

## Sequence variation in the *Toxoplasma gondii* *eIF4A* gene among strains from different hosts and geographical locations

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Genet. Mol. Res. 13 (2): 3356-3361 (2014)

Received January 25, 2013

Accepted June 14, 2013

Published April 29, 2014

DOI <http://dx.doi.org/10.4238/2014.April.29.14>

**ABSTRACT.** *Toxoplasma gondii* is an opportunistic protozoan parasite that infects a wide range of animals, including humans. The *T. gondii* eukaryotic translation initiation factor 4A (eIF4A) protein is expressed in the tachyzoite, but its expression is markedly downregulated in the bradyzoite, and it is therefore considered to be associated with tachyzoite virulence. The present study examined sequence variation in the eIF4A gene among nine strains of different genotypes from different hosts and geographical localities using polymerase chain reaction amplification, sequence analysis, and phylogenetic reconstruction by Bayesian inference. The complete

genomic sequence of the eIF4A gene was 3156 bp in length in the strain TgCgCaI, 3153 bp in the strain MAS, 3152 bp in the strain TgPNY, and 3154 bp in the other six strains. Sequence analysis identified 29 (0-0.8%) variable nucleotide positions among all strains, with 16 of these variations located in the coding region, while the other 12 were distributed between the two introns. Phylogenetic analyses revealed that these eIF4A sequences were not effective molecular markers for intra-species phylogenetic analysis and differential identification of *T. gondii* strains from different hosts and geographical locations. This study demonstrated the existence of low sequence variation in the eIF4A gene, suggesting that *T. gondii* eIF4A may represent a suitable candidate vaccine against toxoplasmosis.

**Key words:** *Toxoplasma gondii*; Toxoplasmosis; Sequence variation; Eukaryotic translation initiation factor 4A protein; Phylogenetic analysis

## INTRODUCTION

*Toxoplasma gondii* is an important zoonotic protozoan with a global distribution infecting virtually all warm-blooded animals, including humans (Montoya and Liesenfeld, 2004; Nardoni et al., 2011; Zhou et al., 2011; Chen et al., 2012; Tian et al., 2012). Normally, infections in humans are asymptomatic or sub-clinical, although they can cause severe diseases in immunocompromised individuals, such as congenitally infected individuals, AIDS patients, and those with tumors or recovering from transplantation operations (Weiss and Dubey, 2009). Additionally, toxoplasmosis can lead to abortion or congenital toxoplasmosis in all types of livestock, especially in sheep and goats (Innes, 2010), and thus cause considerable economic losses to the livestock industry (Fayer et al., 2004; Dubey et al., 2005).

The eukaryotic translation initiation factor eIF4A can control cell proliferation and morphogenesis, which is critical for parasite growth and survival (Tuteja and Pradhan, 2009, 2010). Moreover, the *T. gondii* eIF4A protein is expressed in the tachyzoite, but is markedly downregulated in the bradyzoite, and is therefore considered to be associated with tachyzoite virulence (Gastens and Fischer, 2002). However, little is known regarding sequence variation of the eIF4A gene among different *T. gondii* strains, despite its important biological impacts. Therefore, the objective of this study was to examine sequence diversity in the eIF4A gene among *T. gondii* strains from different hosts and geographical regions.

## MATERIAL AND METHODS

### *T. gondii* isolates

A total of nine *T. gondii* strains originating from different hosts and geographic locations were used for analysis in this study (Table 1). These *T. gondii* isolates were genotyped and genomic DNA (gDNA) was prepared as described previously (Zhou et al., 2009; Su et al., 2010; Zhou et al., 2010).

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

Strain	Host	Geographical origin	Genotype*
GT1	Goat	United States	Reference, Type I, ToxoDB #10
RH	Human	France	Reference, Type I, ToxoDB #10
PTG	Sheep	United States	Reference, Type II, ToxoDB #1
CTG	Cat	United States	Reference, Type III, ToxoDB #2
TgCgCa1	Cougar	Canada	Reference, ToxoDB #66
MAS	Human	France	Reference, ToxoDB #17
TgPNY	Pig	Luying, Henan, China	Type I, ToxoDB #10
Prugniaud (PRU)	Human	France	Type II, ToxoDB #1
QHO	Sheep	Huzhu, Qinghai, China	Type II, ToxoDB #1

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

### Polymerase chain reaction (PCR) amplification

To obtain amplicons of the long genomic sequence of the eIF4A gene more easily, the entire sequence of the gene was separated into two amplified fragments by PCR amplification of the gDNA of individual strains using two pairs of oligonucleotide primers: CJ-eIF4A-1A (forward primer: 5'-AGAAACTGATGCTGCTATA-3') and CJ-eIF4A-1B (reverse primer: 5'-TCTGAGCATCTTACTGGTG-3') were used for the amplification of the first fragment; CJ-eIF4A-2A (forward primer: 5'-AGAAACTGATGCTGCTATA-3') and CJ-eIF4A-2B (reverse primer: 5'-TCTGAGCATCTTACTGGTG-3') were used for the amplification of the second fragment, and 100 bp overlaps between the 5' termination of the first fragment and the 3' termination of the second fragment of the genomic sequence of the eIF4A gene were designed for sequence splicing. The amplification reaction was carried out in a 25  $\mu$ L volume, containing 10 mM Tris-HCl, pH 8.4, 50 mM KCl, 3 mM MgCl<sub>2</sub>, 250  $\mu$ M each dNTP, 0.2  $\mu$ M of each primer, 100-200 ng template DNA, and 0.25 U La *Taq* polymerase (TaKaRa). Amplification of DNA samples from individual strains, which were carried out in a thermocycler (Biometra), were denatured at 94°C for 10 min (initial denaturation), followed by 35 cycles consisting of 94°C for 30 s (denaturation), 58.1°C for 30 s (annealing for the first fragment) or 56.6°C for 30 s (annealing for the second fragment), 72°C for 2 min (extension), and a final extension step at 72°C for 10 min. Confirmation of the PCR amplifications was carried out by electrophoresis on 1% (w/v) agarose gel, stained with GoldenView™, and photographed using a gel documentation system (UVP GelDoc-It™ Imaging System, Cambridge, UK).

### Sequencing of the eIF4A amplicons

To ensure the accuracy and integrity of eIF4A sequences from individual strains, the eIF4A PCR products were purified using the spin columns according to manufacturer recommendations (Wizard™ PCR-Preps DNA Purification System, Promega, USA), ligated with the pMD 18-T vector (TaKaRa), and then transformed into JM109 competent cells (Promega). Following the screening by PCR amplification and enzymatic digestion, the positive colonies were sequenced by the Shanghai Songon Biological Engineering Biotechnology Company on an ABI377 automated DNA sequencer (BigDye Terminator Chemistry).

## Sequence analysis and phylogenetic reconstruction

The eIF4A gene sequences obtained from different *T. gondii* strains were aligned using the multiple sequence alignment program ClustalX 1.83 (Thompson et al., 1997), and sequence variation was determined among the examined *T. gondii* strains. Phylogenetic reconstructions based on the complete eIF4A gene sequences of different *T. gondii* strains were performed using Bayesian inference (BI). BI analyses were conducted with four independent Markov chains run for 10,000,000 metropolis-coupled MCMC generations, sampling a tree every 10,000 generations in MrBayes 3.1.1 (Ronquist and Huelsenbeck, 2003). The first 250 trees were omitted as burn-ins, and the remaining trees were used to calculate Bayesian posterior probabilities (PP).

## RESULTS AND DISCUSSION

The PCR product of the eIF4A gene appeared to be approximately 3000 bp in length on the agarose gel in all nine *T. gondii* strains, and the amplicons of all strains were sequenced subsequently. The entire genomic sequence of the eIF4A gene was 3156 bp in length for the strain TgCgCaI, 3153 bp for the strain MAS, and 3152 bp for the strain TgPNI, and 3154 bp for the other six strains. Analysis of all the nine eIF4A genomic and cDNA sequences revealed two introns in the eIF4A gene (Table 2). There were 35 transitions (C $\leftrightarrow$ T, T $\leftrightarrow$ G, A $\leftrightarrow$ C, and A $\leftrightarrow$ G) and 6 transversions (A $\leftrightarrow$ T and C $\leftrightarrow$ G) in Tg-eIF4A genomic DNA sequences, 14 transitions (A $\leftrightarrow$ G and C $\leftrightarrow$ T) and 3 transversions (A $\leftrightarrow$ T and C $\leftrightarrow$ G) in Tg-eIF4A coding regions, and 11 transitions (A $\leftrightarrow$ G and C $\leftrightarrow$ T) and 2 transversions (A $\leftrightarrow$ T and C $\leftrightarrow$ G) in intron regions (Table 2). The alignment of all nine sequences revealed a total of 29 (0-0.8%) variable nucleotide positions among all strains, 16 of which were in the coding region of the gene and the other 12 were distributed between the two introns, resulting in a low genetic diversity, which was similar to our previous reports of MIC13 (Ren et al., 2012), PLP1 (Yan et al., 2011), and other genes in clonal lineages of *T. gondii* (Khan et al., 2009).

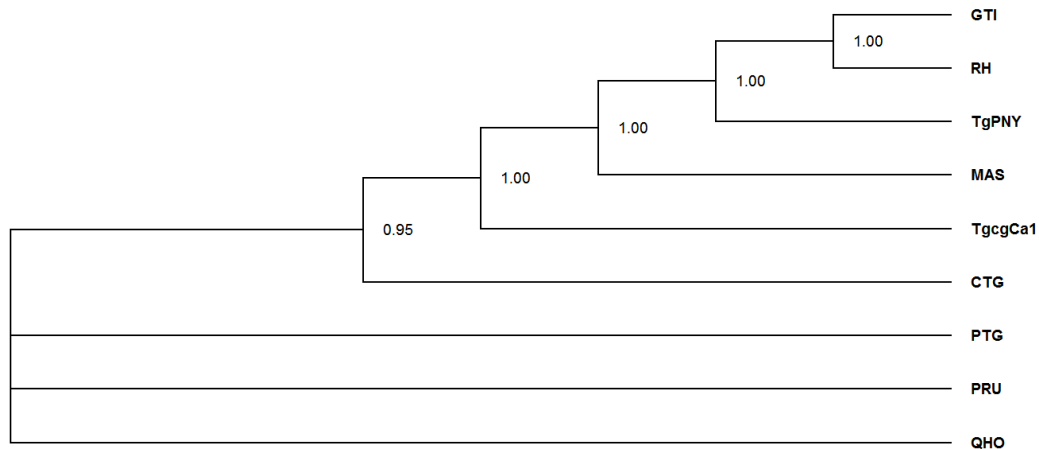
**Table 2.** Characteristics of *Toxoplasma gondii* eIF4A (Tg-eIF4A) gene sequences including coding regions and introns among different strains.

Isolates	Tg-eIF4A DNA	Tg-eIF4A cDNA	Tg-eIF4A introns	
			First	Second
Length (bp)	3152-3156	1239	554-556	242
T+A (%)	46.86-47.07	45.78-46.06	45.29-45.90	47.83-48.74
Transition	35	14	9	2
Transversion	6	3	2	0
R	5.8	4.7	4.5	-
Distance (%)	0-0.8	0-0.8	0.6	0-0.4

R = transition/transversion.

Phylogenetic reconstruction of all nine *T. gondii* strains based on eIF4A sequence data revealed that the eIF4A gene sequences could not readily differentiate *T. gondii* strains belonging to different genotypes (Figure 1), suggesting that the eIF4A DNA sequences were not an ideal genetic marker for intra-species phylogenetic analysis or differentiation of the examined *T. gondii* strains into their respective genotypes. However, the low sequence varia-

tion (0-0.8%) in the eIF4A gene suggests that eIF4A may represent a good candidate vaccine against *T. gondii* infection, and this has been supported by our recent study (Chen et al., 2013).



**Figure 1.** Phylogram of nine *Toxoplasma gondii* strains determined by analysis of the entire sequences of the eIF4A genomic region. The tree was built by Bayesian inference (BI) analysis. The numbers along branches indicate bootstrap values resulting from BI analyses.

In conclusion, the present study demonstrated low sequence variability in the eIF4A locus among *T. gondii* strains of different genotypes from different hosts and geographical regions, indicating that eIF4A is not a suitable marker for studying genetic variation or the population genetic structure of *T. gondii* isolates.

## ACKNOWLEDGMENTS

Research partially supported by the National Natural Science Foundation of China (Grant #31228022, #31230073, #31172316, and #31101812), the International Science and Technology Cooperation Project of Gansu Province (Grant #1204WCGA023), and The Science Fund for Creative Research Groups of Gansu Province (Grant #1210RJIA006).

## Conflicts of interest

The authors declare no conflict of interest.

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