ mice exhibited increased responses to several cytokines.[23] As multiple cytokines, especially interleukin (IL)-18 and IL-21, have been associated with the pathogenesis of AIH in humans[24] and mice models,[25,26] SH2B3 might have a key involvement in one or more of these signal cascades. PBC and AIP in humans have been correlated with IL-8 and IL-4/IL-5/IL-13, respectively.[27,28] SH2B3 might therefore exert different functions in these diseases.

Unexpectedly, haplotype 2 (ATG) of *SH2B3* was also significantly associated with AIP resistance. We have reported SNPs in several genes to be linked to AIP,[29–31] but no GWAS data are available to date. The present study suggested that this haplotype may be an important factor in AIP protection.

We recently identified a significant association between AIH and SNPs in the *PTPN22* gene, which has been linked to several autoimmune disorders in Caucasians. Although an important missense SNP in the *PTPN22* gene was monomorphic in the Japanese, other *PTPN22* SNPs and haplotypes were associated with resistance to AIH.[32] Moreover, susceptibility among HLA genes and other SNPs in Japanese AIH, PBC and AIP patients was different from that in other populations.[4,5,29,30,33–35] We have yet to identify any association of PBC with *SH2B3* SNPs or haplotypes. An earlier GWAS of Japanese PBC patients demonstrated no link between *SH2B3* and PBC susceptibility,[36,37] which was supported by our data.

Table 2 Allele frequencies of SNPs in the *SH2B3* gene in AIH, PBC and AIP patients and in healthy controls

dbSNP	*Position (bp)*	*MA*	*Controls* (n = *325*)		*AIH* (n = *158*)		*PBC* (n = *327*)		*AIP* (n = *160*)		*vs AIH*		*vs PBC*	*vs AIP*
			MAF n *(%)*	*HWE* P-*value*	*MAF* n *(%)*	*HWE* P-*value*	*MAF* n *(%)*	*HWE* P-*value*	*MAF* n *(%)*	*HWE* P-*value*	P-*value*	*OR (95% CI)*	P-*value*	P-*value*
rs2238154	110366868	C	110 (17%)	1.00	44 (14%)	0.83	111 (17%)	0.79	61 (19%)	0.29	0.22	0.79 (0.54–1.16)	0.98	0.40
rs11065904	110368991	T	281 (43%)	0.67	109 (34%)	0.69	265 (41%)	0.29	124 (39%)	0.49	**0.0082**	**0.68 (0.51 – 0.91)**	0.33	0.19
rs739496	110372042	A	110 (17%)	1.00	44 (14%)	0.83	109 (17%)	0.72	59 (18%)	0.29	0.22	0.79 (0.54 – 1.16)	0.90	0.55

Abbreviations: AIH, autoimmune hepatitis; AIP, autoimmune pancreatitis; CI, confidence interval; HWE, Hardy–Weinberg equilibrium; MA, minor allele; MAF, minor allele frequency; OR, odds ratio; PBC, primary biliary cholangitis; SNP, single-nucleotide polymorphisms. Bolded figures indicate statistical significance.

Table 3 Genotype distribution of *SH2B3* gene polymorphisms in patients with AIH, PBC or AIP and in healthy controls

SNP	*Alleles (1>2)*	*Genotype*	*Genotype frequency, %*				*Controls vs AIH*		*Controls vs PBC*		*Controls vs AIP*	
			Controls (n = *325*)	*AIH* (n = *158*)	*PBC* (n = *327*)	*AIP* (n = *158*)	*P-value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*
rs2238154	A>C	AA/AC/CC	68.9/28.3/2.8	73.4/25.3/1.3	69.4/27.2/3.4	63.1/35.6/1.2	0.27	0.45 (0.10–2.11)	0.66	1.22 (0.50–2.99)	0.27	0.44 (0.09–2.08)
rs11065904	A>T	AA/AT/TT	32.9/47.7/19.4	39.2/52.5/8.2	37.0/45.0/18.0	38.1/46.2/15.6	**0.0009**	**0.37 (0.20–0.70)**	0.66	0.92 (0.62–1.36)	0.31	0.77 (0.46–1.28)
rs739496	G>T	GG/GA/AA	68.9/28.3/2.8	73.4/25.3/1.3	70.0/26.6/3.4	64.4/34.4/1.2	0.27	0.45 (0.10–2.11)	0.66	1.22 (0.50–2.99)	0.27	0.44 (0.09–2.08)

Abbreviations: AIH, autoimmune hepatitis; AIP, autoimmune pancreatitis; CI, confidence interval; OR, odds ratio; PBC, primary biliary cholangitis; SNP, single nucleotide polymorphisms; 1, major allele; 2, minor allele.
The model with the smallest Akaike's information criterion value was defined as the most appropriate model for each SNP.
All data were analyzed by the recessive model (11+12 vs 22). Bolded figures indicate statistical significance.

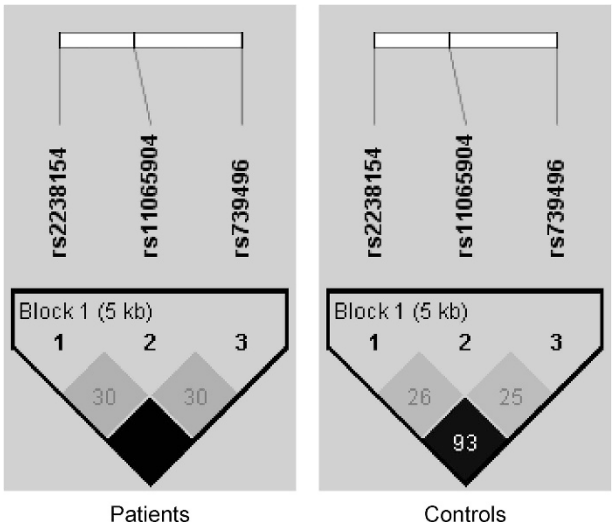

Figure 1 Linkage disequilibrium plot of three single-nucleotide polymorphisms (SNPs) of the *SH2B3* gene in 158 patients with autoimmune hepatitis and 358 healthy controls. Values of r^2 corresponding to each SNP pair are expressed as a percentage and shown within the respective squares.

Although HLA-DRB1*04:05-DQB1*04:01 and rs11065904 in the *SH2B3* gene were associated with AIH, no gene–gene interactions were detected in this study. As the *HLA-DR* and *SH2B3* genes are located on different chromosomes, there is no genetic linkage between them. We previously reported that the HLA-DRB1*04:05-DQB1*04:01 haplotype was related to higher immunoglobulin G levels and a high frequency of anti-smooth muscle antibody positivity in AIH patients.[5] However, rs11065904 was not significantly associated with clinical background or clinical parameters in this investigation.

Variation in gene expression levels is one of the major factors causing phenotypic variation and disease susceptibility. A GWAS demonstrated that the majority of disease-associated SNPs lay outside protein-coding regions and were presumably regulating gene expression as a expression quantitative trait locus (eQTL).[38] The nonsynonymous rs3184504 T allele located in an exon of *SH2B3* exhibited an association with increased expression of SH2B3 as a *cis*-eQTL effect as well as decreased or increased expression in 14 unique genes as a *trans*-eQTL effect.[21] However, rs3184504 is non-polymorphic in Japanese populations and seems not to affect functional gene expression. The AIH risk SNP rs11065904 located in a 3′-untranslated region of SH2B3 is located 2.36 kb downstream from rs3184504 and is in strong LD. According to the East-Asian eQTL database (http://www.genome.med.kyoto-u.ac.jp/SnpDB/), rs11065904 located near rs3184504 might induce eQTL effects (*trans*-eQTL: $P = 3.9 \times 10^{-9}$, *cis*-eQTL: $P = 4.9 \times 10^{-3}$).[39]

The limitations of this study are a small sample size and a narrow focus involving few SNPs. Thus the possibility of type I error cannot be excluded. Additional investigation is needed to validate these newly discovered associations in a larger number of individuals as AIH is rare in Japan, with a prevalence of 15.0 per 100 000 people.[40] However, power calculations based on the study subjects of 158 AIH patients and 325 controls and an OR of 0.68 at rs11065904 demonstrated sufficient genetic power (0.91) at the 0.05 level of significance.

In conclusion, our data implicated that an *SH2B3* SNP and haplotype contributed to AIH protection in the Japanese population. Further studies are needed to clarify the pathogenesis in AIH.

Table 4 Estimated haplotype frequencies of *SH2B3* gene polymorphisms in patients with AIH, PBC, or AIP and in healthy controls

	rs2238154	*rs11065904*	*rs739496*	*Frequency, %*				*Controls vs AIH*		*Controls vs PBC*		*Controls vs AIP*	
				Controls (2n = 650)	*AIH (2n = 316)*	*PBC (2n = 654)*	*AIP (2n = 320)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*	P-*value*	*OR (95% CI)*
1	A	A	G	56.5	65.5	59.2	61.3	—	1	—	1	—	1
2	A	T	G	26.1	20.6	23.8	19.7	**0.021**	**0.67 (0.48–0.94)**	0.26	0.86 (0.67–1.12)	**0.035**	**0.70 (0.50–0.97)**
3	C	T	A	16.5	13.9	16.7	18.4	0.13	0.73 (0.49–1.09)	0.86	0.97 (0.72–1.31)	0.75	1.06 (0.73–1.54)
4	C	T	G	0.5	NA	0.0	0.6			0.20	0.31 (0.0.5–1.82)	0.53	0.59 (0.12–3.00)
5	A	A	A	0.3	NA	NA	NA						
6	A	T	A	0.2	NA	NA	NA						

Abbreviations: AIH, autoimmune hepatitis; AIP, autoimmune pancreatitis; CI, confidence interval; NA, not applicable; OR, odds ratio; PBC, primary biliary cholangitis. Bolded figures indicate statistical significance.

Table 5 Comparisons of demographic and clinical characteristics regarding rs11065904 in 158 patients with type 1 AIH

Clinical feature	*rs11065904 A/A+A/T* (n = *145*)	*rs11065904 T/T* (n = *13*)	P-*value*
Age at diagnosis (years)	59 (50–66)	63 (52–69)	0.360
Female, *n* (%)	128 (88)	13 (100)	0.401
AST (12–37 IU l^{-1})	405 (135–802)	256 (154–859)	0.649
ALT (7–45 IU l^{-1})	423 (150–984)	292 (196–746)	0.531
Bilirubin (0.3–1.2 mg dl^{-1})	1.7 (0.9–6.3)	1.9 (0.9–6.2)	0.721
IgG (870–1700 mg dl^{-1})	2690 (2042–3444)	3313 (2320–4406)	0.195
ANA ($< \times 40$), *n* (%)	138 (9)	12 (92)	0.834
SMA ($< \times 40$), *n* (%)	66/114 (57)	5/9 (56)	0.831

Abbreviations: AIH, autoimmune hepatitis; ALT, alanine aminotransferase; ANA, anti-nuclear antibody; AST, aspartate aminotransferase; IgG, immunoglobulin G; SMA, anti-smooth muscle antibody.
Values are expressed as median (interquartile range) unless otherwise noted.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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