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Full Length Research Paper

Sequence variation in TgROP7 gene among *Toxoplasma gondii* isolates from different hosts and geographical regions

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Toxoplasma gondii **can infect a wide range of hosts including mammals and birds, causing toxoplasmosis which is one of the most common parasitic zoonoses worldwide. The present study examined sequence variation in rhoptry 7 (ROP7) gene among different *T. gondii* isolates from different hosts and geographical localities. Phylogenetic analysis of the examined *T. gondii* isolates was conducted using the maximum likelihood (ML) method. Sequence analysis revealed that 60 nucleotide positions were variable in the ROP7 gene sequences among the 19 examined *T. gondii* isolates, corresponding to sequence variations of 0 to 1.7%, which occurred at the first, second and third codons. Phylogenetic analysis indicated that sequence variation in ROP7 gene was low among the examined *T. gondii* isolates from different hosts and geographical localities, and that the ROP7 sequence was not suitable as genetic marker for the differentiation of *T. gondii* isolates. The results of the present study suggest that ROP7 gene may be a suitable vaccine candidate.**

Key words: Sequence variation, rhoptry 7 (ROP7) gene, *Toxoplasma gondii*, toxoplasmosis, phylogenetic analysis.

INTRODUCTION

Toxoplasmosis caused by *Toxoplasma gondii* is one of the most common parasitic zoonoses worldwide, with a wide range of hosts including animals, birds and humans (Tenter et al., 2000; Montoya and Liesenfeld, 2004; Dubey, 2010; Zhou et al., 2011). In many hosts, *T. gondii* performs asexual replication, whereas its sexual development occurs only in the felid gut. These features contribute to the formation of an unusual population structure (Sibley et al., 2009). Genetic linkage mapping has identified quantitative trait loci that are associated with virulent strains of *T. gondii* (Su et al., 2002), and it is clear that the virulence is related to the genotypic profile of a strain (Howe and Sibley, 1995). The *T. gondii* strains can be classified into three major genotypic groups (Howe and Sibley, 1995; Sibley and Howe, 1996; Sibley et al., 2002), and a fourth clonal lineage in North America was identified recently (Khan et al., 2011). Genotype I strains are virulent strains that cause acute toxoplasmosis with rapid multiplication of tachyzoites. Type II and III strains are less virulent strains and are associated mostly with congenital or chronic toxoplasmosis (Ajzenberg et al., 2002; tableBoothroyd and Grigg, 2002).

The rhoptry is a subcellular organelle of apicomplexan

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Table 1. Details of *T. gondii* isolates used in the present study.

Isolate ID	Host	Geographical location	Genotype*	GenBank accession no.
GT1	Goat	United States	Reference, Type I, ToxoDB 10	JF831536
RH	Human	France	Reference, Type I, ToxoDB 10	JF831545
PTG	Sheep	United States	Reference, Type II, ToxoDB 1	JF831542
CTG	Cat	United States	Reference, Type III, ToxoDB 2	JF831537
MAS	Human	France	Reference, ToxoDB 17	JF831538
TgCgCa1	Cougar	Canada	Reference, ToxoDB 66	JF831550
TgCatBr5	Cat	Brazil	Reference, ToxoDB 19	JF831548
SH	Human	Shanghai, China	Type I, ToxoDB 10	JF831546
ZS1	Human	Zhejiang, China	Type #4	JF831554
TgPXx	Pig	Xishui, Hubei, China	Type I, ToxoDB 10	JF831552
TgPNY	Pig	Luying, Henan, China	Type I, ToxoDB 10	JF831551
NT	Pig	Tanshan, Nanjing, China	Type I, ToxoDB 10	JF831539
NTA	Pig	Tanshan, Nanjing, China	Type I, ToxoDB 10	JF831540
NY11	Pig	Nanyang, Henan, China	Type II, ToxoDB 1	JF831541
QHO	Sheep	Huzhu, Qinghai, China	Type II, ToxoDB 1	JF831544
PRU	Human	France	Type II, ToxoDB 1	JF831549
PYS	Pig	Panyu, Guangdong, China	Type #3, ToxoDB 9	JF831543
ZS	Human	Guangzhou, Guangdong, China	Type #3, ToxoDB 9	JF831553
TgC7	Cat	Guangzhou, Guangdong, China	Type #3, ToxoDB 9	JF831547

*Based on genotyping results of Zhou et al. (2009, 2010).

parasites. It excretes rhoptry (ROP) proteins which are important factors for the host cell invasion, virulence and formation of parasitophorous vacuole (Grimwood and Smith, 1996; SamYellowe, 1996). The ROP7 protein was closely related to the ROP4 protein (71% identity) as characterized by mass spectrometry, and is a ROP2 related rhoptry protein of *T. gondii* (El Hajj et al., 2006).

The objective of this study was to examine sequence variability in the ROP7 gene among *T. gondii* isolates of different genotypes from different hosts and geographical localities.

MATERIALS AND METHODS

Parasite isolates and preparation of genomic DNA

19 *T. gondii* isolates of different genotypes from different hosts and geographic localities were used in the study (Table 1). These isolates had been used in previous studies (Zhou et al., 2009, 2010). Total genomic DNA (gDNA) was extracted from individual isolates by SDS/proteinase K treatment, column-purification (Wizard® SV Genomic DNA Purification System, Promega) and elution to 50 µl volume with distilled water, according to the manufacturer's recommendations. The prepared DNA samples were then stored at -20°C until use.

PCR amplification of ROP7 gene

A pair of primers designated ZYA (5'-ATGGGGCACCCTA-CCTCTTTC-3') and ZYB (5'- TCACGTTTCCGGTGGTGGC-3') were designed to amplify the full length of the ROP7 gene with expected length of 1728 bp. A 25 µl PCR mixture contained 1.5 mM of $MgCl_2$, 0.2 µM of each primer, 2.5 µl Ex *Taq* buffer, 2 mM of each dNTPs, 0.25 U of Ex *Taq* DNA polymerase (TaKaRa) and 2 µl gDNA.

PCR amplification was performed in a thermocycler (Biometra) under the following conditions: 94°C for 4 min (initial denaturation), followed by 30 cycles at 94°C (1 min denaturation), 62°C (1 min annealing), 72°C (2 min extension) and a final extension step of 7 min at 72°C. Murine gDNA was included in each amplification as host control and a negative control (no-DNA) was also included in each run. An aliquot (4 µl) of each amplification was examined on 1% agarose-TBE (65 mM Tris-HCl, 22.5 mM boric acid and 1.25 mM EDTA, pH 9.0) gel. The gels were then stained with ethidium bromide and photographed upon UV light transillumination. The DL 2000 DNA marker (TaKaRa) was used to estimate the size of the amplified PCR products.

DNA sequencing

ROP7 PCR products were purified using spin columns (Wizard™ PCR-Preps DNA Purification System, Promega, USA) and were ligated with pGEM-T Easy plasmid vector (Promega, USA) according to the manufacturer's recommendations. The recombinant plasmids were then transformed into *E. coli* JM109 competent cells (Promega, USA). Positive transformants were selected and confirmed by plasmid DNA extraction using WizardTM Plus Minipreps DNA Purification System (Promega, USA). Cell cultures with confirmed recombinant plasmid were sequenced by Shanghai Songon Biological Engineering Biotechnology Company with ABI 377 automated DNA sequencer (BigDye Terminator Chemistry). *T. gondii* ROP7 sequences obtained from the study were deposited in the GenBank (Table 1).

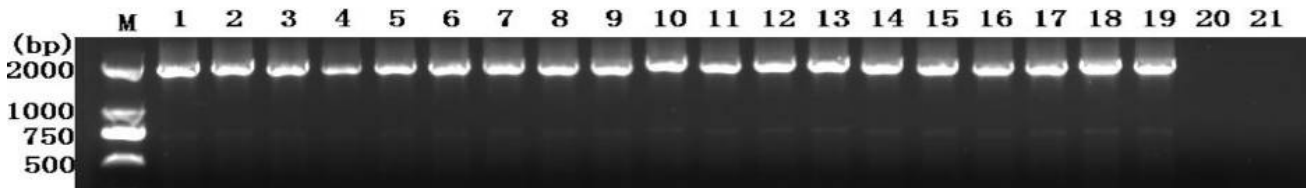

Figure 1. Agarose gel electrophoresis of ROP7 PCR products of *T. gondii* isolates. M represents a DNA size marker. Lanes 1 to 19 represent samples GT, RH, PTG, CTG, MAS, TgCgCa1, ZS1, TgCatBr5, SH, TgPXx, TgPNY, NT, NTA, NY11, QHO, PRU, PYS, ZS and TgC7, respectively (detail of samples is shown in Table 1). Lanes 20 and 21 represent mammalian host (mouse) control and negative control, respectively.

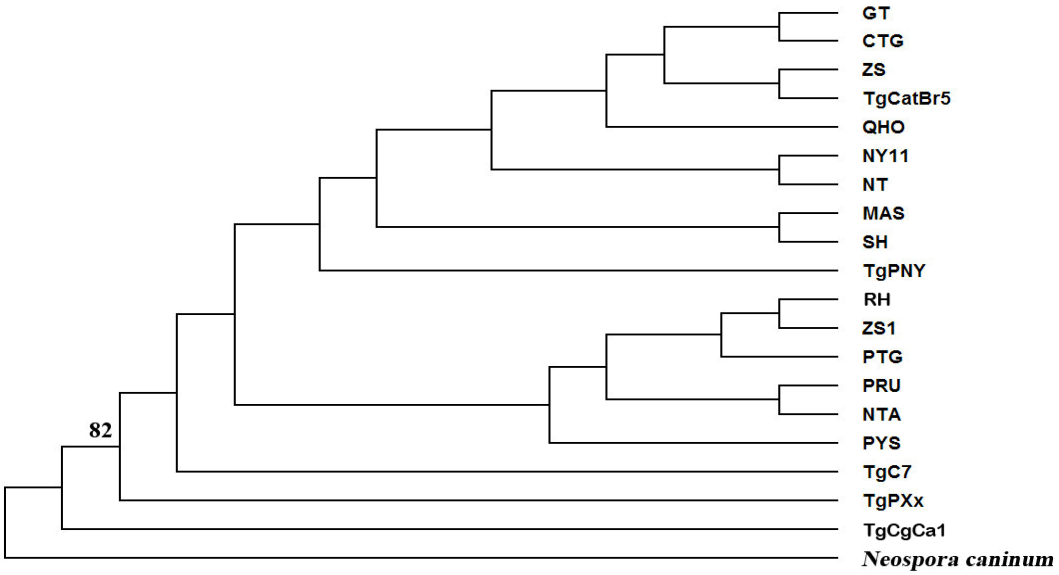

Figure 2. Phylogenetic relationship of the examined *T. gongdii* isolates inferred by maximum likelihood analysis of the ROP7 gene sequences with *N. caninum* as outgroup.

Sequence analysis and phylogenetic reconstruction

The obtained ROP7 gene sequences from different *T. gondii* strains were aligned using Clustal X 1.83 (Thompson et al., 1997), and sequence variation was determined. Phylogenetic analysis among the examined *T. gondii* strains was performed using the maximum likelihood (ML) method by using PhyML 3.0 online (http://www.atgc-montpellier.fr/phyml), and the GTR model with its parameter was determined for the ML analysis using JModeltest (Posada, 2008) based on the Akaike information criterion (AIC). BLAST analysis identified a 64% sequence identity of *T. gondii* ROP7 sequence to the *Neospora caninum* ROP7 sequence (GenBank accession number NCLIV_001950). Therefore, the *N. caninum* ROP7 sequence was used as the outgroup for phylogenetic analysis. Phylograms were obtained by using the Tree View program version 1.65 (Page, 1996).

RESULTS AND DISCUSSION

19 *T. gondii* isolates representing three classical genotypes (I, II and III) and atypical genotypes from different geographical locations and hosts (Table 1) were examined for sequence variation in their ROP7 gene sequences. The amplified PCR products of *T. gondii* ROP7 gene were all approximately 1800 bp on agarose gel (Figure 1). After sequence alignment, a sequence of 1728 bp in length was obtained for each of the 19 *T. gondii* strains. The A+T contents of the ROP7 gene sequences were 45.6 to 46.1%.

A total of 60 nucleotide positions were variable in the ROP7 gene sequences among the 19 examined *T. gondii* isolates, with sequence variations being 0 to 1.7%, which occurred at the first, second and third codons. This is consistent with the overall sequence variation in ROP18 gene and other genes among the clonal lineages of *T. gondii* (Khan et al., 2009). Intra-specific nucleotide variations in ROP7 gene sequences represented transitions (A<–>G or C<–>T; n = 2) and transversions (A<–>C, A<–>T and/or T<–>G; n = 6). Phylogenetic analysis of the examined 19 *T. gondii* isolates based on the ROP7 gene sequences failed to distinguish virulent and avirulent strains or different genotypes of *T. gondii* (Figure 2). Low sequence variation in ROP gene suggests that ROP7 gene may represent a suitable vaccine

candidate for *T. gondii*.

In conclusion, the present study shows that sequence variation in the ROP7 gene sequences among *T. gondii* isolates of different genotypes from different hosts and geographical locations is low. Phylogenetic analysis revealed that ROP7 gene sequence was not a suitable marker for studying genetic variation and population genetics of *T. gondii* isolates from different geographic localities and hosts.

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