

Transcriptome profiling of the fifth-stage larvae of *Angiostrongylus cantonensis* by next-generation sequencing

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Received: 22 March 2013 / Accepted: 7 June 2013 / Published online: 5 July 2013
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Abstract *Angiostrongylus cantonensis* is an important zoonotic nematode. It is the causative agent of eosinophilic meningitis and eosinophilic meningoencephalitis in humans. However, information of this parasite at the genomic level is very limited. In the present study, the transcriptomic profiles of the fifth-stage larvae (L5) of *A. cantonensis* were investigated by next-generation sequencing (NGS). In the NGS database established from the larvae isolated from the brain of Sprague–Dawley rats, 31,487 unique genes with a mean length of 617 nucleotides were assembled. These genes were found to have a 46.08 % significant similarity to *Caenorhabditis elegans* by BLASTx. They were then compared with the expressed sequence tags of 18 other nematodes, and significant matches of 36.09–59.12 % were found. Among these genes, 3,338 were found to participate in 124 Kyoto Encyclopedia of Genes and Genomes pathways. These pathways included 1,514 metabolisms, 846 genetic information processing, 358 environmental information

processing, 264 cellular processes, and 91 organismal systems. Analysis of 30,816 sequences with the gene ontology database indicated that their annotations included 5,656 biological processes (3,364 cellular processes, 3,061 developmental processes, and 3,191 multicellular organismal processes), 7,218 molecular functions (4,597 binding and 3,084 catalytic activities), and 4,719 cellular components (4,459 cell parts and 4,466 cells). Moreover, stress-related genes (112 heat stress and 33 oxidation stress) and genes for proteases (159) were not uncommon. This study is the first NGS-based study to set up a transcriptomic database of *A. cantonensis* L5. The results provide new insights into the survival, development, and host–parasite interactions of this blood-feeding nematode.

Introduction

Next-generation sequencing (NGS) is an important tool in transcriptomic studies. It greatly decreases the time and effort previously required for DNA sequencing by enabling massively sequencing reactions to define millions or billions base positions and allows identification of targeted genes differentially expressed in different tissues, cells, stages, or genders, during development, or on activation of immune responses. In addition, it is able to obtain complete nuclear and organellar genome sequences simultaneously (Shendure and Ji 2008). NGS technologies such as Roche 454 (Margulies et al. 2005), ABI-SOLiD (Pandey et al. 2008), Solexa (Illumina) (Bentley et al. 2008), and Helicos (Harris et al. 2008) change the way we discover and define parasite transcriptomes and genomes (Droegge and Hill 2008; Jex et al. 2010). These sequencing techniques are also involved in the development of enhanced computational methods for the preprocessing, assembly, and annotation of sequence data (Nagaraj et al. 2007a, b). Investigations of the transcriptome by different approaches (Ranganathan et al. 2009; Nisbet et al. 2008) lead to a better understanding of the biochemical and molecular processes

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Electronic supplementary material The online version of this article (doi:10.1007/s00436-013-3495-z) contains supplementary material, which is available to authorized users.

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involved in the development, reproduction, and parasite–host interactions in a number of parasitic nematodes such as *Haemonchus contortus* (Campbell et al. 2008; Cantacessi et al. 2010), *Ascaris suum* (Huang et al. 2008; Cantacessi et al. 2009), *Radopholus similis* (Jacob et al. 2008), *Schistosoma mansoni* (Farias et al. 2011), *Schistosoma japonicum* (Peng et al. 2003), *Leishmania donovani* (Li et al. 2008), *Eimeria brunette* (Aarthi et al. 2011), and *Clonorchis sinensis* (Lee et al. 2003; Cho et al. 2006, 2008).

Angiostrongylus cantonensis, the rat lungworm, is an important zoonotic nematode. This parasite was discovered in the lung of *Rattus rattus* and *Rattus norvegicus* in Guangdong in 1933 (Chen 1933). Human is a nonpermissive host and acquires the infection by eating raw or undercooked intermediate hosts (snails or slugs) or paratenic host (frogs) contaminated with the third-stage larvae (L3) (Alicata 1965, 1988). Although Southeast Asia and the Pacific Islands have been considered to be the main endemic regions, more than 2,800 cases have been recorded in 31 countries up to 2010 (Wang et al. 2008, 2012). After penetrating into the human host, the third-stage larvae migrate to the central nervous system via the bloodstream and develop into fifth-stage larvae (L5) after molting twice. At this site, they cause eosinophilic meningitis and eosinophilic meningoencephalitis. Patients have an insidious or sudden onset of excruciating headache, neck stiffness, nausea, vomiting, and paraesthesia, although fever, cranial nerve palsies, seizures, paralysis, and lethargy are less common. Majority of the patients having cerebral angiostrongyliasis usually have a self-limited course and recover without sequelae. However, fatal outcomes have been reported in severe cases (Punyagupta et al. 1975).

We have submitted 1,226 expressed sequence tags (ESTs) of L5 to the National Center for Biotechnology Information (NCBI) dbEST in 2005. These sequences and some additional ones were analyzed and found to encode proteins participating in metabolism, cellular development, immune evasion, and host–parasite interactions (Xu et al. 2009) and were grouped into 13 categories (Fang et al. 2010). After clustering 1,496 ESTs were generated from a cDNA library of L3 into 161 contigs and 757 singletons, 54.5 % of which were found to have a significant sequence homology with known proteins. Among the identified abundant expressive sequences were cathepsin B-like cysteine protease 1 and 2, metalloprotease I, metalloprotease 1 precursor, and extracellular superoxide dismutase (Chang et al. 2011). Since the information obtained by these transcriptomic studies is very limited, the application of the NGS technologies may provide a global study on the gene expressions of *A. cantonensis* in the central nervous system.

In the present study, we employed a NGS-based approach to set up a transcriptomic database of *A. cantonensis* L5. The CLC bio software was used to assemble the newly generated sequences and bioinformatic techniques to predict the key Kyoto

Encyclopedia of Genes and Genomes (KEGG) pathways, gene ontology (GO) annotations, and groups of molecules involved in the fundamental metabolic pathways of the nematode in this pathogenic stage. In addition, comparative analyses of the dataset predicted a range of proteins that are conserved among nematodes.

This study is the first NGS investigation to explore the gene expression of *A. cantonensis* in a comprehensive manner. The findings provide an invaluable resource to underpin future efforts toward developing new approaches for the intervention against and control of angiostrongyliasis. Understanding the molecular pathways linked to parasite survival in the environment, development, reproduction in the vertebrate host, and host–parasite interactions may provide a guide to the investigations of these pathways and facilitate the identification of new drug targets. Since the genome of *A. cantonensis* has not been sequenced, the establishment of a complete gene expression database is very important for understanding the infection and pathogenicity mechanism of this parasite.

Materials and methods

Establishment and maintenance of *A. cantonensis* in laboratory

The life cycle of *A. cantonensis* was established by isolating more than 10,000 of L3 from an *Achatina fulica* snail collected in Neihu, Taipei in 1985. After infecting these larvae to Sprague–Dawley (SD) rats, the first-stage larvae (L1) were recovered from the rat feces on day 50 postinfection and fed to *Biomphalaria glabrata* snails. To isolate L3, the infected *B. glabrata* snails were killed and the tissues were sonicated with an organization homogenizer (Cole-Parmer Instrument Co., USA) and then digested with artificial gastric juice (0.6 % (w/v) pepsin, pH 2–3) at 37 °C for 45 min on day 20 after infection. By esophageal perfusion, SD rats were infected with the isolated L3. After 42–46 days, L1 were harvested from the rat feces (Wang et al. 1989).

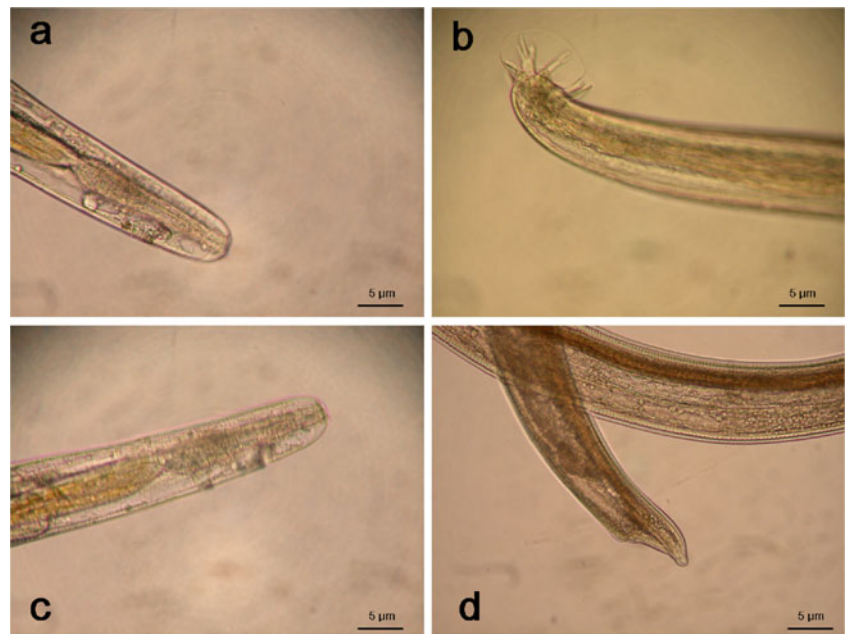
Collection of *A. cantonensis* L5

After collection from infected *B. glabrata*, L3 were fed to SD rats by esophageal perfusion. L5 (Fig. 1) were then isolated and collected from rat brains on day 21 postinfection (Wang et al. 1989).

RNA extraction

The L5 harvested from rat brains were washed with normal saline, PBS, and ddH₂O for three times. The larvae were then disrupted and homogenized in a TRIzol reagent (Molecular Research Center, USA). Chloroform, 75 % (v/v) alcohol (in

Fig. 1 Fifth-stage larvae of *A. cantonensis* collected from SD rats on day 21 postinfection. Differentiation of stage and gender was based on the sexual organs. In male, the bursa was opened (**a** head, **b** tail). The vagina in female was very clear (**c** head, **d** tail). Male worms were about 9.69 mm and females were about 11.48 mm in length



DEPC-H₂O), and isopropanol were used to purify and precipitate RNA samples (Chang et al. 2011).

Next-generation sequencing

Gene expressions in *A. cantonensis* L5 were identified by direct high-throughput sequencing using the next-generation sequencer Solexa (Illumina, USA). Long RNA sequences were first converted into a library of cDNA fragments, and adaptors were subsequently added to each cDNA fragment. This new sequencing technology (Solexa) relies on the attachment of small DNA fragments to a platform, optically transparent surface, and solid-phase amplification to create an ultrahigh-density sequencing flow cell with >10 million clusters, each containing ~1,000 copies of template/cm². These templates were sequenced by a robust four-color DNA sequencing-by-synthesis technology that employs reversible terminators with removable fluorescence. The high-sensitivity fluorescence was then detected by laser excitation. The typical output from a single reaction was approximately 2 GB which contains 20–40 million reads. The Solexa sequence reads were assembled using the CLC bio software. Before assembly, the obtained reads were preprocessed by masking the polyA tails and removing adaptors. This generated datasets were analyzed by the Chang Gung Bioinformatics Center.

BLAST homology searches and sequence annotations

Bioinformatic analyses were conducted using FastAnnotator, an efficient transcript annotation web tool (<http://fastannotator.cgu.edu.tw/>) (Chen et al. 2012). The *Caenorhabditis elegans*

genome database and EST sequences of 18 parasitic or nonparasitic nematodes were obtained from NCBI (<http://www.ncbi.nlm.nih.gov/>). BLASTx homology searches were performed to compare the proteins inferred from the transcriptomes of *A. cantonensis* L5 with those predicted from transcriptomic data of *C. elegans* and the 18 nematodes with an *E* value cutoff at 1×10^{-5} . Identification of GO annotations was carried out using Blast2GO (B2G4Pipe) (Götz et al. 2008). KEGG pathway analysis was performed through the KEGG database (<http://www.genome.jp/kegg/>).

Results

Characterization of the transcriptome

A total of 15,399,691 short-insert Illumina reads were generated for *A. cantonensis* L5 with a mean length of 77.51 nucleotides. After removal of adaptors, these short-insert Illumina reads were de novo assembled by the CLC bio software (<http://www.clcbio.com/index.php?id=1109>). The assembly procedure resulted in 31,487 contigs with a mean length of 617 nucleotides (range 128–18,870) and a G+C content of 43.73 %. The total read count was 13,483,766 nucleotides, and the total contig length was 19,440,921 (Table 1).

Sequence homology

By BLAST analysis, 14,509 (46.08 %) of the 31,487 NGS transcriptome contigs from *A. cantonensis* L5 were found to have significant matches sequences from the *C. elegans* genome database (*E* value $\leq 1 \times 10^{-5}$) (Table 1). Proteins inferred

Table 1 Characteristics of the transcriptome of the fifth-stage larvae of *A. cantonensis*

Contig count	31,487
Type	De novo assembly
Total read count	13,483,766
Mean read length (nucleotides)	77.51
Total read length (nucleotides)	1,045,178,927
Mean contig length (nucleotides)	617
Total contig length (nucleotides)	19,440,921

from the transcriptomes were compared with those predicted from transcriptomic data of 18 nematode ESTs from the NCBI database. Significant matches (36.09–59.12 %) were found with *Ancylostoma caninum*, *Ancylostoma ceylanicum*, *A. suum*, *Brugia malayi*, *Caenorhabditis brenneri*, *Caenorhabditis remanei*, *H. contortus*, *Necator americanus*, *Nippostrongylus brasiliensis*, *Onchocerca volvulus*, *Ostertagia ostertagi*, *Panagrolaimus superbus*, *Parastrongyloides trichosuri*, *Strongyloides ratti*, *Strongyloides stercoralis*, *Toxocara canis*, *Trichinella spiralis*, and *Trichuris muris* (E value $\leq 1 \times 10^{-5}$) (Table 2).

Functional analysis

A total of 3,338 sequences were mapped to 124 KEGG pathways (Table 3). A significant proportion of amino acid sequences were associated with (1) metabolic pathways (1,514 sequences) including carbohydrate metabolism,

Table 2 Sequence homology between *A. cantonensis* and 18 nematodes

Species	Total EST	Matched EST	Percent
<i>Caenorhabditis brenneri</i>	7,857	4,645	59.12
<i>Caenorhabditis remanei</i>	8,170	4,653	56.95
<i>Ancylostoma caninum</i>	8,241	4,572	55.48
<i>Brugia malayi</i>	5,229	2,861	54.71
<i>Trichinella spiralis</i>	5,764	3,040	52.74
<i>Ascaris suum</i>	5,589	2,906	51.99
<i>Onchocerca volvulus</i>	3,704	1,815	49.00
<i>Haemonchus contortus</i>	5,905	2,825	47.84
<i>Strongyloides stercoralis</i>	4,708	2,243	47.64
<i>Ancylostoma ceylanicum</i>	5,007	2,307	46.08
<i>Strongyloides ratti</i>	5,505	2,403	43.65
<i>Panagrolaimus superbus</i>	3,476	1,482	42.64
<i>Nippostrongylus brasiliensis</i>	4,134	1,755	42.45
<i>Parastrongyloides trichosuri</i>	3,696	1,554	42.05
<i>Ostertagia ostertagi</i>	3,686	1,536	41.67
<i>Trichuris muris</i>	3,052	1,192	39.06
<i>Necator americanus</i>	3,987	1,542	38.68
<i>Toxocara canis</i>	2,153	7,770	36.09

Table 3 Metabolic pathways in the fifth-stage larvae of *A. cantonensis* mapped by the Kyoto Encyclopedia of Genes and Genomes (KEGG)

Pathway	No. of sequences
Metabolism	1,514
Carbohydrate metabolism	349
Energy metabolism	141
Lipid metabolism	215
Nucleotide metabolism	180
Amino acid metabolism	312
Metabolism of other amino acids	51
Glycan biosynthesis and metabolism	58
Metabolism of cofactors and vitamins	99
Metabolism of terpenoids and polyketides	14
Biosynthesis of other secondary metabolites	2
Xenobiotics biodegradation and metabolism	93
Genetic information processing	960
Transcription	167
Translation	360
Folding, sorting, and degradation	319
Replication and repair	114
Environmental information processing	358
Membrane transport	7
Signal transduction	320
Signaling molecules and interaction	31
Cellular processes	264
Transport and catabolism	264
Organismal systems	91
Immune system	18
Endocrine system	38
Development	12
Environmental adaptation	23

energy metabolism, amino acid metabolism, lipid metabolism, nucleotide and amino acid metabolism, metabolism of other amino acids, glycan biosynthesis and metabolism, metabolism of cofactors and vitamins, metabolism of terpenoids and polyketides, biosynthesis of other secondary metabolites, and xenobiotic biodegradation and metabolism; (2) genetic information processing (846 sequences) including transcription, translation, folding, sorting and degradation, replication, and repair; (3) environmental information-processing pathways (358 sequences) including signal transduction, membrane transport, and signaling molecules and interaction; (4) cellular processes (264 sequences) including transport and catabolism; and (5) organismal systems (91 sequences) including immune system, endocrine system, development, and environmental adaptation. The KEGG metabolism pathway analysis also showed 63 sequences in eight different metabolism pathways including the glycolysis/gluconeogenesis pathway ($n=14$), citrate cycle

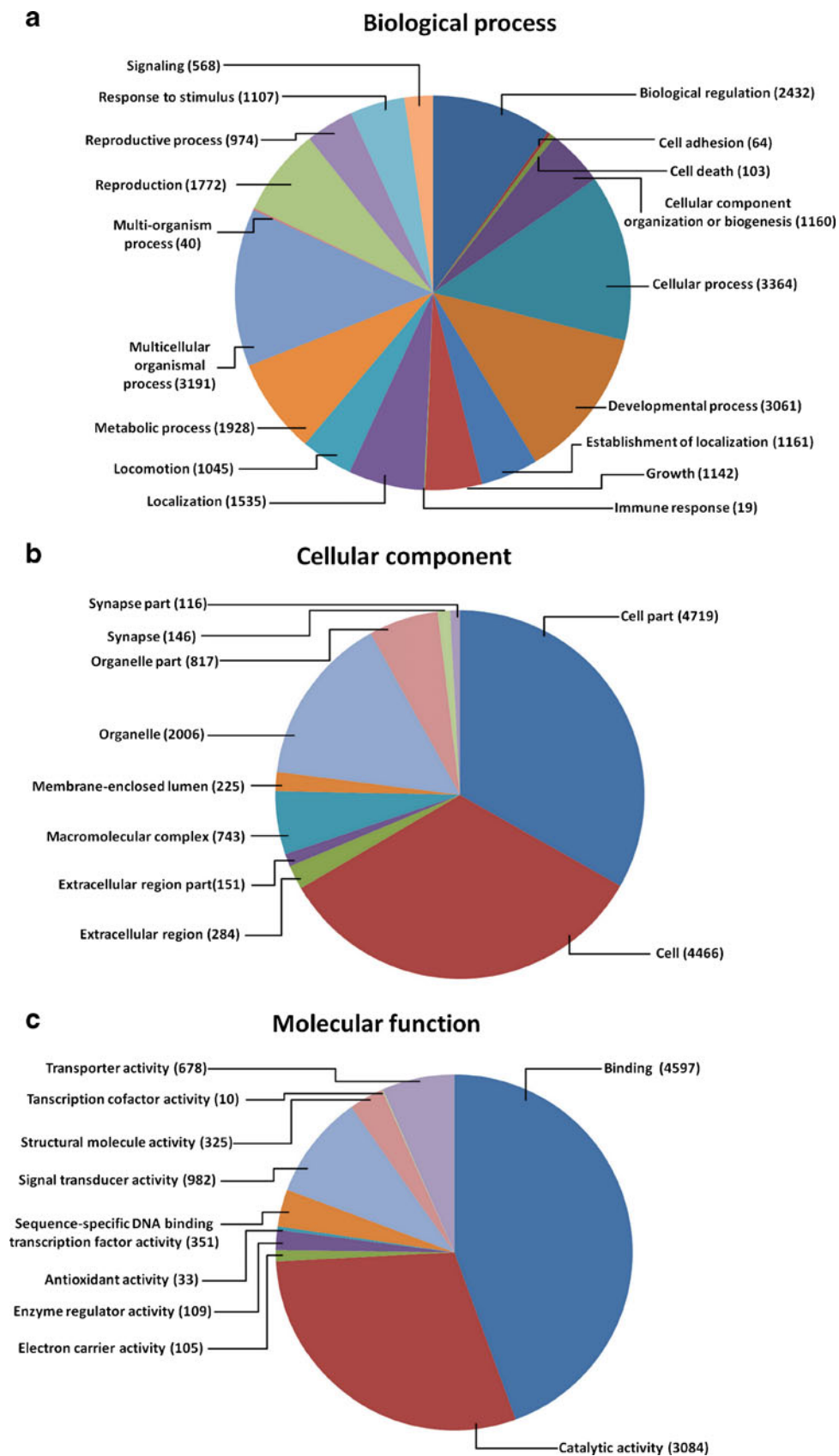


Fig. 2 Functional annotations of the sequences from the fifth-stage larvae of *A. cantonensis* based on gene ontology categories. The pie charts show the general categories of biological process (a), cellular component (b), and molecular function (c)

(TCA cycle) pathway ($n=11$), pentose phosphate pathway ($n=6$), pentose and glucuronate interconversions pathway ($n=9$), fructose and mannose metabolism pathway ($n=9$), galactose metabolism pathway ($n=6$), ascorbate and aldarate metabolism pathway ($n=7$), and fatty acid biosynthesis pathway ($n=1$) (Table S2).

The 30,816 sequences were analyzed with the GO database (Fig. 2). In biological process, 5,656 sequences were expressed and the most expressed sequences are cellular process (3,364 sequences), developmental process (3,061 sequences), and multicellular organismal process (3,191 sequences). In cellular component, 4,719 sequences were expressed including cell part (4,459 sequences) and cell (4,466 sequences). In molecular function, 7,218 sequences were expressed including binding (4,597 sequences) and catalytic activity (3,084 sequences).

In this NGS database, 4,719 sequences were expressed in cellular component including cytoskeletal-related genes. These highly expressed cytoskeletal-related genes are the tubulin-related genes ($n=32$) including the tubulin alpha family ($n=11$), tubulin gamma family ($n=3$), tubulin folding cofactor E-Like protein ($n=4$), and tubulin tyrosine ligase-like family ($n=14$).

Molecules well represented in *A. cantonensis* in stress included antioxidants ($n=32$), heat shock proteins ($n=112$), and proteases ($n=153$) (Fig. 3). The antioxidants highly expressed in *A. cantonensis* life cycle included the peroxiredoxin family ($n=5$), thioredoxin family ($n=7$), glutaredoxin family ($n=1$), and glutathione transferases family ($n=19$) (Table S3).

In addition to molecules related to stress, the NGS database also contained heat shock proteins ($n=112$) including the heat shock factor ($n=5$), heat shock protein 1 family ($n=11$), heat shock protein 3 family ($n=7$), heat shock protein 4 ($n=1$), heat shock protein 6 family ($n=3$), heat shock protein 12 family ($n=4$), heat shock protein 16 family ($n=7$), heat shock protein 17 family ($n=2$), heat shock protein 25

family ($n=2$), heat shock protein 43 family ($n=4$), heat shock protein 60 family ($n=2$), heat shock protein 70 family ($n=1$), and DNaJ domain (prokaryotic heat shock protein) family ($n=63$) (Table S4).

In this study, 153 proteases were found including the ADAM (disintegrin plus metalloprotease) family ($n=7$), aspartyl protease family ($n=15$), CED-3 protease suppressor family ($n=1$), CLP protease family ($n=1$), cysteine protease family ($n=10$), inner mitochondrial membrane protease family ($n=6$), intramembrane protease family ($n=7$), nematode astacin protease family ($n=76$), OTUBain deubiquitylating protease homolog family ($n=1$), paraplegin AAA protease family ($n=3$), trypsin-like protease family ($n=14$), ubiquitin-like protease family ($n=10$), YME1-like (yeast mitochondrial escape) AAA protease family ($n=1$), and zinc metalloprotease family ($n=1$) (Table S4).

Discussion

A. cantonensis is the rat lungworm. Human is a nonpermissive host, and the infection leads to eosinophilic meningitis or eosinophilic meningoencephalitis. After infecting the central nervous system of mice, *A. cantonensis* L5 induce a wide range of immune responses including eosinophilia recruitment and cytokine release (IL-4, IL-5, and eotaxin) via the NF- κ B pathway (Lan et al. 2004). In addition, the migrating L5 have also been reported to cause mechanical injuries (Li et al. 2012). Blood–brain barrier dysfunction and CSF eosinophilia have also been observed in experimentally infected mice (Tsai et al. 2009). Therefore, studying *A. cantonensis* L5 gene expression in host is very important to understand the pathogenic mechanism.

Recent studies in high-throughput sequencing and bioinformatics provide researchers with the much needed tools to study nematodes (Jex et al. 2010; Webb and Rosenthal 2011), trematodes (Young et al. 2011), or other species (Vogel et al. 2011). NGS has been used to analyze *Fasciola gigantica* transcriptomes. From 20 million raw sequence reads, 30,000 contiguous sequences were assembled. These sequences were characterized on the amino acid level based on homology, gene ontology, and/or pathway mapping by KEGG (Young et al. 2011).

In our laboratory, 708 ESTs (159 clusters and 549 singletons) have been generated from *A. cantonensis* L5. In this study, we used NGS to study gene expression in the same developmental stage of this parasite. A total of 31,487 sequences were found and 14,509 (46.08 %) matched the *C. elegans* genome database. Therefore, we can perform a global study on the gene expression of *A. cantonensis* L5 in this study.

Proteins inferred from the transcriptome of *A. cantonensis* were compared with those predicted from transcriptomic data of 18 nematode ESTs from the NCBI database.

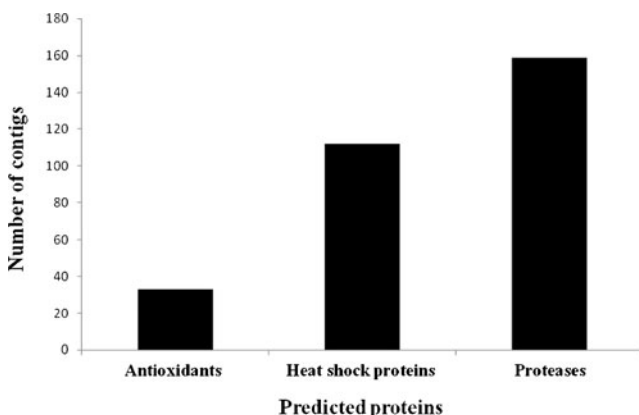


Fig. 3 Distribution of stress-related proteins in the fifth-stage larvae of *A. cantonensis*

Caenorhabditis species were found to be the most similar to *A. cantonensis* (*C. elegans* 97.87 %, *C. brenneri* 59.12 %, *C. remanei* 56.95 %). This result is very interesting since *Caenorhabditis* is a genus of nematodes living in bacteria-rich environments. In addition, it is free-living. *C. elegans* and *Caenorhabditis briggsae* are the most studied species in this genus. Moreover, *C. elegans* is an animal model and it is the first multicellular organism to have its genome completely sequenced and published in 1998. Gene expressions of blood-living parasite (*B. malayi* and *O. volvulus*) are also similar to *A. cantonensis* (52.35 %), since these parasites lived in blood vessels.

In the life cycle of *A. cantonensis*, the conversion of L3 to L5 is a complex differentiation process through regulating various proteins expression. During infection, *A. cantonensis* has to adapt to their new environment in a twofold way: L3 switch from a poikilothermic to a homoiothermic host and from a snail to a mammal. In addition, they are confronted with different types of host defense mechanisms both in snails and in mammals.

Albendazole is a member of the benzimidazole compounds and anticytoskeletal drugs. It has been used to treat a variety of worm infections (Barrère et al. 2012). This drug causes parasite tegument and intestine degenerative alteration by binding to the colchicine-sensitive site of tubulin, inhibiting tubulin polymerization and assembly into microtubules. In *A. cantonensis*, this drug has been reported to be the drug of choice against larvae and it is effective in the treatment of *A. cantonensis* infection in rabbits within 15 days after infection (Wang et al. 2006). Cell motility, growth, motor, and morphological changes in parasites require remodeling of cytoskeleton in response to intracellular and extracellular signals (Huang et al. 2009; O'Hagan et al. 2011; Martínez-Valladares et al. 2012). The reorganization of the microtubule network is important for the survival of nematodes (Banora et al. 2011). The identification of these cytoskeleton genes provided a rationale for the treatment of *A. cantonensis*.

Heat shock proteins are a highly conserved group of proteins in prokaryotic and eukaryotic organisms. They function as chaperones and assist newly synthesized protein folding (Morassutti et al. 2012). Their expressions are induced by the host immune responses such as reactive oxygen species (ROS) generation. After infection, ROS generation is induced in the mouse brain. It has been demonstrated that the expression of ROS-related enzymes such as glutathione reductase, glutathione peroxidase, and glutathione S-transferase (GST) significantly increases in the CSF of mice infected with *A. cantonensis* (Chung et al. 2010).

Antioxidants have been suggested to play a role in host immune modulation. They are highly expressed throughout the life cycle of *A. cantonensis*. Peroxiredoxin, thioredoxin, and glutathione transferases are important ones. Their

expressions protect parasites from the attack of ROS generated by the host immune response (Dzik 2006; Sayed et al. 2006; Donnelly et al. 2008; Morassutti and Graeff-Teixeira 2012). We identified a total of 20 GSTs in the *A. cantonensis* L5 NGS database. GSTs are a large and important family of proteins found in many parasites to play a central role in H₂O₂ detoxification. Parasites contain multiple GSTs belonging to different GST classes and with differing enzyme activities to accommodate a wide range of functions of this enzyme family. GST in *Fasciola hepatica* influences host immune cell activities. It is present near the surface of the fluke and is expressed in eggs and newly excysted larvae as well as in the excretory/secretory fraction of the adults (LaCourse et al. 2012). In *Opisthorchis viverrini*, GST is one of the excretory/secretory products (ESP) and acts as a mitogen. It may also cause tumor development in the host. This antioxidant induces cell proliferation by activating pAKT and pERK (Daorueang et al. 2012).

In ESP of *A. cantonensis*, peroxiredoxin (Prx) is one of the highly expressed proteins (Donnelly et al. 2008). It is an enzyme reported to exist in many parasites and known to play a central role in H₂O₂ detoxification. In *F. hepatica*, Prx in the ESP has been shown to downregulate immune responses and to affect macrophage activation following injection into mice (Sayed et al. 2006). In our unpublished proteomic studies, highly expressed disulfide isomerase 1 and oxidoreductase have been determined by two-dimensional electrophoresis and mass spectrometry species-specific analysis and associated with defense oxidation stress. Inhibition of Prx expression in *A. cantonensis* not only causes the reduction of Th-2 immune responses of the host against *F. hepatica* and *S. mansoni* infection (Sayed et al. 2006) but also reduces their survival in the host (Donnelly et al. 2008).

Proteases are important for the survival of parasitic helminths by facilitating tissue penetration, digestion of food proteins, and immunomodulation. Moreover, the nutrition of blood-feeding nematodes depends on a multienzyme synergistic proteolytic cascade in the intestine, cleaving the ingested host hemoglobin tetramer into successively small fragments (Williamson et al. 2004). Aspartyl protease has been reported to initialize the degradation of host hemoglobin in blood-feeding parasite. It plays an important role in the nutrition of schistosomes (Brindley et al. 2001) and hookworms (Williamson et al. 2004). In our previous study, we identified an EST representing an aspartic protease from an *A. cantonensis* L5 dataset and this protease sequence encodes a protein with a predicted molecular mass of 46 kDa. Variations in the expression level of this aspartic protease gene exist at different development stages in both genders (Hwang et al. 2010). In other parasitic nematodes, highly expressed aspartyl protease has also been reported in *Steinernema carpocapsae* (Balasubramanian et al. 2012) and *T. spiralis* (Park et al. 2012).

By analysis of the ESTs of *A. cantonensis* L5, three cysteine protease genes have been reported. They are cathepsin B-like enzyme gene, 1, 2 (AC-cathB-1, AC-cathB-2), and hemoglobin-type cysteine protease gene (AC-hem) (Fang et al. 2010). A cysteine protease gene has also been isolated and described from the adult worm (Ni et al. 2012). Cysteine protease is a potential new target for the development of novel antimalarial drugs. It has reported that inhibitors of cysteine protease are able to block *Plasmodium falciparum* development in vitro and *P. falciparum*-infected mice could be cured (Sundararaj et al. 2012). In hookworm, a cysteine protease inhibitor may inhibit cysteine protease and has been used for the treatment of hookworm infection. This inhibitor may be developed as a novel anthelmintic to target or treat hookworm infection (Vermeire et al. 2012). RNA interference assays have been used to demonstrate functions of cysteine protease in *B. malayi*, and inhibition of cysteine protease causes the reduction in the numbers of secreted microfilariae in vitro. Moreover, siRNA-treated worms revealed a disruption in the process of embryogenesis (Ford et al. 2009).

This study is the first NGS-based study to set up a transcriptomic database of *A. cantonensis* L5 with identification and characterization of the genes. The results provide new insights into the survival, development, and host–parasite interactions of this blood-feeding nematode. Understanding of these aspects also allows us to carry out new investigations for the pathogenesis, clinical diagnosis, and chemotherapy of this zoonotic disease.

Acknowledgments This work was supported in part by grants from the National Science Council, Executive Yuan, ROC (NSC100-2320-B-182-013 and NSC101-2320-B-182-045) and Chang Gung Memorial Hospital Research Grant CMRPD1B0351. We thank the Chang Gung Molecular Medicine Research Center for the technical supports.

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