CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION

Lymphatic filariasis (LF), a mosquito vector-borne disease is a major public health problem in many parts of the tropics affecting 120 million people majorly in developing regions of India, Southeast Asia and sub-Saharan Africa. *W. bancrofti* is the most common causative agent and accounts for about 90% of cases while *B. malayi* accounts for 10% of cases and is confined to East and Southeast Asia. *B. timori* is found only in Timor and nearby islands. LF is caused by three lymphatic dwelling nematode parasites *viz.*, *Wucheria bancrofti, Brugia malayi* and *Brugia timori*. The disease manifests as progressive lymphoedema leading to disfiguring elephantiasis in both genders and hydrocele, the predominant manifestation in males. In some cases, loss of respiratory function due to tropical pulmonary eosinophilia (TPE) is also observed. Filariasis patients suffer from episodic adenolymphangitis, which causes acute suffering and incapacitation. Other health problems due to filariasis include renal disease, arthritis, endomyocardial fibrosis (Pani et al 1995). In India, major population is affected by bancroftian infection and the single largest tract of brugian infection lies along the west coast of Kerala.

WHO initiated the ‘Global Program to Eliminate Lymphatic Filariasis’ (GPELF) by the year 2020 and it has been successfully implemented in China, Malaysia, Korea and in certain islands of the Pacific
(Ottesen 2000, Burkot et al 2002, Molyneux and Zagaria 2002). GPELF mainly focuses on mass drug administration (MDA) using either diethylcarbamazine (DEC) (Gelbrand et al 1994) or ivermectin (Eberhard 1997, Molyneux et al 2003) in single- or two-dose regime combined with albendazole once a year to interrupt transmission of LF (Molyneux et al 2001, Gyapong et al 2005) and morbidity alleviation. Management of acute and chronic filariasis cases requires treatment of ADL with antibiotics since majority of acute episodes appear to be of bacterial aetiology. Rigorous local hygiene measures like washing of legs with or without local antibiotic and antifungal agents to reduce the severity of ADL. The current anti-filarial drugs are micro-filaricidal drugs which cannot clear the adult worms and there is a need for more macro-filaricidal drugs. The effective control of filariasis lies in the early diagnosis, treatment of the infected individuals, particularly the microfilaraemics, and effective follow-up of drug administration.

The conventional methods of diagnosis of lymphatic filariasis are the microscopic examination of microfilariae (mf) by Giemsa-stained night blood smears, membrane filtration techniques (Schultz 1988, Schuurkamp et al 1990, Sabry 1992). The nocturnal periodicity of mf areas requires nighttime blood collection and low numbers of mf are sequestered in inaccessible sites or completely absent as in pre-patent cases, making these methods ineffective in diagnosis. Recently, immunological and PCR methods have been developed to detect/ differentiate these parasites for individual cases or for epidemiological studies. The circulating filaria-specific antibodies are largely explored to develop antibody-based diagnostic methods that conversely detect mf. Recombinant antigen-based rapid IgG4 antibody ELISA (Rahmah et al 2001) and dipstick test (Rahmah et al 2003) have been developed for the detection of antibodies in sera of patients with brugian infection. Currently, the MAb-based Og4C3 assay and the ICT card test have
been used widely for the early diagnosis of bancroftian filariasis. Although Og4C3 assay is the most sensitive in detecting CFA levels in bancroftian filariasis, it cannot be used for the detection of active filarial infection in brugian filariasis. On the other hand, the ICT card test is only a qualitative test and is found to be specific for bancroftian filariasis (Weil et al 1997).

As no vaccine is available, and vector control does not result in a sustained effect apart from in a small number of locations, the elimination of lymphatic filariasis relies on mass drug administration (MDA) using the three current drugs (Gyapong et al 2005). The eradication of lymphatic filariasis from much of the People’s Republic of China using DEC alone has demonstrated the feasibility of this approach. There is always a threat imposed by the development of drug resistance alleles in the parasite population (Sangster 1996, Prichard 2001). Drug resistance in parasites is observed in the spread of resistance to antimalarial drugs including chloroquine and pyrimethamine- sulfadoxine and in livestock by widespread anthelmintic resistance of parasitic nematodes (Wolstenholme et al 2004). The issue of resistance to anthelmintics used in humans has assumed increasing importance as the global program to eliminate lymphatic filariasis is implemented in larger population groups and the duration of the program increases. Factors that have been considered to lessen the impact against the development of drug resistance in lymphatic filariasis include the combined use of drugs with different modes of action and the long and complex lifecycle of filariae. Our knowledge about the genetics of drug resistance in helminths has a lot of gaps and moreover, the critical question of whether helminth parasites display any form of antigenic variation has not been systematically addressed despite the fundamental implications this would have on the whole of parasite immunology.
The development of vaccine candidate antigens or new drug targets cannot proceed far without knowledge of the distribution and conservation of antigen genes among parasite populations worldwide. Conceptually, it is necessary to understand the population genetic structure of helminth parasites to predict how such resistance alleles may spread in natural populations. This requires more data on allele frequency, interbreeding and molecular variation among all important helminth species (Nadler 1995, Anderson et al 1998). In addition, polymorphism in genes provides a spotlight on proteins with interesting functions in the host-parasite relationship, variation on a single locus in isogenic organism marks a conclusive link between gene and function (Maizel and Atmadja 2002). Our present polymorphic study on filarial parasite, *W. bancrofti* has aimed at one of these aspects. Therefore three candidate genes for studying polymorphism viz., abundant larval transcript-2, glutathione-s-transferase, thioredoxin are carefully chosen which are of functional importance for filarial parasite survival and for the establishment of immunogenicity in the host. Abundant larval transcript-2 (*alt*-2), an essential gene for parasite immune evasion in the infective stage, contribute >1% of L3 cDNA and are highly specific for filarial species, focuses it as a good vaccine candidate. Glutathione-s-transferase, one of the major enzymes involved in the detoxification system as the helminthes lack cytochrome P-450. The significant difference between the tertiary structure of the helminth GSTs and that of the host enzymes make the GSTs promising chemotherapeutic targets (Nathan et al 2005). Thioredoxins are a family of small redox proteins that are very much essential for the survival of the parasites. Therefore studies were done as an attempt to demonstrate polymorphism of these three *W. bancrofti* genes and characterizing the variant alleles to study their functional impact.
1.1.1 Overview of the Thesis

LF is an important cause of physical and social disability that affects over 100 million people in 83 countries in the developing world. The feasibility to eliminate LF is postulated to be mainly on diagnosis to identify endemic areas followed by repeated cycles of mass drug administration (MDA) to reduce both infection prevalence and transmission rates to levels below those required for sustained transmission. Treatment for LF depends almost exclusively on drugs: DEC, ivermectin and albendazole. Vaccines and new drugs are needed, certainly because drug resistance in human helminth parasites would present a major problem for current treatment and control strategies. Without the knowledge of parasite population genetic structure, it’s hard to establish new drugs or vaccines and it is also necessary to look for the existence and distribution of drug resistant alleles in the population. Therefore the present study focuses mainly on the distribution and conservation of new emerging drug targets and vaccine candidates. For polymorphic studies, two antioxidant genes, Glutathione-s-transferase (GST) and thioredoxin (trx) which are very crucial for the parasite survival by overcoming the oxidative stress environment of the host and abundant larval transcript-2 (alt-2), a promising vaccine candidate were investigated.

Indian parasite population was chosen for the polymorphic studies. Samples from four different geographical region viz., Tamil Nadu, Puducherry, Maharashtra and Bhubaneswar, were collected as microfilaria positive (mf+ve) blood samples or blood smear stained mf+ve microscopic slides. Mf was isolated and genomic DNA was extracted from it. The selected genes were amplified using gene specific primers and the PCR products were cloned directly into sequencing vector to avoid missing and mixing of alleles. The sequence was analyzed for variation using BLAST tool. Depending on the presence or absence of variation and new findings
from the genetic structure, the genes are characterized further. Interestingly, 
*Wb-alt*-2 had tandem repeats in intron-2 similar to *Bm-alt*-2 but tandem 
repeats that was already reported in intron-3 of *Bm-alt*-2 was completely 
absent in *Wb-alt*-2. This evolutionarily modified tandem repeat was exploited 
in developing genetic marker that differentiated bancroftian infection from 
brugian.

Compared to *alt*-2, which was conserved in the exonic region, 
*Wb-GST* was found to be highly polymorphic in natural population. Three 
variant alleles designated as *Wb-GST2a, Wb-GST2b, Wb-GST2c* were found 
in the parasite population in India. These alleles were cloned and expressed in 
prokaryotic expression system and the purified recombinant protein was 
further characterized. Enzyme kinetics of the three variant *Wb-GST* proteins 
was compared with the wild-type by its interaction with different substrates 
and inhibitors. *Wb-trx* was found to be conserved in the parasite population 
and proves to be very good drug target. The polymorphic studies has 
provided spotlights for the better understanding of the parasite genes 
importance in relation with the parasite survival, the genetic distribution in 
natural population and a support for the evaluation of new drug targets and 
vaccine candidates. The following are the objectives of the present study.

1.1.2 **Objectives**

i) Genetic polymorphic studies of *Wb-alt*-2, *Wb-trx* and *Wb-
*GST* gene of *W.bancrofti* from different Indian geographical 
areas.

ii) Characterization of sequence variation of *Wb-alt*-2 gene for 
the development of genetic markers for differentiation of 
lymphatic filarial species in co-infection.
iii) Cloning, expression and purification of \(Wb\)-GST and its variants (\(Wb\)-GST2a, \(Wb\)-GST2b, \(Wb\)-GST2c) in prokaryotic expression host.

iv) Kinetic studies of \(Wb\)-GST and its variants (\(Wb\)-GST2a, \(Wb\)-GST2b, \(Wb\)-GST2c) with different substrates and inhibitors.

v) Gene regulation studies of \(Wb\)-alt-2 intron-2 repeat region in eukaryotic cell lines.

1.2 REVIEW OF LITERATURE

1.2.1 Lymphatic Filarial parasites and its transmitting vectors

Humans and animals are infected by tissue dwelling nematode parasites called “Filariae” which are slender, elongated, thread-like, unsegmented, white worms. \(Wuchereria\) and \(Brugia\) are mainly responsible for human lymphatic filariasis in the tropical countries. \(W. bancrofti\) belongs to Kingdom – Animalia, Phylum – Nematoda, Class – Secernentea, Order – Spirurida, Sub-Order – Spirurina, Family – Filarioidea and Genus – \(Wuchereria\). There are two species of \(Wuchereria\) and there are nine species of \(Brugia\). The \(B. malayi\) and \(W. bancrofti\) adult worms reside in the lymphatics and cause brugian and bancroftian filariasis, respectively.

Mosquitoes are the important vectors in filariasis and lymphatic filarial parasites are transmitted by many mosquito species. The principal mosquito species that transmit the lymphatic filariae of humans are found in the genus \(Anopheles\) (\(W.bancrofti, B.malayi, B.timori\)), \(Aedes\) (\(W.bancrofti, B.malayi\)), \(Culex\) (\(W.bancrofti\) and \(Mansonia\) (\(W.bancrofti, B.malayi\)). \(C. quinquefasciatus\) is the principal vector of bancroftian filariasis throughout the mainland of India. Anopheles vectors are important in the transmission of periodic \(W. bancrofti\) in Africa, Southern Asia and the island of New Guinea.
It is also a significant vector of periodic brugian infection in Southern Asia. New transmission and distribution records include *A. gambiae* from the island of Grande Comore and *A. flavirostris* from Sabah, while *A. philippinensis* has very limited distribution in the northeastern part of India. Transmission by *Aedes* is limited only to Andaman and Nicobar islands of India. *Mansonioides* include the important vectors *M. annulifera*, *M. uniformis* and *M. indiana* of *B. malayi* infection in Southern and south-eastern Asia. *Mansonia* vectors are mainly from India, Indonesia, Malaysia and Thailand.

![Global map showing distribution of lymphatic filarial parasite](image)

**Figure 1.1 Global map showing distribution of lymphatic filarial parasite**

### 1.2.2 Life Cycle of the Lymphatic Filarial Parasite

Lymphatic filariae have biphasic life cycle where larval development takes place in the mosquito (intermediate) host, and larval and adult development takes place in the human (definitive) host (Figure 1.2). These parasites have no free-living forms. The infection is initiated by the deposition of the L3 onto the skin of the human host by an infected mosquito during its blood meal. The larvae penetrate into the human host through the bite wound and undergo additional molt to the fourth larval stage (L4). They mature and develop into lymph-dwelling adult male and female worms.
Adults are dioecious and undergo ovoviviparous reproduction and produce first stage larvae (L1 or mf), which are sheathed and have nocturnal periodicity, except the South Pacific mf which have the absence of marked periodicity. The mf migrates into lymph and blood channels moving actively through lymph and blood. A mosquito ingests the mf during a blood meal. After ingestion, the mf lose their sheaths and some of them work their way through the wall of the proventriculus and cardiac portion of the mosquito's mid-gut and reach the thoracic muscles and moults to the second stage larvae (L2) and subsequently moults into L3 after few days. The L3s migrate through the haemocoel to the mosquito's proboscis and can infect another human when the mosquito takes a blood meal.

Figure 1.2 Life cycle of *W. bancrofti* parasite
1.2.3 Morphology of Filarial Parasites

Localization and isolation of *W. bancrofti* adult worms by ultrasonography has facilitated the ultra-structural studies of the surface by scanning electron microscopy (Araujo et al 1995).

![Figure 1.3 (a) *W. bancrofti* mf (b) *B. malayi* mf (c)*B. timori* mf](image)

**Figure 1.3** (a) *W. bancrofti* mf (b) *B. malayi* mf (c)*B. timori* mf

![Figure 1.4 The larval stages of *W. bancrofti*.](image)

**Figure 1.4** The larval stages of *W. bancrofti*.

Light microscopy of living first stage (microfilaria-Mf), third stage (L3), fourth stage (L4) and adult (Ad) of *W. bancrofti* (Ash et al 1971)
Adults are found primarily in lymphatic vessels, less commonly in blood vessels. Adult *W. bancrofti*, *B. malayi* and *B. timori* are minute, thread like and have a smooth cuticle. Adult males measure 40 mm in length and 0.1 mm in diameter, whereas females measure 80–100 mm in length and 0.24–0.3 mm in diameter (Nanduri and Kazura 1989). The mf of both *W. bancrofti* and *B. malayi* are sheathed and measure 244–296 μm and 177–230 μm in length, respectively (Figure 1.3). *B. malayi* mf has two terminal nuclei distinctly separated from each other and the last terminal nucleus is small and is present at the tip of the tail, whereas the body nuclei of *W. bancrofti* mf do not extend to the tip of the tail. The microfilariae of *Brugia timori* are longer and morphologically distinct from those of *Brugia malayi* and *Wuchereria bancrofti*, with a cephalic space length-to-width ratio of about 3:1. Also, the sheath of *B. timori* does not stain pink with Giemsa stain as is observed with *B. malayi* and *W. bancrofti*.

### 1.2.4 Clinical symptoms

The clinical symptoms of filariasis vary from one endemic area to the other and depend upon the species involved in the infection. In Africa, hydrocoele is common compared to lymphedema and elephantiasis. But in other countries such as India, hydrocoele and lymphedema are distributed in equal frequency. Tropical pulmonary eosinophilia is restricted to certain parts of the world such as South Asia, South East Asia and Brazil. The clinical groups are classified as below. **Asymptomatic microfilaraemics** (Endemic Normals, EN), “Endemic normal” is the term given to those individuals living in endemic region and who inspite of constant exposure to filariasis do not exhibit any clinical evidence of the disease. Immune responses to filarial antigens are higher in these individuals compared to other clinical groups (Ottesen et al 1977). The most common clinical manifestation observed among the endemic population is **asymptomatic microfilaraemia** (MF).
Individuals in this clinical group do not exhibit disease symptoms but harbour large number of circulating mf in the peripheral blood. Filarial antigen-specific hyporesponsiveness is the characteristic of these MF individuals (Mahananty et al 1997, Ravichandran et al 1997).

The most common acute manifestation of filariasis is identified by recurrent attacks of fever associated with inflammation in lymphatics termed as adenolymphangitis (ADL), which is characterized by intense lymphangitis. Individuals belonging to this chronic manifestations (CP) group are clinically affected and the manifestations usually develop after 10–15 years from the onset of the first acute attack. They suffer from either acute attacks of lymphatic inflammation such as lymphadenitis, lymphangitis or from chronic obstructive form of the disease characterized by elephantiasis of the legs, hydrocoele or chyluria and lymphuria. Tropical pulmonary eosinophilia (TPE) is the least common manifestation of filariasis and it is a form of occult filariasis. It is manifested by recurrent asthmatic attacks, transitory lung field mottling, low-grade fever and marked leukocytosis and eosinophilia. Other extralymphatic signs include chronic microscopic haematuria and proteinuria, and mild polyarthritis, all presumed to result from immune complex deposition.

1.2.5 Diagnosis of Lymphatic filariasis (LF)

The lymph dwelling filariae infects million of people who may suffer from clinical manifestations such as elephantiasis, hydrocoele and ADL or may have clinically silent manifestations of infection associated with abnormalities in the lymphatic system. The infection goes unnoticed for years in some cases until they develop clinical signs of the disease. It is therefore essential to develop diagnostics that can detect the infection earlier. MDA programme under GPELF is in progress to minimize the rate of LF transmission and it lies on community diagnosis and programs which will
help in deriving appropriate chemotherapy measures. Also there is a need to improve the available diagnostic tests and surveillance tools, especially for lymphatic filariasis (Weil and Ramzy 2007).

1.2.5.1 Parasitological diagnosis by microscopy method

Traditionally, diagnosis of lymphatic filariasis depended on the detection of mf by microscopic examination of the blood. It is the simplest and conventional technique to detect the presence of the parasite by conventional night blood smear stained with Giemsa stain (Schultz 1988, Schuurkamp et al 1990, Sabry 1992). Though it had implication in isolating mf from large volume samples, it cannot be applied in the field as it is a time-consuming method and requires large volumes of blood for examination.

1.2.5.2 General imaging techniques for detection of filariasis

Lymphoscintigraphy is a special type of nuclear medicine imaging that provides pictures called scintigrams, by which the lymphatic abnormalities in both asymptomatic MF and clinical cases can be detected (Freedman et al 1994). Although it is a safe and non-invasive technique, it is costly and not suitable for field evaluations. Another method called lymphangiography is used to study alterations in lymphatic function of filarial patients (Sen and Ellappan 1968). Ultrasound is a non-invasive, highly sensitive and widely available tool to identify adult worms in parasitized lymph vessels. It is laborious, impracticable for mass screening, time consuming, invasive and uses oil-based contrast material for imaging (Miller et al 1990), which can induce local morbidity and aggravate the secondary infections.
1.2.5.3 Detecting lymphatic filariasis using microwaves

The microwave method for detecting LF is based upon the changes in the dielectric properties. This measurement technique is simple and suggests a novel, alternative, *in vitro* method of diagnosing LF using microwaves and is completely independent of time. Although simple, it needs sophisticated laboratories to perform the tests and skill to interpret the results. Moreover, it cannot differentiate between other parasitic co-infections and filariasis. Although it cannot be used in the field, it is encouraging to see the advancement in diagnosing LF.

1.2.5.4 PCR-based diagnosis of filariasis

DNA sequences allow the identification of even single adult worm or mf in the definitive host or a developing larva in the intermediate host. Polymerase chain reaction is a powerful tool in the study of parasitic infections. Species-specific DNA probes have been developed for *B. malayi, W. bancrofti, O. volvulus*, and *Loa loa* (Nutman et al 1994). Such DNA-based diagnostic methods have been recently developed for detecting parasitic DNA in human blood samples and in vector population. The genome-based survey was helpful in differentiating the species of infection. *B. malayi Hha I* DNA repeat sequence was used to develop PCR assays for filariasis (Lizotte et al 1994). A rapid PCR based on *W. bancrofti* 185-bp repeat sequence *Ssp1* was developed and standardized for the detection of *W. bancrofti* in mosquitoes (Williams et al 1996, Hoti et al 2001). Earlier, this genus-specific repeat sequence from *W. bancrofti* was shown to detect L3 in mosquitoes (Zhong et al 1996). Another 969-bp repeat sequence *pWb12* that could detect *W. bancrofti* DNA in human blood samples, hydrocoele fluid and in mosquito vector was developed by Siridewa et al (1996).
There are other modern approaches such as multiplex-PCR where primers specific for brugian and bancroftian species are used together to detect both *W. bancrofti* and *B. malayi* in single step (Mishra et al 2005). The primers designed were novel and have been tested with the parasite DNA. In a single reaction, they amplify a 188-bp fragment specific for *B. malayi* and ~129-bp fragment specific for *W. bancrofti*. The test is highly sensitive for both *B. malayi* and *W. bancrofti* as it detects parasitaemia up to the level of one mf per reaction. PCR is found to be more efficient in comparison to microscopy, as it can detect 8% and 5% more filarial parasites in field-collected blood and mosquito samples, respectively. Thus, several of these DNA-based assays are promising for the diagnosis of filarial infection and are currently in the research phase, and its large-scale field applicability in the endemic areas will require further validation.

1.2.5.5 Immunodiagnosis of Lymphatic filariasis

Excretory–secretory (ES) products are molecules that are released by the living parasites that can also be used in diagnostics since circulating antibodies will appear early during infections. Immunological cross-reactivity was the main drawback for developing diagnostics using crude or ES antigens. Recombinant proteins were used in developing more specific diagnostic tools. Brugia Rapid (BR) test, an immunochromatographic dipstick test, is used to detect circulating IgG₄ antibodies and appears to be a promising tool in GPELF program (Fischer 2007). Recently pan LF-ELISA developed using BmR1 and BmSXP antigens as single or a mixture showed 98% sensitivity (Rohana et al 2007). Likewise, two new rapid immunochromatographic IgG₄ cassette tests have been produced, namely, WB rapid and pan LF rapid, using the recombinant antigens BmSXP and BmR1 for detection of bancroftian filariasis and all three species of lymphatic filarial parasites. Both the IgG₄ rapid tests are highly sensitive and specific,
and would be useful additional tests to facilitate the global drive to eliminate this disease (Noordin et al 2007).

Antigen detection assays reveal the current status of the infection which holds an important implication of MDA. Commercially available Og4C3 and ICT card assays use Mabs directed against *O. gibsoni* adult antigen (More and Copeman 1990) and AD12 of *Dirofilaria immitis*, respectively. They can detect bancroftian filarial infections with high sensitivity and specificity. Both these tests are based on Mabs developed against heterologous antigens. The ICT card test is positive in early stages of the disease when the adult worms are alive and becomes negative once they are dead (Weil et al 1997). Both the tests are currently used in the GPELF programme. Currently mabs for WbSXP-1 are raised in our centre and the process of developing antigen detection kit is in the progress.

### 1.2.6 Chemotherapy for lymphatic filariasis

The elimination of lymphatic filariasis relies on mass drug administration (MDA) using the three drugs currently available for treatment: diethylcarbamazine (DEC), albendazole, and ivermectin (Gyapong et al 2005). The eradication of lymphatic filariasis from much of the People’s Republic of China using DEC alone has demonstrated the feasibility of this approach. The GPELF, launched in 2000, involves community-wide annual treatment with the broad-spectrum ABZ in combination with IVM or DEC. The combination chosen depends on whether or not human onchocerciasis is co-endemic (Zagaria and Savioli 2002). DEC is the oldest of the three drugs used for control of lymphatic filariasis. It is the antifilarial drug with the best activity in single dose against the lymphatic-dwelling adult worm and microfilariae, the life-cycle stage residing in the blood that is taken up by the mosquito vector. DEC 6 mg/kg annual treatment showed reduction in mf prevalence by 74.9% in the annual treatments and 90% in the biannual
treatments (Panicker et al 1991). DEC was not found to be 100% effective against adult worms, with clinical and ultrasound studies demonstrating a variable response, and even refractory infection (Eberhard et al 1991, Dreyer et al 1999).

The activity of ivermectin is largely confined to immature stages of the parasite, including embryos in the uterus of the adult female and microfilariae. Albendazole, a member of the benzimidazole (BZ) class of drugs, is one of the most important anthelmintics in human medicine. It possesses clinically important activity against a wide range of nematode and cestode parasites and even some protozoa such as Giardia sp. Although single-dose albendazole has little effect on microfilariae, when used as part of a combined single-dose regimen with either ivermectin or DEC, synergistic activity is observed, presumably by an effect on adult worm viability. Benzimidazoles and avermectins that have been used extensively in veterinary medicine for over 2 decades led to the development of drug resistance to both types of compounds in many helminth parasites affecting livestock (Prichard et al 1980, Prichard 1990, Wolstenholme et al 2004).

1.2.7 Genetic diversity in filarial parasites

The comprehension of host-parasite interactions represents a major challenge in evolutionary biology. Parasites are responsible for substantial deleterious effects on their hosts, and, therefore, represent a major driving force for host evolution. In parallel, parasites have to cope with the evolving host defense mechanisms, i.e., they must co-evolve with their host to avoid elimination. This adaptation of host–parasite systems predicts that an arms race takes place in which both host and parasite develop mechanisms that generate diversity and polymorphism of molecules that play key roles in the host–parasite interplay (Ebert 2008). Polymorphism in the coding region has been reported in (Table 1.1). The level and pattern of genetic diversity was
reported in filarial parasites are emerging. So it is imperative to know more about the functional genetic diversity of the parasites to identify or to understand its adaptation or evolution in order to develop new diagnostic tools and to interrupt the transmission of the disease.

Table 1.1 Sequence polymorphism in coding genes of nematode parasites (Maizels and Atmadja 2002)

<table>
<thead>
<tr>
<th>Species</th>
<th>Gene</th>
<th>aa</th>
<th>nt</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ancylostoma caninum</em></td>
<td>ASP-1 (VAL-1)</td>
<td>10/424</td>
<td>42/1271</td>
<td>(Qiang et al. 2000)</td>
</tr>
<tr>
<td><em>Brugia malayi</em></td>
<td>ALT-2</td>
<td>0/128</td>
<td>137 nt in intron</td>
<td>(Gregory et al. 2000)</td>
</tr>
<tr>
<td></td>
<td>CPI-2</td>
<td>1/161</td>
<td>1/483</td>
<td>(Gregory and Maizels, unpublished)</td>
</tr>
<tr>
<td></td>
<td>SHP-1</td>
<td>6/205</td>
<td>12/615</td>
<td>Kurniawan-Atmadja et al unpublished</td>
</tr>
<tr>
<td></td>
<td>SHP-5</td>
<td>5/161</td>
<td>9/632</td>
<td>Kurniawan-Atmadja et al Unpublished</td>
</tr>
<tr>
<td></td>
<td>VAL-1</td>
<td>0/220</td>
<td>2/660</td>
<td>(Murray et al. 2001)</td>
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<tr>
<td><em>Haemonchus contortus</em></td>
<td>β-tubulin isotypes I and/or II</td>
<td>4/448</td>
<td>na</td>
<td>(Beech et al. 1994), (Kwa et al. 1994)</td>
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<td><em>Onchocerca Volvulus</em></td>
<td>PDI</td>
<td>0/281</td>
<td>1/843</td>
<td>(Keddie et al. 1999)</td>
</tr>
<tr>
<td></td>
<td>CAR</td>
<td>0/337</td>
<td>0/1011</td>
<td>(Keddie et al. 1999)</td>
</tr>
<tr>
<td></td>
<td>API</td>
<td>0/240</td>
<td>0/720</td>
<td>(Keddie et al. 1999)</td>
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<td>RAL2</td>
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<td>0/420</td>
<td>(Keddie et al. 1999)</td>
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<td></td>
<td>Actin-2</td>
<td>1/376</td>
<td>1/125</td>
<td>(Zeng and Donelson 1992)</td>
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<tr>
<td><em>Teladorsagia circumcincta</em></td>
<td>β-tubulin isotype I</td>
<td>1/371</td>
<td>na</td>
<td>(Elard et al. 1996)</td>
</tr>
</tbody>
</table>

*Ac*-ASP-1 *Ancylostoma* secreted protein-1, *Bm*-ALT-2 Abundant larval transcript-2, *Bm*-CPI-2 Cysteine protease inhibitor-2, *Bm*-SHP-1, SHP-5 Sheath protein-1, -5 of the microfilarial sheath, *Bm*-VAL-1 Vespid venom,
1.2.7.1 Antigenic diversity

To succeed in infection, parasites must have ways to reach the host, penetrate its tissues and escape its defense systems. As they are not necessarily fatal, most helminth parasites remain viable within their host for many years, exerting a strong influence over the host immune function. Proteins released from the parasites, conventionally named excretory/secretory products (ES) have been identified and characterized, particularly from *B. malayi* (Moreno and Geary 2008). There is a difference in the amount of protein expressed in every stage of the life cycle. Surface antigens are the key tools that the parasites utilize for immune evasion. An inverse relationship has been found between detectable antibody binding to the surface of mf and the presence of mf (McGreevy et al 1980). Antigenicity difference in surface antigens has been demonstrated previously (Ravindran et al 1994). The mf sheath is composed of a tightly cross-linked set of repeat-rich proteins, together with some carbohydrate structures (Hirzmann et al 1995). Microfilarial sheath proteins are found to be secreted in mf stages alone (Sasisekhar et al 2009) and antibody-staining studies showed antigenic variation. It is evident that these parasites are likely to be repositories of numerous novel 'immune evasion genes' with no or only weak sequence similarity to known products. Variations are essential for the establishment of the successful parasitic lifestyle and therefore the study of genetic variation may lead to the discovery of novel drug, diagnostic and vaccine targets. Moreover, this will help us to illuminate a path for developing a deeper understanding of how parasite proteins function in immune evasion (Moreno and Geary 2008).

1.2.7.2 Genetic diversity: Implications of drug resistance

Nucleotide diversity is directly related to the mutation rate and population size (Anderson et al 1998). Both of these factors are likely to
contribute to the levels of genetic diversity in parasitic nematodes. It is important to be aware of initial resistance allele frequencies, as these will affect the speed at which resistance spreads, and thus monitoring field situations with appropriate resistance markers. A phenylalanine-to-tyrosine substitution at position 200 on the β-tubulin isotype 1 molecule has been identified in a number of helminth parasites of farmed ruminants including *H. contortus* (Kwa et al 1993, 1994), *Cooperia oncophora* (Njue and Prichard 2004), and *Teladorsagia circumcincta* (Elard and Humbert 1999) and is associated with benzimidazole (BZ) resistance in these species. Recently, it has been shown that a mutation from phenyalanine to tyrosine at position 200 of the β-tubulin gene, causes benzimidazole resistance in veterinary parasites (Prichard 2007).

In *O. volvulus*, differences in allele frequencies between worms obtained from untreated and IVM-treated human populations for β-tubulin, P-glycoprotein and other ABC transporter genes showed the adaptations of the parasites to counteract the drugs (Eng and Prichard 2005, Ardelli and Prichard 2004). The resistance-associated mutation at codon 200 of β-tubulin gene was present in the populations of *W. bancrofti* sampled, especially those from Burkina Faso, at a reasonably high frequency in IVM-treated than in non-treated populations (Schwab et al 2005). Genetic differentiation is determined by inbreeding of the parasite populations. Infra population determines the facets of the species, homogeneity or heterogeneity. The drug-resistant alleles are found to be recessive (β -tubulin tyrosine genes) and have low initial frequency. Inbreeding leads to the selection of the resistant parasites that successfully survive in host. This accumulation of resistant parasite in the infra population will lead to the drug-resistant alleles across the parasite populations. Therefore, it is advisable to implement promptly the monitoring and evaluation of possible changes in the parasite genome, by means of appropriate molecular markers, and to link this to the monitoring of
responses to treatment in the host population. These actions may identify, before anthelminthic resistance becomes a major public health concern.

1.2.8 Methods for Analysis of Variations

1.2.8.1 Isozymes to identify phenotypic variations

Although isozymes are not DNA markers, earlier they have been used extensively to quantify polymorphism in nematode populations. Quantitative and qualitative changes in non-specific isozyme polymorphisms have been demonstrated in *H. contortus* for esterase activity associated with BZ resistance (Sutherland et al 1988). Similar studies of ivermectin-resistant and ivermectin-susceptible strains of *H. contortus* were found to have inter-strain differences (Echevarria et al 1992). Although these studies will not give any information about the genetic makeup of the parasites, they are convenient markers to demonstrate the phenotypic variations.

1.2.8.2 Mitochondrial DNA (mtDNA)

The mitochondrial DNA evolves independently and more rapidly than the nuclear genome (Anderson et al 1998). Mitochondrial DNA (mtDNA) is therefore useful for analyzing genetic variation within and among parasite populations. In a detailed study, Blouin et al (1995) used a PCR-based sequencing approach to analyze the population genetic structure of the parasitic trichostrongylid species from North America, utilizing a long non-coding region of mtDNA which showed high within-population diversities that appeared to be related to large population sizes and a relatively rapid rate of mutations.
1.2.8.3 Microsatellite markers

Minisatellites and microsatellites have been described as being both abundant and ubiquitous in the genomes of all eukaryotes. These sequences consist of tandem repeats of short motifs which are randomly dispersed throughout the genome. They are usually non-transcribed and maintain polymorphism as a consequence of the accumulation of mutations (Tautz 1989). Microsatellite repeat sequences are very informative markers to estimate the evidence of selection at drug-resistance locus and to analyze variation that had occurred in a locus (Nash et al 2005). They are used to measure population genetic parameters such as gene flow and genetic drift. Genetic polymorphism among the *B. malayi* strains was demonstrated using two microsatellite markers, *BMsat1* and *BMsat2* (Underwood et al 2000). They are reliable and sensitive for distinguishing closely related species or subspecies of filarial parasite, such as distinguishing human filarial parasite *B. malayi* from the animal parasite *B. pahangi* in regions where both species are endemic. Genomic DNA amplified from both species was restricted using *HhaI* restriction enzyme and they showed differences between repeat sequences of *B. malayi* and *B. pahangi* that distinguished the two species.

1.2.8.4 Restriction fragment length polymorphism (PCR-RFLP)

Identifying genetic polymorphism through DNA-based restriction fragment length polymorphism is another method to identify variations. PCR-RFLP is a method which detects minor variations in a gene where a single-base substitution has either created a site capable of being digested by a restriction endonuclease or abolished a restriction site. In PCR-RFLP, PCR is used to amplify a region of a gene which contains single- or multiple-base substitutions and the PCR product is subjected to digestion by one or more restriction endonucleases. These digested products are visualized in agarose
gel electrophoresis and the banding pattern is used to determine the particular allele type.

1.2.8.5 Amplified fragment length polymorphism (AFLP-PCR)

AFLP-PCR reaction-based markers for rapid screening of genetic diversity is relatively cheap, easy, fast and reliable method in generating informative genetic markers. It requires no prior knowledge of genome sequence and can be applied to any organism to estimate genetic diversity at species level (Vos et al. 1995). The main disadvantage in this method is the difficulty in identifying homologous markers (alleles), thereby not useful in assigning allelic states, such as heterozygosity.

1.2.8.6 Random amplified polymorphic DNA (RAPD)

Random amplified polymorphic DNA polymerase chain reaction (RAPD-PCR), has been proved useful for analyzing the inter- and intra-specific genetic variations and phylogenetic relationships. The RAPD technique is based on the amplification of a random DNA segment with a single primer of arbitrary nucleotide sequence (Welsh and McClelland 1990, Williams et al. 1990). The RAPD-PCR has been proven to be an easy, reproducible and rapid technique that could be used as a diagnostic tool, which even allows the differentiation of strains of the parasites. RAPD-PCR developed for *W. bancrofti* differentiated Thai and Myanmar strains, which differed in their periodicity and the morphological characters (Nuchprayoon et al. 2007).

1.2.9 Genome organization - filarial genome project

The *B. malayi* nuclear genome is organized as five chromosomes with XY sex chromosome (Sakaguchi et al. 1983). The recent rough draft of
Filarial genomic project revealed its genomic size to be about 90–95 Mb and has 14,500–17,800 protein-coding genes (Ghedin et al 2007). By conventional karyotyping it was found that B. malayi has 10 diploid chromosomes. Male worms contain eight small, one large and one medium element, whereas females contain eight small and two large elements (Sim et al 1987). B. malayi genome is complicated by two factors: an unusually large number of singleton reads (1, 76,099) (i.e., reads that are not incorporated into the assembly), and a wide variation in the depth of coverage by reads at different regions of the genome. Large number of singleton reads suggested a high A+T-rich regions which prevent their inclusion as a unique region of the genome and contains 30.5% G+C content (Ghedin et al 2007). Two major multiple-copy repeats Hhal and MboI named after the restriction enzymes that release monomer size fragments from their tandem arrays (McReynolds et al 1986, Natarajan et al 1990) were identified. The genome analysis still represents a key factor in identifying new targets for drug, vaccine and diagnostics development, as well as dissecting the biological basis of drug resistance, antigenic diversity, infectivity and pathology.

As a part of Brugia genome project, EST sequences from multiple cDNA libraries representing the complete filarial nematode lifecycle were generated and compared with complete genome sequence of the model nematode C. elegans. This is an effective way for gene discovery (Blaxter et al 1999). Data from expressed sequence tag (EST) analysis identified filarial proteins mimicking those of cytokines, chemokines and other immune effector/modulatory molecules of humans (Pastrana et al 1998). These ESTs have been predicted to or shown to promote parasite survival or development, and suggests that the parasites, by secreting or expressing these on surface exposed membranes, have adapted these gene products to promote successful parasitism (Sasisekar et al 2009). There are many representations of single gene or variants such as B. malayi allergen, Bm ALT-2, and cuticular
antigens. The presence of such variants shows their importance in the parasitism (Blaxter et al 2002). Thus a small genome survey of the ESTs can reveal the nature of the gene and their importance in the parasitic life cycle.

1.3 NEED FOR GENETIC POLYMORPHIC STUDIES OF FUNCTIONAL GENES

Ongoing MDA with various combinations of anthelminths creates an immense selective pressure on the parasite survival. Since there is no appropriate animal model, it is very difficult to demonstrate these changes in *W. bancrofti*. It is therefore important to develop tools, such as DNA markers, that can be used to follow gene selection as an indicator of resistance in the field. Gene polymorphism is also an evolutionary indicator of the parasite. ABC transporter genes help the parasite in transporting IVM. An analysis of *O. volvulus* ABC transporter gene (OvPLP) in IVM-treated and non-treated population, showed substitution of isoleucine to valine (Bourguinat et al 2008).

Apart from studying the drug resistant alleles, conceptually, we need to understand the population genetic structure of helminth parasites to predict how such resistance alleles may spread and reach fixation in natural populations. This requires more data on allele frequency, interbreeding and molecular variation among all important helminth species (Nadler 1995, Anderson, Blouin and Beech 1998). In addition, there is the argument that natural variation in genes provides a spotlight on proteins with interesting functions in the host-parasite relationship. Furthermore, comparisons between isogenic organisms differing at only one locus can provide a far more conclusive link between gene and function than any number of analyses of newly-identified sequences in a single isolate.
The development of vaccine candidate antigens cannot proceed far without knowledge of the distribution and conservation of antigen genes among parasite populations worldwide. Moreover, the critical question of whether helminth parasites display any form of antigenic variation has not been systematically addressed despite the fundamental implications this would have on the whole of parasite immunology. The present polymorphic study on filarial parasite, *W. bancrofti* has aimed at one of these aspects. Therefore three candidate genes for studying polymorphism viz., abundant larval transcript-2 (*alt*-2), glutathione-s-transferase (*GST*), thioredoxin (*trx*) are carefully chosen which are of functional importance for filarial parasite survival and for the establishment of immunogenicity in the host.

### 1.3.1 Abundant Larval Transcript

A most intriguing set of L3-associated antigens in filarial nematodes is the Abundant Larval Transcript (ALT) family (Gregory et al 2000, Maizels et al 2001), which represent 1-10% of cDNAs in various filarial species at the L3 stage, but are barely found at other points in the life cycle. The two genes encode proteins with 79% amino acid identity, but have no similarity to any gene of known function, and are characterized by a signal peptide, a variable acidic domain, and a conserved, cysteine-rich domain. ALT-1 and ALT-2 are homologous to an abundant immunogen from larvae of the dog heartworm *Dirofilaria immitis* (Di-20/22L) (Frank et al 1995) and to proteins from the additional filarial parasites *Onchocerca volvulus* (Ov-ALT-1) Joseph et al (1998) and *Acanthocheilonema viteae* (Pogonka et al 1999). The SLAP (secreted larval acidic protein) produced by *O. volvulus* larvae (Bianco et al 1990, Bianco et al 1995) has also been shown to be a member of the ALT family.
Figure 1.5  Sequences of *B. malayi* ALT-1 (U57547) and ALT-2 (U84723) compared to *W. bancrofti* ALT-2 (AF084553, Sabarinathan and Kaliraj 2000) and *C. elegans* cosmid CO8A9.

1.3.1.1  ALT as vaccine candidate

ALT are the front running candidates for vaccine antigens. Humans exposed to *Brugia malayi* showed a high frequency of immunoglobulin G1 (IgG1) and IgG3 antibodies to ALT-1 and -2, distinguishing them from adult-stage antigens, which are targeted by the IgG4 isotype. It has been reported that immunization of susceptible rodents (jirds) with ALT-1 elicited a 76% reduction in parasite survival, the highest reported for a single antigen from any filarial parasite (Gregory et al. 2000). ALT-2 has been demonstrated for protection studies using protein or DNA vaccination (Ramachandran et al. 2004). ALT-2 in combination with other recombinant filarial antigen such as thioredoxin peroxidase has been reported for higher protectivity (Anand et al. 2008).

1.3.1.2  *alt-2* gene structure

ALT family has been characterized by numerous related genes rather than variants at any one locus, and coding polymorphisms have yet to be identified. However, with TRS laboratory strain (TRS laboratories, Athens,
Ga. 30605), alt-2 was found to exhibit variant forms of intron 3. Intron 3 is itself unusual in consisting of a repeat sequence, and two forms of differing repeat numbers are found. Individual worms possess either or both these forms, consistent with the notion that they are Mendelian alleles (Gregory and Gomez-Escobar 2000). Gomez-Escobar et al (2002), demonstrated with transient transfection in *C. elegans* that the high level of alt transcription in filarial L3s is not due to expression from a multi-copy gene family but to a set of strong promoter elements shared between the two *alt* genes. A transgenic of *L. mexicana* expressing the ALT proteins is more virulent in macrophages *in vitro*, and this property is abolished by deletion of the filarial-specific acidic domain (Gomez-Escobar et al 2005). Gene structures of *B. malayi* alt-1 and alt-2, as described by Gregory et al (2000). Alleles shown are both represented in the TRS laboratory strain. Boxes represent exons, thin lines are introns with nucleotide lengths given (Maizels and Kurniawan-Atmadja 2002).

![Figure 1.6 Intronic polymorphism in *B. malayi* abundant larval transcript-2 gene](image)

1.3.2 Thioredoxin

Thioredoxins (Trx) are small proteins (molecular weight about 12 kDa) found in all living cells from archaeabacteria to humans. They are involved in a wide variety of fundamental biological functions (Figure 1.7 and 1.8). All Trx possess a highly active site made up of two neighbouring cysteines in a conserved motif, Cys-Gly-Pro-Cys (CGPC), also referred to as the “Trx-motif”. The oxidized form of the protein is reduced by NADP-linked
system, present in all types of the cells, in which Trx obtains electrons from NADPH via the flavin enzyme Trx reductase (TrxR). The reduced Trx are the major cellular protein disulfide reductases, therefore they also serve as an electron donor for specific enzymes of primary metabolism (Stewart et al 1998). However, the functions of Trx are by far not limited to this reaction and in fact, different Trx within one organism can serve different functions (Stefanková et al 2005). Antioxidant defence plays a crucial role in rapidly growing and multiplying organisms, including parasites and tumor cells. Apart from reactive oxygen species (ROS) produced in endogenous reactions, parasites are usually exposed to high ROS concentrations imposed by the host immune system.

The glutathione and thioredoxin systems represent the two major antioxidant defence lines in most eukaryotes and prokaryotes. These systems are NADPH-dependent and based on the catalytic activity of the flavoenzymes glutathione reductase, trypanothione reductase and thioredoxin reductase (TrxR), respectively (Holmgren 1984). Comparing the thioredoxin systems of different parasites and their respective host cells enhances our understanding of parasite biology and evolution, of parasite-host interactions and mechanisms of drug resistance.

![Diagram of Trx fold](image)

**Figure 1.7   Architecture of Trx fold.**

β-sheet strands are drawn as arrows and α-helices as rectangles.
Figure 1.8 Oxidoreductase activity of Trx system and functions of reduced Trx (Trx-S2) (Stefanková et al. 2005).

1.3.2.1 *Brugia malayi* Thioredoxin (*Bm*-trx)

A majority of the thioredoxins that have been reported to date from eukaryotic and prokaryotic organisms are initially translated as a 12-kDa protein. But it is 16-KDa in *Brugia malayi*. Despite notable differences in mass and the sequence of the catalytic site, appear to be functionally similar to the 12-kDa thioredoxins. The putative active-site sequences from the nematode-derived 16-kDa thioredoxins are variable from the rest, with the dominant sequence being WCPPCR. Despite the lack of a signal sequence, *Bm*-TRX is secreted by the adult and microfilarial stages of *B. malayi* (Kunchithapautham et al. 2003). It is possible that the secreted form of *Bm*-TRX-1 is an important source of reducing equivalents required by these antioxidant enzymes to maintain their activity and protect the parasite from radicals produced by cells of the immune response.
1.3.3 Glutathione-S-transferase

Glutathione-S-transferase (GSTs, EC. 2.5.1.18) are multifunctional proteins that can function as enzymes catalyzing the conjugation of glutathione thiolate anion with a multitude of second substrates or as non-covalent binding proteins for a range of hydrophobic ligands (Mannervik and Danielson 1988).

![Glutathione-S-Conjugate](image)

**Figure 1.9 Schematic representation of conjugation activity by GST**

There are several GST classes identified in mammals are A, M, P, S,T, Z, and O, except for the sigma and omega classes, the rest are subdivided in subclasses. These classes were established according to genetic, biochemical, and sequence/structural properties as well as by antibody cross-reactivity (Armstrong 1997, Eaton and Bammler 1999, Frova 2006, Hayes et al. 2005, Mannervik 1985, Sheehan et al. 2001). The main cGSTs classes identified in parasites are M, P and S, along with some in the A and O classes, although there are parasite cGSTs that cannot be assigned to any class as occurs with *P. falciparum* (Brophy et al. 1990, Chemale et al. 2006, Deponte and Becker 2005, Girardini et al. 2002, Hong et al. 2001).
Table 1.2  Known GST classes

<table>
<thead>
<tr>
<th>Class</th>
<th>Members</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alpha (α)</td>
<td>GSTA1, GSTA2, GSTA3, GSTA4, GSTA5</td>
</tr>
<tr>
<td>Kappa (κ)</td>
<td>GSTK1</td>
</tr>
<tr>
<td>Mu (μ)</td>
<td>GSTM1, GSTM1L, GSTM2, GSTM3, GSTM4, GSTM5</td>
</tr>
<tr>
<td>Omega (ω)</td>
<td>GSTO1, GSTO2</td>
</tr>
<tr>
<td>Pi (π)</td>
<td>GSTP1</td>
</tr>
<tr>
<td>Theta(θ)</td>
<td>GSTT1, GSTT2</td>
</tr>
<tr>
<td>microsomal</td>
<td>MGST1, MGST2, MGST3</td>
</tr>
</tbody>
</table>

1.3.3.1  Glutathione-S-transferase-π during stress response

Several studies from different laboratories suggested that changes in redox conditions can trigger cellular responses through a number of different pathways. Although as yet unproven, the nature and extent of the ROS insult may determine the threshold of the cellular response manifest as proliferation, stress response and damage repair or apoptosis. The link between thiols/GST and stress-activated protein kinases such as C-jun NH2-terminal kinase (JNK) and apoptosis signaling kinase (ASK) has been described (Alder et al. 1999). In an unstressed cellular environment JNK and ASK are kept in an inactive mode by the presence of one or more repressors. Biochemical purification identified GSTP1-1, the most ubiquitous and prevalent of the GST isozymes in non-hepatic tissues as a JNK inhibitor (Tew et al. 1994). In particular, increased expression of the GST-pi isozyme family has been extensively linked to drug resistance and the malignant phenotype of many solid tumors (although absence of expression has also been found in prostate cancer) (Lee et al. 1994).
The protein: protein interactions between GSTP1-1 and JNK serve to extend the principle of the ligand binding properties of the GST isozyme families. Under condition of oxidative stress, GSTP1-1 dissociates from JNK and forms dimers and/or multimeric complexes which activates the stress cascade involving the numerous sequential downstream kinases (Alder et al 1999). In mouse embryo fibroblasts made from mice null for GSTP1-1 expression (Henderson et al 1990), high basal levels of JNK activity were found. This activity was reduced if these cells were transfected with GSTP1-1cDNA. In addition, a specific GSTp inhibitor, TER199, was able to cause an activation of JNK.

1.3.3.2 GST as Drug Target for Filariasis

The GSTs have been considered as good targets for anti-parasitic drug development and studies have identified anti-schistosomal, anti-malarial and anti-filarial activity of compounds known for their GST inhibiting activity. The GSTs appear to be the major detoxification system present in helminths since there is no evidence that the oxygen-dependent P-450 system is expressed in adult worms. Structural studies indicate that nematode GSTs are topologically related to the \( \pi \) GSTs. An interesting variation from the typical \( \pi \) GSTs was noted at the hydrophobic substrate-binding site that it possesses an open and accessible hydrophobic substrate binding cleft suggesting that it is possible for the GSTs to play the role of the major detoxifying system in the parasite. However, the glutathione-binding site is closely related to that found in mammalian enzymes. The significant difference between the tertiary structure of the helminth GSTs and that of the host enzymes make the GSTs promising chemotherapeutic targets (Nathan et al 2005).

Further drug resistance has been correlated to increased GST level in certain nematodes like \textit{Haemonchus contortus}. So far, there have been no
documented problems of antihelminthic resistance in lymphatic filaria. Treatment of large human populations to break transmission of filarial nematodes and the tendency to increase the frequency of DEC treatment in lymphatic filariasis from once per year to two or more times per year will increase selection pressure for resistance. It was sought whether level of GST in *Setaria cervi*, a bovine filarial parasite, is affected by exposure to the antifilarial drug DEC. The results suggest that *S. cervi* GST is inducible in response to the antifilarial drug diethylcarbamazine and may play an important role in parasite’s survival, thus could be a potential drug target.

### 1.3.3.3 Polymorphic studies in GST

GST has been increasing correlated with drug resistance in tumors. Though there is no clear evidence of polymorphism in helminth parasites, in *O. volvulus* three isoforms have been described which differs in their localization and functional properties. huGSTM1, huGSTM3, huGSTT1 and huGSTP1 are polymorphic in the human population. Because these enzymes are involved in the detoxification of a variety of potentially toxic and carcinogenic substrates numerous epidemiology studies have examined association between GST polymorphism and increased risk for disease especially cancer. Homozygous null alleles for *GSTM1* and *GSTT1* genes appear to predispose to certain types of cancer (Ryberg et al 1997). Four allelic variants have been identified for human GSTP1 gene: GSTP1-A, GSTP1B, GSTP1-C and GSTP1-D. GSTP1-B results from a transition mutation in codon 104 that changes Ile to Val and GSTP1-C has same codon104 as GSTP1-B but also has second transition mutation in codon 113 that changes Ala to Val. The codon 113 change has been identified as a variant allele by itself, designated as GSTP1-D. The B and C allele variants appear to be functional since the catalytic efficiency of both these variant enzymes towards CDNB is about 3-4 folds lower than the wild type protein.
Recently, several groups have presented evidence that certain allelic variants of \textit{GSTP1} seen in the human population are associated with oesophageal and bladder, testicular and prostate tumors. This may be due to an inability of these allelic variants to catalyze the efficient conjugation and detoxification of certain carcinogens (Eaton 2005). The GSTP1 gene has been shown to exist in allelic variants (Zimniak et al 1994, Ali-osman et al 1997), and recent epidemiological studies indicated that individuals with the GSTP1* B allele encoding the GSTP1-1 protein with valine (GSTP1-1/V-105) rather than isoleucine at position 105 (GSTP1-1/I-105)1, may be susceptible to tumour formation in organs exposed to PAH (Harries et al 1997).

1.3.4 Need for Polymorphic Studies in Lymphatic Filarial Parasite

Lymphatic filariasis elimination currently depends on chemotherapeutic drugs such as albendazole, ivermectin and diethylcarbamazine. Drug resistance alleles have already been reported in ruminants (Kwa et al 1993 1994, Schwab et al 2005). Apart from studying the drug resistant alleles, conceptually, we need to understand the population genetic structure of helminth parasites to predict how such resistance alleles may spread and reach fixation in natural populations. This requires more data on allele frequency, interbreeding and molecular variation among all important helminth species (Nadler 1995, Anderson et al 1998). In addition, there is the argument that natural variation in genes provides a spotlight on proteins with interesting functions in the host-parasite relationship.

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Anderson et al 1998). In addition, the natural variation in genes provides a spotlight on proteins with interesting functions in the host-parasite relationship. The development of vaccine candidate antigens cannot proceed far without knowledge of the distribution and conservation of antigen genes among parasite populations worldwide. The present polymorphic study on filarial parasite, *W. bancrofti* has aimed at one of these aspects. Therefore three candidate genes for studying polymorphism viz., abundant larval transcript-2 (*alt-2*), glutathione-s-transferase (*GST*), thioredoxin (*trx*) are carefully chosen which are of functional importance for filarial parasite survival and for the establishment of immunogenicity in the host.