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Research article

C316N Polymorphism associated with resistance to HCV polymerase NS5B in treatment-naïve patient with chronic hepatitis C

Noureddine Raiss*[1,2] Azzeddine Annan[2], Sanae Lemrabet[2], Leila Medraoui[1], Hicham Oumzil[2,3]

[1] Research Center of Plant and Microbial Biotechnologies, Biodiversity and Environment. Faculty of Sciences, Mohammed V University. Rabat. Morocco

[2] Virology Department. National Institute of Hygiene. Rabat. Morocco

[3] Pedagogy and Research Unit of Microbiology and Genomic Center of Human Pathologies, School of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco

ABSTRACT

High genetic variability of the hepatitis C virus (HCV) due to copying errors during the viral cycle leads to the development of mutations, and resistance-associated variants (RAVs), even with the advent of direct-acting antivirals (DAAs). Assessment of the presence of these mutations is essential for targeted treatment regimens and proper infection management, as treatment is related to genotypes and developing mutations. The study investigates the presence of resistance mutations in the nonstructural protein 5B (NS5B) region in treatment naïve patients. 100 positive plasma samples from patients presented for a follow-up service of chronic HCV infection (CHC) at the National Institute of Hygiene (NIH) of Rabat-Morocco. NS5B sequencing revealed the presence of C316N in one treatment naïve patient of subtype 1b. Additionally, six treatment-naïve patients with subtypes 2a and 2i exhibited the presence of the M289L mutation.

Keywords: HCV, Direct-acting antivirals (DAAs), NS5B sequencing, C316N.

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Correspondence: M Noureddine raiss ✉ raiss.nouredine@gmail.com, **Orcid Id**: 0000-0002-4075-1525

Research Center of Plant and Microbial Biotechnologies, Biodiversity and Environment. Faculty of Sciences, Mohammed V University. Rabat. Morocco & Virology Department. National Institute of Hygiene. Rabat. Morocco

INTRODUCTION

Hepatitis C infection is a significant public health worldwide; 3% of the world's population is infected with this virus [1], which is asymptomatic, causing fibrosis and/or hepatocellular cancer (hepatocellular carcinoma).

HCV is characterized by very high genetic variability [2], which has resulted in the emergence of eight genotypes [3] and 86 confirmed subtypes [4]. The distribution of genotypes depends on geographical areas but also on populations at risk [5]. Morocco is classified as a low-endemic country, with an estimated HCV prevalence of 1.58% in the general population [6].

The most frequent genotypes in Morocco are genotypes 1 and 2, while genotypes 3 and 4 are less frequent [7]. The reference treatment is based on the combination of ribavirin and pegylated interferon alpha (peg-IFNα), leading to a sustained virological response (SVR) of 50% which depends on the genotype responsible for the infection [8]. Other treatment regimens are possible with Sofosbuvir.

The high level of virus variability, combined with a high virus replication rate and a low RNA polymerase fidelity rate (absence of corrective 5'-3' exonuclease activity), causes the development of resistance mutations [9].

The presence of resistance mutations in treatment naïve patients have already been reported in different countries [10]. Detecting these substitutions is essential for proper treatment management [11]. This study aims to identify NS5B mutations confer resistance in treatment naïve Moroccan patients.

Our findings could provide valuable insights into the clinical relevance of the C316N Polymorphism in HCV treatment-naive patients and inform the development of more effective and personalized HCV treatment strategies in Morocco. In addition, investigating the presence of the C316N Polymorphism in Morocco can contribute to the global effort to control and eliminate HCV.

MATERIALS AND METHODS

Ethics approval

The director of NIH-Rabat approved the study through Dahir N1–15-110 of August 4, 2015, promulgating law N28–13 on the protection of persons undergoing biomedical research. The law

provides special non-interventional or observational research provisions as stipulated in articles 2 and 26. Therefore, no application for authorization to the national Ethics Committees was required.

Study design

Selection and Description of participants

The National Institute of Hygiene, Rabat in Morocco, is the national reference center for monitoring respiratory viral infections, human immunodeficiency virus (HIV), and HCV infections.

Out of thousands of HCV-positive patients present for a blood donation infection monitoring service. 100 sample was collected between 2016 and 2017 and was the subject of this study. HCV viral load was quantified using the Abbott RealTime HCV assay (Abbott, USA). HCV RNA was extracted from 500 µL plasma in an automated extractor (m2000sp),

RNA Extraction and RT-PCR

According to the manufacturer's instructions, plasma collected between 2016 and 2017 was used for viral RNA isolation using Qiagen kits, the Qiamp Viral Mini Kit 250 (Qiagen, France). According to the suppliers ' instructions, HCV-RNA was extracted from 140 µL plasma samples using the Qiamp Viral Mini Kit 250 (Qiagen, France). The viral RNA was eluted on a final volume (60 µL) of elution buffer. Eluates were stored at -20 ° C until analysis.

RT-PCR of the NS5B region was amplified using in the GeneAmp PCR 9700 thermocycler, One-step RT-PCR (Qiagen, France). The pair primer ; Forward (NS5B-F) and reverse (NS5B-R) (Table1) was prepared at a final concentration of 0,6 µM[12–14].

Table 1: primers used for NS5B sanger sequencing

Genotype	ID	Sequence	Position
ALL genotype	NS5B-R	TATGAYACCCGCTGYT TTGACTC	8622-8644
ALL genotype	NS5B-F	GCNGARTAYCTVGTCA TAGCCTC	8256-8278

Sequencing reaction and RASs detection

Reverse transcription PCR (RT-PCR) products were purified using the ExoSAP-IT. And sequenced using The BigDye ™ Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher). The same NS5B-F and NS5B-R primers were used at a final concentration of 3.2µM.

Genotypes were determined by comparing the sequences' similarity with the NCBI database (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The Geno2pheno [HCV] (https://hcv.geno2pheno.org/) also analyzed the nucleotide sequence obtained for resistance to DAAs.

RESULTS

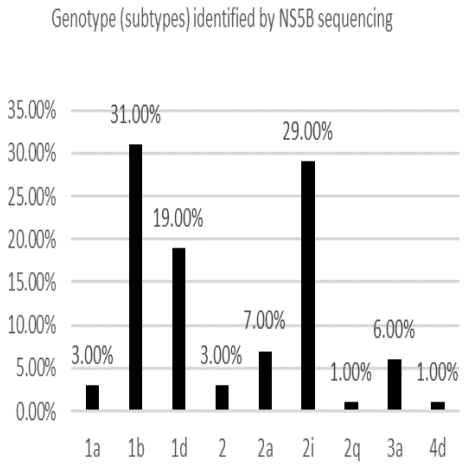
Genotype and subtype identification

Genotype 1 was the most frequent in all cases (53%), followed by genotypes 2 (40%), 3 (6 %), and 4 (1 %).

Different HCV subtypes were also detected, namely the 2a, 2i, 1b, 1a, 3a, 2, 2q, and 4d. The 1b (29%) subtype was the most predominant compared to the other subtypes (figure 1).

Figure 1 : Percentage of Genotypes and subtypes identified by NS5B sequencing

Resistance mutations



Resistance mutations and resistance-associated substitution (RASs) occur spontaneously in different regions of the genomes due to the absence of the corrective exonuclease activity of the viral polymerase. These mutations can occur throughout the viral genome, including the NS3, NS5A, and NS5B [15]. These mutations impact the treatment by affecting the sensitivity of the antiviral molecules.

Seven samples revealed mutations of interest in the NS5B region; 1 sample (MZ418185) in the subtype 1b with the C316N Polymorphism, 5 samples in 2a (MZ356321/ MZ418139/ MZ418155/ MZ418187/ MZ418217) with the 289L mutation and 1 sample in 2i with 289L substitution.

289L

In vitro, the M289L[15] mutation was detected in genotype 2a replicons before and after developing the S282T mutation that confers resistance to sofosbuvir[16].

C316N

The C316N mutation, including Sofosbuvir, has been reported as a variant conferring resistance to DAAs treatment [17].

DISCUSSION

Treatment of HCV has advanced significantly with the advent of direct-acting antivirals [18]. The efficacy, safety, and duration of treatment, typically 12 months [19], have made DAAs the best option for HCV treatment, with fewer side effects than peg-IFNα/Ribavirin therapy. Nonetheless, DAA-based treatments fail to eradicate HCV in 1–10% of patients, which may limit the long-term use of some HCV treatments.

Resistance mutation substitutions are the main cause of DAA failure [20]. These mutations can occur spontaneously before DAAs exposure or after drug exposure in different regions of the genomes, including the domains NS3, NS5A, and NS5B [15] t. These

mutations impact the treatment by affecting the sensitivity of the antiviral molecules.

NS5B is the primary target for DAAs that directly inhibit viral replication. Numerous mutations that reduce susceptibility to NS5B inhibitors have been reported and can occur naturally in treatment naïve patients. These substitutions appear due to the absence of the corrective exonuclease activity of the viral polymerase.

Studies have never been conducted in Morocco to look for mutations associated with drug resistance treatment naïve HCV patients. Analysis of over 1400 full-length HCV sequences published in the NCBI-Genbank database revealed a high mutation frequency in Africa [21].

The Moroccan Ministry of Health has joined the global vision of eradicating HCV infection through a national program that includes diagnosis, virological monitoring, and Therapy. Data on viral circulation remain highly disparate and dependent on the level of resolution of available techniques.

This study investigates the presence of mutations that confer resistance to DAAs, notably C316N Polymorphism associated with resistance to HCV ns5b polymerase in a treatment-naïve Moroccan patient with CHC.

Genotype 1 was the most prevalent. Our results corroborate the distribution reported by a study conducted between 2003 and 2010 at the Pasteur Institute of Morocco and the University Hospital of Casablanca [22]. The predominant subtype was 1b. Sequencing of a 350 bp segment of the NS5B region in our treatment-naive Moroccan patients revealed two substitutions in the NS5B region. One patient with subtype 1b was subject to the C316N Polymorphism associated with resistance to Sofosbuvir [23].

This substitution may prevent binding to Sofosbuvir. The second mutation was identified at position 289. The M289L mutation has been reported in Tunisia and can be the leading cause of Sofosbuvir resistance if associated with other substitutions such as S282T, T179A, and 293L [16].

CONCLUSION

Partial sequencing of NS5B by the reference method (Sanger sequencing) has demonstrated its robustness for HCV genotyping and subtyping. The search for mutations revealed the existence of two polymorphisms in the NS5B region in our Moroccan treatment-naïve patients that result in resistance to Sofosbuvir. Identifying mutations before treatment is a key step; other mutations described in other regions such as NS3 must be taken into account in order provide maximum information before prescribing a treatment regimen to avoid therapeutic failures and predict the response to treatment.

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Conflict of interest:

The authors declare no conflict of interest.

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