Presence of a newly described human DNA virus (TTV) in patients with liver disease

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Summary

Background A newly described DNA virus, named transfusion-transmitted virus (TTV), was recently detected with high prevalence in Japanese patients with fulminant hepatitis and chronic liver disease of unknown aetiology. We investigated the presence of this virus in patients with liver disease in the UK to find out whether TTV infection is associated with liver damage.

Methods We used semi-nested PCR to amplify TTV DNA from serum samples from 126 adults, of whom 72 were patients with a range of chronic liver diseases, 24 had spontaneous resolution of infection with hepatitis C virus (HCV), and 30 were normal controls. Direct DNA sequencing and phylogenetic analysis were used to characterise the TTV isolates.

Findings We detected TTV DNA in 18 (25%) of the 72 patients with chronic liver disease, which was not different from the 10% prevalence in normal controls (p=0.15). The rate of TTV DNA was similar among patients with various liver diseases. The majority of TTV-positive cases had no biochemical or histological evidence of significant liver damage. TTV DNA sequencing of nine isolates showed the same genotypic groups as in Japan: three patients were infected with genotype 1, which showed 4% nucleotide divergence, and six patients were infected with genotype 2 with 15–27% divergence.

Interpretation The high prevalence of active TTV infection in the general population, both in the UK and in Japan, and the lack of significant liver damage, suggest that TTV, similar to hepatitis G virus (HGV), may be an example of a human virus with no clear disease association.


Introduction

Following the discovery of hepatitis C virus (HCV), it became apparent that a proportion of cases with acute and chronic hepatitis are still negative for all known viral markers, which prompted investigations to search for new hepatitis agents. In 1997, investigators in Japan isolated a DNA clone of a novel human virus using a representation-difference analysis from serum samples from a patient (TT) with post-transfusion hepatitis of unknown aetiology and designated TTV for transfusion-transmitted virus. Preliminary data show that TTV is an unenveloped, single-stranded DNA virus with 3739 nucleotides. Two genetic groups of this virus have been identified, differing by 30% in nucleotide sequences. TTV DNA was detected in 47% of patients with fulminant non-A-G hepatitis and 46% of patients with chronic liver diseases of unknown aetiology, suggesting that TTV may be the cause of some cryptogenic liver diseases. So far, TTV has been described only in Japanese patients.

The aim of this study was to gain information about the prevalence of this newly described virus in the UK and its association with liver damage. We used semi-nested PCR with primers directed to conserved regions of the TTV genome and tested serum samples from healthy individuals and patients with a range of chronic liver diseases.

Methods

Study population

The study population comprised 126 adults (83 male, 43 female) in six groups (table 1). The first group of 33 patients had chronic hepatitis C, all seropositive for anti-HCV by enzyme immunoassay (Abbott Diagnostics, Maidenhead, UK) and HCV RNA by Amplicor assay (Roche Diagnostics, Basle, Switzerland). 17 of these patients had acquired HCV through well-documented blood transfusion; 13 had a long period of intravenous drug use, and in three patients the source of infection was not evident.

Since the preliminary data suggested that TTV is transmitted mainly via a parenteral route, we selected as a second group 24 patients with past exposure to HCV via blood transfusion or intravenous drug use, but with spontaneous resolution of HCV infection and repeatedly normal liver-function tests. All were seropositive for anti-HCV, confirmed by an immunoblot assay (Innogenetics, Ghent, Belgium), but HCV RNA was undetectable in the serum on several occasions.
Detection of TTV

We extracted the total DNA from 100 µL serum using QIAamp Blood kit (QIAGEN Ltd, Crawley, UK) and resuspended it in 50 µL elution buffer. TTV DNA was amplified by semi-nested PCR with TTV-specific primers derived from two conserved regions of the published sequences. For the first round, 10 pmol primer A (sense primer 5'-ACA GAC AGA GGA GGC AAC ATG-3') and 10 pmol primer B (antisense primer 5'-TTT TAC CAT TTC CAA AGT T-3') were used in 50 µL PCR mixture containing 1×PCR buffer, 0·2 mM deoxynucleoside triphosphates, 2 mM magnesium chloride, and 0·5 U Taq polymerase (Gibco BRL, Paisley, UK). The amplification was for 35 cycles at 94°C for 30 s, 58°C for 30 s, and 72°C for 45 s, followed by 7 min at 72°C after the last cycle in a Perkin Elmer 2400 thermocycler. The second round was with another sense primer C (5'-GGC AAC ATG TTA TGG ATA GAC TTG-3') and the same antisense primer B. 1 µL of the first round PCR was transferred into another tube and amplified for 25 cycles at 94°C for 30 s, 58°C 30 s, 72°C for 30 s, with a final extension step at 72°C for 7 min giving a 272 bp amplification product. The amplicons were electrophoresed in 2% agarose gel, stained with ethidium bromide, and photographed under ultraviolet light.

We carried out direct sequencing of the amplicons with ABI 310 automated sequencer (PE Applied Biosystems) using primer C as a sequencing primer. Phylogenetic analysis was done with sequences between positions 1964 and 2178 of the published TTV genome (accession number AB008394). We used maximum-likelihood and distance-based methods with the test version 4.0d63 of PAUP*, written by David L Swofford, and PHYLIP* software packages, respectively. The dataset was bootstrap resampled 1000 times to ascertain support for major branches of the tree.

For statistical analyses, we used χ² test with Yates correction or Fisher’s exact test, as appropriate.

Results

TTV DNA was detected in 18 (25%) of 72 patients with chronic liver diseases—not significantly different from the prevalence in normal controls: three (10%) of 30 (table 1, χ²=2·069, p=0·15). When we did this analysis for the individual groups studied, we found TTV infection more frequently in patients with different liver diseases who were seronegative for HBV and HCV markers: five (38%) of 13 patients (group 4, table 1) compared with normal controls (p=0·042, Fisher’s exact test). We got similar results when taking into account all patients in this series who had liver diseases with non-B, non-C aetiology (group 4 plus liver-transplant recipients in group 5; table 1), since nine (31%) of 29 such patients were positive for TTV DNA (p=0·045, Fisher’s exact test). The prevalence of TTV infection did not differ between patients with chronic hepatitis C, patients with spontaneous resolution of HCV infection (serum HCV RNA negative), patients with chronic hepatitis B, the group with non-B, non-C liver diseases, and the group of liver-transplant recipients (table 1).

Of the 24 individuals with TTV DNA in serum, the majority (15 [63%] of 24) have had either blood transfusions or a period of intravenous drug use. Those without a history of parenteral procedures included six patients with chronic liver diseases and the three normal individuals. All but three of TTV-positive patients in our study were of European descent.

A large proportion of TTV-positive patients (14 [58%] of 24) had normal liver-function tests; these 14 included eight patients with chronic liver diseases, three with spontaneous resolution of HCV infection, and the three healthy controls. Of the 14 patients for whom liver histology was available, ten showed no significant liver damage—either no inflammation in four patients, or mild portal inflammation without interface hepatitis in six patients (table 2). The two patients in the non-B, non-C

Table 1: Prevalence of TTV infection in patients with chronic liver diseases and healthy controls

<table>
<thead>
<tr>
<th>Group</th>
<th>TTV DNA positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic hepatitis C (n=33)</td>
<td>7 (21%)</td>
</tr>
<tr>
<td>Blood transfusion (n=17)</td>
<td>4</td>
</tr>
<tr>
<td>IV drug use (n=13)</td>
<td>2</td>
</tr>
<tr>
<td>Sporadic (n=3)</td>
<td>1</td>
</tr>
<tr>
<td>Asymptomatic anti-HCV positive/HCV negative cases (n=24)</td>
<td>3 (12%)</td>
</tr>
<tr>
<td>Chronic hepatitis B (n=10)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Non-B, non-C liver diseases (n=13)</td>
<td>5 (38%)</td>
</tr>
<tr>
<td>Liver-transplant recipients non-A-G (n=16)</td>
<td>4 (25%)</td>
</tr>
<tr>
<td>Healthy controls (n=30)</td>
<td>3 (10%)</td>
</tr>
</tbody>
</table>

Table 2: Liver histology in 14 patients with TTV infection

<table>
<thead>
<tr>
<th>Grading and staging of chronic hepatitis</th>
<th>HBV and TTV</th>
<th>HCV and TTV</th>
<th>Non-A-G post-OLT and TTV</th>
<th>TTV alone and TTV</th>
<th>Alcohol misuse and TTV</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Mild</td>
<td>4</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Moderate</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Severe</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
</tbody>
</table>

Phylogenetic tree of TTV isolates identified in nine patients in UK and relation to representatives of two main genotype groups reported in Japan

A maximum-likelihood tree was calculated with assumption of a constant rate of evolution (tips of all branches being equidistant from root) and nucleotide substitution model of Hasegawa and colleagues.  

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group who had raised aminotransferases of unknown aetiology were positive for TTV DNA. Liver histology showed no inflammation or other liver pathology in one of them, and chronic hepatitis with moderate activity in the other (table 2).

We did DNA sequencing analysis in nine TTV-positive samples, which verified that the amplicons represent the TTV genome. Alignment with the published TTV sequences showed that three of these isolates belong to TTV genotype 1, and that six of the nine belong to genotype 2. The nucleotide divergence of TTV in the UK from the sequences in Japan was between 3-7% and 4-1% for the three patients infected with genotype 1a, whereas for genotype 2a, there was greater variability—between 15% and 27% (figure). Both maximum-likelihood and distance-based methods gave the same basic tree topology. Furthermore, bootstrap support for the two major lineage cluster was 100% in both cases.

Discussion

This study, the first to our knowledge for the UK, shows that the DNA virus, recently described in Japan and named TTV, is present in the UK with a similar prevalence and the same viral genotypes as in Japan, suggesting that TTV has a worldwide distribution. The prevalence of TTV DNA in the general population appears to be high—about 10%, as recorded in this study, and in blood donors in Japan. This rate of TTV infection in normal people is at least three times higher than the prevalence of HGV agent in the general population—1-7% in the USA and 3-2% in Scotland (UK). The majority of patients with TTV infection had a history of blood transfusion or intravenous drug use, confirming the importance of the parenteral route of transmission. However, we also detected TTV in a large proportion of individuals with "community-acquired" infection—38% in our series—so the role of non-parenteral transmission requires further investigation.

Although in our series the prevalence of TTV infection was slightly higher in liver-disease patients with non-B, non-C aetiology than in healthy controls, this result does not necessarily imply a causative role for TTV. More relevant is the fact that the majority of TTV-positive cases had normal liver-function tests and only minor changes in liver histology. The present study suggests, therefore, that TTV is not the causative agent of chronic liver diseases of unknown aetiology, and that neither does it affect the degree of liver damage when present in coinfection with HBV or HCV.

As a single-stranded DNA virus, TTV was suggested to belong to Paroviridae family, though it has no aminoacid similarity to the known paroviruses. The parovirus B19 is the only autonomous human pathogenic parovirus currently recognised. Experimental infection with B19 in healthy adult volunteers was shown to cause early haematological changes with a slight decrease in haemoglobin, lymphopenia, and thrombocytopenia; and, in a second-phase illness, erythematous rash consistent with an adult form of erythema infectiosum. In children, B19 infection may cause acute hepatitis at the time of erythema infectiosum, which resolves without chronic liver damage. Parovirus B19 has also been suggested as a possible causative agent of fulminant liver failure in association with aplastic anaemia in paediatric cases.

During a recent B19 epidemic in Denmark, only two adults were identified with mild liver dysfunction due to acute B19 infection in a general practice incorporating 1200 patients. Whatever the relation between TTV and parovirus B19, our data show that TTV is unlikely to be the long-sought pathogen that causes non-A, non-B, non-C hepatitis.

The scenario for TTV seems to be similar to that for the GB virus C/HGV because the clinical impact of infection with this agent is increasingly doubtful. In 73% of cases, HGV infections were not accompanied by hepatocellular injury, and an additional 16% were associated with only a minor rise in alanine aminotransferase. If our findings that TTV has no pathogenic role for liver diseases are confirmed, it may be another example of a human virus initially identified as a genomic sequence with a high prevalence in the general population—that has no clear disease association. These findings should emphasise the importance of a cautious interpretation of the role of new viral genomes discovered by molecular biological techniques.

Contributors

Nikolai Naoumov was the principal investigator and was responsible for the design of the study and writing of the paper. Nikolai Naoumov, Elena Petrova, and Mark Thomas carried out the laboratory work and did data interpretation. Mark Thomas carried out the phylogenetic analysis. Roger Williams was responsible for the series of patients and contributed to the writing of the paper.

Acknowledgments

Elena Petrova is a recipient of a NATO Postdoctoral Fellowship from the Royal Society, UK. Mark Thomas is supported by the Melford Charitable Trust, UK.

References