A Genome-Wide Association Study Identifying *SVEP1* Variant as a Predictor of Response to Tolvaptan for Cirrhotic Ascites

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Research Article

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Abstract

Background and Aims: Tolvaptan, an orally active 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.

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: GWAS showed 5 candidate SNPs around the *miR818, KIAA1109*, 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 x 10^{-8}). Univariate and multivariate analyses showed that blood urea nitrogen (BUN) and *SVEP1* SNP were significantly associated with the response (OR = 1.03, p = 0.02 and OR = 4.24, p < 0.0001, respectively). Based on a prediction model of logistic regression analysis in a population with the rs2991364 risk allele, the failure probability (= exp (score: 22.234 + BUN*0.077 + Na*-0.179) (1 + exp (score)) 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 can identify tolvaptan non-responders and help to avoid a lengthy period of futile treatment.

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 (160mg furosemide, spironolactone 400mg) 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 suppress 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 to 80mg furosemide and/or 25 to 100mg spironolactone (4) for mild ascites. When ascites is uncontrolled by conventional diuretics, tolvaptan is administered at a dose of 3.75 or 7.5mg. Tolvaptan was first reported to increase serum sodium levels safely and effectively in patients with euvolemic and hypervolemic 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 high blood urine nitrogen (BUN) or high urine osmolality can worsen the treatment response (8, 9), 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 (10). 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 (6, 7). 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.

Material And Methods

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 and after tolvaptan administration from 550 patients who had been treated with tolvaptan for hepatic ascites at each of the participating hospitals (40 hospital liver units with hepatologists). Responders to tolvaptan was 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.

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.

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 ASI 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 2 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.

Statistical analysis

Details of the prediction model based on a logistic regression model in which the backward elimination ($P < 0.05$) method was applied after including age, sex, weight, Child-Pugh classification, platelet, %PT, albumin, AST, ALT, total bilirubin, BUN, Cre, Na, etiology (HBV, HCV, alcohol, and NASH). 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).

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.

Results

Baseline characteristics of enrolled patients

A total of 413 patients were analyzed in this study. Table S1 shows the baseline clinical characteristics of the patients (269 men and 144 women). The mean age was 67.2 years (range: 37–97 years). Etiologies of the patients were viral hepatitis B in 38 patients, viral hepatitis C in 147 patients, heavy alcohol consumption in 126 patients, non-alcoholic steatohepatitis in 50 patients, autoimmune hepatitis in 9

patients, primary biliary cholangitis in 24 patients, and other causes in 28 patients. A total of 163 patients (39.5%) 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. The events included thirst in 43 patients (50.0%), hepatic coma in 21 patients (24.4%), dehydration in 9 patients (10.5%), and other (hypernatremia, muscle cramps, and eruption etc.) in 13 patients (15.1%). 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 BMI ($P = 0.02$), presence of HCC ($P = 0.04$) and BUN ($P = 0.005$).

Genetic variants associated with response to tolvaptan

We conducted a GWAS using 25 cases (non-responders) and 156 controls (responders) by analyzing 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: $P = 5.97 \times 10^{-7}$ and rs10800602: $P = 8.69 \times 10^{-8}$ located on chromosome 1 q32.1) and KIAA1109 (rs3108399: $P = 5.84 \times 10^{-8}$ on chromosome 4 q27) and *SVEP1* (rs9299186: $P = 7.46 \times 10^{-7}$ and rs4978937: $P = 7.46 \times 10^{-7}$ on chromosome 9 q31.3).

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, 3 (rs2991364, rs9299186, and rs4978937 on *SVEP1* intron region) were validated and consistent associations were observed between the GWAS set and replication set (Table 3). One SNP showed a genome-wide significant association (rs2991364: OR = 3.55, $P = 2.01 \times 10^{-8}$) using the combined set (80 cases and 333 controls) (Table 2).

Risk factors for response to tolvaptan

The results obtained by multivariate logistic regression analysis are shown in Table 3. Univariate analysis showed that BUN and *SVEP1* SNPs (rs2991364) were significantly associated with the response to tolvaptan, and multivariate analysis showed that BUN and *SVEP1* SNPs were also associated with the response to tolvaptan (OR = 1.03, p = 0.02 and OR = 4.24, p < 0.0001, respectively). Table S2 shows that 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 area under the curve (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 0.58–0.70. The highest sum of sensitivity and specificity of combination of *SVEP1* SNP and BUN was 0.60 and 0.70, respectively. The AUC for the combination of *SVEP1* SNP and BUN was 0.69, and 95%CI was 0.62–0.76. The difference in AUC between *SVEP1* SNP alone and the combination of *SVEP1* SNP plus BUN was significant ($p < 0.01$) (Figure S3). These results suggest that the combination of BUN and *SVEP1* SNP could predict the response to tolvaptan more accurately and identify non-responders.

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. On the other hand, 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% (Fig. 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 (failure probability = exp (score)/ (1 + exp (score)) was identified for detecting non-responders (Fig. 1b). Assuming a cutoff of failure probability of 38.6%, sensitivity was 84.4%, specificity was 70%, and the AUC was 0.774 (Fig. 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% (Fig. 2). There was a statistically significant difference between the groups ($p < 0.001$).

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 (eats) of rs2991364 using the Genotype-Tissue Expression (GITEX Release V8) project dataset via the website gtexportal.org. (11). In the GITEX 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 using public database to determine whether SNP rs2991364 influences the expression of *SVEP1* downstream signal genes. We analyzed WGS and RNA-seq data from hepatitis C virus-associated cancer patients in ICGC - RIKEN, Japan (LIRI-JP) cohorts (12). 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 (Fig. 3a). *SVEP1* expression was downregulated in the minor CT-allele group (Fig. 3b) [$P = 6.70 \times 10^{-4}$]. *SVEP1* is known to deposit around lymphatic vessels and upregulates Forehead box protein C2 (FOXC2) expression through integrin 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 remodeling (13). 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 (Fig. 3c) [ITGA9: P = 0.011, ANGPT2: P = 0.025, FOXC2: P = 0.015]. These results indicate that SNPs rs2991364 can influence the expression of *SVEP1* and its downstream signal genes, resulting in vascular network fragility (Fig. 3d).

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 harbors sushi (named also complement control protein; CCP), von Willebrand factor (VWF) type A, EGF, and pentraxin-domain motifs (14). SVEP1 is an extracellular matrix protein involved in lymphatic vessel remodeling and plays a critical rule in epidermal differentiation (13). Previously known as Polydom, SVEP1 is also a high-affinity ligand for integrin a9b1. Polydom knockout mice show severe edema and die immediately after birth as a result of respiratory failure due to dysfunction of fluid drainage; these mice also fail to undergo remodeling and formation of collecting lymphatic vessels (13). SVEP1 is expressed in 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 (15).

With respect to clinical data, only BUN was identified as a response marker based on multivariate analysis (Table 3), as previously reported (6, 8). This result showed that renal dysfunction, especially caused by dehydration, may worsen the response to tolvaptan. On the other hand, 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 BUN and *SVEP1* SNP showed a stronger correlation with the response to tolvaptan compared with BUN or *SVEP1* SNP alone. Previous reports showed that various parameters were associated with the tolvaptan response, including BUN (6, 8), BUN/Cre (16), CRP (17), urine Na/K (18) ratio, urine osmolarity (9), urine AQP-2 (9, 17). As it was difficult to collect urine samples in this multicenter study, we could only compare BUN and BUN/Cre with previous reports. BUN showed a stronger correlation compared with BUN/Cre (data not shown); however other urinary parameters could not be evaluated.

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 BUN was 0.86, suggesting that BUN is a good predictive marker for the response to tolvaptan. However, because the NPV of the response to tolvaptan based on BUN was only 0.30, it would be difficult to predict non-responders to tolvaptan using BUN alone. The NPV of the response to tolvaptan based on the combination of BUN and *SVEP1* was 0.63, indicating that this parameter was more accurate for predicting non-responders, but not sufficiently so 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 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 (19). 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.

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 (20–22), 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 Polydor/*SVEP1* mutant mice, causing severe edema (20), which is consistent with the results of our public data analysis. Our results indicate that in the rs2991364 risk allele group, tissue fluid is originally difficult to collect due to the weakness of vascular network remodeling, 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 edema, especially cardiac edema. 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 (15). To elucidate the relationship between the tolvaptan response and rs2991364 allele in cardiac edema 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 from. We excluded patients with a borderline response (BW change − 1.5 < to < 0kg) to tolvaptan. Third, it was not possible to evaluate urine osmolarity or urine AQP-2. 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 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.

Abbreviations

Alb: albumin

ALT: alanine aminotransferase

AFP: alpha fetoprotein

NH_3: ammonia

ANGPT2: angiopoietin-2

AQP: aquaporin

AUC: area under the curve

AST: aspartate aminotransferase

BUN: blood urine nitrogen

BW: body weight

CI: confidence interval

Cre: creatine

EGF: epidermal growth factor

FOXC2: Forehead box protein C2

GWAS: genome-wide association study

ITGA9: integrin alpha 9

IFN: interferon

Na: sodium

NPV: negative predictive value

NASH: nonalcoholic steatohepatitis

OR: odds ratio

PPV: positive predictive value (PPV)

PT: prothrombin time

ROC: Receiver operating characteristics

T-bill: total bilirubin

Declarations

Data Availability: Yes

Animal Research (Ethics): not applicable

Consent to Participate (Ethics): Yes

Consent to Publish (Ethics): Yes

Plant Reproducibility: not applicable

Clinical Trials Registration: not applicable

Author Contribution: Yes (Substantial contributions to study conception and design: HK, HY, YT; substantial contributions to analysis and interpretation of the data: HS, MO, MN, YK; critical review of manuscript, acquisition of data: TK, NS, HU, TN, NM, KI, TI, ST, HM, AK, NH, SS, YE; final approval of the version of the article to be published: HK, HY, YT)

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Tables

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	0.83
Sex (male/female)	55 / 35	214 / 119	0.73
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
BMI	23.1 ± 6.1	24.9 ± 4.9	0.02
Etiology (B/C/Alc/NASH/AIH/PBC/others)	10/26/24/12/3/2/7	28/121/102/38/6/22/21	0.27
Presence of HCC (yes / no)	16 / 64	147 / 186	0.04
Child-Pugh (A / B / C)	0 / 37 / 43	5/ 193 /215	0.61
Serum albumin (g/dL)	2.6 ± 0.5	2.7 ± 0.5	0.25
Prothrombin activity (%)	57.9 ± 20.1	59.8 ± 18.2	0.43
Total bilirubin (mg/dL)	3.0 ± 3.8	2.7 ± 3.0	0.40
BUN (mg/dL)	25.8 ± 14.2	21.5 ± 13.2	0.005
Serum creatine, Cr (mg/dL)	1.08 ± 0.56	0.97 ± 0.46	0.08
Serum sodium, Na (mEq/L)	135.2 ± 5.7	136.4 ± 4.7	0.65
Serum ammonia, NH_3 (mg/dL)	58.1 ± 34.1	64.7 ± 36.0	0.32
Serum AFP (ng/mL)	5.5 (2.8-28.6)	5.2 (2.8-17.9)	0.05

Table 2. Three SNPs associated with tolvaptan's efficacy

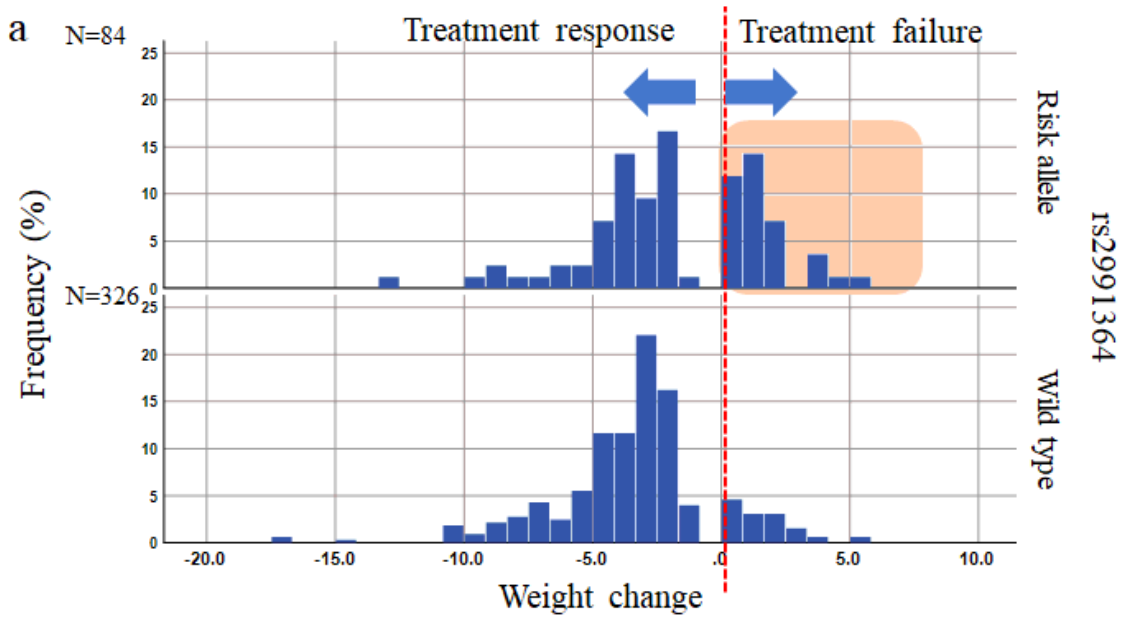
			Cases				Controls					OR
Marker	Allele (1/2)		11	12	22	MAF	11	12	22	MAF	P-value	(95%CI)
rs2991364	T/C	GWAS	1	11	13	0.260	0	20	136	0.064	7.90E-06	5.13 (2.36-11.16)
		replication	2	20	33	0.218	1	30	146	0.090	3.26E-04	2.81 (1.57-5.02)
		combined	3	31	46	0.231	1	50	282	0.078	2.01E-08	3.55 (2.23-5.65)
rs9299186	C/T	GWAS	2	10	13	0.280	0	24	132	0.077	1.37E-05	4.67 (2.22-9.83)
		replication	2	20	33	0.218	2	29	146	0.093	4.88E-04	2.71 (1.52-4.83)
		combined	4	30	46	0.238	2	53	278	0.086	6.36E-08	3.33 (2.11-5.24)
rs4978937	C/T	GWAS	2	10	13	0.280	0	24	132	0.077	1.37E-05	4.67 (2.22-9.83)
		replication	2	20	33	0.218	2	30	145	0.096	7.17E-04	2.63 (1.48-4.66)
		combined	4	30	46	0.238	2	54	277	0.087	9.79E-08	3.27 (2.08-5.14)

Table 3. Univariate and multivariate analysis for tolvaptan response

	Univariate	Multivariate		
	P-value	OR	95%CI	P-value
Age	0.83			
Sex	0.73			
Child-Pugh	0.61			
AFP	0.05	1.00	1.00-1.00	0.05
ALB	0.25			
AST	0.31			
ALT	0.36			
BUN	0.005	1.03	1.01-1.06	0.02
Cr	0.08	0.74	0.32-1.74	0.49
NH_3	0.32			
Na	0.65			
Platelet	0.90			
PT activity	0.43			
SVEP1 rs2991364	0.00000128	4.24	2.23-8.07	0.00001

PT: Prothrombin, OR: odds ratio, CI: confidence interval

Figures



b

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
= exp(score) / {1 + exp(score)}

Optimal cutoff = probability of 38.6%
Sensitivity 84.4%
Specificity 70.0%

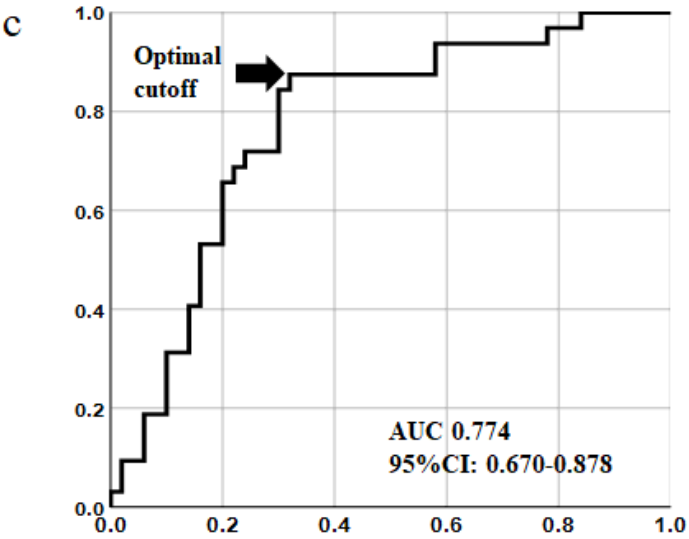


Figure 1

a) Histogram for weight change stratified with or without the rs2991364 risk allele. Treatment failure cases were concentrated in populations with risk alleles (red area). Prediction model construction and evaluation for treatment failure in a population with the rs2991364 risk allele. (b) Details of the prediction model based on logistic regression in which a backward elimination (P<0.05) method was applied after including age, sex, weight, Child-Pugh classification, platelet, %PT, albumin, AST, ALT, total bilirubin, BUN, creatinine, Na, and etiology (HBV, HCV, alcohol, and NASH). (c) ROC analysis of the prediction model.

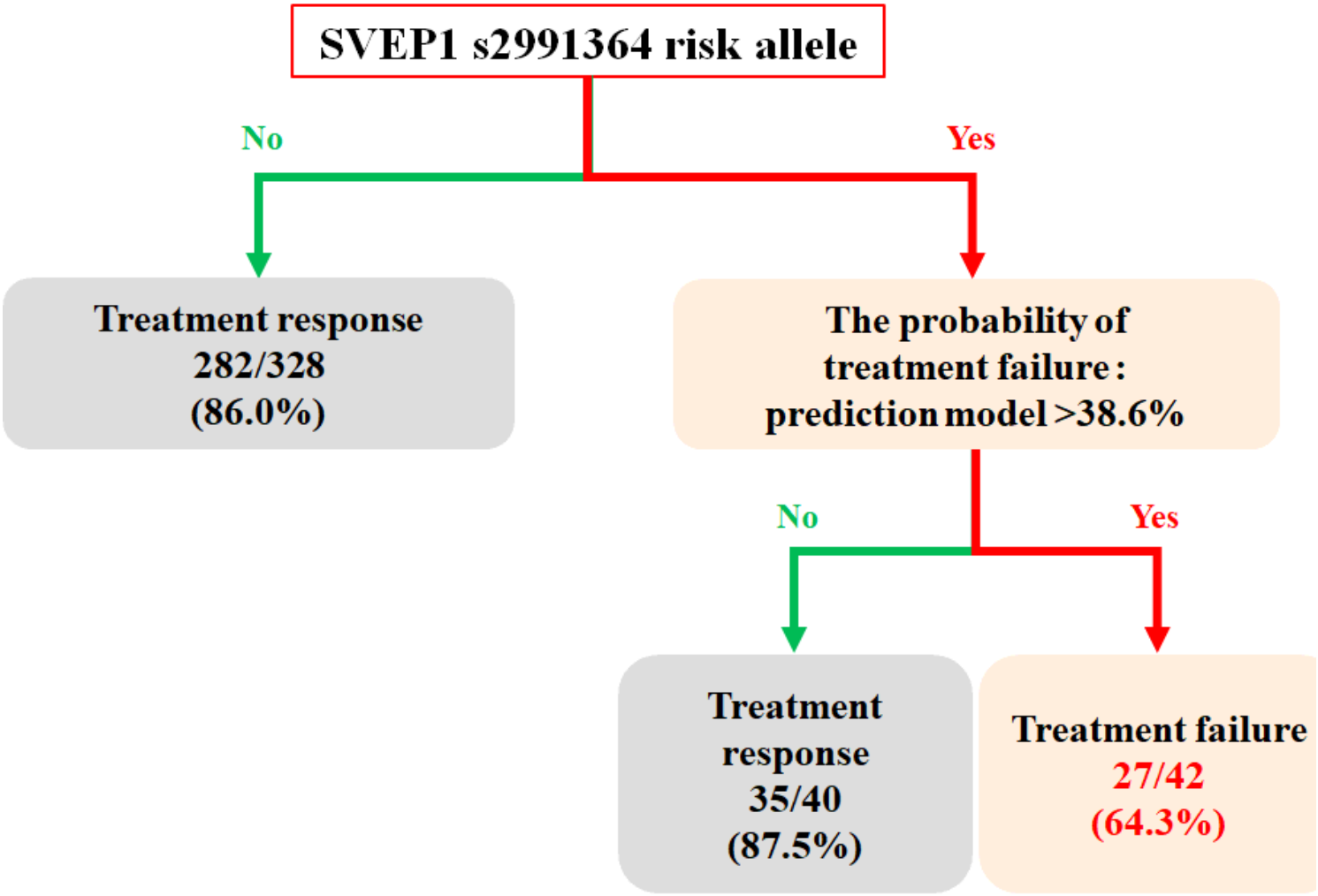


Figure 2

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

Fig. 3

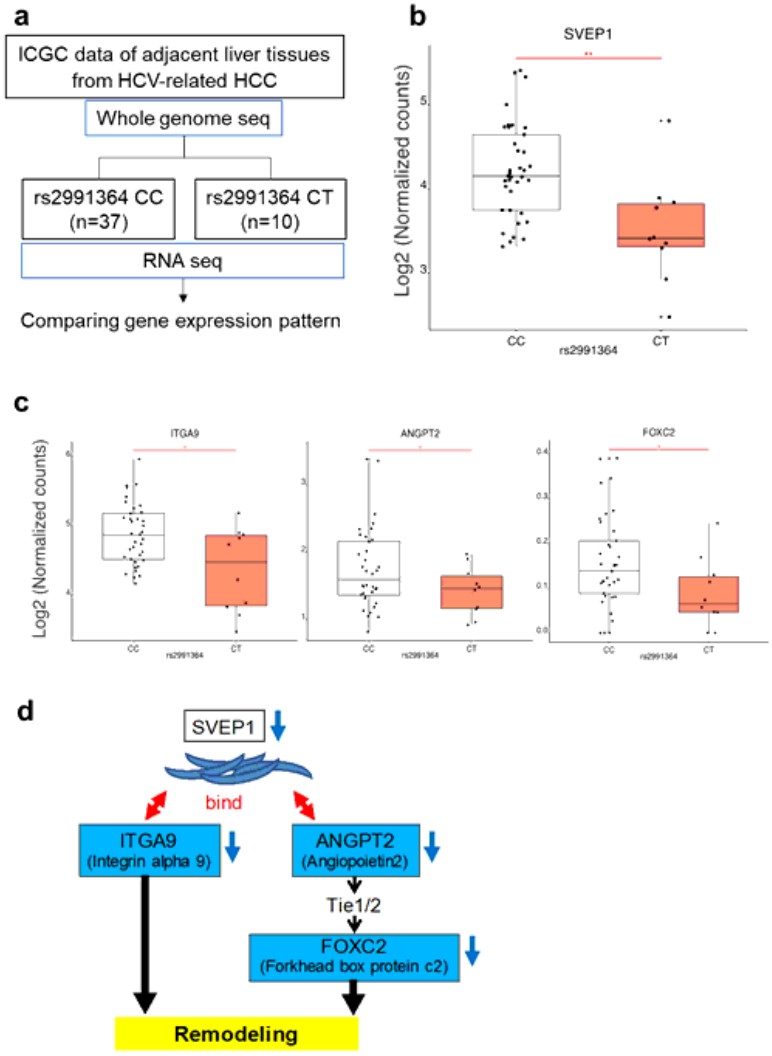


Figure 3

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 × 10−4). Data derived from RNA-seq. Mean and SEM are shown. Level of significance at *P < 0.05, **P < 0.01. (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 = 0.011, ANGPT2: P = 0.025, FOXC2: P = 0.015). (d) Impact of SNP rs2991364 genotypes on SVEP1 signaling pathway. rs2991364 affects the expression of SVEP1 signal genes, resulting in vascular network fragility.

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