Recent evidence of underestimated circulation of hepatitis C virus intergenotypic recombinant strain RF2k/1b in the Rhône-Alpes region, France, January to August 2014: implications for antiviral treatment

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Since the beginning of 2014, hepatitis C virus (HCV) recombinant forms RF2k/1b have been detected in the Rhône-Alpes French region in 10 patients originating from the Caucasus area. Circulation of this particular HCV strain is very likely to be underestimated. It is also prone to be misgenotyped when using genotyping methods based on the 5' region of the viral genome, which may lead to suboptimal treatment.

Here we report the detection of 10 patients infected with a particular recombinant form (RF) of hepatitis C virus (HCV), RF2k/1b, in two virology laboratories in the university hospitals in Lyon and Grenoble in the Rhône-Alpes region, France.

Case description

The first RF was identified in a patient in their 50s born in the Caucasus and followed up in a hepato-gastroenterology unit in Lyon for HCV-induced cirrhosis. This infecting virus was first classified as a genotype 2a/2c HCV strain, based on genotyping results using VERSANT HCV Genotype 2.0 Assay Line Probe Assay (LiPA, Siemens) which uses sequence information from both the 5' untranslated and the core regions of the viral genome. According to current European recommendations [1], a three-month course of sofosbuvir and ribavirin dual therapy was initiated in January 2014. Despite a rapid virological response, relapse occurred four weeks after treatment completion. Genotyping was repeated by sequencing of the NS3 region, which resulted in a clear classification as genotype 1b, highly suggestive of the presence of an HCV inter-genotypic recombinant form. To confirm this hypothesis, analysis

was completed with near full-length sequencing of the HCV genome using next-generation sequencing (NGS) on the same sample. Results confirmed the presence of an RF2k/1b strain with a breakpoint between genomic regions of genotype 2k and 1b localised in the NS2 region between nucleotide position 3,189 and 3,200 on the H77 reference genome (Figure 1), as previously observed in the reference RF2k/1b genome sequence (GenBank accession number: AY587845) [2].

Genotyping

Following this identification, the virology laboratory in Lyon, as well as the collaborating laboratory in Grenoble, decided to perform two genotyping methods, namely NS3 sequencing and either LiPA 2.0 or core sequencing, for each new genotyping request when one of the following criteria were met: patients with previous treatment failure infected with HCV genotype 2 (based on previous LiPA 2.0 results), NS3 or NS5B sequence clustering with available RF sequences in GenBank or patients born in Russia or in the countries of the former Soviet Union, as these regions represent the major source of circulating HCV recombinants described to date. This strategy led to the detection of the RF2k/1b strain in nine additional patients (two in Grenoble and seven in Lyon). Of these 10 patients, six were born in Georgia, three in Armenia and one in Azerbaijan. Five patients were people who inject drugs (PWID) or had been in the past. Two patients had a history of surgical interventions performed in their home country. For the remaining three patients, putative ways of transmission were unclear. Based on anamnestic data, it was very likely that they had acquired

FIGURE 1

Bootscan plot of percentage permuted trees over nucleotide positions for the recombinant hepatitis C virus RF2k/1b strain NA_Armenia, isolated in France, May 2014

Trees were constructed with Kimura 2-parameter distances and the neighbour-joining algorithm. Subtypes with their corresponding colour code are indicated on the right.

HCV before their migration to France, but it was not possible to be absolutely certain on this point for two patients. At the time of the study, treatment had not yet been initiated in any of the nine additional patients, and a survey to test potential contacts, particularly in PWID, was still underway.

When HCV genotyping using LiPA 2.0 was performed, four or five positive bands were detected (numbers 5, 9, 10, 11, +/-12) leading to classification of the strains as genotype 2 or 2a/2c. Phylogenetic analysis of core sequences was performed for seven patients, and of NS3 sequences for all 10 patients (Figure 2). Core sequencing confirmed that the 5' fragment of these genomes belonged to genotype 2k (Figure 2a), whereas NS3 sequencing led to the classification of the 3' fragment of the viral genome region as 1b (Figure 2b). Overall, core and NS3 sequences from all strains clustered with previously described RF2k/1b and were distinct from reference sequences of non-recombinant genotype 2k and genotype 1b, respectively. GenBank accession numbers for all sequences obtained in this study are indicated in the Table.

Since the beginning of 2014, HCV from 21 patients originating from the Caucasus (i.e. born in Armenia, Azerbaijan or Georgia) have been genotyped in the

virology laboratory in Lyon: eight were RF2k/1b, eight were of genotype 1b, four of genotype 3a and one of genotype 4a. Patients in Grenoble are not described here in detail as some of their demographic data were incomplete.

Discussion

The first natural HCV recombinant form, RF2k/1b, was described in Saint Petersburg in 2002 [3]. Since then, 17 recombinant forms of HCV have been identified worldwide [2,4], but the RF2k/1b strain is the only circulating recombinant form for which several isolates with a supposed common origin have been described. Recombinant forms probably emerged in patients exposed to multiple HCV strains [5]. Concerning RF2k/1b, the time of its emergence has been estimated to be between 1923 and 1956, which coincides with the development of blood donation centres in the former Soviet Union [6]. However, despite a high rate of mixed infections among certain risk groups, in particular PWID and haemophiliacs, recombinant strains appear to constitute the minority among HCV circulating strains, probably due to the constraints of viral replication [6].

Among our patients, the RF2k/1b strain was, along with 1b, the most frequent genotype detected in patients

FIGURE 2

Phylogenetic analysis of hepatitis C virus RF2k/1b strains identified in this study and reference strains from GenBank, France, January–August 2014 (n=16)

Two dendrograms are presented based on 423 nt of the core region (A) and 465 nt of the NS3 region (B). Both analyses were performed using MAFFT software (version 7).

originating from the Caucasus area. Thus the recombinant RF2k/1b strain seems to have spread widely and appears to be one of the major strains infecting patients from these countries. Moreover, this RF was identified in 10 new patients during a single eight-month period, whereas only 37 isolates of this particular strain have been reported in GenBank to date since the first description in 2002 [2,4]. Based on these observations, it can be hypothesised that the number of patients infected with the RF2k/1b HCV strain (and maybe other recombinant HCV forms) is underestimated. During the study period, HCV genotyping has been verified by NS3 sequencing in 16 patients not born in the Caucasus region who were infected with HCV previously classified as genotype 2 by either LiPA or sequencing of the 5' non-coding region. No RF was detected in any of these patients; however, it cannot be excluded that this strain has spread outside of the Caucasus population. Indeed, the spread of these RF in Western European countries is poorly described and only a few previous reported cases of HCV RF2k/1b are available in studies from Cyprus, France, Ireland and the Netherlands [6-9]. In all these cases, the patients had their origin in Russia or Georgia. Supplementary studies will be necessary to determine the frequency

of this strain in France and other European regions, in particular among PWID.

HCV genotyping remains an essential criterion when considering the choice of antiviral treatment protocols, and the circulation of recombinant HCV strains must be taken into account. Even when using new highly efficient anti-HCV direct acting agents (DAA) such as sofosbuvir, misgenotyping may lead to a suboptimal treatment choice and eradication failure, as illustrated by the case reported here. Moreover, it has been shown in a recent study by Hedskog et al. that the response to the sofosbuvir and ribavirin combination, a regimen for HCV genotype 2, was similar in patients infected with different HCV RF2/1 recombinant forms and in patients infected with genotype 1 [4]. In particular, three of the four patients infected with HCV RF2k/1b in the study by Hedskog et al. relapsed following treatment with this regimen. HCV genotyping methods based on sequencing of portions of the NS3–3'UTR region of the genome should therefore be recommended and standardised, and particular attention should be paid to patients from the Caucasus region.

TABLE

GenBank accession numbers for hepatitis C virus sequences used in the phylogenetic analysis of this study, France, January–August 2014 (n=16)

Patient	GenBank accession number	
	Core	NS3
AM_Azerbaijan	KM591900	KM591886
KV_Armenia	KM591899	KM591887
ZN_Georgia	KM591898	KM591888
GS_Georgia	KM591897	KM591889
CA_Georgia	KM591896	KM591890
AS_Georgia	ND	KM591892
GA_Armenia	ND	KM591893
OK_Georgia	ND	KM591894
CD_Georgia	KM591895	KM591891
Near full-length genome		
NA_Armenia	KM495736	

ND: not done.

Conflict of interest

None declared.

Authors' contributions

Christophe Ramière, Pauline Trémeaux, Mary-Anne Trabaud, Patrice André, Sylvie Larrat performed laboratory diagnostics and viral characterisation. Christophe Ramière, Pauline Trémeaux, Alban Caporossi, Marie-Ange Thélou, Patrice Morand, Patrice André, Sylvie Larrat performed sequencing data analysis. Fanny Lebossé, François Bailly, Jean Nana, Vincent Leroy provided clinical care. Christophe Ramière, Pauline Trémeaux, Patrice Morand, Patrice André, Sylvie Larrat wrote the paper. All authors reviewed the manuscript before submission.

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