

RESEARCH OF THE GENETIC DIVERSITY OF TT VIRUSES IN BULGARIA

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Abstract

Torque teno viruses (TTVs) are a member of the newly formed family Anelloviridae and contain single stranded circular DNA genome. It is believed that these viruses chronically infect people of any age, yet it is not clear what is their pathogenic potential. Our previous research has shown spread of TT viruses at least 61.87% in voluntary blood donors, 62.74% in patients with respiratory diseases, 65.21% in cases of hepatitis, 62.96% in unknown cases of pathology, 66.66% in patients with brain tumour and 64.28% in patients with renal transplantation. It was the first report demonstrating TT viruses in Bulgaria. The purpose of this work is identification of the main genotypes of TT viruses circulating in Bulgaria. The original viral DNA was isolated from blood samples from clinically healthy blood donors, a HBsAg positive sample and a patient with unexplained febrile condition. Phylogenetic tree analysis shows that most of our sequences are genetically related, highly correlated, and fall into a single cluster. The analysis shows that they are close relatives and most likely have a common ancestor. Part of the sequences could also be a separate cluster or the start of a new clone within that cluster, together with the reference sequences clustered by literature data in genotype 1.

Key words: TT virus, sequence analysis, TTV prevalence

Introduction. In 1997, a new DNA virus was discovered, different from the previously known human viruses. The newly discovered small single-stranded

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virus was isolated and differentiated in Japan from a serum of a patient who had elevated aminotransferase levels after transfusion, without detecting any of the previously known hepatitis viruses [1]. The name TTV was given by its discoverers [1,2] in relation to the initials of the patient whose serum was isolated (T.T.) and the way it was infected – “transfusion transmitted virus”. Following the discovery of the first TTV isolate – N22, various TTV variants with over 50% genetic differences were detected [3,4].

In 2000, a small virus related to TTV was discovered relevant to human pathology, which was originally called the TTV-like mini virus [5]. Later, in 2007, the International Committee on Virus Taxonomy (ICTV) officially registered it as TTMV (Torque teno mini virus) to the Anelloviridae family. The genetic variability of the various TTMV isolates is about 40% [6].

Through SISPA (sequence independent single primer amplification) method, two new small TTV-like viruses were discovered. They are called SAV1 (small anellovirus 1) and SAV2 (small anellovirus 2) [7]. The newly discovered and identified isolates were named torque teno midi virus (TTMDV) [8] in relation to the length of the genomic molecule.

Studies on TTV show its prevalence in a large proportion of the human population, as well as its ability to cause chronic infections. According to some authors [9] the prevalence of human anelloviruses is: TTV up to 100%, TTMV up to 82% and TTMDV up to 75% presence in the human population. These high values are most likely due to the development of more sensitive and selective PCR detection methods [9].

The infections that cause the three human anelloviruses are characterized by lifelong viremia. On the other hand, they have significant genetic variability of the causative agents. TTV infections are thought to be associated with many and many different diseases: lung, liver, hematological disorders and cancer. There is still no clear evidence of a direct link between TTV infections and the specific clinical manifestations of these diseases.

Materials and methods. Pre-amplified TTV [10] sequences from the UTR region of the genome were sequenced using ABI Prism Big Dye Terminator v.1.1 in compliance with the manufacturer's requirements. The products obtained from the Big Dye PCR reaction were analyzed by an ABI 3730xl capillary electrophoresis apparatus. It was done as follows: the samples were transferred to special separate tubes and subjected to a cyclic process underlying the decoding of the sequences obtained from the Big Dye PCR reaction. The apparatus is provided with software that reads and interprets fluorescence information and displays it in the form of an electropherogram. The information was analyzed using ABI Sequence Analysis Software v. 5.2. The genomic sequences obtained were compared with those stored in the gene bank. An alignment file was constructed that reflects the comparative characteristics of the nucleotide changes in the sequenced and reference sequences (NCBI, GenBank). Sequences were aligned by Clustal W

and adjusted manually. The phylogenetic tree was constructed using the Neighbour joining algorithm (NJ) [11] and the two-parameter correction introduced by M. Kimura [12]. The bootstrap probabilities (BPs) of the tree generated were calculated for 1000 bootstrap replicates [13]. For BPs above and about 50%, the distribution in clusters was considered significant. The tree shows the genetic relationships between the sequenced and TTV reference thiols by dividing our sequences into clusters. All of these procedures were performed with the MEGA 5 program. The original viral DNA was isolated from blood samples from clinically healthy blood donors, a patient with a positive HBsAg sample and a patient with an unexplained febrile condition – Table 1.

T a b l e 1

Patient characteristics and sample number from which the amplicons were obtained and their designation in the sequencing study

Sequence No/Patient No	Patient characteristics	Designation of the sequenced fragment
1. No 751	Positive sample for HBsAg	BG1G751
2. No 004/213	Unclear febrile condition	BG2L2013/004
3. No 4P	Clinically healthy blood donor	BG3B4P
4. No 8P	Clinically healthy blood donor	BG4B8P
5. No 15P	Clinically healthy blood donor	BG5B15P
6. No 21P	Clinically healthy blood donor	BG6B21P
7. No 23P	Clinically healthy blood donor	BG7B23P

Sequence analysis used reference TT viral genomes available in the gene bank (Genbank, NCBI, USA). The characteristics of the reference sequences with the corresponding names of the isolates are presented in Table 2.

All sequences were converted and nucleotide aligned with the reference strains.

Results and discussion. Initially, 10 DNA fragments were selected for sequencing. Sequencing samples were selected to include sequences from all representative groups. The best results were obtained by sequencing analysis of 7 TTV DNA fragments. In the other three cases, given the presence of several TTV genotypes in one sample, the data were not reliable enough to process. For this reason, in order to determine the genotypes of the TT viruses circulating most frequently among the study groups of patients, we performed sequential analysis of 7 representative TTV DNA fragments. The original viral DNA was isolated from blood samples from clinically healthy blood donors, a patient with a positive HBsAg sample, and a patient with an unexplained febrile condition – described in the Materials and methods section of Table 1.

T a b l e 2

Reference sequences used in sequence analysis

Name of the relevant isolate	DNA isolation source	Gene bank access code
TA278	blood	AB017610
TUS01	blood	AB017613
SANBAN	blood	AB025946
GH1	blood	AF122913
JA20	blood	AF122914
JA9	blood	AF122915
JA1	blood	AF122916
JA4	blood	AF122917
JA2B	blood	AF122918
JA10	blood	AF122919
US35	blood	AF122920
US32	blood	AF122921
BDH1	blood	AF116842
TTVCHN1	blood	AF079173
TTVCHN2	blood	AF129887

From the obtained alignment files, a phylogenetic tree was constructed using the Neighbour-Joining method – Fig. 1.

Tree analysis shows that most of our sequences: BG4B8P, BG7B23P, BG5B15P and BG3B4P are genetically related, highly correlated, and fall into a single cluster. The analysis shows that they are close relatives and most likely have a common ancestor. It could be a separate cluster or the start of a new clone within that cluster along with the reference sequences clustered on the literature [14] in genotype 1. Samples from healthy blood donors, designated BG4B8P, BG7B23P, BG5B15P, and BG3B4P, respectively, contain similar sequences and are separated into an independent branch. Separation into an independent branch may result from the recombination frequency characteristic of the TTVs or the start of a new genogroup. From the results obtained, we can assume that our TTV sequences from healthy blood donors may be the start of a new clone within said cluster or a separate new cluster. Isolate BG2L2013/004 falls into a cluster of JA4 and JA10 TTVCHN2 isolates, which are representative of genotype 2. The approach used by the program we had access to did not very clearly divide TTV genotype 2 and genotype 3, which is due to the recombinant polymorphism characteristic of human anelloviruses [14]. The analysis of the phylogenetic tree with respect to the other two sequences BG6B21P and BG1G751 shows a great



Conclusion. Most of our sequences: BG4B8P, BG7B23P, BG5B15P and BG3B4P are genetically related. With a high degree of correlation, they fall into a cluster. The analysis shows that they are close relatives and most likely have a common ancestor. From the results obtained, we can assume that our TTV sequences from healthy blood donors may be the start of a new clone within said cluster or a separate new cluster. Isolate BG2L2013/004 is in a cluster with the sequences of isolate JA4, JA10 and TTVCHN2, which are representative of genotype 2. With respect to the other two sequences, the BG6B21P and BG1G751 make a big difference both in ours and to the reference sequences. They do not come close to genotype 1, 2 or 3.

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The ever-changing levels of the three human anelloviruses relative to one another, or the viral load, or combination of the genotypes of each of these viruses, are thought to be associated with the pathogenicity or potential of these viruses to cause disease. For this reason, efforts are being made to develop effective methods for differentiating the genomic DNA of the three anelloviruses.

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