Hepatology Research

Hepatology Research 2018; **48**: 36–44 doi: 10.1111/hepr.12890

Original Article

Impact of hepatitis C virus recombinant form RF1_2k/1b on treatment outcomes within the Georgian national hepatitis C elimination program

Marine Karchava,[1,2] Nikoloz Chkhartishvili,[1] Lali Sharvadze,[1,2,3] Akaki Abutidze,[1,2] Natia Dvali,[1] Lana Gatserelia,[1,2] Lela Dzigua,[1] Natalia Bolokadze,[1,3] Ekaterine Dolmazashvili,[1,2,3] Adam Kotorashvili,[4] Paata Imnadze,[4] Amiran Gamkrelidze[4] and Tengiz Tsertsvadze[1,2,3]

[1]Infectious Diseases, AIDS and Clinical Immunology Research Center, [2]Hepatology Clinic- Hepa, [3]Ivane Javakhishvili Tbilisi State University, and [4]National Center for Diseases Control and Public Health, Tbilisi, Georgia

Aim: Hepatitis C virus (HCV) recombinant form RF1_2k/1b is common in ethnic Georgians. This chimera virus contains genomic fragments of genotype 2 and genotype 1 and is misclassified as genotype 2 by standard genotyping. We aimed to identify RF1_2k/1b strains among genotype 2 patients and assess its impact on treatment outcomes.

Methods: The study included 148 patients with HCV genotype 2 as determined by 5-untranslated region/core genotyping assay. RF1_2k/1b was identified by sequencing the non-structural protein 5B region. Patients were treated within the national hepatitis C elimination program with sofosbuvir/ribavirin (SOF/RBV), interferon (IFN)/SOF/RBV, or ledipasvir (LDV)/SOF/RBV.

Results: Of 148 patients, 103 (69.5%) had RF1_ 2k/1b. Sustained virologic response (SVR) data was available for 136 patients (RF1_ 2k/1b, $n = 103$; genotype 2, $n = 33$). Sustained virologic response was achieved in more genotype 2 patient than in RF1_2k/1b patients (97.0% vs. 76.7%, $P = 0.009$). Twelve weeks of LDV/SOF/RBV treatment was highly effective (100% SVR) in both genotypes. Among RF1_2k/1b patients, LDV/SOF/RBV for 12 weeks was superior (100% SVR) to SOF/RBV for 12 weeks (56.4%, $P < 0.0001$) or 20 weeks (79.2%, $P = 0.05$). Twelve weeks of IFN/SOF/RBV also showed better response than SOF/RBV for 12 weeks (88.9% vs. 56.4%, $P = 0.02$) in these patients.

Conclusions: High prevalence of the RF1_2k/1b strain can significantly affect treatment outcomes. Treatment with IFN/SOF/RBV and especially LDV/SOF/RBV ensured significantly higher SVR in patients infected with RF1_2k/1b strain compared to standard HCV genotype 2 treatment with SOF/RBV. There is a need to reassess existing methods for the management of HCV genotype 2 infections, especially in areas with high prevalence of the RF1_2k/1b strain.

Key words: DAA treatment, HCV recombinant strain

INTRODUCTION

Georgia, a country nested between the Black Sea, Russia, and Turkey, has a population of 3.7 million[1] and is reported to have a high adult hepatitis C virus (HCV) seroprevalence of 7.7% (Ministry of Labor, Health, and Social Affairs of Georgia, unpublished data, 2016).

Along with high prevalence, the HCV-infected population in Georgia is under the scientific spotlight due to the frequency of a natural intergenotypic recombinant form, RF1_2k/1b, among HCV genotype 2 patients.[2,3]

This chimera virus is called a natural intergenotypic recombinant form, due to the possession of genotype 2 sequences in the structural and genotype 1 in the non-structural regions of the HCV virus.[4]

After the report of the first recombinant strain, designated as RF1_2k/1b in Russia,[4] other groups have also described this genotype among patients in Ireland,[5] Estonia,[6] Uzbekistan,[7] Cyprus,[8] France,[9] Germany, and Israel.[10] This strain was later fully sequenced, showing recombination breakpoint positions in non-structural 2 and non-structural 3 (NS2–NS3) regions of the HCV genome.[11] Most of the patients described in these studies are ethnic Georgians. A pilot study carried out in Georgia in 2011 showed that 71.4% of genotype 2 patients by

Correspondence: Dr Marine Karchava, Infectious Diseases, AIDS and Clinical Immunology Research Center, 16 Al Kazbegi Ave, Tbilisi 0160, Georgia. Email: kmarika79@yahoo.com

Conflict of interest: The authors have no conflict of interest.

Received 9 February 2017; revision 2 March 2017; accepted 2 March 2017.

conventional genotyping are indeed infected with the RF1_2k/1b strain; therefore, its actual prevalence is underestimated.[2,3]

Due to the extremely rare occurrence of RF1_2k/1b strain in HCV clinical trial patients worldwide, no official recommendation exists on diagnostic standards or effective treatment.[12] Only a limited number of studies have reported treatment outcomes among patients infected with the RF1_2k/1b strain because this strain was underdiagnosed. In these reports, patients were treated with direct-acting antiviral (DAA) regimens approved for HCV genotype 2, resulting in low sustained virologic response (SVR).[10,13] These authors suggested that the RF1_2k/1b strain behaved as genotype 1 and low cure rates could be due to the fragments of the NS5B region of HCV genotype 1.[13] Thus, the authors proposed treatments based on sofosbuvir (SOF), ribavirin (RBV), and pegylated interferon (IFN) in combination for 12 weeks, which were the optimal regimens for HCV genotype 1 at the time of publication.

Georgia made significant efforts to address the very high burden of HCV infection in the country by implementing a national program to increase the affordability of HCV treatment. In partnership with the US Centers for Disease Control and Prevention (CDC) and commitment from Gilead Sciences to donate DAAs, Georgia launched the world's first hepatitis C elimination program in April 2015. The program primarily builds on the concept of treatment as prevention and sets ambitious targets of 90–95–95, implying that, by 2020, 90% of HCV-infected persons are diagnosed, 95% of those diagnosed are treated, and 95% of persons who receive treatment are cured.[14] By the end of 2016, the elimination program had already treated up to 30 000 patients with either SOF- or ledipasvir (LDV)/SOF-based regimens donated by Gilead Sciences. It should be noted that these DAAs were available within the program.

A national population-based survey conducted in Georgia in 2015 reported that HCV genotype 2 is the third most common genotype, accounting for 24.5% of all HCV infection in the country (Ministry of Labor, Health, and Social Affairs of Georgia, unpublished data, 2016). Given the high prevalence of RF1_2k/1b strain in the country, we can assume that a significant proportion of these patients may receive inadequate treatment if this strain is not identified and treated as HCV genotype 2 within the national hepatitis C elimination program.

The objective of this study was to determine the prevalence of the RF1_2k/1b strain in Georgia and asses its impact on treatment outcomes within the national hepatitis C elimination program.

METHODS

Study settings and patients

THIS OBSERVATIONAL COHORT study was carried out at the Infectious Diseases, AIDS and Clinical Immunology Research Center, which is Georgia's largest provider of HCV care within the national hepatitis C elimination program.

The study enrolled HCV genotype 2 patients receiving care at the Infectious Diseases, AIDS and Clinical Immunology Research Center who started HCV treatment within the elimination program. Eligibility criteria included: (i) age ≥18 years; (ii) confirmed HCV infection with genotype 2 by conventional genotyping; (iii) plasma HCV RNA >3000 IU/mL; and (iv) ability to provide informed consent. Both treatment-naïve and treatment-experienced patients were eligible.

All patients received DAA-based treatment free of charge within the national hepatitis C elimination program in accordance with the national treatment protocols. The regimen was selected by a physician for each patient individually, taking into account various clinical characteristics, including tolerability, degree of liver damage, and previous treatment experience, as well as availability of drugs. From April 2015 through to February 2016, SOF was the only DAA available within the program and the following three regimens were recommended for genotype 2: SOF/RBV for 12 or 20 weeks, and IFN/SOF/RBV for 12 weeks. From March 2016, LDV/SOF was introduced in Georgia and the combination of LDV/SOF/RBV for 12 weeks was prescribed for all genotype 2 patients. Such DAA combinations were approved considering the high prevalence of RF1_2k/1b strain in Georgia that may respond better to genotype 1 DAA regimens than standard genotype 2 combinations. Sequencing analysis carried out within the study did not influence decisions on selection of treatment regimen.

Study approval

The study was approved by the Institutional Review Board of the National Center of Diseases Control and Public Health (NCDC #2015–038).

Hepatitis C virus RNA quantification and standard HCV genotyping

Hepatitis C virus RNA levels were determined by the COBAS TaqMan HCV Test, version 2 (Roche, Basel, Switzerland) with the quantification limit of 25 IU/mL. Patient specimens with detectable HCV viral load of more than 3000 IU/mL were genotyped before initiation of therapy as per national HCV treatment protocols.

Initial HCV genotyping was carried out using the Versant HCV Genotype version 2 Kit (Siemens, Ghent, Belgium). The kit is designed to reverse-transcribe and amplify 240 and 270 base pairs of the structural 5′-untranslated region (5′-UTR) and core region. After amplification, PCR products were immobilized on a nitrocellulose strip, which resulted in a visible banding pattern. The HCV genotyping results were then interpreted using the manufacturer's protocol.

Additional genotyping analyses by NS5B region sequencing

In order to identify possible infection with RF1_2k/1b strain, results from structural 5′-UTR/core and NS5B regions of HCV genome fragments were compared.

Hepatitis C virus RNA extraction and cDNA synthesis

For NS5B sequencing analysis, HCV RNA was extracted from 0.5 mL remnant plasma using manual extraction using the High Pure System Viral Nucleic Acid Kit (Roche, Basel, Germany) as per the manufacturer's RNA extraction protocol.

Amplification and sequencing of the NS5B region

Extracted HCV RNA was subjected to PCR using the Qiagen One-step RT-PCR kit (Qiagen, Foster City, CA, USA) and primers HCV DM 100 (5′-tacctvgtcatagcctccgtgaa-3′) and HCV DM 101 (5′-ttctcrtatgayacccgctgyttt ga-3′).

Secondary PCR conditions were identical to the primary, except that primer HCV DM 101 was substituted with HCV PR 3 (5′-tatgayacccgctgytttgac tc-3′) and AmpliTaq Gold polymerase (5 U/μL) with 10× Buffer II and Magnesium Chloride (25 mM). This amplification step rendered a 337-bp amplicon, which was purified later and visualized on 1% agarose gel. The following sequencing reactions were carried out bidirectionally using the BigDye Terminator v1.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) and analyzed on a 3500xl Genetic Analyzer (Applied Biosystems) using the same primers used in the secondary PCR reactions.

Data collections were undertaken using 3500 series data collection software version 3.0 and sequence analysis software version 5.3 (Applied Biosystems). This protocol provided 337-bp NS5B products, which were manually edited for errors to generate consensus sequence.

A consensus sequence for each sample was saved in FASTA format and then analyzed in a web-based genotyping tool available from the BLAST program of the Los Alamos HCV sequence database (https://hcv.lanl.gov/content/sequence/BASIC_BLAST/basic_blast.html).

Next-generation sequencing of selected RF1_2k/1b specimens

Selected RF1_2k/1b strains were analyzed using next-generation sequencing to obtain near-full genome HCV sequences.

RNA library preparation was carried out using the NEBNext Ultra RNA Library Prep kit for Illumina (New England Biolabs, Hitchin, UK); RNA was reverse-transcribed, amplified with 12 PCR cycles using indexed primers, and then purified using the appropriate volume of Ampure XP beads (Beckman Coulter, Brea, CA, USA). Libraries were quantified (Qubit HS DNA assay kit; Invitrogen, Carlsbad, CA, USA) and assessed for fragment sizes (Bioanalyzer 2100, High Sensitivity kit; Agilent, Santa Clara, CA, USA). Metagenomic (host–pathogen) RNA sequencing libraries were sequenced with 500 cycle sequencing kit on the Illumina MiSeq sequencing system with v3 chemistry. Low-quality bases were trimmed from demultiplexed sequences using CLC Bio Workbench 8.5 (https://www.qiagenbioinformatics.com/). Human sequences were excluded by mapping reads to the human reference genomes available at the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/). Hepatitis C virus-derived paired reads were assembled de novo into contigs, and reads were mapped back to the assembly using CLC Bio Workbench 8.5. Genotypes 2k, 1b and recombinant RF 2k/1b available at the National Center for Biotechnology Information were used for analysis as references.

Statistical analysis

Statistical analyses were carried out using SAS software, Cary, NC, USA. Demographic, clinical, and laboratory data were extracted from medical records. Percentage with 95% confidence interval (CI) using exact binomial methods was used to describe the prevalence.

With regard to treatment outcomes, the end-point was SVR defined as undetectable plasma HCV RNA at least 12 weeks after completing treatment.

Bivariate comparisons were tested using Pearson's χ^2-test or Fisher's exact test as appropriate. Predictors of SVR were assessed in multivariate logistic regression analysis. The main explanatory variable was genotype (RF1_2k/1b vs. genotype 2). Other predictor variables included well-known covariates associated with treatment outcomes such as: age, gender, treatment regimen, cirrhosis, baseline HCV RNA level, alanine aminotransferase, aspartate aminotransferase, total bilirubin, albumin level, and platelet count. Because of the small sample size issue, treatment regimens of IFN/SOF/RBV and LDV/SOF/RBV

(standard regimen for genotype 1) were grouped in one category versus SOF/RBV (standard regimen for genotype 2) for either 12 or 20 weeks grouped in the other category. The results are presented as an odds ratio (OR) with 95% CIs. *P*-values <0.05 were considered statistically significant.

RESULTS

Study population

THE STUDY INCLUDED 148 HCV genotype 2 patients receiving HCV care within the national hepatitis C elimination program. Among them, 103 (69.5%; 95% CI, 61.5–76.9%) patients had RF1_2k/1b strain based on NS5B region sequencing, while the remaining 45 (30.4%; 95% CI, 23.1–38.5%) had HCV 2a, 2k, or 2c subtypes. Selected specimens with suspected RF1_2k/1b strain were also confirmed using HCV full genome sequencing technology. Recombinant breakpoint positions were observed at 3175 bp within the NS2 region, upstream of a 12-nt encoding the conserved region of tyrosine, asparagine/aspartic acid, histidine, and leucine (Fig. 1) as previously reported.[4] Analysis of treatment outcomes was limited to 136 persons (103 with RF1_2k/1b and 33 with genotype 2), who completed treatment and SVR was evaluated. Of 136 patients with complete SVR data, 119 (87.5%) were male, median age was 52.4 years (interquartile range, 46.6–56.3 years) and liver cirrhosis was observed among 47 (34.6%) patients (Table 1). Information on possible HCV transmission routes was not available in our cohort.

The majority of patients were treated with SOF/RBV 12 weeks (39.0%), followed by LDV/SOF/RBV 12 weeks (25.7%), SOF/RBV 20 weeks (20.6%), and IFN/SOF/RBV 12 weeks (14.7%) (Table 1).

Baseline characteristics are shown in Table 1.

Treatment efficacy and predictors of SVR

Sustained virologic response was achieved in 97.0% (32/33) of genotype 2 and 76.7% (79/103) of RF_2k/1b patients ($P = 0.009$), with a total SVR rate of 81.6% (111/136).

Eight patients had been previously treated with IFN/RBV-based regimens and all of them achieved SVR.

The highest SVR rate was observed among patients treated with LDV/SOF/RBV 12 weeks among both genotypes (100% SVR rate).

A statistically significant difference was observed among patients treated with SOF/RBV 12 weeks (100.0% in genotype 2 patients compare to 56.4% in RF1_2k/1b, $P = 0.002$) (Fig. 2).

Among patients with cirrhosis, the SVR rate in genotype 2 was 91.7% (11/12) compared to 80.0% (28/35) in RF1_2k/1b ($P = 0.66$). No genotype-specific differences were found in response to various regimens used in these patients (Fig. 3).

Among non-cirrhotic patients, genotype 2 had better response (SVR 100% [21/21]) compared to RF1_2k/1b (SVR 75% [51/68], $P = 0.009$). Statistically significant difference was observed in response rates to SOF/RBV for 12 weeks (100% genotype 2 vs. 56.4% RF1_2k/1b, $P = 0.002$) (Fig. 3).

Among patients with RF1_2k/1b, treatment with LDV/SOF/RBV for 12 weeks was superior (100%) to both SOF/RBV for 12 weeks (SVR 56.4% [22/39], $P < 00001$) and 20 weeks (SVR 79.2% [9/24], $P = 0.05$). Treatment

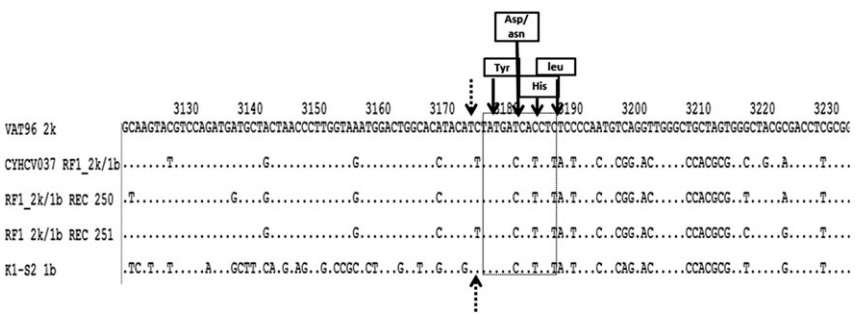

Figure 1 Alignment of amino acid sequences within the non-structural protein 2 region of hepatitis C virus strain RF1_2k/1b. Hepatitis C virus sequences retrieved from GenBank are indicated by the name of the strain or clone; k1-s2 (D50485), VAT96 (AB031663), and CYHCV037 (HQ537005) and study sequences RF1_2k/1b REC 250 and RF1_2k/1b REC 251. Nucleotide positions are numbered according to the subtype 1a H77. Dotted arrows indicate the position of the breakpoint upstream of the tyrosine (Tyr), asparagine/aspartic acid (Asp/Asn), histidine (His), and leucine (Leu) coding regions.

Table 1 Baseline demographic and clinical characteristics among patients with hepatitis C virus (HCV) genotype 2 and RF1_2k/1b

	All ($n = 136$)		Genotype 2 ($n = 33$)		RF 2k/1b ($n = 103$)	
Characteristics	***n***	**%**	***n***	**%**	***n***	**%**
Age, years						
<45	28	20.6	3	9.1	25	24.3
45–59	90	66.2	23	69.7	67	65.0
≥60	18	13.2	7	21.2	11	10.7
Median, IQR	52.4	46.6–56.3	55.8	52.8–59.8	50.5	45.4–55.5
Gender						
Female	17	12.5	4	12.1	13	12.6
Male	119	87.5	29	87.9	90	87.4
Regimen						
SOF/RBV for 12 weeks	53	39.0	14	42.4	39	37.9
SOF/RBV for 20 weeks	28	20.6	4	12.1	24	23.3
IFN/SOF/RBV for 12 weeks	20	14.7	2	6.1	18	17.5
LDV/SOF/RBV for 12 weeks	35	25.7	13	39.4	22	21.4
Cirrhosis						
No	89	65.4	21	63.6	68	66.0
Yes	47	34.6	12	36.4	35	34.0
HCV RNA						
<6 $\log_{10}$ IU/mL	66	48.5	13	39.4	53	51.5
≥6 $\log_{10}$ IU/mL	70	51.5	20	60.6	50	48.5
Median, IQR	6.02	5.4–6.5	6.2	5.3–6.6	6	5.4–6.4
ALT						
<2× ULN	92	67.6	21	63.6	71	68.9
≥2× ULN	44	32.4	12	36.4	32	31.1
Median, IQR	64.9	33.3–89.5	57.8	26.2–89.1	67.0	38.0–90.0
AST						
<2× ULN	105	77.2	26	78.8	79	76.7
≥2× ULN	31	22.8	7	21.2	24	23.3
Median, IQR	52.0	37.0–76.0	44.2	33.3–68.2	54.0	40.0–77.5
Total bilirubin						
<1.1 mg/dL	106	77.9	22	66.7	84	81.6
≥1.1 mg/dL	30	22.1	11	33.3	19	18.4
Median, IQR	0.94	0.63–1.08	0.97	0.77–1.11	0.94	0.62–1.08
Albumin						
≥3.2 g/dL	120	88.2	32	97.0	88	85.4
<3.2 g/dL	16	11.8	1	3.0	15	14.6
Median, IQR	3.7	3.3–4.5	3.7	3.3–4.3	3.8	3.3–4.5
Platelet count						
≥150 × 10^9/L	104	76.5	24	72.7	80	77.7
<150 × 10^9/L	32	23.5	9	27.3	23	22.3
Median, IQR	188	151–222	185	139–226	192	156–220

ALT, alanine aminotransferase; AST, aspartate aminotransferase; IFN, interferon; IQR, interquartile range; LDV, ledipasvir; RBV, ribavirin; SOF, sofosbuvir; ULN, upper limit of normal.

with IFN/SOF/RBV for 12 weeks also showed better response than SOF/RBV 12 weeks (88.9% vs. 56.4%, $P = 0.02$) in these patients.

Predictors of SVR were assessed in multivariate logistic regression analysis (Table 2). The regression model that included all patients (model 1) indicated that independent predictors of SVR were: HCV genotype 2 (OR, 18.01; 95% CI, 1.76–183.90; $P = 0.01$), treatment with LDV/SOF/RBV or IFN/SOF/RBV for 12 weeks among all patients (OR, 26.38; 95% CI, 4.53–153.60; $P = 0.0003$), whereas among other covariates studied only a higher platelet level showed borderline

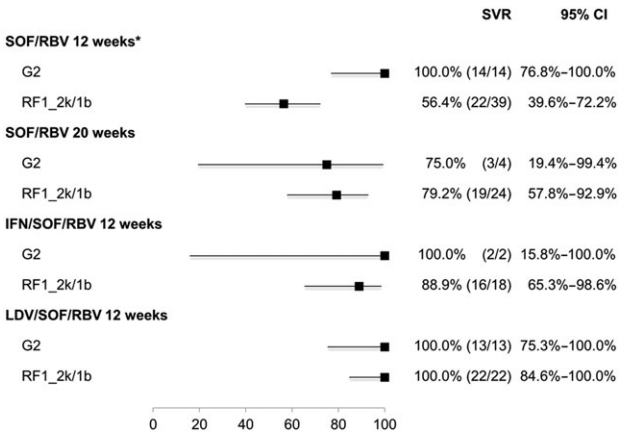

Figure 2 Sustained virologic response (SVR) rates in Georgians infected with hepatitis C virus recombinant form RF1_2k/1b or genotype 2 (G2) by treatment regimen ($n = 136$). *Statistically significant difference. CI, confidence interval; IFN, interferon; LDV, ledipasvir; RBV, ribavirin; SOF, sofosbuvir.

significance (OR, 3.76, 95% CI, 0.88–16.12, $P = 0.07$). Separate analysis among RF1_2k/1b patients (model 2) showed that only the treatment regimen was a highly significant predictor of SVR (OR, 21.42; 95% CI, 3.77–121.59; $P = 0.0005$) (Table 2).

DISCUSSION

IN THIS PROSPECTIVE observational cohort study, samples from HCV genotype 2 patients were re-analyzed for RF1_2k/1b strain. Only one-third of these samples originally typed as HCV genotype 2 were confirmed by NS5B region sequencing. The high discrepancy between the two typing methods was due to the misclassification of the RF1_2k/1b strain by initial conventional genotyping.

Until recently, HCV recombination has been thought to be a rare event that played an insignificant role for global HCV infection. However, it was reportedly detected among ethnic Georgians residing in the European Union[8,9,15] and former Soviet Union,[4] and later in studies undertaken in Georgia, Italy, and Germany.[2,3,10,13] These reports show not only the occurrence of this strain, but also cases of RF1_2k/1b patients failing on standard SOF/RBV therapy.[10,13,16]

Clinical trials as well as real-life studies have revealed the high efficacy of SOF/RBV combination therapy among patients with HCV genotype 2, regardless of liver damage and treatment history.[17–21] However, due to the underestimation of RF1_2k/1b globally it has not been systematically addressed until now. Therefore, current HCV treatment guidelines do not include optimal DAA regimens,[12] thus patients with this strain are being undertreated by either the SOF/RBV combination for 12, 16, or 20 weeks, or IFN-based regimens, depending on their liver damage or treatment history.[22]

In this study, we report the effectiveness of SOF/RBV for 12 and 20 weeks, IFN/SOF/RBV for 12 weeks, and LDV/SOF/RBV for 12 weeks among RF1_2k/1b patients receiving HCV care within the national hepatitis C elimination program in Georgia. Our study has several important implications for HCV epidemiology, care, and treatment. First, to the best of our knowledge, this study showed the highest prevalence of RF1_2k/1b strain among HCV genotype 2 patients in the world. This result is

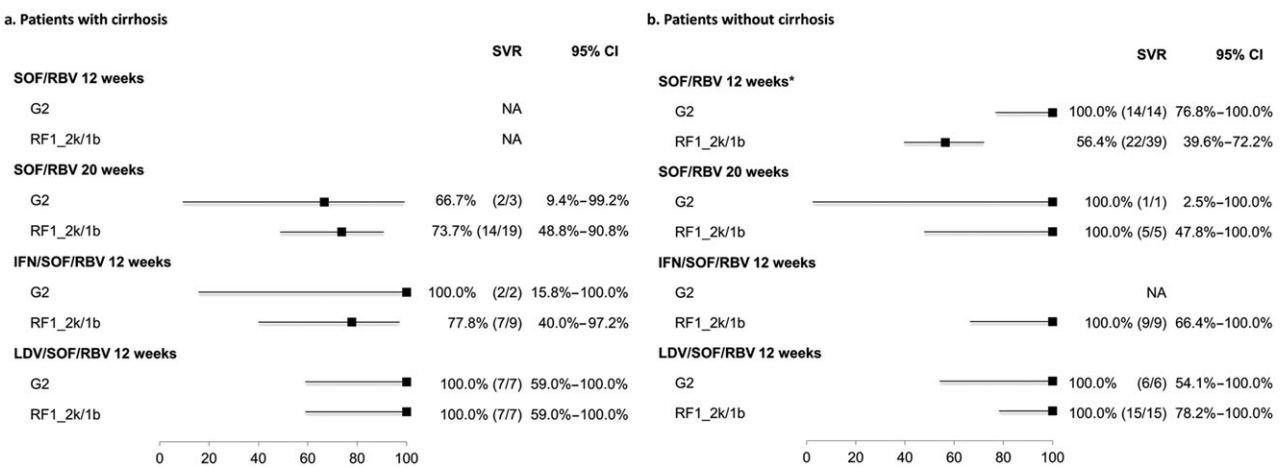

Figure 3 Sustained virologic response (SVR) rates in Georgians infected with hepatitis C virus recombinant form RF1_2k/1b or genotype 2 (G2) by treatment regimen, grouped according to the presence (a) or absence (b) of liver damage ($n = 136$). *Statistically significant difference. CI, confidence interval; IFN, interferon; LDV, ledipasvir; RBV, ribavirin; SOF, sofosbuvir.

Table 2 Factors associated with sustained virologic response (SVR) among patients with hepatitis C virus (HCV) genotype 2 and RF1_2k/1b in multivariate analysis

	Model 1, all patients ($n = 136$)				Model 2, patients with RF1_2k/1b ($n = 103$)			
Characteristics	**Total *n***	**SVR, *n* (%)**	**OR (95% CI)**	***P*-value**	**Total *n***	**SVR, *n* (%)**	**OR (95% CI)**	***P*-value**
Age, years								
<45	28	24 (85.7)	0.50 (0.06–4.52)	0.5400	25	21 (84.0)	0.52 (0.06–4.70)	0.5600
45–59	90	71 (78.9)	0.19 (0.03–1.26)	0.0900	67	49 (73.1)	0.22 (0.03–1.44)	0.1100
≥60	18	16 (88.9)	1.00		11	9 (81.8)	1.00	
Gender								
Female	17	15 (88.2)	4.81 (0.69–33.40)	0.1100	13	11 (84.6)	4.12 (0.61–27.67)	0.1500
Male	119	96 (80.7)	1.00		90	68 (75.6)	1.00	
Regimen								
IFN/SOF/RBV or LDV/SOF/RBV for 12 weeks	55	53 (96.4)	26.38 (4.53–153.60)	0.0003	40	38 (95.0)	21.42 (3.77–121.59)	0.0005
SOF/RBV for 12 or 20 weeks	81	58 (71.6)	1.00		63	41 (65.1)	1.00	
Genotype								
2	33	32 (97.0)	18.01 (1.76–183.90)	0.0100				
2k/1b	103	79 (76.7)	1.00					
Cirrhosis								
No	89	72 (80.9)	1.26 (0.36–4.47)	0.7200	68	51 (75.0)	0.99 (0.27–3.58)	0.9900
Yes	47	39 (83.0)	1.00		35	28 (80.0)	1.00	
HCV RNA								
<6 $\log_{10}$ IU/mL	66	53 (80.3)	0.63 (0.20–1.97)	0.4200	53	41 (77.4)	0.72 (0.23–2.28)	0.5800
≥6 $\log_{10}$ IU/mL	70	58 (82.9)	1.00		50	38 (76.0)	1.00	
ALT								
<2× ULN	92	72 (78.3)	0.47 (0.09–2.58)	0.3800	71	52 (73.2)	0.51 (0,09–2.77)	0.4300
≥2× ULN	44	39 (88.6)	1.00		32	27 (84.4)	1.00	
AST								
<2× ULN	105	83 (79.0)	0.25 (0.03–1.89)	0.1800	79	58 (73.4)	0.29 (0.04–2.11)	0.2200
≥2× ULN	31	28 (90.3)	1.00		24	21 (87.5)	1.00	
Total bilirubin								
<1.1 mg/dL	106	86 (81.1)	0.83 (0.19–3.57)	0.8000	84	65 (77.4)	0.94 (0.21–4.12)	0.9300
≥1.1 mg/dL	30	25 (83.3)	1.00		19	14 (73.7)	1.00	
Albumin								
≥3.2 g/dL	120	99 (82.5)	1.32 (0,30–5.75)	0.7200	88	68 (77.3)	1.29 (0.32–6.06)	0.6600
<3.2 g/dL	16	12 (75.0)	1.00		15	11 (73.3)	1.00	
Platelet count								
≥150 × 10^9/L	104	87 (83.6)	3.76 (0.88–16.12)	0.0700	80	63 (78.8)	3.15 (0.70–14.19)	0.1400
<150 × 10^9/L	32	24 (75.0)	1.00		23	16 (69.6)	1.00	

ALT, alanine aminotransferase; AST, aspartate aminotransferase; CI, confidence interval; IFN, interferon; IQR, interquartile range; LDV, ledipasvir; OR, odds ratio; RBV, ribavirin; SOF, sofosbuvir; ULN, upper limit of normal.

consistent with our previous findings.[2] We also reported that the RF1_2k/1b strain is common (more than 70%) among Georgian HCV genotype 2 specimens retrospectively collected and analyzed between 2003 and 2011.[2] Second, we reported the limited effectiveness of standard SOF/RBV 12-week treatment among patients infected with this strain. Our results are similar to the results reported by colleagues.[10,13] However, the numbers studied were small and statistically not significant. We suggest that the low efficacy of this regimen among RF1_2k/1b patients may be attributed to the incorporation of NS5B regions from HCV genotype 1 virus.[10,13] Third, we assessed DAA success rates with regard to multiple demographic and clinical parameters

such as age, sex, HCV RNA viral load, liver enzymes, total bilirubin, albumin, and platelets as well as cirrhosis status. Our study showed that these parameters did not play significant roles in achieving SVR among the RF1_2k/1b group. Finally, we report that the LDV/SOF/RBV treatment combination represents an excellent option for patients with RF1_2k/1b strain regardless of liver cirrhosis. The LDV/SOF combination with or without RBV for 12 weeks is a first-line DAA regimen recommended for HCV genotype 1, with a high SVR rate ranging between 93.0% and 99.0%.[23–25] Thus, LDV/SOF with RBV is the universal treatment option for genotype 2 as well as RF1_2k/1b patients.

Our study has several limitations. The relatively small patient number studied limits the statistical power for assessing the effectiveness of each DAA regimen. However, the study represents a unique paradigm that identified, analyzed, treated, and followed 103 patients infected with RF1_2k/1b strain. To the best of our knowledge, it is the largest number of RF1_2k/1b patients treated with various DAAs ever reported. Additionally, we did not sequence the NS2 breakpoint or HCV full genome among all suspected specimens to confirm the occurrence of RF1_2k/1b strains. We believe that studying the discrepancy between 5′-UTR/core and NS5B regions is an adequate method for identifying RF1_2k/1b infection, as similar studies rely only on such approaches.[26,27]

In conclusion, our study shows that the high prevalence of RF1_2k/1b strain among Georgian HCV patients can significantly affect treatment outcomes. Treatment combinations using IFN, SOF, and RBV, and especially LDV/SOF/RBV, ensured significantly higher cure rates in patients infected with RF1_2k/1b strain compared to standard HCV genotype 2 treatment with 12 or 20 weeks of SOF/RBV.

Findings of our study underline the need for reassessing existing methods for the management of HCV genotype 2 infections, especially in areas with high prevalence of the RF1_2k/1b strain.

ACKNOWLEDGMENTS

THIS STUDY WAS funded by the Department of Public Health and Human Services, National Center for Diseases Control and Prevention, Georgia (grant number 1U2GGH001658-01).

REFERENCES

1 National Statistics Office of Georgia. *Main statistics: population*: Tbilisi, GeorgiaNational Statistics Office of Georgia, 2016 Available from: http://geostat.ge/index.php?action=page&p_id=152&lang=eng.
2 Karchava M, Waldenstrom J, Parker M *et al.* High incidence of the hepatitis C virus recombinant 2k/1b in Georgia: recommendations for testing and treatment. *Hepatol Res.* 2015; **45**: 1292–8.
3 Tsertsvadze T, Karchava M, Sharvadze L, Gatserelia L, Dolmazashvili E. Discrepancy between HCV structural and non structural genes in Georgian genotype two patients. *Georgian Med News* 2014; **235**: 74–8.
4 Kalinina O, Norder H, Mukomolov S, Magnius LO. A natural intergenotypic recombinant of hepatitis C virus identified in St. *Petersburg J Virol* 2002; **76**: 4034–43.
5 Moreau I, Hegarty S, Levis J *et al.* Serendipitous identification of natural intergenotypic recombinants of hepatitis C in Ireland. *Virol J* 2006; **3**: 95.
6 Tallo T, Norder H, Tefanova V *et al.* Genetic characterization of hepatitis C virus strains in Estonia: fluctuations in the predominating subtype with time. *J Med Virol* 2007; **79**: 374–82.
7 Kurbanov F, Tanaka Y, Avazova D *et al.* Detection of hepatitis C virus natural recombinant RF1_2k/1b strain among intravenous drug users in Uzbekistan. *Hepatol Res* 2008; **38**: 457–64.
8 Demetriou VL, Kyriakou E, Kostrikis LG. Near-full genome characterisation of two natural intergenotypic 2k/1b recombinant hepatitis C virus isolates. *Adv Virol* 2011; **2011**: 710438.
9 Morel V, Descamps V, Francois C *et al.* Emergence of a genomic variant of the recombinant 2k/1b strain during a mixed hepatitis C infection: a case report. *J Clin Virol* 2010; **47**: 382–6.
10 Susser S, Dietz J, Barak M *et al.* Prevalence and clinical importance of hepatitis c virus genotype 2 k/1b chimeras. *J Hepatol* 2016; **64**(Suppl): S136.
11 Kalinina O, Norder H, Magnius LO. Full-length open reading frame of a recombinant hepatitis C virus strain from St Petersburg: proposed mechanism for its formation. *J Gen Virol* 2004; **85**: 1853–7.
12 EASL Recommendations on Treatment of Hepatitis C 2016. *J Hepatol* 2017; **66**: 153–94.
13 Hedskog C, Doehle B, Chodavarapu K *et al.* Characterization of hepatitis C virus intergenotypic recombinant strains and associated virological response to sofosbuvir/ribavirin. *Hepatology* 2015; **61**: 471–80.
14 Mitruka K, Tsertsvadze T, Butsashvili M *et al.* Launch of a Nationwide Hepatitis C Elimination Program – Georgia, April 2015. *MMWR Morb Mortal Wkly Rep* 2015; **64**: 753–7.
15 Ramiere C, Tremeaux P, Caporossi A *et al.* Recent evidence of underestimated circulation of hepatitis C virus intergenotypic recombinant strain RF2k/1b in the Rhone-Alpes region, France, January to August 2014: implications for antiviral treatment. *Euro Surveill* 2014; **19**(43): 20944.

16 Paolucci S, Premoli M, Ludovisi S, Mondelli MU, Baldanti F. HCV intergenotype 2k/1b recombinant detected in a DAA-treated patient in Italy. *Antivir Ther* 2017.
17 Zeuzem S, Dusheiko GM, Salupere R *et al.* Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *N Engl J Med* 2014; **370**: 1993–2001.
18 Lawitz E, Gane EJ. Sofosbuvir for previously untreated chronic hepatitis C infection. *N Engl J Med* 2013; **369**: 678–9.
19 Jacobson IM, Gordon SC, Kowdley KV *et al.* Sofosbuvir for hepatitis C genotype 2 or 3 in patients without treatment options. *N Engl J Med* 2013; **368**: 1867–77.
20 Tacke F, Gunther R, Buggisch P *et al.* Treatment of HCV genotype 2 with sofosbuvir and ribavirin results in lower sustained virological response rates in real life than expected from clinical trials. *Liver Int* 2017; **37**: 205–11.
21 Welzel TM, Nelson DR, Morelli G *et al.* Effectiveness and safety of sofosbuvir plus ribavirin for the treatment of HCV genotype 2 infection: results of the real-world, clinical practice HCV-TARGET study. *Gut* 2016.
22 EASL Recommendations on Treatment of Hepatitis C 2015. *J Hepatol* 2015; **63**: 199–236.
23 Reddy KR, Bourliere M, Sulkowski M *et al.* Ledipasvir and sofosbuvir in patients with genotype 1 hepatitis C virus infection and compensated cirrhosis: an integrated safety and efficacy analysis. *Hepatology* 2015; **62**: 79–86.
24 Afdhal N, Reddy KR, Nelson DR *et al.* Ledipasvir and sofosbuvir for previously treated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1483–93.
25 Afdhal N, Zeuzem S, Kwo P *et al.* Ledipasvir and sofosbuvir for untreated HCV genotype 1 infection. *N Engl J Med* 2014; **370**: 1889–98.
26 De Keukeleire S, Descheemaeker P, Reynders M. Diagnosis of hepatitis C virus genotype 2k/1b needs NS5B sequencing. *Int J Infect Dis* 2015; **41**: 1–2.
27 De Keukeleire S, Descheemaeker P, Reynders M. Potential risk of misclassification HCV 2k/1b strains as HCV 2a/2c using VERSANT HCV Genotype 2.0 assay. *Diagn Microbiol Infect Dis* 2015; **82**: 201–2.