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Research Letter

Characteristics of a Chinese Cohort of Patients with Chronic Hepatitis C Infection (2019–2023) and a Case Report of Resistance-associated Substitutions to Sofosbuvir-velpatasvir Treatment

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Hepatitis C virus (HCV) infection remains a major public health concern, with 56.8 million people chronically infected worldwide and 1.5 million new infections yearly.[1] As a leading cause of liver-related mortality, chronic HCV infection is associated with 19% of hepatocellular carcinoma and 21% of cirrhosis cases.[2] The advent of direct-acting antiviral agents (DAA) in recent decades, which target viral replication proteins and inhibit various steps in the HCV life cycle, has shown remarkable progress in achieving a cure for hepatitis C. DAA regimens block replication complex formation, reduce virion assembly and release, accelerate viral RNA degradation, and are associated with the restoration of liver function and improvement in histology. Pangenotypic DAA regimens, with DAA combinations targeting different and complementary stages of the HCV cycle, have been recommended by current practice guidelines[3] and can be initiated without knowledge of the HCV genotype and subtype. As the global prevalence of chronic HCV infections has decreased by seven million since 2015, the World Health Organization has set the goals of a 90% reduction in new infections, an 80% reduction in diagnosed patients, and a 65% reduction in HCV-related mortality by 2030. To accomplish this, expanded access, active HCV screening, and linkage to care are required.[4]

Sofosbuvir-Velpatasvir (SOF-VEL) is a pangenotypic regimen coformulated with fixed-dose combinations of the nucleotide polymerase inhibitor sofosbuvir (400 mg) and the NS5A inhibitor velpatasvir (100 mg) for adult patients. The treatment with SOF-VEL for 12 weeks resulted in high rates of continuous absence of detectable HCV RNA for at least 12 weeks after the end of therapy (SVR12).[3,4]

The present study retrospectively analyzed the characteristics of a Chinese cohort of chronic hepatitis C patients collected from January 2019 to August 2023. A genotype-based baseline comparison was performed in 358 HCV RNA-positive patients with intact clinical information. The risk factors for hepatocellular carcinoma and cirrhosis were analyzed (Supplementary Tables 1–5). Child-Pugh stage and FIB-4 score were used for the assessment of liver cirrhosis. Child-Pugh stages B and C indicated advanced cirrhosis, and a FIB-4 index score >3.25 indicated cirrhosis most likely. Consistent with our previous study,[5] HCV genotype (GT)-1 infection was the most prevalent (N = 163, 45.5%), followed by GT-2 and GT-3a (both N = 48, 13.4%). The mean age of all patients was 56.6 ± 11.3 years. Male patients predominated among most genotypes (total N = 228, 63.7%). 22.3% of patients (N = 80) had fatty liver, and 7.5% (N = 27) had renal function abnormalities. Furthermore, 20.1% of patients (N = 72) tested positive for antinuclear antibodies. Among patients aged ≥50 years, GT-3a and GT-3b patients had the highest proportions of liver cancer cases [10 of 24 (41.7%) and four of fourteen (28.6%) patients, respectively], as well as the highest proportions of Child-Pugh class B and C (29.2% and 50%), and FIB-4 index ≥3.25 (70.8% and 50%) cases. Considering the relative prevalence of GT-3 has increased dramatically in China,[6] the characteristics of this genotype warrant attention.

Single and multivariate analyses revealed that male gender, age ≥50 years, Child-Pugh grade B+C, and FIB-4 score ≥3.25 were associated with an increased liver cancer risk in patients with hepatitis C [OR (95% CI) = 2.19 (1.00–5.06), 3.48 (1.29–11.23), 1.53 (0.65–3.55), 9.48 (4.35–22.19), respectively]. Similarly, age ≥50 years, comorbidity with diabetes, and antinuclear antibody positivity were associated

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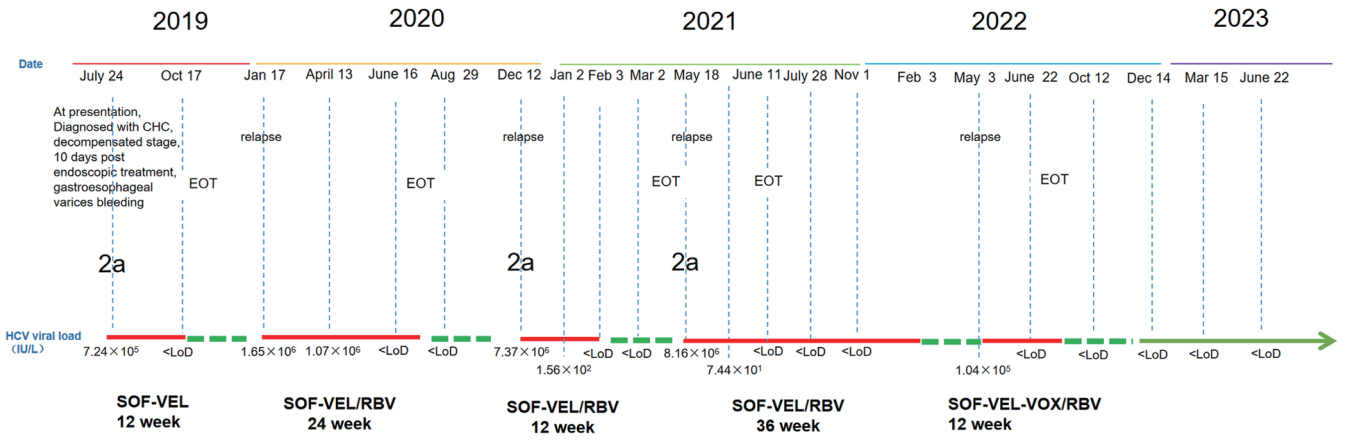

Fig. 1. The treatment timeline of a drug-resistant patient. One patient exhibited an unusual resistance profile and experienced multiple rounds of SOF-VEL or SOF-VEL in combination with ribavirin treatments. These treatments showed effectiveness each time in suppressing viral replication to below the LoD; however, the patient failed to achieve SVR12 after the EOT. CHC, chronic hepatitis C; SOF-VEL, Sofosbuvir-Velpatasvir; RBV, ribavirin; LoD, limit of detection; EOT, end of treatment.

with an increased risk of Child-Pugh Grade B and C [OR (95% CI) = 1.74 (0.82–3.96), 3.04 (1.17–7.53), 2.11 (1.00–4.32), respectively], as well as FIB-4 ≥ 3.25 [OR (95% CI) = 3.46 (1.95–6.41), 2.09 (0.93–4.78), 1.79 (1.00–3.19), respectively] in these patients.

Within the present chronic hepatitis C cohort, 80 patients treated with SOF-VEL (Epclusa, Gilead Sciences Ireland, UC) and with information about the status of HCV clearance were further investigated for efficacy and safety. Compared to their baseline status, the patients exhibited a notable reduction in viral load during the 12-week treatment period and at 12 weeks after the end of treatment, with an SVR12 rate of 95% (N = 76). Additionally, there was a significant improvement in hepatic function. The frequency of adverse events (AEs) during treatment was 22.5% (N = 18). The major AEs were anemia (12.5%, N = 10), liver dysfunction (11.3%, N = 9), hepatitis B reactivation (2.5%, N = 2), and hyperuricemia (1.3%, N = 1). Most of them were mild and controllable. Hepatitis B virus reactivation after DAA therapy could be managed by co-administration with anti-hepatitis B virus drugs.

One patient exhibited an unusual resistance profile was monitored. The patient had a history of substance use disorder and presented after an episode of esophageal gastric varices bleeding. After endoscopic therapy, the patient received a total of four rounds of SOF-VEL treatment (Fig. 1), either alone or in combination with ribavirin (400 mg three times daily). The patient showed effective suppression of viral replication to below the limit of detection each time. However, he failed to achieve SVR12 once the treatment was discontinued. HCV genotyping results from relapses showed the same HCV genotype, GT-2a. Notably, the patient did not revert to drug use after medical treatment, thus avoiding reinfection. The patient was eventually treated with a twelve-week course of SOF-VEL-voxilaprevir (VOX) (Vosevi®, Gilead) in combination with ribavirin, which showed a good salvage effect. His HCV RNA levels have remained undetectable for over ten follow-up visits (every three to four months).

One HCV strain, namely SH-ZSH01, was isolated from the serum samples of this SOF-VEL-resistant patient. The complete genome was obtained through next-generation sequencing with a median depth of 1074 and subsequently de novo assembled using Megahit and submitted to GenBank (GenBank Accession No. PP372686). A phylogenetic tree constructed using full genome sequences of SH-ZSH01 and 27 representative isolates confirmed that SH-ZSH01 belonged to subtype 2a (Fig. 2). SH-ZSH01 shared the highest overall sequence homology (91.76%) with a Japanese isolate, JCH-6 (subtype 2a, GenBank Accession No. AB047645.1). The sequence analysis of SH-ZSH01 revealed amino acid substitutions in NS3, NS5A, and NS5B, with 17, 31, and 21 substitutions, respectively, compared to the reference strain HC-J6 (Fig. 3). There were 2 (F28S, L31M) and 3 resistance-associated substitutions (RASs) (T273A, M289L, and A421V) in NS5A and NS5B, respectively, but no RAS in NS3.

HCV drug resistance can be categorized into four types: null response, partial response, relapse, and breakthrough. Viral resistance *in vivo* is influenced by the genetic barrier to resistance, the *in vivo* fitness of the viral variant population (defined as its ability to survive and grow in the replicative environment), and drug exposure.[7] In the present study, the patient who experienced relapses after SOF-VEL treatment was infected with HCV GT-2a, with resistance sites located within NS3, NS5A, and NS5B. In the NS5A protein region of the isolated viral strain SH-ZSH01, two important reported RASs were identified (i.e., F28S and L31M). GT-2a-based HCV harboring the NS5A F28S mutation had significantly higher replication ability and resistance to high concentrations of approved NS5A inhibitors than the wild-type strain.[8] The NS5A L31M mutation confers resistance to velpatasvir in GT-1a and GT-1b, whereas wild-type GT-2a constitutively contains NS5A 31M. Additionally, compared to the GT-2a reference viral strain HC-J6 (D00944), the viral strain SH-ZSH01 exhibited three RASs in the NS5B region (i.e., T273A, M289L, A421V). Among these, the prevalence of M289L in GT-2 was reported to be 4.0%, while T273A and A421V had not previously been reported for their resistance associations in GT-2a. A421V, which was reported in GT-1 and GT-5,[9,10] can reduce drug affinity, causing drug resistance to Beclabuvir.[11,12] Resistance mutation M289L slightly enhances the binding activity of GS-461203 (the active form of SOF) to NS5B, yet improves the fitness of the virus, diminishing its sensitivity to SOF.[13]

NS5A and NS3 RASs are frequently selected in patients who fail NS5A or NS3 inhibitor-containing regimens. In contrast, NS5B nucleotide RASs are rarely detected. This rarity is likely due to the highly conserved catalytic site region where nucleotides bind, making substitutions in this region extremely rare. Any such substitution would likely render the virus replication incompetent. Compounding the clinical impact of NS5A RASs is their ability to maintain high replication competence (i.e., relative fitness) in the absence of

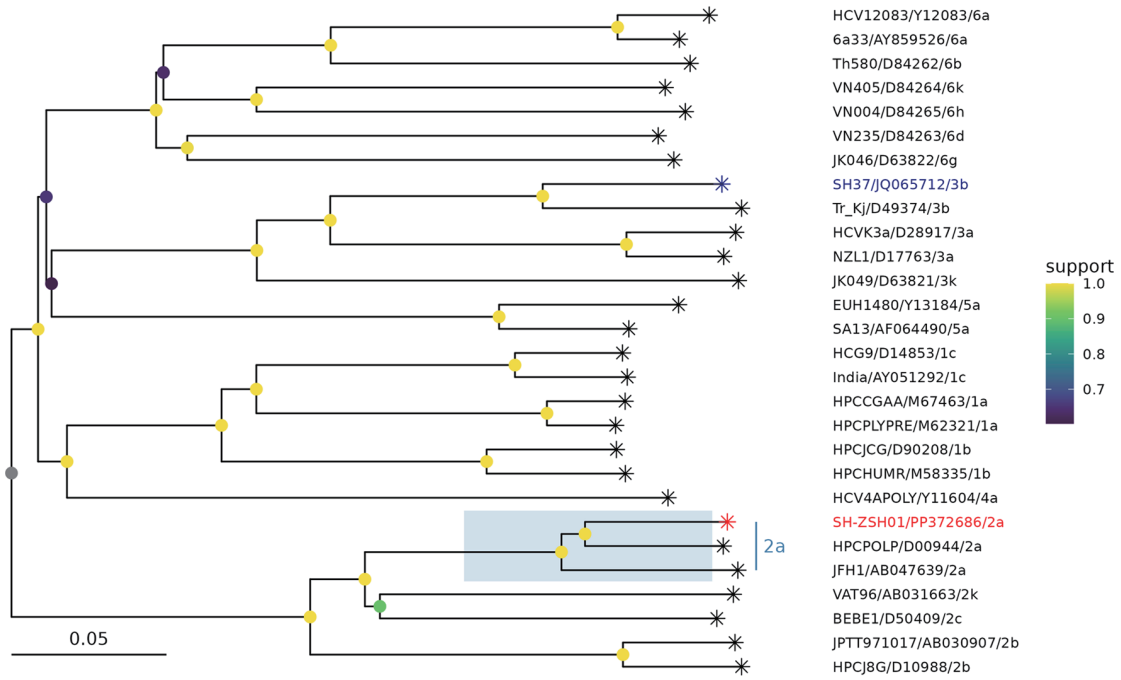

Fig. 2. Phylogenetic analysis of the full genome sequence of isolated SH-ZSH01, belonging to HCV subtype 2a. The phylogenetic tree was constructed with the complete genome of SH-ZSH01 and 27 reference sequences, using the neighbor-joining method and the Jukes-Cantor model in MEGA 11. Bootstrap support for the groupings was tested with 2000 replicates, and support ratios greater than 50% are indicated at the corresponding nodes. The scale bar indicates nucleotide substitutions per position. HCV, hepatitis C virus.

continued drug pressure, allowing them to remain the dominant quasispecies for prolonged periods (years) relative to NS3 protease or NS5B nucleotide polymerase inhibitor RASs, which are typically less fit and tend to disappear over several months, being overcome by more fit wild-type virus species. Although this particular RAS is often considered not clinically relevant, and sofosbuvir may still be used for therapy even when it is present, patients with this HCV variant may need

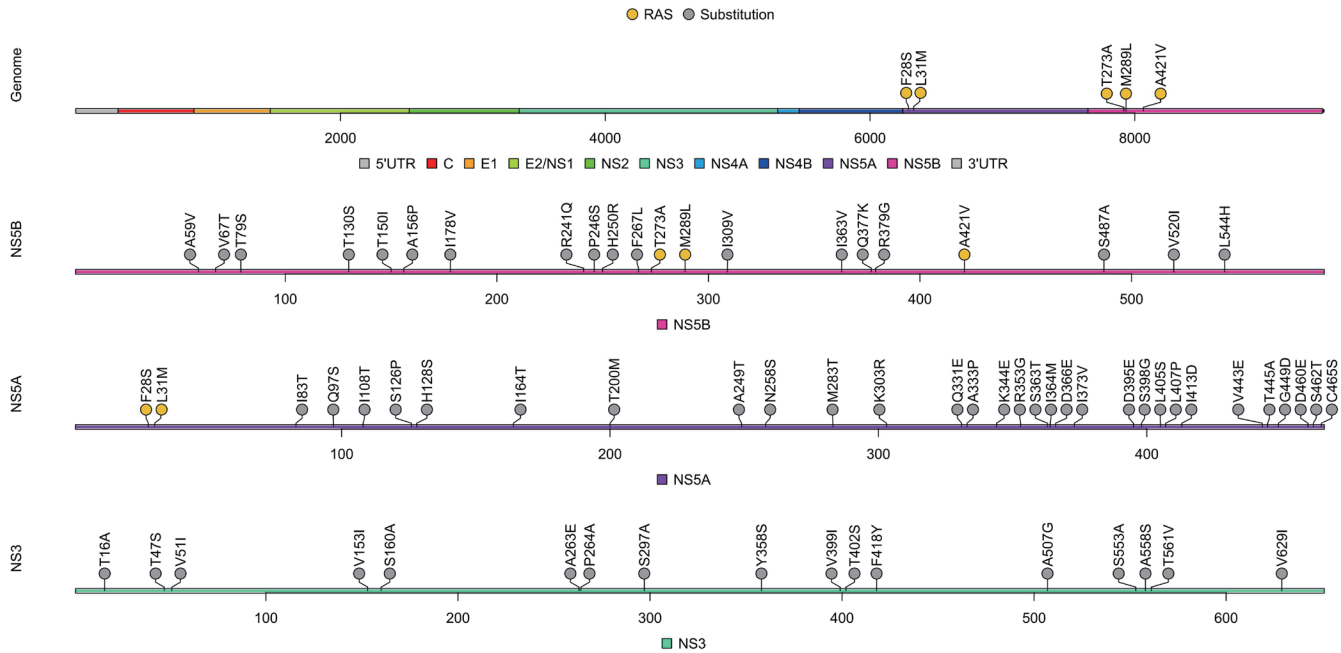

Fig. 3. Genome organization, amino acid substitutions, and RASs of isolated SH-ZSH01, belonging to HCV subtype 2a. The genome of isolate SH-ZSH01 contains 5'UTR, 3'UTR, and a polyprotein consisting of 10 peptides (C, E1, E2, p7, NS2, NS3, NS4A, NS4B, NS5A, and NS5B). All RASs identified in SH-ZSH01 are highlighted with yellow dots within the genome. Substitutions are depicted with grey dots, while RASs in NS3, NS5A, and NS5B are specifically indicated. HCV, hepatitis C virus.

to be continually treated or have their treatment regimen changed to obtain SVR.

SOF-VEL-VOX is a fourth-generation pangenotypic anti-HCV oral combination therapy, coformulated with fixed-dose combinations of SOF 400 mg, VEL 100 mg, and VOX 100 mg. It is indicated for HCV genotypes 1–6 infections, with or without compensated cirrhosis, and for patients who have failed treatment with certain DAA regimens. Recent studies have reported that SOF-VOX plus RBV achieved high SVR12 rates in patients with decompensated cirrhosis and was well tolerated.[14] The primary RASs for HCV GT-2a with VOX use are F43V and A156L/T/V. No reported RAS mutations for VOX were found,[15] and no resistance mutations were associated with VOX in the viral strain SH-ZSH01.

In conclusion, the use of pangenotypic DAA regimens remains the primary approach to meet the treatment goal of reducing the public health burden of hepatitis C. This study confirms the high efficacy of SOF-VEL, with most AEs being mild and manageable. However, the findings also highlight ongoing challenges, particularly the emergence of antiviral resistance. The successful salvage therapy with SOF-VEL-VOX suggests the need for healthcare providers to closely monitor virologic responses, consider potential drug interactions, and select an appropriate treatment regimen based on the individual patient's profile. Larger-scale studies in the future should focus on long-term outcomes and more in-depth explorations of AEs and resistance mechanisms.

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Conflict of interest

JG has been an Editorial Board Member of *Journal of Clinical and Translational Hepatology* since 2023. The other authors have no conflict of interests related to this publication.

Author contributions

Conceptual design of the study (JG, YX), guarantee of the article and manuscript drafting (HX), case collection, data acquisition, statistical analysis (HX, CZ, HS, JG), sequencing, and analysis (SP, YX). All authors made substantial contributions to the conception and design, acquisition of data, or analysis and interpretation of data; they took part in drafting the article or revising it critically for important intellectual content. All authors have approved the final version and publication of the manuscript.

Ethical statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the ethical committee of Zhongshan Hospital affiliated with Fudan University (Approval number B2021-402). Informed consent was obtained from all subjects involved in the study. All authors agreed to submit to the current journal, gave final approval of the version to be published, and agreed to be accountable for all aspects of the work.

Data sharing statement

The genome sequence of the isolated HCV strain, namely SH-ZSH01, has been deposited in GenBank under the accession number PP372686. The sequence information has been publicly available since August 31, 2024. All citations and data included in this manuscript are available upon request by contacting the corresponding authors.

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