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325 Table 1. HCV RNA testing by Roche COBAS AmpliPrep/COBAS TaqMan HCV assay
326 (CAP/CTM) and Abbott RealTime HCV (ART) in 243 sera undetectable HCV RNA
327 using Roche COBAS Amplicor HCV Monitor test, v.2.0.

	CAP/CTM undetectable	**CAP/CTM** detectable
ART undetectable	190	25
ART detectable	11	25

328 Table 2. PPV and NPV for SVR, using undetectable HCV RNA at weeks 4, 8, 12, 16, 20,
329 24 by Roche COBAS Amplicor HCV Monitor test, v.2.0 (CAM), Abbott RealTime
330 HCV (ART) and Roche COBAS AmpliPrep/COBAS TaqMan HCV assay
331 (CAP/CTM) .

	CAM		ART		CAP/CTM	
week	PPV	NPV	PPV	NPV	PPV	NPV
4	5/5 (100%)	34/55 (61.8%)	4/4 (100%)	34/56 (60.7%)	3/3 (100%)	34/57 (59.6%)
8	16/17 (94.1%)	33/43 (76.7%)	13/13 (100%)	34/47 (72.3%)	13/13 (100%)	34/47 (72.3%)
12	26/35 (74.3%)	25/25 (100%)	22/25 (88.0%)	31/35 (88.6%)	20/21 (95.2%)	33/39 (84.6%)
16	26/37 (70.3%)	23/23 (100%)	24/29 (78.1%)	29/31 (93.5%)	23/28 (82.1%)	29/32 (90.6%)
20	26/42 (61.9%)	18/18 (100%)	25/32 (78.1%)	27/28 (96.4%)	24/33 (72.7%)	25/27 (92.6%)
24	26/41 (63.4%)	19/19 (100%)	26/40 (65.0%)	20/20 (100%)	26/36 (72.2%)	24/24 (100%)

332 PPV, positive predictive value;
333 NPV, negative predictive value;
334 SVR, sustained virological response

335 **Legends to the Figures**

336

337 Fig. 1 Proportion of HCV RNA negative by Roche COBAS Amplicor HCV Monitor
338 test, v.2.0 (CAM), Abbott RealTime HCV (ART) and Roche COBAS
339 AmpliPrep/COBAS TaqMan HCV assay (CAP/CTM) at each period after the beginning
340 of treatment..

341

342 Fig. 2. Proportion of SVR according to week after the beginning of therapy when
343 HCV RNA was first undetectable by Roche COBAS Amplicor HCV Monitor test, v.2.0
344 (CAM), Abbott RealTime HCV (ART) and Roche COBAS AmpliPrep/COBAS
345 TaqMan HCV assay (CAP/CTM).

346

347 Fig. 3 Of 26 patients reached SVR, in three patients very low levels of HCV RNA
348 (positive results below the limit of quantification) were detectable by Abbott RealTime
349 HCV (ART) or Roche COBAS AmpliPrep/COBAS TaqMan HCV assay (CAP/CTM)
350 at the end of treatment. The outcome of HCV RNA testing by Roche COBAS Amplicor
351 HCV Monitor test, v.2.0 (CAM) represents positive (+) or negative (-). Positive results
352 of HCV RNA below the limit of quantification represent <1.08 log IU/mL by ART and
353 <1.2 log IU/mL by CAP/CTM. In Case 1, HCV RNA was detectable at the end of
354 therapy by CAP/CTM and at week 12 after the end of therapy by ART, but undetectable
355 afterward. In Case 2, HCV RNA was detectable at the end of therapy by CAP/CTM,
356 and in Case 3, was detectable by ART, but undetectable afterward.

Case 1 50 y.o. female PEG-IFN α 2b+RBV → After the end of therapy

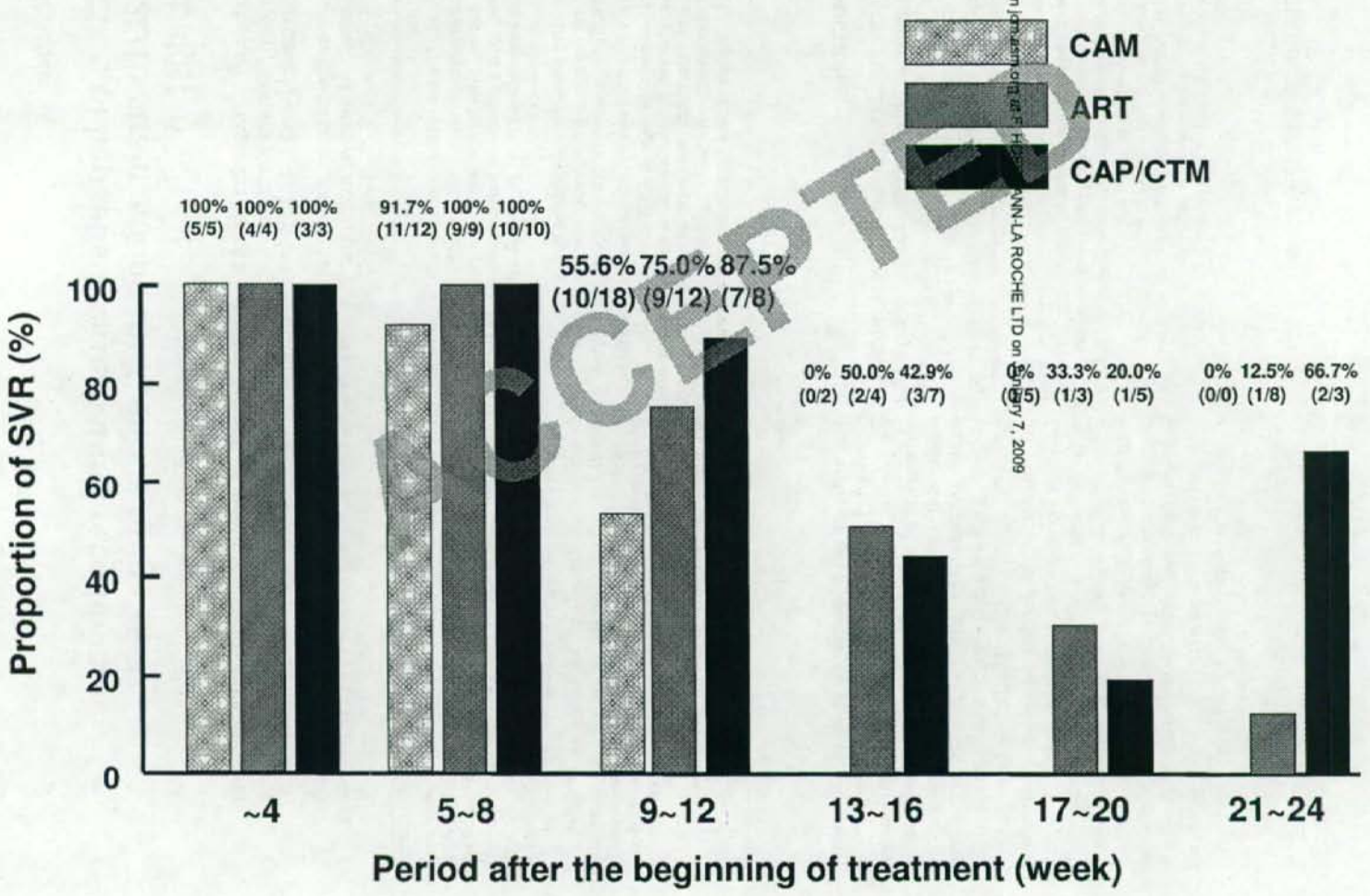
week	4	8	12	16	20	24	48	4	8	12	24	48
CAM	–	–	–	–	–	–	–	–	–	–	–	–
ART (log IU/mL)	–	–	–	–	–	–	–	–	–	<1.08	–	–
CAP/CTM (log IU/mL)	–	–	–	–	–	–	<1.2	–	–	–	–	–

Case 2 40 y.o. male PEG-IFN α 2b+RBV → After the end of therapy

week	4	8	12	16	20	24	48	4	8	12	24	48
CAM	+	–	–	–	–	–	–	–	–	–	–	–
ART (log IU/mL)	<1.08	–	–	–	–	–	–	–	–	–	–	–
CAP/CTM (log IU/mL)	<1.2	–	–	–	–	–	<1.2	–	–	–	–	–

Case 3 52 y.o. male PEG-IFN α 2b+RBV → After the end of therapy

week	4	8	12	16	20	24	48	4	8	12	24	48
CAM	+	–	–	–	–	–	–	–	–	–	–	–
ART (log IU/mL)	1.82	<1.08	–	–	–	–	<1.08	–	–	–	–	–
CAP/CTM (log IU/mL)	1.96	–	–	–	–	–	–	–	–	–	–	–



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Original Article

Detection of hepatitis C virus natural recombinant RF1_2k/1b strain among intravenous drug users in Uzbekistan

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Aim: A series of recent studies have indicated the presence of natural intergenotypic recombinant hepatitis C virus (HCV) strains in distinct parts of the world. The majority of the current genotyping methods are based on analysis of either 5'UTR, structural (Core/E1/E2) or non-structural (NS5B) genomic regions of the virus.

Methods: In the present study, based on both structural and non-structural regions, we determined the genotype of 55 anti-HCV-positive intravenous drug users (IDUs) in Uzbekistan.

Results: HCV-3a (67.3%) was the most prevalent genotype in this cohort, followed by HCV-1b (27.3%). A discrepancy in results was observed between structural and non-structural regions in one case (1.8%). Phylogenetically this strain was related to the previously reported RF1_2k/1b variant. Based on accumulated sequences, specific primers were designed for polymerase chain reaction (PCR) spanning the tentative intergenotypic crossover point of RF1_2k/1b. The sensitivity and specificity of the method were assessed using generated template clones of HCV-1b, 2a, 2k and RF1_2k/1b. The method was applied to 55 cases in the present study and only one case showed a positive result, indicating that in these individuals, the variant is not present as a minor quasispecies clone.

Conclusion: In conclusion, the finding of RF1_2k/1b in Central Asia indicates that the variant has wide geographic distribution. The PCR-based screening method developed in this study should be useful in further epidemiological and clinical studies on the recombination phenomenon in HCV.

Key words: 2k/ 1b, HCV genotyping, HCV, natural recombination

INTRODUCTION

BASED ON NUCLEOTIDE sequence heterogeneity in the genome and phylogenetic relationship, hepatitis C virus (HCV) has been classified into six genotypes (1 to 6) and numerous subtypes (1a, 1b, etc).[1,2] The genotypes are strongly associated with responses to antiviral therapy, thus making genotyping important in the determination of dose and duration of the therapy (reviewed in[3]). Characterization of genotypes and subtypes is also important for vaccine development and epidemiological investigations.[1] A series of recent studies in distinct parts of the world have indicated the presence of natural intergenotypic hybrid strains, having genotype 2 sequence in the structural, and genotype 1,[4–6] 5,[7] or 6[8] in the non-structural half of the genome. Since the majority of the genotyping studies performed to date have been based on analysis of either the structural or the non-structural parts of the HCV genome, there is a substantial possibility that the prevalence of natural recombinant HCV variants is underestimated. Moreover, a recent study on chimpanzees with different genotypes/subtypes of HCV indicated that the intersubtypic recombinant clones were present in two animals as a minor clone of quasispecies population,[9] which may

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The nucleotide sequence data reported in this paper (54 entries) will appear in the DDBJ/EMBL/GenBank nucleotide sequence databases with the accession numbers AB327107–AB327160.
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also result in underestimation of the natural recombinant strains if they present as a minor clone population in infected carriers.

Frequent HCV superinfection has been previously reported in intravenous drug users (IDUs), both intra- and inter-genotype,[10,11] which increases the possibility of the detection of recombinant strains in this category of the population.

In the present study we aimed to investigate the possibility of circulation of the intergenotypic recombinant strains among IDUs in Uzbekistan. Furthermore, we developed and evaluated an easy, sensitive and specific method allowing the detection of the recombinant RF1_2k/1b strain even when it is present in a minority among populations of quasispecies in a specimen.

METHODS

Studied population

THE ANTI-HCV-POSITIVE SERUM samples were collected in 2006, in the State Reference Laboratory of the Ministry of Public Health, Tashkent City, Uzbekistan. The sera were obtained from IDUs who were undergoing hospitalization at the National Narcological Clinic of Public Health, Tashkent City, Uzbekistan, during 2005 and 2006. The material collection was approved by the Institutional Ethics Committee and was conducted according to the Declaration of Helsinki. Risk factors for HCV infection were assessed during interview with each participant. Serum specimens were obtained only from patients who had no history of antiviral treatment. Finally, a total of 55 HCV-RNA-positive IDUs were enrolled in this study. The mean age in this cohort was 31.8 years (standard deviation ± 6.7, range 18–49); 48 subjects were male; the male/female ratio was 6.9.

Serological and molecular confirmation of the HCV infection

The presence of anti-HCV was determined on LUMIPULSE automated Chemiluminescence Enzyme Immunoassay system (Fujirebio, Tokyo, Japan) using the kit produced by Ortho Clinical Diagnostics, Tokyo, Japan. Total RNA was extracted from 200 µL of anti-HCV-positive sera using the SepaGene RV-R kit (Sanko Junyaku, Tokyo, Japan) and reversely transcribed into complementary DNA (cDNA) using SuperScript II RNase H⁻ transcriptase (Invitrogen, Carlsbad, CA, USA) and random hexamer primer (Takara Shuzo, Shiga, Japan) in total reaction volume of 20 µL. Two microliters of the obtained cDNA were used as a template in 50 µL reaction volume for polymerase chain reaction (PCR)-amplification of the HCV 5′UTR by previously described primers KK30 (nucleotides [nt.] 58–77) and KM3 (nt. 313–294).[12] The threshold of the method was as low as 100 copies per milliliter; therefore the primers were used to define HCV-RNA-positive cases.

HCV genotyping

The HCV genotype in each case was determined in both structural (Core/E1) and non-structural (NS5B) viral genes using the corresponding genotyping PCR, as described previously.[13,14] Cases showing an unexpected size of PCR bands were further subjected to PCR and direct sequencing using the genotype-universal primers targeting Core/E1 and NS5B regions.[15]

Phylogenetic analyses

The PCR products were directly sequenced using Prism Big Dye ready reaction kit (Applied Biosystems, Foster City, CA, USA) on the ABI 3100 DNA automated sequencer.

The phylogenetic relationship of the strains sequenced in this study, as well as those reported previously, were investigated by the neighbor-joining method using tools available online from HCV databases (http://s2as02.genes.nig.ac.jp/).[16] Genetic distances were corrected by the Tamura–Nei method, and the bootstrap test was performed on 1000 resamplings.

Primers for HCV type RF1_2k/1b-specific detection

New primers were designed to amplify a part of the HCV genome between nt. 2986 and 3270, spanning the tentative intergenotypic crossover point in the RF1_2k/1b strains.[4] The selection of appropriate primer sites was accomplished manually using alignments of the sequences previously published in the DDBJ/GenBank, including five strains of the recombinant RF1_2k/1b type (accession numbers AY070170–AY070172, AY070214 and AY070215),[4,17] and selected reference sequences of other HCV genotypes.[16]

The sequences of the primers used in this study are shown in Table 1. Amplification using the primers was optimal for our samples under the following PCR conditions: total reaction volume 25 µL, including 2.5 µL of 10 × PCR buffer (Roche, Indianapolis, IN, USA) containing 15 mM $MgCl_2$, 2.0 µL of dNTPs (2 mM each), 1.0 µL of forward and 1.0 µL of reverse primer, 2 units of Taq polymerase (Ampli-Taq Gold; Roche) and 1.0 µL of cDNA. Thermal cycle conditions were as follows: hot start at 96°C with 7 min hold followed by 40 2-step

Table 1 Oligo-nucleotide primers for PCR

Primer ID	Direction	5'-3' sequence and nucleotide position†		Target genotype	
NS2_uni_2415f	Forward	2415	CTC CAC CAA AAC ATC GTG GA	2434	1 & 2
NS2_2ak_2948f‡	Forward	2948	CCG YGA YGG CAT CAT ATG GG	2968	2 (2a & 2k)
NS2_2ak_2994f‡	Forward	2994	GTG TTT GAC ATA ACC AAG TGG	3014	2 (2a & 2k)
NS2_1b_3100f	Forward	3100	GTG CAT GCA TGT TGG TGC GGA	3120	1 (1b)
NS2_1b_3295r‡	Reverse	3295	GTG ATG ATC TTG GTC TCC ATG TCR GA	3270	1 (1b)
NS2_1b_3377r	Reverse	3377	CAC AAG TAT CTC CCT CCC CCT	3357	1 (1b)
NS2_2ak_3417r	Reverse	3417	AGT TTC CAC CCC TTG GAA GT	3398	2 (2a & 2k)

†Relative to the reference strain H77 (NC_004102).
‡The primers used in hemi-nested screening polymerase chain reaction (PCR) are emboldened.

cycles at 95°C for 1 min and 60°C for 1 min, with ramp speed at approximately 1°C per sec. The second PCR products were visualized by electrophoresis in 3% agarose gel with ethidium bromide staining. The approximate size of the target product is 347 bp after the first PCR round (with outer sense primer) and 300 bp after the second "hemi-nested" (with inner sense primer) (Fig. 3a).

Preparation of the "competitive" PCR templates

To evaluate the specificity and sensitivity of the screening primers, the following three clones were generated from the stored environmental serum samples collected at the Department of Molecular Informative Medicine of Nagoya City University: Cln_1b (Japan), Cln_2a (Japan), Cln_2k (Altai, Russia). Obtained cDNA was amplified using the relevant set of primers: ***NS2_uni_2415s*** along with ***NS2_1b_3377as*** or ***NS2_2ak_3417as*** (Table 1), then directly inserted into a plasmid vector (pCR2.1-TOPO; Invitrogen) and cloned in *E. coli* (DH 5 alpha; Toyobo, Osaka, Japan). The resulting colonies were further transferred from LB agar type medium into LB medium and cultivated for 16 h. The obtained clones were purified using QIAprep kits (Qiagen, Valencia, CA, USA) and quantified by measuring optical density and molecular purity on Beckman spectrophotometer (Beckman Instruments, Fullerton, CA, USA). Based on the obtained values, aliquots were prepared using serial dilution.

Statistical analyses

Statistical differences were evaluated by Fisher's exact probability test and χ^2-test with Yates' correction, where appropriate, using the STATA software version 8.0 (StataCorp LP, College Station, TX, USA). Differences were considered significant for *P*-values less than 0.05.

RESULTS

HCV genotypes

THE PREVALENCE OF HCV genotypes in the studied cohort was determined in both Core and NS5B regions. The results are summarized in Table 2. The most prevalent genotype was HCV-3a, determined in 37/55 (67.3%) cases. All of these had concurring genotyping result in both Core and NS5B regions. The second most common genotype was HCV-1b, determined in 15/55 (27.3%) cases in the Core and 16/55 (29.1%) cases in the NS5B, revealing a 1/55 (1.8%) untypable case by Core-genotyping-PCR. The third genotype found in this cohort was HCV-2a, detected in 2/55 (3.6%) cases showing mutual agreement of results between the Core and NS5B PCR. One case (identified in this study as "*UZ-IDU19*") showing an undetermined result with Core-PCR was subjected for E1 region sequencing. Additionally, 38 cases were also subjected

Table 2 HCV genotypes determined in Core/E1 and NS5B coding region

Core[13]	NS5B[14]			Total
	1b	2a	3a	
1b	15			15 (27.3)
2a		2		2 (3.6)
3a			37	37 (67.3)
ND	1			1 (1.8)
Total	16 (29.1)	2 (3.6)	37 (67.3)	55 (100)

HCV, hepatitis C virus; ND, not determined.

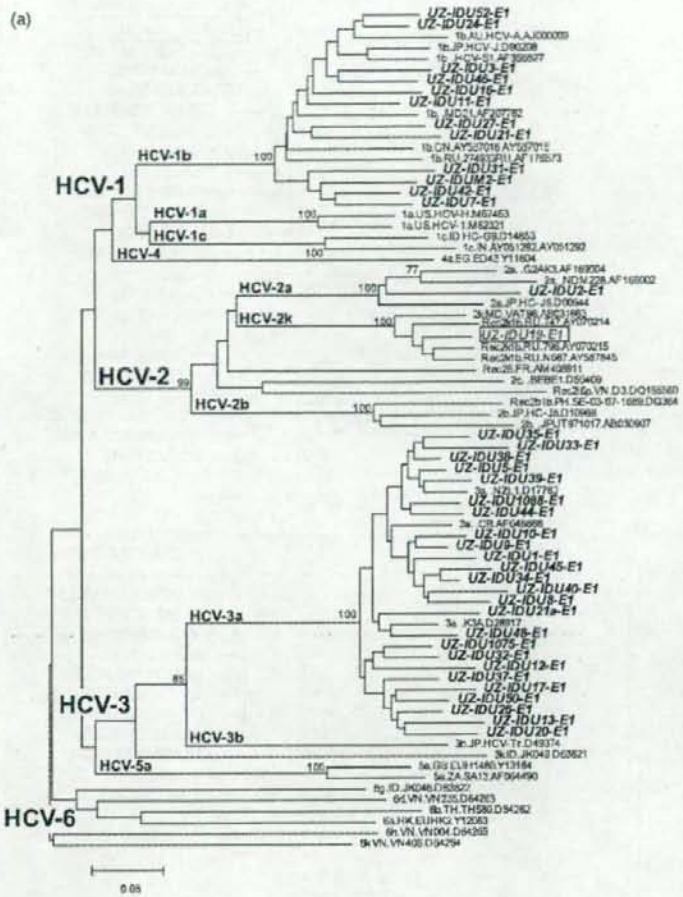

Figure 1 Phylogenetic relation of the hepatitis C virus strains. NJ tree constructed on the basis of 438 nucleic acids sequence of the Core/E1 coding region (nt. 861–1298) (a) and 286 nt. of the NS5B coding region (nt. 8283–8568) (b). Distances were estimated on the basis of synonymous substitutions under a modified Nei–Gojobori model. Bootstrap re-sampling indices exceeding 70% are indicated near to the roots of the corresponding cluster. Strains isolated in this study are indicated in bold italic font. Framed is the strain (*UZ-IDU19*) which was untypable by core genotyping.

for E1 amplification and direct sequencing with the aim of confirming genotyping results. The phylogenetic relationship of the strains sequenced in this study and those reported previously is depicted in Figure 1a. Interestingly, specimen *UZ-IDU19* was clustered phylogenetically together with the HCV-2k[18] and RF1_2k/1b strains from Russia.[4] Of the remaining 38 strains, 25 were clustered with HCV-3a references, 12 with HCV-1b and one with HCV-2a, thus indicating agreement with the results that had been obtained by Core and NS5B PCR-genotyping (Table 2). Furthermore, 14 specimens including the *UZ-IDU19* and 13 other cases in which genotypes 3a ($n = 9$) or 1b ($n = 4$) were determined by all Core-PCR, NS5B-PCR (Table 2) and E1 phylogenetic analysis (Fig. 1a), were subjected to sequencing and phylogenetic analysis in the NS5B region. The resultant tree (Fig. 1b) indicates clustering of the *UZ-IDU19* strain with 1b reference strains along with the four strains obtained in this cohort. The remaining nine strains were clustered with HCV-3a reference sequences, confirming the results obtained by the genotyping methods outlined above.

The collective result of the genotyping revealed that one case in this cohort (*UZ-IDU19*) had phylogenetic evidence of the intergenotypic recombination previously reported as RF1_2k/1b. To confirm this, the *UZ-IDU19* case was subjected to direct sequencing using primers spanning the intergenotypic crossover point previously

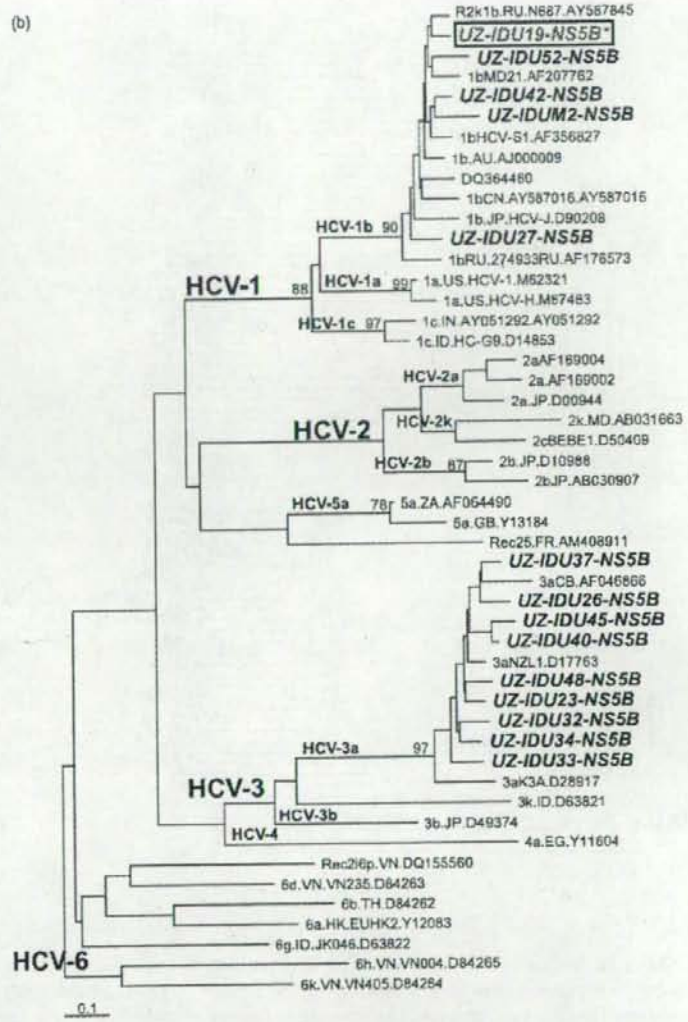

Figure 1 *Continued*

reported within the NS2 coding region (nt. ~3175). The resulting nucleotide sequence was aligned with those of RF1_2k/1b and references of HCV-2 k and HCV-1b previously reported, and the crossover point has been confirmed within the same nucleotide positions (Fig. 2).

Clinical and epidemiological characteristics of the RF1_2k/1b-infected carrier

As was defined by retrospective epidemiological investigation, the carrier of the recombinant HCV variant strain (specimen # *UZ-IDU19*) was a 32-year-old male Caucasian who had no clinical symptoms of active hepatitis (total bilirubin, alanine aminotransferase and aspartate aminotransferase were all within the normal range). The carrier reported neither a history of blood transfusion, nor travel to Russia or other countries (except Kazakhstan), nor any contact with subjects from foreign countries. The carrier has been registered at Republic Narcological Center since 2001, and had a reported history of intravenous drug use since 1994. No

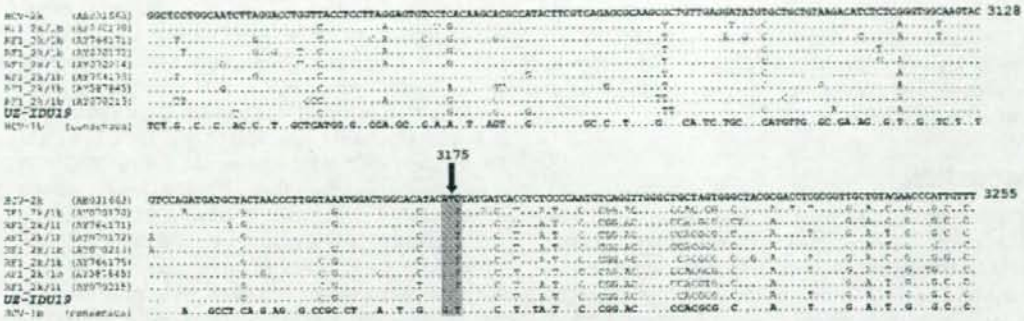

Figure 2 Alignment of 255 nucleic acids sequences of the NS2 coding region (nt. 3015–3269) of the hepatitis C virus (HCV) strains. The RF1_2k/1b strain determined in this study is indicated in bold italic font. Previously published HCV reference sequences identified by previously established genotype and accession number (in parentheses). The tentative crossover point is indicated by an arrow and the corresponding codon is shaded in gray.

records prior to this are available for serological investigation of the subject.

Sensitivity and specificity of the RF1_2k/1b-specific primers

To examine the hypothesis that the recombinant strain may be present in HCV-infected subjects carrying non-recombinant HCV genotypes (i.e. those with determined HCV-1b, 2a and 3a genotypes in this study), we designed a specific PCR amplification strategy, which is graphically depicted in Figure 3a and primers included in Table 1. To evaluate the specificity and sensitivity of the method we used different concentrations of the target PCR templates. The established detection limit of the method was 10 copies per assay. As shown in Figure 3b, specific amplification was successful even when the RF1_2k/1b clone was present in a tested sample along with the HCV-1b clone in a concentration ratio $10–10^5$ copies per assay, respectively. This was confirmed with clones of genotypes 3a, 2a and 2k used as a competitive control template (not shown).

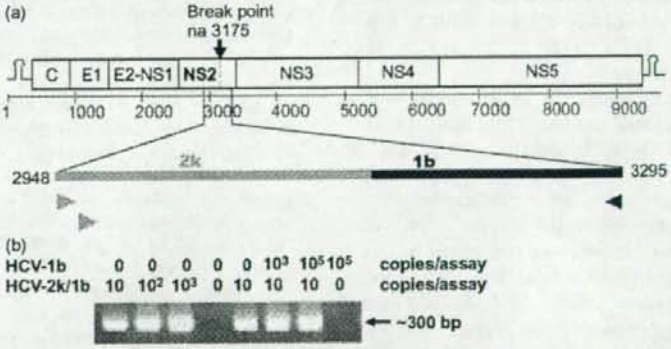

Figure 3 An outline of the amplification strategy of the polymerase chain reaction (PCR)-based detection method for the hepatitis C virus (HCV) type RF1_2k/1b strain (a), sensitivity and specificity of the method (b). (a) HCV genomic region corresponding to nt. 2948–3295 is targeted by the designed primers. A part of the amplicon corresponding to HCV-2 k sequence (in RF1_2k/1b strain) and 2k-specific primers (outer and inner sense) are depicted in gray; a part corresponding to the HCV-1b sequence and 1b-specific antisense are depicted in black. (b) Agarose gel picture showing specific bands obtained after the amplification of RF1_2k/1b template alone, or mixed with non-recombinant strain in different concentrations.

This amplification strategy was applied to 55 cases in the present study and only one case (*UZ-IDU19*) showed a positive result, indicating that the variant is not present as a minor clone of quasispecies population among these individuals.

DISCUSSION

THE RESULTS OF this study indicate the circulation of the RF1_2k/1b natural recombinant strain among IDUs in Uzbekistan (one of the former Soviet Union countries located in Central Asia). This genetic variant of HCV was previously reported only in European parts of Russia[4] and in Russian immigrants to Ireland[6] and Estonia.[19] The detection of the strain within the relatively small cohort of Uzbek IDUs in the present study may indicate a substantial possibility that its actual prevalence is underestimated. Because little is known about the epidemiological and clinical impact of the natural HCV recombinants, further studies are required on RF1_2k/1b. The PCR-based strategy designed in this study allows specific and sensitive detection of the RF1_2k/1b strain and could be used in further large-scale screenings of an HCV infected population.

To date, only three other reports on the complete or nearly complete HCV genome analysis have described natural intergenotypic recombinant strains.[5,7,8] Interestingly, all of these reports concur with the initial report[4] in respect to the following observations: (i) the intergenotypic breakpoint is located between the structural and non-structural genomic parts of the HCV, within the NS2 coding region near to the NS2/NS3 junction (nt. 3175–3455); and (ii) all of the chimeric variants had genotype 2 sequences (subtype 2b, 2i and 2k) in the structural part of the genome.

The HCV genotype distribution in this study was similar to previous reports, suggesting that genotype 3a is frequent among IDUs in Uzbekistan[20] and Russia,[21] with genotype 1b being the second most common. Interestingly, no evidence of recombination between the prevalent HCV genotypes (i.e. 3a and 1b) was observed in this cohort. Furthermore, genotype 2 in this study was found to be in the minority, and none of the strains was phylogenetically related to HCV-2k. These observations may suggest that, unlike hepatitis B virus, recombination in HCV may not be associated with the cocirculation of genotypes in a population. This hypothesis concurs with previous reports that have demonstrated rare dual genotype coinfection among HCV infected individuals.[22,23] One of the possible explanations could be the superinfection exclusion phenomenon, depriving the viral strain of its translational and/or replication ability inside a cell that is primarily infected by another homologous virus. This was recently proven for HCV by delicate *in vitro* studies using genotypes 1a, 1b and 2a.[24,25] Collectively, these studies may indicate the possibility that minor HCV variants such as 2b, 2k, 2e and 2i may possess the unique ability to overcome the superinfection exclusion. Further experimental studies are required to confirm this hypothesis.

In conclusion, this study demonstrated that the first of the reported natural HCV recombinants, RF1_2k/1b, is widely scattered and not associated with cocirculation of the predominant genotypes. The specific PCR-based detection method developed in this study could prove useful in further investigations of the epidemiological and clinical impact of the recombination in HCV.

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厚生労働科学研究費補助金　(肝炎等克服緊急対策研究事業)
研究協力報告書

C型慢性肝炎難治例に対する新たな取り組み

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研究要旨: C型慢性肝疾患においては抗ウイルス療法を行うことで、ウイルスの排除を行うことが予後の改善に最も寄与しており臨床医にとっては究極の目的である。1992 年にインターフェロン治療が開始されてから、完全著効を目指して新しい治療の登場と様々な工夫により治療効果は著明に向上した。しかしながら、未だにウイルスの排除がなされない症例も厳然として存在することも事実である。このような症例を対象とし、いかにその著効率をあげるかについて検討を行い、今後の治療の選択肢の幅を広げることで更なる治療効果の向上を目指すことが研究の目的である。今年度は、全体研究として最難治例である genotype1b かつ高ウイルスに対するペグインターフェロン＋リバビリン併用療法におけるデータマイニング解析を行っているため、我々は著効がえられなかった原因としての副作用や合併症の問題をいかにして解決するかにつき検討した。

A. 研究目的

C型慢性肝炎に対するインターフェロン(IFN)治療の目的は、ウイルス血症を改善し肝臓の組織学的進展(主に繊維化の進行)を改善し、患者の予後の向上を図ることである。このために我々は、治療効果の上がる方法を見いだすため、効果的に予後を予測する方法や、投与期間の延長、他剤との併用といった検討を行い報告してきた。難治例となる要因には、大きく分けて以下の三点があげられる。すなわち、(1) IFN治療を充分に行ってもウイルスの陰性化がえられない。(2)副反応でIFNが予定どおりできない。(3)高齢や合併症のためIFN治療を行えない。などである。今年度は(2)、(3)に焦点を絞り、我々が治療効果向上を目指して行ってきた取り組みにつき検討を行うと共に、これに関わる要因の解析も行い、各個人にあった治療法を見出すことを目的とした。

B. 研究方法

対象は、2008年12月現在までにIFN治療を施行し効果判定可能な症例とし、retrospectiveに解析を行った。これらのうち副反応で中止になった症例を治療別、年齢別に検討し、中止になった理由や要因を解析した。その上で、いかにしてIFN治療の再投与を行い治療効果を挙げるかにつき、個々の症例について詳細な検討を行うことで新たな治療法を模索した。また、高齢者やうつ病などの合併症症例を対象としどのような工夫をすることでIFN治療が可能となり、治療効果の向上が期待しうるのかについても検討した。対象症例は全例において、HCV-RNA量、genotypeを測定し、治療効果判定は厚生労働省の治療効果判定基準に準じて行った。

C. 研究結果

これまでに我々が報告してきたIFNの種類別に見た副反応による治療の中止率を表1に示す。既報のごとく高齢者であること、リバビリン併用例に中止例が多いことが明らかである。また、低ウイルス症例に限って検討を行うと、1b、2a、2bのいずれにおいてもαIFNに比べ、βIFNの方が完全著効率が高くな