A genome-wide association study identifying SVEP1 variant as a predictor of response to tolvaptan for cirrhotic ascites

Hideto Kawaratani1 | Hiromi Sawai2 | Masaya Onishi3 | Tomomi Kogiso4 | Noritomo Shimada5 | Haruki Uojima6 | Tomoaki Nakajima7 | Naoki Matsumoto8 | Kenichi Ikejima9 | Toru Ishikawa10 | Shuji Tera11 | Hiroyuki Motoyama12 | Atsumasa Komori13 | Noboru Hirashima14 | Satoru Saito15 | Yuichiro Eguchi16 | Masanori Nojima17 | Yosuke Kawai18 | Masakuni Tateyama19 | Hitoshi Yoshiji1 | Yasuhiro Tanaka3,19

1Department of Gastroenterology, Nara Medical University, Nara, Japan
2Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
3Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan
4Institute of Gastroenterology, Department of Internal Medicine, Tokyo Women's Medical University, Tokyo, Japan
5Division of Gastroenterology and Hepatology, Ootakanomori Hospital, Kashiwa, Japan
6Department of Gastroenterology, Internal Medicine, Kitasato University School of Medicine, Sagamihara, Japan
7Department of Hepatology, Sapporo Kosei General Hospital, Hokkaido, Japan
8Division of Gastroenterology and Hepatology, Department of Medicine, Nihon University School of Medicine, Tokyo, Japan
9Department of Gastroenterology, Juntendo University Graduate School of Medicine, Tokyo, Japan
10Department of Gastroenterology, Saiseikai Niigata Hospital, Niigata, Japan
11Division of Gastroenterology and Hepatology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan
12Department of Gastroenterology, Graduate School of Medicine, Osaka City University Osaka, Japan
13Clinical Research Center, Nagasaki Medical Center, Nagasaki, Japan
14Department of Gastroenterology, National Hospital Organization, Nagoya Medical Center, Nagoya, Japan
15Department of Gastroenterology, Yokohama City University Graduate School of Medicine, Yokohama, Japan
16Liver Center, Saga University Hospital, Saga, Japan
17Center for Translational Research, The Institute of Medical Science, the University of Tokyo, Tokyo, Japan
18Genome Medical Science Project (Toyama), National Center for Global Health and Medicine, Tokyo, Japan
19Department of Gastroenterology and Hepatology, Kumamoto University, Kumamoto, Japan

Correspondence
Yasuhiro Tanaka, Department of Gastroenterology and Hepatology, Faculty of Life Sciences, Kumamoto University, Chuo-ku, Kumamoto, Japan.
Email: ytanaka@kumamoto-u.ac.jp

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Abstract
Background & Aims: Tolvaptan, vasopressin V2-receptor antagonist, has been used for patients with difficult-to-treat ascites in Japan. In this study, we conducted a genome-wide association study (GWAS) in the Japanese population to identify genetic variants associated with tolvaptan's efficacy for patients with hepatic ascites.

Abbreviations: AFP, alpha fetoprotein; AIH, autoimmune hepatitis; Alb, albumin; ALT, alanine aminotransferase; ANGPT2, angiopoietin-2; AQP, aquaporin; AUC, area under the curve; BUN, blood urea nitrogen; BW, body weight; CI, confidence interval; Cre, creatine; EGF, epidermal growth factor; FOXC2, Forehead box protein C2; GWAS, genome-wide association study; IFN, interferon; ITGA9, integrin alpha 9; Na, sodium; NASH, nonalcoholic steatohepatitis; NH3, ammonia; NPV, negative predictive value; OR, odds ratio; PBC, primary biliary cholangitis; PPV, positive predictive value; PT, prothrombin time; ROC, Receiver operating characteristics; T-bil, total bilirubin; RBV, ribavirin.

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Methods: From 2014 through 2018, genomic DNA samples were obtained from 550 patients who were treated with tolvaptan. Of those, 80 cases (non-responder; increase of body weight [BW]) and 333 controls (responder; >1.5 kg decrease of BW) were included in the GWAS and replication study.

Results: Genome-wide association study showed 5 candidate SNPs around the mir818, KIAA11109, and SVEP1 genes. After validation and performing a replication study, an SNP (rs2991364) located in the SVEP1 gene was found to have a significant genome-wide association (OR = 3.55, \( P = 2.01 \times 10^{-6} \)). Multivariate analyses showed that serum sodium (Na), blood urea nitrogen (BUN) and SVEP1 SNP were significantly associated with the response (OR = 0.92, \( P = .003 \); OR = 1.02, \( P = .02 \) and OR = 3.98, \( P = .000008 \), respectively). Based on a prediction model of logistic regression analysis in a population with the rs2991364 risk allele, the failure probability (\( \text{exp} \left( \text{score} : 22.234 + \text{BUN}^*0.077 + \text{Na}^-*0.179 \right) \left( 1 + \text{exp} \left( \text{score} \right) \right) \) was determined for the detection of non-responders. Assuming a cutoff of failure probability at 38.6%, sensitivity was 84.4%, specificity was 70% and AUC was 0.774.

Conclusion: SVEP1 rs2991364 was identified as the specific SNP for the tolvaptan response. The prediction score (>38.6%) can identify tolvaptan non-responders and help to avoid a lengthy period of futile treatment.

Keywords: blood urine nitrogen (BUN), genome-wide association study (GWAS), hepatic ascites, liver cirrhosis, non-responder, Polydom

1 | INTRODUCTION

Hepatic ascites, the accumulation of fluid in the abdominal cavity due to liver disease, is a difficult condition to treat. In European guidelines, high-dose diuretics (160 mg furosemide, spironolactone 400 mg) are used for uncontrolled hepatic ascites.\(^1\) However, Japanese patients are usually intolerant to high-dose conventional diuretics (furosemide and spironolactone) because of dehydration or hyponatremia. Since December 2013, tolvaptan, an orally active vasopressin V2-receptor antagonist, has been used in Japan for patients with ascites that are difficult to treat with conventional diuretics.\(^2\) Tolvaptan suppresses the expression of aquaporin (AQP)-2 and inhibits water reabsorption in the renal collecting ducts. Tolvaptan does not stimulate sodium channels, unlike other diuretics, and increases free water excretion without affecting urinary sodium levels.\(^3\) Japanese guidelines recommend administering 20-80mg furosemide and/or 25-100 mg spironolactone\(^4\) for mild ascites. When ascites is uncontrolled by conventional diuretics, tolvaptan is administered at a dose of 3.75 or 7.5 mg. Tolvaptan was first reported to increase serum sodium levels safely and effectively in patients with euolemic and hyponatremic hyponatremia in a study that explored ascending doses of tolvaptan (SALT-1 and SALT-2 studies) in 2006.\(^5\) Other studies have also demonstrated the efficacy and safety of tolvaptan.\(^6\) In a Japanese multicenter retrospective study, responders to tolvaptan were defined as patients who had body weight (BW) loss of 1.5 kg/week which reflect the improvement of ascites volume and symptoms.\(^7\) Since this paper was published, BW loss of 1.5 kg/week criterion has been applied to determine the efficacy of tolvaptan. About two-thirds of patients showed an extreme increase in urine volume and/or decrease in BW. Conversely, roughly one-third of patients showed no increase in urine volume and/or decrease in BW. Several studies showed that low serum sodium,\(^8\) high blood urine nitrogen (BUN),\(^9\) or high urine osmolality\(^10\) can worsen the treatment response, and that creatine (Cre) does not necessarily influence the response to tolvaptan.

The genome-wide association study (GWAS) method has been used to predict the treatment response, such in the analysis of IL28B variants strongly associated with the response to pegylated-interferon (IFN) plus ribavirin (RBV) therapy for chronic hepatitis C patients.\(^11\) We hypothesized that the response to tolvaptan differs based on clinical characteristics and host genetics. The reason for the difference in the effect of tolvaptan with respect to clinical characteristics has been discussed previously.\(^5\) In this study, to determine whether to extend administration to tolvaptan non-responders in the hope that they will eventually respond, we conducted a GWAS in the Japanese population to identify genetic variants associated with tolvaptan’s efficacy in patients with difficult-to-treat ascites, and to identify non-responders to tolvaptan.


2 | MATERIAL AND METHODS

2.1 | Patients

This study was conducted nationwide in Japan from 2014 through 2018. The protocol was registered to the clinical trials registry managed by the University Hospital Medical Information Network in Japan (registration no. UMIN000025905). Genomic DNA samples were obtained before or after tolvaptan administration from 550 patients who had been treated with tolvaptan add on to conventional diuretics (20-80 mg/day furosemide and/or 25-100 mg/day spironolactone) for hepatic ascites at each of the participating hospitals (40 hospital liver units with hepatologists). As per previous reports,7 responders to tolvaptan were defined as those who had a greater than 1.5-kg decrease in BW after 1-week tolvaptan treatment; and non-responders, as those with an increase in BW after 1-week tolvaptan treatment. A total of 80 cases (non-responders) and 333 controls (responders) were included in the GWAS and replication study, and 137 borderline patients whose BW change after 1-week tolvaptan treatment was 0 kg to −1.49 kg, were excluded from the study. To identify genetic variants associated with the response to tolvaptan in patients with cirrhotic ascites, we performed a cohort GWAS recruiting 181 patients (25 cases and 156 controls) from 2014 to 2016 for SNP candidates with $P < 10^{-4}$. A replication study recruiting 232 patients (55 cases and 177 controls) from 2017 to 2018 was also performed.

2.2 | Evaluation of GWAS

Genomic DNA was extracted from peripheral blood leukocytes using a standard method. In the GWAS stage, we genotyped 253 patients using the Affymetrix Axiom Genome-Wide AS1 1 Array (Thermo Fisher Scientific) according to the manufacturer’s instructions, and determined the genotype calls of 600,307 SNPs using Genotype Console v4.2.0.26 software. All samples used for genotyping passed a Dish QC $>0.82$ and we excluded 1 sample with an overall call rate $<97%$. We recalled the remaining 252 samples with Genotyping Console software. The average Dish QC for 252 samples was 0.953 (0.877-0.988) and the average call rate reached 98.87 (97.02-99.86). All genotyped samples passed a heterozygosity check and identity by descent testing. A principal component analysis found two outliers that could be excluded by the Smirnov-Grubbs test, and we showed that all the remaining samples formed a single cluster with the HapMap Japanese (JPT) samples but not with the Han Chinese (CHB), Northern and Western European (CEU), and Yoruba (YRI) samples. We then applied the following thresholds for SNP quality control in data cleaning: SNP call rate $>95\%$, minor allele frequency (MAF) $>5\%$, and Hardy-Weinberg equilibrium (HWE) $P$ value $>0.001$. A total of 411,709 SNPs on autosomal chromosomes passed the quality control filters and were used for subsequent GWAS.

2.3 | Statistical analysis

To assess risk factors associated with tolvaptan response, univariate and multivariate logistic regression was performed. Factors with $P < .1$ in univariate analysis were included in multivariate analysis with backward elimination ($P < .05$). Receiver operating characteristics (ROC) analysis was used to assess the prediction ability of the model. All analyses were carried out using SPSS for Windows version 25.0 (IBM, Armonk, NY, USA). The prediction model for the risk allele group was constructed based on a logistic regression model in which backward elimination ($P < .05$) was applied after including age, sex, height, weight, Child-Pugh classification, presence of HCC, platelet, PT activity, Alb, AST, ALT, T-bil, BUN, Cre, Na, NH3 and aetiology (HBV, HCV, alcohol, NASH, AIH, PBC and others). For these factors, missing rate was less than five percent and frequency in categorical factors was less biased (proportion was more than 10% even in the lesser category). The prediction score was then calculated using coefficients of selected factors based on the definition of the logistic regression.

In the GWAS (including genome-wide imputation data) and replication study, the chi-square test was applied to a 2-by-2 contingency table in the allele frequency model. The odds ratio (OR) and the confidence interval (CI) were calculated using the major alleles as references. We considered $P < 5 \times 10^{-8}$ as the threshold for genome-wide significance in the combined analysis.

In the replication stage, we selected 134 SNPs with $P$ values $<10^{-5}$ and linkage disequilibrium (LD) $<0.9$ from the results of the chi-square test in the GWAS using genome-wide imputed data. We additionally selected 49 SNPs located on the functionally interested genes. DigiTag2 assay and TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA, USA) were used to confirm the genotypes at each SNP. We genotyped 80 cases and 333 controls to validate the GWAS results and for the replication study.

3 | RESULTS

3.1 | Baseline characteristics of enrolled patients

A total of 413 patients were analysed in this study. The baseline clinical characteristics of the patients was 269 men and 144 women. The mean age was 67.2 years (range: 37-97 years). Aetiologies of the patients were viral hepatitis B in 38 patients, viral hepatitis C in 147 patients, heavy alcohol consumption in 126 patients, NASH in 50 patients, AIH in 9 patients, PBC in 24 patients and other causes in 28 patients. A total of 171 patients (41.4\%) had hepatoma. The baseline Child Pugh classification was A in 5 patients, B in 193 patients and C in 215 patients. Adverse events occurred in 86 patients (20.8\%). The events included thirst in 47 patients (54.7\%), hepatic encephalopathy in 21 patients (24.4\%), dehydration in 9 patients (10.5\%) and other (hypernatremia, muscle cramps and eruption etc) in 14 patients (16.3\%). There were 333 (80.6\%) patients who were responders to tolvaptan and 80 patients (19.4\%) who were non-responders.
Table 1 showed the comparison of clinical characteristics by the tolvaptan’s response. Comparing between 80 non-responders and 333 responders, there were statistical differences in BUN (P = .003) and Na (P = .03).

3.2 | Genetic variants associated with response to tolvaptan

We conducted a GWAS using 25 cases (non-responders) and 156 controls (responders) by analysing 411,709 autosomal SNPs. Figure S1 shows a genome-wide view of the SNP association data based on allele frequencies. There were 80 SNPs with P values <10^{-4} in the GWAS. Of the 80 SNPs, 20 and 5 SNPs showed P values <10^{-5} and <10^{-6} respectively. The 5 candidate SNPs were identified around miR818 (rs16827413: P = 5.97 × 10^{-7} and rs10800602: P = 8.69 × 10^{-8} located on chromosome 1 q32.1) and KIAA1109 (rs3108399: P = 5.84 × 10^{-6} on chromosome 4 q27) and SVEP1 (rs9299186: P = 7.46 × 10^{-7} and rs4978937: P = 7.46 × 10^{-7} on chromosome 9 q31.3).

3.3 | Imputation-based GWAS and replication study

We performed genome-wide imputation-based GWAS in order to find additional candidate SNPs associated with the response to tolvaptan (Figure S2). There were 2,127 SNPs with P values <10^{-4} in the imputation-based GWAS, and 497 SNPs with P values <10^{-5}. Of the 497 SNPs, 134, 102 and 87 SNPs showed LD <0.9, <0.7 and <0.5 respectively. For validation and replication, we selected 192 SNPs including 134 SNPs with P values <10^{-5} and LD <0.9, 9 SNPs on AXIOM ASI1 array, and 49 SNPs located on the functionally interested genes. The original GWAS set of 181 samples (25 cases and 156 controls) and an independent set of patients (55 cases and 177 controls) were genotyped and used in a subsequent replication analysis. Of the candidate SNPs, three (rs2991364, rs9299186 and rs4978937 on SVEP1 intron region) were validated and consistent associations were observed between the GWAS set and replication set (Table 2). One SNP showed a genome-wide significant association (rs2991364: OR = 3.55, P = 2.01 × 10^{-8}) using the combined set (80 cases and 333 controls) (Table 2).

3.4 | Risk factors for response to tolvaptan

The results obtained by multivariate logistic regression analysis are shown in Table 3. Univariate analysis showed that AFP, BUN, Cre, Na and SVEP1 SNPs (rs2991364) were significantly associated with the response to tolvaptan, and multivariate analysis showed that BUN, Na and SVEP1 SNPs were also associated with the response to tolvaptan (OR = 1.02, P = .02, OR = 0.92, P = .003 and OR = 3.98, P < .00001 respectively). Table S1 shows that the highest sum of sensitivity and specificity of Na was 0.44 and 0.73 respectively. Using the best cutoff value of Na (134.0), the area under the curve (AUC) of Na was 0.64, and 95%CI was 0.58-0.65. The highest sum of sensitivity and specificity of BUN was 0.51 and 0.71 respectively. Using the best cutoff value of BUN (22.4), the AUC of BUN was 0.64, and the 95%CI was 0.57-0.70. The highest sum of sensitivity and specificity of SVEP1 SNP was 0.43 and 0.85 respectively. The AUC of SVEP1 was 0.64, and 95%CI was 0.58-0.69. And, the highest sum of sensitivity and specificity of combination of Na, BUN and SVEP1 SNP was 0.40 and 0.94 respectively. The AUC for the

| TABLE 1 | Comparison of clinical characteristics by the tolvaptan’s response |
|-----------------|-----------------|-----------------|-----------------|
| Variables       | Non-responder(n=80) | Responder(n=333) | P-value         |
| Age (years)     | 66.8 ± 12.4      | 67.3 ± 11.4      | 0.75            |
| Sex (male/female) | 55 / 25         | 214 / 119        | 0.46            |
| Height (cm)     | 161.7 ± 9.7      | 161.0 ± 9.3      | 0.58            |
| Body weight (kg) | 63.3 ± 14.9      | 64.8 ± 14.9      | 0.44            |
| Etiology (B/C/Alc/NASH/AIH/PBC/others) | 10/26/24/12/3/2/7 | 28/121/102/38/6/22/2/1 | 0.27 |
| Presence of HCC (yes / no) | 38 / 42       | 133 / 200        | 0.23            |
| Child-Pugh (A / B / C) | 0 / 37 / 43 | 5 / 156 /172      | 0.57            |
| Serum albumin (g/dL) | 2.6 ± 0.5     | 2.7 ± 0.5        | 0.37            |
| Prothrombin activity (%) | 57.9 ± 20.1 | 60.3 ± 17.6      | 0.34            |
| Total bilirubin, (mg/dL) | 3.0 ± 3.8    | 2.6 ± 2.8        | 0.40            |
| Blood urine nitrogen (mg/dL) | 25.8 ± 14.2 | 20.5 ± 12.8      | 0.003           |
| Serum creatine (mg/dL) | 1.08 ± 0.56   | 0.97 ± 0.46      | 0.08            |
| Serum sodium, (mEq/L) | 135.2 ± 5.7  | 136.7 ± 4.5      | 0.03            |
| Serum ammonia, (mg/dL) | 58.1 ± 34.1   | 66.2 ± 36.3      | 0.07            |
| Serum AFP (ng/mL) | 5.5 (2.8-28.6) | 5.2 (2.8-17.9)   | 0.12            |

AFP, alpha-fetoprotein; HCC, hepatocellular carcinoma.
combination of Na, BUN and SVEP1 SNP was 0.69, and 95%CI was 0.61-0.77. The difference in AUC between Na and the combination of Na, BUN and SVEP1 SNP was significant (P < 0.01). As well, the difference in AUC between SVEP1 SNP alone and the combination of Na, BUN and SVEP1 SNP was also significant (P < 0.05) However, there were no difference in AUC between BUN alone and the combination of Na, BUN and SVEP1 SNP (P = .12) (Supplement Figure S3). These results suggest that the combination of Na, BUN and SVEP1 SNP could predict the response to tolvaptan more accurately and identify non-responders than a single entry.

### 3.5 | Constructing a prediction model for detecting non-responders

In the non-risk allele group, 86.0% of the patients achieved a treatment response, which is sufficient to consider the risk to be low. The percentage of unsuccessful treatment cases was around 40% in the risk allele group (red area), and the number of successful cases was as high as 60% (Figure 1A). Therefore, it is important to conduct a detailed risk assessment in the risk allele group. A logistic model, using the variable reduction method, structured the prediction model based on BUN and
Na, and the predictive score (score) = 22.234 + BUN*0.077 + Na*(-0.179) was calculated. The probability score (failure probability = \[\frac{1}{1 + \exp(-\text{score})}\]) was identified for detecting non-responders (Figure 1B). Assuming a cutoff of failure probability of 38.6%, sensitivity was 84.4%, specificity was 70%, and the AUC was 0.774 (Figure 1C). When the failure probability was more than 38.6%, the treatment failure rate was 64.3%, while the treatment response was observed in 87.5% of the patients with a failure probability ≤38.6% (Figure 2). There was a statistically significant difference between the groups (\(P < .001\)).

3.6 Functional analysis of SNPs rs2991364 in SVEP1 gene

To determine the effect of the rs2991364 genotype on SVEP1 mRNA expression levels, we evaluated the expression quantitative trait loci (eQTLs) of rs2991364 using the Genotype-Tissue Expression (GTEx) project dataset via the website gtexportal.org.12 In the GTEx data, rs2991364 minor CT/TT-allele variants led to lower SVEP1 gene expression in brain tissue [brain – cerebellar hemisphere: \(P = 1.40 \times 10^{-6}\), brain – cerebellar: \(P = 2.00 \times 10^{-6}\)].

We next used a public database to determine whether SNP rs2991364 influences the expression of SVEP1 downstream signal genes. We analysed WGS and RNA-seq data from hepatitis C virus-associated cancer patients in ICGC – RIKEN, Japan (LIRI-JP) cohorts.13 After searching for SNP rs2991364 in germline data from HCC patients, we selected SNPs rs2991364 major TT-allele patients (\(n = 37\)) and minor CT-allele patients (\(n = 10\)), and compared the gene expression level of adjuvant liver tissue between TT-allele and minor CT-allele patients (Figure 3A). SVEP1 expression was down-regulated in the minor CT-allele group (Figure 3B) [\(P = 6.70 \times 10^{-7}\)]. SVEP1 is known to deposit around lymphatic vessels and upregulates Forehead box protein C2 (FOXC2) expression through integrin

| Parameter | P value | Beta  | OR     |
|-----------|---------|-------|--------|
| BUN       | 0.003   | 0.077 | 1.08 (1.026–1.136) |
| Na        | 0.005   | -0.179 | 0.836 (0.738–0.948) |
| Constant  | 0.009   | 22.234 | -      |

Score = 22.234 + BUN*0.077 + Na*(-0.179)
Probability for treatment failure = \[1 / (1 + \exp(-\text{score}))\]
Optimal cutoff = probability of 38.6%
Sensitivity 84.4%
Specificity 70.0%
alpha 9 (ITGA9), angiopoietin-2 (ANGPT2) and tyrosine kinase with immunoglobulin-like and epidermal growth factor (EGF) like domain 1/2 (TIE-1/2) receptors, thereby facilitating lymphatic vascular remodelling. Importantly, the expression levels of these SVEP1 downstream signaling genes were significantly downregulated along with the decrease in SVEP1 expression in the minor CT-allele group (Figure 3C) [ITGA9: \( P = .011 \), ANGPT2: \( P = .025 \), FOXC2: \( P = .015 \)]. These results indicate that SNPs rs2991364 can influence the expression of SVEP1 and its downstream signal genes, resulting in vascular network fragility (Figure 3D).

4 | DISCUSSION

In this study, we found SVEP1 SNPs correlated most closely with the response to tolvaptan. This is the first report showing a strong correlation at a genome-wide level of significance between a genetic variant (SVEP1) and the response to tolvaptan. The SVEP1 gene is located on 9q32, spans 214 kb of genomic DNA, and consists of 48 exons, encoding a secreted multi-domain protein which harbours sushi (named also complement control protein; CCP), von Willebrand factor (VWF) type A, EGF, and pentraxin-domain motifs. SVEP1 is an extracellular matrix protein involved in lymphatic vessel remodelling.

**FIGURE 2** Diagnostic flow diagram for treatment failure based on the rs2991364 risk allele and the prediction model. This flow diagram can be used to identify high-risk patients for tolvaptan treatment failure.

**FIGURE 3** rs2991364 genotype affects the expression of SVEP1 and its downstream genes. (a) Workflow showing sampling, sequencing, and analysis process for ICGC liver data. (b) Impact of SNP rs2991364 genotypes on expression of the SVEP1 gene in ICGC data (TT-allele; \( n = 37 \), CT-allele; \( n = 10 \)). SVEP1 expression was downregulated in the minor CT-allele group (\( P = 6.70 \times 10^{-4} \)). Data derived from RNA-seq. Mean and SEM are shown. Level of significance at *\( P < .05 \), **\( P < .01 \). (c) The effect of SNP rs2991364 genotypes on expression of SVEP1 downstream signal genes. ITGA9, ANGPT2, and FOXC2 were downregulated in the minor CT-allele group (ITGA9: \( P = .011 \), ANGPT2: \( P = .025 \), FOXC2: \( P = .015 \)). (d) Impact of SNP rs2991364 genotypes on SVEP1 signaling pathway. rs2991364 affects the expression of SVEP1 signal genes, resulting in vascular network fragility.
and plays a critical role in epidermal differentiation.\textsuperscript{14} Previously known as Polydom, SVEP1 is also a high-affinity ligand for integrin α9β1. Polydom knockout mice show severe oedema and die immediately after birth as a result of respiratory failure due to dysfunction of fluid drainage; these mice also fail to undergo remodelling and formation of collecting lymphatic vessels.\textsuperscript{14} SVEP1 is expressed in the heart, lung, skeletal tissue, placenta, stomach, intestine, stromal osteogenic tissues and so on. A recent report showed that missense variants of SVEP1 were significantly related to coronary arterial disease.\textsuperscript{16}

With respect to clinical data, BUN and Na were identified as a response marker based on multivariate analysis (Table 3). This result showed that renal dysfunction, especially caused by dehydration, and hyponatremia may worsen the response to tolvaptan. The SVEP1 SNP (rs2991364), which was associated with SVEP1 expression levels, would influence the vulnerability of lymphatic vessels, which could lead to fluid retention. The combination of Na, BUN and SVEP1 SNP showed a stronger correlation with the response to tolvaptan compared with Na or SVEP1 SNP alone ($P < .01$, $P < .05$ respectively) (Table S1). Previous reports showed that various parameters were associated with the tolvaptan response, including Na,\textsuperscript{8} BUN,\textsuperscript{6,7} BUN/Cre,\textsuperscript{17} CRP,\textsuperscript{18} urine Na/K\textsuperscript{19} ratio, urine osmolality,\textsuperscript{10} urine AQP-2,\textsuperscript{10,18} the existence of HCC\textsuperscript{20} and HCC stage.\textsuperscript{21} Our data also showed that the AFP correlated with the tolvaptan response. However, there are no statistical differences by the multivariate analysis, suggesting that the existence of HCC, and HCC stage may not strongly involve in the early tolvaptan response. As it was difficult to collect urine samples in a multicenter study, the other urinary parameters could not be evaluated. We could only compare Na, BUN and BUN/Cre with previous reports.

It is important to identify tolvaptan non-responders to avoid a lengthy period of futile treatment. The PPV of the response to tolvaptan based on Na and BUN was 0.84 and 0.86 respectively suggesting that Na and BUN is a good predictive marker for the response to tolvaptan. However, because the NPV of the response to tolvaptan based on Na and BUN was only 0.30 and 0.30, respectively, it would be difficult to predict non-responders to tolvaptan using Na or BUN alone. The PPV of the response to tolvaptan based on the combination of Na, BUN and SVEP1 was 0.90, and the NPV of the response to tolvaptan based on the combination of Na, BUN and SVEP1 was 0.75, indicating that this parameter was more accurate for predicting non-responders than the single entry, but not sufficient for clinical practice. We next proposed an additional prediction model based on logistic regression analysis of a population with the rs2991364 risk allele. The failure probability based on Na and BUN was identified for detecting non-responders. Assuming a cutoff of failure probability of 38.6%, sensitivity was 84.4%, specificity was 70%, and AUC was 0.774, suggesting that patients with the risk allele and a $>38.6\%$ probability of failure would be expected to have a poor treatment response. This may be a good marker for identifying non-responders to tolvaptan and avoid a lengthy period of futile treatment.

We propose the following strategy for the use of tolvaptan in clinical practice (Figure S4). When a patient has a BW decrease after 1 week of tolvaptan treatment, tolvaptan should be continued. However, when a patient has a BW increase after 1 week of treatment and is therefore a possible non-responder, SVEP1 SNP (rs2991364) should be measured. For patients without an SVEP1 SNP risk allele, measures should be employed to improve renal blood flow by reducing diuretics or performing large volume puncture with consideration of the late tolvaptan response.\textsuperscript{22} For patients with a risk allele, the prediction score should be determined. When the predictive score is over 38.6% (27/32, 84.4%), suspension of tolvaptan treatment should be considered as well as a change to a different treatment option, such as large volume puncture. In our present study, suspension of tolvaptan treatment was estimated at 6.5% (27 of 413 patients).

Our bioinformatic analysis showed that the rs2991364 genotype affects the expression of the SVEP1 gene in the cerebellum, although
the phenotype varies depending on the tissue. Furthermore, decreased SVEP1 expression caused a decrease in the expression of downstream genes. In particular, the expression level of the FOXC2 gene, which is known to play an important role in the development of the lymphatic vascular system, is reduced with the downregulation of SVEP1 expression. It has also been reported that FOXC2 expression is decreased through the ANGPT2 and TIE1/TIE2 receptor system in Polydom/SVEP1 mutant mice, causing severe oedema, which is consistent with the results of our public data analysis. Our results indicate that in the rs2991364 risk allele group, tissue fluid is difficult to collect due to the weakness of vascular network remodelling, and suggest that rs2991364, which affects SVEP1 gene expression, may serve as an SNP marker for predicting the effect of tolvaptan in other types of oedema, especially cardiac oedema (Figure 4). A significant association between coronary artery disease and missense variants in the SVEP1 gene was reported based on a large-scale exome-wide association study. To elucidate the relationship between the tolvaptan response and rs2991364 allele in cardiac oedema patients, further studies are required.

This study had several limitations. First, a limited number of SVEP1 SNP variants were studied. Second, the number of non-responders was relatively small and difficult to draw conclusions, as we excluded patients with a borderline response (BW change -1.5 < to <0 kg) to tolvaptan. Third, it was not possible to evaluate urine osmolality or urine AQP-2. Fourth, portal hypertension (PH) would influence ascites or the response to treatment of ascites, as well as serum albumin. Unfortunately, we did not collect data on the presence of PH and the portal vein invasion/thrombosis in this study. Lastly, all enrolled patients in this study were Japanese and further studies are needed to confirm the data in other populations.

In conclusion, we identified an association between SVEP1 SNPs and the response to tolvaptan among patients with difficult-to-treat hepatic ascites in the Japanese population. The combination of Na, BUN and SVEP1 SNP was predictive of the response to tolvaptan, and the use of the predictive score can further help to identify non-responders and avoid prolonged use of tolvaptan in patients who will not ultimately benefit.

ETHICS APPROVAL STATEMENT AND PATIENT CONSENT STATEMENT

The study protocol was conducted in accordance with the ethical guidelines of the Declaration of Helsinki and was approved by each of the participating Institutional Ethics Review Committees for our human genome projects. Written informed consent was obtained from all individual participants.

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CONFLICT OF INTEREST

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AUTHORS’ CONTRIBUTIONS

HK, HY and YT made substantial contributions to study conception and design. HS, MO, MN and YK made substantial contributions to analysis and interpretation of the data. TK, NS, HU, TN, NM, KI, TI, ST, HM, AK, NH, SS and YE made critical review of manuscript, acquisition of data. HK, HY and YT contributed to the final approval of the version of the article to be published.

All authors have made major contributions to the manuscript and agree with its content. We declare that this article is original, has not been published before, and is not currently being considered for publication elsewhere.

ORCID

Hideto Kawaratani https://orcid.org/0000-0002-4361-0592
Haruki Uojima https://orcid.org/0000-0003-1719-1352
Yasuhiro Tanaka https://orcid.org/0000-0002-2473-6966

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SUPPORTING INFORMATION
Additional supporting information may be found online in the Supporting Information section.

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