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(第一年)※

※ Mother-to-Infant Transmission of TT-Virus (TTV) and Viral Interactions Among ※

※ TTV. Hepatitis C Virus (HCV) and Hepatitis G Virus C (HGV) (1^{st} year) ※

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執行期間：88 年 8 月 1 日至 89 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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執行單位：台大醫學院婦產科

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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。

關鍵語：TT 病毒，母子感染，G 型肝炎病毒，多聚合酶連鎖反應

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

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

<ABSTRACT>

Objectives: To assess the prevalence of TT virus (TTV) viremia in pregnant women and to evaluate the role of maternal transmission in early acquisition of TTV in infants in Taiwan.

Study design: We screened two independent groups of pregnant women- 135 pregnant women attending regularly at our obstetrics department (group A) and the stored sera from 25 HGV-infected mothers (group B) by using polymerase chain reaction with primers from open reading frame 2 of TTV. For infants born to TTV-infected mothers, serial serum samples at regular intervals until 1-year-old were taken and tested for TTV DNA.

Results:, Forty percent (54/135) in group A and 56% (14/25) in group B pregnant women were positive for TTV DNA (P=0.137). Of 54 TTV-infected mothers in group A, 29 and their 30 infants received regular follow-up. The positive rate of TTV DNA in infants was 40% (12/30) in group A and 29% (4/14) in group B (P=0.463). All but 2 of the 16 TTV viremic 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.

Conclusions: TTV viremia is prevalent in pregnant Taiwanese women and their

<List of Abbreviations>

ALT	Alanine aminotransferase
DNA	Deoxyribonucleic acid
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HGV	Hepatitis G virus
PCR	Polymerase chain reaction
RNA	Ribonucleic acid
TTV	TT virus

<INTRODUCTION>

Perinatal transmission of blood-borne viruses, such as hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis G virus (HGV) and human immunodeficiency virus, is well documented.[1-7] This mode of transmission plays a key role in maintaining HBV transmission from generation to generation.[1,2,8,9] 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.[10] 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.[11] Its 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.[11,12] TTV has a wide range of sequence divergence, allowing classification into genotypes (1 and 2) differing by approximately 30%, each of which divides into subtypes (a and b) differing by approximately 10%.[11,12] 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.[13] In literature, the prevalence of TTV in blood donors in different areas ranged from 1% to 36%,[11,14-17] and 12% to 63% in chronic liver diseases in different areas.[11,14-20]

Although early acquisition of TTV infection in infants has been reported in an

endemic area,[21] the role of mother-to-infant transmission remains largely unknown. Thus, we performed a prospective study to assess the prevalence of TTV infection in pregnant women and to evaluate the role of maternal transmission in early acquisition of TTV in infants. The influence of HGV co-infection on TTV-infected mothers and infants was also evaluated.

genome. The sense primer was T801 5'-GCTAC GTCAC TAACC ACGTG-3', and antisense primer was T935 5'-CTTCG GTGTG TAAAC TCACC-3'. PCR was done under the same conditions described before.[22] The end product of gel electrophoresis for PCR was 199 bp. Serum HGV RNA was detected by reverse transcription-polymerase chain reaction (RT-PCR) with primers from the 5'-untranslated region of the viral genome described before.[5] To avoid false-positive results, the precautions described by Kwok and Higuchi[23] were strictly followed.

Amplification and sequencing of the open reading frame 2 of the TTV genome. For viremic mother-infant pairs, a part 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 after the program of neighbor-joining method (PHYLIP[Phylogeny Inference Package], version 3.5c; J. Felstein, University of Washigton, Seattle, USA) based on the nucleotide sequence of the amplified open reading frame 2 region of the TTV genome. Isolates from mother-infant pairs were compared with those from nonrelated subjects with TTV viremia.

Statistical analysis. The categorical groups were analyzed by Pearson chi-square

test. $P<0.05$ was considered statistically significant.

<RESULTS>

The prevalence of TTV viremia in pregnant women (group A) was 40% (54/135) and 56% (14/25) in HGV-infected pregnant women (group B). No statistically significant difference was noted between them (P=0.137). During the follow-up period, the carrier rate was 82% (24/29) and 83% (12/14) in group A and group B, respectively. None of the 29 mothers in group A was HGV RNA-positive.

The positive rate of TTV DNA in infants born to TTV-infected mothers was 40% (12/30) in group A due to twin delivery by one mother, and 29% (4/14) in group B. There was no statistically significant difference between the two groups (P=0.463). All the 16 TTV-infected infants were viremic for TTV DNA, and once detected, the TTV viremia persisted in all 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, it was born to an HBeAg-positive mother and HBsAg antigenemia was positive after birth and persisted thereafter. Thus, the cause of the elevated serum ALT level may be 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 open reading frame 2 of the TTV genome could be determined and compared in 7 of the 16 mother-infant pairs from

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). In contrast, the remaining 5 mother-infant pairs showed 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 TTV genome in 1997.[10] Later, a new set of primers of PCR method to increase the sensitivity for detecting TTV DNA was developed in 1999.[22] Thus, we adopted this new primer for PCR method in this study. Our data showed that the prevalence of TTV viremia was 40% in pregnant Taiwanese women, which is comparable to previous reports with a range from 48% to 61%.[21,24,25] Furthermore, we found that the carrier rate of TTV infection in pregnant women was 82%. The results were consistent with those of previous reports.[21] Similarly, the prevalence of TTV viremia and carrier rate of TTV infection was 56% and 83%, respectively, in HGV-co-infected pregnant women.

The positive rate of TTV DNA in infants was 40% and 28% in TTV-infected mothers and TTV and HGV co-infected mothers, respectively. Our data were comparable to a recent report from an area endemic for TTV infection.[21] By 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 fact

indicates that maternal transmission plays a minor role in the early acquisition of TTV in infants in Taiwan where TTV infection is not so endemic. Thus, other modes of transmission including nonparenteral exposure may be involved.[21,26] This situation is different from other blood-borne hepatitis viruses such as HBV, HCV and HGV.[1,2,8,9] We further explored the issue whether the mode of delivery can influence the positive rate of TTV infection in their infants. Our results showed that there was no correlation between mode of delivery and TTV infection in their infants. Because TTV infection could be through both parenteral and nonparenteral routes as previously reported,[13] the mode of delivery and maternal viremia level might not be the key roles for transmission of TTV, which are very important for other blood-borne viruses.[3,5]

In this 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) born to an HBeAg-positive, 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 srum ALT level during the follow-up period. These data suggested that TTV infection may not be harmful to the liver.[16-20]

The group B pregnant women in this study were infected with HGV. However, the frequency and carrier rate of TTV infection in group B cases as well as frequency of perinatal TTV transmission were not different to those infected with TTV alone (group A). Thus, co-infection of HGV does not influence the epidemiological features of TTV infection.

In conclusion, the prevalence of TTV viremia in pregnant Taiwanese women is 40% with a carrier rate of 82%, and the positive rate of TTV DNA in their infants is 40% with a carrier rate of 94%. Maternal transmission plays a minor role in early acquisition of TTV in infants in Taiwan.

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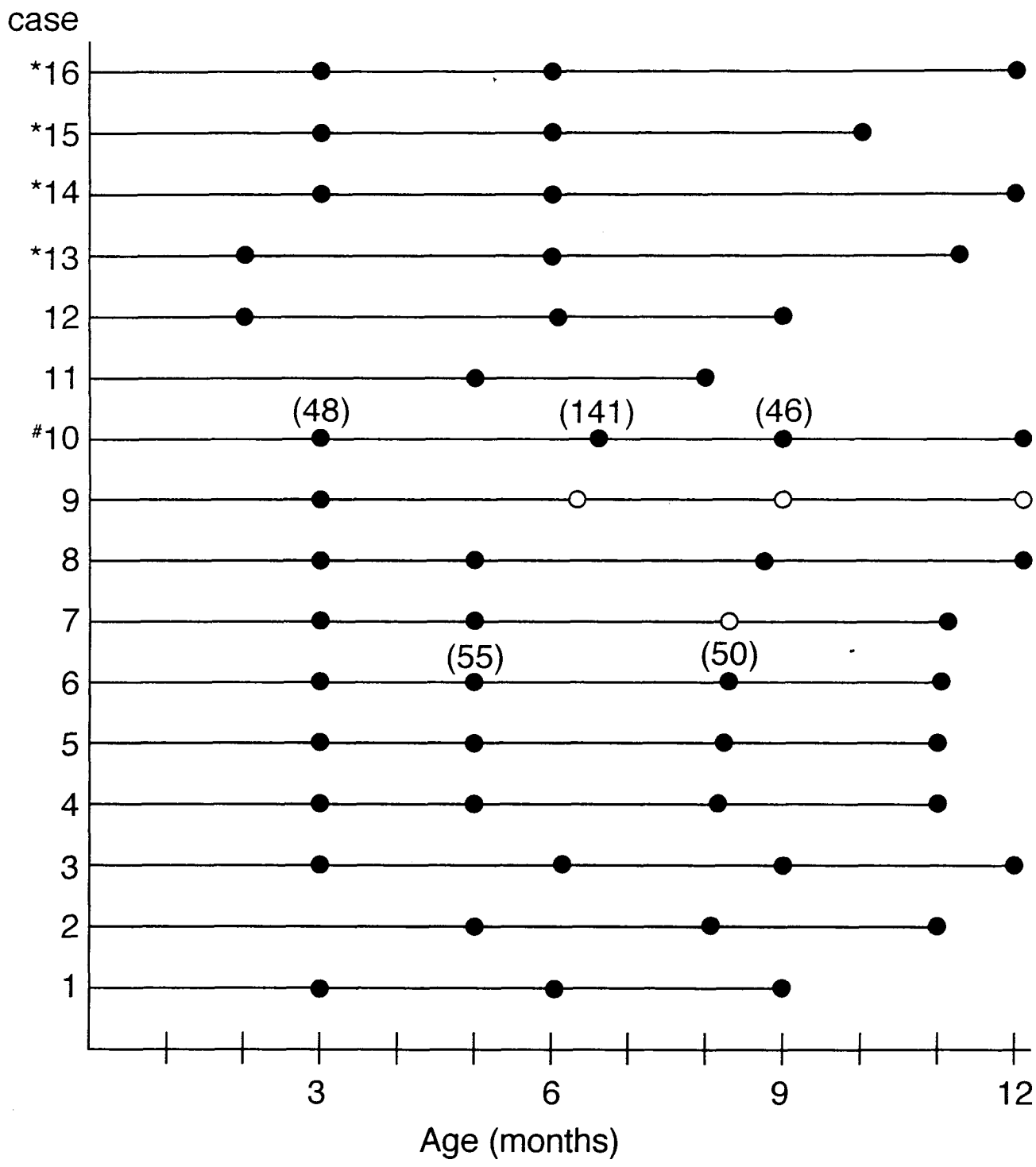
<FIGURE LEGENDS>

Figure 1. Follow-up of serum TTV DNA (●) in 16 infected infants born to TTV-infected mothers. (○) represents negative TTV DNA. Case 10 ($^{\#}$) is also HBsAg-positive. Cases 13-16 (*) whose mothers are 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, based on nucleotide sequence of open reading frame 2 of the TTV genome. Phylogenetic tree was constructed by neighbor-joining method in PHYLIP package (version 3.5c). Open circle represents nonrelated TTV isolate from other source. M: mother; B: baby. Nos. represent case nos. of TTV-infected mother-infant pairs.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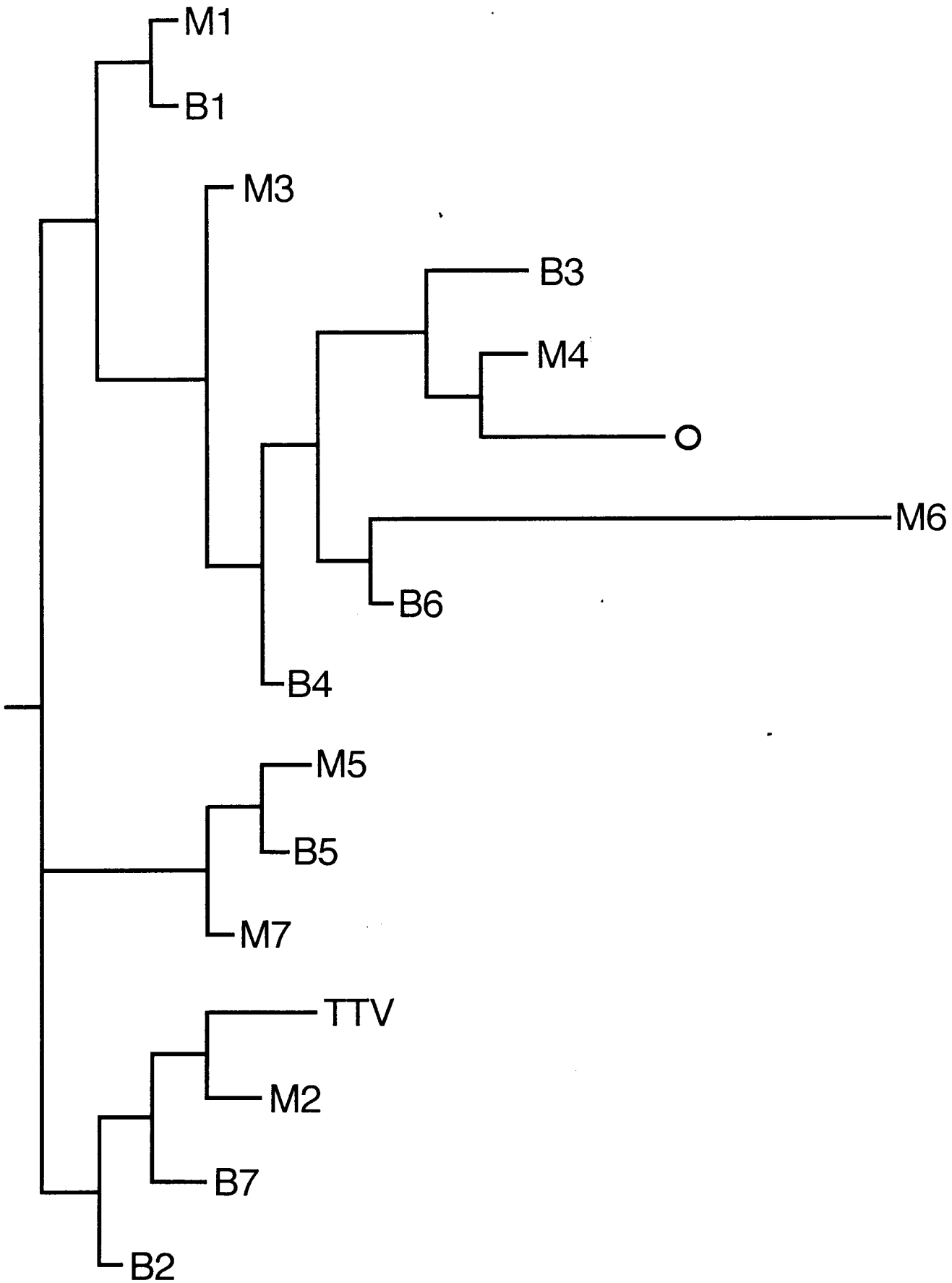
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