Hepatitis C virus genotypes in Tirana, Albania

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1. Introduction

Hepatitis C virus (HCV) genotype identification is clinically important because it predicts the rate of sustained virological response and contributes to the determination of treatment duration. In addition, HCV genotyping is a powerful tool to describe HCV epidemiology. Very few data exist on HCV infection in Albania. The prevalence of HCV antibodies was found to be 0.07% in blood donors, 2.3% and 0.3% in Albanian emigrant populations in Greece and southern Italy, respectively, and 14% and 11% in 1995 and 2005, respectively, for patients with viral and/or alcoholic liver disease at the University Hospital of Tirana, the capital city. To our knowledge, HCV molecular epidemiology has been assessed only once in Albania, using a line probe genotyping assay. We determined HCV genotype prevalence in Albanian patients by sequencing.

2. Materials and methods

Serum samples collected from 61 HCV-infected patients from January 2011 through May 2012 at the University Hospital Center (UHC) of Tirana, Albania, were analyzed. The HCV-infected patients were mostly on the following wards: hepatology (n = 22), nephrology including dialysis (n = 12), infectious diseases (n = 9), pediatrics (n = 4), and hematology (n = 2). Thirty-nine patients were men (64%). HCV genotyping was performed using in-house assays targeting initially the NS3 protease gene (543 nucleotides), then, in the case of negative PCR amplification of this gene, a 195-nucleotide-long fragment of the NS5b gene or an approximately 200-nucleotide-long region of the 5' untranslated region (UTR), HCV sequences were retrieved using a 3130XL Genetic Analyzer (Applied Biosystems, Branchburg, NJ, USA) then analyzed using Seqscape v2.5 (Applied Biosystems). HCV RNA sequences were
Figure 1. Phylogenetic tree based on partial sequences of the NS3 protease gene of the HCV genome (513 nucleotides; nucleotides 3420–3932 in reference to HCV genome accession number AF011751). HCV sequences obtained in the present study are indicated with the black boxes. Sequences with the highest BLAST score recovered from the NCBI GenBank nucleotide sequence database (indicated in boldface, underlined and labeled with BBH, GenBank accession number, country, and year of collection or submission) for sequences obtained in the present study have been incorporated in the phylogeny reconstruction in addition to sequences with known genotypes and subtypes (http://www.ncbi.nlm.nih.gov/projects/genotyping/view.cgi?db=3). Nucleotide alignments were performed using ClustalX v2.0 (http://www.clustal.org/download/current/). The tree was constructed using MEGA v5.0 software (http://www.megasoftware.net/) and the neighbor-joining method. Branches with bootstrap values ≥50%, obtained from 1000 resamplings of the data, are labeled on the tree. The HCV sequence EF108306 (genotype 7) was used as an outgroup. The scale bar indicates the number of nucleotide substitutions per site. (BBH, best BLAST hit; Ref, reference sequence.)
Figure 2. Phylogenetic tree based on partial sequences of the NS5b encoding gene of the HCV genome (195 nucleotides; nucleotides 8287–8481 in reference to HCV genome accession number AF011751). HCV sequences obtained in the present study are indicated with the black boxes. Sequences with the highest BLAST score recovered from the NCBI GenBank nucleotide sequence database (indicated in boldface, underlined and labeled with BBH, GenBank accession number, country, and year of collection or submission) for sequences obtained in the present study have been incorporated in the phylogeny reconstruction in addition to sequences with known genotypes and subtypes (http://www.ncbi.nlm.nih.gov/projects/genotyping/view.cgi?db=3). Nucleotide alignments were performed using ClustalX v2.0 (http://www.clustal.org/download/current/). The tree was constructed using MEGA v5.0 software (http://www.megasoftware.net/) and the neighbor-joining method. Branches with bootstrap values $\geq 50\%$, obtained from 1000 resamplings of the data, are labeled on the tree. The HCV sequence EF108306 (genotype 7) was used as an outgroup. The scale bar indicates the number of nucleotide substitutions per site. (BBH, best BLAST hit; Ref, reference sequence.).
aligned using ClustalX v2.0 (http://www.clustal.org/download/current/) with sequences from the reference set of the NCBI genotyping tool (http://www.ncbi.nlm.nih.gov/projects/ncbi/ClustalW2/index.cgi?db=3) and the two best matches obtained through BLAST searches against the NCBI sequence database for each sequence recovered here. Pairwise nucleotide similarities were generated using BioEdit (http://www.mbio.ncsu.edu/bioedit/page2.html). Phylogeny reconstructions were performed using MEGA v5 (http://www.megasoftware.net/).

3. Results

HCV RNA was obtained from 50 blood samples, PCR amplification being negative in 11 cases. HCV NS5b protease sequences were obtained for 28 sera (Figure 1) and the NS5b gene fragment (Figure 2) and 5’UTR were obtained for 18 and four additional sera, respectively. The predominant genotype was 1b, found in 25 patients (50%). Other genotypes, in decreasing prevalence, were genotype 2c (n = 9, 18%), 4a (n = 7, 14%), 3a (n = 4, 8%), 1a (n = 3, 6%), and 2a and 4d (n = 1, 0.2% each). Best BLAST matches for HCV-1b had been recovered in the USA, France, Switzerland, the UK, Brazil, Australia, and Japan (Figures 1 and 2). Best matches for HCV-2c had been obtained in Japan, Argentina, Italy, the UK, and France, while those for HCV-4a had been obtained in Egypt, Cyprus, and France. HCV-1b NS3 and NS5b sequences from the present study showed a mean ± standard deviation identity of 91.3 ± 1.6% and 94.2 ± 2.2%, respectively. HCV-1b NS3 RNA from two patients (numbers 860 and 639) were clustered together and showed 98.8% identity (Figure 1). These sequences had been recovered in nephropathy and dialysis units. In addition, HCV-1b NS5b RNA from three patients in a pediatrics unit (numbers 623, 188 and 290) were clustered and showed 97.5–99.0% identity, and HCV-4a RNA from four patients including three dialysis patients (numbers 33p, 839, 795, and 323) were clustered and showed 97.0–99.5% identity. Overall, five of eight patients (62%) sampled in the dialysis ward were infected with HCV-4a. Of note, amino acid substitution V55A within the HCV NS3 protease was observed in one patient naive to anti-HCV therapy (number 369) and infected with HCV-1a.

4. Discussion

To our knowledge, we have provided the first HCV sequences from patients in Albania. Half of these patients were infected with HCV-1b. In a previous study conducted on 18 patients with chronic liver diseases in Tirana using a line probe genotyping assay, HCV-1b was identified in 11 cases (61%), then HCV-2a/2c and HCV-3a were identified in five and two patients, respectively. This distribution of HCV genotypes is similar to that observed here. In addition, the predominance of HCV-1b has been reported in other southeastern European countries, including Greece, Serbia, Montenegro, Slovenia, Croatia, and Bosnia and Herzegovina (http://hcv.lanl.gov/components/sequence/HCV/geo/geo.comp). Best matches for HCV RNA obtained here were obtained in multiple countries on the five continents, but not in southeastern Europe, apart from Cyprus. This finding may be explained by the small number of HCV sequences available from southeastern Europe, especially when regarding the NS3 protease region.

The population studied here may not be fully representative of that of HCV-infected patients in Albania. Notably, only a few patients likely acquired HCV through intravenous drug use, as this population is managed in other centers. In one HCV-1a-infected patient, amino acid 55A was observed within the NS3 protease in the absence of any prior anti-HCV therapy. The HCV NS3-V55A substitution was associated with minor drug resistance to linear HCV NS3 protease inhibitors and compromised viral fitness. Further studies, conducted on larger and different populations and in different regions, are needed to strengthen knowledge on HCV genotypes that circulate in Albania.

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References