

Sequences Analysis and Identification of Motifs in TTMV and TTMDV Viruses: A Systematic Review

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Objective

The goal of this study is to examine and assess the literature on the consensus sequence(s) in TTMV and TTMDV viruses, as well as their heterogeneity and features.

Methods

Published, complete sequences of TTMV and TTMDV were identified and retrieved using BLAST search. Bioinformatics tools such as ClustalW, phylogenetic mapping, and MEME were used to align and identify consensus sequences of these Anelloviruses.

Results

The complete genome and ORF demonstrated general sequence differences at the nucleotide and protein levels. The phylogenetic tree of TTMV and TTMDV revealed a cluster of strains that diverge significantly. Furthermore, common consensus sequences (motifs) were also identified.

Conclusion

These findings reveal significant structural variations between TTMV and TTMDV strains. They have a similar identity in terms of consensus or MOTIF in the sequences but are not organized in the same region. Instead, they are unique to all sequences that may lead to the expression of analogous proteins, even though their genomic length and genetic identity are vastly different.

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The non-enveloped, circular, single-stranded DNA viruses Torque teno mini virus (TTMV) and Torque teno midi virus (TTMDV) were found in the Anelloviridae family in 2000 and 2007, respectively.¹ These viruses are defined by their extremely high prevalence, relatively uniform worldwide distribution, increased genomic variability, and apparent pan-tropism at the host level. TTMDV has a genome size of 3.24-3.25 kb, while TTMV has a genome size of 2.86-2.91 kb.² Both viruses have a similar genome structure, including an untranslated region (UTR), an open reading frame (ORF), and a short G/C rich region (Digital Supplementary File, Figure 1).^{3,4}

Polymerase chain reaction (PCR) with Torque Teno Virus(TTV)-specific primers that partially matched homologous sequences in TTMV was used to identify TTMV, which is distantly related to TTV.¹ In cultured cells transfected with recombinant TTV DNA as well as in bone marrow cells taken from an infected patient, three different mRNAs of 2.9-3.0 kb, 1.2 kb, and 1.0 kb with common 50 and 30 termini were found, all transcribed from the genomic, minus-strand of TTV DNA.^{5,6} These three mRNAs share a brief splicing sequence of about 100 nucleotides (nt). Furthermore, the 1.2-kb and 1.0-kb mRNAs have 1700 nt and 1900 nt of extra splicing, respectively, resulting in the development of two unique open reading frames (ORF3 and ORF4). TTV or TTMV infection is ubiquitous, although the link between TTV and disease is unknown.^{7,8} TTMDV was reported by Ninomyia et al⁸ as a cross between TTV and TTMV; nonetheless, despite current databases containing numerous entire genomic sequences of the virus, information on TTMDV remains scant. TTV, TTMV, and TTMDV have quite different genomic structures, and all three viruses have four ORF regions and a GC-rich UTR with few highly conserved or consensus sequences. Variation in genome organization caused by modest variations in sequence identity across the genomes has been reported often among isolates of the same type, raising concerns about the functionality of these variant genes. Given that these viruses function similarly in the host, the current work attempts to examine and further analyze their behavior using various bioinformatics tools. TTV received the majority of research and understanding, while little or no research on TTMV and TTMDV has been done thus far, justifying the need for conducting the study on the current two viruses in particular.

METHODS

Sequences used in the present study

The National Center for Biotechnology Information (NCBI) tool was used to perform a random search on

the TTMV and TTMDV viruses and to identify and retrieve the full-length genome sequences. Viral ORF and protein sequences were retrieved for the same viruses. Digital Supplementary File, Tables 1 and 2 (a and b) show the DNA and ORF's sequences used in the study for TTMV and TTMDV, respectively. The NCBI search yielded nine full-length genomes of TTMV variants and two ORF's (ORF 1 and ORF 2) for both DNA and proteins, which were used for this study (Digital Supplementary File, Table 1).

Phylogenetic analyses of TTMV and TTMDV

MEGA 10 software⁹ was used to align the genomic sequences for both TTMV and TTMDV. With default parameters, the ClustalW method in MEGA10 was used to align DNA sequences. The distance matrix was calculated by using distance and the maximum composite likelihood with Gap/Missing data treatment was conducted by 1000 replicates. Obtained distance matrix and maximum likelihood was used for constructing Molecular phylogenetic analysis. Bootstrap proportions were determined using 1000 replicates to assess the analysis's robustness. A similar procedure was used with the ORF sequences of the other two viruses.

MOTIF Detection and Measurement of Similarities

Multiple EM for Motif Elicitation (MEME), an EM-based technique, was utilized to screen for motif sequences in the TTMV and TTMDV.¹⁰ Genome and ORF sequences of each virus (Digital Supplementary File, Figure 1, Tables 1 and 2) were submitted into MEME (<http://meme.nbrc.net/meme/version 5.1.1>). Each motif set's information content value (IC - relative entropy of the motif compared to a uniform background frequency model) was measured.

RESULTS

The sequences clustered in distinct clades, according to phylogenetic analyses (Digital Supplementary File, Figure 2 for TTMV and Digital Supplementary File, Figure 3 for TTMDV). In this review, we looked at 9 full TTMV sequences and their associated ORF DNA and protein sequences, as well as 11 complete TTMDV sequences and their respective ORF DNA and protein sequences. The 11 TTMDV isolates found in the current investigation and the 9 previously described TTMV isolates were compiled into a phylogenetic tree, which likewise showed a high level of genetic divergence between the various strains. The DNA and protein sequences of both TTMV and TTMDV were found to be significantly different. However, the difference is more noticeable in protein sequences than in nucleotide sequences. In TTMV and TTMDV, MEME analysis

indicated the presence of three MOTIFS for each set of sequences (Digital Supplementary File, Figure 3a, 4a 5a, Table 3a and 3b). The hypothesized genomic organization and transcription profile of TTMDVs are comparable to TTMVs, as evidenced by the maintenance of the coding capability for ORF1 and ORF2 proteins as well as two joint proteins (ORF3 and ORF4).

DISCUSSION

Studies have revealed up to a 30% heterogeneity in genomic sequences between TTV, TTMV, and TTMDV.^{2,11–13} There are several distinct genotypes can infect the same person at the same time, and different viral genotype combinations frequently predominate in different organs. *Anelloviruses* infect healthy humans, and co-infections with numerous distinct lineages are widespread^{14–17} and these lineages make up a person's "personal anellome." Within the *Anelloviridae* family, there are three genera of human anelloviruses: *Alphatorquevirus* (torque teno viruses (TTVs)), *Betatorquevirus* (torque teno mini viruses (TTMVs)), and *Gammatorquevirus* (torque teno midi viruses (TTMDVs)), which share just 35% pairwise nucleotide identity. According to the findings, the differences between TTMV and TTMDV are substantially bigger in protein sequences than in nucleotide sequences. To illustrate this further, TTMV revealed 99% similarity in DNA sequences but only 51% similarity in protein sequences, implying that viruses carry 50% heterogeneity within the same species. TTMDV, on the other hand, has a 56% similarity in DNA sequences and a 50% similarity in protein sequences, which is rather interesting and unusual.

The reason for the wide genomic variation in these viruses and the process by which it happens are unknown. One theory is that these viruses mutate quickly. Many studies have looked at the nucleotide sequences of different isolates, indicating either the same sequence's persistence over time or alterations in the host's sequence.^{18–20} DNA viruses like human herpesviruses^{21–26} JC virus²⁷ and papillomaviruses^{28–30}, have some genetic heterogeneity, but *Anelloviruses* have plenty. There are at least two possibilities for why this is the case. If its replication is carried out by a host DNA polymerase complex due to the absence of a polymerase gene in the TTV genome,³¹ such significant genetic variability would indicate that it has been there for hundreds of thousands, if not millions, of years.

Second, only a small number of these viruses contain all of the necessary components to properly infect hosts, or co-infections with other isolates may bring

the required components together.³² Concurrent infections with hepatitis viruses (HBV and HCV, for example) have been extensively studied, and high viral loads in chronic hepatitis C patients have been linked to the development of hepatocellular carcinoma.³³ The histological grade in hepatocellular patients with TTV-HCV co-infection appeared to be higher than in those infected with just HCV.³⁴ Furthermore, it has been proposed that ORF3 of TTV genotype 1a encodes a protein that is comparable to HCV's nonstructural protein 5A (NS5A). This protein is vital in the antiviral response generated by IFN³⁴. Furthermore, Matsubara et al³⁵ discovered TTMV in infant cord blood, implying that, like other intrauterine viruses such as the human immunodeficiency virus 1, rubella virus, cytomegalovirus, and parvoviruses, TTMV could be transferred from mother to child.

In comparison to TTV, little is known regarding the prevalence, infection, recombination, and co-infection of TTMV and TTMDV in healthy and infected individuals. However, these *Anelloviridae* viruses have a similar genome organization, including an untranslated region (UTR), an open reading frame (ORF), and a short G/C rich region, with nucleotide and amino acid levels in the ORF portions that are quite variable. However, while these assertions are theoretically supported, more specific experimental evidence is needed to assess the pathogenicity of these viruses. Because they replicate through the rolling circle method, the preceding statement supports consensus sequences are thought to play a vital role in replication and recombination.

Third, the process by which viruses evolve through recombination is critical to occur on a regular basis^{36,37} for these viruses. TTV, for example, shares some characteristics of *Circoviridae*³⁸ viruses, such as a negative-stranded circular DNA genome, but it is otherwise unique from them. On the other hand, the TTV genome is nearly twice as massive as that of *Circoviridae* members. A recent study suggested that intra-genomic recombination is a cause of TTV genome diversity³⁹ and that frequent recombination processes mask differences between viral populations³⁷

However, Jelcic et al⁴⁰ have cast doubt on this, stating that the reported recombination events are merely PCR artifacts.

Furthermore, a recent study suggested that the *Epstein-Barr* virus, a herpesvirus, functions as a helper for TTV in terms of recombination during co-infection.^{41,42} DNA viruses lack their own replicative machinery, as they rely on the host's DNA

polymerases, which have a high level of proofreading capacity. However, the ssDNA genomes of parvoviruses have been found to evolve fast, and this enhanced mutation rate could be explained by the single-stranded genome's ability to encode replication proteins.⁴³ In the current study, the first phylogenetic tree² based on TTMV genomic sequences revealed a large cluster of strains with ORF1 nucleotide and amino acid divergences of roughly 42% and 67%, respectively.^{1,44} On the other hand, the evolutionary distances between these sequences are within the range. Although the TTMDV data is scarce; nevertheless, new database searches have turned up multiple whole genomic sequences.

For the three anelloviral genera, the International Committee on Taxonomy of Viruses (ICTV) classification is entirely based on ORF1 analysis, with a species demarcation threshold of 35% nucleotide sequence identity (Alphatorquevirus, Betatorquevirus, and Gammatorquevirus). Alphatorquevirus, which includes 29 species (Torque teno virus 1–29), Betatorquevirus, which includes 12 species (Torque teno mini virus 1–12); and Gammatorquevirus, which includes 15 species (Torque teno midi virus 1–15), are other related viruses that may be members of these genera but have not been assigned to separate species. Based on the frequency pattern of evolutionary distances in the phylogenetic analysis, this observation strongly suggests that these TTV and TTMDV represent different strains that may belong to the same genotype. The fact that primate isolates cluster phylogenetically shows that anelloviruses constitute a long-lived family with genetic variety resulting from millions of years of evolution. The clustering of these viruses was discovered to be similar to that of human and primate HPVs.⁴⁵

The phylogenetic groups of TTMV and TTMDV are poorly understood⁴⁶, although a comparison of TTMV ORF1 sequences revealed considerable divergence, and whole-genome analysis of TTMDV revealed sequence clustering.⁴⁶ Furthermore, despite the three viruses having a similar genome architecture, their ORFs are significantly diverse at both the nucleotide and amino acid levels.⁷ The central area of ORF regions⁴⁷, whose translation product comprises an arginine-rich sequence in the N terminus, has the highest rate of sequence divergence resulting in amino acid conversions, which helped us analyze and comprehend the ORF regions of TTMV and TTMDV. More intriguingly, the ORF1 proteins of the N-terminal region contains arginine repeats.⁴⁸ This

arginine-rich region is remarkably similar to the ARM motif seen in *circovirus* Cap proteins, which has been shown to bind DNA.^{48,49} This trait could indicate that ORF1 protein, like *circovirus* Cap proteins, is important for ssDNA packing.⁴⁹ ORF2 protein, on the other hand, is most likely a regulatory protein that aids the virus in disrupting the host's immune response.^{50,51} Recently, potential new species have been discovered mostly in the TTMV and TTMDV genera. The latter is presumably more difficult to detect in methods without viral enrichment and, as a result, are less well understood.

Furthermore, based on its N-terminal arginine-rich region, which is shared with the circovirus capsid proteins, Nishizawa et al and Erker et al^{sup>48,52} postulated that ORF-1 encodes the viral capsid protein, although the functions of the proteins expressed by ORF-2 and ORF-3 are still unknown. An origin of replication in the conserved area is used to identify anelloviruses to learn more about their special characteristics on host-specific reactions.^{39,53} These genomes contain conserved motifs similar to those found in *Circoviridae* family rolling-circle replication related proteins (Rep proteins).⁵⁴ A conserved mononucleotide necessary for rolling-circle replication has also been identified, which is already present in single-stranded DNA viruses.^{55,56} In silico investigations of potential proteins expressed by these sub-viral components, as well as virus transcripts extracted from TTV-infected cultures, have identified signature motifs of proteins involved in autoimmune illnesses.⁵⁷

CONCLUSION

It is important to note that viruses sometimes use many motifs for a single activity. If the primary motif is absent, they might be able to evolve systems to use alternate ones. Within host cells, these motifs mediate connections and molecular functions. As a result, a growing body of evidence points to themes as possible targets for therapeutic drugs. It is crucial to stress that understanding functional motifs will aid in the prediction of protein properties, cellular connections, or the potential function of a protein. Future study should establish the relationship between functional motifs and protein functional analysis and/or prediction. Additionally, these investigations may help in defining virus tropism and researching newly developing viruses and motifs that can infect living organism.

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