Available online at www.sciencedirect.com
SCIENCE DIRECT®
Antiviral Research 60 (2003) 27–33
Antiviral Research
www.elsevier.com/locate/antiviral

Genomic analysis of a recently identified virus (SEN virus) and genotypes D and H by polymerase chain reaction

Hiroshige Kojima [a], Kelly D.E. Kaita [a], Manna Zhang [a], Antonio Giulivi [b], Gerald Y. Minuk [a,*]

[a] *Department of Medicine, Liver Diseases Unit, Health Sciences Centre, John Buhler Research Centre, University of Manitoba, 803-715 McDermot Avenue, Winnipeg, Man., Canada MB R3E 3P4*
[b] *Bureau of Infectious Diseases, Health Protection Branch, Health Canada, Ottawa, Canada*

Received 26 November 2002; accepted 22 May 2003

Abstract

Background/Aims: SEN virus (SENV) was discovered in 1999 as a DNA virus with hepatotropic properties. Nine genotypes (A–I) have been identified with genotypes D and H being more prevalent in cases of chronic hepatitis. Attempts to determine whether SENV causes liver disease have been hampered by limited diagnostic testing. *Methods*: In the present study, we developed two PCR based assays; a general SENV screening and genotype-specific assay. *Results*: By screening PCR, the specificity for all SENV genotypes and SENV-related sequences was 20/20 (100%) with confirmation of the results being provided by genomic sequencing. With the genotype-specific PCR, specificities for SENV-D and SENV-H were 7/7 (100%) and 7/11 (64%), respectively. All screening PCR products were cloned and sequenced. The results of sequencing showed high genetic diversity in representative SENV genotypes. Five of twenty patients (25%) had mixed infections with several SENV genotypes. *Conclusions*: The screening PCR was useful for identifying cases of SENV infection. However, because of high genetic divergence and mixed co-infection, it was difficult to establish a specific method for genotype distinction. Hence, sequencing is still required for further investigations of SENV as a potential cause of liver disease.

Keywords: SENV; Polymerase chain reaction; Hepatitis; Liver disease

1. Introduction

Discovered in 1999, the SEN virus (SENV) is a new family of single-stranded DNA viruses with hepatotropic properties (Primi et al., 2000). To date, at least nine genotypes (A–I) have been identified with genotypes D and H being more prevalent in cases of chronic hepatitis (Umemura et al., 2000a,b, 2001; Tanaka et al., 2001). Phylogenetically, SENV most closely resembles the TT virus (TTV) another family of DNA viruses that may cause hepatocellular injury (Tanaka et al., 2001; Mushahwar, 2000).

Presently, the ability to diagnose SENV is limited to a commercially available assay which involves polymerase chain reaction (PCR) with SENV-specific primers followed by hybridization of amplified DNA (DNA enzyme immunoassay, DEIA) with SENV-D or SENV-H-specific 5′-biotinylated probes (Primi et al., 2000; Umemura et al., 2000a,b, 2001; Tanaka et al., 2001). Because it remains unclear whether other SENV genotypes cause liver disease, it would be advantageous to employ an assay that screens for all SENV genotypes prior to genotype-specific testing.

The purpose of the present study was to develop such an assay and document its sensitivity and specificity in DEIA confirmed positive and negative cases.

2. Material and methods

2.1. Serum samples

Serum samples from 33 patients with chronic hepatitis (anti-HCV(+), 12; HBsAg(+), 19; NBNC, 2) were tested for SENV DNA by PCR. These samples had been randomly selected (by a computer generated numbering system) from one of the investigator's (GYM) serum bank of 330 samples (10%) where they had been stored at −70 °C without repeated freeze/thaw cycles. All subjects contributing to the serum bank had given permission at the time of sample collection for subsequent testing of new hepatotropic viruses and testing was approved by the University's Conjoint Ethics Committee for Human Studies.

* Corresponding author. Tel.: +1-204-789-3204; fax: +1-204-789-3971.
E-mail address: gminuk@cc.umanitoba.ca (G.Y. Minuk).

0166-3542/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0166-3542(03)00133-5

2.2. Extraction of nucleic acids from the serum

Viral DNA was extracted from serum by High Pure Viral Nucleic Acid Kit (Roche Diagnostics GmbH, Mannheim, Germany). To extract DNA, 200 μl of serum was added to 200 μl of 6 M guanidine–HCl, 10 mM urea, 10 mM Tris–HCl (pH 4.4), 20% Triton X-100 (v/v), and 16 μg poly(A) carrier RNA and subsequently added to 50 μl of 18 μg/μl proteinase K and mixed well. The mixture was incubated for 10 min at 72 °C. After incubation, 100 μl of isopropanol was added and the mixture was passed through the supplied filter which was washed once by 38% ethanol, 5 M guanidine–HCl, and 20 mM Tris–HCl (pH 6.6) and twice by 80% ethanol, 20 mM NaCl, and 2 mM Tris–HCl (pH 7.5). DNA was then eluted in 50 μl of distilled water.

2.3. Detection of SENV DNA

For general SENV screening, a primer pair was designed to the conserved region among all SENV genotypes A–I (AI) according to SENV sequences submitted to the patent (WO 00/28039) (1) and GenBank database: AX025667 (SENV-A); AX025677 (SENV-B); AX025718 (SENV-C); AX025730 (SENV-D); AX025761 (SENV-E); AX025822 (SENV-F); AX025830 (SENV-G); AX025838 (SENV-H); and SENV-I (personal communication). For genotype-specific testing, a nested primer pair was designed for genotypes D or H (Fig. 1) (Table 1).

Fifty microliters of the PCR mixture contained 0.5 μM forward primer, 0.5 μM reverse primer, Advantage 2 Polymerase Mix, Advantage 2 PCR Buffer (Clontech Laboratories, Inc. Palo Alto, CA), 0.2 mM dNTP Mix, and template DNA. PCR amplification was started with 60 s at 95 °C for denaturation followed by 35 cycles amplification at 95 °C for 30 s, 68 °C for 60 s. All reactions were performed in a PTC-150 MiniCycler (MJ Research, Inc. Waltham, MA).

The first-round PCR was performed with sense primer AI-1F and antisense primer AI-1R for all genotypes of SENV. The second round PCR was performed with sense primer D-1148F and antisense primer D-1341R for SENV-D detection, and with sense primer H-1020F and antisense primer H-1138R for SENV-H detection (Table 1).

```
      131                                                                190
SENV-D   FSLFVLYDQYTRHLNRWSYPNDQLDLARYKGCKFKFYRDTNTDFIVTYDINPPMKNTELS
SENV-H   ...Q......Q.FM.K...S.........F..T.W...HPEV..VAQF.NV....MD.NT
SENV-A   ...L.....HE......TF.......V...HTR.....SKD......FN.K....MN.TT
SENV-B   ...RA......K.M...TFS.........R.Y..R...HPTC...IH.NLI..L.INQFT
SENV-C   ...K......Q.F..K.................I...HPE...V.Q..NV....MD.FT
SENV-E   .N.R.....HQ.G....TF..............T...NKM....AQ...VA.YALDRN.
SENV-F   ...Y.......X.M...................S.RL..HPT....LQ..N.......I.
SENV-G   I..Q....EF.........S.E.......R..TL.V..QPKV...M.FNTI...QMN..T
SENV-I   ...K..F..HQ.G..K..F.......V..R....Y...TKQ..W.GQ...SE.Y.LDKY.

      191                                                                250
SENV-D   SPNTHPGMLMQQKRKILVPSWDTYPRGRKYVLAKIPPPKLFEDHWYTQPDLCKVPLVTLR
SENV-H   A.....SF.L.N.HRVKI..FK.K.F...K.RVTVG.......K..S.H........SW.
SENV-A   ...A.......M.H......FQ.R.G..R..SV..G.......K..P.A.F......S.T
SENV-B   .......L..LT.H..II..FL.R.G..RF.KI.L........K....Q....Q.....T
SENV-C   A.....SL.L.A.HRRKI..FK.R.F.K.A.AIRVG.......K..P.S........SW.
SENV-E   ..SYA..IM..A.N...I..YN.R....QKISV........V.K..S.E...S.N..S.A
SENV-F   ............RH.......Q.........KV..........................
SENV-G   A.........I..M...I..FE.R.K.K..KRV..H.......K..S.S...R....S..
SENV-I   C..Y...NMIKA.H.F.I..Y..N...KQKIII.....D..V.K....E...S.N..S.A

      251                                  284
SENV-D   STAADLKHPFCSPQTNNPCTTFQALREQYNDMIG
SENV-H   L....FRF.......D...Y...V.H.E.YPV..
SENV-A   A....FR................V......KV..
SENV-B   A...S.RY..........NC...V..KN..KV..
SENV-C   V.....RF.......D...F...V.H.E.YNVL.
SENV-E   VS...FV...G....D...V...VCESFL.SV..
SENV-F   .....FR................V...N..EV..
SENV-G   F...SF.Y.......VT.I....V.Q.P..RY..
SENV-I   VS..SFL...G....D...Y...V.KDF.YQA..
```

Fig. 1. Amino acid (aa) alignment of the putative open-reading frame (ORF) 1 of SENV. The aa sequences of SENV-H, A–C, E–G, and I were compared with the aa sequence of SENV-D. Pairwise percent homology of aa sequences within entire ORF 1 in nine SENV isolates was between 39 and 77%. General primers (AI-1F and AI-1R) for all SENV genotypes were located within conserved regions (149–156 and 259–265 aa; underlined). Numbers indicate aa position in SENV-D sequences.

Table 1
Primers and probes used to detect SENV

Sense primer (sequence)	Antisense primer (sequence)	Region amplified (nucleotides)
AI-1F (5′-TWCYCMAACGACCAGCTAGACCT-3′)	AI-1R (5′-GTTTGTGGTGAGCAGAACGGA-3′)	1028–1377[a] 987–1336[b]
D-1148F (5′-CTAAGCAGCCCTAACACTCATCCAG-3′)	D-1341R (5′-GCAGTTGACCGCAAAGTTACAAGAG-3′)	1148–1341[a]
H-1020F (5′-TTTGGCTGCACCTTCTGGTT-3′)	H-1138R (5′-AGAAATGATGGGTGAGTGTTAGGG-3′)	1020–1138[b]
D10S (5′-GTAACTTTGCGGTCAACTGCC-3′)	LUCKY 2AS (5′-CCTCGGTTKSAAAKGTYTGATAGT-3′)	1322–1544[a]
C5S (5′-GGTGCCCCTWGTYAGTTGGCGGTT-3′)	LUCKY 2AS (5′-CCTCGGTTKSAAAKGTYTGATAGT-3′)	1271–1500[b]
Probe for DEIA (sequence)		Region detected (nucleotide)
SENV-D-specific probe (5′-ATGATAGGCTTCCCYTTTAACTATAACCCA-3′)		1427–1456[a]
SENV-H-specific probe (5′-CCCCTTCCAGGTATTGCATGAAGAGTATTAC-3′)		1411–1442[b]

W: A or T, Y: C or T, M: A or C, K: G or T, S: C or G.
[a] Obtained from the sequence of SENV-D (AX025730).
[b] Obtained from the sequence of SENV-H (AX025838).

2.4. Determination of SENV genotypes

The phylogenetic tree based on comparisons of partial fragments of SENV A–I which were amplified with AI-1F and AI-1R primers, was identical to the tree based on a comparison with the full-length genome (Fig. 2A and B).

Products in the first-round PCR were inserted into a pCR2.1-TOPO vector (Invitrogen BV, Groningen, Netherlands) and used to transform *Escherichia coli*. At least four clones per sample were processed with Dye Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems, Foster City, CA) and their sequences determined with an ABI PRISM 310 Genetic Analyzer (PE Applied Biosystems). The sequences of the PCR fragments were aligned with Clustal W (version 1.81; San Diego Supercomputer Center) to all SENV genotypes and representative TTV, SANBAN, YONBAN, TLMV isolates (TA278, JA20, JA2B, T3PB, SANBAN, TTVsan-IR1031, TUS01, TUPB, PMV, TTVyon-LC011, TTVyon-KC186, and TLMV-NLC030). The genotype of SENV was determined by the phylogenetic tree and the highest sequences aligned score.

2.5. Sequencing of PCR products

All screening PCR products amplified with AI-1F and AI-1R general primers were successfully cloned and more

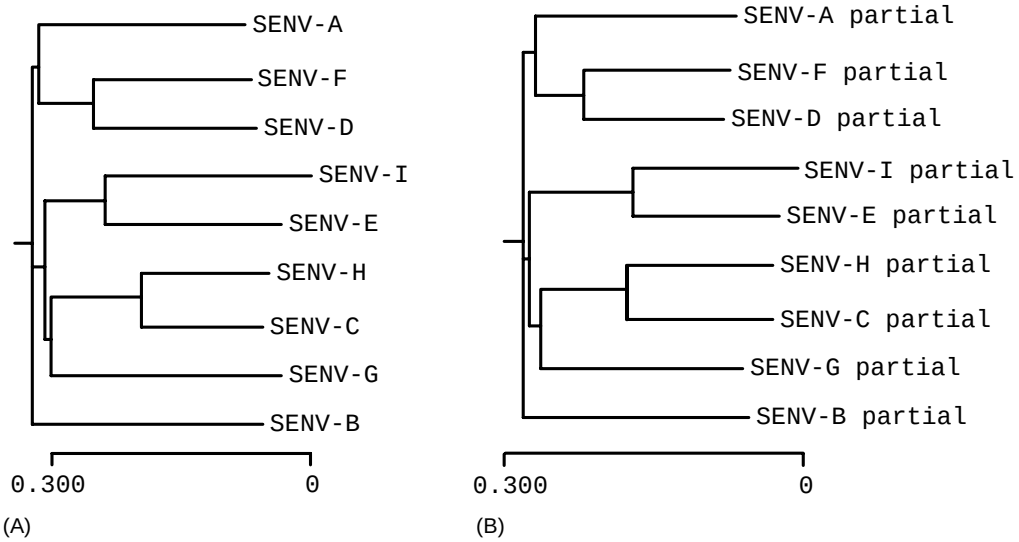


Fig. 2. Phylogenetic tree of SENV by neighbor-joining method. (A) The tree based on a comparison with the full length and (B) the tree based on a comparison of partial fragments of SENV A–I which were amplified with AI-1F and AI-1R primers. The phylogenetic tree based on comparisons of partial fragments of SENV A–I was identical to the tree based on a comparison with the full-length genome. The horizontal bar indicates number of nt substitutions per site.

than four clones per patient were sequenced. According to manufacture's instructions, the Advantage 2 Polymerase Mix exhibits an error rate of 25 errors per 100,000 bp after 25 PCR cycles. In our study, the error rate of the PCR product was estimated to be 0.12 errors per 350 bp. For the purpose of this study, clones with less than 5% differences in sequence were defined as the same clone.

3. Results

3.1. Screening PCR with general primers

As shown in Table 2, among the 33 patients studied, SENV DNA was detected in 20 by screening PCR with AI-1F and AI-1R general primers. Of the 20 patients who

Table 2
Results of SENV testing by DEIA and PCR assays for individual cases

Case number	Background		DEIA	PCR assay			Sequencing
	HBsAg	Anti-HCV		A–I general	Specific		
					D	H	
1	+	−	D	+	+	−	SENV-D (91%)
2	+	−	D	+	+	−	SENV-D (90%)
3	+	−	D	+	+	−	SENV-D (92%)
							SENV-F (86%)
							SENV-E (71%)[a]
4	+	−	D	+	+	−	SENV-D (92%)
							SENV-E (86%)
							SENV-F (86%)
							SENV-B (81%)
							SENV-E (72%)[a]
5	+	−	D	+	+	−	SENV-D (91%)
							TUS01 (99%)
6	+	−	D	+	+	+	SENV-D (91%)
							SENV-H (74%)
							SENV-E (73%)[a]
7	+	−	D	+	−	+	SENV-E (73%)[a]
8	+	−	D	+	−	−	TTVsanIR1031 (95%)
9	−	+	D	+	−	−	TTVsanIR1031 (82%)
10	−	+	D	+	−	+	SENV-H (94%)
11	+	−	H	+	−	+	SENV-H (94%)
12	+	−	H	+	+	−	SENV-D (92%)
13	+	−	H	+	−	+	SENV-I (78%)
							SENV-E (72%)[a]
							SENV-E (71%)[a]
14	+	−	H	+	−	+	SENV-H (93%)
15	+	−	H	+	−	+	SENV-E (89%)
16	−	+	H	+	−	+	SENV-H (93%)
17	−	+	H	+	−	+	SENV-H (95%)
18	−	+	H	−	−	−	
19	−	−	H	+	−	+	SENV-H (90%)
20	−	−	H	−	−	−	
21	+	−	−	+	−	+	SENV-H (80%)
22	+	−	−	−	−	−	
23	+	−	−	−	−	−	
24	+	−	−	−	−	−	
25	+	−	−	−	−	−	
26	+	−	−	−	−	−	
27	−	+	−	+	−	−	SENV-I (71%)
28	−	+	−	−	−	−	
29	−	+	−	−	−	−	
30	−	+	−	−	−	−	
31	−	+	−	−	−	−	
32	−	+	−	−	−	−	
33	−	+	−	−	−	−	

Cases 1–10, cases 11–20, and cases 21–33 had been previously diagnosed as SENV-D positive, SENV-H positive, and SENV negative, respectively by DEIA. In cases 3–6 and 13 clones which had sequence variation over 5%, were found.

[a] The nucleic acid alignment of these six clones which had relatively low homology (less than 80%) with SENV but did not have closer homology with other known sequences submitted to GenBank database is shown in Fig. 4.

Table 3
Comparison between DEIA and PCR assay for SENV infection

	PCR assay		
	A–I general	D-specific	H-specific
DEIA			
SENV-D	10/10 (100%)	6/10 (60%)	3/10 (30%)
SENV-H	8/10 (80%)	1/10 (10%)	7/10 (70%)
Negative for D or H	2/13 (15%)	0/13 (0%)	1/13 (8%)

had been diagnosed as SENV positive (either D or H) by DEIA, SENV was detected in 18 (90%) by screening PCR. Of the 13 patients diagnosed as SENV negative by DEIA, SENV was detected in 2 patients (16%) by screening PCR (Table 2).

The specificity of the screening PCR was confirmed by sequencing. Twenty of 20 patients (100%) had close homology with SENV or SENV-related TTV (TTVsanIR1031), 15 of 20 (75%) had high homology (>80%) to SENV. Two (cases 8 and 9) of 20 patients (10%) had closer homology with TTVsanIR1031, which has homology with SENV-I. Three (cases 7, 13, and 27) of 20 patients (15%) had limited homology (<80%) with SENV but no closer homology with other known sequences submitted to GenBank database.

3.2. Genotype-specific PCR with specific primers

By genotype-specific PCR with D-specific primers, SENV-D was detected in 6 of 10 patients (60%) diagnosed as SENV-D positive by DEIA. SENV-D was also detected in 1 of 10 patients (10%) diagnosed as SENV-H positive by DEIA (Table 3).

With H-specific primers, SENV-H was detected in 7 of 10 patients (70%) diagnosed as SENV-H positive by DEIA. SENV-H was also detected in 3 of 10 patients (30%) diagnosed as SENV-D positive by DEIA (Table 3).

Of the 13 patients diagnosed as SENV negative by DEIA, 1 of the 2 who were positive by screening PCR was SENV-H positive by genotype-specific PCR with H-specific primers while the other was negative for both SENV-D and SENV-H (Table 3).

The specificity of the genotype-specific PCR was also confirmed by sequencing. Here, 7 of 7 patients (100%) diagnosed as SENV-D by the genotype-specific PCR had homology with SENV-D while 7 of 11 patients (64%) diagnosed as SENV-H by genotype-specific PCR had homology with SENV-H (Table 4).

Table 4
Comparison between sequencing and PCR assay for SENV infection

	Sensitivity	Specificity
PCR		
D	7/7 (100%)	7/7 (100%)
H	7/7 (100%)	7/11 (64%)[a]

[a] Eleven cases were diagnosed as SENV-H positive by PCR assay, however, in four cases, no clone similar to SENV-H was found.

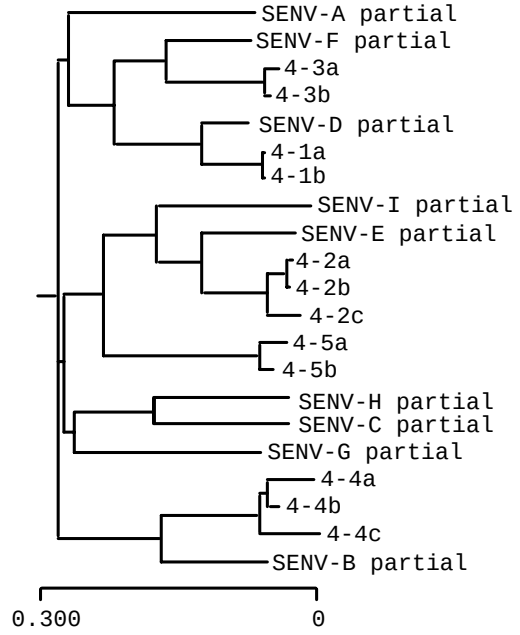


Fig. 3. The phylogenetic tree of nucleic acid sequences from Case 4 obtained by PCR with AI-1F and AI-1R primers. At least five different SENV species appear to be present in this patient.

3.3. Determination of SENV genotypes

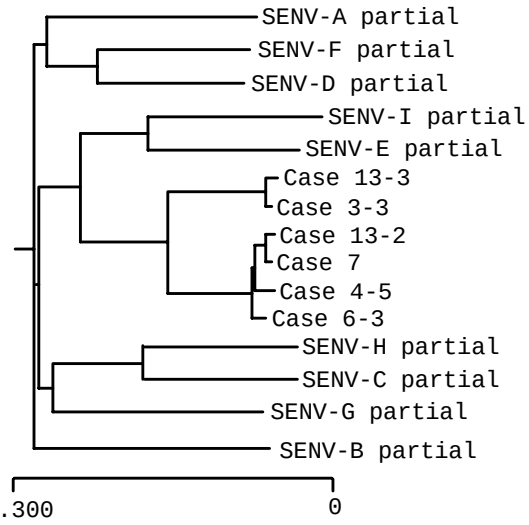
In cases 3–6 and 13, sequence variation exceeded 5% among the four clones. One representative case (Case 4) is shown in Fig. 3. At least five different SENV species might be present in this patient. All 31 representative clones had close homology with SENV or SENV-relative TTV genotypes (TUS01, TTVsan IR1031). Nine clones had relatively low homology (less than 80%) with SENV but did not have closer homology with other known sequences submitted to GenBank database. Of these nine clones, six clones isolated from cases 3, 4, 6, 7, and 13 had high homology to each other, suggesting yet another SENV genotype (Fig. 4).

4. Discussion

Whether SENV causes liver disease remains unclear. Supportive data include the appearance of SENV-DNA in the blood of blood transfusion recipients who develop post transfusion hepatitis and high prevalence rates of SENV infection among patients with chronic liver disease and/or risk factors for the same (Primi et al., 2000). On the other hand, high SENV prevalence rates also exist in the general population, the majority of whom are not thought to have liver disease (Umemura et al., 2000a,b). Moreover, liver enzyme abnormalities and hepatic function are similar in patients with chronic viral hepatitis regardless of their SENV status (Wong et al., 2002). Together, these findings suggest that like the closely related TTV, only certain SENV genotypes are capable of causing acute and/or chronic liver disease

```
Case 4-5        TTCCCCAACGACCAGCTAGACCTGGCCAGGTACAAGGGGGCCAAGTTCACCTTCTACAGA
Case 6-3        ····························································
Case 7          ····························································
Case 13-2       ····························································T·············
Case 3-3        ····························A····G···CTG··CT··············
Case 13-3       ·····················A··········G···CTG··CT··············

Case 4-5        CACAAAAAGACTGACTTTATAGTGTACTTTAACAGAAAGCCTCCCTTTAAGCTAAACAAG
Case 6-3        ········A···················································
Case 7          ········A·············A·····································
Case 13-2       ········A·············A·····································
Case 3-3        ·····················GG·GG·····················A·········
Case 13-3       ·····················GG·GG·····················A·········

Case 4-5        TACAGCTCCGCTTCGTATCACCCCGGCATGCTCATGCAACAGAAACACAAGATCCTAATA
Case 6-3        ·············C·····················G·····················
Case 7          ·············C·····················A·G···················
Case 13-2       ·············C·····················A·G···················
Case 3-3        CTG····G·C·G··C·TC·····························G·G·····C
Case 13-3       CTG····G·C·G····TC·····························G·G·····C

Case 4-5        CCCAGCTACGACACTAAACCCAAGGGCAGGCCAAAAATAACAGTTAGAATAAAGCCCCCC
Case 6-3        ····························································
Case 7          ····························································
Case 13-2       ····························································
Case 3-3        ··T····TT···········GG·····AG·T·····T·G···C·················
Case 13-3       ··T····TT···········GG·····AG·T·····T·G···C·················

Case 4-5        ACTCTCTTAGAAGACAAGTGGTACACCCAGCAAGACCTGTGCAACGTTAACTTGTTGCAA
Case 6-3        ····························································
Case 7          ····························································
Case 13-2       ····························································
Case 3-3        ·····················T·········T····TCCG··A···C··GC····
Case 13-3       ·····G···············T·········T····TCCG··A···C··GC····

Case 4-5        CTTGTGGTTACTGCGGCTGACTTTCAACATCCGTTCTGCTCACCACAAAC
Case 6-3        ···········································
Case 7          ···········································
Case 13-2       ··········C··································
Case 3-3        ···········································
Case 13-3       ···········································
```

(A)



(B)

Fig. 4. (A) Alignment and (B) phylogenetic tree of nucleic acid sequences from cases 3, 4, 6, 7, and 13 obtained by PCR with AI-1F and AI-1R primers. These clones had high homology to each other, suggesting yet another SENV genotype.

in humans. Until the genotypes are identified, it would be prudent to employ an initial screening assay that would identify all known SENV genotypes prior to proceeding to genotype-specific assays.

The results of the present study indicate that screening PCR products amplified with AI-1F and AI-1R general primers could serve as a useful SENV screening assay. Indeed, in addition to being able to identify infection with all known human SENV genotypes, the specificity and sensitivity of the assay as confirmed by genomic sequencing are greater than those obtained by DEIA testing. Moreover, because all components of the assay are readily available (specific antibodies are not required), viral testing can be performed in most laboratories that perform PCR.

Like TTV, SENV also has high genetic diversity (Okamoto et al., 1999). Among SENV genotypes, the pairwise percent homology of nucleotide and amino acid sequences of open-reading frame 1 are 52–78 and 39–77%, respectively (Tanaka et al., 2001). Results from our sequencing of PCR products confirm this high genetic diversity in representative SENV genotypes. Of interest, several clones were found to have low homology with known SENV genotypes and the TTV family. Whether these clones represent a new genotype of SENV requires further study.

Because it remains unclear as to which (if any) SENV genotypes are responsible for causing liver disease, genotype-specific PCR, and/or sequencing analysis remain essential to investigations regarding the relationship between SENV and liver disease. While, the D-specific primers utilized in our assay have sufficient sensitivity and specificity to detect infection with the SENV-D genotype, the H-specific primers cross react with other genotypes of SENV (especially SENV-E) and need to be improved.

In conclusion, A–I general primers were useful as a screening test for SENV infection in humans. However, because of high genetic divergence and mixed co-infection, it was difficult to establish a genotype-specific method for genotype distinction. Sequencing is still required for further investigations of SENV as a potential cause of liver disease.

Acknowledgements

The authors would like to thank Mrs. S. Zdanuk for her prompt and accurate typing of the manuscript. This work was supported by the Health Sciences Centre Research Foundation, Winnipeg, Manitoba, Canada.

References

Mushahwar, I.K., 2000. Recently discovered blood-borne viruses: are they hepatitis viruses or merely endosymbionts? J. Med. Virol. 62 (4), 399–404.

Okamoto, H., Takahashi, M., Nishizawa, T., Ukita, M., Fukuda, M., Tsuda, F., et al., 1999. Marked genomic heterogeneity and frequent mixed infection of TT virus demonstrated by PCR with primers from coding and noncoding regions. Virology 259, 428–436.

Primi, D., Fiordalisi, G., Lorenzo, M.G., Mattioli, S., Sottini, A., Fabrizio, B., et al. (2000). Identification of SENV genotypes. International Application Number: PCT/EP99/08566 and WO 00/28039 (International application published under the patent cooperation treaty).

Tanaka, Y., Primi, D., Wang, R.Y.H., Umemura, T., Yeo, A.E.T., Mizokami, M., et al., 2001. Genomic and molecular evolutionary analysis of a newly identified infectious agent (SEN virus) and its relationship to the TT virus family. J. Infect. Dis. 183, 359–367.

Umemura, T., Yeo, A.E.T., Wang, R.Y.H., Shih, J.W., Donahue, P., Primi, D., et al., 2000a. The incidence of SEN virus infection in transfusion-associated hepatitis (abstract). Hepatology 32 (Suppl.), 52A.

Umemura, T., Yeo, A.E.T., Shih, J.W., Matsumoto, A., Orii, K., Tanaka, E., et al., 2000b. The prevalence of SEN virus infection in Japanese patients with viral hepatitis and liver disease (abstract). Hepatology 32 (Suppl.), 125A.

Umemura, T., Yeo, A.E.T., Sottini, A., Moratto, D., Tanaka, Y., Wang, R.Y.H., et al., 2001. SEN virus infection and its relationship to transfusion-associated hepatitis. Hepatology 33, 1303–1311.

Wong, S.G., Kojima, H., Primi, D., Sottini, A., Giulivi, A., Minuk, G.Y., 2002. Insights into SEN-V prevalence, transmission and treatment. Clin. Infect. Dis. 35, 789–795.