行政院國家科學委員會補助專題研究計畫成果報告

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※ TT病毒母子感染及TT病毒、C型肝炎病毒和G型肝炎病毒間相互作用(第二年) ※
※ Mother-to-Infant Transmission of TT-Virus (TTV) and Viral Interactions Among ※
※ TTV. Hepatitis C Virus (HCV) and Hepatitis G Virus C (HGV) (2nd year) ※

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計畫類別：☑個別型計畫　□整合型計畫

計畫編號：

NSC 89－2314－B－002－267

執行期間：89年8月1日至90年7月31日

計畫主持人：林 鶴 雄

共同主持人：高 嘉 宏

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中　華　民　國　九十　年　十　月　二十五　日

行政院國家科學委員會專題研究計畫成果報告

國科會專題研究計畫成果報告撰寫格式說明

Preparation of NSC Project Reports

計畫編號：NSC 89-2314-B-002-267

執行期限：89年8月1日至90年7月31日

主持人：林鶴雄　台大醫院婦產科

共同主持人：高嘉宏

一、中文摘要

目的：評估 TT 病毒(TTV)在台灣地區孕婦受到感染之盛行率，以及 TT 病毒在嬰兒早期感染中母親傳染的角色。

研究方法：我們篩檢不同兩組的孕婦，A 組為 135 位在我們產科門診接受定期產檢之孕婦；B 組為 25 位已知感染到 G 型肝炎病毒孕婦之儲存血清。使用的方法為 TT 病毒的 open reading frame 2 的 primers，作多聚合酶連鎖反應，測量 TTV DNA。對於感染到 TT 病毒孕婦所生下的嬰兒，定期抽血測 TTV DNA 直到 1 歲為止。

結果：A 組中 40% (54/135)和 B 組中 56% (14/25)之孕婦可測出 TTV DNA (p=0.137)。在 A 組 54 位受到 TT 病毒感染的孕婦，其中 29 位孕婦和其所生下 30 位嬰兒接受追蹤檢查。在 A 組的嬰兒中，40% (12/30)以及 B 組嬰兒中，29% (4/14)可測出 TTV DNA (p=0.463)。全部 16 位受到 TT 病毒感染的嬰兒中，除了 2 位以外，其餘的肝功能檢查均屬正常。在 7 組母子配對中，以 phylogenetic 方法分析，發現在同一配對中其核酸相同性非常相異，僅在 2 組配對中發現有十分相近的遺傳相關性。

結論：TT 病毒感染台灣地區的孕婦及其嬰兒之盛行率相當高，但是對於嬰兒早期受到 TT 病毒感染時，其母親所扮演的角色極小。

關鍵詞：TT 病毒，母子感染，G 型肝炎病毒，多聚合酶連鎖反應

摘　要

目的：評估 TT 病毒(TTV)在台灣地區孕婦受到感染之盛行率，以及 TT 病毒在嬰兒早期感染中母親傳染的角色。

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關鍵語：TT 病毒，母子感染，G 型肝炎病毒，多聚合酶連鎖反應

Journal of Medical Virology

PROFESSOR A.J. ZUCKERMAN, MD, DSc, FRCP, FRCPath, FMedSci
Editor-in-Chief

Editorial Office, Royal Free and University College Medical School
Rowland Hill Street, London NW3 2PF (England)
Telephone: (0)20 7830 2579 Fax: (0)20 7830 2070 E-mail: j.m.vir@rfc.ucl.ac.uk

July 18, 2001

Professor Ho-Hsiung Lin
Dept. of Obstetrics and Gynecology
National Taiwan University Hospital
No. 7, Chung-Shan South Road
Taipei 100
Taiwan

Dear Professor Lin,

Our Ref: **JMVir/2001/4903/3**

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Early Acquisition of TT Virus in Infants: Possible Minor Role of Maternal Transmission

Ho-Hsiung Lin,[1] Jia-Horng Kao,[2,3] Ping-Ing Lee,[4] and Ding-Shinn Chen[2,5]

From the Departments of Obstetrics and Gynecology,[1] and Internal Medicine,[2] and Pediatrics,[4] and the Graduate Institute of Clinical Medicine,[3] and Hepatitis Research Center,[5] College of Medicine and the Hospital, National Taiwan University, Taipei, Taiwan

The financial support was from National Science Council (NSC89-2314-B-002-165 and NSC89-2314-B-002-267), Executive Yuan, R.O.C.

Correspondence and reprint requests should be addressed to Prof. Ho-Hsiung Lin, Department of Obstetrics and Gynecology, National Taiwan University Hospital, No.7, Chung-Shan South Road, Taipei, Taiwan 100

Tel: +886-2-2312-3456 ext 5156

Fax: +886-2-2393-4197

E-mail: hhlin@ha.mc.ntu.edu.tw

<**Running Title**> Maternal Transmission of TTV

ABSTRACT

This study assessed the prevalence of TT virus (TTV) viremia in pregnant women and evaluated the role of maternal transmission in early acquisition of TTV in infants in Taiwan. Two groups of pregnant women were screened for TTV using polymerase chain reaction. The first group included 135 healthy pregnant women attending the obstetrics department for routine prenatal care and the second group from 25 GB virus-C/hepatitis G virus (GBV-C/HGV)-infected mothers. In both groups, when TTV infection was found in mothers, serial serum samples were collected for the infants at regular intervals until 1 year of age and were tested for TTV DNA. The results showed that 40% (54/135) of the women undergoing routine prenatal care and 56% (14/25) of GBV-C/HGV-infected pregnant women were positive for TTV DNA (P=0.137). Of the 54 TTV-infected mothers in the routine prenatal group, 29 and their 30 infants received regular follow-up. The positive rate of TTV DNA in infants was 40% (12/30) in the routine prenatal group and 29% (4/14) in the group with GBV-C/HGV-infected mothers (P=0.463). All but 2 of the 16 TTV-infected infants had normal serum alanine aminotransferase levels during follow-up. The phylogenetic analysis in 7 mother-infant pairs showed that the homology was diverse in each pair and a close genetic relatedness was found in 2 mother-infant pairs. In conclusion, TTV viremia is common in pregnant Taiwanese women and their infants.

However, our results suggest that maternal transmission may play only a minor role in early acquisition of TTV in infants.

<**Key words**> TT virus (TTV), mother-to-infant transmission, GB virus-C/hepatitis G Virus (GBV-C/HGV), polymerase chain reaction (PCR)

INTRODUCTION

Perinatal transmission of blood-borne viruses, such as hepatitis B virus (HBV), hepatitis C virus (HCV), GB virus-C/hepatitis G virus (GBV-C/HGV) and human immunodeficiency virus, is well documented [Stevens et al., 1975; Okada et al., 1976; European Collaborative Study, 1992; Lin et al., 1994; Ohto et al., 1994; Peckham & Gibb, 1995; Simons et al., 1995; Linen et al., 1996; Zuckerman, 1996; Lin et al., 1998]. This mode of transmission plays a key role in maintaining HBV transmission from generation to generation [Stevens et al., 1975; Okada et al., 1976; Chen et al. 1987; Hsu et al., 1988]. In 1997, a new DNA virus was isolated from a patient with posttransfusion hepatitis of unknown etiology and designated TT virus (TTV) for the initials of the index patient [Nishizawa et al., 1997]. In addition, TTV genome was detected in patients with cryptogenic posttransfusion hepatitis and the emergence of viremia coincided with the modest increase of serum alanine aminotransferase (ALT) levels [Okamoto et al., 1998a]. The TTV genome is circular and negative stranded, and comprises 3,852 bases with a particle size of 30-50 nm, suggesting TTV is similar to the Circoviridae [Okamoto et al., 1998a; Mushahwar et al., 1999]. TTV has a wide range of sequence divergence, allowing classification into genotypes (1 and 2) differing by approximately 30% initially, each of which divides into subtypes (a and b) differing by approximately 10% [Okamoto et al., 1998a; Mushahwar et al., 1999].

Recently, this classification is now exceeded since at least 16 genotypes or even several virus species have been defined [Okamoto et al., 1999]. Besides, TTV DNA can also be detected in the feces of TTV-infected patients, suggesting TTV can be transmitted not only parenterally but also nonparenterally by a fecal-oral route [Okamoto et al., 1998b]. The reported prevalence of TTV in blood donors in different areas has ranged from 1% to 80% [Charlton et al., 1998; Naoumov et al., 1998; Okamoto et al., 1998a; Simmonds et al., 1998; Tanaka et al., 1998; Desai et al., 1999; Biagini et al., 2000] and 12% to 63% in patients with chronic liver diseases in different areas [Charlton et al., 1998; Naoumov et al., 1998; Okamoto et al., 1998a; Simmonds et al., 1998; Tanaka et al., 1998; Desai et al., 1999; Hsieh et al., 1999; Kao et al., 1999; Biagini et al., 2000; Kao et al., 2000].

Although early acquisition of TTV infection in infants has been reported in an endemic area [Davidson et al., 1999], the role of mother-to-infant transmission remains largely unknown. This prospective study assessed the prevalence of TTV infection in pregnant women and evaluated the role of maternal transmission in early acquisition of TTV in infants. The influence of GBV-C/HGV co-infection on TTV-infected mothers and infants was also evaluated.

MATERIALS AND METHODS

Subjects. A total of 135 consecutive pregnant women who attended the prenatal clinic at National Taiwan University Hospital during the first or early second trimester for routine examinations from July 1999 to August 1999 were enrolled in this study. A group of 25 GBV-C/HGV-infected mothers who had participated in our previous study [Lin et al., 1998] served as a second study group. Serum samples from both groups were collected during prenatal examinations and assay for hepatitis B surface antigen (HBsAg) and then assay for hepatitis B e antigen (HBeAg) was performed if specimens positive for HBsAg. Mothers were defined as carriers of TTV when serum specimens were positive for viral DNA at least twice within an interval of ≥3 months. Blood samples were collected from infants born to carrier mothers and were tested for TTV DNA and ALT starting from 3 months after birth until 1 year of age (usually every 2-3 months) [Lin et al., 1998].

The mode of delivery was classified as elective cesarean section, emergent cesarean section, or normal spontaneous delivery [Lin et al., 1996].

Serologic assays. Serum HBsAg and HBeAg were tested by EIA (Abbott, Abbott Park, IL). The serum ALT level was determined by an autoanalyzer (Hitachi, Tokyo). An abnormal serum ALT level was defined as >40 IU/L.

Detection of TTV DNA and GBV-C/HGV RNA. DNA or RNA was extracted

form 100 uL of each serum sample as previously described [Lin et al., 1994]. Serum TTV DNA was detected by polymerase chain reaction (PCR) with primers from the open reading frame 2 of the TTV genome. The sense primer was T801 5'-GCTAC GTCAC TAACC ACGTG-3', and the antisense primer was T935 5'-CTTCG GTGTG TAAAC TCACC-3'. PCR was done under the same conditions as previously described [Takahashi et al., 1998]. The end product of gel electrophoresis for PCR was 199 bp. Serum GBV-C/HGV RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) with primers from the 5'-untranslated region of the viral genome as previously described [Lin et al., 1998]. To avoid false-positive results, the precautions described by Kwok and Higuchi [1989] were strictly followed.

Amplification and sequencing of the TTV genome. For viremic mother-infant pairs, a segment surrounding the TATA signal region localized upstream of the open reading frame 2 of the viral genome was amplified with the two primers described above. The amplified DNA was directly sequenced with an automatic DNA sequencer (model 373A; Applied Biosystems, Foster City, CA).

Phylogenetic analysis. A phylogenetic tree was constructed using a computer program of the neighbor-joining method (PHYLIP[Phylogeny Inference Package], version 3.5c; J. Felstein, University of Washington, Seattle, USA) based on the

nucleotide sequence of the amplified TTV genome. The dataset was bootstrap re-sampled 100 times to ascertain support for major branches of the tree. Isolates from mother-infant pairs were compared with those from nonrelated subjects with TTV viremia. The TTV sequences were deposited in the DDBJ/EMBL/GenBank data base (accession no. AF380373 – AF380386).

Statistical analysis. The groups were analyzed by Pearson's chi-square and Fisher's exact tests. A value of $P<0.05$ was considered statistically significant.

RESULTS

The prevalence of TTV viremia was 40% (54/135) in the women undergoing routine prenatal care and 56% (14/25) in the GBV-C/HGV-infected pregnant women. No significant difference was noted between these two groups (P=0.137 by Pearson's chi-square test). During the follow-up period, the carrier rate in the routine prenatal group was 82% (24/29) and in the GBV-C/HGV-infected group was 83% (12/14). None of the 29 mothers in the routine prenatal group was GBV-C/HGV RNA-positive, and none of the total of 44 TTV-infected mothers in the two groups were drug users.

The positive rate for TTV DNA in infants born to TTV-infected mothers was 40% (12/30) in the routine prenatal group (twin delivery by one mother), and 29% (4/14) in the GBV-C/HGV-infected group. There was no significant difference in the positive rate for TTV DNA in infants between the two groups (P=0.463 by Pearson's chi-square test). All of the 16 mothers of TTV-infected infants had normal spontaneous delivery/emergent cesarean section except for one (mother of Case 3 in Figure 1) who had elective cesarean section, while the remaining 28 mothers of TTV-uninfected infants all had normal spontaneous delivery/emergent cesarean section. Only 1 of the 16 TTV-infected infants was breast-fed for a 2 month duration, whereas 5 of the 28 TTV-uninfected infants were breast-fed for 1 to 4 months.

TTV viremia, once detected, persisted in all the 16 infants but Case 9 (Figure 1). Case 6 showed transient and mild elevation of serum ALT level. Case 10 had mild elevation of serum ALT level by 9 months of age with values ranging from 46 to 141 IU/L. However, this infant was born to an HBeAg-positive mother and was positive for HBsAg antigenemia after birth and this condition persisted at the follow-up at age of 12 months. Thus, the cause of the elevated serum ALT level in this patient may have been due to perinatally transmitted HBV. In total, the carrier rate of TTV-infected infants was 94% (15/16) during the follow-up period.

The nucleotide sequences (199 bp) of the TTV genome could be determined and compared in 7 of the 16 mother-infant pairs for whom the amount of serum samples was adequate for this study. The homology was diverse in each pair, and phylogenetic analysis showed a close genetic relatedness only in two mother-infant pairs (Nos. 1 and 5, Figure 2) [29%, (95% CI, 3.7-71%)]. In contrast, the remaining 5 mother-infant pairs showed a low percentage of homology at the molecular level.

DISCUSSION

The first report which described the discovery of this novel DNA virus (TTV) used the primers of nested PCR from the open reading frame 1 of the TTV genome in 1997 [Nishizawa et al.]. Later, development of a new set of PCR primers in 1998 enabled the detection of a large number of genetically distant TTV isolates [Takahashi et al.]. We adopted this new primer in the PCR of this study. Our data showed that the prevalence of TTV viremia was 40% in pregnant Taiwanese women undergoing routine prenatal care, which is comparable to previous reports which have ranged from 48% to 61% [Davidson et al., 1999; Schroter et al., 2000; Sugiyama et al., 2000], and is similar to our data for age-matched women (42%) (unpublished data). In this study, the carrier rate of TTV infection in pregnant women was 82%, which is consistent with the results of a previous report [Davidson et al., 1999]. Similarly, in GBV-C/HGV-infected women, the prevalence of TTV viremia and the carrier rate of TTV infection were 56% and 83%, respectively.

The first blood samples from TTV-infected infants were collected when the infants were 2-3 months of age. Whether TTV intrauterine infection is possible remains unclear. Several studies have demonstrated the absence of intrauterine infection without TTV DNA in cord blood or blood obtained 1 week after birth [Toyoda et al., 1999; Kazi et al., 2000]. In contrast, other studies found TTV DNA in

cord blood samples [Saback, et al., 1999; Goto et al., 2000]. In the present study, the positive rate of TTV DNA in infants of TTV-infected mothers was 40% and in GBV-C/HGV co-infected mothers was 28%. Our data were comparable to that of a recent study from an area endemic for TTV infection [Davidson et al., 1999]. Using phylogenetic analysis of TTV isolates from 7 viremic mother-infant pairs, we found a close genetic relatedness in 2 mother-infant pairs, suggesting the 2 infants were indeed infected by their mothers. In contrast, the remaining 5 infants were not infected by their corresponding TTV-positive mothers as revealed by the low percentage of homology at the molecular level in each pair (Figure 2). This finding indicates that maternal transmission may play a minor role in the early acquisition of TTV in infants in Taiwan. Thus, other modes of transmission including nonparenteral exposure may be involved [Davidson et al., 1999; Sugiyama et al., 1999]. This situation is different from other blood-borne hepatitis viruses such as HBV, HCV and GBV-C/HGV [Stevens et al., 1975; Okada et al., 1976; Chen et al., 1987; Hsu et al., 1988; Lin et al., 1998]. Our results also showed that no correlation between mode of delivery and TTV infection in infants. Because TTV infection could occur through both parenteral and nonparenteral routes as previously reported [Okamoto et al., 1998b], the mode of delivery and maternal viremia level might not play key roles in the transmission of TTV, which are very important for other blood-borne viruses [Lin

et al., 1994; Lin et al., 1998]. Besides, our data also suggest that breast-feeding had no correlation with TTV infection in infants. Although we did not investigate whether TTV DNA was present in the breast milk, a previous study indicated that infection in infants was not due to milk-borne virus [Kazi et al., 2000], and another study indicated that most infants were infected with TTV even before breast-feeding began [Sugiyama et al., 2000].

In the present study, 2 of the 16 TTV-infected neonates had mild and transient elevations of serum ALT levels during the follow-up until 1 year of age. One TTV viremic infant (Case 10) who was born to an HBeAg-positive and TTV DNA-positive mother was also co-infected with HBV and had HBsAg antigenemia which persisted during the follow-up period. Therefore the elevation of serum ALT level in this infant was likely due to HBV infection. The other TTV-infected infant (Case 6) had mild and transient elevation of serum ALT level during the follow-up period. These data suggest that TTV infection may not be harmful to the liver [Charlton et al., 1998; Naoumov et al., 1998; Hsieh et al., 1999; Kao et al., 1999; Kao et al., 2000].

The pregnant women infected with GBV-C/HGV in this study had a frequency and carrier rate of TTV infection cases as well as a frequency of perinatal TTV transmission that were not different to those mothers infected with TTV alone in the routine prenatal care group. This result suggests that GBV-C/HGV co-infection does

not influence the epidemiological features of TTV infection.

In conclusion, the prevalence of TTV viremia in pregnant Taiwanese women in this study was 40% with a carrier rate of 82%, and the positive rate for TTV DNA in their infants was 40% with a carrier rate of 94%. Our data suggest maternal transmission may play a minor role in early acquisition of TTV in Taiwanese infants.

<ACKNOWLEDGEMENTS>

We would like to thank Mrs. Donna Y. Lee for her skillful technical assistance in this study.

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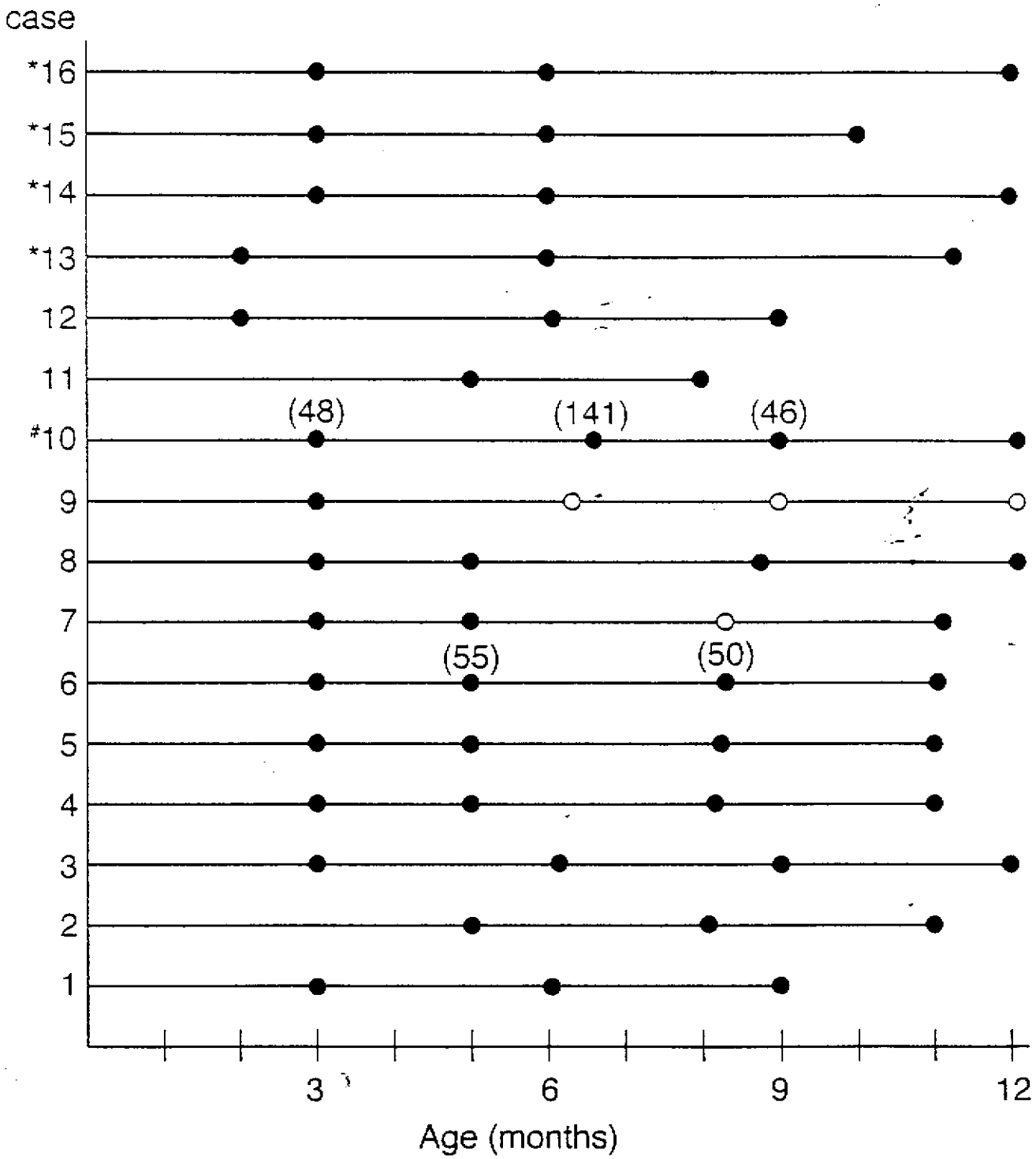
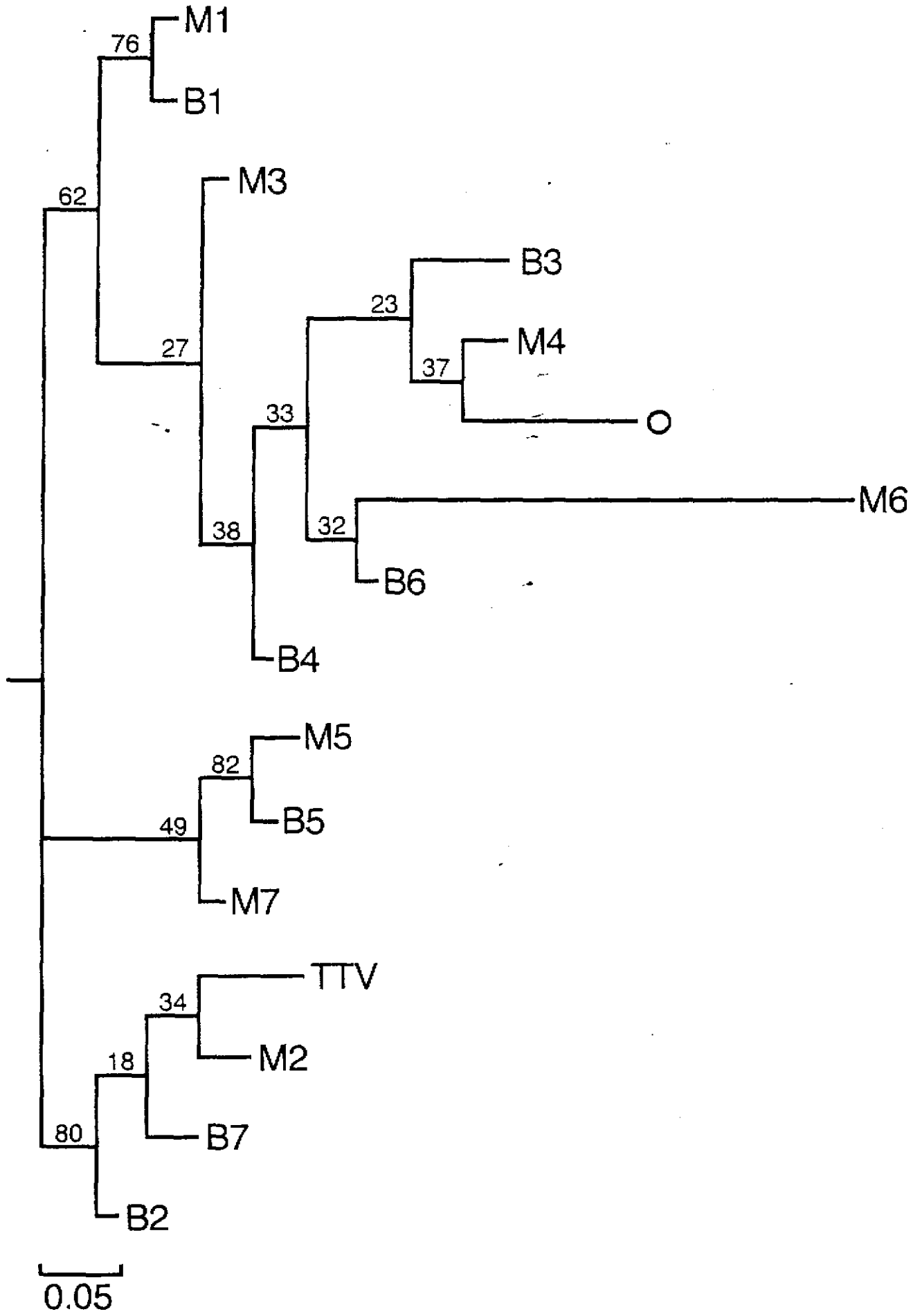
<FIGURE LEGENDS>

Figure 1. Follow-up of serum TTV DNA (•) in 16 infected infants born to TTV-infected mothers. (○) represents negative TTV DNA. Case 10 (#) was also HBsAg-positive. Cases 13-16 (*) whose mothers were co-infected with HGV. Nos. in parenthesis represent elevated serum alanine aminotransferase level (IU/L). Serum alanine aminotransferase levels were normal (<40 IU/L) for all but 2.

Figure 2. Phylogenetic analysis of TTV isolates from 7 viremic mother-infant pairs (M1,B1 to M7,B7), 1 non-implicated TTV-infected patient (open circle), and 1 reported TTV clone (GH1: DDBJ/EMBL/GenBank accession no. AB017613) based on the nucleotide sequences of a segment surrounding the TATA signal region localized upstream of open reading frame 2 of the TTV genome. The phylogenetic tree was constructed using a computer program of the neighbor-joining method (PHYLIP [Phylogeny Inference Package], version 3.5c; J. Felstein, University of Washington, Seattle). The dataset was bootstrap re-sampled 100 times, and the tree is not rooted. The horizontal bar indicates the number of nucleotide substitution per site. M: mother; B: baby.


case
*16
*15
*14
*13
12
11
#10
9
8
7
6
5
4
3
2
1
(48)
(141)
(46)
(55)
(50)
3
6
9
12
Age (months)


M1
76
B1
62
M3
B3
23
M4
27
37
O
33
M6
38
32
B6
B4
M5
82
B5
49
M7
TTV
34
M2
18
B7
80
B2
0.05