Prevalence of hepatitis C virus and distribution of its genotypes in Northern Eurasia

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Summary. We tested hepatitis C virus (HCV) antibody in 4,216 sera collected from healthy people living in European part of Russia (including Northern, North-Western, Central, Central-Blacksoil, Volga-Vyatka, Volga, and North-Caucasian regions), non-European part of Russia (the Urals, East-Siberia, and the Far-East regions) and Mongolia. Prevalence of HCV antibody varied significantly by regions, ranging from 0.7% in Central region of European part of Russia to 10.7% in Mongolia. Genotyping of HCV (into la, lb, 2a, 2b, and 3a) was performed on 469 sera from blood donors and patients (in Russia, Moldova, Turkmenistan, and Mongolia) who were positive for both HCV antibody and RNA. Genotype 1b was the most dominant genotype irrespective of regions (68.9%), with the highest rate in Moldova (96%). HCV unclassifiable into genotypes la-to-3a was found in 28 (6.0%) samples: particularly 4 of 10 samples from Lipetzk were untypable. Overall, HCV genotypes in European part of Russia were more similar to those in European countries, while those in Eastern part of Russia more similar to China or Japan. Genotype distribution was not associated with the clinical expression of HCV disease: acute hepatitis, chronic hepatitis or liver cirrhosis.

Introduction

Discovery of hepatitis C virus (HCV) genome in 1989 [4] invoked thereafter a wide spectrum of studies on nucleotide sequence of HCV isolates obtained worldwide, leading to our understanding that there exist various genotypes of HCV. According to Simmonds et al. [20] and Tokita et al. [24], there are 6 to
9 major genetic groups of HCV, each of which could be further branched into some subtypes. Among the genotypes so far recognized, there are 5 most popular genotypes designated as la, Ib, 2a, 2b, and 3a by Simmonds and his colleagues [20] (corresponding, respectively, to I, II, III, IV, and V by Okamoto and his colleagues’ nomenclature [13]).

Geographical distribution of HCV genotypes in the world, in particular, of the 5 popular genotypes from la to 3a, has been roughly outlined by now [10]. However, only scarce information is available for HCV genotypes in Northern Eurasia. In 1987, we began collecting sera from general populations in various regions within the former USSR to assess the prevalence of HCV infection by testing HCV antibody. At the end of 1993, however, we renewed our study and set forth a research program aimed for assessment of HCV genotypes, covering almost entire area of the former USSR: not only Russia but also Moldavia, Turkmenistan, and Mongolia that are contiguous to Russia.

In this report, we present data so far obtained through the program on the prevalence of HCV infection in the general population, and also a result of genotyping on HCV isolates from blood donors and patients of Northern Eurasia.

**Materials and methods**

**Blood sampling and antibody testing**

During the period from 1987 to 1993, sera were collected from a total of 4,216 individuals (students, healthy volunteers, and blood donors; 18 to 40 years of age; male/female ratio approx. 1:1) of general populations in European part of Russia (i.e., Northern, North-Western, Central, Central-Blacksoil, Volga-Vyatka, Volga, and North-Caucasian regions of Russia), non-European part of Russia (the Urals, East-Siberia, and the Far-East regions) and Mongolia. These sera were tested for HCV antibody using commercially available reagents (2nd generation ELISA, Ortho Diagnostic Systems). Storage conditions for sera collected during the period were not suitable for HCV RNA detection and/or genotyping.

Since the end of the 1993 until now, sera collected from blood donors and patients in Russia, Moldavia, Turkmenistan, and Mongolia have been tested for HCV antibody with use of another assay system (2nd generation ELISA, Pasteur Diagnostics) combined with a confirmatory test (RIBA-II, Ortho Diagnostic Systems). Antibody-positive samples were then tested for HCV RNA by RT-PCR, and RNA-positive samples were kept frozen thereafter at −20 °C or lower temperature for later analysis of HCV genotype.

**HCV RNA detection by nested RT-PCR**

RNA was extracted from 0.2–0.3 ml of antibody-positive sera using a “guanidine-isothiocyanate-phenol-chloroform” method [3]. cDNA synthesis was performed using HCV-specific antisense primer, #194, reported by Okamoto et al. [14] and AMV-derived reverse transcriptase (Promega) under the conditions similar to those described previously [2]. Amplification of HCV RNA-specific sequence was carried out by PCR with use of primers derived from a well-conserved 5’ untranslated region (5’ UTR) sequence of HCV genome, according to Okamoto’s method [14]. Briefly, the 1st-round PCR amplified a 5’ UTR sequence of 254 bp in length with primers #32/#194 (35 cycles of 94 °C–30 sec/55 °C–30 sec/72 °C–90 sec), and the 2nd-round PCR amplified an inner sequence of 207 bp in length.