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Review paper

System pharmacogenomics application in infectious diseases

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Abstract

The new era in systems pharmacology has revolutionized the human biology. Its applicability, precise treatment, adequate response and safety measures fit into all the paradigm of medical/clinical practice. The importance of mathematical models in understanding the disease pathology and epideomology is now being realized. The advent of high-throughput technologies and the emergence of systems biology have resulted in the creation of systems pharmacogenomics and the focus is now on personalized medicine. However, there are some regulatory issues that need to be addresssed; are we ready for this universal adoption? This article details some of the infectious disease pharmacogenomics to the developments in this area.

Key words: system pharamcogenomics; biological networks; infectious disease

Introduction

Systems's pharmacogenomics encompases disease characterization at the genomic level and its relation with modern technologies of drug discovery. Computational modeling of molecular pathways, deciphering sequence to structure activity relationships, gene and whole genome assembly techniques have been possible owing to advancement in computational biology and high-throughput technologies. Today computational methods are increasingly being used to understand gene expression and protein–protein interactions and to carry out genome-wide association studies [1]. The focus is on the identification of pathogenic pathways and the disease phenotypes to improve target identification, drug selection and clinical trial design. Owing to the large-scale data generation as a result of high-throughput technologies, computational pharmacogenomics has become an evolving science, which not only aims at sequence retrieval but also focusses on the data mining. This has made the prediction of drug targets, mode of action of a drug and its response relatively easier. In this process, one of the major challenges faced is the interpretation of the large amount of data being generated. To achieve this, various data mining techniques are now being developed. These techniques use methods derived from computational linguistics, language processing and artificial intelligence to retrieve the data in a much faster manner, enabling the understanding of patterns underlying a particular disease [2]. This would particularly prove to be useful in drug designing, as the reasons behind drug failure or success can be easily understood.

Network medicine

It is known that drugs interact with the molecular targets like receptors and enzymes. They thus can act as agonists and enhance the acitivity of the molecular targets or also act as antagonists and inhibit the functioning of the target proteins. This results in the alteration of not only the function of several biological networks but also tissue- or organ-level modifications [3–5]. Therefore drug interactome modeling is essential to derive a

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systems level understanding about any particular drug and its effect on the body.

It is imperative that drugs have their own set of adverse effects along with the required therapeutic effect. For example, the side effects of non-nuceloside reverse-transcriptase inhibitors (NNRTIs) that are used as antiretroviral medication are that they also cause skin rashes. To avoid these effects, appropriate dosing of the drug has to be determined. During the dose optimization process, genetic differences of an individual have to be accounted for, as genomic variations could significantly alter the metabolism and therapeutic response of a drug. This also justifies the need for identifying and classifying genetic changes and genomic variations in individuals for the design of personalized therapy [6]. Owing to the similarity of the molecular targets in different tissues and organs, side effects of drugs can be extended to multiple tissues and organs. For example, anticancer drugs cause hair loss by affecting the hair follicles. Some drugs can induce harmful off-target effects like the antihistaminic drug terfenadine, which was withdrawn by the Food and Drug Administration, as it was found to interfere with cardiac potassium channel currents, leading to fatal arrythmias. Thus, the effect of any particular drug and the patients ability to tolerate the dose and side effects of a drug depends on many factors, including the patient's age, genetic makeup, preexisting illness if any, the dose of the drug administered and the interaction of the drug with any other drugs the patient might be taking [7].

Systems pharmacogenomics in the area of personalized medicine

Pharmacogenomics and pharmacogenetics deal with the study of the role of inheritance and its role in inducing variation in drug response. Herein, studies usually focus on the monogenetic traits, i.e. genetic variation in drug metabolism. The rapid advances in the field of human genomics has resulted in the evolution of pharmacogenetics to pharmacogenomics. At the same time, with the increasing importance of pharmocotranscriptomics and pharmacometabolomics, systems pharmacogenomics has begun to emerge [8, 9]. Systems pharmacogenomics deals with not only the effect of a drug on one particular target. The aim is to derive a systemic view of the possible interactions a drug may have. This helps to understand not only the effect of drug but also the associated side effects of a drug by looking at the effects of a drug in context of cellular networks as well as exploring relationships between drugs [10, 11]. Thus the individual patient susceptibility and the side effects can be accounted for and the off-target effects can be reduced.

Bioinformatics analysis of data sets that arise as a result of *de novo* genome sequencing, gene expression studies or targeted resequencing helps in the identification of druggable targets in the system under question. The massive data generated as a result of sequencing of genes and genomes have resulted in the identification of genotype/phenotype correlation with drug responses [12]. Thus, the basic mechanism behind the therapeutic intervention for any disease can be dissected in a piecewise manner by comparing the genomic scenario with the transcripiton and translation patterns of genes. In this regard, techniques such as hierararchial clustering, k means of clustering, deterministic annealing, self-organizing maps and graph theory approaches are being used to analyze reproducibility of data and correlate expression patterns with the disease progression [13].

Models such as Artificial Neural Networks (ANNs) and Gene Regulatory Networks (GRNs) are capable of statistically validating the data sets, recognizing patterns and the interplay of thousands of genes/omics data sets [14]. The nonlinear dynamics behind biological systems and the associated genomic control are well solved with interaction networks and mathematical models such as GRN, Protein-Protein Interactions (PPI) and metabolic networks. Quantitative network diagrams, probabilistic graphical models such as Bayesian networks, prediction models based on differential equations and state space models aid in better understanding about biological pathways at systems level [15]. This is particularly useful for therapeutic interventions and drug design, as the focus is not on the one target–one drug model but rather on deriving a systemic view of a particular disease.

Biological networks

Network approaches in biology are useful for organizing high-dimensional biological data sets and extracting meaningful information. Biological networks are composed of the nodes and edges; nodes can be either genes, proteins or metabolites, which are in turn connected by edges. Edges represent the regulatory interactions, relations and other shared relationships between the nodes. A biological network is generally built to describe a biological process. To obtain relevant information pertaining to the network, networks have to be modeled, visualized and interpreted. Network visualization is a key method, which helps biologists understand the network and interpret the information that lies within the large data sets. Networks are built from the basic building blocks like genes, proteins, and metabolites according to which a network is classified. While the gene regulatory networks and signaling networks describe how genes can be activated or repressed, protein–protein interaction networks describe the interaction among the proteins, and the metabolic networks depict the flow of metabolites and how they are transformed [16–18] (Figure 1). Networks are visualized in the form of network graphs, which can be analyzed using several algorithms that are largely based on the graph theory. While performing the analysis, properties such as the degree of nodes, betweeness and centrality measures are used, which provide information about the importance of different nodes within the network. Network analysis is particularly useful in the identification of important nodes, which are generally referred to as the 'hubs'. Hubs are classified either as party or date hubs based on their connectivity and their ability to influence other components in the network. The 'party hubs' generally coordinate a specific cellular process or protein complex, while the 'date hubs' are co-expressed, link together and convey information between modules or complexes (Figure 2).

Systems biology for infectious diseases

Infectious diseases are a huge social, economic and health burden especially in the tropical countries. With the lack of adequate drugs to treat the diseases and the growing problem of drug resistance, it has become essential to identify druggable targets in the causative organism. The role and importance of mathematical models in increasingly being realized in the field of infectious diseases. These predictive models provide a framework for understanding the initiation, progression and outcome of a disease. Using integrated data sets generated from a combination of 'omics' technologies, system-wide host/pathogen interaction networks can be generated. By studying such models, the overall complexity of the molecular processes within the microbial organisms and their interaction with the host can be easily understood. A model is an abstract representation of

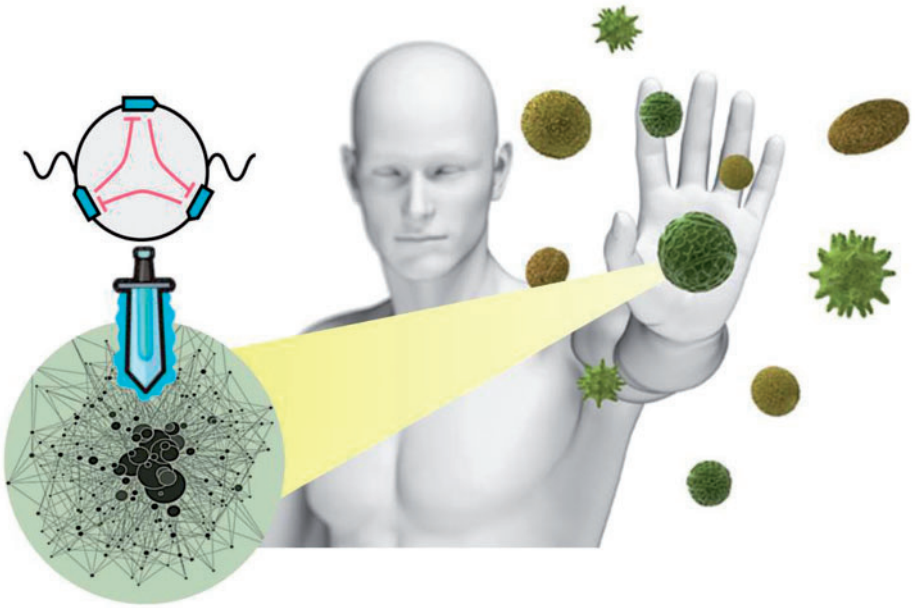

Figure 1: System pharmacogenomics and biological networks. (A colour version of this figure is available online at: https://academic.oup.com/bfg)

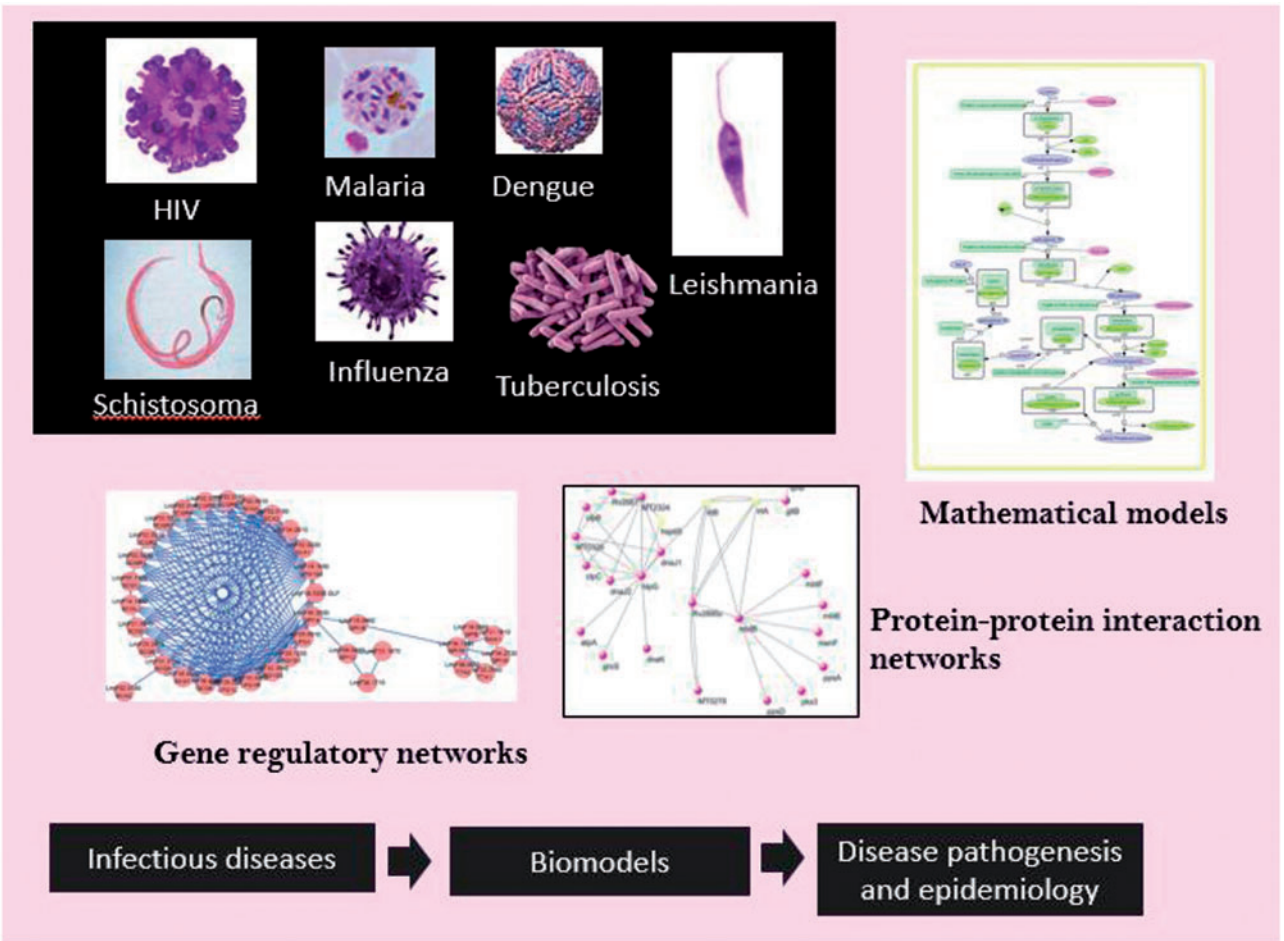

Figure 2: Application of biomodels in dealing with infectious diseases. (A colour version of this figure is available online at: https://academic.oup.com/bfg)

the disease progress and dynamics and its complex structure needs to be resolved. Models are used for a variety of purposes but by large are used to make clinical and biological observations about a particular disease. In the present review, we aim to discuss the possibility of using systems biology as a tool for guiding research toward personalized medicine in the field of infectious diseases [19].

HIV

HIV is an infectious viral disease that affects nearly 36.7 million people worldwide. The mortality rate of this disease is 1.1 million (www.who.org). It is known now that viral proteins that play a major role in the infection frequently interact with the host genes. Dickerson, 2010 [20] has proved that proteins with a high degree of nodes are likely to be HIV-interacting proteins and are likely to be essential. Several HIV proteins like Gag, VPR, Tat, Env, Nef, Pol, Vpu, Rev and Vif are known to interact with several host proteins. These proteins are hubs and bottlenecks of the host [20]. Of all the HIV proteins, Tat and Gp120 proteins have more connections than other proteins. Based on the centrality, betweeness and number of connected nodes, the virus–host dynamics are defined. Some of the other proteins that are highly connected are the HIV dependency factors. Network analysis indicated that HIV reprioritizes cellular processes such as transcription and proteasome activity by hijacking the transcriptional machinery of the host. Also, the rate of replication of the virus is much faster than the immune response of the host [21]. This is probably the reason why development of vaccine for HIV is proving to be difficult. Studies carried out in macaques have revolved around systems analysis of RNA expression profiling of peripheral blood mononuclear cells (PBMCs) and lymph nodes, which was useful to predict the CD4 and the CD8 T cell responses. Similarly, analysis also revealed that viral load was lesser in people who carry B27, B57 and B58 HLA alleles. This was associated with more enhanced CD8 response [22]. Systems analysis can also demonstrate the association of antibody titers with immune pathways like the toll-like receptor (TLR) pathway. It is an onus to identify the protective genetic variations and the functional differences in the HIV-specific CD8 T cells and their controlling molecular networks for a more rational vaccine design.

Tuberculosis

Mycobacterium tuberculosis is the causative agent of tuberculosis. This infectious disease affects nearly 2 million people worldwide every year. The disease is prominent in parts of India where extensively drug resistant (XDR) cases of tuberculosis are also reported. Tuberculosis is generally treated with drugs like isoniazid, ethambutol, pyrazinamide and rifampicin. There are around 12 vaccines that are currently in clinical trials to treat tuberculosis. The most frequently used vaccine is Bacillus Calmette-Guerin (BCG). However, this vaccine fails to induce immunity especially in adults who have a weakened immune system. To improve tuberculosis vaccines, it becomes essential to re-engineer BCG to achieve better priming. Progress in this direction would need a systems level understanding of the mechanisms that are involved with the immune protection against Mycobacterium tuberculosis (MtB). This would involve understanding the transcriptional signatures of infected individuals. The whole genome sequencing of the virulent strain of MtB (H37Rv) by Cole and his group has been a landmark in the field of tuberculosis (TB). Since then, focus was laid on using 'omics' approaches to better understand the disease progression and the associated immune responses of the host. Berry *et al.* [23] have identified 393 transcript signatures for active TB in the intermediate and the high burden settings and around 86 transcript signatures that discriminate active TB from other inflammatory and infectious diseases. Pathway analysis indicated that TB signature was dominated by interferon (IFN)-inducible gene profile, which consisted of both IFN-γ and type I IFN-$\alpha\beta$ signaling [23]. Maertzdorf *et al.* [24] have studied a set of differentially expressed genes from a systems point of view and identified Fc gamma receptor 1B as one of the most differentially expressed genes. The group has also identified that higher expression of particular gene clusters involved in apoptosis and natural killer (NK) cell activity in latently infected TB cases are likely to be controlling the dormancy of tuberculosis bacilli in the host [24]. In addition to the transcriptomics, metabolic pathways such as mycolic acid synthesis, which is essential for the infectivity of MtB, have been modeled in the form of mathematical model. A detailed model of the mycolic acid synthesis pathway has been built by Raman *et al.*, and the pathway was studied by flux balance analysis. *In silico* gene knockouts and exposure of the system to various drugs have been done *in silico*, which has led to the identification of genes that are essential to the pathway [25]. Similarly, Singh and Ghosh [26] have developed kinetic models of the tricarboxylic acid (TCA) cycle and the glyoxylate cycle of Mtb to understand the importance of various enzymes in the pathway. Their observation indicates that isocitrate dehydrogenase 2 is required for a flux through the glyoxylate bypass in the persistent mycobacteria. Thus, isocitrate dehydrogenase could be a good drug target for treating tuberculosis [26]. Studies have also focused on modeling the host–pathogen interactions in tuberculosis. Boolean networks such as the model developed by Raman and his group have focused on understanding and predicting the outcome of tuberculosis based on the host–pathogen interactions. It was observed that processes such as phagocytosis and phagolysosome maturation can be impaired by reducing the levels of cytokines such as tumor necrosis factor alpha (TNFalpha) and IFN gamma. In addition, enhancing the production of IL10 and defense proteins like SapM favors clearance of MtB from the host macrophages [27]. Even a multi-level workflow in terms of genome-wide gene expression profile, growth analysis by flux balance analysis (FBA), studying the protein–protein interaction networks and identifying the evolutionary conservedness of the set of genomes to trace the functional basis of essential genes can be done. Study by Ghosh *et al.* has identified 283 genes that are highly essential for MtB survival [28]. Last but not the least, even the problem of drug resistance can be solved by developing a drug–target interaction network. One of the examples in this direction is the identification of RecA, Rv0832c, Rv0892 and DnaE1 proteins, which can be drug targets for combating drug resistance in MtB [29].

Influenza

A decade of high-throughput screening of human viruses has led to the construction of intraviral and virus–host interaction networks. Theories on various factors governing the interactome organization have led to the computational analysis of various intraviral networks such as that of Epstein-Barr Virus (EBV), Influenza Virus Type A (FLUAV), Hepatitis C Virus (HCV), herpes simplex virus type 1 (HSV1), Kaposi's sarcoma-associated herpes virus (KSHV), SARS coronavirus, Vaccinia virus (VACV) and Varicella zoster virus (VZV). The host and viral interactome and their interplay determine the rate of virus

replication and eventually the pathogenesis of a virus. One notable example of a model developed to determine the phenotypic consequences of viral–host interactions is by Gulbahce *et al.* [30]. Together the team has developed a model of two DNA tumor viruses, i.e. the Epstein–Barr virus and the human papillomavirus, for understanding the viral–host interactions of these two viruses. Analysis of the interactome gives insight into various proteins targeted by the virus. Chassey *et al.* [31] have identified two of the proteins expressed by influenza (NS1 and NS2), which interact with around 79 different proteins belonging to the host. The interacting host proteins were seen to regulate viral replication. The major protein that was found after interactome analysis was ADAR1, which colocalizes with NS1 protein of the virus and helps in improving viral infectivity [31]. Similarly, Neveu *et al.* [32] have developed viral–host interactome networks of E6 and E7 oncoproteins belonging to 11 distinct human papillomaviruses [32].

Malaria

Malaria caused by the parasite plasmodium is one of the oldest infections known to mankind. Today, around 243 million people are affected by this disease. Lack of effective vaccine and reduced efficacy of antimalarial drugs with the increasing drug resistance has contributed to increase in the incidence of malaria. One of the earliest mathematical model built pertaining to the infectious diseases is the Ross model, which clearly highlighted the power of prediction of disease transmission using mathematical models. Since then, a lot of complex models have been developed that deal with various epidemiological aspects of malaria as well as drug target identification. These models are a function of host- and parasite-specific factors, their interactions and environmental conditions. While the basic Ross model focused on the susceptibility of humans to malaria, models such as the Macdonald, Anderson-May models have gone deeper into the parasitic life cycle within the host [33, 34]. More complex models have accounted for the age-specific immunity [35] and the immune response and reoccurrence of the disease [36]. In addition to the disease transmission, models have also been used to depict drug sensitivity of the host [37] and the environmental factors affecting the mosquito population [38]. In addition to the epidemiological models, recent trend has been the identification of druggable target proteins that are directly associated with the pathogenesis of malarial parasite [39].

Dengue

Dengue is an infectious disease prevalent in the tropical and the subtropical regions of the world. As many as 400 million people are infected with dengue annually. It is a viral disease that is transmitted by mosquitoes. The most effective protective measures so far used to prevent the disease has been to avoid mosquito bites. Mathematical models such as the Derouich model have highlighted that partial vaccination along with vector control is essential for controlling the disease. They have shown that number of mosquitoes and the vaccination level of the susceptible population appear to be the two most important variables in the model [40]. Other models such as that of Feng model suggests that mosquito activity levels appear to have higher impact on the outbreak [41]. The probability of getting bitten by the mosquito and its relevance has been explored in the model developed by Syafruddin and Noorani in the year 2013 [42]. Currently there are no therapeutics used to treat dengue, and the only ways and means of controlling dengue have been the vector control. Hence, most of the models developed have laid their focus on the population dynamics of the susceptible, infected, exposure and removed vector transmission of dengue fever.

Schistosomiasis

Schistosomiasis, an infectious disease caused by the nematode Schistosoma affects nearly 210 million people in 76 countries. The parasites reside in water bodies and can penetrate the skin of people who come in contact with the contaminated freshwater. Lipids play an important part of parasitic membranes. In a study done by Shinde *et al.*, genome scale reconstruction of the lipid metabolism of the parasite has resulted in the identification of choline-phosphate cytidyltransferase (CCT) as an important drug target [43]. It was observed that targeting CCT would cause result in developmental defects in the parasite. Schistosomiasis is a relatively understudied disease and there is an urgent need to identify drug targets in the parasite, as there are reports that the parasite is developing resistance to existing drugs such as Praziquantel [42].

Leishmaniasis

Leishmaniasis is an infectious disease that affects nearly 12 million people worldwide (www.who.org). Identification of novel drug targets has become increasingly important for this disease, as the existing chemotherapy suffers from drawbacks like side effects and the problem of drug resistance. Targeting metabolic networks could lead to the identification of probable chemotherapeutics. This could be done by assessing the role of enzymes in the production of metabolites, which directly regulates the growth and survival of the parasite. Essential reactions in the pathways can be probed for categorizing the drug targets. In this direction, models such as iAC560 and iAC142 developed by Subramanian *et al.* and Chavli *et al.* quantitatively access the importance, essentiality and the role of chemotherapeutic targets in the growth of parasites like Leishmania. These studies based on the amastigote stage of the parasite have predicted by flux balance analysis the reactions that exert the maximum effect on the parasite survival. Further *in silico* reaction knockout analysis has resulted in identification of a minimum inhibition threshold that a chemotherapeutic drug needs to exert to reduce the parasite growth. One of the major challenges faced in the construction of the models is that *Leishmania major* is not well-characterized experimentally. Hence, models need to be updated as and when adequate information is obtained. Mandlik *et al.* have shown the importance of mathematical models in the identification of drug targets. Analysis of the model pertaining to the sphingolipid metabolism of the parasite has revealed that enzymes such as Inositol phosphorylceramide synthase (IPCS), Sphingosine-1-P lyase (SPL) and Serine palmitoyl transferase (SPT) are crucial for the production of metabolites like ethanolamine phosphate and sphingolipids like inositol phosphoryl ceramide [44]. Subramanian *et al.* have reported that reactions catalyzed by glutamate dehydrogenase and phosphosphogluctamate that are essential for the parasite growth. It was observed that any reduction in the enzyme activity and or reaction flux leads to reduction in the survival rate of the parasite, suggesting that both these enzymes could be good druggable targets. In addition to the *in silico* predicted flux profiles of *L. major* metabolism, the importance of evolutionary rates of these metabolic enzymes has also been highlighted [45]. On similar lines, in a study done by Chavli *et al.*, evaluation of fluxes through the F_0F_1-ATP synthase reaction resulted in the

construction of iAC560 MODEL. Lethal double deletions were identified, which are some of the promising drug targets in the leishmania genome. A comparison with the metabolic networks of the host created a framework for probing the difference in the host parasite metabolome. This type of novel network analysis is particularly useful for the identification of unique drug targets, which can be targeted for controlling the emergence of diseases like leishmaniasis [46]. Models can also be built for epidemiological purpose such that the transmission dynamics of leishmania can be understood. Stauch *et al.* have built an ordinary differential equation-based model for *Leishmania donovani*, which highlights the infection dynamics in sand-fly, the animal hosts and immunocompromised patients. This model provides information about the various stages of the infection and relates it to the immune response of the host, thereby improving existing chemotherapeutic interventions [47].

Future prospects

Systems level understanding of biological systems has led to the advent of P4 (personalized, preventive, predictive and participative) medicine. As models represent an abstraction of real-world systems, the importance of mathematical models has been increasing among biologists. To obtain a clear understanding of the disease pathology, its advent, transmission and impeding problem of drug resistance, a clear logical understanding about a systems behavior is important. Model analysis would help in understanding hitherto unknown scenarios that are important in disease progression and spread. The effect of variables and parameter changes directly correlate with the predictions made from a model. One of the challenges faced in model building is the non-availability of several parameters. It is therefore essential to estimate the variables well and build models that can incorporate the essentials of host–parasite–vector interactions and clinical outcomes of any infectious disease. The importance of mathematical models has been first realized in the field of infectious diseases and it is imperative that our efforts further facilitate research in this area.

Conclusion

Although the integration of systems biology with pharmacology has its own facets, opening a myriad of biological questions to the researchers at present, the coming age has evolved on its own with expert clinicians playing a prominent role. Clinical candidates need to expand vertically by licensing technologies for product development. The systems pharmacogenomics has the potential to herald into a new era with financially sustaining deals. Collaboration may impact the commercial development trajectory centering on systems pharmacogenomics.

Key Points

- The central task of systems biology is to gather information, integrate and analyze data sets in the form of biological networks.
- Disease-specific gene regulatory, metabolic and protein–protein interaction networks can throw light on the various nodes that are perturbed in the diseased state.
- Aiding systems pharmacogenomics with the interdisciplinary approaches may herald into a new area of therapeutic intervention against infectious disease.

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