

# GB Virus C Infection among Lithuanian Population with Hepatitis C (HCV) Virus Infection

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## Abstract

**Background:** GB virus C (GBV-C), also known as hepatitis G virus (HGV), is an enveloped virus with about 9,4Kb genome-length single-strand RNA. Based on similarity in genome structure with HCV, the GBV-C virus is classified as the *Flaviviridae* family virus. The GBV-C virus has a worldwide distribution and virus infections are common among healthy blood donors as well as among immunocompromised individuals. Previous studies showed the high GBV-C co-infection rate in hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) positive individuals. Based on genetic differences between the 5' untranslated region (5'UTR) sequences of GBV-C isolates, virus is classified into seven major genotypes and in several subtypes. The distribution of GBV-C genotypes varies geographically and information is still incomplete. The aim of this study was to determine the frequency the frequency of GBV-C and the GBV-C genotypes among the HCV positive individuals in Lithuania.

**Methods:** In this study, GBV-C RNA was isolated from serum samples of 170 patients with known HCV infection. The nested reverse transcriptase reaction was used to synthesize the complementary DNA (cDNA) and the fragment of 210 bp from 5'UTR region was amplified by nested RT PCR. The PCR products were sequenced and subjected to phylogenetic analysis by using reference sequences from each genotype obtained from GenBank (n=46). The analysis was computed using CLC bio version 6.6.5 and MEGA 5.2.

**Results:** Among 170 HCV positive patients, 36 (21.17%) were positive for GBV-C. Based on phylogenetic analysis of a short region (210 bp) in 5'UTR region two genotypes, 2a and 3 were classified.

**Conclusions:** In this study it was found a high frequency of the GBV-C genotype 2a in Lithuanian HCV positive patients. The presence of the genotype 3 may be correlated to the geographical location and history of Lithuania.

**Keywords:** GBV-C; HCV; Co-infection; Lithuania

## Background

GB virus C (GBV-C), also known as hepatitis G virus (HGV), is an enveloped virus with about 9.4 Kb genome-length single-stranded RNA [1]. The genome contains a long reading frame with an internal ribosomal entry site (IRES) at the genomic 5' region leading translation of a polyprotein. The polyprotein of close to 3000 amino acids is cleaved by cellular and viral proteases into two structural and five nonstructural proteins [2]. The 5' region coded structural proteins consist of the E1 and E2 surface glycoproteins. Although enveloped GBV-C particles were visualized by electron microscopy, the complete structure of nucleocapsid is still unknown [3]. Nonstructural proteins NS2 (protease), NS3 (serine protease/RNA helicase), NS4, NS5A, and NS5B (RNA-dependent RNA polymerase) are located at the 3' region [4].

GBV-C is classified as a member of the *Flaviviridae* family. Since the discovery of GBV-C, the virus was not assigned to any of three *Flaviviridae* genera (flavi, pesti and hepaci). Based on phylogenetic relationships, genome organization and pathogenic features of GB viruses, recently it has been proposed to classified GBV-C, together with GBV-A and GBV-D, as a members of a fourth *Flaviviridae* genus, named *Pegivirus* [5].

Although GBV-C has spread worldwide and virus infections are very common among healthy blood donors as well as among immunocompromised individuals, the virus appears to be nonpathogenic with beneficial effects. The results of recent studies showed that co-infection with GBV-C in the HIV-infected causes a reduction in mortality rates and better clinical outcomes [6]. Moreover

the similar structure of the genome, transmission routes and other properties of GBV-C and HCV suggest that GBV-C could be a promising model to study HCV [7].

GBV-C is classified into seven major genotypes and in several subtypes based on genetic differences between the 5' untranslated region (5'UTR) sequences of GBV-C isolates. The distribution of GBV-C genotypes varies geographically and information is still incomplete [8]. Genotype 1 is predominantly found in Western Africa, 2 in Europe and USA, 3 in Asia, 4 in Southeast Asia, 5 in South of Africa, 6 in Indonesia, and, more recently discovered, the subtype 7 in China [8-15].

The aim of this study was to determine the frequency of GBV-C RNA and the GBV-C genotypes among the HCV positive individuals in Lithuania. This is the first study that detects the presence of GBV-C in Lithuania.

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**Received** August 05, 2013; **Accepted** September 02, 2013; **Published** September 04, 2013

**Citation:** Valinciute A, Kiveryte S, Mauricas M (2013) GB Virus C Infection among Lithuanian Population with Hepatitis C (HCV) Virus Infection. J Antivir Antiretrovir 5: 132-136. doi:10.4172/jaa.1000076

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## Methods

### Study population

One hundred and seventy patients with hepatitis C were studied. The patients were sent by doctors to diagnose hepatitis C infection. All had RNA-positive quantitative test for HCV (artus<sup>®</sup> HCV RG RT-PCR Kit, Qiagen). A source of viral RNA was left-over extracted viral RNA samples that were stored at -80°C. The patients do not have co-infections with other viruses except HCV.

The protocol used in this study was approved by the Lithuanian Bioethics Committee.

### Detection of GBV-C RNA

The RNA was converted into cDNA with the use of reverse transcriptase SuperScript<sup>®</sup> III First-Strand Synthesis System (Invitrogen) according to the manufacturer's instructions. The reaction mixture together with 5 µl RNA was first allowed to react at room temperature for 5 minutes and then incubated at 50°C for 60 minutes. After incubation the samples were stored at -24°C.

Highly conserved nested primers for the 5'UTR of GBV-C genomes amplification were used to perform the nested PCR [16]. Briefly, 5 µl of the cDNA was added to 20 µl of a first round PCR mix containing: 12.5 µl AmpliTaq Gold<sup>®</sup> 360 PCR Master Mix (Invitrogen), 5.5 µl sterile distilled water and 1 µl of each primer (5 µM): GBV-C Fw1 (5'-GGC CAA AAG GTG GTG GAT GG-3', sense, outer) and GBV-C Rv1 (5'-ATT GAA GGG CGA CGT GGA CC-3', antisense, outer). Three microlitres of the first round PCR product was added to 22 µl of a second round PCR mix containing: 12.5 µl AmpliTaq Gold<sup>®</sup> 360 PCR Master Mix (Invitrogen, USA), 7.5 µl sterile distilled water and 1 µl of each primer (5 µM): GBV-C Fw2 (5'-GTG ATG ACA GGG TTG GTA GG-3', sense, inner) and GBV-C Rv2 (5'-GTA CGT GGG CGT CGT TTG CC-3', antisense, inner). Amplification consisted of 35 cycles for first and second round of PCR using the following temperatures: 95°C 30 s, 55°C 30 s and 72°C 30 s for both rounds. Ten microliters of the second-round 210 bp PCR products were detected on a 1.5% agarose gel prepared in 1×TBE buffer stained with SYBR<sup>®</sup> Safe DNA Gel Stain (Invitrogen) (Figure 1).

Amplified cDNA was purified using the QIAquick Gel Extraction Kit (Qiagen). All the purified PCR products were subcloned into the pCR2.1-TOPO vector (Invitrogen). Positive clones were identified and sequenced with M13 forward primer (5'-GTCGTGACTGGGAAAACCCCTGGCG-3'). Sanger sequencing was performed at Microsynth AG.

### Phylogenetic analysis

Nucleotide sequences were aligned using CLC Genomics Workbench 5.0 (CLC Bio). The distances of the nucleotide were estimated by the Jukes-Cantor method [17] and phylogenetic tree was constructed by the neighbor-joining method [18] using MEGA software v. 5.2 [19] based on 1000 bootstraps.

The sequences obtained in this work were subjected to phylogenetic analysis by using reference sequences from each genotype obtained from GenBank (n = 45) that have been published elsewhere. The accession numbers of each genotype were as following: Genotype 1: U59547, U59546, U59540, U59545, U59541, U59543, U59544, U59542; Genotype 2a: AF006959, AF006960, AF006963, AF006965, AF006957, AF006964, AF006958, AF006961, AF006962, AF031828; Genotype 2b: U59531, U59534, U59535, U59532, U59536, U59533, AF006976,

AF006975; Genotype 3: U86126, D90601, D87710, D87263, D87713, D87714, U94695, D87709, D87712, D87711, D87262; Genotype 4: DH021, DH019; Genotype 5: AY949771; Genotype 6: AB003292; Genotype 7: DH028, DH044, DH084, DH071.

## Results

GBV-C RNA was detected in 36 (21.17%) out of the 170 HCV RNA positive samples. The samples consisted of 72 females, 14 of them positive for GBV-C RNA, and 98 males, 22 of them positive for GBV-C RNA (Table 1). The alignment of the 5' UTR sequences from GBV-C isolates with representative isolates from each group is shown in Figure 2.

Phylogenetic analysis was performed based on 210 bp GBV-C 5'-UTR nucleotide sequence. Phylogenetic tree was reconstructed by the neighbor-joining method with 1000 bootstrap replicates. The 45 reference sequences of GBV-C isolates were included. The most GBV-C isolates present in this study fall within genotype 2a 97.22% (35/36) and one isolate was found to belong to the genotype 3 (2.78% (1/36)) (Figure 3).

## Discussion

GBV-C is common, non-pathogenic virus. The co-infection rate with GBV-C is frequent in patients with HCV and HIV infections due to similar routes of transmission [6]. In the present study GBV-C RNA was identified in 36 (21.17%) among the patients with HCV. Other studies have reported the rate of GBV-C in patients suffering from HCV infection varies from 11% to 43.6% [20-29].

To date seven genotypes have been identified in different regions. The genotypic distribution of GBV-C is associated with human evolution and migration in antiquity [30]. The distribution of genotypes on the world map corresponds to the human migration from Africa to Europe (genotype 2), northern Asia (genotype 3), and southern Asia (genotype 4) [31,32]. In this study the phylogenetic analysis revealed the presence of GBV-C genotypes 2a and 3 among Lithuanian population with HCV infection. Genotype 2a corresponds to the human and GBV-C co-evolution theory and genotype 3 could be correlated to the geographical location and history of Lithuania.

GBV-C has been shown to have no effect on the development of HCV infection, related chronic liver disease, or response to antiviral therapy [33]. However, GBV-C has a close sequence homology and genome organization to HCV and this offers the possibility to use GBV-C as a less expensive model for HCV study and vaccine

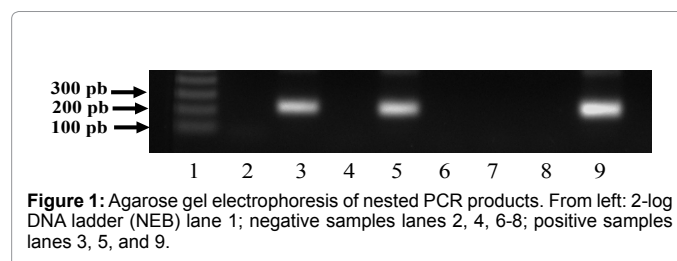
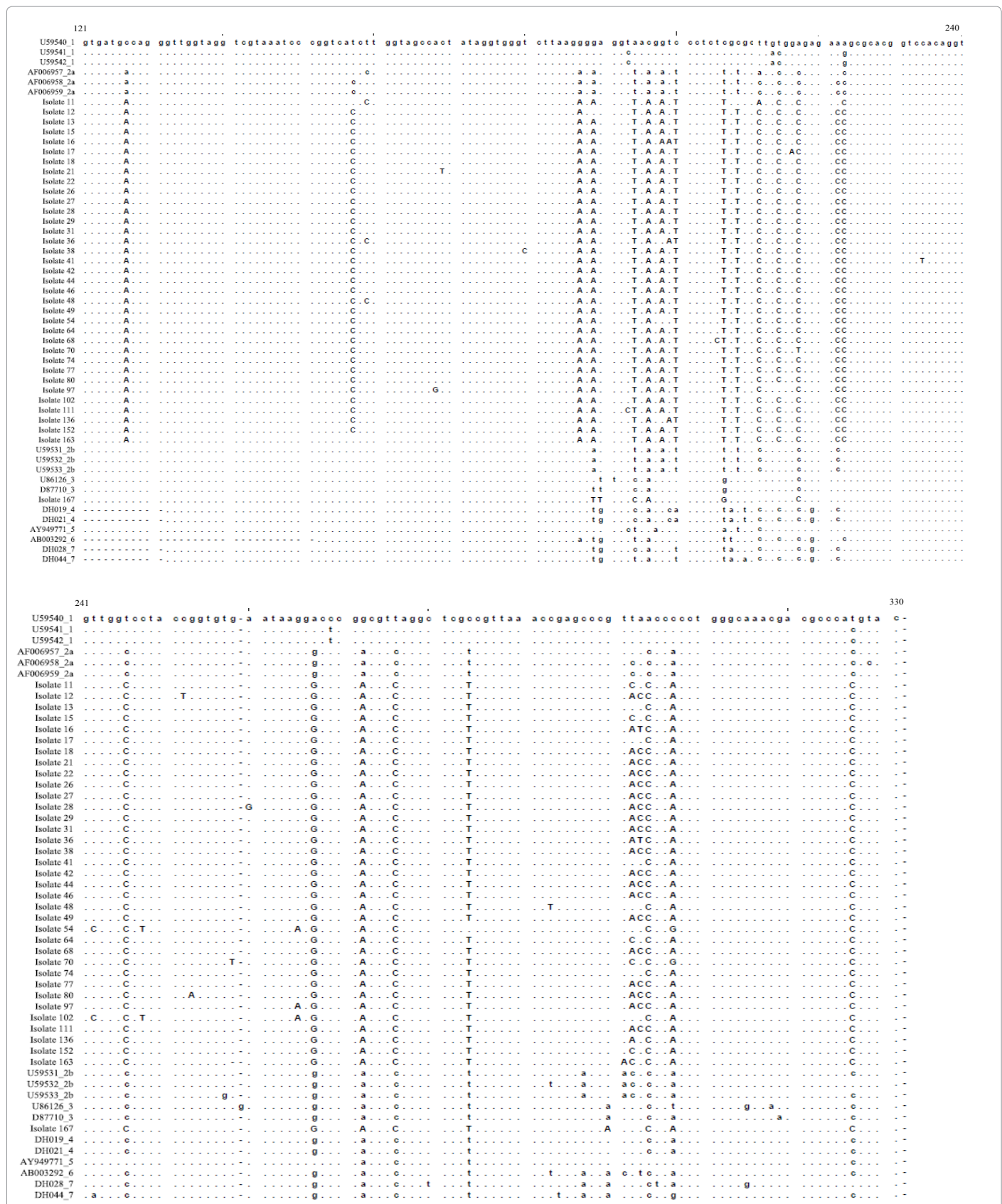


Figure 1: Agarose gel electrophoresis of nested PCR products. From left: 2-log DNA ladder (NEB) lane 1; negative samples lanes 2, 4, 6-8; positive samples lanes 3, 5, and 9.

Sex	Total HCV patients	HCV and GBV-C co-infected patients	HCV genotype					GBV-C genotype	
			1a	1b	2a/2c	3a	NA	2a	3
Male	98	22	11	-	9	2	22	-	
Female	72	14	8	1	-	5	13	1	

Table 1: HCV-infected and HCV, GBV-C co-infected patients' characteristics.



**Figure 2:** Alignment of nucleotide sequences of the GBV-C 5' UTR. The isolates with the accession numbers are described in the text. The nucleotide positions are based on the GBV-C isolate NC\_001710. Dots indicate bases identical to the top sequence (U59540) and dashes indicate deleted nucleotides.

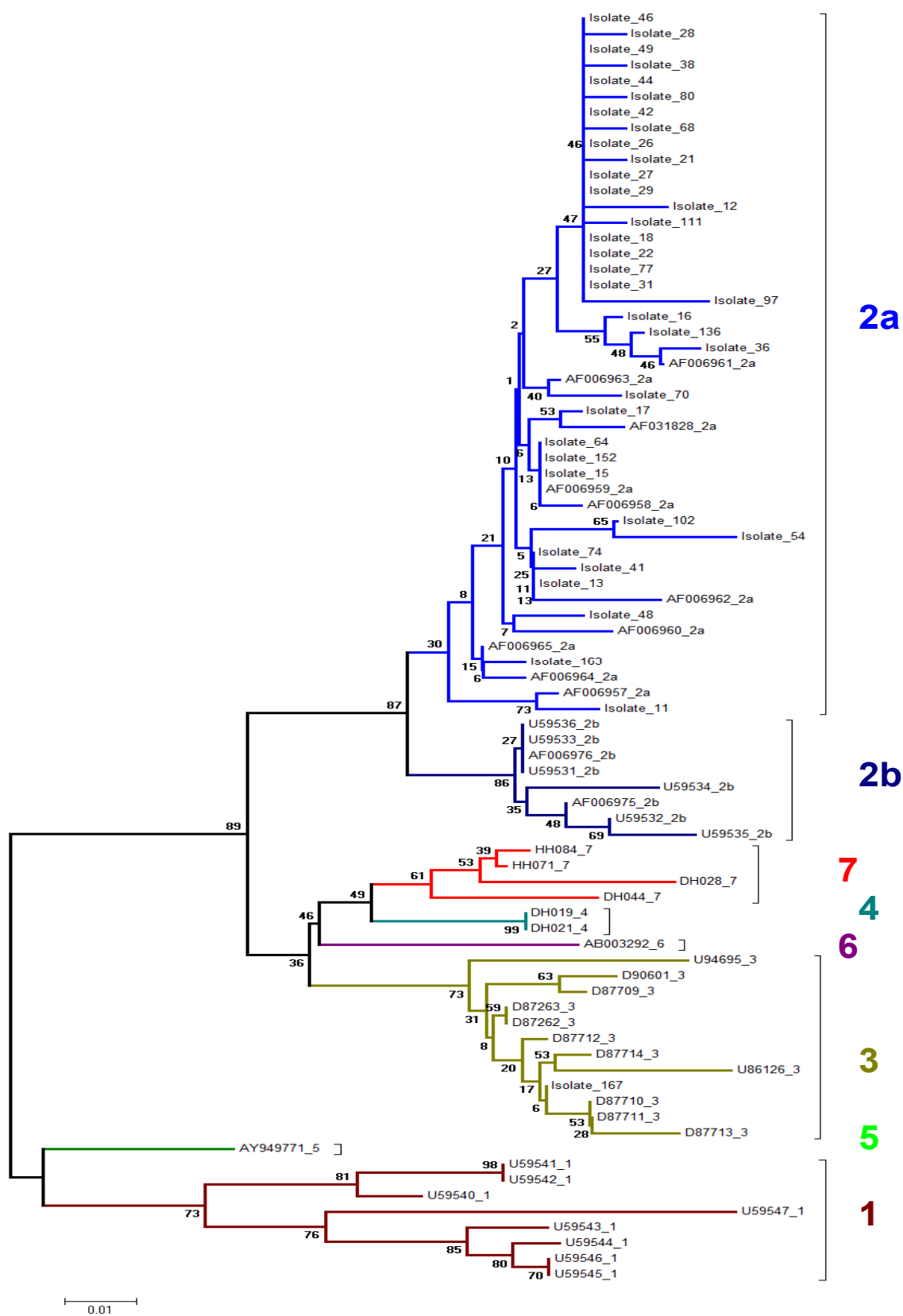


Figure 3: Phylogenetic tree generated by neighbor-joining analysis of genetic distances in the 5' UTR of GBV-C isolates. Bootstrap values are shown next to the branches.

development [6]. Information about the geographical distribution of the genotypes is still limited and the larger studies may be required.

## Conclusions

In conclusion, this is the first study that reported the GBV-C and HCV co-infection frequency among Lithuanian population and the first report presented data of GBV-C and HCV co-infection in Baltic countries. The prevalence of GBV-C in Lithuanian patients with HCV infection is similar to the findings in other countries and the genotype 2a is the most common among HCV patient in Lithuania.

## Competing interests

The authors declare that they have no competing interest.

## Acknowledgements

The authors acknowledge the support of State Research Institute Centre for Innovative Medicine for this study.

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