Pharmacogenomics and Personalized Medicine for Infectious Diseases

27

Nirmal Kumar Ganguly and Gautam Kumar Saha

Abstract

Humans have been plagued by the scourge of invasion by pathogens leading to infectious diseases from the time in memoriam and are still the cause of morbidity and mortality among millions of individuals. Trying to understand the disease mechanisms and finding the remedial measures have been the quest of humankind. The susceptibility to disease of an individual in a given population is determined by ones genetic buildup. Response to treatment and the disease prognosis also depends upon individual's genetic predisposition. The environmental stress induces mutations and is leading to the emergence of ever-increasing more dreaded infectious pathogens, and now we are in the era of increasing antibiotic resistance that has thrown up a challenge to find new treatment regimes. Discoveries in the science of high-throughput sequencing and array technologies have shown new hope and are bringing a revolution in human health. The information gained from sequencing of both human and pathogen genomes is a way forward in deciphering host-pathogen interactions. Deciphering the pathogen virulence factors, host susceptibility genes, and the molecular programs involved in the pathogenesis of disease has paved the way for discovery of new molecular targets for drugs, diagnostic markers, and vaccines. The genomic diversity in the human population leads to differences in host responses to drugs and vaccines and is the cause of poor response to treatment as well as adverse reactions. The study of pharmacogenomics of infectious diseases is still at an early stage of development, and many intricacies of the host-pathogen interaction are yet to be understood in full measure. However, progress has been made over the decades of research in some of the important infectious diseases revealing

N.K. Ganguly (✉) • G.K. Saha
Policy Centre for Biomedical Research, Translational
Health Science and Technology Institute
(Department of Biotechnology Institute, Government
of India), Office@National Institute of Immunology,
New Delhi, India
e-mail: nkganguly@nii.ac.in;
gautamkumarsaha@gmail.com

how the host genetic polymorphisms of drug-metabolizing enzymes and transporters affect the bioavailability of the drugs which further determine the efficacy and toxicology of the drugs used for treatment. Further, the field of structural biology and chemistry has intertwined to give rise to medical structural genomics leading the way to the discovery of new drug targets against infectious diseases. This chapter explores how the advent of "omics" technologies is making a beginning in bringing about a change in the prevention, diagnosis, and treatments of the infectious diseases and hence paving way for personalized medicine.

1   Introduction

Over the millennia with the progression of human civilization, the condition of human health has changed considerably. The lifespan of the human has increased considerably with the advent of vaccines against several diseases which has eradicated small pox, and now we are embarking on the global campaign to eradicate poliomyelitis and have controlled the disease in most parts of the world. The treatment of infectious diseases got a boost with the discovery of penicillin by Alexander Fleming in the earlier part of the twentieth century. As a range of antibiotics were later discovered, infectious diseases such as meningitis, bacterial pneumonia, sepsis, and other life-threatening bacterial infection were treatable. Also the survivability of patients undergoing operative procedures and aggressive chemotherapy was feasible and their recovery high. The last 50 years of the twentieth century have been eventful with the discovery of antimicrobials that had given us the hope that we shall eradicate all infectious diseases. Despite all the efforts and progress we have made in medical science, infectious disease remained a major health problem.

But the golden era of the antibiotics will not be long if we go on unregulated administering and promoting rampant use of antibiotics, as is evident from the rise of antibiotic resistance (Caramia and Ruffini 2012), and the new emerging diseases have posed major challenge (Table 27.1). In the last few decades with the advent of field of genomics, there is a new hope in prevention, diagnostics, and treatment of infectious diseases. We will explore in this chapter

Table 27.1 Timeline of pathogen discovery

Year	Microbe
1973	Rotavirus
1975	Parvovirus B-19
1976	*Cryptosporidium parvum*
1977	Ebola virus
1977	*Legionella pneumophila*
1977	Hantaan virus
1977	*Campylobacter jejuni*
1980	Human T-lymphotropic virus I (HTLV-I)
1981	Toxin producing strains of *Staphylococcus aureus*
1982	*Escherichia coli* O157:H7
1982	HTLV-II
1982	*Borrelia burgdorferi*
1983	Human immunodeficiency virus
1983	*Helicobacter pylori*
1985	*Enterocytozoon bieneusi*
1986	*Cyclospora cayetanensis*
1988	Hepatitis E virus
1989	*Ehrlichia chaffeensis*
1989	Hepatitis C
1991	Guanarito virus
1991	*Encephalitozoon hellem*
1991	New species of *Babesia*
1992	*Vibrio cholerae* O139
1992	*Bartonella henselae*
1993	Sin nombre virus
1993	Encephalitozoon cuniculi
1994	Sabia virus
1995	HHV-8
1999	Nipah virus
2002	SARS virus

The table highlights the organisms that are of public health importance and their year of discovery
Source: World Health Organization: Newly discovered organisms of public health importance: Page 6. From WHO Regional Office East-Asia: Combating Emerging Infectious Diseases in South East Asia Region (2005)

how the advent of new technologies is bringing about a change in medical treatment of the infectious diseases. For the use of "omics" technology to be successful requires considerable information of pathogen genome as well as genome information of the host. The pathogen genomic and proteomic information helps to identify antigens that can give us information necessary for making a diagnostic tool and vaccine design. The pathogen genome on one hand gives us the information about the important genes conferring disease pathogenesis as well as drug resistance, while the genome of the host on the other hand will reveal the susceptibility genes, and the further knowledge of polymorphisms in genes of the host metabolic and immune system will lead to the new vaccine strategies, drugs targets, and also their treatment outcomes.

Rapid advances of biotechnological and informatics tools in the past few decades mainly in fields of genetics, genomics, and proteomics are leading the way in identifying treating and thus improving the health of human beings. The effective treatment in a patient can only be achieved by first rapid diagnosis of the disease and also identifying its causative agent that is particularly important in the cases of infectious diseases. New insights gained by the analysis of genome and structural feature of pathogen macromolecules have brought about new hope in the treatment of the dreaded diseases. The knowledge of system biology in respect to the microbial infections is still in development, and data available is mostly for few human infections. The rapid development of new generation sequencing technologies have led to generation of new knowledge base and with more advancement of such technologies in the coming years has brought in a hope that all diseases will conquered. In the near future, we will have complete sequences of the total transcriptomes, like genome sequences a decade earlier, and proteomic technologies will attain the throughput and sensitivity of microarrays. Other technologies like metabolomics, glycomics, lipidomics, and phosphoproteomics when referred to in the context of infectious diseases are still in various stages of development, but we are taking the right steps in the direction of development of such technologies (Antony et al. 2012).

2   Host-Pathogen Interactions: Technologies Enhancing Our Understanding

The technologies of transcriptomics and functional genomics are transforming our understanding of microbial infections and helping us decipher the reason of infections susceptibility in the humans.

2.1   Transcriptomics in Infectious Disease

Transcriptomics have been developed and used by scientists to broaden our understanding of infectious diseases. To elucidate the host-pathogen interaction, cDNA microarrays have been widely used. The studies have focused on how the pathogens effect the host cell gene expression. The wild-type virulent strains and isogenic mutants have been used to gauge the responses of the host cell. The major findings of these studies have shown how the pathogen virulence factors modify host cell factor expression (Roy and Mocarski 2007). The role of pathogen recognition receptors (PRR) affecting host-pathogen interaction has been studied. These studies have shown that the host cell responses have an alarm signal (Jenner and Young 2005). Studies have also shown that gene cluster signals are responsible for generating the alarm signals that are the target of attack by the invading pathogens (Hamon and Cossart 2008). Array technologies are being used with molecular probes of host human (also animals/plants) and microbial genes to monitor and at the same time point the expression of genes from host cells and those of the pathogens to better understand the full complexity of host-pathogen interaction.

2.2   Functional Genomics in Infectious Diseases

Functional genomics have also led to the development of tools to decipher infectious diseases

by manipulating the cellular mechanisms. The technology of the RNA interference (RNAi) has undergone tremendous development in the last decade which has led to the large-scale reverse genetic screens in human cells and model organisms (Boutros and Ahringer 2008). RNAi technology uses double-stranded ribonucleic acid (dsRNA) with having complementary sequence to the target mRNA sequence and is used to silence or downregulate the gene expression of its target. Long dsRNA induces interferons or other unspecific responses in mammalian cells which is avoided by the use of small interfering dsRNA (siRNA) directly or small hairpin RNA (shRNA). RNAi screening using RNA probes, which induces loss of function of host genes, leads to discovery of host resistance factors (HRF). It is made possible when silencing this restrictive factors leads to invading pathogen replication enhancement and may also identify host susceptibility factors (HSF) and also identify permissive factors, that when silenced will decrease the pathogen replication. The RNAi screens still have some limitations due to off-target siRNA effect (Echeverri et al. 2006). Thus, the primary screening validation is made by using additional siRNA screens. From the several RNAi screenings in human and in fruit fly cells, only 300 host factors were validated from about 10,000 and 20,000 targets identified in the initial screen (Agaisse et al. 2005; Brass et al. 2008; Konig et al. 2008; Krishnan et al. 2008; Zhou et al. 2008).

To make the system biological tools like RNAi more effective in finding mechanisms in host-pathogen interaction and thus finding cure for the microbial infections, there is a need for integrations of all data obtained from the omics technologies. In addition several rounds of biological experimentations are required by using mutant pathogens, cellular RNAi knockdowns, or humanized animal models using mice or primate infection model. The resulting inferences from the validated data would help us build predictive models which could lead us to the better understanding of pathogen interactions with the host.

2.3 Susceptibility to Infection Is Determined by Host Genes

It is evident from human history of infectious diseases that not everybody in a given population is affected by an infective disease. For an infective organism to cause an infection, both the virulence of the pathogen and the host susceptibility are important. The identification of genetic factors of host innate and adaptive immunity that determine the protection from pathogen is an important endeavor of scientists. Animal models of infectious diseases especially mouse models have been used to find the genetic factors and biochemical mechanism of disease susceptibility (Marquet and Schurr 2001). The identification of candidate genes responsible for disease susceptibility or resistance and the occurrence of genetic polymorphism in them give us the best possible biological scenario of the disease. Researchers have found that in bacterial diseases, tuberculosis and leprosy seem to have similar genetic susceptibility determinants in the host as exemplified from the finding that higher incidence of these diseases was found in the monozygotic twins than in dizygotic twins and siblings (Abel et al. 1995; Vidal et al. 1995). Mouse model of infections has revealed that gene encoding the natural resistance-associated macrophage protein 1 (*Nramp1*) confers natural resistance to infections caused by *Mycobacterium*, *Salmonella*, and *Leishmania*. *Nramp1* is found in the membrane of the phagosome of the macrophages where it seems to be probably affecting the replication of the infecting intracellular bacterium (Gruenheid et al. 1997). The genomic analysis in humans has found a similar gene to that of mice which is also having similar pattern of expression. Hence, it has been inferred that humans too carry similar susceptibility gene. There have been further studies which have shown that polymorphisms found in the *Nramp1* gene are related to the infectivity of leprosy and tuberculosis (Abel et al. 1998). In one of the studies, it was found that persons who carry an *Nramp1* heterozygous variant will be four times more likely to be infected by tuberculosis than persons who are

carrying more common variants of the *Nramp1* (Bellamy et al. 1998).

Cell-mediated immunity plays an important role in the context of tuberculosis and is well studied. Research has further gone ahead to find links between tuberculosis susceptibility and polymorphism in the gene coding for receptors of interferon-γ and interleukin-12, which are cytokines belonging to T-helper cell type 1 (Th 1). The absence of functional copies of either of these genes in families and of isolated patients leads to high susceptibility to *M. tuberculosis* infection (Jouanguy et al. 1996, 1997; Newport et al. 1996; Altare et al. 1998).

The highly polymorphic human leukocyte antigen (HLA) system is the name of the major histocompatibility complex (MHC) in humans. The HLA class I glycoproteins are highly expressed on the surface of every nucleated human cell, and they present endogenous peptides derived from the cell to the cytotoxic T cells. HLA class I glycoprotein plays a major role during the viral infection as it presents intracellular viral peptides on the surface which leads to cell-mediated immune response, which further leads to the destruction of virus-infected cells. HLA class II glycoproteins which are present on the surface of antigen-presenting cells (APCs) on the other hand present 9–14 amino acids long peptides that are derived from the engulfed pathogen and displayed on the surface which then are recognized by T cell as foreign antigens, and it will elicit an immune response to the antigen. The length of antigen as well as composition are important in deciding if the antigenic peptide will bind to the antigenic peptide-binding cleft. Polymorphisms occur almost solely in the peptide-binding cleft of HLA class I and II in the glycoproteins. The diversity of HLA-binding region ensures that some pathogenic peptides will be preferentially presented compared to the others. Thus, in a given population, the HLA diversity ensures the advantage that some of the HLA glycoprotein peptide-binding clefts will be able to bind and present the pathogenic antigen peptide which will lead to an immune response to any invading pathogen. This ensures the survivability of the species against an infection.

Thus, genomic studies have focused on the identification of susceptibility genes which would lead to the better management of infectious diseases in the population. The tuberculosis susceptibility has been associated with HLA class II genetics. The association is evident from the studies that have been found between pulmonary tuberculosis and class II HLA antigens in several populations (Marquet and Schurr 2001).

As is now clear, the knowledge of the mechanism of action of the pathogen and the identification of the susceptibility genes goes a long way in the management of the disease in context of a public health perspective to prevent, diagnose, identify, and target the vulnerable populations against a given infectious disease.

3   Pharmacogenomics in Infectious Disease Management

The requirement of the genomic information of both the host and pathogen is important to fully carry out infectious disease management. The first sequence map of human genome being completed in June 2000 (Lander et al. 2001; Venter et al. 2001), followed by discovery of genome-wide single-nucleotide polymorphisms (Sachidanandam et al. 2001) and further genomic sequencing of several pathogens by institutes like the J. Craig Venter Institute (www.jcvi.org) gradually opened the pathway to create new treatment and disease management methods. The ultimate aim of genomic technologies to bring personalized medicine for every infectious disease scenario is still decades away, but here we will focus only on important breakthrough which has shown us the way forward in respect to infectious disease identification and treatment.

3.1   The Use of Pathogen Genome for Antigen Identification

Neisseria meningitidis is a bacterium that can cause meningitis and other forms of meningococcal disease such as meningococcemia, a life-threaten-ing sepsis (www.cdc.gov/meningococcal/).

N. meningitidis is a major cause of morbidity and mortality during childhood in industrialized countries and has been responsible for epidemics in Africa and in Asia. *Neisseria meningitidis* serogroup B is responsible for causing about one third of infection. The genome sequence of *N. meningitidis* has opened a new way for disease management (Pizza et al. 2000; Tettelin et al. 2000). The vaccine that was used only contained capsular polysaccharides from the serogroups A, C, Y, and W135 only. Serogroup B polysaccharide contained elements that resemble human polysialic acid and hence is poorly immunogenic and might generate autoantibodies (Hayrinen et al. 1995). In this scenario the *N. meningitidis* serogroup B was sequenced, and 350 potential antigens from the serogroup B were expressed in *Escherichia coli* to find the potential vaccine candidate. The expressed proteins are injected into mice to find immunogenic antigens that can be developed into a vaccine. Similar strategies are being used to find vaccine candidates for other serotypes of Neisseria species and for other pathogenic organisms.

Pathogen genomic information is also being used to find the immunologically important peptides for cytotoxic T lymphocytes (CTLs) epitopes. The response of CTLs is to seek out virally infected cells by recognizing the peptides presented by human leukocyte antigen (HLA) glycoproteins on the cell surface and killing the infected cells. CTL epitopes are the viral peptide that is presented by the HLA and recognized by CTLs. The peptides are of the length of 10 amino acids, and genome sequence is used to find out and synthesize these peptides for immunogenic evaluation. Amino acids are divided into segments of peptide, measuring 10 amino acids in length and overlapping the previous peptides 9 amino acids, for example, West Nile virus genome translates into 3,433 amino acids which can be segmented into 3,424 peptides that are 10 amino acids in length.

Immunoinformatics, a field of bioinformatics, is speeding up the finding of CTL epitopes for the scientist working in the field. Algorithms on computer softwares are being used to match the viral peptides with the HLA glycoproteins in silico for binding based on previously known results and are being tested (De Groot et al. 2001). Informatics based algorithms help eliminate

99 % of the peptides that would not be used in the experimental screens. Thus, the time and effort to screen for the CTL epitopes have been reduced drastically. CTL epitopes may be used for making subunit-based vaccines and diagnostic tests. Virus-specific antibody found using CTL epitopes can be used in enzyme-linked immunosorbent assay. It may be even possible to use CTL epitopes to test for the antigen itself.

3.2 Genomic Information for Identifying Infectious Pathogens

Basically as of now only four types of molecular diagnostic tests are carried out to detect infection in laboratory setup. First is by direct detection where the pathogens can be detected directly by imaging technologies of microscopy and cell culture. Second method of diagnosis is by the detection of proteins produced by pathogens by the use of specific antibodies, like that used in enzyme-linked immunosorbent assay (ELISA). Third method is by detection of the specific antibodies IgA, IgM, and IgG against the pathogens and the changes in their titers using antibody capture assay. Fourth method uses detection of nucleic acid of the pathogens and amplifying their signal using techniques like polymerase chain reaction. Latest diagnostic technologies have been developed on these basic four biotechnological technologies (Speers 2006).

Pathogen genome can also be used to identify the infecting organism itself. Microbial DNA in the clinical specimen can be used to identify the disease-causing pathogens. Human immunodeficiency virus (HIV), hepatitis virus, *Borrelia burgdorferi* (causative agent of Lyme disease), and mycobacteria are few examples of pathogens that can be identified by their genomic sequences. *Mycobacterium tuberculosis* antimicrobial resistance strain-caused infections are becoming quite common, and genomic information has deciphered few potential candidates like *katG* gene mutations in resistant strains (Siqueira et al. 2009a, b; Marahatta et al. 2011). The traditional culture test for mycobacterium which is time-consuming and less sensitive is giving way

to restriction fragment length polymorphism (RFLP), a specific technique used in DNA fingerprinting (Van Soolingen 2001). The technology uses restriction enzymes that cut DNA at the places having certain particular nucleotide sequences. The nucleotide pattern that is obtained is then compared to the previously identified specific nucleotide pattern of the genome of the pathogen DNA. DNA patterns can be separated on the basis of length, and the pattern of DNA fragments in the DNA fingerprint is characteristic of particular isolate, and each particular pathogen has a unique pattern. DNA fingerprint technology is faster and reliable than culturing of the mycobacteria, ideal for discovering new drug-resistant strains from unique genomic sequences of each mycobacterium. The technology is very useful for identification of strains during the time of outbreaks and further epidemiological studies.

The knowledge of viral load in patients is also important for dosage determination in drug therapy; hence, detection of the viral pathogenic DNA and RNA in clinical specimens is of paramount importance. Treatment of viral diseases like HIV, chronic hepatitis B, and hepatitis C often depends on the knowledge of viral load (Revets et al. 1996). For example, HIV viral loads are detected by enzymatic amplification of the viral nucleic acid and detection of the signal from the labeled probes that hybridizes with them. The signal usually is either a color signal conjugated to the probe or a chemiluminescent probe, and the intensity of the signal corresponds to the number of copies of the nucleic acid RNA. Capillary electrophoresis detects hybridized probes at a very high sensitivity with detecting as low as 2,000 copies of HIV RNA in milliliters of plasma (Kolesar et al. 1997).

3.3 Pathogen Genomic Information Determines Antimicrobial Resistance

The rampant uncontrolled use of antimicrobials has led to increased number of antibiotic bacterial strains. Genomic mutations allow the certain bacterial strains to overcome the effects of antimicrobials and are able to propagate in spite of the presence of antibiotics. The pathogenic bacteria have started showing resistance and have become a major problem for human health. *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa,* and *E. coli* are a few examples of such bacteria.

Mechanisms of gaining resistance have been elucidated by means of omics technologies. Here are few examples. Fluoroquinolones are drugs that act on the bacterial DNA replication by binding to bacterial enzymes involved in bacterial DNA replication, that is, DNA gyrase and topoisomerase. The bacterial resistance to quinolone occurs due to mutation in the quinolone-binding site in the enzymes mentioned above. The mutation leads to change in the amino acid at the site of binding of fluoroquinolones to the enzymes. If both the bacterial enzymes are mutated, then high-level resistance occurs to the quinolone drug affecting the treatment of infection as compared to when either of the enzymes is mutated (Hooper 2001).

Now genetic test is available to detect antimicrobial resistance in the infecting pathogens. The information is important because it would lead to a better treatment management of the infection. The methicillin-resistant *Staphylococcus aureus* phenotype is detected when cultured in the presence of oxacillin after a period of 24 h. Before the era of omics technology, the only means of resistance detection was by culture test which is a very time-consuming test. Methicillin resistance in *S. aureus* is controlled by alternations of penicillin-binding protein PBP2a. Gene *mecA* controls the production of PBP2a. Polymerase chain reaction test is used to detect the presence of *mecA* in reference laboratories, while commercially developed kit can detect the same using a fluorescein-labeled *mecA* probe. Both DNA probe and PCR technology when used for analysis can detect *mecA*-resistant gene in a given sample in less than 3 h. The rapid detection of antimicrobial resistance in pathogens helps patients in providing adequate treatment opportunities (Louie et al. 2000).

3.4 Genomic Factors Determine Response to Therapy in Infectious Diseases

The study of the host genome becomes important to fully understand the drug effects and as such design more effective methods of treatments. Although the ultimate goal is to decipher the system biological effect, the trend of single gene effects is also very important.

Cytokines play a very important role in human immunity (Paul and Seder 1994). In hepatitis C infection, interferon alpha is used to stimulate cell-mediated immunity against the viral infection and is the primary treatment. However, studies have shown that response to interferon-alpha treatment is only 50 % in some cases even when combined with other antiviral treatment (Manns et al. 2001). Further studies have shown that if chronic hepatitis C patients have IL-10 polymorphism variant, it leads to the reduction in expression of IL-10 itself, and they will have five times more chance of effective treatment with interferon alpha than those who do not carry the polymorphism (Edwards-Smith et al. 1999).

Interleukin-10 (IL-10) is a polymorphic cytokine and is a T-helper cell type II (Th2) cytokine that is associated with the induction of the production of large amount of antibodies in body's immune response. Th1 cytokines which promote cell-mediated immunity inhibit Th2 response and vice versa. Thus, people with high-expressing IL-10 genotype if infected and suffering with chronic hepatitis C infection are less likely to respond to interferon-alpha treatment. New treatment regimes have to be developed for patients suffering from chronic hepatitis C infection and carrying IL-10 polymorphism associated with high cytokine expression.

Vaccine responses can be used as a system of gauging the state of immune system (Poland 1999a, b). Vaccines are administered to large number of population as an integral part of public health system. Vaccines are used to mimic the infective disease conditions that induce immunological memory to protect the individual against subsequent exposure to the pathogen and lead to prevention of disease. The phenomenon to gain protective immunity against a pathogen upon being vaccinated for the particular pathogen depends on individual genetic build. As studies have shown, not all healthy individuals are able to generate a protective immune response upon vaccination. It has been observed in the case of measles vaccination that only 10 % of the population was seronegative and clustered in family (Poland 1999a, b; Poland et al. 1999c). Both HLA I and HLA II class alleles have been responsible for the measles vaccine response, while HLA-B7, HLA-B51, HLA-DRB1*13, and HLA-DQA1*01 are associated with positive measles vaccine response, and HLA-B, HLA-DR, and HLA-DQA1 have been responsible for the vaccine being noneffective (Hayney et al. 1996, 1998; Poland et al. 1998).

4 Infection Treatment: Response to Drug Treatment Determined by Host Genomics

Drugs used for targeting any pathogenic infection can only be successful if we are aware how it is affecting the host and pathogen at genomic level and hence are able to explain the host efficacy and toxicity. We look at few important infectious diseases where pharmacogenomic research has been bringing a landscape change in the disease treatment.

4.1 Leishmania: Pharmacogenomics in Disease Management

4.1.1 The Disease

Leishmaniasis is a very complex major tropical infection transmitted by the vector Sand fly is all right. The infection is caused by intracellular protozoan parasites of Leishmania genus. There are more than 20 species of *Leishmania*. The type of infective species, virulence factors, and host immune responses and depending on the clinical symptoms, the disease is categorized into cutaneous leishmaniasis (CL) and visceral leishmaniasis (VL). VL is also known as kala-azar; the origin of the name is from the eastern and

Fig. 27.1 Geographical distribution of visceral leishmaniasis in the Old and New World (*Source: World Health Organization:* URL: http://www.who.int/leishmaniasis/leishmaniasis_maps/en/, Data source and Map production with permission of WHO headqauters: WHO/NTD/IDM HIV/AIDS, Tuberculosis and Malaria (HTM) World Health Organization, October 2010, Accessed February 2013)

northeastern part of the Indian subcontinent where the disease is endemic. Depending upon the place where one has acquired the infection, CL is further categorized into "New World" from Central America and South America and "Old World" if from Asia, Middle East, Africa, or southern Europe. More than 1–1.5 million cases of leishmaniasis occur worldwide (about 80 countries are affected) with major countries being the developing nations of Asia, Africa, and Latin America (www.who.int/topics/leishmania-sis/en/).

Species of Leishmania are several causing different clinical manifestations of the infectious disease. *L. donovani* produces primary cutaneous disease as well as gives rise to visceral leishmaniasis (VL) and also post-kala-azar dermal leishmaniasis (PKDL) that is manifest after the treatment of the initial visceral disease. Visceral leishmaniasis main causative pathogen is the *L. donovani* complex with Old World VL

disease being caused by the species *L. donovani and L. infantum,* and New World disease is mainly caused by different species of *L. chagasi* (Fig. 27.1).

Old World CL causative Leishmania species are *L. tropica* which are mainly found in urban areas and *L. major* being prevalent in the desert areas, while the New World CL disease is caused by *L. mexicana complex* (includes *L. mexicana, L. amazonensis,* and *L. venezuelensis*) and *Leishmania (Viannia subgenus) braziliensis complex* (*L.(V.) braziliensis, L.(V.)colombiensis, L.(V.) panamensis, L.(V.) guyanensis, and L.(V.) peruviana*). In the cases of infection caused by *L. braziliensis complex,* there is always a chance that the infection dissemination to mucosal region can occur to give rise to mucocutaneous leishmaniasis (MCL) (Herwaldt 1999).

The complex disease is manifested due to multiple factors ranging from environmental factors such as time and number of exposure with

infected vector sand flies, species of the infecting Leishmania pathogen, to host genetic factors that include immune status of both innate and adoptive immune systems that determine the clinical outcome of the disease. Other reasons for *Leishmania* disease susceptibility are malnutrition, immunodeficiency with HIV coinfection, and young age. The protection against invading pathogenic Leishmania protozoa and even the curative resolution of the disease is provided by Th1 cytokine response involving cytokine interferon gamma (IFN-γ), interleukin (IL)-12, and tumor necrosis factor alpha (TNF-α), whereas Th2 response cytokines IL-10, transforming (TGF)-β, and IL-4 have been implicated in increasing susceptibility to the disease in the experimental animals (Reed and Scott 1993; Sacks and Noben-Trauth 2002). Nonhealing lesions and diffused lesions in CL have been implicated to Th2 response, while self-healing lesion has been associated with Th1 response (Melby et al. 1994). However, in some situation IL-4 (a Th2 cytokine) has been implicated to induce IL-12 production and lead to Th1 cytokine response, and it has also been found in some cases that Th2 response occurs independent of IL-4 (Alexander and Bryson 2005; Mansueto et al. 2007). Leishmania infection is a complex infection depending on host factors as well as strain polymorphism. *Leishmania mexicana* cysteine proteases which target IL-12 that prevents Th1 protective response (Buxbaum et al. 2003), while the Leishmania analogue of activated C kinase (LACK) from the *Leishmania major* induces Th2 response that leads to host parasitization (Kelly et al. 2003). Polymorphism of *L. braziliensis* also affects disease outcomes (Cupolillo et al. 2003; Schriefer et al. 2004). PKDL is a complication arising after treatment of VL, affecting 50 % of VL patients in Sudan (study carried out in United Sudan) and also 5–10 % patients in India. PKDL has been found to be associated with increased levels of IL-10 (Zijlstra et al. 2003; Ganguly et al. 2008).

4.1.2 Disease Treatment

The major treatment regime of CL which has propensity of dissemination towards VL and MCL is with parenteral antimonials like sodium stibogluconate or meglumine antimoniate, pentamidine, and oral miltefosine (Olliaro et al. 2005; Ameen 2007; Amato et al. 2008), whereas CL with low risk of spread is treated with local and physical therapies such as intralesional antimonials, topical paromomycin, cryotherapy, and thermotherapy or by oral azoles. However, when the disease progresses to MCL, treatment is prolonged, and toxicity from such long-duration drug use is a common occurrence (Marsden 1986; Amato et al. 2008).

Vaccine is still elusive in the case of Leishmania. Some trials with DNA vaccines do have shown a way forward. These vaccines have shown the promise to be effective as they have been able to induce 1L-12 production, which was in response from the persistent antigen exposure from the DNA vaccine (Requena et al. 2004). In Venezuela killed *Leishmania* promastigotes along with bacillus Calmette-Guerin (BCG) used as immunotherapy have shown results with a high cure rate in clinical trials by inducing Th1 response (Convit et al. 2003). *L. major* vaccine trial with BCG and parenteral antimony combined have been successfully used for treatment of PKDL (Mansueto et al. 2007). The search for effective vaccine for Leishmania had got a boost with knowledge from genome sequence data of several Leishmania strains. More vaccine candidate genes will be evaluated in the future (Stober et al. 2006).

4.1.3 Genetic Susceptibility and Pharmacogenetic Implications

In the absence of an effective vaccine with recurring infection such as PKDL, dissemination infection to mucosa leads to aggravating of the disease. Prolonged treatment with parenteral antimonials that give rise to high-level risk of toxicity with high morbidity and mortality from the disease is a problem of concern (Convit et al. 2003; Muse et al. 2008). The new technologies are trying to address these very problems. Epidemiological studies in different ethnic populations in several countries have shown the variation susceptibility to the disease be it CL, MCL, or VL which caused different strains of Leishmania. Variation in disease presentation and progression, familial clustering, in a

population indicates the underlying genetic reason of susceptibility. Recent studies have shown that *L. donovani* cause of CL has been found to affect exclusively a particular ethnic group in Sri Lanka (Samaranayake et al. 2008). *L. donovani* though normally associated with causing VL is shown in few places, Kenya, Yemen, Cyprus, and the Himalayan region of North India, and is the main causative pathogen of CL (Mebrahtu et al. 1993; Pratlong et al. 1995; Sharma et al. 2005; Antoniou et al. 2008).

To deduce the genetic susceptibility of the Leishmania disease, experimental murine animal models along with clonal parasite line (to control environmental variable) have been used to find the genes responsible for disease progression along with their human homologues of disease susceptibility (Handman et al. 2005). First genes that were used to deduce from such analysis in murine model were *NRAMP 1* and the H-2 locus had been implicated in *L. donovani* infection (Blackwell et al. 1980). HLA class II antigen HLA-PQ3 is found to be associated with CL in Venezuela (Lara et al. 1991) and MCL in Brazil caused by *L. braziliensis* (Petzl-Erler et al. 1991). PCR genotyping studies in Mexico on Leishmania patients has found an association with HLA class II genes with Cutaneous Leishmaniasis (CL) (Olivo-Diaz et al. 2004). High blood TNF has been found to be associated with MCL (Castes et al. 1993) and acute VL (Barral-Netto et al. 1991a, b). A Venezuelan study has implicated that allele 2 of TNF-β polymorphism with high risk of developing MCL caused *L. braziliensis* and higher frequency of allele 2 of TNF-α polymorphism was also associated with MCL (Cabrera et al. 1995). In Brazil by using family-based disequilibrium test analysis (TDT), investigation has shown that TNF polymorphism has been linked to *L. chagasi* infection (Karplus et al. 2002). In asymptomatic patients having positive skin test, *L. chagasi* has been associated with TNF-1 allele of TNF-α gene, while in case of symptomatic *L. chagasi* VL patients, TNF-2 allele is implicated. Due to parasite heterogeneity, this TNF polymorphism association has not been correlated to infection by other Leishmania species such as in *L. infantum* VL (Meddeb-Garnaoui et al. 2001) and *L. major* CL (Kamali-Sarvestani et al. 2006). Variation in

promoter of *IL-4* and IFN-γ gene polymorphism has been found to be linked to *L. major* CL disease susceptibility and progression respectively in an Iranian study, while in a Sudanese VL patient study, *IL-4* polymorphism has been shown to increase disease susceptibility (Mohamed et al. 2003). Polymorphism in promoter region of *IL-10* gene leads to higher IL-10 production which has been shown to increase the risk of having skin lesions during an infection of *L. braziliensis* (Salhi et al. 2008). IL-6 can diminish the high Th1 proinflammatory response that occurs when *L. braziliensis* CL progresses to MCL (Hatzigeorgiou et al. 1993; Bacellar et al. 2002). IL-6 polymorphism plays an important role in the progression of *L. braziliensis* CL to MCL, and this finding is important since their genetic markers have high prognostic value (Castellucci et al. 2006).

Genome-wide linkage have been performed for *L. donovani*-infected VL patients in Artinga ethnic group in Sudan to help identify loci on chromosome 22q12 and is associated with disease susceptibility genes (Bucheton et al. 2003a, b). IL-2 receptor β chain (IL2RB) gene is present in the highly susceptible loci on chromosome 22q12 that was identified from this study. IL-2 receptor has been detected in high levels during VL infection and plays a critical role in T cell genetic responses (Barral-Netto 1991b). Further studies have shown *IL2RB* polymorphism in association with *L. donovani* VL (Bucheton et al. 2007).

Another candidate gene found is *SLC11A1* (formerly NRAMPI) on chromosome 2q35, an innate resistance gene that regulates macrophage activation and contributes to increased VL risk in Sudanese population (Bucheton et al. 2003a, b; Mohamed et al. 2004) as well as increased susceptibility to several intracellular pathogens (Blackwell et al. 2001). Other studies have shown that genotypes having significantly high level of mannan binding lecithin occur more prominently in patients with clinical VL. An opsonin, mannan-binding protein, is known to enhance pathogen infection. Polymorphism in mannan-binding gene has been shown to increase risk of developing *L. chagasi* VL in Brazilian study population (Alonso et al. 2007). In PKDL there is elevated level of IFN-γ. Polymorphism of

IFN-γ receptor 1 from study in Sudan has been implicated in PKDL (Salih et al. 2007). The IFN receptor expression is important for the activation of macrophages via IFN-γ.

Drug treatments are not very efficient in the treatment of leishmaniasis disease; more effective treatment regimes can be developed by thoroughly knowing the genetic factors that lead to disease progression. Thus, unnecessary drug use and adverse reaction can be avoided. As various genetic susceptibility studies have shown, cytokine response determines the disease progression in leishmaniasis. Role of IL-10 in pathogenesis of leishmaniasis is known and is well established, and IL-10 polymorphisms have shown to increase risk of lesions in *L. braziliensis* infection. In a study with *L. guyanensis* infected CL patients from French Guiana, high level of mRNA IL-10 within lesions leads to poor chemotherapy response and treatment failure (Bourreau et al. 2001). It is hypothesized that IL-10 might be regulating the response to chemotherapy by blocking the Th1 response. The increased level of IL-10 has been linked to the active VL (Nylen and Sacks 2007) and PKDL (Saha et al. 2007) and also associated with persistent CL infection occurring from *L. major* (Melby et al. 1996) and *L. mexicana* (Louzir et al. 1998). Success of VL treatment with amphotericin B and the complete elimination of IL-10 are associated with one another (Saha et al. 2007). On the other hand, MCL is associated with low IL-10 receptor expression and low IL-10 secretion that decrease the ability for modulation of proinflammatory response (Faria et al. 2005).

Progress has been made to find the susceptibility genes and will provide further insight into disease pathogenesis and will lead to progress in the field of diagnostic markers, drug targets, and vaccine development to control, treat, and eradicate leishmaniasis.

4.2 Improving the Treatment of Malaria by Pharmacogenomics

4.2.1 Disease Burden

Malaria is vector-borne (mosquito) disease that has been one of the top causes of mortality in the world for generations especially in tropical countries of Asia and Africa. Even after renewed global efforts, still there is high infectivity and mortality. Three billion people are at risk with 1–2 million deaths attributed to malaria each year (www.who.int/topics/malaria/en/). Four species of protozoan parasite are involved from genus *Plasmodium,* i.e., *P. falciparum*, *P. vivax*, *P. malariae,* and *P. ovale*. These malaria-causing combination parasitic species occur in human population and occur in infected individuals (Gurarie et al. 2006). In respect to prevalence, virulence, and multidrug resistance, *P. falciparum* has been a major cause of mortality and morbidity. *P. falciparum* accounts for about 80 % cases of malaria in Africa (Roca-Feltrer et al. 2008). Next to it is *P. vivax* which causes 100–300 million cases annually (Price et al. 2007). The most commonly used drugs are chloroquine (CQ) and sulfadoxine-pyrimethamine (S-P Fansidar®) that are becoming less effective due to the development of resistance in malaria parasite by *P. falciparum,* and the species has become predominant and become a threat to travelers and people alike (Schlagenhauf and Petersen 2008). In the absence of vaccine and in addition, development of resistance even in the mosquito vector control against chemical methods using insecticides has thrown new challenges for the researchers (Greenwood et al. 2008) (Fig. 27.4a).

4.2.2 Malarial Therapy

Some of the recent developments in malarial treatment using pharmacogenomics are bringing about improvements in the efficacy of treatment regime of malaria. Current treatment regimes have recommended artemisinin combination treatments (ACTs) in cases of uncomplicated falciparum malaria in nonpregnant adults (Lin et al. 2010). The drug regime is highly efficacious and has reduced development to resistance. In cases of uncomplicated malaria, the ACT is being used in 88 countries by 2009. In the coming years, a number of patients including women and children will be brought under ACT therapy regime as per World Health Organization.

Like the treatment of HIV and tuberculosis, combination therapy is now being used for malaria treatment too, which reduces resistance among

the highly efficacious drug the artemisinins which rapidly eliminate the parasite from blood and thus limit the number of parasites so that the other more bioavailable drugs given in combination act on the parasite. Unrelated mode of action of two or more combination drugs also reduces the chances of resistance (Yeung et al. 2004).

4.2.3 Pharmacogenomics Way Forward to Effective Treatment

Among many other factors which contribute to drug effectiveness, malarial drug bioavailability and tolerability are depended upon the host metabolic mechanisms. The severe drug reaction to primaquine in the 1950s used in antimalarial treatment was instrumental in the discovery of glucose-6-phosphate dehydrogenase (G6PD) deficiency in 1956; thus, importance of the use of pharmacogenetics in malarial treatment was realized (Alving et al. 1956). The polymorphism leading to variation G6PD or even its deficiency is a grave problem in designing the effective drugs. Even now during malarial terminal prophylaxis to decrease transmission, primaquines are administered. Thus, the G6PD status of patient becomes quite important (Luzzatto 2010).

Knowledge of both the host and parasitic genetics is necessary to designing drugs and dosage for effective treatment regimes. Parasitic genetics helps us in deciphering the modes of resistance, and host genetics help us in giving the information about host drug bioavailability and explain adverse reaction to the drugs. G6PD polymorphisms and genetic variation in CYP2C8 can play pivotal role in point of care diagnostics, but these genetic testings will have to be incorporated into the laboratories and national health programs. The knowledge of this important genetic variations in population would ultimately reduce cost and make the treatment regime more effective and with lesser adverse reaction and ultimately reduce the suffering of the patients.

The pharmacogenetic drug policy in context of malaria is slowly becoming a reality as per efforts of the WHO and other agencies. Genetics is becoming a guide to new drug policy. Amodiaquine was generally known to be tolerated in malarial treatment, but later when it was found in the Caucasian population during the decades of 1980 and 1990 to be responsible to cause agranulocytosis with fatalities and also cause hepatotoxicity (Hatton et al. 1986; Raymond et al. 1989; Phillips-Howard and West 1990), the drug was first removed from the list of essential drugs against malaria but then had to be added back to the list as alternate drugs started showing resistance. Amodiaquine induced adverse reaction in individuals was attributed to genetic make up of the individual. The genotypes of individuals harboring CYP2C8, CYPIA1, and CYP1B1 have been reported in studies to show immunogenic adverse reaction to amodiaquine (Li et al. 2002; Kerb et al. 2009). Some population in Africa has shown hepatotoxicity and leucopenia with only two doses given 3 weeks apart (Orrell et al. 2008).

Amodiaquine when administered to an individual with reduced CYP2C8 activity impairs the metabolism of the drug and hence leads to the cause of hepatotoxicity and agranulocytosis. Other common variants of the enzymes CYP2C8*2 and CYP2C8*3 have been associated with decrease in the metabolizing activity of CYP2C8 enzyme as is evident from studies with anticancer drugs (Dai et al. 2001). Individuals with CYP2C8*3 genotype have no CYP2C8 enzymatic activity in vitro (Parikh et al. 2007). In a study from Burkina Faso, patients carrying CYP2C8*2 genotype showed common adverse effects to Amodiaquine and in addition patients have also reported to experience more abdominal pain when compared to healthy individuals. The study from Burkina Faso and Ghana could not clearly establish the relation between drug efficacy and CYP2C8 genotype (Adjei et al. 2008). Though the inactivated gene of CYP2C8 is not very high in population, estimates have shown that CYP2C8*2 and CYP2C8*3 occur in about 2.1 % of the population in Zanzibar, United Republic of Tanzania, which was about 30,000 patients of the total malarial patients ~100,000 (Cavaco et al. 2005). In Ghana it was found that 1.5 % of the population has been estimated to have metabolic variants of CYP2C8. Hence, due to high disease burden, the study of pharmacogenomics for drug metabolism was carried out in large patient samples from the population to get a clear correlation between genotype and efficacy of drug treatment as well as adverse reaction.

Fig. 27.2 World map showing frequencies of the CYP2C8*2 allele in different populations. x=allele frequency in reference population (US Caucasians), y=allele frequency in country with data analysis refer (*Source:* Roederer et al. (2011), Map by: Pharmacogenetics for every nation initiative: Accessed Feb 2013)

Fig. 27.3 World map showing frequencies of the CYP2C8*3 allele in different populations. x=allele frequency in reference population (U S Caucasians), y=allele frequency in country with data analysis refer (*Source:* Roederer et al. (2011), Map by: Pharmacogenetics for every nation initiative: Accessed Feb 2013)

These pharmacovigilance studies further reduced the effective treatment cost incurred on public health both monetary and from the point of view suffering of the patients (Figs. 27.2 and 27.3).

4.2.4   Pharmacogenomics of ACTs

Major active antimalaria metabolite of artemisinin is dihydroartemisinin (DHA) (Ilett et al. 2002). Artesunate is rapidly converted to its active metabolite catalyzed via CYP2A6 which is a major enzyme; conversion to DHA also includes minor enzymes CYP2B6, CYP1A1, and CYP1A2 (Li et al. 2003). CYP2A6 has about 40 variant forms of which at least 13 have been implicated as slow metabolizing enzymes, and 5 have been reported to show no activity in vitro (Di et al. 2009). Hence, lower level of CYPBA6 enzymes in patients will have reduced bioavailability of DHA the major antimalarial metabolite and hence have lower antimalarial activity.

Major endemic areas of malaria like sub-Saharan Africa, Ghana, Sabah region of Malaysia have been evaluated for the presence of CYP2A6 genotype. Among these population of Ghana has high wild-type CYP2A6 along with 80 % alleles being CYP2A6*1A (Gyamfi et al. 2005), whereas Malaysian population has an allele CYP2A6*1A frequency of 32 % with only 8 % wild-type enzyme (Yusof and Gan 2009). Other Asian populations have been reported to carry several other alleles of CYP2A6 with even alleles that do not show any CYP2A6 enzyme activity at all. No activity variant of CYP2A6 is found about 11.5–20.1 % in Japanese, Chinese, and Thai populations (Gyamfi et al. 2005). Hence, artesunate is expected to be more effective in population of Ghana. In some parts of Thailand, about 10 % of patients have shown resistance to artemisinins (White 2008). Though it has been found by study that about 14 % frequency of CYPZ86 alleles have no activity in the Thai population and the antibiotic resistance is indicative to be related to CYP2A6 activity and ability to convert artesunate to DHA (Noedl et al. 2009), more studies require to be done to clearly establish the relation between the genotype and resistance to artemisinin-based therapy.

4.2.5   Malarial Parasite Resistance Genes

Several mutations in gene targeted by antimalarial drug have been identified which led to the resistance in vivo of ACT drug partners such as mefloquine, lumefantrine, amodiaquine, and chlorproguanil (Kerb et al. 2009; Mehlotra et al. 2009). Identification of genes and mechanism is important for controlling the infection. Research has yielded the information regarding the gene responsible and underlying mechanism of action resistance of some drugs against *P. falciparum* and *P. vivax*.

Chloroquine resistance (CQR) in *P. falciparum* has been linked to point mutation CQ resistance transporter gene (Pfcrt chromosome 7). The mutation *Pfcrt*-K76T is a reliable marker for CQR. While CQ-sensitive strain carries wild-type allele *CVMNK*, the variant *CQR* alleles are S_{agt}*VMNT* (Asia, South America, Africa), S_{tct}*VMNT* (South America), *CVMNT* (South America, Philippines), *CVIET* (Southeast Asia, Africa), and *CVMET* (Colombia). Another multidrug resistance gene (*pfmdr1* chromosome 5) is a parasite transporter gene. Polymorphism, point mutation, and copy number variation have been implicated in multidrug resistance. In different geographic regions, the *pfmdr1* two mutant alleles have been reported, namely,   86Y_184Y_1034S_104N_1246D found mostly in Asia and Africa and 86N_184F_1034C_1042D_1246Y predominantly from South America (Valderramos and Fidock 2006). The *pfcrt-76* and *pfmdr1-86Y* mutations have been related jointly to contribute in giving rise to CQR phenotype in addition to other likely parasite genes (Hayton and Su 2004).

P. falciparum DHPS enzyme (*pf-dhps*, chromosome 8) has been linked to resistance to the sulfa class of antimalarial drugs, while mutations in DHFR (*pf-dhfr*, chromosome 8) domain have been linked to high level of pyrimethamine resistance. Combination of sulfadoxine-pyrimethamine (S-P) treatment failure has been found to be associated with *pf-dhps* double mutant (437G with either 540E or 581G), combined with the *pf-dhfr* triple mutant (108N_511_59R) (Hayton and Su 2004; Hyde 2007).

Point mutations in *P. vivax* ortholog of *pfcrt* *(pcvg10)* are associated with clinical CQR. *pfmdr1 P. vivax* ortholog that is *pvmdr1* has been proven and has also been identified. Y97CF point mutation of *pvmdr1* has been linked to CQR. *pv-dhs* and *pv-dhr* gene point mutations have been identified and are suspected to link to clinical resistance in antimalarial S-P treatment (Hayton and Su 2004).

More data is required for new mutations in the parasite genes, and in addition more data is needed for therapy of other ACT drug partners like sulfadoxine-pyrimethamine and lumefantrine. A new rejuvenation is taking place in pharmacology and pharmacokinetics development of databases of antimalarial pharmacogenetics. Worldwide Antimalarial Resistance Network (http://www.wwarn.org/) has set up a module together with high-quality pharmacological research data for optimum drug dosage in light drug resistance information and adverse event reporting. The aim to achieve global cooperation will go a long way to personalized malarial treatment as per population needs.

4.3   Pharmacogenomics in Tuberculosis Treatment

4.3.1   Disease Burden

Infectious diseases are still a major challenge to our society; however, newer technologies have brought in new hope for control to this dreaded disease. Tuberculosis is caused by pathogenic bacterium *Mycobacterium* tuberculosis (MTB) and still infects about one third of the population of the world (www.who.int/topics/tuberculosis/en/). A person with active tuberculosis will infect about 10–15 persons in a year. For decades now we have very efficacious treatment regimes, but still we have not been able to eradicate the disease from the population. Now with the rise of human immunodeficiency (HIV) infection in the last few decades, people infected with AIDS are more at risk due to diminished immunity. The year 2010 saw as per estimates about ~8–8 million cases of tuberculosis, of which ~1.1 million deaths were reported among HIV negative

patients, while about ~0.35 million deaths in HIV-related TB were also reported.

During the last half a century (for about 50 years), the most effective treatment regimes have been the combination therapies of drugs that was because a single drug treatment was found to be in invariantly leading to resistance for the drug, leading to much more severity and complications (Crofton 1994). Due to rampant and unregulated use of tuberculosis drugs, however, this has led to emergence of multidrug resistant tuberculosis (MDR) (Fig. 27.4b).

4.3.2   Treatment Regimes

Now the treatment course is usually for 6 months with the combination of isoniazid, rifampicin, pyrazinamide, and ethambutol for the first 2 months. This has to be followed up by the next 4 months with isoniazid and rifampicin treatment. If the treatment is taken up with diligence and patient completes the whole drug course, then it has been reported that efficiency of the treatment is very high with more than >95 % patients getting cured and relapse is in less than 5 % of patients (Menzies et al. 2009). Another advantage of multidrug treatment is that the treatment regime helps in treating different population of tubercle bacilli. For the last 20 years, knowledge from the field of genetic molecular basis of drug treatment outcomes has helped us in the better management of and understanding of treatment efficiency and of drug. The difference in drug response is found among different individuals of the population. The individual person tends to show similar type of response to tuberculosis drugs that do not change over time. Thus, in light of above observations, we say that there is a huge variation in drug response among individuals due to variation in genes involved in drug metabolism, drug transporters, and drug targets compared to minimal within-subject variation as found from studies. Further studies on drug response revealed that 20–95 % of variation in drug pharmacokinetics is due to genetic factors (Kalow et al. 1998). The sequence variation in drug-metabolizing enzymes, drug transporters, or drug targets leads to the variation in drug response among individuals (Evans and Relling 1999; Evans and Johnson 2001).
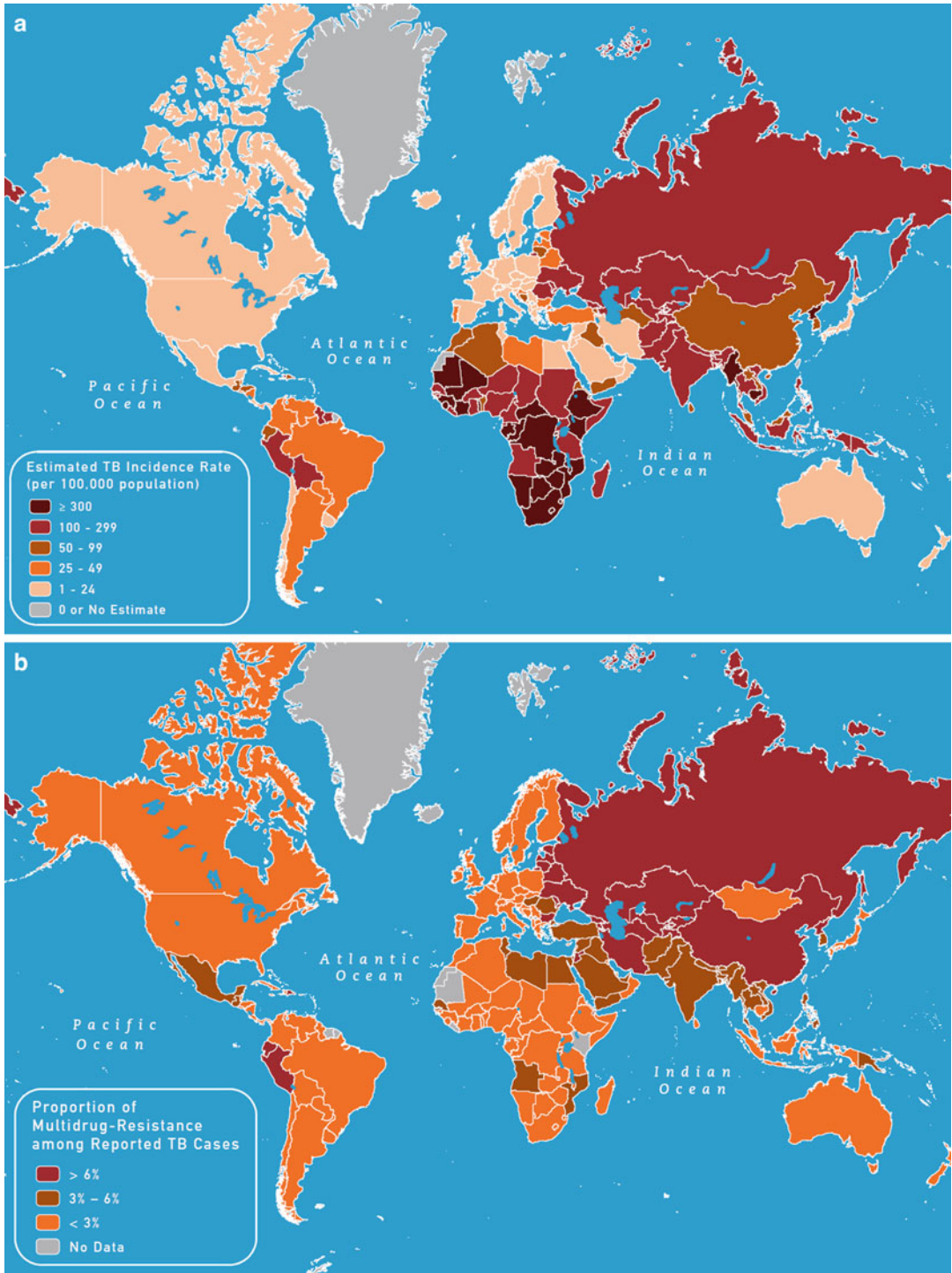
Fig. 27.4 (**a**) Estimated incidences of tuberculosis 2009. (**b**) Proportion of MDR TB among new TB cases 2009 (Map Source: Centre for Disease Control, USA- Health Information of International Travel 2012)

Some nongenetic factors such as nutrition organ function, age and other concomitant therapies, nature of disease, and drug interaction can also effect in drug response, but genetic determinant remains constant throughout the lifetime of the individual. Pharmacogenomics have played an important role in deciphering therapeutic efficacy of drug metabolism and occurrence of adverse events. Though research is still being pursued to decipher the intricacies of how genetic differences play an important role in regard to clinical application of the drug however, through research we have gained information on the role of genetic polymorphism with respect to drug efficacy for the treatment of tuberculosis. In this section we will discuss the knowledge we have gained through newer technologies in regard to different drugs being used for tuberculosis.

4.3.3   Isoniazid Pharmacogenomics

Since isoniazid has been in use for antituberculosis treatment since 1952, it is the most well studied of the lot (Ellard and Gammon 1976). This drug has been found to be tuberculosis specific in its action against tubercle bacilli and has relatively minimal toxicity. Now pharmacogenomics is playing a very important role in making isoniazid the first-line treatment drug.

Acetylation of isoniazid takes place mainly in the liver and gut mucosa. For any drug ingested in the body, it is absorbed and metabolized and then its soluble by-products are released or excreted out of the body. The drugs have specific retention and metabolizing rates depending upon their chemical composition and the genetic polymorphisms of the metabolizing enzymes.

The activation of isoniazid is catalyzed by highly polymorphic enzyme N-acetyltransferase (NAT2) and leads to formation of acetyl isoniazid. This is formed by the transfer of acetyl group from the acetyl coenzyme A to acceptor amine leading to formation of an amide. Acetyl isoniazid combines with several other cellular compounds to give a variety of metabolites which do not have any antituberculosis activity. The level of acetylating isoniazid that will be subjected to during metabolism in the body determines the

disease outcome. The level of bioavailability of the drugs determines whether the drug would be effective for elimination of the invading pathogen or toxic to the human body. Acetylation of isoniazid varies from individual to individual depending as per his or her genetic predisposition. Genetics determines the amount of active NAT2 enzyme that an individual expresses. The metabolism of isoniazid is catalyzed by NAT2 enzyme which takes place in liver or gut mucosa. Thus, the level of NAT2 gene expression is controlled by the type of polymorphism in *NAT2* that particular individual carries. Thus, for the pharmacogenomic and personal medicine in effect to succeed, the dosage for the drugs that are metabolized by NAT2 should be tailor made as per the enzymatic activity depending upon the polymorphic variant (Roy et al. 2008).

The enzymatic activity being highly variable Cascorbi and Roots (1999) has been studied over the years in human subjects who have been categorized as slow or rapid inactivators (http://www.brti.co.zw). The categorization has been based on the measure of capacity of NAT2 enzyme to acetylate isoniazid to acetyl isoniazid thus inactivating it. Here the rapid inactivators are those who have more concentration of active NAT2 enzymes than slow inactivators. Based on the new technologies, genotypic studies have led to further classification depending upon enzymatic activity NAT2 variant as rapid acetylators, that is, the wild type gene which codes for the completely active enzyme. Rapid acetylators are highly active forms of the enzyme denoted by *NAT2*4 allele. Patients harboring these alleles can tolerate conventional dosage of drug that is rapidly metabolized by NAT2 enzyme. Individuals who carry *NAT2* heterozygous alleles where only one of the allele is active/functional should be administered lower than average drug (those are NAT2 metabolized) dosage to get an optimum effective drug response without adverse drug response. Mutations in NAT2 enzyme in human individuals designated as *NAT2*5A*, *NAT2*5B*, *NAT2*6A,* and several others which lead to rendering the NAT2 gene activity are termed as slow acetylators which can lead to diminished drug clearance and toxicity.

The variation of frequency of slow acetylator gene is depended on the race, population type, and the ethnicity from one country to the next. It is found in a study that 90 % of Middle Eastern, 60 % in South Indian population, Caucasian and Negroid, and 72 % of the US population harbor slow acetylator gene. In mongoloid populations like the Eskimos, Japanese, and the Chinese, slow acetylators are found in only 10 % of study subjects. In another study carried out in a population of 18 healthy Caucasian, there is variability in isoniazid clearance. While isoniazid preparation is responsible for only 2 % variation and body weight accounted for only 3 % variation in isoniazid clearance, the majority variation of 88 % in isoniazid clearance was due to *NAT2* genotypes (Kinzig-Schippers et al. 2005). High-activity *NAT2* allele-carrying individuals have higher isoniazid clearance. Other studies have shown that 4–6 times more isoniazid concentration in individual is carrying slow acetylator NAT2 genotype (Parkin et al. 1997). A study estimating the comparison of urinary isoniazid excretion in Japanese patients to normal, healthy individuals showed that persons with higher number of active *NAT2* alleles had higher level of isoniazid acetylation (Kita et al. 2001).

The relation between isoniazid concentration in blood with drug efficacy and toxicity knowledge is important. Peak isoniazid concentration to minimum inhibitory concentration ratio has been proposed to serve as a means to outcome tuberculosis treatment (Mitchison 1984). Mean early bactericidal activity of isoniazid depends on its level in the plasma which in turn depends upon the variant *NAT2* genotype carried by an individual. Comparatively the mean bacterial activity is lower in rapid acetylators than in the slow acetylators (Donald et al. 2004). Therapeutic failure or relapse of infection is thus attributed to the lower plasma level due to rapid metabolism of isoniazid in rapid acetylator genotypes, while on other hand high level of isoniazid in slow acetylators may lead to the high level of toxicity (Weiner et al. 2003). NAT2 allele genotyping of tuberculosis patients prior to the treatment with isoniazid is the way forward. Dosage adjustment of isoniazid could be carried out depending upon

if the patient is harboring none, one, or two alleles NAT2 rapid acetylators. Thus, isoniazid would be more pharmaceutically viable for treatment of tuberculosis.

In pulmonary tuberculosis patients with known acetylator state, the response to isoniazid treatment analysis was carried out when it is administered alone or in combination with p-aminosalicylic acid. The study compared isoniazid response between *NAT2* slow and fast acetylators, and the study revealed that there is association with treatment response and bacteriological negativity (Selkon et al. 1961). Tuberculosis treatment trials used dosage regimes of daily, twice weekly (Tuberculosis Research Centre Madras, study 1970, 1973), or three times weekly drug regimes (Ellard and Gammon 1976). By means of controlled clinical trials, it was observed that using once a week uptake of isoniazid showed better clinical response to treatment compared to rapid acetylators, with cure rate of 20–35 %. It was postulated that metabolic status of isoniazid may have lesser clinical significance for daily isoniazid treatment regime as compared to thrice weekly or twice weekly treatments. In slow acetylator individuals, the peak concentration of isoniazid was higher than rapid acetylators, and the level of isoniazid decreased more gradually. The effectiveness of a drug in tuberculosis treatment is determined in terms of coverage and the exposure. Coverage has been defined as the number of hours for which bacteriostatic concentration of isoniazid is (0.2 μg/ml) maintained in the blood, while exposure is defined as area under concentration time curve. Both parameters have been found to be significantly greater in slow acetylators. Hence, in rapid acetylator individuals, there is a suboptimal concentration of isoniazid which leads to failure of once-weekly regime of isoniazid (Sarma et al. 1975). Other studies using once-weekly isoniazid-rifapentine were compared with twice-weekly isoniazid-rifampicin; treatment also showed that in case of once-weekly treatment regimes, treatment outcome was poor and was associated with isoniazid acetylator status of the patients (Weiner et al. 2003).

The clinical studies have shown that rapid acetylators having infected from combined

tuberculosis and HIV infection are at a further disadvantage since it has been found that antituberculosis drug bioavailability becomes suboptimal in those individuals (Gurumurthy et al. 2004). Tuberculosis patients having chronic renal failure are also at a risk from adverse drug reactions if they also happen to harbor slow acetylator genotypes of *NAT2*. Studies have shown that slow acetylators have higher peak isoniazid concentration, exposure, and half-life compared to rapid acetylators and healthy subjects (Gurumurthy et al. 1992). Hence, in the case of pulmonary tuberculosis patient also suffering from chronic renal failure, the isoniazid dosage should be administered based on their *NAT2* genotypes status. In adult pulmonary patients, studies were carried out to determine correlation between isoniazid dosage and *NAT2* genotypic and phenotypic status. Determination of isoniazid therapeutic dosage has shown that the fast acetylators need higher drug dosage to have an optimum positive treatment response. Fast acetylators tuberculosis patients when administered with 6 mg/kg isoniazid had similar exposure level as 3 mg/kg isoniazid administered to slow acetylators does (Donald et al. 2007).

In a further study in a population of South African tuberculosis patients, it was found that current treatment regimes were causing suboptimal exposure of isoniazid in patients having rapid acetylator status (Wilkins et al. 2011). Several field studies have further suggested that there is a need for calibration of isoniazid dosage as per the individual tuberculosis patient's age, acetylator status, and disease process for an effective antimicrobial outcome of drug treatment (Jeena et al. 2011). In children affected with tuberculosis, it was shown through several studies that the exposure of isoniazid was reduced in the rapid acetylators when compared to the slow acetylators and thus likely to affect the outcome of the treatment of tuberculosis (Cranswick and Mulholland 2005; Schaaf et al. 2005; McIlleron et al. 2009).

Though isoniazid has been found to be nontoxic during conventional regimes, two types of adverse reactions to isoniazid have been reported. The most common isoniazid toxicity reported is hepatotoxicity which affects 2–28 % of the patients (Tostmann et al. 2008). Another isoniazid-associated adverse event is peripheral neuropathy. Neuropathy usually occurs in slow acetylators due to administration of high doses of isoniazid (Devadatta et al. 1960). Hepatotoxicity is the major adverse reaction of isoniazid, and the factors that are responsible are NAT2 acetylation, oxidation by cytochrome P450s oxidation (CYP) 2EI, and detoxification by glutathione S-transferase (GST) enzyme activity (Roy et al. 2008).

Accumulation of acetyl hydrazine, a toxic metabolite of isoniazid, has been implicated in peripheral neuropathy, and the condition in humans is reversible by concomitant administration of pyridoxine (Zilber et al. 1963). Further, it has also been deduced that hepatotoxicity occurs due to hydrazine metabolites of isoniazid. Rifampicin also causes induction of isoniazid metabolism and inducing isoniazid hydrolase to produce isonicotinic acid and hydrazine. The rifampicin induction is more pronounced in slow acetylators compared to rapid acetylators (Sarma et al. 1986).

In some populations studies have established association with *NAT2* acetylator status and isoniazid-induced hepatotoxicity, while in other studies it has not. Studies in Japanese and Taiwanese populations have shown that the acetylator status of *NAT2* increased the risk factor for hepatotoxicity by 28-fold isoniazid-induced hepatotoxicity (Ohno et al. 2000; Huang et al. 2002). In another study on the Korean population, the *NAT2* slow acetylator status has been implicated to increase isoniazid-induced hepatotoxicity by two- to eightfold, and hence the *NAT2* acetylator genotype could serve as predictor of hepatotoxicity (Cho et al. 2007). *NAT2*53/5B*, *NAT2*6A/6A*, *48IT/T,* and *590A/A* diplotypes have been indicated and could be used as biomarkers for prediction of antituberculosis drug-induced toxicity (Ben Mahmoud et al. 2012). Slow acetylator *NAT2* alleles have been attributed to increase 3–8-fold to 28-fold higher risk in isoniazid-induced hepatotoxicity. But other studies in tuberculosis patients have not been able to find any association of *NAT2* acetylator status and the drug-induced hepatotoxicity. Case studies of Caucasian origin

patients with tuberculosis (Leiro-Fernandez et al. 2011), genotyping in an Indian population (Roy et al. 2001), and study on heterogeneous population of Hispanics, Africans, Caucasian, South American, and Asian have not reported any linkage between *NAT2* acetylator status and isoniazid-induced hepatotoxicity polymorphisms (Vuilleumier et al. 2006).

Cytochrome P450 2E1 is one of the enzymes of the hepatic microsomal enzyme system. *CYP2E1* gene encodes a member of the cytochrome P450 superfamily of enzymes. The cytochrome P450 proteins are monooxygenases which catalyze many reactions involved in drug metabolism and synthesis of cholesterol, steroids, and other lipids. CYP2E1 is an enzyme which brings about conversion of acetyl hydrazine to hepatotoxins, such as acetyl diazone and ketene, and brings about conversion of acetylonium ion (Nelson et al. 1976).

Polymorphism in CYP2E1 has been linked with increasing the risk factor associated with isoniazid-induced liver injury (Lee et al. 2010). The enzyme relocates to the endoplasmic reticulum and can be induced by isoniazid or its metabolite hydrazine. In animal model studies using rat, it has been found that CY2PE1 activity is linked to blood isoniazid levels (Yue et al. 2004). In the presence of variant genotype of *CYP2E1,* isoniazid could on the other hand inhibit the activity of the cytochrome P450 2E1 enzyme. Enhanced cytochrome P450 2E1 activity leads to the increased production of hepatotoxins and hence causing hepatotoxicity. Both NAT2 and CYP2E1 polymorphisms have been shown to be associated with susceptibility of first-line drug-induced hepatitis. CYP2E1 polymorphisms have been related to increase in risk of antituberculosis drug-induced liver toxicity. The common *1A/*1A* genotype of CYP2E1, in tuberculosis patients from Taiwan, has been linked to increase in the liver damage risk by 2.5 times. Presence of both slow *NAT2* acetylator status and the *1A/*1A* genotype further increases risk of hepatotoxicity when compared to presence of either of the single polymorphism (Huang et al. 2003).

The *CYPLE1 *6* and *1A*6*1D* haplotypes in Indian pediatric patients have been shown in sep-

arate study and have shown to increase the liver toxicity. Further, the common *1A* allele at *CYP2E1* has been implicated to hepatotoxicity in various heterogeneous population comprising of Asians, Africans, Caucasians, Hispanics, and South Americans (Leiro-Fernandez et al. 2011), but study done on a Korean population on the other hand could not find any association between *CYP2E1* polymorphism and liver toxicity (Huang et al. 2002).

The glutathione S-transferases are class of two distinct supergene families of proteins located in cytosolic and membrane-bound forms. Glutathione S-transferases are a class of enzymes that are responsible for detoxification of therapeutic medication, carcinogens, therapeutic medication, and toxic chemicals that are mostly electrophilic in nature. GSTs are present both in eukaryotes and prokaryotes. At present, eight distinct classes of the soluble and cytoplasmic mammalian glutathione S-transferases have been identified: alpha, kappa, mu, omega, pi, sigma, theta, and zeta. The cytosolic GST enzymes are encoded by at least five different loci coding for GST enzymes, distantly related gene families (designated class alpha, mu, pi, sigma, and theta GST), whereas the membrane-bound enzymes, microsomal GST, and leukotriene C4 synthetase are encoded by single genes and both have arisen separately from the soluble GST (Simon et al. 2000; Strange et al. 2000).

Glutathione S-transferase catalyzed elimination of toxic chemicals from the human body is carried out by making the toxic chemical soluble by conjugation with glutathione. In context of isoniazid-related hepatotoxicity, studies have indicated that deletions of GST mu 1 (*GSTM1*) and GST theta 1(*GSTT1*) are associated with liver damage (Cho et al. 2007; Huang et al. 2007). GST enzymes play an important role in removing the harmful metabolites of isoniazid from the body. The toxic metabolites generated by isoniazid metabolisms are from intracellular free radicals that are scavenged by conjugation with glutathione in reactions catalyzed by GST enzymes. Now, studies in Indian patients suffering from tuberculosis show that those harboring homozygous *GSTM1* mutations have higher risk

of hepatotoxicity. It was also found in a study on Taiwanese tuberculosis that patients have twice the risk of isoniazid-induced hepatotoxicity if they have homozygous *GSTM1* deletion. Thus, it can be inferred from similar studies that identification of GSTM1 deletion in patients will lead to the better management of isoniazid-induced hepatotoxicity.

4.3.4 Manganese Superoxide Dismutase Role in Drug-Induced Hepatotoxicity

Reactive oxygen species as we have been aware is a causative agent for damage to hepatic tissue. It has been deduced that level of mitochondrial oxygen species is reduced by the action of manganese superoxide dismutase (MSD). As the name suggests, MSD catalyzes the dismutation of superoxide into oxygen and hydrogen peroxide and is the first line of defense against reactive oxygen species. Polymorphism in the MSD enzyme has been found in a study in the population in Taiwan, where genotypes having T>C polymorphism in codon 47 lead to variant amino acid valine in place of alanine which increases in risk associated with antituberculosis drug-induced hepatotoxicity (Huang et al. 2007). The presence of valine at codon 47 causes the increased activity in the enzyme manganese superoxide dismutase which leads to the accumulation of the toxic byproduct hydrogen peroxide which can cause hepatotoxicity.

4.3.5 Rifampicin in Tuberculosis Treatment

Rifampicin has been proven to show concentration-dependent activity against *M. tuberculosis* and is a very important first-line drug against tuberculosis (Ji et al. 1993; Jayaram et al. 2003). Drug transporters P-glycoprotein and OATP1B transporters uptake rifampicin as a substrate and hence play an important role in distributing the drug throughout the body. The drug transporters are transcriptionally regulated by the nuclear receptors, i.e., pregnane X receptor and constitutive androstane receptor.

The phenomenon variation in bioavailability of rifampicin among individuals in a population on administration of standard dosage has been subjected to investigation by the scientists. The pharmacokinetics of rifampicin depends upon the uptake of machinery of the cells in the body. It has been found that there is a relation between pharmacokinetics and polymorphisms of genes that is responsible for drug efflux and influx. A study group of individuals suffering from tuberculosis who were categorize as per place of origin Africans versus non-Africans, it was observed that in drug transporter gene, *SLCO1B1* 463 C>A polymorphism leads to reduced rifampicin exposure and bioavailability (Weiner et al. 2010). The people of African origin (black subjects) that carry *SLCO1B1* 463 C>A gene polymorphisms have been associated with more pronounced reduced rifampicin exposure compared to people from other races. The study thus showed for the very first time that marked interindividual variation in rifampicin exposure can be attributed to *SLCO1B1* polymorphism. Another study in South Africa has also highlighted that the variant allele of *SLCO1B1* rs 4149032 polymorphism reduced the bioavailability of rifampicin in the body of the patients when present both in the homozygous and heterozygous states (Chigutsa et al. 2011). This finding has been attributed to the observation that there is about 21 % variability in drug clearance among the patients. Polymorphisms in the *ABCB1*, PXR, and CAR genes have not been found to affect the pharmacokinetics of rifampicin in the patients in any significant manner. Researchers have further predicted by means of stimulation that increasing the dosage of rifampicin in patients carrying *SLCO1B1* rs 4149032 would increase the plasma availability of the drug and thus would have a positive impact on the treatment outcome. However, more studies needed to be carried out to know the exact association of *SLCO1B1* gene polymorphisms between rifampicin bioavailability to provide an effective treatment regime.

As has been already mentioned, the variation in human leukocyte antigens also is known cause of disease susceptibility and the response to treatment has also in Indian patients the lack of human allele *HLA DQAI* 01201,* while the presence of DQB1*0207 has been reported to be associated with antituberculosis-induced hepatotoxicity (Sharma et al. 2002).

4.3.6 Aminoglycosides: Genetics of Adverse Reaction in Tuberculosis Treatment

Aminoglycosides are antibiotics which are molecules that consist of amino-modified sugars, and some of the drugs of this class have been used for treatment of tuberculosis. Aminoglycosides such as kanamycin, streptomycin, and amikacin have been used to treat tuberculosis. Aminoglycosides have known to cause ototoxicity. Ototoxicity is term used when there is damage to the ear (oto-), specifically the cochlea or auditory nerve and sometimes the vestibular system due to toxins. The association between aminoglycoside-induced ototoxicity and mitochondrial mutations has been found in a study in Chinese family. The deafness phenotype was found to be associated with C>T 1494 12S rRNA gene polymorphisms which could be induced with the administration of aminoglycosides or even get more aggravated (Zhao et al. 2004).

There is still no clear relationship of ethnicity and genetic background and response to antituberculosis treatment, and no single variant of *NAT2* and *CYP2E1* genes is associated with significant liver damage (Yamada et al. 2009). More extensive pharmacogenomic research is still needed for realization of robust personalized medicine for tuberculosis.

5 Case of Amphotericin B Toxicity

The antifungal medicine amphotericin B has been found to be effective and well quite toxic. Further investigations revealed that the immuno-modulatory role of amphotericin B also involves the induction of production of proinflammatory cytokine. In human cell the amphotericin-induced higher mRNA expression and cytokine production have been detected in studies (Rogers et al. 2000). The discovery of induction of proinflammatory cytokine production was able to explain the infusion-related toxicity effect like nausea, fever, chills, and hypotension that are characteristics of this cytokine release. It was also able to explain the mechanism of action of amphotericin B since the proinflammatory cytokines are responsible for the activation of monocytes, macrophages, and promote chemotaxis that led to enhanced immune response to the infection.

6 Omics on the Path of Drug Discovery

Since the advent of the era of omics technology, the number of drugs that have been discovered have not delivered as was first predicted especially in the case of infectious disease. Some of the shortcoming and the remedial measures have already been discovered in the previous sections. It has been found that even with high-throughput screening of number of drug, candidates have not been successful always (Payne et al. 2007). The field of scientific research which has become all encompassing and interdisciplinary has added strength along the way and opened new avenues. The field of biology is intertwined with structural biology and chemistry has given rise to the field of medical structural genomics. The exact causes of failure of high-throughput screens have not been well defined. The field of structure-based drug discovery has tried to overcome these limitations in the availability of chemical libraries and absence of structural information of many of the targets. The field of structure-based drug discovery has its origin from the field of X-ray crystallography and nuclear magnetic resonance (NMR) technology. With the availability of human genome sequences and pathogen genome sequence databases, the field of structural genomics has gained importance, and hence over the past decade, more than 20 such projects have been taken up. The field of structural biology has got a boost with the coming together of robotics and informatics in the biological research sphere (Haquin et al. 2008).

For the synthesis of an effective drug, by means of medical structural genomics, the protein which drug will affect should be well defined experimentally in both structure and functional aspects as the potential target. The protein should not only be well characterized structurally but also should be well defined as essential for the survival of the pathogen. Once drug and its protein target in a microorganism is identified, the field of medical structural genomics provides

rapid mechanisms using high-throughput X-ray crystallography and NMR assay system to find the ligand-bound structures. To identify such drug targets, it is very essential to know the complex host and pathogen interaction. The mode the pathogen uses to cause the infections is very difficult to elucidate and is a long process. The technologies of RNA interference and other gene knockout techniques should be complimented with experimental chemical biology approaches as microorganisms adopt multiple mechanisms for survival.

This has been emphasized for the fact examples of efforts of scientist for targeting the fatty acid biosynthesis pathways of bacteria. At first drugs were found to have high bioavailability and are potent against the bacterial replication in vitro. These compounds were subjected to be tested in animals and have been found to be not effective, the reason being that the bacterium utilizes the fatty acids present in their host vertebrates (Brinster et al. 2009). Hence, this study proves that there is need for more effective screening using the services of scientist from several spectra of biology like microbiologist, biochemists along with structural biologist, and chemical biologists to find effective molecules and compounds which can eliminate the pathogen under proper infective conditions (Hoon et al. 2008).

In pharmaceutical research scenario, it might also be possible that the drug target for a cell active compound is not known and then medical structural genomics provides a number of purified protein targets which can be assayed for binding interaction with bioactive compound by means of number of biophysical techniques like thermal stability (Ericsson et al. 2006). Such efforts have already been carried out in the field of protozoan pathogens. The program of Medical Structural Genomics of Protozoan Pathogens (http://msgpp.org/description.shtml) has been initiated to screen for drugs for ten protozoan diseases. The initiative has screening of thousands of potential antimalarial drugs against about 67 putative *Plasmodium falciparum* protein targets by expressing them in bacterial expression system in the laboratory and deciphering their 3D structures. Further, the com-

pounds are assayed for their effectiveness in live organisms and further validated in appropriate disease model. The terms chemical validation and drugability are often used in conjunction in such cases. Drugability is meant to be used how tractable a given drug target is for the development of a drug candidate, while chemical validation means that drugs have been found to be active in live organism. Drugs which fulfill the abovementioned criteria are worth the effort, time, and resources. In the future more collaborative efforts between medical structural genomic centers and the chemical biology institutes would be possible with the availability of collection of phenotypically defined compound that would have proven anti-pathogen activity resulting in the synergistic target validation and hit to lead development using structure-based drug design.

Pharmaceutical industry has now taken fragment-based drug discovery methodology as an alternatively less expensive and at times more effective than high throughput screening. Variety of methods like X-ray crystallography, NMR, surface fluorescence polarization, plasmon resonance yield, and differential thermal denaturation have been used to obtain macromolecular structure to screen libraries of small fragment that are obtained from compounds that are building blocks of drugs and hence can be more drug like. The fragment-based drug discovery is based on the screening libraries of small molecules on the rule of three which has the molecular weight <300 Da, the calculated log of octanol/water coefficient (Clog P)<3, and $3 \leq$ rotatable bonds and hydrogen bonds (Rees et al. 2004; Congreve et al. 2008).

Protein-protein interactions are important for all biological processes. Metabolic activities in the biological system are catalyzed by protein-based enzyme where in certain cases their activities are regulated by modulation of an equilibrium of an alternate, nonadditive, functionally distinct oligomeric assemblies (morpheeins) that have now been described as mode allostery. The oligomerization from the protein-protein interaction need not lead to gain in free energy, and it has been found that small molecules can block or

disrupt any protein-protein interaction that is necessary for biological systems, for example, being in the development of potent peptidomimetic inhibitor of HSV ribonucleotide reductase with antiviral activity (Liuzzi et al. 1994). The discoveries have opened avenues where structure-based information can be used to develop small novel antimicrobial molecules that can be made which can target protein-protein interfaces (Wells and McClendon 2007).

An example of this technology has been used to find small-molecule species-specific allosteric drugs for porphobilinogen synthase (PBGS). The oligomeric equilibrium for porphobilinogen synthase (PBGS) consists of high-activity octamers, low-activity hexamers, and two dimer conformations. In silico docking analysis from a small molecule library helped in selecting suitable compounds and molecules that had more affinity for docking PBGS allosteric site and thus were subjected to testing in vitro. In one compound whose inhibition mechanism is species specific, conversion of PBGS octamers to hexamers was thus identified (Lawrence et al. 2008). The above findings have led the way of targeting of oligomeric enzymes in pathogenic organism bacteria. Prime example is bacterial inorganic pyrophosphatases, which function as hexamer (Kankare et al. 1996). On the other hand, the eukaryotic, cytosolic, and mitochondrial pyrophosphatases function as homodimers (Oksanen et al. 2007) and hence have different interfaces than its bacterial counterparts as evident from the study of evolutionary aspects of inorganic phosphatases. In this context the strategy has been to target the oligomeric state of the bacterial inorganic pyrophosphatase enzymes to inhibit their activity rather than their conserved active site (Sivula et al. 1999). The technology has opened a novel pathway where more antibiotics can be developed.

The amount of knowledge of protein structures being generated is enormous; the need of the hour is dissemination of the knowledge databases among scientists and academic researchers on a worldwide scale. The protein structural know-how should be in the public domain without any constrains and copyright restrictions; also in addition the databases should be available free of any monetary charge. Structural genomic projects the world over have solved the structures of many proteins and have made the knowledge available for world community by submitting the structures to Protein Data Bank {PDB; http://www.wwpdb.org/}. Worldwide Protein Data Bank is the site whose mission is to maintain a single protein structural public database which can be accessed by the global community (Berman et al. 2007).

There is lot of structural data of protein-ligand complexes that is in private pharmaceutical industries not in the public domain. The economic incentives of drug discovery are driving force for this secrecy, but in this process there are a lot of valuable data that are duplicated and lots of valuable resources and energy efforts. The learning process from failures and successes in pharmaceutical corporate sectors is never known to the scientific community, and a major loss is of most valuable time. Hence, as we can see, the drug discovery resources are not being adequately utilized across the academia and industry, so there is suggestion to have open-access industry-academia partnerships as possible mechanisms to overcome the problem. A frame work is need where both financial and intellectual properties of the innovators are safeguarded when there structural data are deposited in the databases like PDB. A simple proposition would delay the release date of such structural data so that protection of intellectual property is feasible. Policies which can bring into the public domain structural data from the corporate world could only be possible by the concerted efforts of all stakeholders from industry, national, and international research funding agencies from all nations (Edwards et al. 2009).

Apart from easier dissemination of structural information related to infectious diseases and collaboration of structural biologist with medical chemist and molecular biologist, there is need for development of automation in several technologies to bring about unprecedented growth in the new drug discovery. Fragment-based drug design needs the support of high throughput technologies such that along with structural genomics, there will be more success in the determining protein-ligand structure determination.

7   Conclusion

Decades of experience have shown that the infectious diseases would emerge with more vigor and virulence. When the diseases are not controlled, then it would take a considerable toll of human health both in terms of mortality and morbidity. The life would be affected by emerging microbial disease-causing pathogen whatever the region, ethnicity, lifestyle, socioeconomic status, and ethnic background. Hence, the threat from infectious diseases is real and the situation is overtly challenging. The great advances in the genetics, genomics, and proteomics have the potential to take up the challenge in the coming decades. It is evident that these technologies have the potential to change the field of diagnostics, treatment, and discovery of drugs and vaccines. The need of the hour is to strengthen the public health programs at both the national and the international levels to strengthen research in the field of omics to fully realize the potential of scientific technologies that would usher in the era of pharmacogenomic-based personalized medicine.

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