are required to determine at what point in evolution this loss of specificity occurred.

Only a single α-chymase gene is present in humans and baboons (*8*) and only a single α-chymase has been described in dogs. In contrast, five chymase isoenzymes have been identified in mice and two in rats. Four of the five mouse chymases are of the β subtype, and one is of the α subtype; both rat chymases are of the β subtype (*13*). One possibility that could explain this distribution of chymase isoenzymes is that the α- and β-chymases split apart when rodents branched off from other mammals. We believe, however, that the division into α- and β-isoenzymes occurred long before mammals branched off from therapsids because mouse chymase-5 segregated from other rodent chymases as an α-chymase (bootstrap value, 98%; Fig. 1B). This hypothesis implies that humans and baboons have lost their β-chymase genes and that rats have lost their α-chymase gene. Ang II contributes to cardiovascular regulation in several nonmammalian species (*15*), but pathways for its synthesis have been explored largely in mammals. Because mast cells occur in frogs, birds, and lizards (*16*) and because the reconstructed ancestral chymase is an efficient Ang II–forming enzyme, we speculate that a chymase-dependent pathway of Ang II formation occurred early in vertebrate evolution.

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17. Abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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Molecular Cloning and Disease Association of Hepatitis G Virus: A Transfusion-Transmissible Agent

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An RNA virus, designated hepatitis G virus (HGV), was identified from the plasma of a patient with chronic hepatitis. Extension from an immunoreactive complementary DNA clone yielded the entire genome (9392 nucleotides) encoding a polyprotein of 2873 amino acids. The virus is closely related to GB virus C (GBV-C) and distantly related to hepatitis C virus, GBV-A, and GBV-B. HGV was associated with acute and chronic hepatitis. Persistent viremia was detected for up to 9 years in patients with hepatitis. The virus is transfusion-transmissible. It has a global distribution and is present within the volunteer blood donor population in the United States.

Although sensitive and specific tests for detection of the known hepatitis viruses are available (*1*), the etiology of a substantial fraction of post-transfusion (*2*) and community-acquired hepatitis (*3*) cases has remained undefined, suggesting the existence of additional causative agents. To identify such an agent, designated hepatitis G virus (HGV) (*4*), molecular cloning was initially performed with plasma from a patient designated PNF2161, who was originally identified as having non-A, non-B viral hepatitis through the Centers for Disease Control and Prevention (CDC) Sentinel Counties Study of Viral Hepatitis (*3*). Patient PNF2161 was initially believed not to be infected with hepatitis C virus (HCV), on the basis of consistently negative results with a first-generation immunoassay (the Ortho HCV ELISA Test System; Ortho Diagnostics, Raritan, New Jersey). However, subsequent testing with a second-generation HCV immunoassay (also from Ortho) and a polymerase chain reaction (PCR) assay based on HCV 5′ untranslated region primers (*5*) demonstrated that PNF2161 was infected with HCV.

Library construction and immunoscreening with plasma from PNF2161 were performed as described (*6*). Sequence analysis of immunoreactive clones isolated from the PNF2161 λ gt11 library revealed HCV-related sequences as well as several sequences that did not match any in the GenBank database (*7*). PCR primers designed from these nonmatching sequences were used to determine that they were exogenous to

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Escherichia coli, *Saccharomyces cerevisiae*, or human genomes (*8*). Amplification by reverse transcription (RT)–PCR was used to show that an exogenous clone, 470-20-1, was present in the cloning source plasma but not in healthy control subjects (*9*). RT-PCR analyses of serial threefold dilutions of RNA derived from PNF2161 plasma yielded a 470-20-1 amplification product that was detected by probe hybridization at a dilution corresponding to an estimated titer of 10^6 viral genome copy equivalents per milliliter (*10*).

Starting from the initial 470-20-1 complementary DNA (cDNA) isolate, an anchored PCR (*11*) approach was used to generate multiple overlapping cDNA clones whose sequences were determined and combined to create a 9392-nucleotide (nt) sequence (GenBank accession number U44402). At least three independent cDNA clones were used to establish a consensus sequence for each region of the genome. The extreme 5′ and 3′ ends of the genome were isolated by modified rapid amplification of cDNA ends (RACE) methods (*12*).

The consensus sequence derived from the PNF2161 plasma specimen indicated that it contains a continuous open reading frame (ORF) that could encode a viral polyprotein of 2873 amino acids. The long ORF is preceded by an apparent 5′ untranslated sequence of 458 nucleotides and is followed by a 3′ untranslated sequence of 315 nucleotides. The amino acid sequence encoded by the immunoreactive 470-20-1 clone is not present in the polyprotein encoded by the putative positive strand. Instead, the 470-20-1 amino acid sequence is found as part of a small ORF of 119 amino acids encoded in the complementary strand. Antibodies contained in the sera of several additional patients with suspected non–A through E (non–A-E) hepatitis reacted with the 470-20-1 protein in protein immunoblot assays (*13*). These observations suggest that the negative-strand ORF may be expressed during infection.

Using the sequences of the cDNAs isolated from patient PNF2161, we isolated overlapping cDNAs from the plasma of a second patient, R10291 (an asymptomatic individual with a history of intermittent elevations in liver enzymes), and determined a second consensus sequence of 9103 nucleotides (GenBank accession number U45966). The specimen from R10291 had no serologic evidence of infection with hepatitis B virus (HBV) or HCV; HCV infection was also excluded by PCR testing (*5*). The nucleotide sequences derived from PNF2161 and R10291 are 90.5% identical, whereas an alignment of the encoded amino acid sequences revealed a 97.5% identity (*14*).

As compared with that isolated from PNF2161, the HGV variant isolate derived from R10291 is predicted to encode a slightly larger polyprotein of 2910 amino acids. PNF2161 contains a single nucleotide deletion that introduces a frame shift, resulting in the shorter continuous ORF.

Comparison of HGV amino acid sequences with entries in a nonredundant sequence database revealed statistically highly significant similarity (probability of matching by chance, $P < 10^{-100}$) (*15*) to the amino acid sequences of the GBV-A and GBV-B viruses recently isolated from tamarins (*16*) and to the numerous sequenced isolates of HCV. Limited similarity (P values between 0.001 and 0.05) was observed between HGV amino acid sequences and those in the genera *Pestivirus* and *Flavivirus*, that together with HCV belong to the family *Flaviviridae*, and with those from tobacco necrosis virus and tomato bushy stunt virus, whose RNA-dependent RNA polymerases are related to those of the member viruses of the family *Flaviviridae* (*17*). The regions of conservation among HGV, GBV-A, GBV-B, and HCV covered the whole nonstructural region of the polyprotein as defined for HCV (*18*).

In pairwise alignments (*14*), the greatest global sequence identity was detected between the polyproteins of HGV and GBV-A (43.8%), whereas the identity of the HGV polyprotein with GBV-B and the HCV-1 isolate of HCV was 28.4 and 26.8%, respectively. The sequence identity was, however, considerably higher in the RNA-dependent RNA polymerase (RDRP) and helicase domains (*17*) (about 60% for HGV and GBV-A, and 40 to 50% for HGV and GBV-B or HCV). A comparison with the 331 nucleotides of available sequence from the putative NS3 region of the recently identified human virus GBV-C (*19*) showed an 85.5% nucleotide identity and a 100% amino acid identity over the corresponding region of HGV. It appears that HGV and GBV-C are very closely related, but a comparison of their entire genomes will be necessary to address this issue definitively. Thus, database screening indicated that HGV, along with HCV and the GBV-A, -B, and -C viruses, belong to a distinctive group of hepatitis-associated viruses within the family *Flaviviridae*.

Multiple alignment analysis (*20*) confirmed the presence of the full complement of conserved motifs typical of the RDRP, helicase, and chymotrypsin-like protease of member viruses of the family *Flaviviridae*, as well as several motifs that appear to be unique for the new group of hepatitis-associated viruses (Fig. 1).

The NH_2-terminal extremity of the HGV polyprotein that would correspond to the capsid protein of HCV showed no similarity to any other viral sequences. Nevertheless, the presence of the conserved domain that includes the COOH-terminal portion of envelope protein E1 and the NH_2-terminus of envelope protein E2 (Fig. 1) allowed us to position the NH_2-terminus of the HGV polyprotein relative to that of HCV. The sequence from patient R10291 contains 71 amino acid residues, and that from patient PNF2161 contains only 34 residues in the domain corresponding to the NH_2-terminal 175 residues of the HCV polyprotein, indicating that the capsid protein may be absent or defective in HGV. Although there is no sequence identity, the amino acid composition of the short NH_2-terminal region of the HGV polyprotein is similar to that of the HCV capsid protein (*21*), which is compatible with the possibil-

E1

HGV	71	(34)	LLLLLVVEAGAILAPATHACRANGQYFLTNCCAPEDIGFCLEGGCLVALGCTICTDQ	746
GBV-A		10	WLLVCFPLAGGVLFNSRHQCFNGDHYVLSNCCSRDEVYFCFGDGCLVAYGCTVCTQS	793
GVB-B		141	LFVVCLLSLACPCSGARVTDPDTNTTILTNCCQRNQVIYCSPSTCLHEPGCVICADE	660
HCV		175	IFLLALLSCLTVPASAHEVRNASGVYHVTNDCSNSSIVFEAADLIMRTPGCVPCVRE	708
Consensus			UUUU.U.....U$N.C....U.U.....U...GC..C...	

NS2 Zn protease

HGV	KMCARGAYLFDHM	20	LSFTRTDCRIIRDAARTLSCGQCVMGLPVVARRGDEVLIGVFQDVNHLPPGFVPTAPV
GBV-A	VLRKRGVLLYEHA	20	VSITKEDCAIVRDSARVLGCGQLVHGKPVVARRGDEVLIGCVNSRFDLPPGFVPTAPV
GVB-B	VFGENGVFFYKHL	18	FFPFEGKARVYRNEGRRLACGDTVDGLPVVARLGDLVFAGLAMPPD----GWAITAPF
HCV	LGALTGTYVYNHL	20	VTFSDMETKIITWGADTAACGDIILGLPVSARRGREILLGPADSLV--GQGWRLLAPI
Consensus	.....G.UUU.H.		U.....UU.....CG..U.G.PV.AR.G..UU.G.....GU...APU

NS5A

HGV	LCPVVSLPLWHCGEGWSGEWLLDGHVESRCLCGCVITGDVLNGQLKEPVYSTKLCRHYW	327	ETLASFSYIWSGVPL
GBV-A	ALPRLRLPLIGCSTGWGGPWEGNGHLETRCTCGCVITGDIHDGILHDLHYTSLLCRHYY	370	ETPTSYSYIWSGAPL
GVB-B	MVNIPGCPFYSCQKGYKGPWIGSGMLQARCPCGAELIFSVENGFAKL-YKGPRTCSNYW	309	EFSCSMSYTWTDVIS
HCV	LPRLPGVPFLSCQRGYKGVWRGDGIMHTTCPCGAQIAGHVKNGSMR--IIGPKTCSNTW	362	VVCCSMSYTWTGALI
Consensus	.....PUU.C..GU.G.W...G.U...C.CG..U.....G...		.....C...U ....SUSY.W$.

Fig 1. Alignment of selected conserved domains in hepatitis-associated viruses in the family *Flaviviridae* (*24*). The alignment was constructed with the use of the MACAW program (*20*). GBV-A, GBV-B, and HCV sequences are from the GenBank database (*7, 25*). The consensus shows the amino acid residues that are conserved in all aligned sequences. U, bulky hydrophobic residue (I, L, V, M, F, Y, W); $, S or T; a dot indicates any residue; a dash indicates a gap. The catalytic residues of the nonstructural protein NS2 protease (*26*) and the conserved cysteine residues in E1 and nonstructural protein NS5A are highlighted by bold type. The distances between the alignment blocks and the distances from the polyprotein termini are indicated by numbers. For HGV, the number in parentheses is for PNF2161.

ity that these regions are truncated derivatives of an ancestral capsid protein. In GBV-A, the capsid protein appears to be completely deleted.

A diagnostic RT-PCR procedure was developed for detection of unique HGV RNA sequences in plasma or serum specimens (*22*). In experiments with purified in vitro transcripts as the target template, the assay had a threshold sensitivity of 10 HGV RNA copy equivalents per reaction, corresponding to approximately 200 copy equivalents per milliliter of starting specimen plasma or serum.

Serum specimens from 12 cases of prospectively studied post-transfusion hepatitis, in which hepatitis A through E had been excluded by serologic testing and HCV RT-PCR (*5*), were tested for HGV RNA by RT-PCR. Two of our 12 patients tested negative for HGV RNA before transfusion and were HGV-positive in multiple post-transfusion samples. In addition, plasma samples from a patient with transfusion-associated non–A-E hepatitis, diagnosed at another institution, were referred to us for testing. This patient was also found to have converted from HGV RNA–negative before transfusion to HGV RNA–positive after transfusion. The biochemical and HGV RNA profile of two post-transfusion hepatitis cases is shown in Fig. 2. Initial positive results for HGV RNA corresponded to an elevation of liver enzymes that is characteristic of the onset of hepatitis. HGV RNA persisted for a minimum of 1 year in all three cases. One patient (Fig. 2, case 1) had detectable HGV RNA and elevated alanine aminotransferase (ALT) levels until his death from unrelated causes 4 years after the onset of infection. In the other two patients, viral RNA became undetectable 12 to 18 months after the onset of infection. In one case (Fig. 2, case 2), HGV RNA persisted for approximately 30 weeks after the normalization of serum ALT. Donor plasma samples were available from cases 1 and 2 and in each an HGV RNA–positive donor was identified.

To examine whether HGV is associated with acute disease, serum specimens from patients with community-acquired non–A-E hepatitis in four U.S. counties (*3*, *23*) were tested by RT-PCR for HGV RNA. Of 38 patients identified from 1985 to 1993, 5 (13%) were HGV RNA–positive at the time of their acute illness. None developed chronic hepatitis, but four remained HGV RNA–positive over a follow-up period of 2 to 9 years. Of 107 patients with acute hepatitis C identified over the same period, 19 (18%) were also HGV RNA–positive. The clinical characteristics of acute hepatitis in patients with HGV infection alone did not differ from those of patients co-infected with HCV. In addition, risk factors for infection did not differ between the two groups, and included transfusion, injection drug use, and multiple sexual partners.

We also studied plasma and serum specimens from patients with a variety of liver diseases, who were broadly distributed geographically, and from people at high risk for infection with parenterally transmitted viruses (Table 1). The observed frequency of plasma HGV RNA was similar to the frequency of serologically diagnosed HCV infection in the same populations, although examples of both single HGV infection and HGV co-infection with HBV or HCV were observed. HGV infection was demonstrated in patients with biopsy-documented chron-

Table 1. Frequency of HGV viremia in people who have hepatic disease, are at risk for exposure to parenterally transmitted infectious agents, or are volunteer blood donors. US, United States; AUS, Australia; SA, South America; EU, Europe.

Condition	Origin	Patients (*n*)	Total HGV+	HGV only	HGV+ and HBV+	HGV+ and HCV+	HGV+, HBV+, and HCV+
		Liver disease					
Post-transfusion non–A-E hepatitis	US	12	2	2	0	0	0
	AUS	1	1	0	0	0	0
Chronic non–A-C hepatitis	SA	48	6	6	0	0	0
	EU	110	9	9	0	0	0
Suspected non–A-E hepatitis	EU	12	1	1	0	0	0
Chronic HBV	EU	72	7	0	7	0	0
Chronic HCV	EU	96	18	0	0	18	0
Hepatocellular carcinoma	EU	30	2	0	1	1	0
Alcoholic hepatitis	EU	49	5	5	0	0	0
Autoimmune hepatitis	EU	53	5	5	0	0	0
Primary biliary cirrhoses	EU	58	1	0	0	1	0
		Parenteral exposure risk					
Hemophilia	EU	49	9	0	0	8	1
Multiply transfused anemia	EU	100	18	11	1	6	0
Intravenous drug use	EU	60	20	6	1	11	2
		Volunteer blood donors					
Accepted donations	US	769	13	13	0	0	0
Rejected donations (ALT > 45 IU/ml; fresh)	US	214	5	4	0	0	1
Rejected donations (ALT > 45 IU/ml; frozen)	US	495	6	4	0	1	1

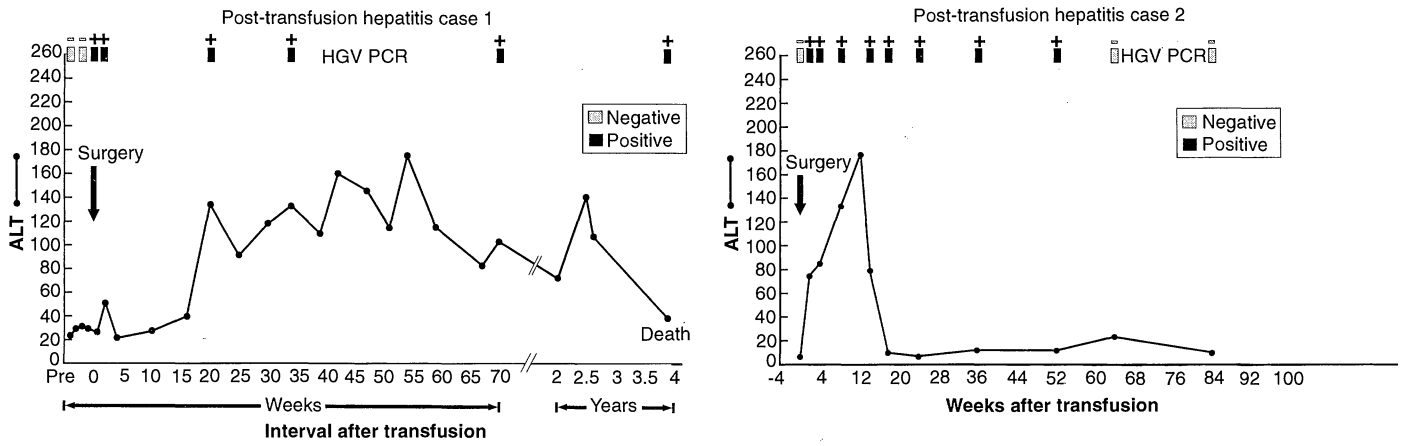

Fig. 2. Serial ALT levels and HGV RNA status of two transfusion recipients previously diagnosed with non–A-C hepatitis are shown (*27*).

ic hepatitis with cirrhosis of presumed viral etiology, in whom HBV and HCV infection had been excluded (Table 1).

In assessing the potential risk of transfusion-mediated transmission of HGV, we evaluated serum and plasma samples from volunteer blood donors whose donations were either accepted or withheld from transfusion because of elevated ALT levels. Among 779 consecutively screened volunteer donors with normal ALT values (<45 IU/ml), 13 (1.7%) were HGV RNA–positive as determined by PCR (Table 1). In testing of specimens from 709 donors excluded from donation because of an elevated ALT level (>45 IU/ml), 11 (1.5%) were HGV RNA–positive. Thus, donors with elevated ALT levels were no more likely to be carriers of HGV than were donors with normal ALT levels. None of the donors implicated in transfusion-mediated HGV transmission (Fig. 2) had an elevated ALT level at the time of donation.

Demonstration of replication of the virus in liver tissue, animal transmission studies, and characterization of changes in viral sequences and immune responses over the course of infection will be helpful steps in understanding the full pathogenesis of HGV infection. Epidemiological studies based on immunoassay approaches will help to establish the overall prevalence and clinical significance of HGV infection. Further studies are required to determine the natural history of HGV infection and to establish its full clinical significance, both in the setting of single infection and in relation to other known and recently described hepatitis viruses.

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4. We designated the virus hepatitis G virus (HGV) because an agent called hepatitis F virus (HFV) has been described [N. Deka, M. D. Sharma, R. Mukerjee, *J. Virol.* **68**, 7810 (1994)].
5. RT-PCR analysis was performed for detection of HCV RNA sequences essentially as described by J. H. Han *et al.* [*Proc. Natl. Acad. Sci. U.S.A.* **88**, 1711 (1991)] with the following primer sequences: primer UTR-1: 5′-TTCACGCAGAAAGCGTCTAGCCAT-3′ and primer UTR-2: 5′-TCGTCCTGGCAATTCCGGTGTACT-3′.
6. For isolation of RNA used in both the construction of the λ gt11 cDNA library [R. A. Young and R. W. Davis, *Proc. Natl. Acad. Sci. U.S.A.* **80**, 1194 (1983)] and the cloning of the HGV genome by anchored PCR, viral particles present in 1 ml of undiluted PNF2161 plasma were precipitated by the addition of polyethylene glycol (PEG, molecular weight 6000) to 8% (v/v) and were centrifuged at 10,000*g* for about 15 min at 4°C. RNA was extracted from the resulting pellet essentially as described by P. Chomczynski [*Biotechniques* **15**, 532 (1993)]. The cDNA was synthesized according to standard methods [U. Gubler and B. J. Hoffmann, *Gene* **25**, 263 (1983)]. After synthesis, the cDNA was amplified with the use of sequence-independent single primer amplification (SISPA) as previously described [G. R. Reyes and J. P. Kim, *Mol. Cell. Probes* **5**, 473 (1991)].
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8. To evaluate whether the clone 470-20-1 sequence was exogenous to the human, *E. coli*, and yeast genomes, PCR was performed with primers 77F (5′-CTCTTTGTGGTAGTAGCCGAGAGAT-3′) and 211R (5′-CGAATGAGTCAGAGGACGGGGTAT-3′), derived from the 470-20-1 sequence. Templates were human genomic DNA, *E. coli* DNA, and *S. cerevisiae* DNA. After initial denaturation at 94°C for 1 min, 30 cycles of amplification were performed at 94°C for 75 s, at 55°C for 75 s, and at 72°C for 60 s. Specific products were detected with a 470-20-1 ^{32}P-labeled oligonucleotide probe (152F: 5′-TCGGTTACTGAGAGCAGCTCAGATGAG-3′) used in solution hybridization, followed by polyacrylamide gel electrophoresis and autoradiography. Results from PCR were confirmed by Southern (DNA) blot analysis.
9. To determine the presence of the 470-20-1 sequence in the cloning source, 125 μl of plasma from PNF2161 was ultracentrifuged (Beckman 70.1 Ti rotor at 40,000 K for 1 hour). RNA was extracted from the pellet (*6*) and subjected to RT-PCR analysis with the use of RNA corresponding to the equivalent of 50 μl of plasma per reaction. Reverse transcription was performed essentially as described (*5*), except that only random hexamers were used for priming. Forty-five cycles of PCR amplification followed by solution hybridization were performed essentially as described above (*8*) with the primers 77F and 211R and hybridization probe 152F. No product was obtained from PNF2161-derived plasma if the PCR reactions were performed without prior reverse transcription, which indicated that the 470-20-1 sequence was of RNA origin.
10. J. Wages Jr. *et al.*, unpublished results.
11. Extension cDNA clones were generated by anchored PCR with a ligation reaction containing SISPA-amplified PNF2161 cDNA (*6*) and λ gt11 DNA as template for PCR amplification. A λ gt11 reverse primer (5′-TGGTAATGGTAGCGACCGGCGCTCAGC-3′) was used in combination with HGV-specific primers for 35 to 40 cycles of PCR amplification. All primer concentrations for PCR were 0.2 μM. Amplification products were agarose gel–purified, identified by hybridization to an HGV-specific oligonucleotide probe, and cloned into the plasmid vector pCR II (Invitrogen, San Diego, CA).
12. The cDNAs derived from the 5′ end of the HGV genome were obtained by a RACE method modified from that originally described [M. A. Frohman, M. K. Dush, G. R. Martin, *Proc. Natl. Acad. Sci U.S.A.* **85**, 8998 (1988); A. Belyausky, T. Vinogradova, K. Rajewsky, *Nucleic Acids Res.* **17**, 2919 (1989)]. First-strand cDNA synthesis was primed with random hexamers and synthesis was carried out with the use of either Superscript II (Perkin-Elmer/ABI, Foster City, CA) or *rTth* reverse transcriptase (Perkin-Elmer). Other reagents used in the RACE procedures were obtained from Clontech (Palo Alto, CA) as part of the AmpiFinder RACE kit. The cDNA clones derived from the 3′ end of the HGV genome were obtained by a modified anchored RT-PCR method. Polyadenylate [poly(A)] polymerase (Gibco/BRL, Gaithersburg, MD) was used to catalyze the addition of a poly(A) tail to PNF2161 RNA before cDNA synthesis. The poly(A) addition was performed according to the manufacturer's recommendations. After purification of the poly(A) modified RNA, reverse transcription with Superscript II (Gibco/BRL) was carried out with primer GV-5446IRT (5′-CGGTCCCTCGAACTCCAGCGAGTCTTTTTTTTTTTTTTT-3′). The resulting cDNA was amplified by PCR with primers GV59-5446F (5′-CTGAGCGACCTCAAGCTCCCTGGC-3′) and GV-5446IR (5′-CGGTCCCTCGAACTCCAGCGAGTC-3′). After amplification, the products of interest were identified by hybridization to an HGV-specific oligonucleotide probe.
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21. The NH_2-terminal 58–amino acid residues of R10291 contain 19% arginine residues and have a net positive charge of 9, as compared with 18.3% arginine and a net positive charge of 21 in the capsid protein of HCV1 that consists of 191 residues. Compositional bias analysis performed with the SEG program [J. C. Wootton, *Comput. Chem.* **18**, 269 (1994)] indicated that both proteins are likely to have a nonglobular conformation.
22. RNA was extracted as described (*9*) or with Purescript (Gentra Systems, Minneapolis, MN) according to the manufacturer's instructions. PCR was carried out as described (*8*, *9*). Initial product analysis was performed with a Perkin-Elmer QPCR 5000 system according to the manufacturer's instructions and with the use of an oligonucleotide probe (probe 152F). Analysis was performed in duplicate for each specimen. Specimens were scored as initially positive if both duplicate reactions were positive; if one of two duplicate reactions was positive, the result was considered indeterminate. Most specimens that gave indeterminate or positive results were retested in duplicate. Specimens that gave positive results with both duplicates were considered positive. Most specimens scored as positive were confirmed by RT-PCR analysis with the use of a second, nonoverlapping primer set (GV57-4512MF: 5′-GGACTTCCGGATAGCTGARAAGCT-3′ and 5′-GCRTCCACACAGATGGCGCA-3′) and a hybridization probe (5′-CYCGCTGRTTTGGGGTGTACTGGAAGGC-3′) (R = A or G; Y = C or T).
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24. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
25. GenBank accession numbers are U22303 for GBV-A (*16*), U22304 for GBV-B (*16*), and S62220 for HCV [N. Hayashi *et al.*, *J. Hepatol.* **17** (suppl. 3), S94 (1993)].
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27. Patient 1 received blood from nine donors. Stored samples were available from eight, and two of the eight were found to be HGV RNA–positive. No samples were available from the two donors to case 2. All 14 donors from case 2 were tested and 1 was found to be HGV-positive. All of the donors to these cases were negative for HBV and HCV markers and all had a normal ALT level.

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