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Full Length Research Paper

Genetic diversity among *Toxoplasma gondii* isolates from different hosts and geographical locations revealed by analysis of ROP13 gene sequences

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***Toxoplasma gondii* can infect almost all the warm-blooded animals and human beings, causing serious public health problems and economic losses worldwide. Rhoptry protein 13 (ROP13) plays some roles in the invasion process of *T. gondii*. In this study, sequence variation in ROP13 gene among 14 *T. gondii* isolates from different geographical locations and hosts was examined. The ROP13 gene was amplified from individual isolates and sequenced. Results show that the length of the ROP13 sequences was 1203 bp. In total, there were 44 variable nucleotide positions in the ROP13 sequences, and sequence variations were 0.1 to 2.0% among the 14 examined *T. gondii* isolates, representing higher rate in transversion than in transition. Intra-specific nucleotide variations were mainly at the second codon positions. Phylogenetic analysis of the 14 examined *T. gondii* isolates indicate that the ROP13 sequence was not a suitable genetic marker to differentiate *T. gondii* isolates of different genotypes from different hosts and geographical regions. Low variation in ROP13 gene sequence may suggest that ROP13 gene could represent a good vaccine candidate against toxoplasmosis.**

Key words: *Toxoplasma gondii*, toxoplasmosis, rhpotry protein 13 (ROP13), sequence variation, phylogenetic analysis.

INTRODUCTION

Toxoplasma gondii is an important obligate intracellular parasite infecting humans and a wide range of warm-blooded animals (Montoya and Liesenfeld, 2004; Dubey, 2010; Zhou et al., 2011). *T. gondii* infection is normally asymptomatic, but it may be lethal for immuno-compromised patients, such as AIDS patients and organ transplant recipients (Montoya and Liesenfeld, 2004; Dubey, 2010). Moreover, toxoplasmosis can cause abortions, stillbirths and neonatal deaths in all kinds of livestock which can result to significant economic losses (Kijlstra and Jongert, 2009; Dubey, 2010).

Rhoptry proteins (ROPs) are unique secretory/ excretory proteins found exclusively in the Apicomplexa. They are discharged during invasion and are critical for the establishment of a productive infection (Grimwood and Smith, 1996; SamYellowe, 1996). A previous study reported that *T. gondii* ROP13 was a novel rhoptry protein which showed no homology with any known protein and lacked identifiable domains (Bradley et al., 2005), and it may be involved in host response to the parasite (Turetzky et al., 2010), playing an important role in the invasion process of *T. gondii*. However, little is known about sequence variation in ROP13 gene among *T. gondii*

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Table 1. Details of *T. gondii* isolates used in this study and the GenBank accession numbers of their ROP13 gene sequences.

Isolate ID	Host	Geographical location	Genotype*	GenBank accession no.
GT1	Goat	United States	Reference, Type I, ToxoDB 10	JN051285
RH	Human	France	Reference, Type I, ToxoDB 10	JN051278
PTG	Sheep	United States	Reference, Type II, ToxoDB 1	JN051284
CTG	Cat	United States	Reference, Type III, ToxoDB 2	JN051283
MAS	Human	France	Reference, ToxoDB 17	JN051286
TgCatBr5	Cat	Brazil	Reference, ToxoDB 66	JN051289
TgPxd	Pig	xiangfan, Hubei, China	Reference, Type I	JN051291
TgPNY	Pig	Luying, Henan, China	Type I, ToxoDB 10	JN051290
NT	Pig	Tanshan, Nanjing, China	Type I, ToxoDB 10	JN051287
NY11	Pig	Nanyang, Henan, China	Type II, ToxoDB 1	JN051288
QHO	Sheep	Huzhu, Qinghai, China	Type II, ToxoDB 1	JN051279
PRU	Human	France	Type II, ToxoDB 1	JN051281
PYS	Pig	Panyu, Guangdong, China	Type #3, ToxoDB 9	JN051280
TgC7	Cat	Guangzhou, Guangdong, China	Type #3, ToxoDB 9	JN051282
Neospora caninum (NC)	Cattle	Beijing, China		JN051292

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

isolates from different hosts and geographical regions.

The objective of this study was to examine sequence variability in ROP13 gene among *T. gondii* isolates of different genotypes from different geographical locations and hosts.

MATERIALS AND METHODS

T. gondii isolates

A total of 14 *T. gondii* isolates from different geographical locations and hosts were used in the study (Table 1). These isolates have been genotyped previously (Zhou et al., 2009, 2010).

Enzymatic amplification and sequencing of ROP13 gene

The *T. gondii* ROP13 gene was amplified from individual DNA samples by using a pair of primers; ROP13F, 5′-CGCGGATCCATGAAGAGAACAGAGCTTTG-3′ and ROP13R, 5′-GCTCTAGATCACAATAGCCTCAAGGAATTC-3′. PCR reactions (25 µl) were performed in a mixture with 2.5 mM of $MgCl_2$, 2.5 µM of each primer, 2.5 µl Ex *Taq* buffer, 0.2 mM of each dNTPs, 1.25 U of Ex *Taq* DNA polymerase (TAKARA) and 2 µl of DNA sample in a thermocycler (Biometra) under the following conditions: initial denaturation at 94°C for 4 min, followed by 36 cycles of 94°C for 1 min (denaturation), 55°C for 1 min (annealing), 72°C for 1.5 min (extension) and a final extension of 72°C for 7 min. PCR mixture without genomic DNA (no-DNA controls) was included in each amplification run. Each amplicon (5 µl) was examined on 1% agarose-TBE (65 mM Tris-HCl, 22.5 mM boric acid and 1.25 mM EDTA, pH 9.0) gel to assess amplification efficiency. Sizes of ROP13 PCR products were estimated by using a DNA marker (DL2000, TAKARA).

ROP13 PCR products were then purified using the Wizard™ PCR-Preps DNA Purification System (Promega), ligated with pGEM-T Easy vector (Promega) and sequenced according to the methods reported previously (Huang et al., 2004).

Sequence analysis and phylogenetic reconstruction

The obtained *T. gondii* ROP13 sequences were aligned with the computer program ClustalX 1.81 (Thompson et al., 1997). Sequence variation among the examined *T. gondii* isolates was determined using the formula D = 1-(M/L), where M is the number of alignment positions at which the two sequences have a base in common, and L is the total number of alignment positions over which the two sequences are compared (Chilton et al., 1995).

Phylogenetic reconstruction of the examined *T. gondii* strains was performed by maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ) analyses using ROP13 gene sequence of *Neospora caninum* (NC) as the outgroup. The NJ and MP analyses were done using the PAUP 4.0 Beta 10 programme (Swofford 2002), while the ML analysis was carried out using the PUZZLE 4.1 (Strimmer and von Haeseler, 1996) under the default setting. The consensus tree was obtained after bootstrap analysis with 1000 replications, with values above 50% reported.

RESULTS AND DISCUSSION

PCR amplified ROP13 gene was approximately 1200 bp in length and no size variation was detected among the amplicons examined. No products were amplified from the host (mouse) or no-DNA controls (Figure 1).

Pairwise comparison revealed a total of 44 variable nucleotide positions in the ROP13 sequences among the examined *T. gondii* isolates. The A+T contents were 46.30 to 46.88%, which were lower than G+C contents (53.12 to 53.70). Sequence variations in ROP13 gene among the 14 examined *T. gondii* isolates were 0.1 to 2%, which were similar to that reported for ROP18 and

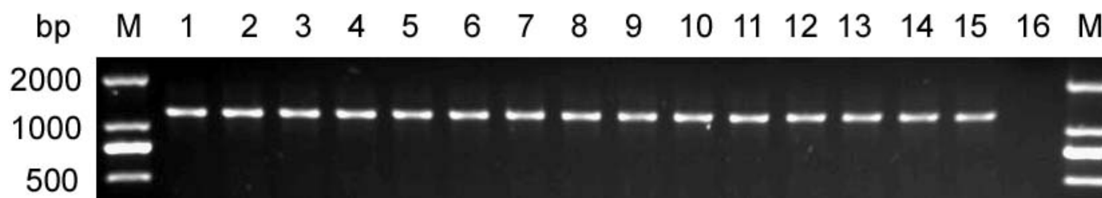

Figure 1. ROP13 PCR products amplified from 14 *T. gondii* isolates from different geographical locations and hosts. Lanes 1 to 14 represent *T. gondii* isolates/strains RH, QHO, TgPg, PYS, TgPNY, PRU, TgC7, CTG, TgPxd, GT1, NY11, TgcatBr5, MAS, NT and PTG. Lane 15 represent *N. caninum*. Detail of samples is shown Table 1. Lane 16 represents no-DNA control. M represents a DNA size marker (ordinate values in bp).

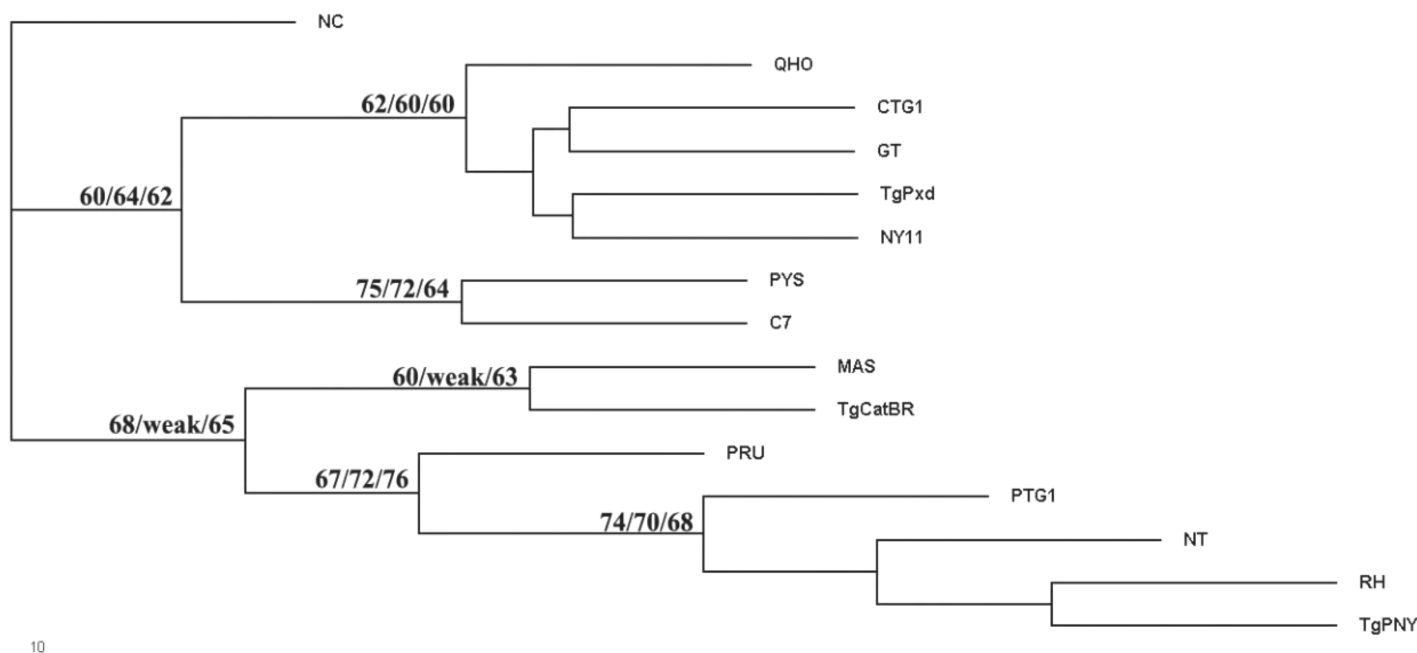

Figure 2. Inferred phylogenetic relationships of 14 *T. gondiii* isolates from different geographical locations and hosts based on ROP13 gene sequences by maximum parsimony (MP), maximum likelihood (ML) and neighbor joining (NJ) analyses using *N. caninum* (NC) as the outgroup. Bootstrap values (in percentage) above 50% from 1,000 pseudo-replicates are shown for the MP (the first value), ML (the second value) and NJ analyses (the third value). Weak = node resolved by the method but very weak (<50%). Scale bar indicate an evolutionary distance of 10 substitutions per site in the sequence.

other genes among the clonal lineages of *T. gondii* (Khan et al., 2009). The intra-specific nucleotide variations mainly occurred at the second codon positions.

Phylogenetic analysis of the 14 examined *T. gondii* isolates using the ROP13 gene sequence dataset was not able to readily differentiate *T. gondii* isolates belonging to different genotypes (Figure 2) or according to the host and geographical origins because they clustered in different clades.

In summary, the results of this study reveal that sequence variability in the ROP13 gene sequences among the 14 *T. gondii* isolates of different genotypes from different hosts and geographical locations was low. ROP13 gene sequence was not an ideal genetic marker for studying the population genetic structure of *T. gondii* isolates of different geographic and host origins.

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