



Polymorphism of gp15/400 allergen gene of *W. bancrofti* from different regions of India endemic for lymphatic filariasis

S.L. Hoti^{*}, R. Thangadurai, K.P. Patra, P.K. Das

Molecular Biology and Bioinformatics Unit, Vector Control Research Centre (ICMR), Medical Complex, Indira Nagar, Pondicherry 605 006, India

Received 23 May 2006; received in revised form 17 July 2006; accepted 18 July 2006

Abstract

Nematode polyprotein allergens (NPA) are lipid binding/transport molecules that elicit elevated levels of IgE response in the infected host, leading to Th2 type of immune response. They also transport arachidonic acid and its metabolites that are known to be involved in the action of antifilarial drug, Diethylcarbamazine and hence are of great significance for the control of lymphatic filariasis. We investigated the polymorphism of gp15/400 polyprotein of 35 isolates of lymphatic filarial parasite *Wuchereria bancrofti* collected from different geographic locations of India. The repeat sub-unit of the gene was found to be highly conserved in all the isolates with only two nucleotide synonymous changes at positions 286 (A-G) and 337 (C-T). Since this molecule is highly conserved and has multifarious roles in the survival and pathogenesis of the parasite it has good potential as a target for drug, immunomodulation tool and immunotherapy development.

© 2006 Elsevier B.V. All rights reserved.

Keywords: NPA; gp15/400; *Wuchereria bancrofti*; Polymorphism; Evolution

1. Introduction

The nematode polyproteins are allergen antigens (NPAs) involved in binding small lipids and transportation to muscles and gonads via coelomic cavity (Kennedy, 2000). First characterized in *Ascaris suum* and *A. lumbricoides* and named ABA-1 protein of *Ascaris*, they have been found in worms belonging to numerous species (e.g. *Onchocerca ostertagi*, *Dictyocaulus viviparus*, *A. suum*, *D. imitis*) since then (de Graaf et al., 1995) and have no counterparts in mammals. They are produced in the gut of parasites as large precursor protein complex comprising of 10–50 tandemly repeated polypeptide units, depending upon the species and have a short hydrophobic leader sequence. The repeat units get cleaved at cleavage sites (Arg-Arg-Lys-Arg) of subtilisin serine protease at the C-terminal into functionally similar repeat sub-units of approximately 15 kDa. The allergen, found in all stages of the parasite (Kennedy and Qureshi, 1986), was located as the most abundant protein species in the body fluid of parasites and secreted into that of infected animals (Christie et al., 1992; Kennedy and Qureshi, 1986; McGibbon et al., 1990; Spence

et al., 1993; Tomlinson et al., 1989). Very importantly, nematode parasites are unable to synthesize their own complex lipids and derive them entirely from the hosts. A homologue of this polyprotein, called ladder or gp15/400 protein, has been found in filarial nematodes, which is also a complex of approximately 400 kDa with 20 tandemly arranged repeats sub-units of 132 amino acid residues and encoded by a single gene. gp15/400 protein of *Brugia malayi* is associated with the surface and also distributed in all tissues of the parasite (Tweedie et al., 1993). Similar to its counterparts in other nematodes, it is associated with elevated levels of IgE antibodies, thereby inducing allergic type-II (Th2) response in elephantiasis cases (Hussain et al., 1992) and exhibiting strong genetic control of immune response (Kennedy et al., 1990; Allen et al., 1995).

Paxton et al. (1993) have reported that the repeat sub-unit of the gp15/400 gene was highly conserved between brugian filarial parasites, *B. malayi* and *B. pahangi*, but exhibited small degree of divergence in *Wuchereria bancrofti* with 21 nucleotides (and 7 amino acids) variation (94% identity) and great degree of divergence in *Dirofilaria immitis* with only 57% identity. However, information on the diversity in the structure of lymphatic filarial antigens sequenced to date is extremely limited, both between closely related species and (presumably)

^{*} Corresponding author. Tel.: +91 413 2279073; fax: +91 413 2272041.

E-mail addresses: slhoti@yahoo.com, vcrc@vsnl.in (S.L. Hoti).

between different strains of the same species (Paxton et al., 1993). We therefore sequenced the repeat units of gp15/400 protein gene from 35 *W. bancrofti* isolates collected from different geographic areas of India in order to see the extent of nucleotide polymorphism, compared its nucleotide sequence with that of other filarial, non-filarial and parasitic nematodes and examined the evolutionary relationship in relation to its host inhabitation and parasitic life.

2. Materials and methods

2.1. Study areas, sample collection and purification of microfilaria and DNA

A total of 35 blood samples were collected from microfilaria (mf) carriers residing in locations representing different geographic regions of India (Hoti et al., 2003). Eighteen of these were from Pondicherry town in southern peninsular India, one each from two villages near Pondicherry (Chinnanergunam and Athipakkam, Tamil Nadu state), three from Rajahmundry town in southern state of Andhra Pradesh, five from Jagadapur town in Chattisgarh state (central India) and seven from Varanasi town in northern region (Uttar Pradesh state) of India. While Varanasi and Kozhikode are highly endemic for bancroftian filariasis, the other locations are moderately endemic (Sabesan et al., 2000). Five milliliters of venous blood was collected from each mf carrier during night hours (20.00–22.00 h), after obtaining written consent from him/her. EDTA (final concentration 1 mM) was added to the blood sample to prevent it from clotting and the sample was stored at 4 °C. The mf were separated from the blood samples by membrane filtration technique (Dennis and Kean, 1971) followed by Percoll-Sucrose gradient centrifugation technique (Chandrasekar et al., 1984). The genomic DNA from mf was extracted following the method described earlier (Hoti et al., 2003).

2.2. PCR reaction condition and sequencing

Single repeat unit of polyprotein allergen (gp15/400) gene of isolates of *W. bancrofti* from different geographical regions was amplified using gene specific primers (Wbgp15f-5'-TGGCTT-ACGGATGCCCAAAGG-3' and Wbgp15r-5'-ACTTAGATG-CGTCCGAAAATAG-3') (Paxton et al., 1993). The PCR mixture consisted 100 ng of purified DNA, 5 µl of 10× buffer, 20 pmol of each primer, 20 mM dNTP, 2.5 mM MgCl₂ and 2 units of Dynazyme II Ext Taq DNA polymerase (Finnzymes, Finland), in reaction volume of 50 µl. Amplification was carried out in a Master Cycler Gradient (Eppendorff, USA) and the temperature cycles consisted of an initial denaturation at 96 °C for 4 min followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 2 min with a final extension step of 72 °C for 7 min. The amplified products were resolved in 1.5% Agarose gel, stained in ethidium bromide and observed under UV transilluminator. The amplified product was purified using nucleotide removal kits (Qiagen, German) and sequenced in an automated DNA sequencer (ABI 7000). The nucleotide sequences of the isolates

have been deposited in the GenBank and have the accession numbers from DQ 321502 to DQ321536. The sequences of the NPA gene of other nematodes were obtained from the GenBank (Fig. 4). The sequences of *W. bancrofti* gp15/400 sub-unit, which are 396 bp in length were aligned with those of other nematodes using BioEdit programme (Hall, 1999), and edited by trimming off those that extended beyond the length of the former. The dendrogram was constructed using the sequences thus edited employing MEGA 3.1 programme (Kumar et al., 2004). MEGA 3.1 (Molecular Evolutionary Genetic Analysis) programme is an integrated tool for automatic and manual sequence alignment, inferring phylogenetic trees, mining web-based databases, estimating rates of molecular evolution, and testing evolutionary hypotheses. Pairwise DNA matrices were generated using the Kimura two-parameter model and phylogenetic analyses, using program and tree topologies were inferred by the unweighted pair-group method with arithmetic mean (UPGMA) with the Kimura two-parameter distance matrices. Bootstrap resampling (1000 data sets) of the multiple alignment tested the statistical robustness of the trees.

3. Results and discussion

NPA, apart from binding small lipids, have also been reported to bind to haeme and divalent metal ions, arachidonic acid and its metabolites, lyso-platelet activating factor, lysophospholipids and retinoids, as also sequestering pharmacologically active lipids (Kennedy, 2000). The binding property of these proteins with arachidonic acid and its metabolites and platelet activating binding factor are of great significance to lymphatic filarial parasites as Diethylcarbamazine, the only available antifilarial drug, acts through interfering with the arachidonic acid pathway (Maizels and Denham, 1992). Principally, our interest in the polymorphism of this gene stems from facts that it is involved in (a) pathogenesis of lymphatic filariasis (Paxton et al., 1993) and hence may have potential to serve as effective components for immunodiagnostics and immunomodulation for controlling pathology, (b) transport of Arachidonic acid and metabolites (Kennedy, 2000), which in turn are involved in the action of antifilarial drug, Diethylcarbamazine and hence polymorphism of this gene, if exists, would affect the disease outcome of the infection and the response of the parasite to DEC treatment, and lastly (c) transports lipids from host to parasite (Barrett et al., 1997) and has no counterpart in mammals (Kennedy, 2000) and therefore forms a potential drug/vaccine candidate. In fact recent studies have shown that it is a potential candidate for vaccine development (McGibbon and Lee, 1990; Vercauteren et al., 2004).

Amplification using gp15/400 gene specific primers (Paxton et al., 1993) yielded a ladder like profile on gel with amplicons ranging from approximately 400–1200 bp, in multiples of 400 bp (Fig. 1) from all the *W. bancrofti* isolates collected from different geographic locations of India. A faint band of 1600 bp was also observed in the case of some isolates. But, upon repetition of amplification, it was found in all the isolates. This is similar to that reported for Indonesian isolate of *W. bancrofti* and other filarial nematodes, *B. malayi* and *B. pahangi*, by other

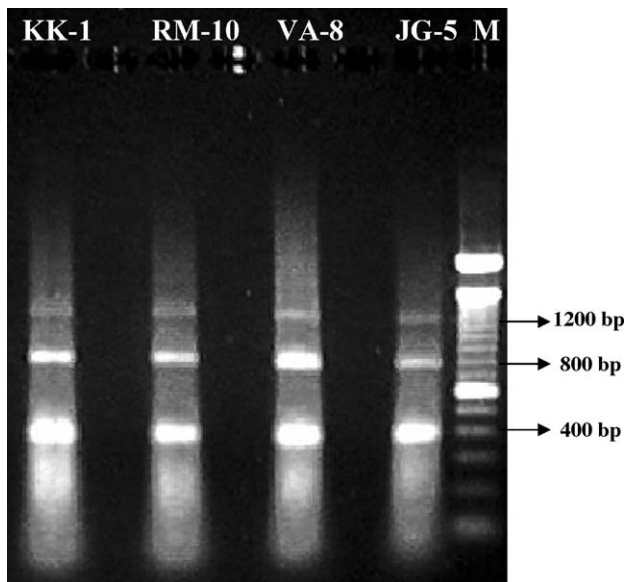


Fig. 1. Amplification of gp15/400 repeat sub-unit of polyprotein allergen gene from *Wuchereria bancrofti* mf collected from different geographic regions of India (KK-1: Kozhikode, RM-10: Rajahmundry, VA-8: Varanasi and JG-5: Jagadalpore. M-100 bp ladder).

workers (Paxton et al., 1993). These results thus showed that the polyprotein gene had no size polymorphism among *W. bancrofti* isolates originating from wide geographic areas of India. The amplicons of the gp15/400 gene from geographical

isolates were sequenced and compared after alignment. Since sequences of many isolates were truncated at 5' and 3' ends, sequences nucleotide positions between 144 and 359 only were compared. It was evident from the comparison that the sub-units of the gene are highly conserved within *W. bancrofti* isolates, with very minimum nucleotide variation. The variations were mainly at nucleotide positions 286 (A-G) and 337 (C-T), which are synonymous. Thus, nucleotide sequences of individual repeat units of the gene, although from 35 different isolates of *W. bancrofti*, were found to be highly conserved between sub-units of an isolate, as well as between sub-units of different isolates. The nucleotide sequence alignment of the repeat sub-unit of gp15/400 gene of *W. bancrofti* from Pondicherry (PD8) isolate with that of Indonesian isolate (Paxton et al., 1993) showed that the latter also had only three additional nucleotide variations, at positions 62 and 63, which lead to a change in amino acid (from K to I) and at position 369 with no amino acid change (Fig. 2). The consequence of change of an amino acid at this position needs to be investigated for functional (phenotypic) changes at protein level and at this stage it is difficult to predict such changes. But for these variations the gp15/400 gene of *W. bancrofti* might be conserved Pan-Asian region.

However, close examination of the chromatograms of the nucleotide sequence data revealed the existence of that two peaks at positions 286 and 337 (Fig. 3), indicating that at these positions there might be (1) heterozygous alleles within an

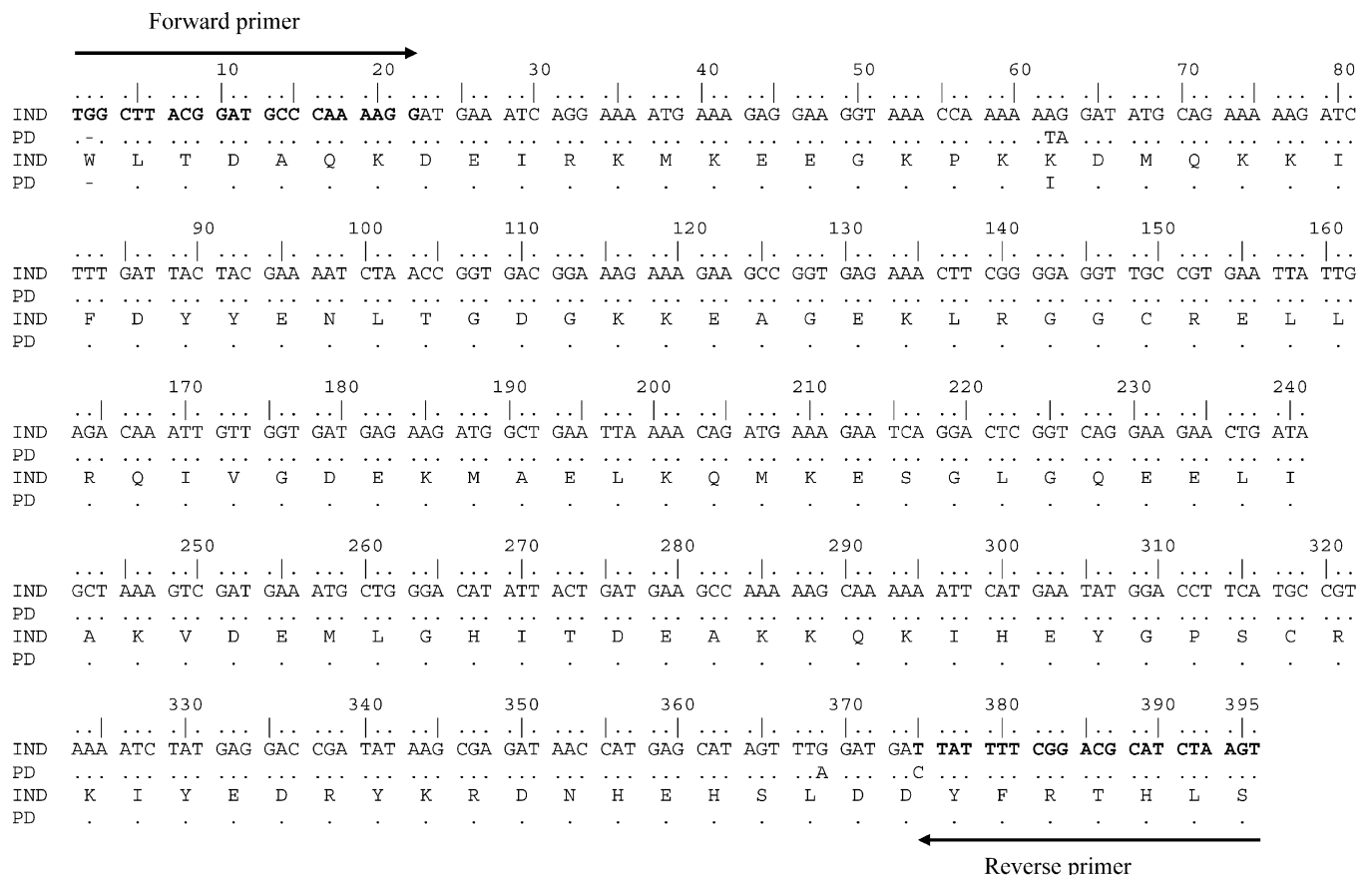


Fig. 2. Alignment of nucleotide and derived amino acid sequences of gp15/400 repeat sub-unit of *W. bancrofti* from Jakarta, Indonesia (IND) and Pondicherry (PD), India.

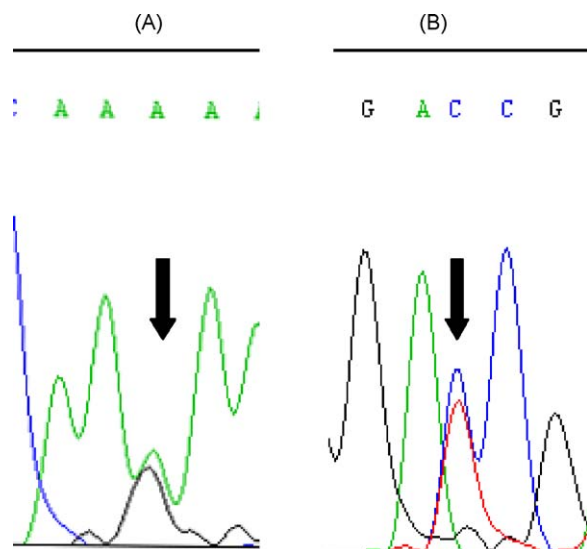


Fig. 3. Chromograms of nucleotide sequences of gp15/400 repeat sub-unit of *W. bancrofti* isolates showing two peaks at positions 286 (A) and 337 (B).

individual mf, (2) nucleotide variation between mf populations (as the template DNA used for amplifications was from a mixture of mf), or (3) nucleotide variation between different sub-units of a gp15/400 gene. In any case when the nucleotide variation within the gene is considered it appears that there could be only two variations (either of the two or both) and only at these positions. This is because, assuming that the gene is a 20-mer, as many as 35 sequences of the sub-units from Indian isolates and also the Indonesian isolate exhibited variation only at these positions. Further, since these variations are synonymous they might not have any structural consequences to the allergic protein, although can cause structural alterations at mRNA level and hence expression (King et al., 2002). Also, the two variations had no relevance to the geographic locations as the isolates with the two different SNPs were observed in parasite isolates from all geographic locations.

Thus, in the present study, a sub-unit of gp15/400 gene of *W. bancrofti* was investigated for polymorphism among parasite isolates collected from wide geographic areas of India, spanning about 3000 km. The Indian isolates, as also the Indonesian isolate differed from one another only at two nucleotide positions, thus, indicating that the gene is highly conserved across geographic boundaries. Since the nucleotide variations are synonymous the protein structure of gp15/400 of *W. bancrofti* isolates from different Indian regions is highly conserved and this also is in agreement with that reported for *B. malayi* and *B. pahangi* (Paxton et al., 1993). In cDNA sequences of these parasites Tweedie et al. (1993) also found that the two complete copies of these repeated segments were identical. The present study has shown that the conformity of gp15/400 sub-unit and hence the allergic peptide, is complete within *W. bancrofti* populations of wide geographic areas of India. Antigenic conformity is advantageous in that defined preparations of such immunogens may well have a broad spectrum of efficacy against different strains and species of filariae. Such cross-protection has already been observed

following immunization with irradiated infective larvae (Storey and Al-Mukhtar, 1982). Immunization of mice with ABA-1 conferred about 50% protection against challenge with *Ascaris* species (McGibbon and Lee, 1990) and hence it will be interesting to assess the immunogenicity of gp15/400 of *W. bancrofti* in experimental models of filariasis and in putatively immune individuals in filariasis endemic areas. Also, there is a report of variation in the clinical spectrum of lymphatic filariasis between northern and southern Indian regions (Rao et al., 1977), which was contended by Srividya et al. (1991) as due to sampling error. Lack of polymorphism of gp15/400 protein between isolates from across the country also indicates lack of support for such geographic variation in clinical spectrum. Heterogeneity in response to DEC among micro-filaria carriers is well known (Eberhard et al., 1991; Sankari et al., 2006). Since this protein is involved in the transport of Arachidonic acid and metabolites through which the antifilarial drug is known to act, it is possible that its polymorphism protein may affect the action of drug. However, the absence of polymorphism of gp15/400 of *W. bancrofti* shows that the heterogeneity in response to drug is not at least due to the polymorphism of this protein but may be due to that of other proteins of the parasite or the host.

The alignment of NPA sequences of different nematodes exhibited great variation, with a few to several transitions, transversions and indels. Kennedy (2000) has hypothesized that the NPAs might have evolved through duplications occurred in independent lineages or successive deletions or additional gene conversion events, without affecting function. Diversity of nucleotide sequence of sub-units of NPA's appears to have a relation with their life style and ancestral condition of the progenitor of the species (Kennedy, 2000). Free living species such as *Coenorhabditis elegans*, have units which are diverged while parasitic forms have very similar or even identical units. The diverse units appear to represent older amplifications while more similar units indicate recent amplifications. Diversity of units of the gene may provide alternative strategies for the nematode to negotiate with the changing environments, such as in the case of free living ones and opportunistic parasites that infect more than one host. If this is the case the highly conserved nature of units within a gp15/400 gene and between genes of several geographically distant isolate of *W. bancrofti* correlates well with highly obligate parasitic and the most recent evolutionary nature of this parasite (Fig. 2). Interestingly, the information on the nucleotide changes at various positions of the repeat sub-unit of the gene of different nematode species, even between lymphatic filarial parasites, and multicopy nature has significant value in terms of diagnostic development, such as development of simple PCR assays for differentiating parasite species. The evolutionary pattern of the gene (Fig. 4) also suggests that it is in parallel with location/site of infection of parasites. The soil dwelling nematodes, such as strongylids and rhabditids (including free living *C. elegans*), appear to have anteceded the nematode as that inhabited abdomen and the organs therein, which in turn gave rise to parasites (ascarids, and early spirurids) which found place in further deep seated organs such as heart and eyes. Human filarids represent

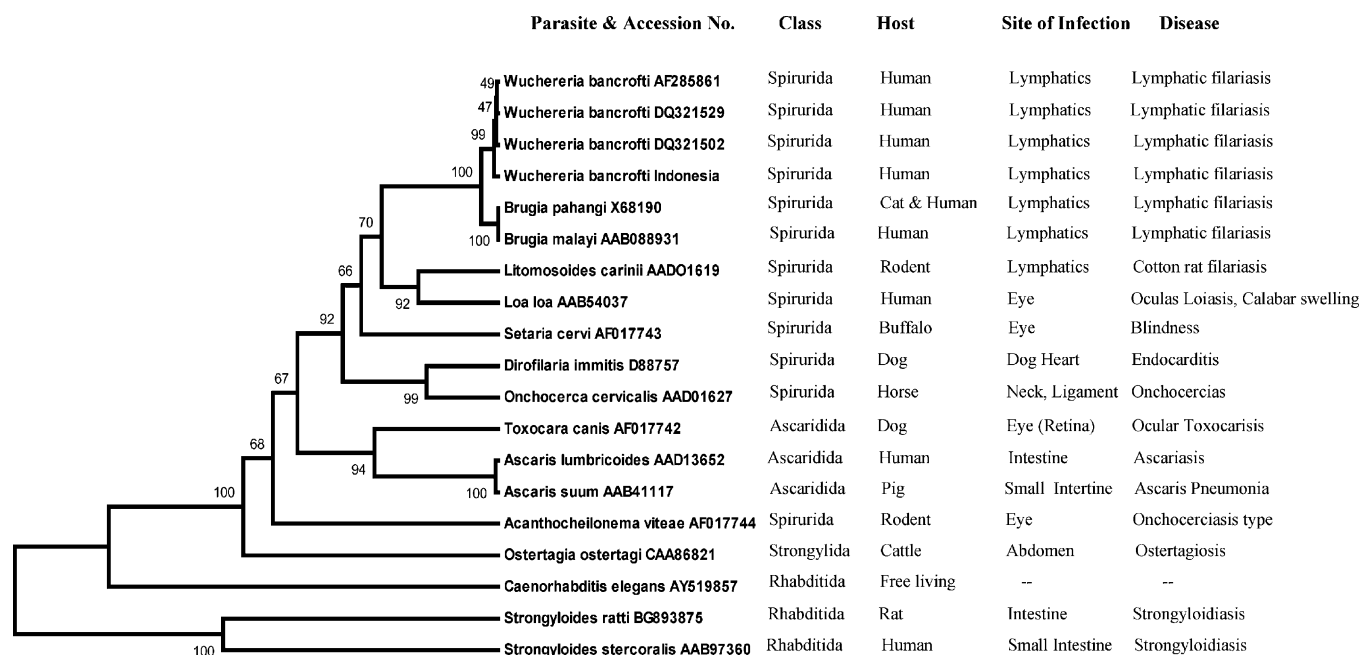


Fig. 4. Dendrogram of nucleotide sequences of NPAs from different parasitic nematodes and free living *Coenorhabditis elegans*. It was constructed using MEGA 3.1 programme and with 1000 replicates and employing unweighted pair-group method with arithmetic mean (UPGMA).

probably the highest order of parasitic strategy and appear to have evolved to dwell in a place free from immune challenge of the host, viz., the lymphatics.

The phylogenetic tree (Fig. 4) constructed using nucleotide sequences of NPAs of other nematodes, along with that of filarial nematodes exhibited two clades, with one branching earlier and consisting of *Strongyloides* species and the other consisting of rest of the nematodes falling under in two sub-branches. One of the sub-branches had lone free living member, *C. elegans* and the other with all other parasitic nematodes. Within the latter branch, lymphatic filarial parasites formed a distinct and most recent clade, while animal filarids formed early branching clades. Interestingly, within lymphatic filarial parasites the zoonotic *B. pahangi* and *B. malayi* formed a group distinct from strict human parasite, *W. bancrofti*. Human eye dwelling parasite *Loa loa* and intestinal parasite *Ascaris lumbricoides* grouped along with the animal filarids. The second major clade comprised parasites that infect rodents, canines, cattle, equines as well as humans. Generally, latest branches showed tendency towards parasitizing humans. Further, it appears that there is a similarity in the trend of evolution of polyprotein genes and small sub-unit rDNA genes of parasitic nematodes. Blaxter (1998) have proposed a molecular evolutionary frame for the phylum nematode based on the comparison of SSU rDNA nucleotide sequences. The Phylogenetic tree constructed using the nucleotide sequence of polyprotein gene of mammalian parasitic nematodes analyzed in the present study also depicts similar picture, with few exceptions. The filarial parasites and ascarids formed sister clades, also similar to that observed with SSU sequence analysis. This indicates that the two genes are evolving more or less in a synchronous manner, possibly because both are repeats and house keeping genes.

In any case the conserved nature of gp15/400 protein of *W. bancrofti*, its function as a lipid carrier, property of binding to molecules involved in the action of antifilarial drug, DEC and involvement in the elicitation of immune reaction (Th2 type) make it a good target for chemo/immunotherapy development. Currently, work is in progress in our laboratory to express this protein and explore it as a drug/vaccine target.

Acknowledgements

This investigation received funding from the Task Force on Lymphatic Filariasis, Indian Council of Medical Research, New Delhi. The authors are thankful to Mr. V. Ramu and Mr. R. Balasubramaniam, Laboratory technicians for their assistance in the study.

References

- Allen, J.E., Lawrence, R.A., Maizels, R.M., 1995. Fine specificity of the genetically controlled immune response to native and recombinant gp15/400 (polyprotein allergen) of *Brugia malayi*. *Infect. Immun.* 63, 2892–2898.
- Barrett, J., Saghir, N., Timanova, A., Clarke, K., Brophy, P.M., 1997. Characterisation and properties of an intracellular lipid-binding protein from the tapeworm *Moniezia expansa*. *Eur. J. Biochem.* 250, 269–275.
- Blaxter, M.L., 1998. *Caenorhabditis elegans* is a nematode. *Science* 282, 2041–2046.
- Chandrasekar, R., Rao, U.R., Rajasekhariah, G.R., Subrahmanyam, D., 1984. Isolation of microfilariae from blood on iso-osmotic gradients. *Indian J. Med. Res.* 79, 497–501.
- Christie, J.F., et al., 1992. Comparison between the MHC restricted antibody repertoires to *Ascaris* antigens elicited by adjuvant-assisted immunization or infection. *Parasite Immunol.* 14, 59–73.
- de Graaf, D.C., Peelman, L.J., Claerebout, E., Hilderson, H., Schallig, H.D., Vercruysse, J., 1995. Cloning and sequencing of an excretory/secretory antigen from *Ostertagia ostertagi* fourth-stage larvae containing multiple tandem repeats. *Mol. Biochem. Parasitol.* 72, 239–241.

- Dennis, D.T., Kean, B.H., 1971. Isolation of microfilariae: report of a new method. *J. Parasitol.* 57, 1146–1147.
- Eberhard, M.L., Lammie, P.J., Dickinson, C.M., Roberts, J.M., 1991. Evidence of nonsusceptibility to diethylcarbamazine in *Wuchereria bancrofti*. *J. Infect. Dis.* 163, 1157–1160.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.* 41, 95–98.
- Hoti, S.L., Subramaniam, K., Das, P.K., 2003. Detection of codon for amino acid 200 in isotype 1 beta-tubulin gene of *Wuchereria bancrofti* isolates, implicated in resistance to benzimidazoles in other nematodes. *Acta Trop.* 88, 77–81.
- Hussain, R., Poindexter, R.W., Ottesen, E.A., 1992. Control of allergic reactivity in human filariasis. Predominant localization of blocking antibody to the IgG4 subclass. *J. Immunol.* 148, 2731–2737.
- Kennedy, M.W., 2000. The polyprotein lipid binding proteins of nematodes. *Biochim. Biophys. Acta* 1476, 149–164.
- Kennedy, M.W., Qureshi, F., 1986. Stage-specific secreted antigens of the parasitic larval stages of the nematode *Ascaris*. *Immunology* 58, 515–522.
- Kennedy, M.W., Tomlinson, L.A., Fraser, E.M., Christie, J.F., 1990. The specificity of the antibody response to internal antigens of *Ascaris*: heterogeneity in infected humans, and MHC (H-2) control of the repertoire in mice. *Clin. Exp. Immunol.* 80, 219–224.
- King, L.M., Ma, J., Srettabunjong, S., Graves, J., Bradbury, J.A., Li, L., Spicker, M., Liao, J.L., Mohrenweiser, H., Darryl, C.Z., 2002. Cloning of *CYP2J2* gene and identification of functional polymorphisms. *Mol. Pharmacol.* 61, 840–852.
- Kumar, S., Tamura, K., Nei, M., 2004. MEGA3, Integrated software for Molecular Evolutionary Genetics Analysis and sequence alignment. *Brief. Bioinform.* 5, 150–163.
- Maizels, R.M., Denham, D.A., 1992. Diethylcarbamazine (DEC): immunopharmacological interactions of an anti-filarial drug. *Parasitology* 105 (Suppl), S49–S60.
- McGibbon, A.M., Christie, J.F., Kennedy, M.W., Lee, T.D., 1990. Identification of the major *Ascaris* allergen and its purification to homogeneity by high performance liquid chromatography. *Mol. Biochem. Parasitol.* 39, 163–172.
- McGibbon, A.M., Lee, T.D.G., 1990. The major *Ascaris* allergen. *Bull. Soc. Fr. Parasitol.* 8, 492.
- Paxton, W.A., Yazdanbakhsh, M., Kurniawan, A., Partono, F., Maizels, R.M., Selkirk, M.E., 1993. Primary structure of and immunoglobulin E response to the repeat subunit of gp15/400 from human lymphatic filarial parasites. *Infect. Immun.* 61, 2827–2833.
- Rao, C.K., Sen, T., Narsimham, M.V.V.L., KrishnaRao, C.H., Sharma, S.P., 1977. Variation in clinical pattern of bancroftian filariasis in Kerala and Uttar Pradesh. *J. Com. Dis.* 9, 203–205.
- Sabesan, S., Palaniyandi, M., Das, P.K., Michael, E., 2000. Mapping of lymphatic filariasis in India. *Ann. Trop. Med. Parasitol.* 94, 591–606.
- Sankari, T., Subramanian S., Hoti, S.L., Das, P.K., 2006. Heterogeneous response of *Wuchereria bancrofti* infected persons to diethylcarbamazine citrate and its implications for GPELF Filaria J., communicated.
- Spence, H.J., Moore, J., Brass, A., Kennedy, M.W., 1993. A cDNA encoding repeating units of the ABA-1 allergen of *Ascaris*. *Mol. Biochem. Parasitol.* 57, 339–343.
- Srividya, A., Pani, S.P., Rajagopalan, P.K., Bundy, D.A., Grenfell, B.T., 1991. The dynamics of infection and disease in bancroftian filariasis. *Trans. R. Soc. Trop. Med. Hyg.* 85, 255–259.
- Storey, D.M., Al-Mukhtar, A.S., 1982. Vaccination of jirds, *Meriones unguiculatus*, against *Brugia malayi* and *B. pahangi* using irradiated larvae of *L. carni*. *Tropenmed. Parasitol.* 33, 23–24.
- Tomlinson, L.A., Christie, J.F., Fraser, E.M., McLaughlin, D., McIntosh, A.E., Kennedy, M.W., 1989. MHC restriction of the antibody repertoire to secretory antigens, and a major allergen, of the nematode parasite *Ascaris*. *J. Immunol.* 143, 2349–2356.
- Tweedie, S., Paxton, W.A., Ingram, L., Maizels, R.M., McReynolds, L.A., Selkirk, M.E., 1993. *Brugia pahangi* and *Brugia malayi*: a surface-associated glycoprotein (gp15/400) is composed of multiple tandemly repeated units and processed from a 400 kDa precursor. *Exp. Parasitol.* 76, 156–164.
- Vercauteren, I., Geldhof, P., Vercruysse, J., Peelaers, I., Van Den Broeck, W., Gevaert, K., Claerebout, E., 2004. Vaccination with an *Ostertagia ostertagi* polypeptide allergen protects calves against homologous challenge infection. *Infect. Immun.* 72, 2995–3001.