Epidemiol. Infect. (2002), **129**, 307–313. © 2002 Cambridge University Press
DOI: 10.1017/S0950268802007264 Printed in the United Kingdom

The prevalence of TT virus and GB virus C/hepatitis G virus infection in individuals with raised liver enzymes but without HBV or HCV infection in Taiwan

C. Y. DAI[1], M. L. YU[1], W. Y. CHANG[1], C. H. TSENG[2], C. HOU[1], Z. Y. LIN[1], S. C. CHEN[1], M. Y. HSIEH[1], L. Y. WANG[1], J. F. TSAI[1] AND W. L. CHUANG[1]*

[1] *Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, Kaohsiung, Taiwan*
[2] *School of Nursing, Kaohsiung Medical University, Kaohsiung, Taiwan*

(*Accepted 20 March 2002*)

SUMMARY

The prevalence of TT virus (TTV) and GB virus-C/hepatitis G virus (GBV-C/HGV) infection and the association with raised liver function tests in 546 Taiwanese with negative HBsAg, anti-HCV and HCV RNA was elucidated. They were tested for serum alanine aminotransferase (ALT), GBV-C/HGV RNA, anti-envelope protein 2 antibody (anti-E2) and TTV DNA. Direct sequencing and phylogenetic analyses were performed on 58 isolates for TTV genotype determination. The prevalence of TTV DNA, GBV-C/HGV RNA, anti-E2 and over all GBV-C/HGV exposure was 24·9, 3·4, 8·2 and 11·1%, respectively. Using uni- and multi-variate analyses, male gender and TTV viremia were associated significantly with raised ALT values. Sixty-nine percent of TTV isolates were deduced to be TTV genotype 1 and they had significantly lower mean age than genotype non-1 isolates. In the population, raised ALT may be related to male gender and be attributable to TTV infection but not to GBV-C/HGV among individuals with no evidence of current HBV and HCV infection. TTV genotype 1 is the most prevalent genotype and associated with younger age.

INTRODUCTION

A flavivirus-like virus named GB virus C (GBV-C) and another isolate of GBV-C named hepatitis G virus (GBV-C/HGV) have been identified as possible etiological agents of viral hepatitis in humans [1, 2]. GBV-C/HGV has been claimed to be associated with posttransfusion [2], acute community-acquired [1, 2], or fulminant or chronic hepatitis [3]. However, other reports have indicated that GBV-C/HGV is not associated with liver disease [4, 5]. The pathogenicity and hepatopathic effects of GBV-C/HGV infection remain controversial.

In 1997, DNA was cloned from an acute phase serum of a Japanese patient with posttransfusion hepatitis of unknown etiology [6]. It was derived from a novel non-enveloped, single-stranded DNA virus [6, 7] designated TT virus (TTV) [7]. It has a circular genome resembling the circoviridae [8], but the clinical significance of TTV infection is still far from definite. Some reports suggest that TTV might be associated with elevated ALT levels [6, 7, 9, 10] and play a role in the development of chronic liver diseases of unknown etiology [6, 11, 12]. By contrast, numerous investigations revealed different results i.e. that the presence of TTV DNA was not correlated with abnormal alanine aminotransferase (ALT) levels [13, 14] liver injury [15–17] or cryptogenic liver disease [18, 19]. With a wide range of sequence divergence, TTV has been classified into at least 16 genotypes separated by an evolutionary distance of $> 0{\cdot}30$ [20].

Taiwan is a hepatitis B and C endemic area [21, 22] and chronic hepatitis B virus (HBV) or chronic

* Author for correspondence: Hepatobiliary Division, Department of Internal Medicine, Kaohsiung Medical University Hospital, No. 100, Shih-Chuan 1st Rd, Kaohsiung 807, Taiwan.

hepatitis C virus (HCV) infection account for the majority cases with abnormal liver biochemistry. Nevertheless, the causative factors can not be identified definitely among some individuals with elevated ALT values. To shed light on the roles of TTV and GBV-C/HGV infection in raised ALT values among individuals who were not infected with HBV and HCV, we used polymerase chain reaction (PCR) methods and an immunoassay system to investigate the epidemiology and clinical characteristics of TTV and GBV-C/HGV infection. Direct sequencing and phylogenetic analyses were performed to elucidate the distribution and clinical characteristics of TTV genotypes.

METHODS

Subjects

Serum samples from 546 Taiwanese were studied retrospectively in the present study. All the patients were negative for both hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus antibody (anti-HCV). To exclude chronic HCV infection with negative anti-HCV, all samples were also tested for HCV RNA with negative results. All serum samples were collected in 1999 from individuals screened for chronic hepatitis (405 men, 141 women; mean age: $33{\cdot}0 \pm 11{\cdot}1$ years, range 20–72 years), and were stored at -20 °C in Kaohsiung Medical University Hospital, a medical centre in southern Taiwan. The possible causes of elevated ALT levels including drugs or herbal remedies were excluded among individuals with abnormal ALT levels.

Laboratory tests

The ALT values (normal upper limit of serum ALT = 25 IU/litre) were measured on a multichannel autoanalyser. HBsAg and second-generation anti-HCV were detected with commercially available enzyme-linked immunosorbent assay (ELISA) kits (Abbott, North Chicago, IL).

Detection of HCV RNA in serum

The nested reverse-transcription polymerase chain reaction (RT-PCR) to detect serum HCV RNA was performed using 5′-noncoding region specific primers as described previously [23].

Detection of GBV-C/HGV RNA and anti-E2 antibody in serum

GBV-C/HGV RNA was detected by nested reverse transcription PCR (RT–PCR) using primers targeting the 5′ UTR as described previously [24]. The anti-E2 antibody (anti-E2) was measured according to the manufacturer's instructions by an ELISA from Boehringer Mannheim (GmbH, Germany) [25]. Overall exposure was assessed as reactivity in either the PCR or the anti-E2 test.

Detection, nucleotide sequencing and phylogenetic analysis of TTV DNA

TTV DNA was detected by nested PCR using semi-nested primers targeting the N22 region in the coding region as described previously [7]. TTV DNA genomes from 58 samples were sequenced directly and the phylogenetic analysis was carried out. Both strands of DNA from nested PCR products were purified by QIAquick PCR purification kit (Qiagen Ltd) and directly sequenced from both directions using an ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction (PerkinElmer Cetus, Norwalk, CT) with an ABI 310 Genetic Analyzer (Applied Biosystems). Sequence comparison and phylogenetic analyses were done using the Clustal method with a weighted-residue weight table (DNAstar) with the aid of computer software.

Statistical analyses

The data were expressed as mean $\pm$ S.D. By using univariate analyses, frequency was compared between groups using the χ^2 test or Fisher's exact test, and group means were compared using the *t*-test. The presence of a statistical significance was inferred when P was less than 0·05. Stepwise logistic regression method was used to analyse the study data. Odds ratio (OR) and their associated 95% confidence intervals (CI) were used to quantify the magnitude of their associations.

RESULTS

Prevalence of TTV viremia and GBV-C/HGV infection

Of 546 Taiwanese without HBsAg and anti-HCV, 136 were positive for TTV DNA, showing an overall

https://doi.org/10.1017/S0950268802007264 Published online by Cambridge University Press

Table 1. *Comparison of clinical characteristics between individuals with and without raised alanine aminotransferase values in Taiwanese not infected with HBV or HCV*

		No. (%) of patients with raised serum ALT* values		
	No.	Positive (> 25 IU/l) ($n = 57$)	Negative (⩽ 25 IU/l) ($n = 489$)	*P*
Sex				< 0·01
Male	405	51 (12·6)	354 (87·4)	
Female	141	6 (4·3)	135 (95·7)	
Age (year, mean ± S.D.†)		34·0 ± 9·1	32·9 ± 1·3	NS
TTV DNA				< 0·05
Positive	136	21 (15·4)	115 (84·6)	
Negative	410	36 (8·8)	374 (91·2)	
GBV-C/HGV‡ RNA				
Positive	18	1 (5·6)	17 (94·4)	NS
Negative	528	56 (10·6)	472 (89·4)	
Anti-E2§				
Positive	45	5 (11·1)	40 (88·9)	NS
Negative	501	52 (10·4)	449 (89·6)	
HGV exposure**				
Positive	61	6 (9·8)	55 (90·2)	NS
Negative	485	51 (10·5)	434 (89·5)	

* ALT, alanine aminotransferase.
† Mean ± S.D., mean ± standard deviation.
‡ GBV-C/HGV, GB virus C/Hepatitis G virus.
§ Anti-E2, anti-E2 antibody of GBV-C/HGV.
** GBV-C/HGV exposure, positive for GBV-C/HGV RNA and/or anti-E2 antibody.

Table 2. *Stepwise logistic regression analysis of factors significantly associated with raised alanine aminotransferase values and TTV viraemia in Taiwanese not infected with HBV or HCV*

Dependent variable	Independent variable	Comparison	OR (95% CI)*
Raised ALT** values	Gender	Male *vs.* female	3·29 (1·37–7·88)
	TTV viraemia	Positive *vs.* negative	1·93 (1·08–3·47)
TTV DNA	Age	Each year-old increase	1·03 (1·01–1·05)
	ALT†	Abnormal *vs.* normal	1·87 (1·04–3·37)

* OR, Odds Ratio; CI, confidence interval.
† ALT, alanine aminotransferase.

prevalence of 24·9%. GBV-C/HGV RNA and anti-E2 antibodies were present in 18 (3·3%) and 45 (8·2%) of the studied samples. With two (0·4%) positive for both serum GBV-C/HGV RNA and anti-E2 antibodies, the prevalence of HGV exposure, defined as positive for serum GBV-C/HGV RNA and/or anti-E2, was 11·2%.

Factors associated with raised ALT values

Raised ALT values were found in 57 (10·4%) (mean ALT value: 37·0 ± 17·3 IU/litre range 26–118 IU/litre) of 546 Taiwanese without HBsAg, anti-HCV and HCV RNA. The clinical characteristics between individuals with and without raised ALT values are shown in Table 1. By univariate analyses, the prevalence of raised ALT values was significantly higher among male than female subjects (12·6% *vs.* 4·3%, $P < 0·01$) and also among individuals positive for TTV DNA compared with those negative for TTV DNA (15·4% *vs.* 8·8%, $P < 0·05$). The mean age and status of GBV-C/HGV infection were not observed to be different between two groups with normal and raised ALT values. Based on the results of multiple logistic regression analyses, two factors including male gender and TTV viremia were significantly associated with raised ALT values with OR and their corresponding 95% CI shown in Table 2.

https://doi.org/10.1017/S0950268802007264 Published online by Cambridge University Press

Table 3. *Comparison of clinical characteristics between individuals with and without TTV viraemia in Taiwanese not infected with HBV or HCV*

	No.	No. (%) of patients with TTV DNA		*P*
		Positive ($n = 136$)	Negative ($n = 410$)	
Sex				NS
Male	405	100 (24·7)	305 (75·3)	
Female	141	36 (25·5)	105 (74·5)	
Age (year, mean ± S.D.*)		35·8 ± 12·2	32·0 ± 10·5	< 0·001
GBV-C/HGV† RNA				
Positive	18	4 (22·2)	14 (77·8)	NS
Negative	528	132 (25·0)	396 (75·0)	
Anti-E2‡				
Positive	45	8 (17·8)	37 (82·2)	NS
Negative	501	128 (25·6)	373 (74·4)	
HGV exposure§				
Positive	61	12 (19·7)	49 (80·3)	NS
Negative	485	124 (25·6)	361 (74·4)	

* Mean ± S.D., mean ± standard deviation.
† GBV-C/HGV, GB virus C/Hepatitis G virus.
‡ Anti-E2, anti-E2 antibody of GBV-C/HGV.
§ GBV-C/HGV exposure, positive for GBV-C/HGV RNA and/or anti-E2 antibody.

Table 4. *Comparison of clinical characteristics of 58 TTV-viraemic individuals with genotype-1 and genotype non-1 infection*

	No.	No. (%) of patients with TTV viraemia		*P*
		Genotype-1 ($n = 40$)	Genotype non-1 ($n = 18$)	
Sex				NS
Male	46	34 (73·9)	12 (26·1)	
Female	12	6 (50·0)	6 (50·0)	
Age (year, mean ± S.D.*)		32·0 ± 11·7	39·0 ± 12·5	< 0·05
ALT† (IU/l, mean ± S.D.)		14·7 ± 10·4	20·9 ± 24·5	NS
Normal (⩽ 25 IU/l)	48	33 (68·8)	15 (31·2)	NS
Raised (> 25 IU/l)	10	7 (70·0)	3 (30·0)	
HGV exposure‡				
Positive	3	3 (100)	0 (0)	NS
Negative	55	37 (67·3)	18 (32·7)	

* Mean ± S.D., mean ± standard deviation.
† ALT, alanine aminotransferase.
‡ GBV-C/HGV exposure, positive for GBV-C/HGV RNA and/or anti-E2 antibody.

Factors associated with TTV viremia

The clinical characteristics of individuals with and without TTV DNA were analysed and are shown in Table 3. In addition to a positive association between TTV infection and ALT abnormality, the mean ages of the 136 TTV DNA-positive individuals were significantly higher than those of 410 negative ones (35·8 ± 12·2 *vs.* 32·0 ± 10·5 years, $P < 0·001$). No significant difference was observed between positive rates of TTV DNA among males and females and the status of GBV-C/HGV infection was not associated with TTV viraemia. Based on the results of multiple logistic regression analyses, two factors, increased age and raised ALT values, were significantly associated with TTV viraemia (OR and their corresponding 95% CI shown in Table 2).

TTV genotype

As regards subtypes of TTV of the 58 isolates, 23 (39·7%) were related to genotype 1a, 17 (29·3%) to

https://doi.org/10.1017/S0950268802007264 Published online by Cambridge University Press

genotype 1b, 2 (3·4%) to genotype 2a, 12 (20·7%) to genotype 2b and 4 (6·9%) to genotype 4. Therefore 69% (40/58) of TTV isolates were deduced to be TTV genotype 1. The mean age was significantly lower in individuals with genotype-1 infection than in those infected with genotype non-1. Factors including gender, mean ALT levels, the prevalence of raised ALT values and GBV-C/HGV exposure were not related to TTV genotype 1 infection (Table 4).

Factors associated with GBV-C/HGV infection

The mean age of the 61 GBV-C/HGV-infected individuals was significantly higher than the 485 negative ones ($38{\cdot}8 \pm 13{\cdot}5$ *vs.* $32{\cdot}2 \pm 10{\cdot}6$ years, $P < 0{\cdot}001$). No significant difference was observed between positive rates of GBV-C/HGV RNA, anti-E2 and GBV-C/HGV exposure among male and female subjects.

DISCUSSION

Taiwan is a hepatitis B and C endemic area and the majority of persons with raised ALT values are infected with HBV or HCV. In the present study involving more than 500 individuals without HBV and HCV infection, we tried to clarify the association of TTV and GBV-C/HGV infection with raised ALT values. The prevalence of GBV-C/HGV exposure among individuals with raised ALT values, having excluded HBV and HCV infection in southern Taiwan, was similar to that in blood donors reported previously [26]. GBV-C/HGV viraemia was not associated with raised ALT values, confirming the minimal pathogenic effect of GBV-C/HGV infection observed in previous reports [5]. The mean age of GBV-C/HGV-exposed Taiwanese was significantly higher than negative ones indicating increased exposure of GBV-C/HGV with ageing. This result was not found in our previous study on a hepatitis C hyperendemic community located on southern Taiwan [27]. There are two possible causes of this discrepancy. Firstly, presence of HCV infection; we found a close association between GBV-C/HGV and HCV infection in a hepatitis C hyperendemic community, and the cases of the present study were all negative for anti-HCV. Secondly, the younger ages of cases in the present study compared to our HCV-positive hyperendemic persons. It is probably due to high prevalence of HCV infection and older age of the residents that the trend of increased GBV-C/HGV exposure with increased age could not be found.

With a 24·9% rate of TTV-viraemia, which was higher than the 11% among eastern Taiwan aborigines [13] and the 10% among healthy subjects in northern Taiwan [16], TTV infection was endemic among non-B, non-C-infected individuals with raised ALT values in southern Taiwan. When considering the factors related to TTV infection, an increased age was an independent factor associated with TTV viraemia, as in previous reports [15, 28]. With the annual rate of spontaneous clearance for TTV reported to be 7–8·2% in our [29] and other previous studies [30, 31], the higher prevalence of TTV with increased ages indicate that re-infection of TTV possibly occurs. On the other hand, TTV may have non-parenteral transmission routes such as the faecal-oral route [28, 32] that might contribute to the increased TTV viraemic rate with ageing.

Not only the clinical significance and the pathogenesis of TTV infection but also the association between TTV infection and raised ALT values has been controversial [6, 7, 13]. We found that the raised ALT values were independently related to TTV viraemia among Taiwanese who were not infected with HBV and HCV. TTV infection seemed to be responsible for raised ALT values and to hint positive hepatopathic effects. Tuveri et al. suggested that TTV might be implicated in a few cases of acute and chronic non A-non G hepatitis [33]. However, Nakano et al. reported that TTV was not the main causative agent of cryptogenic liver disease [18]. Further efforts at confirming the pathogenicity of hepatocyte damage by TTV are necessary.

If TTV infection is to be precisely related to raised ALT values and hepatocyte damage, some problems should be considered. Firstly, it will become necessary to detect TTV DNA among patients with cryptogenic liver disease. Secondly, treatment for those TTV-infected patients with interferon-α (IFN) should be considered subsequently because IFN therapy has been documented to be effective against TTV with 45–55% of TTV eradication rates reported previously [34, 35] and in our recent study [36]. Thirdly, the screening of blood donors for TTV should be taken into account to avoid viral transmission by transfusion.

In the present study male gender was related to raised ALT values. We supposed the reasons that male individuals may have a higher occurrence of other pathogenic aetiologies responsible for raised

https://doi.org/10.1017/S0950268802007264 Published online by Cambridge University Press

ALT values, were alcohol abuse, nonalcoholic steatohepatitis [37] or exposure to other toxins, even though drugs or herbal remedies were excluded. Further studies are needed to search for the causative factors of raised ALT values, even though it seems to be partially attributable to TTV infection.

TTV genotype 1, distributed widely and most prevalent throughout the world, especially in Asia [38], is also the most prevalent genotype among isolates from Taiwanese with raised ALT values and not infected with HBV or HCV. A difference of pathogenesis compared with other TTV genotypes has been reported for genotype 1 that may be associated with pathogenetic potential [39]. Nevertheless, like other previous studies [16, 17], we did not find significant association between TTV genotype 1 infection and liver damage in this study. However, we made the distinct finding that the mean age was significantly lower in individuals with genotype-1 than genotype non-1 infection. The result indicated either a natural evolution of TTV from genotype 1 to non-1 or a difference of viral clearance by the host immunity for TTV genotype-1 compared with non-1 infection.

In conclusion, among Taiwanese without HBV or HCV infection, TTV infection is prevalent. Raised ALT values are associated with male gender and attributable to TTV infection but not GBV-C/HGV. Both TTV and GBV-C/HGV infection are associated with increased age. Genotype 1 of TTV is most prevalent and is associated with younger age.

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