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Research Note

Sequence variation and bioinformatics analysis of *Toxoplasma gondii* GRA16 Gene

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Abstract. Toxoplasmosis is caused by the intracellular protozoan *Toxoplasma gondii*. It is anopportunistic zoonosis in warm-blooded animals and humans, with a worldwide distribution. *Toxoplasma gondii* dense granule protein 16 (TgGRA16) can modulate some functions in host cells and is considered a significant virulent factor of the parasite. The present study reports sequence variation in TgGRA16 gene among *T. gondii* strains from different hosts and geographical locations, and the construction of phylogenetic relationships of these *T. gondii* strains based on sequences of TgGRA16, and analysis of B cell epitopes in TgGRA16. Our results showed that all TgGRA16 gene sequences were 1518 bp and the C+G contents ranged from 52.17% to 52.59%. Sequence variation in the TgGRA16 gene was 0-1.51%. Phylogenetic analysis revealed that TgGRA16 gene sequence could not be used to differentiate the different *T. gondii* genotypes. Six B cell epitopes were predicted in TgGRA16. These results indicated that TgGRA16 gene is not an ideal marker for studying genetic relationships of *T. gondii* isolates, but may represent a good vaccine candidate against toxoplasmosis.

Toxoplasmosis, a zoonotic disease with a worldwide distribution, is caused by the obligate intracellular pathogen *Toxoplasma gondii*. This parasite can infect all warm-blooded animals and humans (Dubey, 2010; Zhou *et al*., 2011; Robert-Gangneux *et al*., 2012). *Toxoplasma gondii* infection in humans is usually asymptomatic or presents only mild symptoms in immune-competent individuals (Montoya *et al*., 2004). However, toxoplasmosis in immune-compromised patients or pregnant women may lead to psychiatric disorders, abortion, stillbirth and some other serious health problems or even death (Trees *et al*., 2005; Mwambe *et al*., 2013; Khademvatan *et al*., 2014). In animals, toxoplasmosis may result in abortion and neonatal loss especially in goats and sheep, which cause great economic losses to the livestock industry (Dubey *et al*., 2005; Dubey, 2010).

Toxoplasma gondii infections in humans are acquired mainly through ingestion of cysts in undercooked or raw meat or ingestion of water or food contaminated with sporulated oocysts (Hill *et al*., 2002, 2013). Nearly one third of the world's population is reported to be seropositive for *T. gondii* (Dubey, 2008). However, chemical drugs cannot completely eliminate *T. gondii* from infected hosts or prevent the parasite from reactivation (Coombs *et al*., 2002; Innes,

2010). Development of vaccines against the parasite infection is thus a high priority (Kur *et al*., 2009; Zhang *et al*., 2013). No effective vaccines have been licensed for human use. A commercialized vaccine (Toxovax®) has been produced for use in sheep (Buxton, 1993). Evaluation of antigens in the life stages of *T. gondii* as vaccine candidates is an important strategy in the development of effective vaccines against toxoplasmosis (Zhang *et al*., 2013).

The *T. gondii* dense granule protein 16 (TgGRA16) can restrict inflammatory response in the host through maintenance of p53 level, which is a key molecule in controlling the inammation during glucocorticoid responses *via* regulation of the expression of NF-κB gene (Bougdour *et al.*, 2014). TgGRA16 is also considered a virulent factor for *T. gondii* genotype II strains with functions related to metabolism and cell cycle regulation (Bougdour *et al*., 2013). However, little is known about sequence variation on TgGRA16 genes among *T. gondii* isolates from different hosts and geographical regions. The objectives of the present study were to examine the sequence variation in TgGRA16 gene among *T. gondii* strains and to analyse its potential antigenicity, which would provide foundation for development of *T. gondii* vaccines.

Twelve *T. gondii* strains from different hosts and geographic locations were used for analysis in this study (Table 1). All the strains had been genotyped in previous studies and their genomic DNA (gDNA) was prepared as described previously (Zhou *et al*., 2010, Su *et al*., 2010, Huang *et al*., 2012).

The TgGRA16 gene of *T. gondii* strains from different hosts and geographic locations was amplified by PCR using a pair of oligonucleotide primers (G16F: 5'-CGGGGTACCATGTATCGAAACCACTCAG-3' and G16R: 5'-CCGGAATTCTCACAT CTGATCATTTTTC-3') designed using Primer 5.0 software. The amplification reaction was carried out in a final volume of 25 µl, containing 12.5 µl Premix *ExTaq* DNA enzyme (TaKaRa), 0.2 µM of each primer and 100-200 ng of template DNA. The PCR condition was started at 94ºC for 10 min, followed by 35 cycles of 94ºC for 35 sec (denaturation), 56ºC for 30 sec (annealing) and 72ºC for 35 sec (extension). After a final extension of 72ºC for 10 min, the PCR products were analyzed by electrophoresis on a 1.0% (w/v) agarose gel, stained with GoldenView™. The DL 2000 marker (TaKaRa) was used to estimate the sizes of the TgGRA16 PCR products.

The TgGRA16 PCR products were purified using the PCR-Preps DNA

Table 1. Details of *Toxoplasma gondii* strains used in the present study

Strain	Host	Geographical origin	Genotype
RH	Human	France	Reference, Type I, ToxoDB #10
GT1	Goat	United States	Reference, Type I, ToxoDB #10
SH	Human	China	Type I, ToxoDB #10
Prugniaud (PRU)	Human	France	Type II, ToxoDB #1
QHO	Sheep	China	Type II, ToxoDB #1
CTG	Cat	United states	Reference, Type III, ToxoDB #2
MAS	Human	France	Reference ToxoDB #17
TgC7	Cat	China	ToxoDB #9
PYS	Pig	China	ToxoDB #9
TgCgCa1	Cougar	Canada	Reference, ToxoDB #66
TgCatBr64	Cat	Brazil	Reference, ToxoDB #19
TgWtdSc40	Deer	United states	Type12, ToxoDB #5

*based on genotyping results of Zhou *et al.* (2010), Su *et al.* (2010) and Huang *et al.* (2012).

Purification System (Promega, USA) according to the manufacturer's recommendations, and then ligated to pMD18-T vector (TaKaRa). The recombinant vectors were then transformed into competent *Escherichia coli* JM109 cells (Promega, USA). Positive colonies were identified by bacterial PCR amplification, and their plasmid DNA was sent to Shanghai Sangon Engineering Biotechnology Company, China for sequencing.

The TgGRA16 gene sequences determined in the present study plus the corresponding sequences from *T. gondii* VEG and ME49 strains, and the corresponding sequence from *Neospora caninum* (GenID: NCLIV_003340) available in ToxoDB database were aligned using Clustal X 1.81 software (Thompson *et al.*, 1997). The sequence variation was determined. Phylogenetic construction was performed using maximum parsimony (MP) and namely maximum likelihood (ML) using PAUP* 4.0 with indels treated as missing character states (Swofford, 2010). A total of 1000 random addition searches using tree bisection-reconnection (TBR) branch swapping were performed for each MP and ML analysis. Bootstrap probability (BP) was calculated from 1000 bootstrap replicates with 10 random additions per replicate in PAUP. The nucleotide composition, transition, transversion and the genetic distance calculation were performed using MegAlign program in the software DNA Star Version 8.0.

The TgGRA16 gene sequence from *T. gondii* RH strain obtained in the present study was translated into amino acid sequence by DNAStar. The hydrophilicity/hydrophobicity was analyzed with ProtScale. The flexibility and accessibility of the protein were analyzed by the Hopp and Woods methods (Hopp *et al.*, 1981). The potential epitopes of TgGRA16 were predicted by DNAStar 8.0 (DNAStar, USA) software with the Jameson-Wolf index.

The amplicons of TgGRA16 gene from 12 *T. gondii* isolates were approximately 1500 bp in length on agarose gel. No length difference was detected among the TgGRA16 gene sequences. Their C+G contents ranged from 52.17% to 52.59%. Sequence variation varied from 0 to 1.51%, which was lower than that in GRA5 (Chen *et al.*, 2014), ROP7 (Zhou *et al.*, 2012) and ROP13 (Wang *et al.*, 2012) genes. There were 21 transitions (A<->C, C<->T, A<->G, T<->G) and 2 transversions (C <->G and A<->T). Prediction of the amino acid sequences showed a classical signal peptide sequence in the N-terminal with a cleavage site between 23 aa and 24 aa.

Phylogenetic analysis showed that the *T. gondii* isolates of the same genotypes were clustered in different clades (Figure 1). Topologies of all trees inferred by ML and MP were similar with only the minor difference in bootstrap values. These results suggested that the TgGRA16 gene sequence may not be an ideal genetic marker for population genetic studies of *T. gondii* isolates. This is consistent with previous studies using other genes, such as eIF4A (Chen *et al.*, 2014), ROP7 (Zhou *et al.*, 2012), and ROP38 (Xu *et al.*, 2014).

B-cell epitopes are the sites of molecules that are recognized by antibodies of the immune system. Predicting the epitopes can help in the development of diagnostic reagents and design of new vaccines (Bai *et al.*, 2012, Zhang *et al.*, accept). Linear B cell epitopes are usually constituted by amino acids with hydrophilic and flexible characters. Analysis of amino acid hydrophilicity/hydrophobicity can help understand protein folding, interaction sites, and predict protein secondary structure and antigenic epitopes (Bai *et al.*, 2012). The hydrophilic/hydrophobic regions of TgGRA16 were predicted at 59-95, 125-170, 298-328, 337-363, 370-400 and 480-501 aa. From these analyses, some major B cell epitopes were predicted at 60-96, 127-170, 188-205, 298-362, 386-397 and 479-506 aa (Figure 2).

In conclusion, the present study revealed low sequence variation in the TgGRA16 gene of *T. gondii* strains from different hosts and geographical locations. Phylogenetic analysis indicated that TgGRA16 gene sequence is not a suitable marker for population genetic studies, but the prediction of B cell epitopes in TgGRA16 suggested that it may represent a potential vaccine candidate against toxoplasmosis model.

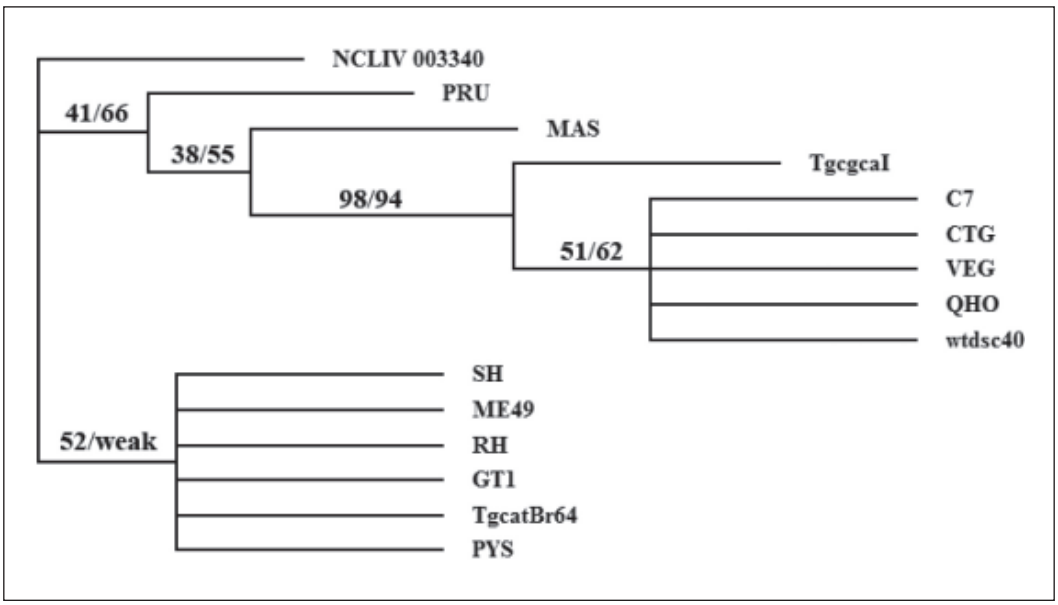

Figure 1. Phylogenetic analysis of 14 *Toxoplasma gondii* strains based on analysis of the TgGRA16 gene sequences. The tree was built by maximum parsimony (MP) and maximum likelihood (ML) analyses. The numbers at nodes indicate bootstrap values resulting from different analyses in the order: MP/ML. The topologies of all trees inferred by ML and MP were similar with only the small difference of bootstrap values.

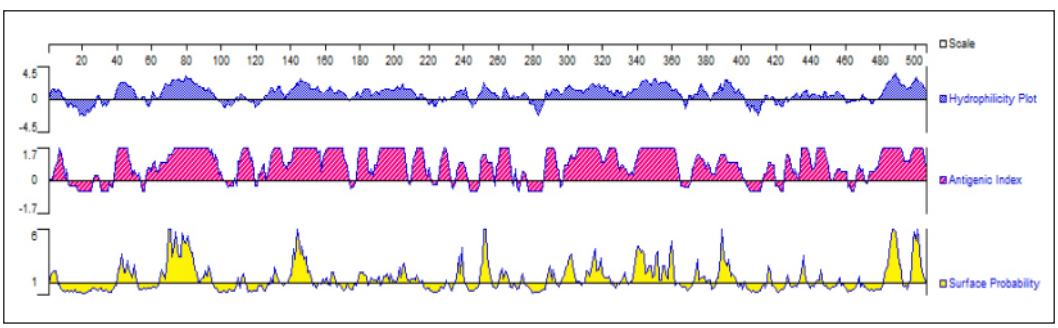

Figure 2. The prediction of the potential epitopes of *T. gondii* TgGRA16 by DNAStar 8.0 (DNAStar, USA) software with the Jameson-Wolf index. Hopp & Woods. The major B cell epitopes are predicted on 60-96, 127-170, 188-205, 298-362, 386-397, and 479-506 aa.

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