Intraspecific genetic differentiation of *Angiostrongylus cantonensis* based on the complete mitochondrial genome

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Background

Angiostrongylus cantonensis (also known as rat lung worm) is the primary pathogen of eosinophilic meningitis in tropical region[1]. Up to date, over 2800 cases due to *A. cantonensis* infections had been documented in more than 30 countries[2]. In China, more than 380 cases were reported in the past decade and 88% of them were involved in 9 outbreaks[3]. More than three quarters were definitely attributed to two invasive snail species, i.e., *Pomacea canaliculata* and *Achatina fulica*[3]. The recent study showed that the two snail species had become the intermediate hosts of *A. cantonensis* in China[4]. Along with biological invasion of these snails, the parasites were probably transmitted beyond its original habitats. This project was performed in order to reveal the potential role of these snails in transmission of *A. cantonensis* based on genetic variation of *A. cantonensis*.

Materials and methods

A. cantonensis from China and *A. costaricensis* from Brazil were prepared for sequencing complete mitochondrial (mt) genome. Total genomic DNA was extracted from individual nematodes using sodium dodecyl-sulphate/proteinase K treatment[5] with a little modification. The primers were constructed according to the conversed sequences of current available mitochondrial genomes, i.e., those of *Ancylostoma duodenale* and *Necator americanus*[6]. The PCR products were sequenced by the dideoxynucleotide termination method. The sequences were assembled and edited using Vector NTI package.

The primers for intraspecific variation were designed based on the comparison between complete mt genomes of *A. cantonensis* and *A. costaricensis*. 28 isolates of *A. cantonensis* from different counties in China were used to reveal the genetic variation. The methods to determine DNA sequence were similar to those in complete mt genomes.

Results

Five complete mitochondrial genomes, including 4 isolates of *A. cantonensis* and one isolate of *A. costaricensis*, were determined. The mt genome size (around 13495 bp) of *A. cantonensis* isolates is smaller than that (13585 bp) of *A. costaricensis*. The main difference in size between the two *Angiostrongylus* species lies in AT-rich region. All the mt genomes contain 12 protein-coding genes (NADH dehydrogenase subunit 1~6, cytochrome c oxidase subunit I~III, cytochrome b, ATP synthase F0 subunit 6), two rRNA (rrL, rrS) genes and 22 tRNA genes. Like the majority of known nematode mt genomes, they lack of ATP synthase F0 subunit 8.

NADH1 gene is determined as the best genetic marker for intraspecific variation by comparing complete mt genomes of *A. cantonensis* and *A. costaricensis*. The targeted fragment was sequenced in 28 isolates. Phylogenetic analysis was performed based on NADH1 gene sequence. Three clusters were identified and the distance within group is respectively 0.0031, 0.0063 and 0.0064 (with overall mean of 0.0412). The isolates in the same genetic group are not necessary to be geographically clustered.

Conclusion

A. cantonensis and *A. costaricensis* are the first member of family metastrongylidae whose complete mitochondrial genomes were determined. The findings in our experiment will supply basic data for phylogenetic analysis of nematode and further study on the pathogens of neuro- and abdominal angiostrongyliasis. Variation analysis showed that the isolates with the same genetic feature might be dispersed at a relatively long distance, which indicated that the invasion of leading vector, i.e., *Pomacea canaliculata*, potentially play an important role in transmission of this parasite. Meanwhile it is worth to further study morphology and pathogenicity of isolates from three clusters, after all no anigostrongliasis case was reported from Hainan province.

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Although we selected several worm samples from some isolates, which were sampled at random, for sequencing and analysis, it is still difficult to determine the genetic structure of one population. Therefore, more worm samples from the same population should be assessed in order to reveal potential genetic variation in population.

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