# 8 Genetic Diversity, Molecular Markers, and Population Genetics of Human Lymphatic Filarial Parasites

S. L. Hoti and R. Dhamodharan

**Abstract**

One of the most disfiguring diseases is lymphatic filariasis (LF) which is a major public health problem in tropical world. The disease is caused majorly by *Wuchereria bancrofti* and to smaller extent by *Brugia malayi* and *B. timori*. It is transmitted by mosquito vectors belonging to genera *Culex*, *Anopheles*, *Aedes*, and/or *Mansonia*. Currently, LF is targeted for elimination with mass drug administration of a combination of diethylcarbamazine/ivermectin and albendazole as a tool. Several rounds of MDA have already been administered in endemic communities, and questions have arisen pertaining to the continuance of parasite prevalence in some areas despite repeated rounds of MDA. This could be due to variations in the parasite strains that may not be responding to the anti-filarial drug administered. These variations could be the result of various factors such as geographic isolation, infra-population or refugia, environmental factors, and drug pressure. The long-term administration of the drug in the elimination program itself might have led to this phenomenon, or wide geographic distribution spanning continents might have affected variation. Investigating genetic variations among these variants may reveal the differential response to the anti-filarial drugs, and such studies are important for devising the drug administration strategies. In summary, there is a need to understand the genetic variation among the

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parasite populations in different LF endemic areas for which there is a need to develop appropriate markers. This review discusses the biological, physiological, and genetic variations among LF parasites.

## 8.1 Introduction

Lymphatic filariasis (LF) is considered as a neglected tropical disease caused by mosquito-borne nematode parasites, viz., *Wuchereria bancrofti*, *Brugia malayi*, and *B. timori* (Routh and Bhowmik 1994). It affects around 120 million people in over 73 countries (WHO, 2014), and *W. bancrofti* is responsible for ~90% cases of LF (WHO 1992, 2002). The infection and disease caused by these worms are commonly termed as bancroftian filariasis, brugian filariasis, and timorian filariasis, respectively. The parasites are transmitted among humans by the bites of female mosquitoes belonging to genera *Culex*, *Anopheles*, *Aedes*, and/or *Mansonia* (Table 8.1). These mosquitoes act as vectors of specific filarial parasites by developing their larval stages and carrying the infective stage to human hosts (Manson-Bahr and Bell 1995; Simonsen 2003).

Filariasis is widely distributed in the tropical and subtropical regions of Africa, Asia, Northern South America, Western Pacific, and Eastern Mediterranean region. Approximately 70% of infections occur in India, Nigeria, Bangladesh, and Indonesia, and more than 1 billion people worldwide are at risk of acquiring LF infection (Molyneux and Zagaria 2002). Of these, 95% are infected with *W. bancrofti* and the remainder with *B. malayi* and *B. timor*i (WHO 1995). A population of 44 million is affected with various clinical forms of the disease, while 76 million carry the parasites with silent clinical damages of lymphatics and renal systems (Ottesen et al. 1997; Melrose 2002; Remme et al. 2002; Hotez et al. 2006). Reports until the year 2006 showed that an estimated 1.254 billion people in 83 endemic countries and territories are at the risk of infection of LF. The WHO Southeast Asian region has the highest proportion of cases (64%), followed by the African region (32%). The European region remains free from LF (WHO 2007, 2014, http://www.who.int/wer).

In India, data available until the year 2000 showed that up to 27.09 million microfilaremic and 20.83 million symptomatic filariasis cases existed, and about 429.32 million individuals were at the risk of infection (Sabesan et al. 2000). India alone accounts for 40% of the global burden of LF (Michael and Bundy 1997), and at least one-third of the people affected with the disease live in India. Bancroftian filariasis caused by *W. bancrofti* accounts for about 98% of the national burden, and brugian filariasis by *B. Malayi* is responsible for the remaining 2% which is distributed mainly in Odisha and Kerala. The recent report showed that an estimated 554.2 million people are at risk of LF infection in India (WHO 2007).

The clinical and immunological outcomes of infection and disease vary from individuals living in different endemic regions due to genetic polymorphisms, environmental factors, or inherent innate immunity (Tisch et al. 2001; Hise et al. 2003;

**Table 8.1** Important discoveries and events related to disease, parasite life cycle, pathogenesis, and genetic diversity of human lymphatic filariasis *W. bancrofti*

| S. no. | Name of the scientist/project | Year | Discovery/event |
|---|---|---|---|
| 1 | William Prout | 1849 | Chyluria |
| 2 | Jean-Nicholas Demarquay | 1863 | Mf of *W. bancrofti* (hydrocele fluid) |
| 3 | Otto Henry Wucherer | 1868 | Mf of *W. bancrofti* (urine) |
| 4 | Timothy Lewis | 1872 | Mf of *W. bancrofti* (blood) |
| 5 | Joseph Bancroft | 1876 | Adult female of *W. bancrofti* |
| 6 | Thomas Spencer Cobbold | 1877 | Adult worm of *W. bancrofti* |
| 7 | Timothy Lewis | 1877 | Adult worm of *W. bancrofti* |
| 8 | Patrick Manson | 1877 | Life cycle of *W. bancrofti* |
| 9 | Bourn | 1888 | Adult female of *W. bancrofti* |
| 10 | Sibthorpe and Bourne | 1888 | Adult male of *W. bancrofti* |
| 11 | Bancroft | 1889 | The mode of transmission |
| 12 | George Carmichael Low | 1900 | Mf in the mouthparts of mosquitoes |
| 13 | Low | 1900 | The mode of transmission |
| 14 | Lichtenstein and Brug | 1927 | *Filaria malayi* (*Brugia malayi*) |
| 15 | Poynton and Hodgkin | 1939 | Zoonotic filariasis |
| 16 | Hewitt et al. | 1947 | DEC as filaricidal compound |
| 17 | David and Edeson | 1965 | *B. Timori* |
| 18 | Buckley | 1958 | *B. malayi* – Nomenclature |
| 19 | WHO | 1997 | MDA |
| 20 | WHO | 2000 | GPELF |
| 21 | Aboobaker and Blaxter | 2003 | RNAi in *B. malayi* |
| 22 | Foster et al. | 2005 | Complete genome of *Bm-Wolbachia* |
| 23 | Ghedin et al. | 2007 | Mitochondrial genome of *B. malayi* |
| 24 | Daub et al. | 2008 | Complete Mt. genome of *B. malayi* |
| 25 | Filarial genome project | 2009 | *B. malayi* whole genome sequencing |
| 26 | McNulty et al. (2012) | 2012 | Mitochondrial genome of *Wuchereria bancrofti* |
| 27 | *Wuchereria bancrofti* WGS project | 2016 | *Wuchereria bancrofti* whole genome shotgun sequencing project |

Choi et al. 2001). The clustering of filarial infection within the household (Ottesen et al. 1981) showed the evidence of genetic polymorphism in different outcomes of the disease.

## 8.2 Strains Based on Periodicity of Microfilariae

Filarial parasites, both *W. bancrofti* and *B. malayi*, occur as different physiological strains in different geographical regions of the world (Sasa 1974). In most areas (Asia, Africa, Malaysia, the Philippines, Papua New Guinea), the periodicity of *W. bancrofti* is nocturnal, i.e., presence of microfilariae (mf) in peripheral circulation during night time (Sasa and Tanaka 1974; Shriram et al. 2002; Pichon and Treuil 2004; Bockarie

et al. 2009). In some areas it is diurnally sub-periodic (South Pacific, Andaman & Nicobar Islands) or nocturnally sub-periodic (Thailand), where *mf* are present continuously in the peripheral blood, where the concentrations are higher than the average during the day and midnight, respectively (Kalra 1974; Pothikasikorn et al. 2008). It is governed by a biological rhythm inherent in the *mf* of a particular strain but is influenced by the circadian rhythm of the specific mammalian host. Also, the periodicity is in agreement with the feeding behavior of the vector mosquito (Weerasooriya et al. 1998) enabling them to ingest the *mf* in large numbers by presenting themselves in high density in the peripheral blood during the biting period of the mosquito.

## 8.3 Diagnosis and Genotyping of the Disease

Filariasis can be diagnosed by detection of *mf* through microscopic examination of blood smear or membrane filtration of the blood, taken during the time of circulation of *mf* depending upon its periodicity. However, the method of diagnosis based on finger prick thick blood smear test for *mf* has low sensitivity and is costly and not well accepted by either community or program personnel (Weil et al. 1997; Das et al. 2005). As an alternative and, more importantly, as a replacement for the night blood collection, more rapid immunological tests such as monoclonal antibody (Og4C3)-based ELISA or immuno-chromatographic test (ICT) are in use. In an attempt to detect antigens in day blood samples collected on filter paper, through Og4C3 test, Itoh et al. (1998) could get high sensitivity and specificity in the immune reaction. The ICT test kit (Weil and Ramzy 2007), though costly, is claimed to be specific and sensitive and could provide on-the-spot results (Weil et al. 1997; Pani et al. 2000). Detection of IgG4 antibodies using recombinant antigen also has been proved sensitive for the detection of filarial infection (Rahmah et al. 2003; Supali et al. 2004; Weil et al. 2011; Athisaya Mary et al. 2011). Apart from these, molecular tests based on DNA (PCR, PCR-RFLP, RT-PCR, and DNA sequencing) are also in place for detection of active infection both in human and mosquito vectors (Abbasi et al. 1996; Williams et al. 1996; Hoti et al. 2008; Vasuki et al. 2003, 2012). Molecular tests for diagnosis and species differentiation were mainly targeted HhaI repeat (McReynolds et al. 1986; Rao et al. 2006; Triteeraprapab et al. 2001), SspI repeat (Fischer et al. 1999; McCarthy et al. 1996; Williams et al. 1996; Zhong et al. 1996), glutathione peroxidase, cytochrome oxidase I and internal transcribed spacer-1 (Fischer et al. 2002; Nuchprayoon et al. 2005; Thanomsub et al. 2000), cofactor-independent phosphoglycerate mutase isoform-1, and abundant larval transcript-2 genes (Fong et al. 2013; Sakthidevi et al. 2010; Dhamodharan et al. 2012).

## 8.4 Treatment and Drug Resistance

Currently, diethylcarbamazine (DEC) or combination with ivermectin (IVM) and albendazole (ALB) is used to target the *mf*, to reduce or interrupt transmission, and these drugs have low macrofilaricidal activity (Ottesen et al. 1997; Ottesen et al. 1999;

Plaisier et al. 2000; Meyrowitsch et al. 2004). Interruption of transmission is expected to be achieved by mass annual drug administration (MDA) of DEC to entire communities at risk of infection when community drug consumption rates are adequate. Recently, treatment with doxycycline to kill the symbiotic bacterium of filarial parasite has also drawn attention of many workers (Hoerauf et al. 1999, 2008; Smith and Rajan 2000; Casiraghi et al. 2002; Rao 2005; Taylor et al. 2005). An earlier trial of doxycycline against *Wolbachia* of *W. bancrofti* has shown complete clearance of *mf* and significant level of reduction in adult worm activity (Taylor et al. 2005). In India, recently the National Task Force for Elimination of Lymphatic Filariasis (NTF-ELF) decided to modify its existing strategy of MDA using DEC alone to DEC + albendazole co-administration based on the recommendation of the Indian Council of Medical Research (ICMR). MDA with DEC or its combination with other microfilaricides has its own limitation as there are instances of recurrences of microfilaremia, possibly due to reproduction from surviving female adult worms (Fernando et al. 2011). There is also a possibility of resurgence of infection and the emergence of drug-resistant strains after MDA due to the strong selective pressure on parasites (Grant 1994; Anderson and Jaenike 1997; Schwab et al. 2007). Though it was not possible to assess resistance to DEC, resistance to IVM has been reported in animal filarial parasites as well as in the human filarial parasite, *Onchocerca volvulus* (Prichard 1990; Awadzi et al. 2004). Resistance to ALB has been seen in many parasitic nematodes of animals, and many workers demonstrated the possibility of ALB resistance in *W. bancrofti* too due to phenylalanine to tyrosine mutation at 200th and 167th positions, responsible for resistance existing in *W. bancrofti*, and also developed allele-specific PCR assays (Prichard 2007; Schwab et al. 2005, 2007; Hoti et al. 2009) for screening resistance alleles in treated parasite populations.

## 8.5 Molecular Biology and Genomics of Filariasis/Genetic Variation

The development of molecular methods and techniques has led to the advancement in isolation, purification, and characterization of nucleic acids and proteins from filarial parasites. Many structural and functional genes from the genome of *B. malayi* and *W. bancrofti* and their endosymbiont, *Wolbachia*, have been characterized and used for phylogenetic analysis (Williams et al. 2000). The entire mitochondrial genome of *B. malayi* (13.67 Kb) and *W. bancrofti* (13.63 Kb) (Ghedin et al. 2007; McNulty et al. 2012) and endosymbiont genome of *Wolbachia* from *B. malayi* (~1 Mb) and *W. bancrofti* (~1 Mb) (Foster et al. 2005; http://www.ncbi.nlm.nih.gov/genome/11274?project_id=199733) have also been characterized. The draft genome of *B. malayi* (93.65 Mb) (Ghedin et al. 2007) and whole genome short gun sequences of *W. bancrofti* (81.5 Mb) (McNulty et al. 2012, http://www.ncbi.nlm.nih.gov/Traces/wgs/?val=ADBV01#contigs) have been released into the public domain. The sequence data for the genomes of major human filarial pathogens, *W. bancrofti*, *O. volvulus*, and *Loa loa*, have been made publically available (http://

www.broadinstitute.org/annotation/genome/filarial_worms/MultiHome.html). The genome annotation and analysis are essential for the determination of the complete sequence of the chromosomal genome, identification of the coding genes, prediction of function of structural/functional genes, and investigation of genetic variation in the genome, population structure, and gene polymorphism in relation to drug resistance and other selective forces. Genomics of filariasis is mainly focused on identifying new drug/vaccine targets, diagnostic development and biological basis of drug resistance, antigenic diversity, infectivity and pathology, and gene polymorphism (Unnasch and Williams 2000; Blaxter et al. 1999).

## 8.6 *Wolbachia* Endosymbiont of Filarial Parasites

*Wolbachia pipientis* is a bacterial endosymbiont, maternally transmitted and associated with arthropods and filarial nematodes (Werren 1997). Most of the filarial parasites, including human parasites such as *W. bancrofti*, *B. malayi*, and *O. volvulus*, are infected with the intracellular *Wolbachia* (Sironi et al. 1995; Bandi et al. 1998; Taylor et al. 2000). The genus *Wolbachia* of filarial nematode and arthropods was identified based on DNA sequence data (Henkle-Duhrsen et al. 1998). *Wolbachia* plays an important role in the biology of the host and in the immunopathology of filariasis (Brattig et al. 2000; Taylor 2002; Keiser et al. 2002). The phylogenetic analysis of *Wolbachia* endosymbiont of the lymphatic filarial parasite showed that their phylogeny agrees with the phylogenetic structure of their host-parasite such as *B. malayi*, *W. bancrofti*, *O. volvulus*, etc., which indicates that they have evolutionary significance on parasite-endosymbiont association (Taylor et al. 1999; Casiraghi et al. 2004, 2005; Foster et al. 2005; Ferri et al. 2011; Sharma et al. 2013). Many genes of *Wolbachia* of human lymphatic filarial parasite have been characterized to focus on *Wolbachia*-based control strategies of filariasis (Brattig et al. 2000; Hoerauf et al. 2000; Taylor 2000; Dhamodharan et al. 2011; Sharma et al. 2013; Slatko et al. 2014; Shahab et al. 2014).

## 8.7 The Nuclear Genome

The draft genome size of *B. malayi* is 100 Mb and encodes about ~20,000 genes (Ghedin et al. 2004; Parra et al. 2007; Scott et al. 2013). It consists of five pairs of chromosomes (four autosomal pairs and one sex determination pair), and the genome is rich in adenosine and thymidine (75% AT). *Brugia* genome consists of a single 322 bp repetitive sequence, the HhaI repeat which occupies 10–12% of the genome. This HhaI repeat is genus-specific and exists in around 30,000 copies per haploid chromosome set, organized in tandem arrays (Mcreynolds et al. 1986). The draft genome of *W. bancrofti* (81.5 Mb) (McNulty et al. 2012) is yet to be annotated (http://www.ncbi.nlm.nih.gov/sra/?term=SRP000772).

## 8.8 Mitochondrial Genome Diversity

The complete genome of *B. malayi* mitochondria was characterized by Ghedin et al. (2007). Recently, the complete mitochondrial genome of *W. bancrofti* was sequenced (McNulty et al. 2012), and the genetic variation among the mitochondrial genome of three *W. bancrofti* strains from India, West Africa, and Papua New Guinea is analyzed (Ramesh et al. 2012). The Mt. genomes of nematodes are usually smaller than other metazoans, and the size varies from ~13.6 to 26 kb. Most of the nematode Mt. genomes contain 12 protein-coding genes, 22 trn genes, and 2 rrn genes, and they usually lack an atp8 gene. The arrangements of nematode Mt. genes are more variable and AT-rich (Okimoto et al. 1992; Keddie et al. 1998; Lavrov and Brown 2001). High mutation rate and maternal inheritance make mitochondrial genome a good source of molecular marker for studying population genetic structure (Avise et al. 1994; Wallace 1999). Cytochrome oxidase subunit 1 (COI) has been used as a taxonomic and population genetic marker to analyze the genetic diversity of *W. bancrofti* strains (de Souza et al. 2014).

## 8.9 Genetic Variation and Molecular Markers in LF

### 8.9.1 Genetic Diversity of Human Lymphatic Filarial Parasites

Nematode parasite populations are genetically heterogeneous (Nadler 1987, 1990), and genetic variation in parasitic nematodes is a prerequisite for a genetic response to a selection pressure. Proper identification of genetic variation in parasitic nematodes is essential to understand the genetic structure (e.g., effective population size, heterozygosity) or as epidemiological markers. Molecular methods have proven to be useful for assessing both inter- and intra-genetic variation in parasite populations (Nadler 1990; Grant 1994; Gasser and Newton 2000).

The analysis of genetic diversity and heterogeneity of filarial parasites is essential for understanding the phylogeny, the evolutionary history, and the emergence of drug-resistant phenotypes after treatment with antifilarial drugs (Hoti et al. 2008; Dhamodharan et al. 2008; Hoti et al. 2003. The presence of polymorphism in parasites can be identified from biological (e.g., morphology, infectivity, and periodicity), biochemical (e.g., enzymatic and drug sensitive/resistance), immunological (e.g., difference in immune response due to antigenic/antibody diversity), or molecular biological (e.g., variation in DNA and proteins sequence that leads to functional differences) characteristics. Genetic diversity of parasitic nematodes has been studied by analyzing the variability using molecular markers such as isozymes, mitochondrial DNA (mt DNA), random amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), microsatellites, and internal transcribed spacers (ITS) of ribosomal DNA. All these markers have been used in different combination to characterize the genetic diversity of nematodes.

### 8.9.2 Morphological and Behavioral Variations in Filarial Parasites

Almost all the developmental stages of the filarial parasites show differences in morphological features, with which they can be differentiated up to the species level. Since *W. bancrofti* and *B. malayi* coexist in many places (Raina et al. 1990; Rajendran et al. 1997), their identification to species level is very important in diagnosis as well as epidemiological surveys. The size of adult *W. bancrofti* worm is larger than the adult *B. malayi*. Adult male worms of *B. malayi* also show morphological variation due to their posterior cuticular structure (Maizels and Kurniawan-Atmadja 2002). Apart from differences in body size, cephalic space, and distance from head to nerve ring, the *mf* of *B. malayi* and *W. bancrofti* can be distinguished, very clearly, by the presence of caudal nuclei at the tip of the tail of the former which is not seen in the latter. The infective L3 stage of these species can be differentiated by examining their caudal papillae. There are three caudal papillae, two lateral and one terminal. All the three caudal papillae are distinctly protruding in *W. bancrofti* as compared to *B. malayi*. The lateral papillae of *B. malayi*, under electron microscopy, show a gutter-like indentation around their bases, and this character is absent in *W. bancrofti* (Zaman and Narayanan 1986).

Behavioral variations occur between these two species, in terms of *mf* periodicity and localization of the adult worms in the mammalian hosts. Microfilaria periodicity, which has been discussed in an earlier section, leads to strain variations, which ultimately decide the vector adaptation (Thorpe 1896; Basu 1958; Kalra 1974; Russel et al. 1975). Regarding adult worm localization, *W. bancrofti* adults are most commonly localized both in the lymphatics of limbs and the intrascrotal lymphatic vessels as against *B. malayi* worms, which are found mostly localized in the lymphatic system of limbs (Dreyer et al. 2006; Partono, 1987).

### 8.9.3 Biochemical Variations

Biochemical properties, structures, and roles of parasite proteins/proteases vary due to variation in amino acid sequence. Identification of biochemical variation is essential for the development of suitable diagnostic markers and vaccine and drug targets for the control of parasitic diseases. Though isoenzymes (or allozymes) are the essential biochemical markers and have been used for analyzing genetic variation in *Ascaris*, *Anisakis simplex*, and many nematodes (Leslie et al. 1982; Anderson et al. 1998), till now no such studies are reported for lymphatic filarial parasites, probably due to the scarcity of parasite materials. However, there are strong indications of interspecific as well as stage-wise intraspecific biochemical variations in filarial parasites (Maizels and Kurniawan-Atmadja 2002). Protein variations among species could be the reason for the enhancement of specificity of certain diagnostic filarial antigens such as *Bm*-SXP-1 and *Wb*-SXP-1, when the same species molecules are used (Rao et al. 2000; Pandiaraja et al. 2010).

### 8.9.4 Immunological Variations

Antigenic diversity and polymorphism in parasite populations are evolutionary strategies to escape from the host immune response (Maizels 2009). Filarial parasite antigenic surface binding to human antibody varies (Ravindran et al. 1994; Maizels and Kurniawan-Atmadja 2002), and this variation has direct effect on the recognition of immune system, immunity, and parasite immune evasion mechanisms because the survival of parasite is associated with the immune response stimulated in the host (Piessens et al. 1980). The outcome of in vitro analysis with sera samples collected from *mf* carriers also showed that *mf* could express a variety of surface antigens (Ravindran et al. 1994). The variations in *mf* sheath proteins (SHPs) which are composed of the tightly cross-linked set of repeat-rich proteins, with some carbohydrate structures, were reported (Hirzmann et al. 1995; Zahner et al. 1995). And variants of SHP-1, SHP-2, and SHP-3 are the key proteins identified, but the level of host immune response due to these variations is yet to be reported. Many studies have already reported the diversity of genes coding for immune immunogenic proteins in filarial parasites (Choi et al. 2001; Hise et al. 2003; Jaoko et al. 2007; Hoti et al. 2007).

### 8.9.5 Molecular Variations

Analysis of polymorphism in coding sequences of structural and functional genes, regulatory elements (promoter and enhancer sequences), RNA elements, and introns of protein-coding genes have received more attention because of their potential in treatment and diagnosis. DNA sequence-based assays have become easier to use and more efficient at screening for nucleotide sequence-based polymorphisms. Polymorphism in abundant larval transcript-2, cysteine protease inhibitor-2, and sheath proteins (SHPs) has been reported from filarial parasites *B. malayi* and *W. bancrofti* (Gregory et al. 1997; Gomez-Escobar et al. 2002; Maizels and Kurniawan-Atmadja 2002). Molecular variations of filarial antigens have also been reported from *B. malayi* cDNA clones sequenced under Filarial Genome Project (Maizels et al. 2001). Hoti et al. (2007) have studied polymorphism of lipid binding/transport molecules (gp15/400 allergen gene) of *W. bancrofti* from different regions of India and found that the parasite populations from different geographical locations, viz., Thanjavur and Tiruvannamalai (two districts in Tamil Nadu), are heterogeneous with three to five genotypes, and influence of drug pressure is associated with the gene diversity of the populations studied.

### 8.9.6 Molecular Markers/Methods for Analyzing Genetic Variation of Filarial Parasites

Currently, only a few molecular markers are available for filarial parasitic nematodes, which include RAPD, AFLP, RFLP, and rDNA. Molecular markers serve as effective tools for analyzing inter- and intraspecific genetic variations and

phylogenetic relationships (Williams et al. 1990; Cameron et al. 1988; Patra et al. 2007; Thangadurai et al. 2006; Dhamodharan et al. 2008; McNulty et al. 2013; Small et al. 2014).

#### 8.9.6.1 Random Amplification of Polymorphic DNA (RAPD)

Random amplification of polymorphic DNA (RAPD) is an effective marker to differentiate the parasite species, as well as to determine the gene polymorphism within the genus. Nucleic acid sequence variation due to point mutations, inversions, deletions, and additions will determine the number and size of the RAPD bands. This technique is very rapid and simple and does not depend on previous knowledge of the target DNA sequences. It is mainly used for phylogenetic inference among closely related species through distance and parsimony analyses (Morgan et al. 1993; Sire et al. 2001).

Several studies have reported the use of the RAPD markers in analyzing the genetic diversity of *W. bancrofti* populations. The analysis of genetic polymorphism by RAPD markers in few studies with the selective population (from LF endemic areas) from India has revealed different levels of inter- and intra-genetic diversity of *W. bancrofti* populations (Pradeep Kumar et al. 2002; Patra et al. 2007). Thangadurai et al. (2006) used RAPD marker for inferring phylogeography of *W. bancrofti* in India and found it to be complex with high genetic divergence and varying gene flow between populations. Genetic heterogeneity of *W. bancrofti* populations at spatially hierarchical levels in south India (Hoti et al. 2008) showed the high degree of variability associated with human populations. Phylogenetic analysis of *W. bancrofti* microfilariae isolated from dry blood smears collected from microfilaria carriers residing in villages under MDA and selective chemotherapy with DEC was also analyzed using RAPD markers (Bisht et al. 2006). Dhamodharan et al. (2011) attempted to elucidate the influence of anti-filarial chemotherapy strategies on the genetic structure of *W. bancrofti* populations using RAPD marker-based population genetic analysis. Also, these investigators have used RAPD marker for differentiating periodic and sub-periodic *W. bancrofti* (Das et al. 2011). Analysis of genetic diversity of diurnally sub-periodic *W. bancrofti* in the Andaman and Nicobar Islands using RAPD marker showed that the parasites transmitted by *Cx. quinquefasciatus* and *Ochlerotatus* (*Aedes*) *niveus* from Car Nicobar Island and from neighboring islands, respectively, are phylogenetically distinct (Dhamodharan et al. 2008). Nuchprayoon et al. (2007) developed RAPD assay to differentiate a nocturnally subperiodic Thai strain from nocturnally periodic Myanmar strains of *W. bancrofti*.

#### 8.9.6.2 Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is used both as diagnostic and phylogenetic marker (Gasser et al. 1996; Thanomsub et al. 2000; Nuchprayoon et al. 2003a, b) in nematode parasites. It is achieved by digesting either the genomic DNA directly or PCR amplified fragments (PCR-RFLP) with a suitable restriction enzyme followed by generating RFLP profile using gel electrophoresis (Nuchprayoon 2009). Bhandari et al. (2005) used RFLP of the internal transcribed spacer (ITS) region of the ribosomal RNA to study the intraspecific variation in *W. bancrofti*

isolates collected from different endemic zones but found that they were genetically highly similar. Nuchprayoon et al. (2005) used PCR-RFLP for the digestion of the ITS1 product with the restriction endonuclease Ase I, and based on the genetic diversity, they differentiated species-level identification of filarial parasites such as *W. bancrofti*, *B. malayi*, *B. pahangi*, *Dirofilaria immitis*, and *D. repens*.

#### 8.9.6.3 Amplified Fragment Length Polymorphism (AFLP)

Amplified fragment length polymorphism (AFLP) is a powerful molecular marker, and this technique also doesn't require prior sequence data. It is a combination of RFLP and RAPDs, and the basic methodology includes digestion of genomic DNA using two different restriction enzymes (a rare and a frequent cutter), ligation of adapters to the digested fragments, and PCR amplification with primers homologous to the adapters plus one to four additional random bases at its 3′ end. The subset of digested DNA fragments amplified will give distinct bands that can be analyzed on a polyacrylamide gel. Patra et al. (2007) have identified suitable AFLP marker for genotyping filarial parasite, *W. bancrofti*, collected from India.

#### 8.9.6.4 Microsatellite Markers

The hypervariable repeat length of microsatellites is used to study DNA polymorphisms among related organisms (Makova et al. 2000). Microsatellite markers are ideal for studying genetic diversity (Rosenthal et al. 2002) because it is highly polymorphic, selectively neutral, distributed throughout the genome, reproducible, and expressed co-dominantly. Microsatellites have been characterized in animal parasitic nematodes such as *Strongyloides ratti*, *Trichinella spiralis*, and *Haemonchus contortus* (Zarlenga et al. 1996; Fisher and Viney 1996; Hoekstra et al. 1997), in potato cyst nematode *Globodera* spp. (Thiery and Mugniery 2000), and in *B. malayi* (Tautz 1989). Underwood et al. (2000) have studied microsatellite-based polymorphism among *B. malayi* isolates from Indonesia and Malaysia. Currently, no existing report on microsatellite markers for *W. bancrofti* is available and is yet to be studied/developed.

#### 8.9.6.5 Genetic Markers Based on DNA Sequencing

Mitochondrial markers are more suitable for investigating the population genetics of nematodes in inter- and intra-genetic variations, differentiating sibling species, because they are maternally inherited and have higher substitution rates than nuclear genes (Anderson et al. 1998). Many studies were reported already on the phylogenetic analysis of nematode populations based on mtDNA markers (Tarrant et al. 1992). Ribosomal DNA (rDNA) markers have also been used extensively for studying genetic variation within and between species of nematodes (Dame et al. 1991; Nadler 1992). Internal transcribed spacers (ITS) of rDNA as genetic markers for phylogenetic and phylogeographical identification of nematode species, including filarial parasites, have been reported recently (Zhu and Gasser 1998). Ribosomal RNA (rRNA) such as 16S rRNA (for Wolbachia) and 18 s rRNA (for nematode) have also proven to be used as molecular marker for species identification and phylogenetic analysis (Gogarten et al. 1996; Jain et al. 1999). Recently Bhandari et al.

(2005) studied the polymorphism of the 18S rRNA gene in *W. bancrofti* parasites collected from three different zones in India which showed that these parasites were genetically similar. Fong et al. (2013) phylogenetically differentiated the *B. malayi* isolated from Northeast Borneo Island and Thailand using ITS1 nucleotide sequences (Fong et al. 2013).

The polymorphism of gp15/400 of *W. bancrofti*, a polyprotein allergen (NPA), was investigated through sequencing a single repeat subunit of this 10-mer gene from 35 isolates of *W. bancrofti* collected from different geographic locations of India (Hoti et al. 2007). The repeat subunit was found to be highly conserved in all the isolates with only two nucleotide changes at positions 285 (A-G) and 336 (C-T), which are synonymous. This gene is multifunction in nature ranging from lipid binding and transportation to elicitation of elevated levels of IgE and Th2 type of immune response in the infected host. It is also reported to be involved in the transportation of arachidonic acid and metabolites, which are known to be the mode of action of anti-filarial drug, diethylcarbamazine. It is a good target for the development of anti-filarial drugs and immunomodulation and immunochemotherapy.

## 8.10 Phylogeny of Filarial Parasites

The phylum Nematoda has a large number of parasitic groups, and all the filarial parasites belonging to this phylum were grouped under the family Onchocercidae and the subfamily Onchocercinae. Molecular phylogenetic analysis using rDNA and mtDNA showed that the human lymphatic filarial parasite, *W. bancrofti*, is more closely related to *B. malayi* than *B. pahangi*, the animal filarial parasite, and *O. volvulus*, the human cutaneous filarial parasite (Casiraghi et al. 2001). Phylogenetic tree constructed recently by aligning complete mtDNA of nematodes available from online databases also clearly indicates this phylogenetic position of filarial parasites.

In a recent study, the existence of parallelism between the evolution of the polyprotein genes and small subunit (SSU) rDNA genes of parasitic nematodes has been observed. This was also evidenced by the phylogenetic tree constructed using the nucleotide sequence of the polyprotein gene of mammalian parasitic nematodes. Recently mitochondrial cytochrome oxidase 1 sequence-based population genetics of the *W. bancrofti* in Papua New Guinea with respect to posttreatment (Small et al. 2013) showed that parasite diversity was similar among people residing within the same village and clustered within transmission zones.

## 8.11 Phylogeography of *W. bancrofti*

Genetic analysis of *W. bancrofti* populations collected from 71 *mf* carriers from different geoclimatic regions of India through RAPD profiles showed that the parasite populations are comprised of at least five genotypes (Thangadurai et al. 2006). Generally, parasite populations from urban areas are genetically heterogeneous exhibiting two

to four different genotypes, possibly due to the convergence of infected individuals from different areas for trade and employment and consequent intermixing of parasite populations (Hoti et al. 2008). In their study, analysis of parasite populations collected from 31 *mf* carriers residing in Pondicherry and surrounding villages showed that *W. bancrofti* populations of rural area are highly homogenous comprising one or two genotypes, compared to the urban populations, which showed high genetic divergence and varying levels of gene flow between populations of different areas. The diversity of parasites in relation to different age groups has also been investigated, and the results showed that the parasite heterogeneity increases with age of the carrier, possibly due to acquiring of new infections at different time points in life (Hoti et al. 2008).

All these studies on the phylogeography of *W. bancrofti* populations in India have been indicative of the following:

a. High genetic divergence and gene flow between populations.
b. The route of entry of the parasite into Indian subcontinent possibly appeared to be from an ancient origin from the countries of the Southeast Asian archipelago, through the eastern coastal line of the southern peninsula.
c. The Western Ghats would have played a major role in the selection process by geographic isolation leading to the genetic drift between the two strains on its western and eastern side. Also, the chemotherapeutical pressure would have contributed to the high genetic heterogeneity of the populations in the eastern side of the country (Thangadurai et al. 2006).

## 8.12 Conclusion

Parasite populations respond to selective pressures like drug pressure and are genetically heterogeneous. Understanding the genetic variability and diversity of genes and genome within and between populations of the filarial parasite is essential for appropriate treatment schedules and designing control programs. Currently, molecular markers have been successfully used in analyzing the population genetic structure of filarial parasites in India on a limited scale. Such efforts need to be undertaken on national and further on a global scale.

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